FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

FORENSIC TOXICOLOGICAL SCREENING AND CONFIRMATION OF 800+ NOVEL PSYCHOACTIVE SUBSTANCES BY LC-QTOF-MS AND 2D-LC ANALYSIS.

A dissertation submitted in partial fulfillment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

CHEMISTRY

By

Melanie Nicole Eckberg

2018

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

This dissertation, written by Melanie Nicole Eckberg, and entitled Forensic Toxicological Screening and Confirmation of 800+ Novel Psychoactive Substances by LC-QTOF-MS and 2D-LC Analysis, 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.

_____
Jose Almirall

_____
Yong Cai

_____
Georg Petroianu

_____
Stanislaw Wnuk

_____
Anthony DeCaprio, Major Professor

Date of Defense: August 24, 2018

The dissertation of Melanie Nicole Eckberg is approved.

_____
Dean Michael R. Heithaus
College of Arts, Sciences, and Education

_____
Andrés G. Gil
Vice President for Research and Economic Development
and Dean of the University Graduate School

Florida International University, 2018

DEDICATION

This dissertation is dedicated to my friends and family, especially my parents, without whom, none of this would have been possible. Thank you for all of your support, your patience, and your love. I am excited to close this chapter of my life and begin the next with all of you by my side.

"For last year's words belong to last year's language and next year's words await another voice. And to make an end is to make a beginning."

-T.S. Eliot

ACKNOWLEDGMENTS

First, I wish to express my gratitude to Dr. Anthony DeCaprio for serving as my research advisor and allowing me to be a part of his research laboratory. I would like to thank Dr. José Almirall, Dr. Yong Cai, Dr. Stanislaw Wnuk, and Dr. Georg Petroianu for serving as my committee members. I am extremely grateful for your constructive criticism, words of advice, and willingness to help me become a better scientist. Thanks to Dr. Luis Arroyo for introducing me to the QTOF and helping me to learn most of what I know about that instrumentation. Special thanks to Ralph Hindle for your invaluable assistance and guidance throughout all of my work and for teaching me a lot about everything. I also wish to thank Dr. Dwight Stoll of Gustavus Adolphus College for welcoming me to his laboratory for a week in order to teach me the basics of 2D-LC and help in my preliminary data acquisition. Without your help, my 2D project would have never gotten off the ground.

Words cannot express how grateful I am to my friends and family for coming with me on this journey. I could not have made it through these past five years without all of you. When I came to Miami I had no idea what I was in for. I am so lucky to have found a family here that loves and supports me and can understand what I'm going through. Vanessa (aka V$), you were the first to really welcome me into the lab and although we only shared that space for a few months, you quickly became one of the most important people in my life. Thank you for always being so real and for keeping me grounded and for letting me be a part of your family (kthxluvyoubye). Erika, I literally could not have done this without you. I am so happy to have been adopted into your life.

You've kept me sane (and well fed) and were always there for me to talk to. You and Mitch have truly become family and I am so excited to be Auntie Mel from now until forever (love you J!). James, what would I ever do without you? I'm so lucky to have found another brother that gets me and shares in my love of all things pop culture. Thank you for being my outlet when school got tough and I needed a break. I look forward to many more years of Disney adventures, personalized theme park plans and guides, Harry Potter madness, all the sports, geeking out over movies and TV, going to cons, and so, so much more! To W.E., thank you for helping me learn to accept my flaws and embrace them as sources of strength. You have shown me that I don't have to be perfect to do great things and helped me to truly appreciate the value of my Found Family. Haley, you have been such a great support even as you were going through your own grad school experience. Thank you for being my link to home and to the Ramily and I can't wait to be that support for you! Joanna. You have literally been there since Day One all the way back in our dorms at VCU. Even though we have been thousands of miles apart as you've undergone your own PhD adventure, your unwavering love and support has always been felt. Who knew that we would both end up on this journey and I can't imagine the last nine years without you! D.H., look at where we are, look at where we started. You have been such a source of strength and support for so many years. I definitely could not have kept any amount of sanity without your friendship. Thank you for always being there for me and for always politely nodding and listening to my science woes. I don't think I would have been able to grow as a person as much as I have without you and I look forward to so many more (non-science) adventures!

To my lab family, I'm not sure how I will get through the days without you. Allen, we started this journey together and I am so lucky to have had your unwavering support throughout the last five years. Your humor, your advice, your candor, and your willingness to always help have gotten me through more days than you may realize and I will always treasure our Taco Bell visits. Ashley, you were our first "junior" student but have become much more of a partner in the lab and outside of it. Your friendship has meant so much to me and I know for sure that I definitely could not have gotten to this point without you. Jenna, to say that you have made my life interesting is a vast understatement but I wouldn't change any of it. You've been a great sounding board and support for me and I am really going to miss our coffee runs. Josh, thank you so much for teaching me how to do almost everything in the lab. Even though we may not agree on our sports teams, I guess you are a pretty good friend and I know I will be learning from you for years to come. Quentin, you may not officially be part of the lab, but we might as well have given you access for all the time you spend there. Thank you for being another voice of reason amongst our group, and for making sure I was having fun and relaxing. I will never forget all of our adventures.

Kat, when we first met back at Visitation, I knew I would be fortunate to have your friendship. The last 5+ years have never been dull and I am so glad that we got assigned to room together to start with. I will never forget our adventures of living in Doral together and all of our years of friendship after that. Thad, thank you for always being another sounding board for me and for being another "adult" here. I am so grateful for your help whenever I asked and for your friendship throughout.

To my FIU family, thank you for your incredible support! I absolutely would never have been able to get through any of these years without you. Maggie, you were always there to help me navigate FIU and the Department and I am forever grateful for your help. Pupi, you have been so supportive and helpful and I am going to miss you and your chats so much. Jackie, thank you for always being another resource for me to go to, but also someone I could vent with and call a friend.

To my family, thank you so much for all of your little shows of support. From the little postcards, to the letters, to the text messages sent every now and then, these little bits of love were extremely appreciated and helped pick me up when I really needed an extra boost.

To my mom and dad, your unwavering love and support (and attempts at understanding when I vented to you about science) helped keep me going more than you could ever imagine. You have always been there for me and even more so during my time in Miami when I have had some of my highest highs and my lowest lows. You have sacrificed so much for me to get to this point and those sacrifices and efforts do not go unnoticed or unappreciated. This dissertation is for me, but also belongs to you. I never would have gotten this far without you and I'm proud to call you my parents. Nick, I could not be more proud of you or more grateful to have a little brother like you. We have always been close, but even when we were separated by a continent, I have always felt your love and support and I can't wait to be back in the same state with you.

I love you all and I am so thankful to have had your love and support in my life. Thank you being a part of my journey and this is for you as much as it is for me.

ABSTRACT OF THE DISSERTATION

FORENSIC TOXICOLOGICAL SCREENING AND CONFIRMATION OF 800+ NOVEL PSYCHOACTIVE SUBSTANCES BY LC-QTOF-MS AND 2D-LC ANALYSIS

by

Melanie Nicole Eckberg

Florida International University, 2018

Miami, Florida

Professor Anthony DeCaprio, Major Professor

Novel psychoactive substances (NPS) represent a great challenge to toxicologists because of the ability of illicit drug manufacturers to alter NPS chemical structures quickly and with ease to circumvent legislation regulating their use. Each time a new structure is introduced, there is a possibility that it has not been previously recorded in law enforcement or scientific databases. Many toxicology laboratories use targeted analytical methods that rely on libraries of known compounds to identify drugs in samples. However, these libraries do not include large numbers of NPS, which could result in non-identification or detection.

High-resolution mass spectrometry (HRMS) has been suggested as a method for screening a wide variety of analytes because of its high sensitivity and mass accuracy as compared to some other forms of mass spectrometry. The technique can generate characteristic MS/MS spectral data for use in compound identification. The main goal of

this research was to create a high-resolution mass spectrometry (HRMS) library of NPS and metabolites, as well as to validate a method for screening and confirmation of these substances. The study consisted of three main tasks which included; 1) the development of a large high-resolution MS/MS spectral library and database, 2) validation of a method for screening and confirmation of over 800 NPS and metabolites, and 3) screening of blind-spiked and authentic urine specimens to determine real-world applicability of the HRMS library and method.

During validation, several isomeric and structurally related NPS were observed which could not be adequately separated using traditional LC methods. A fourth task was therefore added to investigate improved separation using two-dimensional liquid chromatography (2D-LC). Increased resolving power is achieved in 2D-LC through the coupling of multiple orthogonal separation systems. Ultimately, an on-line, comprehensive method was developed using orthogonal reversed-phase columns in each dimension (RP x RP) for improved separation of isomeric and structurally similar synthetic cannabinoids.

This work can aid laboratories in the identification of NPS through the use of a validated LC-QTOF-MS method for screening and confirmation and HRMS spectral library. In instances where isomeric and structurally related NPS are not sufficiently separated, RP x RP methods can be explored.

TABLE OF CONTENTS

CHAPTER PAGE

LIST OF TABLES

TABLE PAGE

LIST OF FIGURES

FIGURE PAGE

LIST OF ABBREVIATIONS, ACRONYMS, AND SYMBOLS

^{1}D	First Dimension
^{2}D	Second Dimension
1D	One-dimensional
2D	Two-dimensional
ACN	Acetonitrile
AF	Ammonium Formate
APCI	Atmospheric Pressure Chemical Ionization
CB1	Cannabinoid Type 1 Receptor
CB2	Cannabinoid Type 2 Receptor
CV	Coefficient of Variation
DAD	Diode Array Detector
DEA	Drug Enforcement Administration
DFC	Drug Facilitated Crime
DI	Deionized
DUI	Driving Under the Influence
EI	Electron Ionization
EIC	Extracted Ion Chromatogram

ESI	Electrospray Ionization
FA	Formic Acid
FBF	Find by Formula
GC	Gas Chromatography
H_2O	Water
HILIC	Hydrophilic Interaction Liquid Chromatography
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
IMS	Ion Mobility Spectrometry
IS	Internal Standard
LC	Liquid Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantitation
MDPV	Methylenedioxy Pyrovalerone
ME	Matrix Effect
MeOH	Methanol
mg	Milligram
mL	Milliliter

MRM	Multiple Reaction Monitoring
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
m/z	Mass to Charge Ratio
ng	Nanogram
NP	Normal Phase
NPS	Novel Psychoactive Substances
OSAC	Organization of Scientific Area Committees
PCDL	Personal Compound Database Library
PCP	Phencyclidine
PDA	Photodiode Array
ppm	Parts Per Million
QC	Quality Control
QQQ	Triple Quadrupole Mass Spectrometer
QTOF	Quadrupole Time-of-Flight Mass Spectrometer
RP	Reversed-phase
RSD	Relative Standard Deviation
SAMHSA	Substance Abuse and Mental Health Services Administration

SEC	Size-exclusion Chromatography
SWGTOX	Scientific Working Group for Forensic Toxicology
TIC	Total Ion Chromatogram
TFA	Trifluoroacetic Acid
UV	Ultraviolet
µg	Microgram
µL	Microliter

1. INTRODUCTION

1.1 Statement of the Problem

The emergence of many novel psychoactive substances (NPS) has caused great concern in the areas of public health and law enforcement. NPS include a wide variety of diverse compounds such as synthetic cannabinoids, designer opioids, designer hallucinogens, and synthetic stimulants for which multiple reports of overdoses and even fatalities are available.[1-4] These compounds are mainly created with the purpose of circumventing existing drug laws. Manufacturers achieve this goal by simply altering the existing structures of NPS and other drugs (*i.e.,* adding or removing a functional group, moving a functional group, etc.). When changes are made to the structures, the new compounds no longer match the illegal structure and therefore do not fall under the regulation of controlled substance laws.[5] As a result, there exist thousands of these compounds whose effects can range from having no pharmacological effect to exhibiting significant toxicity.

Changing the chemical structures of these compounds can also make existing methods of detection unreliable, since these methods are generally designed to identify specific functional groups or structures.[6] In the fields of clinical and forensic toxicology, the changing structures can especially cause issues when trying to identify which compound was used in instances of overdose and emergency department cases in order to properly treat patients. To combat the public health issue of NPS, a reliable method for detection and identification of the multitude of NPS potentially present in biological specimens must be available.

One solution for detecting and identifying NPS that might be missed by other screening methods involves the creation of drug libraries for chromatographic and mass spectrometric data. These libraries contain characteristic spectral information generated using mass spectrometry (MS) that can help identify NPS in specimens. Currently, there exist a number of libraries for gas chromatography (GC) generated via electron ionization (EI) methods, but fewer for liquid chromatography (LC) and electrospray ionization (ESI) methods. For example, Ojanpera, Broecker, and Rosano have all created libraries for LC, but they are either mostly theoretical or do not contain many NPS which can hinder identification efforts during screening and confirmation.[7-9]

The lack of suitable screening methods and support libraries has hindered the ability of forensic and clinical laboratories to quickly and reliably identify NPS. Typically, these compounds are not detected and identified until there are incidences of mass-intoxications or a series of deaths in a singular region. To rapidly identify such incidents as they occur, laboratories need to have access to constantly updated mass spectral databases that consist of accurate precursor mass and fragmentation data.[10]

1.2 Rationale for Research

In most toxicology laboratories, drug screening typically involves the use of immunoassays. While these methods are fairly effective in screening for common drugs of abuse, they are not well suited for the screening of specimens containing NPS.[6,10-12] A major goal of the present work was to develop a reference standard-based spectral library containing high resolution mass spectrometry (HRMS) data for more than 800 NPS and related compounds for use with an LC-quadrupole time-of-flight (QTOF)-MS based screening method. The developed library will help enable the rapid detection and

identification of NPS in biological specimens such as urine. The library can also be used with retrospective data searching in order to detect and identify previously unreported NPS in specimens.

Following validation of the LC-QTOF-MS method, blind-spiked urine specimens were screened in order to establish the applicability of the method for use with real-world specimens. The specimens used for this part of the research were created in a "blind" manner, meaning that they were designed and made by a third party directly involved with the analysis.

It was also important to develop a comprehensive screening and confirmation method capable of detecting and identifying several hundred NPS in a single analytical run with high specificity at minimal (*i.e.* low ng/mL) concentrations. In order to accomplish this goal, a rapid, sensitive, and specific LC-QTOF-MS based analytical method was developed and validated in conjunction with the compound database and HRMS libraries. In some cases, however, it can be extremely difficult to chromatographically separate certain NPS to facilitate identification via mass spectrometry, such as in the case of chemically similar and isobaric compounds which have the same or significantly related chemical formulae. This lack of separation can be especially problematic when attempting to identify a previously unknown or unreported NPS using its mass spectra alone. To address the challenge presented by lack of separation, a two-dimensional (2D)-LC separation system was investigated to separate isobaric and non-isobaric co-eluting SC.

1.3 Significance of Study

The body of work presented herein has applicability to forensic science, toxicology, law enforcement, and even pharmaceutical development. The research provides a large HRMS library for the identification of over 800 common and uncommon NPS, metabolites, and related compounds, generated using ESI. A validated, comprehensive LC-QTOF-MS method for screening and confirmation was also developed in conjunction with the library and tested using blind-spiked urine specimens to ensure applicability. A possible solution to the separation challenge presented by the co-elution of some NPS, both isobaric and non-isobaric, was also investigated through 2D-LC analysis of mixes containing co-eluting synthetic cannabinoids (SC).

The NPS used in the research were identified and selected on the basis of the reference standards available from commercial suppliers, as well as citations in government documents, peer-reviewed literature, and online drug-user forums. In order to complete this research, the work was divided into three major tasks.

1.3.1 Task 1 – Development of database and spectral library

Comprehensive libraries are widely used in analytical toxicology for the identification of analytes present in specimens, however, these libraries often do not contain many NPS. In order to identify NPS, a database and library containing characteristic spectra of these compounds must be developed. This report details the creation of a database containing over 800 NPS, metabolites, and related compounds, as well as the generation and collection of tandem mass spectrometry (MS/MS) data for each compound using commercially available reference standards. The database and

library were then used to qualitatively screen blind-spiked urine specimens to ensure applicability to real-world samples.

1.3.2 Task 2 – Comprehensive LC-QTOF-MS method validation

In order to detect and identify NPS and related compounds in biological specimens, a comprehensive LC-QTOF-MS method for screening and confirmation of said compounds was developed and validated according to accepted analytical method development guidelines.

1.3.3 Task 3 – Investigation of a 2D-LC method

Chemically related and isobaric NPS are not uncommon and can present challenges to identification using mass spectral data alone. Throughout the development of the library and LC-QTOF-MS method, several such compounds were identified which demonstrated similar chromatographic retention times by standard 1D-LC. Consequently, 2D-LC was investigated as a possible approach for separation of co-eluting, chemically related and isobaric SC in order to analyze each compound individually.

2. BACKGROUND

2.1 Novel Psychoactive Substances

Novel psychoactive substances (NPS), also known as "designer drugs", are compounds that have been created to circumvent controlled substance laws. When the Comprehensive Drug Abuse Prevention and Act, also known as the Controlled Substances Act (CSA), was passed into legislation in 1970, it created a protocol for regulating substances depending on their potential for abuse, potential for addiction, and accepted medical usage. Compounds that had high potentials for abuse and addiction, but with no or limited accepted medial use were classified as Schedule I or II substances, respectively.[13]

Typically, NPS have been synthesized to be structurally or pharmacologically comparable to substances that have been identified as Schedule I or II drugs by the U.S. Drug Enforcement Administration (DEA).[5,10] In the late 1980s, an addition to the CSA, known as the Federal Analogue Act, was made to allow for the scheduling of NPS that were structurally and pharmacologically "substantially similar" to Schedule I or II substances under DEA control.[14,15] In order to get around these legal controls, illicit laboratories turn to a variety of sources to guide the synthesis of new compounds that are not yet under government control. These sources may include scientific journal articles, patents, and books published by pharmaceutical companies, academic or research institutions, and other organizations as part of the legitimate scientific process.[15]

For classification purposes, NPS are generally divided according to their chemical structure. These classes include, but are not limited to, piperazines, phenethylamines, tryptamines, cathinones, opioids, and synthetic cannabinoids.[16,17] With the rise of

internet access over the past two decades, not only has synthesis information become more widely available, but the ability to market and traffic these compounds has resulted in an explosion of new, untested NPS on the market. In addition to the issues with legality, NPS also represent a significant risk to public health, given the untested nature of these compounds and the potential for adverse effects associated with their usage as compared with more traditional drugs of abuse.[5,17,18]

One example involves the emergence of illicit fentanyl and its derivatives as part of the on-going opioid crisis in the United States. Fentanyl is a synthetic opioid that is approximately 100 times as potent as morphine. When the analog α-methylfentanyl was classified as a Schedule I drug by the DEA in 1981, it took just a few years for another analog, α-acetylfentanyl, to appear in 1984, demonstrating the efficiency with which illicit drug manufacturers have historically been able to introduce new compounds.[5] New fentanyl derivatives now appear on the street in a time frame of weeks to months rather than years.

More recently, numerous synthetic cannabinoids have been scheduled, only to have analogues appear on the market shortly thereafter. The synthetic cannabinoid JWH-018 was sold as a component of herbal incense products such as 'Spice' and 'K2,' and was labeled "not for human consumption" in order to avoid regulation. However, JWH-018 exhibits effects at a higher potency than Δ^9-tetrahydrocannabinol (THC), which is the psychoactive component in cannabis.[19] As a consequence of these effects, the DEA temporarily placed JWH-018 on the Schedule I list in 2011. That very same year, NutraGenomics, a company that sold synthetic cannabinoids, stopped selling JWH-018 products. Instead, they began selling analogs of JWH-018 such as AM-2201, whose

structure differs only by the substitution of a fluorine for a hydrogen at the end of the alkyl chain. It was not until 2012 that AM-2201 was controlled as a Schedule 1 substance.[20,21]

Synthetic cannabinoids are not the only class of NPS that have been subjected to fast turn-around times by illicit manufacturers. The phenethylamine compound 4-methyl-N-methylcathinone, also known as mephedrone, was placed on the temporary Schedule 1 list in October 2011 because it can cause seizures, increase blood pressure and heart rate, result in delusions, and even cause death.[22] After permanent scheduling occurred in July 2012, the compound 4-fluoro-N-methylcathinone (4-FMC) appeared exhibiting similar effects after substitution of a fluorine for the *para*-position methyl group of the benzene ring. This compound was not placed on a schedule by the DEA until March 2014.[23]

2.2 Analysis of Drugs in Biological Specimens

The analysis of drugs of abuse, including NPS, in biological specimens represents a large challenge for toxicologists. Methods for such analyses need to take into account not only the analytes of interest, but also any potential interferences or sample preparation issues that arise as a result of biological matrices and any endogenous or naturally occurring compounds within the specimen.

The most common matrices encountered in forensic toxicological analyses include blood and urine.[6,24] Drug concentrations in both matrices can provide valuable information about a person's exposure to a drug, including the identity of the compound and, in some cases, an approximate time since exposure. Analysis of urine also presents the option of detecting and identifying metabolites, providing another option for targeted analysis in cases when the parent compound cannot be detected using current methods.

For example, synthetic cannabinoid metabolites are the most prevalent in urine with minimal or even no parent compound present. The presence of any one of these metabolites can then indicate exposure to the parent drug.[25]

Both urine and blood are fairly easy to obtain, however, urine collection is much less invasive and is thus a very common specimen collected for analyses, especially those for antemortem toxicology. Larger volumes of urine than blood may also be collected at any one time, providing a significant advantage in the ability for multiple analyses and re-analyses to be conducted on a single specimen.[8,26] These benefits make urine a good option for screening in clinical and forensic toxicology, including cases such as compliance monitoring, workplace drug testing, drug rehabilitation, child welfare, doping control, drug-facilitated crime (DFC), driving under the influence (DUI), and more.[27]

In clinical and forensic toxicological analyses, urine is an important matrix for several other reasons. A major advantage of conducting analyses with urine is that there is a much longer window of detection for drug compounds in the matrix. This window can extend to several days after exposure, as opposed to blood concentrations which typically dissipate after a few hours. Both parent drug and metabolites can often be detected in urine providing more targets to analyze to indicate extent and timing of exposure.

Another key advantage to using urine is that drug compounds and their metabolites tend to be more concentrated. As substances are metabolized, they accumulate in the bladder and when excreted in urine produce a specimen in which the analytes of interest are present at higher concentrations, even if the user was exposed to only a low dose of the compound.[28] Concentrated samples present a huge benefit for

qualitative analysis, however, quantitation for the purposes of correlation to levels of intoxication is much more difficult as compared to blood specimens.

From a sample preparation standpoint, urine is much easier to work with than blood since many analyses can be conducted simply by diluting the urine specimen with water, as opposed to the sometimes laborious extraction procedures required for the analysis of blood (*i.e.,* solid-phase extraction). Liquid-liquid extraction (LLE), which involves several steps in order to selectively remove acidic and basic compounds, can also be applied to urine, but for purposes of simplicity, dilution of the specimen is a valid technique for urinalysis.[29-32]

Consequently, while there are some drawbacks to urine analysis, including the inability to correlate drug concentration with levels of intoxication, urine is a very valuable matrix for the qualitative analysis of small molecules and drug substances in both clinical and forensic toxicology settings.[33]

Presumptive screening tests are the first step in the detection and identification of analytes of interest in biological specimens. These screening methods are needed for fast delineation of negative samples from positive ones, and to also generate preliminary information as to what analytes might be present in any positive specimens.[34] Screening techniques look for selected analytes present at concentrations above a specific level known as the "cutoff" concentration. A predetermined set of analytes to be searched, or screened for, in a single run is known as a drug "panel". One of the most commonly used panels for drug screening, particularly in workplace drug monitoring, is known as the Substance Abuse and Mental Health Services Administration (SAMHSA) Five. This panel includes tests for five common categories of drugs: amphetamines, cocaine,

marijuana, opiates, and phencyclidine (PCP). Other categories that might be included are barbiturates, benzodiazepines, hydrocodone, methylenedioxy-methamphetamine (MDMA), and methadone.[35] However, screening tests typically include few, if any, NPS. The need for accurate screening methods cannot be understated, especially since many current screening methods are not reliable for use in detecting NPS; oftentimes generating a negative result when NPS are actually present.[36,37]

The type of screening method used depends on the matrix being analyzed, but typical methods for urine specimens include immunoassays to determine which class of drugs or NPS may be present, as well as liquid chromatographic (LC) and gas chromatographic (GC) methods coupled with mass spectrometry (MS). Screens that result in positive results then undergo confirmatory testing in order to accurately identify the compound(s) present and provide quantitation when required. However, this process of screening and confirmation can only be followed if compounds are known and if the immunoassay is capable of detecting NPS.[36,38]

Immunoassays are a type of immunochemical technique which can provide a simple answer to whether or not a specific drug or drug class is present above cut-off concentrations. These assays represent a relatively quick, inexpensive, and user-friendly method to determine which drug or class of drug is present, if any at all.[12,39,40] When a compound is detected and tentatively identified using the results of the immunoassay screen, a more selective confirmatory technique, such as mass spectrometry, is then used for specific identification. However, immunoassays use antibody-antigen interactions with the analytes of interest serving as the antigen. The antibodies employed by the assays are engineered to detect specific chemical structures characteristic of one or more

drug classes. As a result, the slight structural alterations present in NPS can be enough to cause a negative result, or no cross-reactivity, regardless of whether the NPS has been previously identified or not.[12,41] In these instances, specimens are determined to be "negative" for drugs and are not submitted for further analyses.[39] For example, mephedrone and methylenedioxy pyrovalerone (MDPV) are two of the most common illicit stimulant-type substances. When immunoassays were used to try and screen for these compounds, there was little to no cross-reactivity demonstrated, meaning that these samples would be determined to be negative and likely discarded.[41]

A published work by Swortwood *et al.* sought to determine the level of cross-reactivity present when 16 commercial immunoassay kits were used to screen for 30 NPS from the phenethylamine, piperazine, and tryptamine classes of compounds in human serum. The kits chosen included some designed to detect amphetamine and/or methamphetamine-like compounds, as well as a few more specific kits such as one solely for the detection of mephedrone and methcathinone. Ultimately, the commercial kits demonstrated little to no cross-reactivity with the NPS chosen for evaluation. Those that demonstrated minimal cross-reactivity did so at concentrations too high to be practical for forensic or clinical applications.[6] Results from such studies have shown that immunoassays, as they currently exist, cannot be reliably used as a presumptive detection method for NPS within the same drug class, let alone comprehensively. While some newer immunoassay kits have been designed for the presumptive identification of a few NPS classes, specific immunoassays are not widely available for many NPS or their derivatives.

As the structures of NPS are constantly changing, it is not feasible to create the specific antibodies needed for immunoassays rapidly enough to keep pace with introduction of new varieties of NPS. In addition, existing kits cannot be expected to demonstrate cross-reactivity with newer NPS as they emerge on the market. Therefore, different methods must be employed for comprehensive screening of NPS in biological specimens to account for the lack of reliable detection with immunoassay-type methods.

2.3 Liquid Chromatography Mass Spectrometry

In order to overcome some of the limitations of screening with immunoassays, and to increase specificity, methods involving GC or LC coupled to MS have been used for both targeted and non-targeted screening purposes of analytes in biological specimens. Targeted screening occurs when the presence of a specific known compound or set of compounds is searched for in the sample matrix and only MS data associated with the selected compound(s) are collected. Non-targeted analyses involves the use of broad screening methods in which all MS data are collected and then analyzed afterwards to identify any compounds that might be present. Non-targeted methods are ideal for unknown screening, since analytes that might be of interest are not unintentionally excluded during data collection as they might be in targeted methods.[28,40]

Both targeted and non-targeted methods involve the chromatographic separation of compounds from the specimen matrix, as well as from other compounds that may be present in a mixture, before detection by MS. Within the mass spectrometer, analytes are ionized and can be detected as the original molecule, or can be subjected to an energy source and broken into fragments. These fragmentation patterns are characteristic of the

original molecule and can then be used for the structural elucidation and identification of analytes present.[42-45]

When using MS-based techniques in conjunction with either GC or LC separation to identify an analyte, mass spectral libraries are required. Typically, ion fragmentation patterns are generated via GC- or LC-MS and these patterns are then compared with those present in existing libraries which can contain information for several thousand different compounds.[42,46,47]

Gas chromatography with mass spectrometry (GC-MS) has been described as the "gold standard" for drug screening and identification. As an established and well-understood technique, GC-MS is used by many labs for the detection and identification of NPS and many other compounds in both toxicological and seized material analyses. Large spectral libraries containing tens of thousands of spectra have been built over the course of 40+ years using electron ionization (EI) techniques; a hard ionization technique which typically produces fragment-rich, characteristic spectra.[42,48] In the 2017 iteration of the EI mass spectral library released by the National Institute of Standards and Technology (NIST), over 260,000 unique compounds are represented by over 300,000 EI spectra.[49]

While there exist large databases of reproducible spectral data generated through the use of EI sources, the use of GC-MS is not without its restrictions, particularly in the types of analytes that can be evaluated with this technique. In order to generate spectra using EI sources, compounds of interest must be volatile, non-polar, and thermally stable. Lengthy sample preparation processes such as derivatization are sometimes required to make some compounds suitable for analysis using EI, while the above suitability criteria

for analysis might prevent some analytes from being detected at all.[50] The lengthy sample preparation restriction is especially true for the analysis of urine specimens that require cleavage of the glucuronic acid or sulfonate acid conjugates of the phase I metabolites that may be present during analysis via GC.[8]

In contrast, liquid chromatography with mass spectrometry (LC-MS) is suitable for the analysis of non-volatile, polar, and thermally labile compounds and does not mandate that specimens undergo derivatization or other chemical modifications prior to analysis.[8,36,38,42,47,51] Electrospray ionization (ESI), commonly used in LC-MS, is a soft ionization technique that is effective in ionizing analytes contained in aqueous specimens without requiring the derivatization often needed in GC-MS. As a result, LC-MS screening has increased in popularity for clinical and forensic toxicological case work because of the increasing polarity and low volatility of many new relevant substances which are difficult, if not impossible, to analyze via GC-MS.

2.3.1 Electrospray Ionization Spectral Libraries

Since the use of LC-MS for screening is much newer than the use of GC-MS, the spectral libraries that have been created are not as extensive. The very nature of the ionization techniques used with LC-MS are also not as reproducible as those used with GC-MS, and require complete standardization of source parameters in order for libraries to be used across different laboratories and instruments.[42,50,52] Additional work is required in the development of large, comprehensive mass spectral libraries to support the identification of compounds using LC-MS methods and their corresponding ionization techniques, particularly electrospray ionization (ESI).[25,28,45,53]

Techniques using LC-MS for identifying and quantifying NPS require the use of libraries with known compounds and their known masses, spectral data, or ion transitions.[18,54] If a NPS has not been previously included in such a database or library then detection may be possible with a non-targeted analytical method, however, identification and thus quantification will not be achievable.[10,18] Lack of analyte detection due to absence in a database or library is also true for NPS metabolites, which are of particular interest in forensic toxicological analyses, especially those from the synthetic cannabinoid classes which can be abused in the same manner as the parent compound.[55]

Electrospray ionization (ESI) is a soft ionization technique commonly used with LC-MS to generate ions detectable by the MS detectors. Libraries created using ESI coupled to tandem mass spectrometry (ESI-MS/MS) have been shown to be much more reproducible than those created with "in-source" collision induced dissociation (CID), such as with methods utilizing a single mass analyzer, so long as collision energy and collision gas pressure are consistent.[47,53,56,57] This is because the first mass analyzer can be used as a filter to isolate a particular range of masses and exclude ions from background contaminants and matrix components before CID fragmentation occurs. Selective ion fragmentation then occurs in the collision cell placed after the first mass analyzer. The resultant product ions then move through the second mass analyzer where they are analyzed using mass-to-charge (*m/z*) ratios.[53,56,58] The first mass analyzer can also be used to detect analytes before undergoing a mass dependent scan which provides the MS/MS spectra needed for identification via a library search.[38] The use of ESI-MS/MS has the added benefit of virtually always revealing the molecular ion (*i.e.,* the

ionized original compound) in resultant spectral data, leading to increased confidence in identification from library searching.[42,54]

Libraries for ESI-MS/MS techniques are typically generated using triple quadrupole (QqQ) or hybrid quadrupole time-of-flight (QTOF) mass analyzers with fragmentation patterns collected at more than one collision energy to account for differences between mass analyzers and brands.[42,47,53,58] Most ESI libraries exist in-house, but there have been published works creating reproducible and shareable libraries.[8,9,28,34,36,38,50,57,59,60]

The poor availability of reference standards for many NPS and metabolites presents a large challenge to forensic toxicology laboratories when trying to detect and identify both known and unknown NPS and other xenobiotics. In an attempt to address this issue, the Ojanperä group created a database containing theoretical monoisotopic masses for over 7,500 toxicologically relevant compounds and metabolites. Their database was then used in conjunction with a method for LC-TOF-MS and applied to postmortem human urine specimens. Each search of the database resulted in no more than three potential elemental formulas which resulted in a significantly more manageable list of possible identifications. The greatest asset to this database was that it could be updated with new formulas and theoretical masses as soon as they appear in the literature or are noted by law enforcement and/or public health officials.[27] While this approach simplified the list of possible identification significantly, the use of accurate mass data alone was not enough to explicitly identify the compounds present. Retention data could have been used to corroborate identifications, but reference standards would have been needed in order to acquire those data.[8]

Another approach to theoretical databases was employed by Polettini *et al.* when they developed a screening procedure utilizing a subset of compounds curated from those available in PubChem. The PubChem database contained accurate masses for over 50,000 toxicologically relevant compounds including pharmaceutical and illicit drugs, pesticides, poisons, and over 6,000 metabolites. The database was then used to screen for compounds present in a variety of postmortem biological specimens. The resulting number of possible identifications from this work ranged from one to 39 per analyte.[60] While Polettini's work indicated the potential of a theoretical database to help narrow down possible identifications, unambiguous identifications could not be generated for each analyte. Other information that would assist in improving the confidence of an identification include chromatographic retention data, isotope patterns, and/or fragmentation patterns.[60,61]

Another possible way to address the issue of the lack of available reference standards, especially of NPS metabolites, involves the use of *in silico* methods to predict metabolites of certain compounds and what their characteristic fragmentation patterns might be. For example, Pelander *et al.* used metabolite prediction software to predict the phase I metabolites of the anti-psychotic drug quetiapine. Using another software tool, fragmentation patterns of these metabolites were also predicted. The resulting data were used to screen authentic urine specimens in which several of the predicted major metabolites were detected, however, there were several metabolites detected that had not been predicted, particularly the hydroxylated metabolites. The predicted fragmentation patterns were also helpful in differentiating between isobaric metabolites, but the work was not conclusive enough to serve as a reliable substitute for identification using CID

spectral profiles collected from reference standards.[62] Predicted fragmentation patterns would not be suitable for use in forensic cases as they would not meet standards for admissibility in court.

In a recent paper by Colby *et al.*, the efficiency of using certain workflows for screening via LC-QTOF-MS was evaluated when using databases and/or spectral libraries to identify compounds of interest. Four different workflows were assessed in the identification of 170 drugs and metabolites: one targeted and three involving "suspect screening." The first involved analysis of a reference standard followed by targeted searching of the sample. In contrast, suspect screening does not utilize a reference standard but instead bases identification on predicted and/or intrinsic characteristics such as accurate mass, isotope pattern, and product ion spectrum. The study focused on which combination of these three characteristics carried the most weight when identifying compounds without direct use of a reference standard. It was found, unsurprisingly, that the most effective methods included the use of product ion spectra that had been previously collected from reference standards and included in a searchable library. When product ion spectra were utilized, in addition to accurate mass and isotope patterns, over 80% of the drugs in human urine specimens were correctly identified with a minimal number of false identifications. These results, combined with the fact that retention times were not required, indicated the potential of building large screening methods and libraries for screening of toxicologically relevant compounds, including NPS.[63]

The utility of spectral libraries for identification of compounds in screening procedures has been demonstrated in other works as well. Lee *et al.* created a screening method for toxicologically relevant compounds present in urine which utilized a spectral

library developed with the use of reference standards. Spectral data were collected using ultra performance liquid chromatography (UPLC) coupled to a TOF MS then searched against a library containing spectra for 300 compounds, 102 of which were metabolites. The substances in the library originated from pure reference standards, pharmaceutical materials, and from metabolites present in authentic urine specimens. The library was created using retention data, exact mass, and fragmentation patterns collected at two collision energies (10 and 45 eV) for each of the compounds. The inclusion of spectra containing a pseudomolecular ion in addition to one CID fragmentation pattern provided additional confidence in the identification of the compounds present in the samples and improved the specificity of the method.[50]

The largest MS library, containing CID mass spectral data for over 2,500 toxicologically relevant substances, was created by Broecker *et al.* A hybrid quadrupole time-of-flight mass spectrometer (QTOF-MS) was used to collect the CID fragmentation patterns generated in the collision cell located between the quadrupole and the TOF analyzer. Substances were subjected to three different collision energies (10, 20, and 40 eV) and the data were then curated and checked for suitability before being added to an existing database of theoretical fragment masses and molecular formulas for 7,500+ additional toxicologically relevant substances. The compounds in the combined spectral library and database represented substances such as illicit and therapeutic drugs, pesticides, alkaloids, and other toxic chemicals and metabolites.[7] However, there was not a significant presence of NPS and NPS metabolites as is true for many other existing ESI-MS/MS libraries and databases. This lack of NPS representation in existing libraries can hinder the detection and identification of such substances in systematic clinical and

forensic toxicological analyses. Thus the development of larger, more comprehensive libraries that include NPS is needed to improve detection and identification of compounds during screening efforts.

Historically, QqQ-MS, a unit resolution technique, is more often used for drug screening than HRMS because of its lower cost of operation and its robustness. However, since the mass spectra generated are of low resolution, compounds must be known prior to analysis. Libraries for QqQ-MS are built by collecting data generated from multiple reaction monitoring (MRM), which is suitable for use with targeted screening methods.[7,37,54] High resolution MS methods, such as those utilizing QTOF-MS are recognized as having higher resolution and allowing for the collection of all ion spectra, an approach that is ideal for non-targeted screening. High-resolution mass spectra can also be collected for the screening of known targets using data-dependent acquisition methods.[7,36,40,64] Compounds are then identified using corresponding libraries through the comparison of accurate masses or the characteristic spectral data.[8,65]

Methods using QTOF mass analyzers are recognized as having high mass accuracy and high-resolution capabilities, meaning that collected data have mass accuracy better than 5 ppm and resolution greater than 20,000 full width half maximum (FWHM), respectively. However, high mass accuracy is not required for a method to be considered high-resolution.[28] These qualities are vital in building HRMS libraries to ensure the collection of accurate masses, as well as the ability to resolve isobaric compounds or those with very similar chemical formulas and similar product ion fragmentation patterns. An added benefit of using QTOF mass analyzers is that the accurate masses of analytes can be recorded over a wide range of abundances. The

ability to record over a wide abundance range is especially important for conducting both targeted and non-targeted analyses, which enables scientists to conduct screening for known analytes and also to collect ion data that can be retroactively searched for previously unknown substances as libraries are updated.[18,28,66] The ability to conduct non-targeted analyses and collect data that can be searched later on are crucial to the future of forensic toxicological analysis of NPS, as the potential to create new drug compounds within synthetic chemistry is practically unlimited.[67,68]

Employing QTOF mass analyzers also enables users to search for common fragments or use mass-defect filtering to investigate unknown compounds that share common functional groups or structural components with known NPS.[69] When such collected spectra are searched against a library, a "hit list" of possible compound identities is generated. These lists are comprised of "scores" which reflect the likelihood that the collected spectra and the library spectra are from the same compound.[42,43,47] In instances where the collected spectrum is from a compound not yet included in the library, modern algorithms will include library compounds on the "hit list" that may differ by a simple insertion, deletion, or replacement of a structural group. These "nearest neighbor" identifications are extremely useful when screening unknown NPS and are much more impactful when using HRMS spectra.[44]

There are two main search methods used when comparing collected spectra and library spectra: forward and reverse searching. Forward searching involves comparing the collected spectrum of an unknown with spectra contained in a library. The base mass peak or each ion within the spectrum is identified and then compared to those contained in the library for potential matches. Reverse searching is essentially the same technique

except that the collected spectrum is searched through using spectra in the library. More simply, in forward searching, the collected spectrum is searched for in the library spectra and in reverse searching library spectra are searched for in the collected spectrum.[7,48]

2.4 Co-elution Challenges

Even with the increased resolving power of HRMS, there may still be instances in which isobaric compounds or those that are too structurally related to be identified solely by mass spectral data are present in specimens. This is particularly true with NPS as many are simple variations on existing compounds with minor or novel structural alterations. In cases of mixtures or true unknown substances, resolution of compounds using spectral data alone may not be possible.

Typically, chromatographic separation systems are employed to isolate individual compounds prior to MS analysis. However, there are instances in which the structural or physiochemical differences between NPS are so slight that they cannot be separated using traditional chromatographic techniques (*i.e.* LC or GC) and will therefore not be detected as individual compounds.[70-72] To resolve this issue, two-dimensional liquid chromatography (2D-LC) has been proposed as a method to improve separation and resolution of complex mixtures prior to further mass spectral analysis.[73,74]

2.4.1 2D-LC

When conducting toxicological analyses that rely on searching MS library data, screening for unknown or previously unreported NPS can be problematic because of the large number of isomers and chemically related compounds that exist with similar accurate masses, fragmentation patterns, and/or chromatographic retention times. The alteration of a single functional group may result in the inability to separate such

compounds using traditional chromatographic methods which can further hinder proper detection and identification of each as an individual substance. As more and more NPS are added to an analytical method in order to keep up with the growing number of possible analytes and the increasing complexity of mixtures, more separation issues are likely to arise. These challenges to separation and thus identification will only increase as more NPS are synthesized and introduced to the illicit market.[12,70,72,75,76] In these instances, it is important to perform an effective initial separation so that each substance may be analyzed via MS individually.[45,77] One proposed solution to issues of coelution is the use of two-dimensional liquid chromatography (2D-LC).

Two-dimensional LC combines the separation and resolving power of two independent, orthogonal LC dimensions in order to improve chromatographic separation of analytes from matrices and/or each other and to increase the maximum number of peaks that can be equally resolved in a separation space, also known as peak capacity.[78] The two dimensions can consist of several columns, but typically each dimension contains a single column with different selectivity, or orthogonality, than the other.[71,79,80] There have been various applications of 2D-LC ranging from analysis of proteins and peptides, determination of pesticides, separation of chiral compounds, and the separation of pharmaceuticals and other small molecules. Several of these applications also include using the improved separation and resolving powers to differentiate analytes of interest from toxicologically relevant biological matrices such as urine, whole blood, serum, and saliva.[72,76,78,79,81-84]

The ability of a chromatographic system to separate constituents of a mixture is commonly described by its peak capacity, which is a measure of the theoretical maximum

number of peaks that can be equally resolved within a separation space. Traditional 1D-LC systems generally achieve peak capacities of only a few hundred, whereas 2D-LC can achieve maximums over 1000. As peak capacity increases, so too does the resolution of the separation system.[78,85,86]

The most basic model for calculating peak capacity is for the "comprehensive" 2D-LC approach (discussed below), where the peak capacities of each dimension are multiplied as demonstrated in Equation 1, where $n_{c,tot}$ is the theoretical peak capacity of the 2D-LC system, and ${}^{1}n_c$ and ${}^{2}n_c$ represent the peak capacities of the first and second dimensions, respectively.[87]

$$n_{c,tot} \approx {}^{1}n_c \, x \, {}^{2}n_c \qquad (1)$$

However, there is no general consensus as to how peak capacity can be best calculated, since the ideal capacity values are never fully realized as a consequence of the unique designs and applications of each 2D-LC system.[78,86] In contrast, "heart-cutting" 2D-LC techniques (discussed below) do not require peak capacity calculations since the only relevant chromatographic fraction is the targeted one, and thus calculations of peak capacity, which are employed to determine the maximum number of peaks that can be resolved in a separation window, hold no significant value.[79]

In addition to the increased power of separation, one of the greatest advantages of 2D-LC is the decreased amount of time needed for sample preparation. The potential loss of analytes during preparation can also be minimized by using 2D-LC techniques in which the first dimension is used to separate analytes of interest from complex matrices,

as well as from other analytes present which can also be particularly useful when removing endogenous compounds from the biological matrices relevant to toxicological analyses.[88-91] Using a dimension in which both cleanup and initial separation can be conducted also minimizes the amount of time required for sample preparation prior to analysis.[92]

There are two main modes of operation for 2D-LC: heart-cutting (LC-LC) and comprehensive (LC x LC). Heart-cutting is a method which involves taking selected fractions or peaks of the effluent from the first dimension (^{1}D) and subjecting these to additional separation in the second dimension (^{2}D) with the remaining effluent going to waste. Heart-cutting is useful for conducting targeted analyses of analytes in complex matrices and biological specimens, since the known analytes or peaks eluting from ^{1}D are the only fractions subjected to further separation by ^{2}D.[93-96]

Heart-cutting methods are commonly used in pharmaceutical laboratories to help separate target compounds and any possible impurities of a developed drug compound. Sandra *et al.* used a multiple heart-cutting (mLC-LC) system for the characterization of antibody-drug conjugates (ADCs) consisting of monoclonal antibodies, cytotoxic small molecule drugs, and linkers which are used in the treatment of tumor cells.[94,97] The mLC-LC configuration involved using multiple sample loops, or parking decks, between the dimensions where up to 12 fractions from ^{1}D could be stored before transfer to ^{2}D for analysis. Multiple heart-cutting was a very effective method, but the fractions that were selected to go to the "parking decks" had to be known or expected prior to analysis.[97] Because peaks of interest must be known in order to be transferred to ^{2}D in LC-LC, it is extremely difficult to automate a heart-cutting 2D system. Another potential issue is that

if the incorrect fraction is collected, peaks of interest could be lost between dimensions.[86,98]

Pandohee *et al.* used a heart-cutting method with two RP columns in order to separate the constituents of cannabis/hemp plants. This matrix is extremely complex, which complicates work in pharmacological settings where isolation of the pure compounds is important. Fractions of 200 µL were collected after they eluted from ^{1}D before injection into ^{2}D. Once the sample had gone through separation in both dimensions, another sample could be injected. However, this process resulted in a total analysis time of 12 h, which is not conducive to routine screening, nor does it lend itself to automation.[99]

The separation of samples containing illicit drugs has been improved through the use of LC-LC. Andrighetto *et al.* used an *in silico* technique to optimize a 2D separation system with C_{18} columns in both dimensions in order to differentiate ephedrine and pseudoephedrine-based methamphetamine in seized samples. Following optimization, authentic samples were analyzed and co-eluting peaks were transferred to the second dimension.[82] However, selection of the peaks to be transferred to the second dimension used simulation data, which presents the risk of missing potential peaks of interest should the simulation be incorrect.

Heart-cutting 2D-LC can be a very valuable tool for improving separation of compounds from complex matrices and mixtures.[78] The potential peak capacities that can be achieved through heart-cutting methods are impressive, however, there are some clear drawbacks which do not make it an ideal choice for all multi-dimensional separation applications. In particular, LC-LC is not the best choice for the separation of

mixtures in which there may be unknown targets or in situations where automation is desired. Long analysis times are also common with heart-cutting methods which can slow down productivity.[78,86,100-102]

Alternatively, comprehensive 2D-LC, referred to as LC x LC, offers greatly improved peak capacities as compared to 1D-LC while also allowing for full automation and analysis of the entire sample. Comprehensive 2D-LC methods involve the complete transfer of the ^{1}D effluent to ^{2}D for separation, which is ideal for non-targeted analyses and samples with low concentrations of analytes, since the entire effluent from the first dimension undergoes separation in the second dimension, thus preventing the loss of any sample that might occur when sampling only certain fractions and/or peaks as in LC-LC.[85,98,103,104] Another key benefit to transferring the entire effluent from ^{1}D to ^{2}D is observed when separating extremely complex mixtures such as plant material associated with pharmaceutical or therapeutic samples. In these instances, constituents that are considered to be the target fractions could be even more difficult to isolate because of interferences from endogenous compounds or other interfering analytes in the sample.[105] The potential for contamination is also minimized in a comprehensive system since samples do not leave the system once injected; typically going from the injection, through the first dimension then into sample loops before elution on to the second dimension.[78,85]

The ability to easily combine LC x LC systems with conventional LC detectors such as MS and UV is also an important advantage over LC-LC methods. Many LC x LC systems include a reversed-phase (RP) column in the second dimension which uses mobile phases conducive to detection with MS.[106] Using RP columns in each dimension is a popular choice for several 2D-LC applications as result of easier method optimization

because of mobile phase compatibilities and the ease with which MS detectors can be coupled, resulting in robust methods, particularly for use with pharmaceuticals and derivatives.[81,85,97,104,107-109]

Earlier applications of comprehensive 2D-LC included the use of the first dimension column as an on-line extraction step before achieving chromatographic separation in the second dimension column. Rao and Shinde used a restricted access material (RAM) column followed by a RP C_{18} column for the LC x LC determination of antiretroviral drugs in rat serum and urine. Liquid-liquid extraction (LLE) and solid-phase extractions (SPE) were first tried with the samples in an off-line technique, however, acceptable recoveries were not achieved for low concentrations of drugs. These extraction procedures were also tedious to conduct. Comprehensive 2D-LC was then successfully investigated for rat serum and urine samples by employing a RAM column in ^{1}D as an on-line extraction step to remove proteins and large endogenous molecules, followed by chromatographic separation in ^{2}D. The method enabled fast extraction and separation of the samples within 20 min with only a filtering step required prior to direct injection into the 2D-LC system. The method was also sensitive enough to detect low ng/mL concentrations of the antiretroviral drugs in rat plasma and serum.[89]

Mallet *et al.* used a comprehensive 2D-LC method, called sequential 2D extraction, in which the first dimension included a sample extraction technique with the purpose of providing decreased sample preparation time and increased separation within a single analysis. A mixture of common illicit drugs and drugs of abuse, including amphetamine, MDMA, mescaline, lidocaine, cocaine, THC, and heroin, were spiked into urine and subjected to the 2D extraction and analysis. The method was successful in

extracting and detecting the illicit drugs and drugs of abuse at concentrations as low as 10 pg/mL. Limits of quantitation were set to 1 ng/mL in a 1 mL urine sample and the average recovery was 88% achieved within a 15 min time frame.[92]

The LC x LC methods can also be implemented with analytical columns in each of the dimensions. One such example was presented by Holčapek *et al.* for the analysis of lipidomic samples. A RP column was placed in the first dimension followed by a hydrophilic interaction chromatography (HILIC) column in the second dimension. The RP column enabled the separation of lipid species using the hydrophobic part of the molecule first, then any co-eluting species were separated in the second dimension on the basis of their differing polarities. Although Holčapek's work was a proof-of-concept study, it did demonstrate the potential of a comprehensive HILIC x RP method for the identification and lipids from human plasma and porcine brain samples. In total, 143 lipid species were identified in a run of <2.5 h.[110]

Additional column combinations that have been reported in LC x LC methods, including size exclusion chromatography (SEC) x RP, ion-exchange chromatography (IEX) x SEC, IEX x RP, normal-phase (NP) or HILIC x RP, and HILIC x SEC. However, the most common combination is that of two reversed-phase columns (RP x RP).[108] Combinations of two RP columns have received a lot of attention because of the potential for high peak capacities and suitability for use with pharmaceuticals and other small molecules. Although combinations using RP columns in each dimension may not be highly orthogonal, variation of mobile phases and gradients can result in desirable peak capacities and resolving power for analytes of interest.[111]

Methods using RP x RP have been used for the determination of antioxidants, separation of biological compounds, food analysis, and analysis of natural products.[108,112,113] Natural products typically originate from plant materials which present a very complex sample matrix and contain biologically active substances, thus making them of interest for toxicological analyses. Natural products present in Chinese herbal medicine (CHM) are of particular importance.[105,114,115] The effectiveness of CHMs is believed to come from the combined properties of multiple biologically active components. Therefore CHMs often present a complex mixture of compounds for analysis that requires adequate separation before each can be identified.[116] For example, Hu *et al.* developed a LC x LC system for the separation of *Rhizoma chuanxiong* and *Angelica sinensis* which represent two of the most commonly used drugs in the prescription of CHM. The method used a cyano (CN) column in the first dimension to separate polar compounds and a silica monolithic ODS column in the second dimension for the separation of the less-polar compounds. About 120 compounds from *R. chuanxiong* and 100 in *A. sinensis* were successfully separated.[115]

Krieger used an RP x RP separation method in the analysis of Si-Wu-Tang; a CHM made from four different herbs, each with its own set of characteristic compounds that are used for identification purposes. The high complexity and variability within Si-Wu-Tang make it an ideal choice for separation using 2D-LC. Detection was conducted with QTOF-MS and peaks were matched with library templates for each of the individual compounds. Over 75% of the template peaks were matched to those detected in Si-Wu-Tang, demonstrating the utility of this RP x RP method in the analysis of CHMs.[105]

There have been many successful applications of LC x LC, however, the development and optimization of such methods present many challenges. The two dimensions used must be compatible while also being orthogonal. In order to achieve this goal, there are a multitude of parameters in each dimension, including column type and particle size, mobile phase selection, analysis time, gradient, and flow rate, that must first be optimized individually before they are optimized as part of the whole system.[73,85,98,108]

The parameter that can be most influential and the most challenging to optimize is the solvent selection for the mobile phases in each dimension. Solvent selection is particularly difficult when two different types of columns are used in the two dimensions (*e.g.,* normal-phase and reversed-phased) that require different solvent types that may be incompatible.[85,98,111] If solvents are not compatible, columns could be damaged by the use of improper solvents. A major risk is that compounds separated in the first dimension can remix during transfer to the second dimension, or might not be eluted onto the second dimension at all, and thus 2D separation will not occur.[85,106]

Another potential cause of effluent remixing can occur when the sampling time in the second dimension is longer than the peak width of the effluent transferring from the first dimension, thus causing separable peaks to elute in the same fraction. The discrepancy between sampling time and peak width is referred to as "undersampling" and can lead to decreased peak capacity of the method. Undersampling can be minimized through the appropriate selection of column dimensions for use in the second dimension, as well as optimization of the amount of time set for collection of the effluent from the

first dimension before transfer to the second, also referred to as the sampling time.[78,85,98,117]

Dilution of an analyte or analytes is an inherent issue of chromatographic methods as the sample disperses along the column. Negative effects of dilution are further compounded when two LC systems are combined, as in 2D-LC. Solvent gradients can be used in each dimension as a way to minimize the effects of sample dilution.[74,78] In RP x RP systems, gradients can also be used to improve separation when the columns chosen are not completely orthogonal.[111] The length of time over which a gradient runs, referred to as the "gradient stop time," is the maximum duration of the gradient in the second dimension. The gradient stop time must always be less than the modulation or sampling time which is equal to the gradient stop time plus the time needed for the second dimension column to equilibrate.[86]

Second dimension gradients in LC x LC are used to generate higher peak capacities, eliminate possible carryover effects, and to improve bandwidth suppression. The samples being analyzed by the 2D-LC system should be taken into consideration whenever a gradient type is chose and optimized. Overall, gradients should be quick and have a steadily increasing slope to allow for better separations. The use of gradients also ensures effective separation of complex mixtures, as the components are likely to have a variety of retention factors that must be accounted for.[70,73] In 2D-LC, there are four common gradient types employed in the second dimension (Figure 1); full, segment, shift, and parallel.[108]

Full gradients cover a very steep and wide gradient over the span of a very short amount of time. These gradients provide high bandwidth suppression, which leads to

greater peak capacities. However, there is a greater chance of carry-over, or wrap-around, from compounds that are more strongly retained. Another drawback of using a full gradient, particularly with RP x RP methods, is that compounds with lower (or higher) retention in the first dimension also have lower (or higher) retention in the second dimension. These retention behaviors result in a diagonal appearance of eluted compounds in the final 2D contour plot.[108]

Segment gradients are less steep than full gradients but still have significant bandwidth suppression effects, leading to increased peak capacity. Instead of using a wide, continuous gradient over the entire separation period, a lower gradient coverage is used in the early section of the separation and a higher gradient coverage in the later section. The alteration of gradients throughout the separation, though minimal, results in lower probabilities of wrap-around effects since the gradient range is not continuous as in a full gradient.[108]

A "shifted gradient" is implemented when the second dimension uses a narrow gradient with a changing range or concentration throughout the analysis time. Shifted gradients facilitate compression of peak bandwidths and increased peak capacity in the second dimension. The continuous changing of the gradient minimizes wrap-around effects and demonstrates higher peak capacities than the other gradient types. Through the use of a shifted gradient, more of the 2D separation space can be used, making it a valuable technique for improving separation and spreading peaks further apart.[104] When a shifted gradient is used, though, there is the possibility that a peak from a single compound might elute in two adjacent separations, thus appearing to have two different

retention times. Care should be taken to ensure that such peaks are correctly assigned as to one compound.[86]

Parallel gradients are quasi-isocratic gradients which utilize a longer second dimension elution time and eliminate the need for post-gradient equilibration time, but result in larger bandwidth and lower peak capacities. Parallel gradients are best used in accordance with the retention characteristics of the first dimension separation which requires more time and effort to optimize before the gradient can be used effectively.[108]

Second dimension gradients in LC x LC are used to increase peak capacities, eliminate possible carryover effects, and to improve bandwidth suppression. The samples being analyzed by the 2D-LC system should be taken into consideration whenever a gradient type is chosen and optimized. The number of parameters that must be optimized during development of a 2D-LC method is several times greater than the number of parameters in a traditional 1D separation. However, once a comprehensive LC x LC method has been optimized it can be easily automated for use in high throughput applications.

The improved separation and resolving powers offered by 2D-LC can make such methods extremely attractive for the analysis of complex samples and mixtures. Benefits of 2D-LC systems include increased peak capacities,[70,103,118] separation of isomers,[89] and increased separation of compounds and metabolites.[72,88] Other important attributes of 2D-LC include decreased sample preparation time and decreased potential for loss of analytes during preparation as the first dimension can be used to separate proteins and other unwanted substances from analytes, which is especially important in removing endogenous compounds from the biological matrices common in toxicological

analyses.[18,78,88-90,94] There are currently no reports in the literature on the separation of coeluting or isomeric NPS using 2D-LC. Examples of existing heart-cutting and comprehensive methods are given in Table 1 (see below). Further investigations into the use of 2D-LC to resolve complex mixtures of NPS in biological specimens is therefore a major goal of the present project.

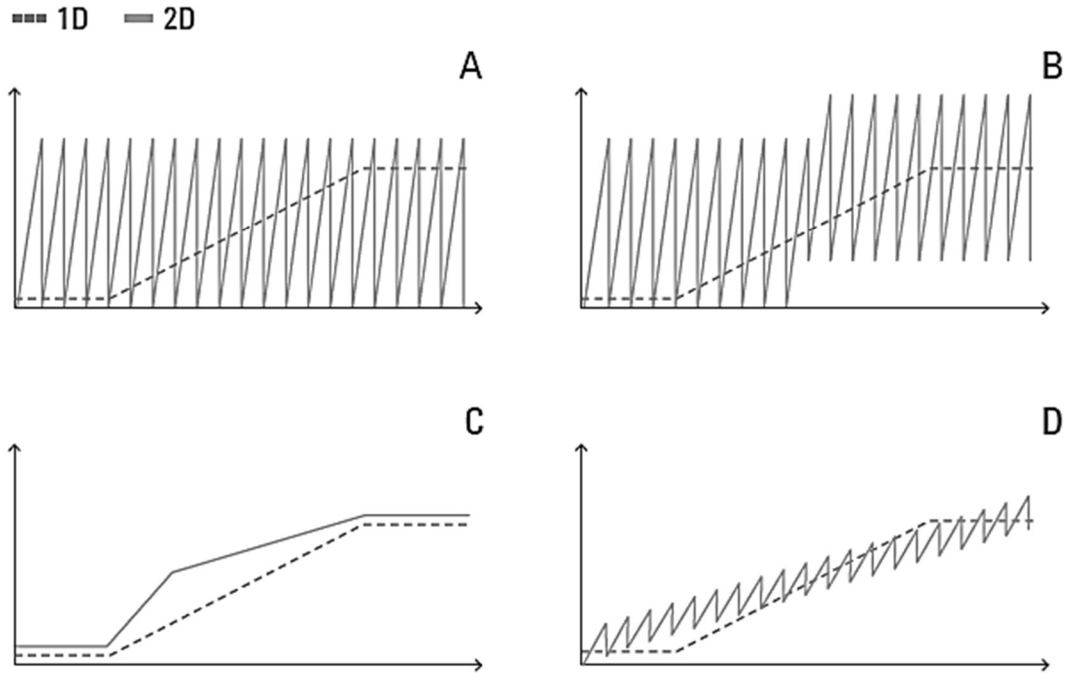

Figure 1. Second-dimension gradient types over time for LC x LC separations: A) full gradient, B) segment gradient, C) parallel gradient, and D) shift gradient.

Table 1. Examples of 2D-LC method applications found throughout the literature.

Matrix	2D-LC Mode	Columns	Detector(s)	Reference
ADCs	LC x LC	HIC x RP	UV-MS	[119,120]
ADCs	LC - LC	SEC - RP	DAD; MS	[94]
antibody digest products	LC x LC	SCX x RP; RP x RP; HILIC x RP	UV; MS	[121]
antiretroviral drugs	LC x LC	RAM x RP	ion-trap MS	[89]
benzenes	LC x LC	RP x RP	UV	[122]
cannabinoids	LC - LC	RP - RP	ESI-TOF-MS	[99]
CHMs	LC x LC	HSA x RP	Ion-trap MS	[123]
drug impurities	LC x LC	RP x RP	DAD; TOF-MS	[124]
drug metabolites	LC x LC	RP x RP	ESI-MS/MS	[72]
E. coli and *S. cerevisae* metabolomic products	LC x LC	SCX x HILIC	ESI-MS/MS	[88]
E. coli proteins	LC x LC	SEC x RP	ESI-MS	[84]
EO-PO (co)oligomers	LC x LC	NP x RP	ELSD	[125]
isomeric oligostyrenes	LC x LC	RP x RP	UV	[126]
isomeric oligostyrenes	LC x LC	RP x RP	UV	[127]
lipids	LC x LC	RP x HILIC	ESI-MS/MS	[110]
low MW components of maize	LC x LC	RP x RP	UV	[128]
oligostyrenes	LC x LC	RP x RP	UV	[129]
paracetamol and ketorolac enantiomers	LC - LC	RP x chiral	Ion-trap MS	[93]
peptides	LC - LC	RP - RP	MALDI-TOF-MS	[130]
peptides	LC - LC	SCX - RP; RP - RP	UV-MS	[131]
peptides	LC x LC	SEC x RP	ESI-MS	[132]

peptides	LC x LC	RP x HILIC	UV	[133]
pharmaceuticals	mLC - LC	RP - RP	UV	[97]
pharmaceuticals and citrus oils	LC x LC	NP x RP	DAD	[134]
phenolic antioxidants	LC x LC	RP x RP	DAD	[135]
phenolics	LC x LC	HILIC x RP	PDA; ESI-MS	[136]
proteins	LC x LC	IEX x RP	UV; ESI-MS	[83]
sertraline enantiomers	LC x LC	RAM x chiral	ESI-MS/MS	[76]
steroids, lemon oils	LC x LC/LC	NP x RP/RP	DAD	[137]
TCMs	LC x LC	RP x RP	DAD; APCI-MS	[107]
TCMs	LC x LC	RP x RP	DAD	[115]
triacylglycerols in lipids	LC x LC	NP x RP	APCI-MS	[138]
warfarin stereoisomers	mLC - LC	RP x chiral	QTOF-MS	[96]
warfarins/hydroxywarfarins	LC x LC	RP x chiral	ESI-MS/MS	[139]

*mLC – LC = multiple heart-cutting

3. DEVELOPMENT OF DATABASE AND SPECTRAL LIBRARY

3.1 Introduction

The presence of novel psychoactive substances (NPS) on the illicit drug market and therefore present in toxicological specimens is not a new occurrence. However, as NPS continue to be developed, analytical methods for detection and identification of such compounds must adapt to keep pace.[140,141] Novel psychoactive substances are altered by illicit manufacturers to circumvent federal, state, and local legislation intended to control their usage in public. Preventing a substance from falling under legal control can be achieved through an action as simple as altering a functional group through its removal, addition, or movement along the chemical structure. As these changes are made, the structures of the resultant compounds no longer match those of substances that are illicit, thus placing them outside the purview of the controlled substance laws.[2,5,18]

The constant emergence of new NPS also presents analytical challenges, since existing methods of detection are typically designed for specific functional groups or structures.[6] Identification of analytes of interest in samples generally begins with the use of a screening technique to tentatively identify possible compounds or classes of compounds present in the sample.[45,63,142] Methods using GC-MS and LC-MS have been developed for screening purposes but both typically require the use of libraries, which contain characteristic mass spectra generated using electron ionization (EI) or electrospray ionization (ESI) techniques, respectively, to make identifications.

Using characteristic mass spectra to identify analytes in a sample is not a new concept. Libraries exist containing spectra for as few as a couple dozen compounds to as many as several hundreds of thousands.[7,38,49,53,143] The most common libraries contain

spectra generated through EI, because of their high degree of reproducibility and because EI-based libraries have been established for longer than those that are ESI-based. Libraries containing spectra from ESI sources have been increasing in popularity among forensic toxicology laboratories.[47,53,56,144,145] There are several benefits to using ESI over EI, including the ability to maintain an intact molecular ion, which leads to increased confidence in identification because of the ionization in ESI being a "softer" or less intense technique than EI. Techniques using ESI are also not limited to use with only volatile, thermally stable molecules as with EI sources in GC.[42,52] Currently, there exist a number of GC libraries, but large LC libraries are lacking, and those that do exist do not contain many NPS or use theoretical accurate masses rather than masses measured from reference standards.[7-9]

Development of ESI-based libraries for LC methods has historically involved the use of triple quadrupole mass spectrometry (QqQ-MS) but these instruments are considered to have low resolution, which makes differentiation of some NPS difficult, particularly those with extremely similar accurate masses.[28,40] In contrast, high-resolution mass spectrometry (HRMS) techniques, such as those using hybrid quadrupole time-of-flight mass spectrometers (QTOF-MS), are generally sensitive enough to distinguish between compounds with minute differences in accurate mass.[40,66] Another benefit to using HRMS techniques is the ability to collect information for all ions while still maintaining high resolution and mass accuracy, which is ideal for comprehensive screening. Collection of all ion data also allows for retrospective screening without the need for reanalysis of the sample; allowing for newly reported compounds to be searched for in previously analyzed samples.[40,46,146]

The present study reports the development of a comprehensive compound database for 499 unique chemical entities considered to be possible NPS, metabolites, and related compounds. A full HRMS spectral library was created for 410 of these compounds, with partial spectral information for another 25 compounds also included. A comprehensive compound database was also created for 76 deuterated internal standard compounds.

Materials and Methods

3.1.1 Chemicals

Optima LCMS grade methanol (MeOH), acetonitrile (ACN), HPLC water, and formic acid were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Ammonium formate (99%) was also purchased from Fisher Scientific.

The ESI-L tuning mix (p/n: G1969-85000) and 0.1 mM HP-0321 (I8720263) were obtained from Agilent Technologies (Santa Clara, CA, USA) and used to prepare the tuning solution for the LC-QTOF-MS.

3.1.2 Standards

Reference standards for the NPS and internal standards were obtained from Cayman Chemical (Ann Arbor, MI, USA). Standards that were received as neat solid material were put into solution with methanol (MeOH) for storage at -20°C. Compounds that were not readily soluble in MeOH were put into solution with dimethyl sulfoxide (DMSO). Each standard was assigned a unique identifying number for in-house usage (*i.e.,* FIU-nnnn) in order to track usage of the compounds throughout sample preparation and analysis.

An arginine reference standard was also obtained from Cayman Chemical for use as a quality control standard.

3.1.3 Sample Preparation

Reference solutions were prepared from the reference standards of 499 NPS and 76 internal standards at concentrations of 10 µg/mL in MeOH. Working solutions were then prepared from the reference solutions at concentrations of 1 µg/mL in MeOH. A 1.6 ng/µL working solution of arginine was prepared in HPLC water for use as a quality control to ensure proper tuning and calibration of the instrument prior to FIA.

3.1.4 Instrumentation and Software

Instrumentation used for analysis included an Agilent 1290 Infinity UHPLC system coupled to an Agilent 6530 Accurate-Mass QTOF-MS (Agilent Technologies, Santa Clara, CA, USA). A Zorbax Eclipse Plus C_{18} Rapid Resolution HD column (2.1 x 50 mm, 1.8 µm; Agilent Technologies, Santa Clara, CA, USA) was used for separation during the database and library applicability test. The QTOF-MS was operated in positive-ion electrospray mode with Jet Stream ESI technology.

Agilent MassHunter LC/MS Acquisition software for the 6200 series TOF/6500 series QTOF (Version B.06.00) was used to acquire spectral data. MassHunter Qualitative Analysis software (Version B.06.00) was used to process the data. MassHunter Personal Compound Database Library (PCDL) Manager software (Version B.07.00, Build 7024.0) was used to create the compound database and high-resolution MS/MS spectral library. ChemBioDraw Ultra (Version 14.0.0.117; PerkinElmer,

Waltham, MA, USA) was used to create the 2D chemical structure of each NPS for use in the PCDL.

3.1.5 Methods

Collection of spectral data for the MS/MS spectral library was done via flow injection analysis (FIA). Diluted standards were individually injected directly into an Agilent 6530 series Accurate-Mass Quadrupole Time-of-Flight (QTOF) mass spectrometer with Jet Stream ESI ion source coupled to an Agilent 1290 Infinity Series Binary Pump system. Most injection volumes were 1 µL, however, some compounds had to be injected at volumes up to 10 µL to produce spectra with base peak intensities over the 1000 count threshold. A small percentage of compounds also had to be injected at concentrations of 2 µg/mL.

A 50:50 isocratic mobile phase system was used at a flow rate of 0.2 mL/min for 2 min with aqueous (A) 5 mM ammonium formate in HPLC water with 0.1% formic acid, and organic (B) acetonitrile with 0.1% formic acid. A positive mode electrospray ionization (ESI) targeted MS/MS method was used to collect the data. The quadrupole used a narrow isolation mass window of 1.3 amu. The ESI source parameters were: drying gas temperature 325°C; drying gas flow rate 5 L/min; nebulizer 30 psi; sheath gas temperature 375°C; sheath gas flow 12 L/min. Scanning source parameters were: VCap voltage 4000 V; nozzle voltage 0 V; fragmentor voltage 140 V; skimmer voltage 65 V.

Compounds were fragmented at three standard collision energies (10, 20, and 40 eV) to produce characteristic fragmentation patterns. The MS range was set to 50-1700 *m/z* with an MS acquisition rate of 10 spectra per second. The MS/MS range was set to

25-1700 *m/z* with an MS/MS acquisition rate of 3 spectra per second. Spectral data were added to the MS/MS spectral library when the compound fragmentation produced a base peak of at least 1000 counts and had a mass accuracy within 5 ppm. If these criteria were met, the compound information and fragment ion spectrum from each collision energy was imported into the PCDL using PCDL Manager software. An arginine standard was run with each batch of standards to ensure that the instrument was properly tuned and calibrated.

The "Find by Formula" (FBF) function of the Qualitative Analysis software was used to isolate a targeted compound from the FIA chromatogram. The MS/MS spectra were then extracted and exported into the PCDL using the "Send Spectra to PCDL" function.

3.2 Results and Discussion

Development of libraries for use with ESI-based methods are typically generated using QqQ-MS because of the greater availability of such instrumentation in laboratories. In these instruments, the first quadrupole (Q1) is typically used to scan for a specific precursor ion or range of ions of interest. The selected ions then move to a collision cell (Q2) where they are fragmented using a neutral collision gas. These ion fragments then pass to the third quadrupole (Q3) where selected fragment ions, or product ions, are allowed to pass to the detector while all other ions are filtered out.[79] The fragmentation of ions, or transitions, from the precursor to the product ions is recorded using multiple reaction monitoring (MRM). These MRM transitions are included in QqQ-MS libraries and serve as the characteristic fragmentation data for identification of compounds. However, the collision energy required to generate these characteristic MRM transitions

must be optimized for each compound prior to collection. The need for optimization introduces another step and requires more time to add new compounds to the library. Libraries of MRM transitions also have low degrees of resolution as compared to HRMS techniques, which may result in significant challenges when trying to differentiate between compounds with similar accurate masses.[66,146]

Building a library using an HRMS technique such as QTOF-MS does not require collision energies and fragmentor voltages to be optimized prior to data collection. Instead, multiple collision energies can be employed and collection of all resultant spectra can be conducted simultaneously. The capability to comprehensively collect spectra makes the rapid addition of spectra for new compounds possible and much easier to do than with MRM transition libraries. The ability to collect high resolution full scan MS and MS/MS fragmentation data presents a large advantage for QTOF-MS instrumentation over QqQ-MS, since information about potential unknown compounds can be collected in addition to the high mass accuracy information of known compounds.

In the present research, the major goal was to create an HRMS spectral library for as many NPS standards from the synthetic cannabinoid, stimulant, hallucinogen, and other related classes using a QTOF-MS approach (Table 2 displays the number of compounds represented from each class of NPS; Table 3 displays the types of molecules included in the database). These spectra were later combined with an existing HRMS library containing spectral data for an additional 260 compounds.[147] The new compound database was first created using the PCDL software. This database contained information for 499 entries including NPS, metabolites, and related compounds. A second database was also created containing entries for 76 deuterated internal standards (see Appendices 1

and 2 for a complete listing of compounds contained in each respective database). Compounds selected for inclusion in the database were chosen from a variety of sources, including lists of commercially available standards, government documents and reports, scientific literature, and online drug-user forums. Standard information input into the database included the compound common name, the IUPAC name, the molecular formula, calculated accurate mass, a 2D structure, as well as the CAS registry and ChemSpider numbers when available. Reference standards for each entry were obtained from Cayman Chemical and the manufacturer's product number was also included in the entry for traceability. Figure 2 presents an example of the compound database generated using the MassHunter PCDL software with information for the compound acetyl fentanyl displayed. Under the column labeled "Spectra", the number of successfully acquired HRMS spectra for that compound is presented.

Table 2. Distribution of compounds in database by class.

Drug Class	Number in Database
Synthetic Cannabinoid	295
Other*	89
Cathinone	67
Phenethylamine	29
Tryptamine	14
Piperazine	5

*includes opioids, amphetamines, benzofurans, and other compounds.

Table 3. Distribution of compounds in database by molecule type.

Molecule Type	Number in Database
Precursor Compound	293
Metabolite	109
Isomers	71
Analogs*	21
Glucuronides	5

*derivatives of NPS that are not considered metabolites or isomers.

High resolution mass spectra were generated by direct injection of 1 µL volumes of the 435 individual compound solutions into the ESI source of the QTOF-MS at concentrations of 1 µg/mL in MeOH. After preparation in MeOH, the compounds were ionized in positive mode and targeted ions were subjected to three standard collision energies (10, 20, and 40 eV). All resultant MS/MS spectral data were collected then processed to determine suitability for inclusion into the database. Once MS/MS spectral data were included in the database, it was more properly referred to as the HRMS spectral library.

In order to be accepted into the library, there were several criteria that the collected data had to meet. For each compound data file, the "Find by Formula" (FBF) function in MassHunter Qualitative Analysis was used to isolate the individual compound. The FBF function was linked to the compound database which assigned identification using the accurate mass and generated chemical formula. A secondary function within FBF was the "Extract MS/MS spectrum" function. Using this, the MS/MS spectrum of the compound at each collision energy was extracted. For inclusion in the HRMS spectral library, compounds identified using FBF needed to have a database search "score" >90 and a mass accuracy within ± 5 ppm. The match "score" of a

compound is generated through the use of both a forward search (when data in the sample are matched against those in the library) and a reverse search (when library data are searched against data in the sample). The MS/MS spectral data at each collision energy were then required to have base peak counts of at least 1000 to avoid inclusion of ion peaks from the background. A small percentage of compound solutions needed to be injected at concentrations of 2 µg/mL or at volumes up to 10 µL in order to meet these criteria for inclusion in the HRMS spectral library.

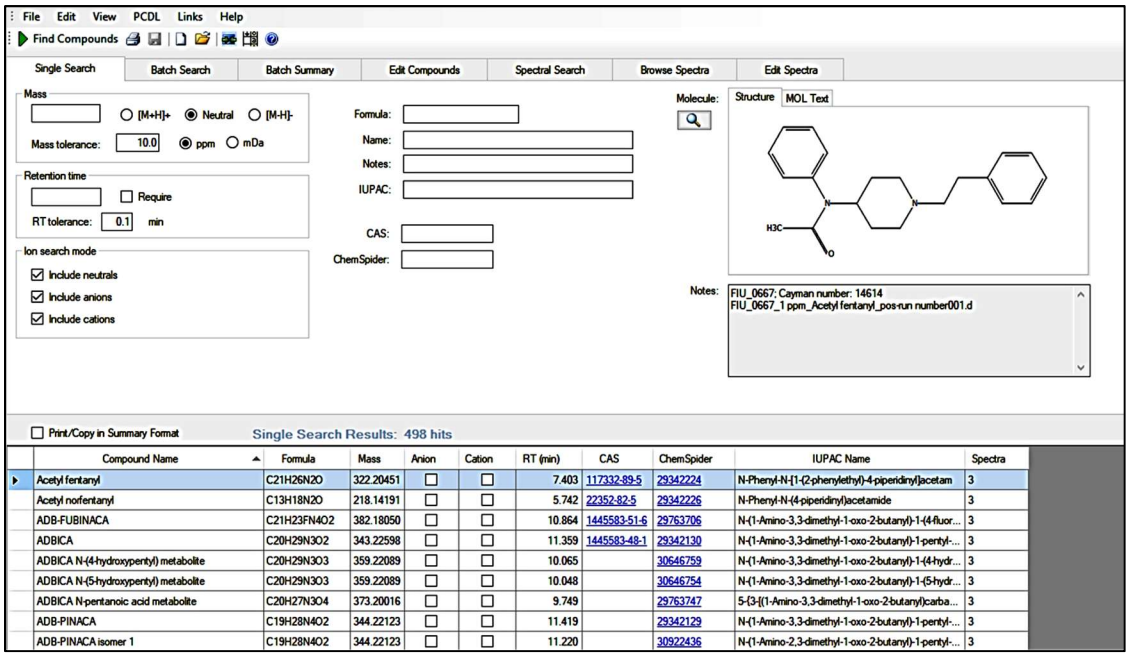

Figure 2. An image of the PCDL software used to create the compound database and MS/MS spectral library.

On the basis of the FBF scores >90, mass accuracy within ±5 ppm, and the 1000-count MS threshold, full MS/MS spectral information at all three collision energies was included in the library for 410 of the 435 NPS, metabolites, and related compounds. A representation of collected MS/MS spectra for the compound acetyl fentanyl is presented

in Figure 3. Of the remaining 25 compounds, MS/MS spectra collected at two of the three collision energies were included for 19 compounds with another six compounds having only a single acceptable MS/MS spectrum. The ions represented at relative abundances >10% for each MS/MS spectrum accepted into the library are presented in Appendix 3.

3.3 Conclusion

The compound database and HRMS spectral library were successfully created containing approximately 550 compounds with MS/MS spectral data for over 470 compounds at three distinct collision energies (10, 20, and 40 eV). Retention time data were also included in the database for in-house use to help differentiate among compounds with similar fragmentation patterns. The database and library were combined with another library that had been created as part of a previous project which included MS/MS spectra for an additional 260 compounds, bringing the number of compounds in the library with MS/MS data to over 700. Entries for which complete MS/MS spectra could not be collected were still included in the database to be used for compound identification using accurate mass data. Ultimately, over 800 compounds were represented in the database.

The high resolution and high mass accuracy of LC-QTOF-MS presents a significant advantage for screening and confirmation of NPS with a high degree of confidence in correct identification. Through the use of a MS/MS spectral library, the confidence in identification is further increased. Further work was conducted to evaluate the practicality of this technique for routine forensic toxicological screening of NPS following standard validation parameters.

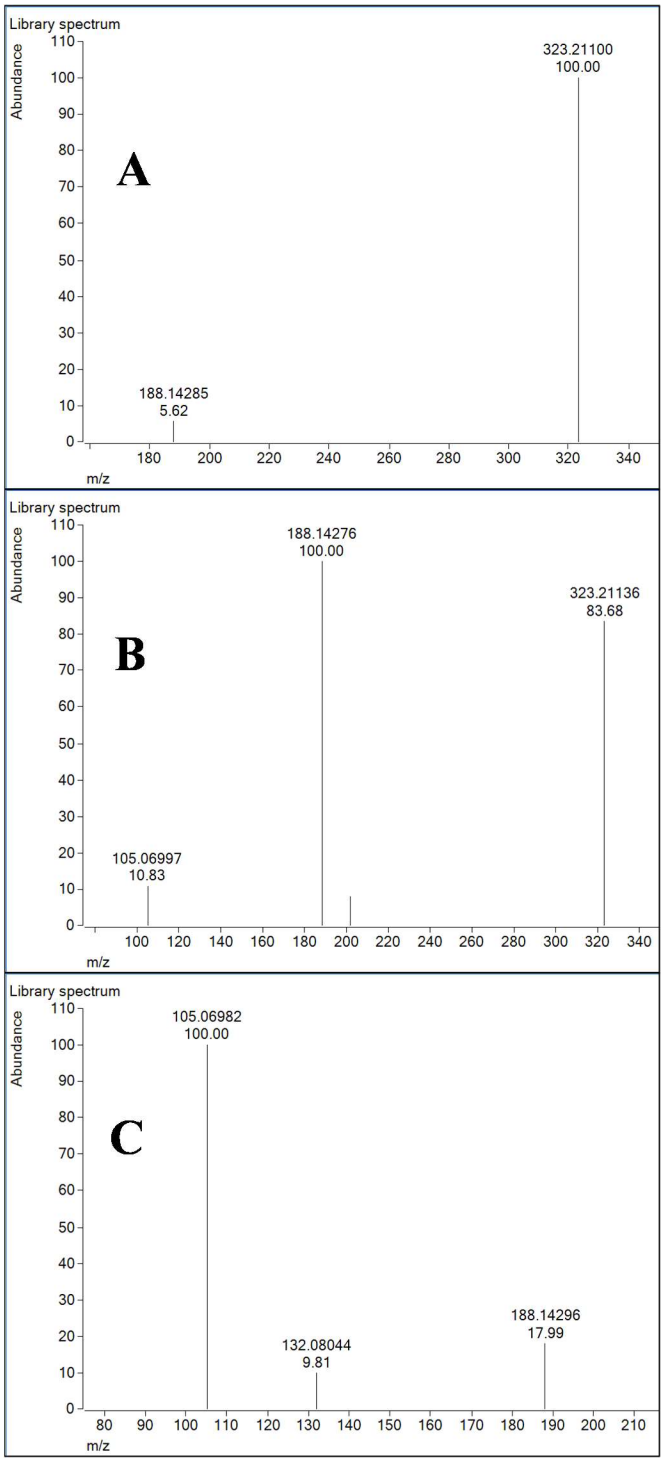

Figure 3. Images of PCDL software showing the MS/MS spectral data of acetyl fentanyl. MS/MS spectral data is shown at collision energies: A) 10 eV, B) 20 eV, and C) 40 eV.

4. COMPREHENSIVE LC-QTOF-MS METHOD VALIDATION

4.1 Introduction

Before the developed compound database and HRMS spectral library can be implemented for routine comprehensive screening, the method to be used in conjunction with the library must be fully validated. Validation ensures that analysis using the method produces reliable data that are not false or susceptible to misinterpretation. In clinical and forensic toxicology, unreliable data may be contested in court and could also result in mistreatment of patients or improper consequences of defendants in legal proceedings.[148]

Standard validation practices have been set forth by the Toxicology Subcommittee of the Organization of Scientific Area Committees (OSACs). These standard practices evolved from previously established standard practices from the Scientific Working Group for Forensic Toxicology (SWGTOX) which disbanded in 2014. The SWGTOX guidelines drew heavily from work published by Peters in 2007, who recommended a series of experiments to validate a method including the following criteria; selectivity, linearity, accuracy (bias), precision, and the limit of quantitation (LOQ). Other parameters such as the limit of detection (LOD), recovery, reproducibility, ruggedness, stability, and matrix effects were also suggested by Peters for inclusion in method validation procedures.[148,149]

A goal of the present work was to validate a method for screening and confirmation of over 800 NPS and related compounds. In order to do so, validation experiments were initially designed following the SWGTOX guidelines and later updated following the release of the OSAC guidelines.[150] Validation experiments for the present

work were conducted for the following parameters; linearity, accuracy, precision, LOD, LOQ, freeze/thaw stability, matrix effects, and carryover.

Validation of a method for toxicological screening and confirmation is typically conducted for a single class of compounds or for a small set of compounds, as the process can require a significant amount of time and resources. In the present work, method validation was conducted using a mixture approach in which each mixture of standards was validated as if it were a single compound. Three validation mixtures were created, each containing between 27-33 compounds, and validated according to the guidelines set by the Toxicology Subcommittee of OSAC.

The validated method was also used in the qualitative investigation of the applicability of the developed compound database and HRMS library for screening of blind-spiked urine specimens. This investigation used 20 blank urine samples that were spiked with 0-1 NPS represented in the database and library. The results of this qualitative investigation indicated that the database and library were suitable for screening purposes.

4.2 Materials and Methods

4.2.1 Chemicals

Optima LCMS grade methanol (MeOH), HPLC water, and formic acid were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Ammonium formate (99%) was also purchased from Fisher Scientific.

The ESI-L tuning mix (p/n: G1969-85000) and 0.1 mM HP-0321 (I8720263) were obtained from Agilent Technologies (Santa Clara, CA, USA) and used to prepare the tuning solution for the ESI-QTOF-MS.

A reference mass solution to ensure proper instrumental calibration throughout the analyses was created using the ESI TOF Mass Kit (p/n: G1959-85001) obtained from Agilent Technologies. The kit contained 100 mM ammonium trifluoroacetate ($TFANH_4$) in ACN:H_2O (90:10), 5 mM purine in ACN:H_2O (90:10), and 2.5 mM hexakis in ACN:H_2O (90:10).

4.2.2 Standards

Reference standards used in the method validation are the same as those used in creation of the compound database and HRMS spectral library for both the compounds and for the deuterated internal standards. All reference standards were obtained from Cayman Chemical (Ann Arbor, MI, USA).

4.2.3 Preparation of Mixtures

The same working solutions that were prepared for the creation of the compound database and HRMS library were used for method validation. The working solutions contained individual reference standards at concentrations of 10 μg/mL in MeOH. Three validation mixtures were created containing 29, 28 and 33 individual compounds, respectively, with each compound present at a concentration of 200 ng/mL in MeOH. The identity of the compounds contained in each mixture are displayed in Table 4, Table 5, and Table 6. The mixtures were designed so that compounds from a variety of NPS classes were represented in each and that no two compounds in a single mixture exhibited co-elution.

An additional mixture was created to serve as the internal standard in each validation mixture for quantitation purposes. The internal standard (IS) mixture

contained 22 deuterated internal standards representing a variety of NPS classes, each present in the mixture at a concentration of 200 ng/mL in MeOH. The compounds contained in the IS mixture are presented in Table 7. A compound from the IS mixture was matched with each NPS in the validation mixtures for quantitation purposes. These "matches" were based on similarities between the NPS and IS compound structures since the IS should be chemically similar to the analyte of interest. The IS compounds used in this research were chosen to represent a variety of drugs, with the majority from the SC class of compounds due to the relevance of SC in real-world samples as well as the large representation of SC in the database and library developed prior to the validation studies.

To validate the method in matrix, blank, pooled human urine was obtained from UTAK Laboratories (Valencia, CA, USA). For all analyses in matrix, the urine was diluted with aqueous mobile phase at a 1:5 dilution.

4.2.4 Instrumentation and Software

Instrumentation used for analysis included an Agilent 1290 Infinity UHPLC system coupled to an Agilent 6530 Accurate-Mass QTOF-MS (Agilent Technologies, Santa Clara, CA, USA). A Zorbax Eclipse Plus C_{18} Rapid Resolution HD column (3.0 x 100 mm, 1.8 μm; Agilent Technologies, Santa Clara, CA, USA) was used for separation of compounds in the urine matrix. The QTOF-MS was operated in positive-ion electrospray mode with Jet Stream ESI technology.

Agilent MassHunter LC/MS Acquisition software for the 6200 series TOF/6500 series QTOF (Version B.06.00) was used to acquire the data. MassHunter Qualitative Analysis software (Version B.06.00) was used to process acquired retention time data and

to process the blind-spiked urine specimens. MassHunter Quantitative Analysis software for QTOF (Version B.07.00, Build 7.0.457.0) was used for quantitation of the data. The compound database and HRMS spectral library created and managed using MassHunter PCDL software (Version B.06.00) was used to identify compounds in the blind-spiked urine specimens.

Table 4. Compounds contained in validation Mix 1, their accurate masses, and retention times.

Compound Name	Accurate Mass (Da)	Retention Time (min)
25I-NBMD	441.0437	9.17
3-Methylbuphedrone	191.1310	7.29
4-APDB	177.1154	6.43
4-fluoro-α-Pyrrolidinobutiophenone	235.1372	6.83
4'-fluoro-α-Pyrrolidinopropiophenone	221.1216	6.17
4-hydroxy MET	218.1419	5.21
4-methoxy PV8	289.2042	8.97
4-methoxy-α-Pyrrolidinopentiophenone	261.1729	7.70
4-Methyl-α-ethylaminobutiophenone	205.1467	7.49
5-fluoro SDB-006	338.1794	10.80
5-Fluoropentylindole	205.1267	11.21
5-MAPB	189.1154	6.99
AB-005	352.2515	9.58
AM-2233 azepane isomer	458.0855	8.66
AM694 N-(5-hydroxypentyl) metabolite	433.0539	10.45
AMT	130.0565	1.63
Benocyclidine	299.1708	9.14
Flubromazepam	331.9961	10.07
JWH 031 2'-isomer	305.1780	13.32
JWH 081 N-(5-hydroxypentyl) metabolite	387.1834	10.94
JWH 200 5-hydroxyindole metabolite	400.1787	8.61
JWH 203	339.1390	12.22
N-Methyltryptamine	174.1157	5.73
NPB-22	359.1634	11.53
PB-22 6-hydroxyisoquinoline isomer	358.1681	11.98
PCMPA	247.1936	8.25
THCA-A	358.2144	19.14
UR-144 N-heptyl analog	339.2562	15.45
α-Pyrrolidinopentiophenone metabolite 1	233.1780	7.65

Table 5. Compounds contained in validation Mix 2, their accurate masses, and retention times.

Compound Name	Accurate Mass (Da)	Retention Time (min)
2,3-methylenedoxy pyrovalerone	275.1521	7.39
25I-NBF	415.0445	9.11
2C-T-2	241.1136	7.90
2C-T-4	255.1293	8.51
2-fluoromethcathinone	181.0903	5.22
3,4-DHMA	181.1103	4.21
3,4-dimethoxy- α-pyrrolidinopentiophenone	291.1834	7.19
3-methyl-α-pyrrolidinopropiophenone	217.1467	6.93
4'-methyl-N-methylhexanophenone	219.1623	8.56
4-ethyl-N,N-dimethylcathinone	205.1467	7.44
4-fluoroisocathinone	167.0746	5.66
4-hydroxy MiPT	232.1576	5.67
4-MMC	177.1154	6.57
A-796260	354.2307	10.90
AB-005 azepine isomer	352.2515	9.78
AB-FUBINACA 3-fluorobenzyl isomer	368.1649	10.49
ADB-PINACA isomer 1	344.2212	11.20
AKB48 N-(5-fluoropentyl) analog	383.2373	13.22
Clencyclohexerol	318.0902	6.10
EG-018	391.1936	16.98
JWH 018 N-(5-hydroxypentyl) metabolite	357.1729	10.74
JWH 018 N-propanoic acid metabolite	343.1208	10.31
KM 233	362.2246	14.08
Loperamide	476.2231	9.52
MAM2201 N-pentanoic acid metabolite	385.1678	10.99
N-Ethylbuphedrone	191.1310	6.53
PB-22	346.1681	11.88
PCPr	217.1830	8.35
RCS-4 2-methoxy isomer	321.1729	11.60
SER-601	434.2933	15.46
UR-144 Degradant	311.2249	12.97

XLR11 N-(2-fluoropentyl) isomer	329.2155	12.46
Δ8-THC	314.2246	14.79

Table 6. Compounds contained in validation Mix 3, their accurate masses, and retention times.

Compound Name	Accurate Mass (Da)	Retention Time (min)
(R)-(−)-MT-45	348.2565	9.48
2,3-Dichlorophenylpiperazine	230.0378	8.31
25H-NBOMe	301.1678	8.41
2C-T	227.0980	7.50
2-Methoxyamphetamine	165.1154	7.95
3,4-Dimethylethcathinone	205.1467	7.64
3C-P	253.1678	8.10
4-Methoxyamphetamine	165.1154	7.42
5-fluoro NNEI	374.1794	11.09
9-octadecenamide/oleamide	281.2719	14.77
AB-CHMINACA	356.2212	11.50
AKB48 N-pentanoic acid metabolite	395.2209	11.81
BB-22 4-hydroxyquinoline isomer	384.1838	13.46
Cathine	151.0997	5.50
Diclofensine	321.0687	9.87
FUB-PB-22	396.1274	11.29
HMA	181.1103	5.11
JWH 018 2-hydroxyindole metabolite	357.1729	15.25
JWH 251 3-methylphenyl isomer	319.1936	12.51
MBZP	190.1470	5.47
Mephedrone	177.1154	6.58
Methylenedioxy Pyrovalerone metabolite 2	263.1521	5.92
N-methyl-2-AI	147.1048	5.83
NRG-3	241.1467	8.76
PB-22 N-(5-hydroxypentyl) metabolite	374.1630	10.45
RCS-4 N-(4-hydroxypentyl) metabolite	337.1678	10.33
UR-144 N-(2-chloropentyl) analog	345.1859	13.07
Δ9-THC	314.2246	14.44

Table 7. Compounds contained in the internal standard (IS) mixture and their accurate masses.

Compound	Accurate Mass (Da)	Retention Time (min)
(-)-11-nor-9-carboxy-Δ^9-THC-d3	347.2176	12.57
(±)-CP 47,497-C8-homolog-d7	339.3155	14.49
25I-NBOMe-d3	430.0833	9.39
3,4-Methylenedioxy pyrovalerone-d8	283.2024	7.51
AB-FUBINACA-d4	372.1900	10.62
AB-PINACA-d9	339.2621	11.18
Acetyl norfentanyl-d5	223.1733	5.91
ADB-PINACA-d9	353.2777	11.53
AM 2201 N-(4-hydroxypentyl) metabolite-d5	380.1948	10.69
Benocyclidine-d10	309.2335	9.27
Butylone-d3	224.1240	6.51
cis-Tramadol-d6	269.2262	7.17
JWH 007-d9	328.2501	12.98
JWH 018-d9	350.2345	12.65
JWH 073 5-Hydroxyindole metabolite-d7	350.2012	11.21
JWH 081 N-pentanoic acid metabolite-d5	406.1941	10.99
MAM 2201 N-pentanoic acid metabolite-d5	390.1992	11.11
Norsufentanil-d3	279.2026	8.05
PB-22-d9	367.2246	11.99
RCS-4 N-(5-hydroxypentyl) metabolite-d5	342.1992	10.46
UR-144 N-(4-hydroxypentyl) metabolite-d5	332.2512	11.38
XLR11-d_5	334.2469	12.10

4.2.5 LC-QTOF-MS Method

The LC separation was conducted with a gradient with 5 mM ammonium formate (AF) in HPLC water with 0.1% formic acid (FA) as the aqueous mobile phase (A) and MeOH with 0.1% FA as the organic mobile phase (B). The mobile phase gradient was employed at a flow rate of 0.3 mL/min with composition beginning at 5% B from 0-1 min, then increasing to 95% B from 1 – 9.5 min, and remaining at 9.5 min for the remainder of the analysis time. An equilibration time of 3 min was also incorporated into the LC method. Chromatographic column temperature was maintained at 40°C.

Detection of the chromatographic data was completed using QTOF-MS in full scan mode. The ESI source parameters were: drying gas temperature 325°C; drying gas flow rate 8 L/min; nebulizer 35 psi; sheath gas temperature 400°C; sheath gas flow 12 L/min. Scanning source parameters were: VCap voltage 3500 V; nozzle voltage 0 V; fragmentor voltage 125 V; skimmer voltage 65 V.

Two reference ions, provided by the reference mass solution, were monitored with mass correction to ensure proper instrumental calibration throughout analysis; 121.0509 *m/z* and 922.0098 *m/z*.

4.2.6 Retention Time Collection

Prior to validation of the method, retention data for all compounds were collected to prevent inclusion of any co-eluting compounds in the validation mixtures. Individual compounds were injected at concentrations of 1 µg/mL in MeOH at volumes of 5 µL. Separation was conducted over 20 min using an Agilent Zorbax Eclipse Plus C_{18} Rapid Resolution HD column (3.0 x 100 mm; 1.8 µm) and the LC-QTOF-MS method described

above. Retention data was collected using Full Scan mode with the MS range set to 50-1700 *m/z* with an MS acquisition rate of 10 spectra per second.

The FBF 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 interfering with identification and quantitation of the compounds.

4.2.7 Preparation of Samples in Urine

Urine samples were prepared for all aspects of validation. Calibrators were prepared in urine at seven different concentrations ranging between 2 – 120 ng/mL for each of the three validation mixes. Samples included blank urine diluted at a ratio of 1:5 using aqueous mobile phase. The working validation mixtures containing compounds at concentrations of 200 ng/mL in MeOH were spiked into the diluted urine at appropriate volumes to make the calibrators. Each sample was also spiked with the IS mixture at a concentration of 40 ng/mL.

Quality control (QC) samples were created at three concentration levels within the calibration range; low, medium, and high (5, 20, and 80 ng/mL) in urine diluted (1:5) with aqueous mobile phase. The QCs were also spiked with the IS mixture at a concentration of 40 ng/mL. Blank urine samples (matrix blanks) were also prepared using pooled, blank urine diluted (1:5) with aqueous mobile phase. The calibrators, QCs, and matrix blanks were used throughout the method validation process.

4.2.8 Preparation of Blind-Spiked Specimens

Two sets of 20 specimens were prepared by a third party not involved in the analysis using blank, pooled urine from UTAK Laboratories. The urine samples were spiked with 0-1 NPS and diluted with aqueous mobile phase at a 1:5 dilution. No internal standard was included as this was a purely qualitative investigation.

4.3 Results and Discussion

4.3.1 Linearity

Linearity experiments were conducted at seven calibration concentrations between 2 – 120 ng/mL. These concentrations were set at 2, 5, 10, 20, 50, 100, and 120 ng/mL and five replicates were run for each concentration level. Measured concentrations were then modeled using linear regression and a weight of *1/x* was applied. An example weighted calibration curve for 25I-NBF from validation mix 2 is presented in Figure 5. An example of an unweighted calibration curve for 25I-NBF is presented in Figure 4. There were no major differences between the unweighted and weighted calibration curves, but per OSAC guidelines, a weighted model should be used when there are notable differences between variances at the lowest and highest concentrations. This is the case when the concentration range is larger than one order of magnitude, as was the case with the calibration levels chosen in this study.

Calibration curves were created for validation mixes 1, 2 and 3. Mix 1 contained 29 compounds, all of which had linear calibration curves. Mix 2 contained 33 compounds, of which 30 had linear calibration curves. Mix 3 contained 28 compounds, of which 24 had acceptable linearity based on their calibration curves. The data collected

during the linearity studies were also used in the calculation of the limit of detection (LOD) and the limit of quantitation (LOQ) for each compound.

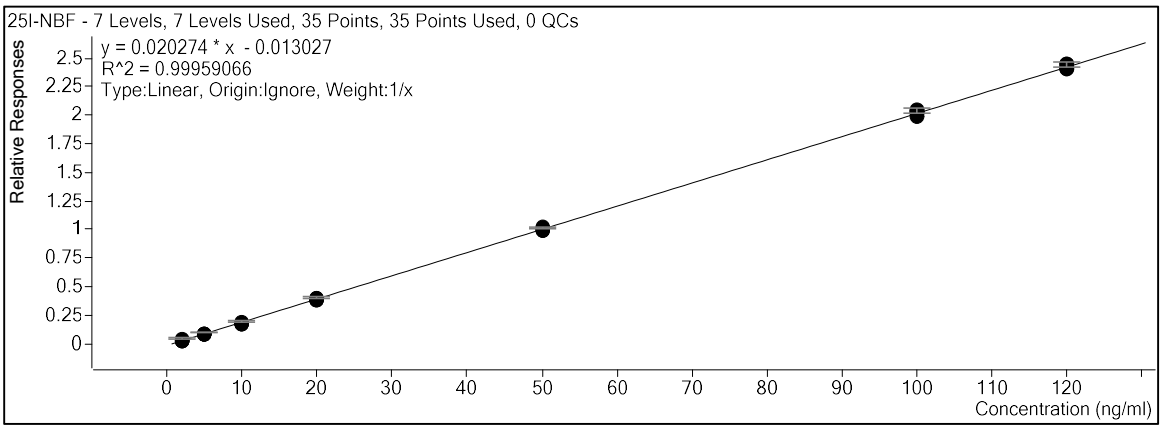

Figure 5. Weighted calibration curve for 25I-NBF from validation Mix 2.

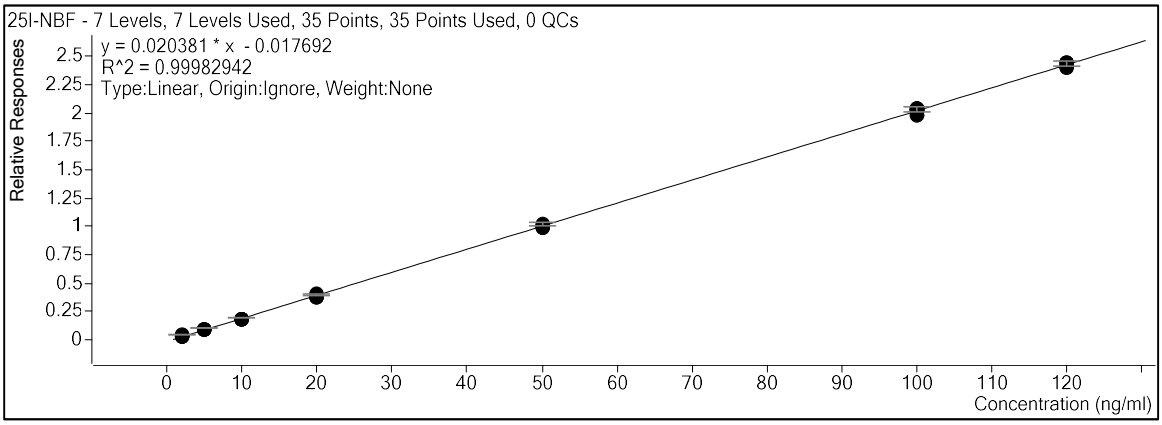

Figure 4. Unweighted calibration curve for 25I-NBF from validation Mix 2.

4.3.2 Accuracy and Precision

Accuracy (bias) is the determination of how closely a compound's calculated concentration corresponds to its actual concentration. Precision is a measure of how closely the calculated concentrations of a compound compare with each other within a

single analysis and between separate analyses. Accuracy and precision studies were preformed concurrently with the same samples known as the QC samples.

Samples for the accuracy and precision studies were created at three different concentrations, representing the low, medium, and high range of the concentrations used in the calibration curves. Three replicates of each concentration level were prepared on each of five consecutive days and analyzed on the day they were prepared.

Accuracy was then calculated for each compound in each mixture using the following equation:

$$Bias\ (\%)\ at\ Concentration =$$

$$\left[\frac{Gran\ \ Mean\ of\ Calculated\ Concentration - Nomina\ \ Concentration}{Nominal\ Concentration}\right] x\ 100 \qquad (2)$$

where the calculated concentration is the measured concentration of the compound during analysis and the nominal concentration is the concentration set for that calibration level. At each concentration, the maximum acceptable bias is ±20%. The data used in bias studies were also used to calculate precision, as per the OSAC guidelines.[150]

Precision studies were conducted to determine variability between runs on the same day (intra-day variability) and between runs conducted on separate days (inter-day variability). General precision values are expressed in terms of the coefficient of variation (% CV) as seen in Equation 3 following the calculation of the mean and standard deviation (*s*) of the response at each concentration.

$$\%CV = \frac{s}{mean\ response} \qquad (3)$$

Acceptable precision values must have a % CV within a range of ±20% at each concentration. Calculation of within-run precision was done using data from each of the three replicates at each concentration level (Equation 4). The largest % CV value calculated at each concentration was used to assess the within-run precision.

$$Within - Run\ CV(\%) = \frac{std\ deviation\ of\ a\ single\ run\ of\ samples}{mean\ calculated\ value\ of\ a\ single\ run\ of samples}\ x\ 100 \qquad (4)$$

Calculation of between-run precision was conducted with data collected at each concentration over the course of five runs completed on separation days (Equation 5).

$$Between - Run\ CV(\%) = \frac{std\ dev\ of\ all\ observations\ for\ each\ concentration}{grand\ mean\ for\ each\ concentration}\ x\ 100 \quad (5)$$

Bias and precision data for Mix 1 are presented in Table 8 with all calculated bias values at each concentration within ±10.1%. Overall precision values at each concentration were within ±10% CV, with all within-run and between-run precision values within ±20%. Most of the within- and between-run precision values were within ±10% with the exception of the low concentration within-run values.

The bias and precision data were calculated for the 30 compounds in Mix 2 which demonstrated linearity. The bias and precision data are presented in Table 9 with all values at each concentration within the limits of ±20%. All bias values were within ±10%. Overall precision values were all within the limits of ±20% CV, with the majority within ±10%. For the within-run and between-run precision values, all compounds had calculated values within ±20%.

The bias and precision data were calculated for the 24 compounds in Mix 3 with demonstrated linearity. The bias and precision data are presented in Table 10 with all values at each concentration within the limits of ±20%. All bias values were within ±10%. Overall precision values were all within ±10% CV. The within-run and between-run precision values were all within ±20%, with the majority within ±10%.

4.3.3 Limits of Detection and Quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) represent the lowest concentrations at which an analyte may be detected by a method and quantitated, respectively. Both values were calculated using the slope (m) and y-intercept values generated during the linearity studies. The LOD was calculated using the following equation:

$$LOD = \frac{3.3 s_y}{Avg_m} \quad (6)$$

where s_y is the standard deviation of the y-intercept and Avg_m is the average slope. The LODs for compounds in Mix 1 were all in the low ng/mL (ppb) range with values ranging from 0.2 to 2.9 ng/mL (Table 11). The LODs for compounds in Mix 2 were all in the low ng/mL (ppb) range with values ranging from 0.1 to 2.2 ng/mL (Table 12). The LODs calculated for compounds in Mix 3 were also in the lower ng/mL range with values between 0.2 and 1.6 ng/mL. Most values were below 1.1 ng/mL (Table 13).

The LOQ values were calculated in a similar manner to the LOD values using the following equation:

$$LOQ = \frac{10\ y}{Avg_m} \quad (7)$$

All of the calculated LOQs for Mix 1 are shown in Table 11 with a range of values from 0.6 to 8.8 ng/mL, with most values ≤ 5.0 ng/mL. The LOQ values for Mix 2 are shown in Table 12 with a range of values from 0.3 to 6.8 ng/mL, with most values at less than 5.0 ng/mL. The LOQ values for Mix 3 are shown in Table 13 with values between 0.6 and 4.7 ng/mL.

The calculated LOD and LOQ values demonstrate that the method is sensitive enough to detect low concentrations of analytes in human urine and can be applied to specimens with compounds present at pharmacologically relevant levels. A wide range of NPS concentrations in human specimens have been reported in the literature. For some SC, such as JWH 018, concentrations have been reported in postmortem cases between 0.1 and 199 ng/mL with an average concentration of 17.5 ng/mL,[151] which is within the range of detection of the method presented in this research for related JWH 018 compounds whose LOD and LOQ values were as low as 0.4 and 1.1 ng/mL, respectively. Another case reported in the literature involved acute fatal poisoning by NNEI, an analog of JWH-018. The concentrations in whole blood collected postmortem ranged from 0.64-0.99 ng/mL depending on from where in the body the samples were collected.[152] The method validated in the present work achieved LOD and LOQ values for 5-fluoro NNEI of 0.2 and 0.6 ng/mL, respectively, indicating that the method would be suitable for use in detecting NNEI and related analogs in real-world samples. A third case of NPS in postmortem samples was from a fatal case of multiple drug intoxication caused by the SCs AB-CHMINACA and 5-fluoro-AMB, combined with diphenidine. Postmortem tissue concentrations for AB-CHMINACA ranged from 7.55-38.9 ng/g.[153] The validated method obtained LOD and LOQ values of 0.8 and 2.3 ng/mL, respectively,

for AB-CHMINACA. The values reported in the literature demonstrate that the LOD and LOQ values obtained by the method from the present research are relevant for use in detection of NPS in both antemortem and postmortem human specimens.

Table 8. Bias and precision data for compounds in Mix 1. Concentrations for low, med, and high are 5, 20, and 80 ng/mL, respectively.

	Bias (%)			Precision (% CV)			Within-Run (%)			Between-Run (%)		
Compound Name	**low**	**med**	**high**	**low**	**med**	**high**	**low**	**med**	**high**	**low**	**med**	**high**
α-Pyrrolidinopentiophenone metabolite 1	1.6	2.0	0.4	6.4	3.2	0.7	12.9	4.0	1.3	6.4	3.2	0.7
25I-NBMD	1.6	2.0	0.4	5.6	3.1	0.7	10.0	4.6	1.3	5.6	3.1	0.7
3-Methylbuphedrone	1.7	2.1	0.4	3.5	2.6	0.7	6.5	4.8	0.7	3.5	2.6	0.7
4-APDB	0.8	1.0	0.2	3.6	2.5	0.9	7.8	3.6	2.0	3.6	2.5	0.9
4-fluoro-α-Pyrrolidinobutiophenone	1.4	1.8	0.4	4.4	3.7	1.0	5.1	3.6	1.7	4.4	3.7	1.0
4'-fluoro-α-Pyrrolidinopropiophenone	1.6	2.0	0.4	5.1	4.0	0.9	9.3	2.0	1.4	5.1	4.0	0.9
4-hydroxy MET	2.8	3.2	0.6	5.0	3.1	0.7	10.2	3.0	1.1	5.0	3.1	0.7
4-methoxy PV8	1.2	1.5	0.3	4.5	2.3	0.8	9.0	4.3	1.7	4.5	2.3	0.8
4-methoxy-α-Pyrrolidinopentiophenone	1.9	2.4	0.5	5.5	3.0	0.8	12.0	3.5	1.7	5.5	3.0	0.8
4-Methyl-α-ethylaminobutiophenone	3.3	4.2	0.8	3.5	2.7	1.0	5.6	3.9	1.4	3.5	2.7	1.0
5-fluoro SDB-006	2.2	2.7	0.5	8.4	4.6	1.1	15.5	3.8	1.4	8.4	4.6	1.1
5-Fluoropentylindole	3.4	4.2	0.8	5.3	2.5	0.9	11.1	3.6	2.1	5.3	2.5	0.9
5-MAPB	1.2	1.5	0.3	6.5	3.0	1.1	12.0	6.1	2.5	6.5	3.0	1.1
AB-005	0.5	0.6	0.1	6.7	3.2	1.4	10.9	6.0	3.5	6.7	3.2	1.4
AM-2233 azepane isomer	2.3	2.8	0.6	7.6	5.2	1.0	11.8	9.4	1.6	7.6	5.2	1.0
AM694 N-(5-hydroxypentyl) metabolite	8.1	10.1	2.0	5.0	5.5	1.5	6.4	7.7	3.1	5.0	5.5	1.5
AMT	0.3	1.0	0.2	4.3	2.8	0.6	6.0	2.7	0.8	4.3	2.8	0.6
Benocyclidine	3.9	4.8	1.0	5.3	4.4	0.8	7.0	4.0	0.7	5.3	4.4	0.8
Flubromazepam	1.7	2.1	0.4	5.1	4.2	1.1	9.2	5.9	2.1	5.1	4.2	1.1
JWH 031 2'-isomer	1.0	1.2	0.2	7.3	4.9	1.2	11.4	7.6	2.5	7.3	4.9	1.2

JWH 081 N-(5-hydroxypentyl) metabolite	1.2	1.5	0.3	7.0	4.7	0.6	11.4	10.7	1.1	7.0	4.7	0.6
JWH 200 5-hydroxyindole metabolite	1.5	1.9	0.4	8.8	3.4	1.5	18.1	4.8	3.2	8.8	3.4	1.5
JWH 203	1.0	1.3	0.3	7.6	4.3	0.7	13.2	8.2	1.3	7.6	4.3	0.7
N-Methyltryptamine	0.01	0.01	0.003	4.8	2.4	0.7	6.9	4.1	1.5	4.8	2.4	0.7
NPB-22	1.0	1.3	0.3	6.5	4.1	1.7	10.5	5.5	3.9	6.5	4.1	1.7
PB-22 6-hydroxyisoquinoline isomer	0.04	0.05	0.009	7.6	3.9	1.0	11.1	5.2	2.1	7.6	3.9	1.0
PCMPA	3.7	4.7	0.9	7.4	3.3	0.5	16.4	4.2	0.6	7.4	3.3	0.5
THCA-A	2.7	3.1	0.6	7.2	4.8	0.8	15.3	7.6	1.1	7.2	4.8	0.8
UR-144 N-heptyl analog	1.8	2.2	0.4	6.1	5.5	1.0	7.9	5.8	0.7	6.1	5.5	1.0

Table 9. Bias and precision data for compounds in Mix 2. Concentrations for low, med, and high are 5, 20, and 80 ng/mL, respectively.

	Bias (%)			Precision (% CV)			Within-Run (%)			Between-Run (%)		
Compound Name	**low**	**med**	**high**	**low**	**med**	**high**	**low**	**med**	**high**	**low**	**med**	**high**
2,3-methylenedoxy pyrovalerone	2.7	0.6	0.3	7.7	2.6	1.5	17.0	2.9	2.5	7.7	2.6	1.5
25I-NBF	1.8	1.8	0.7	3.5	3.0	2.5	6.6	5.1	2.6	3.5	3.0	2.5
2C-T-2	1.3	1.0	3.2	6.2	4.4	6.9	11.0	4.3	1.9	6.2	4.4	6.9
2C-T-4	3.0	4.4	2.7	9.7	6.6	7.4	6.7	6.1	2.8	9.7	6.6	7.4
2-fluoromethcathinone	1.8	3.0	1.7	9.2	6.4	4.3	10.1	9.1	4.4	9.2	6.4	4.3
3,4-dimethoxy- α-pyrrolidinopentiophenone	0.5	1.5	1.3	5.6	5.6	4.5	8.7	8.4	3.8	5.6	5.6	4.5
3-methyl-α-pyrrolidinopropiophenone	4.1	0.9	3.1	5.6	8.7	5.7	6.9	7.0	3.0	5.6	8.7	5.7
4'-methyl-N-methylhexanophenone	2.5	0.1	0.5	5.3	5.4	2.0	9.3	7.5	2.6	5.3	5.4	2.0
4-ethyl-N,N-dimethylcathinone	3.6	0.8	0.6	5.8	5.2	3.0	7.6	6.9	3.2	5.8	5.2	3.0
4-hydroxy MiPT	5.1	0.5	3.7	5.5	6.8	6.8	5.8	2.6	1.5	5.5	6.8	6.8
4-MMC	1.5	1.7	2.7	6.3	8.6	5.2	8.7	6.8	3.2	6.3	5.6	5.2
A-796260	4.5	5.8	2.3	9.9	5.7	6.4	13.6	3.0	1.4	9.9	5.7	6.4
AB-005 azepine isomer	4.1	0.8	2.8	5.0	6.0	4.8	8.3	3.3	3.9	5.0	6.0	4.8
AB-FUBINACA 3-fluorobenzyl isomer	4.0	1.3	3.3	5.6	5.1	6.0	8.4	4.7	4.0	5.6	5.1	6.0
ADB-PINACA isomer 1	4.0	3.7	0.6	6.3	1.8	3.2	11.0	2.9	4.6	6.3	1.8	3.2
AKB48 N-(5-fluoropentyl) analog	3.5	2.0	0.7	6.5	5.5	2.0	6.4	2.9	2.5	6.5	5.5	2.0
Clencyclohexerol	6.1	3.4	0.4	15.7	1.6	1.1	9.6	2.3	1.7	15.7	1.6	1.1
EG-018	5.2	7.0	0.8	6.9	6.0	3.2	3.7	2.5	3.6	6.9	6.0	3.2

JWH 018 N-(5-hydroxypentyl) metabolite	4.1	0.9	2.5	5.6	8.0	4.0	6.0	2.9	1.4	5.6	8.0	4.0
JWH 018 N-propanoic acid metabolite	0.6	0.2	3.3	5.4	4.8	6.4	11.5	4.7	3.4	5.4	4.8	6.4
KM 233	2.6	9.8	2.4	11.4	7.2	7.5	4.3	2.8	4.1	11.4	7.2	7.5
Loperamide	0.9	2.9	0.01	3.9	3.0	1.50	5.5	4.2	2.2	3.9	3.0	1.5
MAM2201 N-pentanoic acid metabolite	1.5	3.7	1.5	6.0	2.6	2.2	12.1	3.6	2.3	6.0	2.6	2.2
N-Ethylbuphedrone	2.5	2.3	0.8	8.1	8.1	4.2	4.3	6.5	7.2	8.1	8.1	4.2
PCPr	5.6	0.001	3.7	6.9	7.0	6.5	8.5	10.4	8	6.9	7.0	6.5
RCS-4 2-methoxy isomer	7.5	3.6	1.5	6.2	4.9	1.2	7.4	3.0	1.6	6.2	4.9	1.2
SER-601	3.5	3.3	2.3	4.6	2.5	5.0	6.6	2.2	1.5	4.6	2.5	5.0
UR-144 Degradant	6.9	4.1	1.4	5.4	1.8	4.0	8.7	2.7	1.5	5.4	1.8	4.0
XLR11 N-(2-fluoropentyl) isomer	7.3	2.4	0.3	7.5	4.0	2.2	8.5	2.7	0.7	7.5	4.0	2.2
Δ8-THC	6.1	6.2	0.5	10.3	6.3	3.5	10.6	5.2	4.4	10.3	6.3	3.5

Table 10. Bias and precision data for compounds in Mix 3. Concentrations for low, med, and high are 5, 20, and 80 ng/mL, respectively.

	Bias (%)			Precision (% CV)			Within-Run (%)			Between-Run (%)		
Compound Name	**low**	**med**	**high**	**low**	**med**	**high**	**low**	**med**	**high**	**low**	**med**	**high**
(R)-(−)-MT-45	0.6	0.7	0.2	1.3	1.4	0.5	1.9	1.4	0.7	1.3	1.4	0.5
2,3-Dichlorophenylpiperazine	3.3	4.1	0.8	3.5	4.5	0.9	2.6	2.5	0.4	3.5	4.5	0.9
25H-NBOMe	1.0	1.2	0.3	1.2	1.0	0.6	2.1	1.0	1.1	1.2	1.0	0.6
2C-T	0.4	0.5	0.1	1.4	1.3	0.5	2.6	0.9	0.8	1.4	1.3	0.5
2-Methoxyamphetamine	0.5	0.7	0.2	1.1	1.3	0.6	0.8	0.7	0.9	1.1	1.2	0.6
3,4-Dimethylethcathinone	0.5	0.7	0.1	0.9	1.1	0.5	0.9	1.9	0.7	0.9	1.1	0.5
3C-P	0.3	0.3	0.1	1.5	1.4	0.5	2.7	1.2	0.9	1.5	1.4	0.5
4-Methoxyamphetamine	0.6	0.8	0.2	1.6	1.0	0.5	3.7	0.7	0.8	1.6	1.0	0.5
5-fluoro NNEI	2.1	2.6	0.5	2.6	3.1	0.7	1.4	1.3	0.7	2.6	3.1	0.7
9-octadecenamide/oleamide	0.2	0.2	0.04	1.7	2.4	0.8	2.2	4.3	1.1	1.7	2.4	0.8
AB-CHMINACA	0.4	0.5	0.1	0.7	0.7	0.5	1.1	0.7	0.8	0.7	0.7	0.5
AKB48 N-pentanoic acid metabolite	1.0	1.3	0.3	1.0	0.9	0.4	1.3	0.9	0.6	1.0	0.9	0.4
Cathine	0.4	2.2	0.5	7.3	4.4	1.3	11.6	5.8	2.0	7.3	4.4	1.3
Diclofensine	0.2	0.2	0.04	0.5	0.6	0.5	0.7	0.8	0.8	0.5	0.6	0.5
FUB-PB-22	1.3	1.6	0.3	1.2	1.5	0.6	1.5	1.6	1.0	1.2	1.5	0.6
HMA	1.1	0.3	0.01	4.4	1.7	0.7	10.2	3.8	1.1	4.4	1.7	0.7
MBZP	5.3	1.6	0.1	5.1	2.1	0.3	2.6	2.6	0.4	5.1	2.1	0.3
Mephedrone	0.4	0.4	0.1	1.0	1.2	0.5	1.1	1.9	0.7	1.0	1.2	0.5
Methylenedioxy Pyrovalerone metabolite 2	0.5	0.6	0.1	1.3	0.9	0.5	2.4	1.2	1.0	1.3	0.9	0.5
N-methyl-2-AI	0.7	0.9	0.2	1.3	1.3	0.5	1.9	1.5	0.8	1.3	1.3	0.5
NRG-3	0.03	0.04	0.01	0.7	0.9	0.7	0.8	1.3	1.1	0.7	0.9	0.7

PB-22 N-(5-hydroxypentyl) metabolite	1.0	1.3	0.3	1.8	1.6	0.5	3.2	0.9	0.7	1.8	1.6	0.5
RCS-4 N-(4-hydroxypentyl) metabolite	0.5	0.7	0.1	1.7	1.1	0.6	3.6	1.0	1.1	1.7	1.1	0.6
UR-144 N-(2-chloropentyl) analog	0.03	0.03	0.01	0.8	0.4	0.3	1.7	0.5	0.4	0.8	0.4	0.3

4.3.4 Freeze-Thaw Stability

Storage conditions can affect analyte stability in samples and in forensic toxicology it is common for laboratories to freeze urine specimens upon receipt to preserve them until a time when they may be analyzed. Thus, analyte stability must be determined using a series of freeze and thaw cycles. In accordance with OSAC method validation guidelines, urine samples fortified with the analytes underwent freeze-thaw cycles in which they were frozen for 24 hours then removed from the freezer and allowed to thaw unassisted at room temperature. This cycle was then repeated two more times. An analyte was considered stable when the average signal was within the method's acceptable bias, which in these studies was ±20% of the time zero average signal.

Samples in this series of studies were prepared in four sets of blank urine diluted with water (1:5) with Mixes 2 and 3 at high and low calibration concentrations (*e.g.,* 5 and 120 ng/mL). These sets were labeled as T_0, T_1, T_2, and T_3, representing each time point of the three freeze-thaw cycles, with T_0 as the time zero signal where the sample has not been subjected to any freeze-thaw cycles. The analyte signal was determined using the analyte response determined using the Quantitative Analysis software. The averages of these values at each concentration from each time point were then compared to the corresponding time zero value average using Equation 2. Results for Mix 2 after three freeze-thaw cycles are displayed in Table 14 and the results for Mix 3 are displayed in Table 15.

The majority of the analytes in Mix 2 fell within ±20% bias with only a few compounds at the low concentration having a bias value greater than 20%. The seven compounds in Mix 2 at with significant stability problems at the low concentration after

three freeze-thaw cycles were all synthetic cannabinoids. Stability of synthetic cannabinoids has not been extensively studied, although there have been reports of instability for several compounds.[26,154]

All compounds in Mix 3 at the high concentration had bias values within ±20% after three freeze-thaw cycles. At the low concentration, only two compounds had bias values >20%: oleamide and AKB48 N-pentanoic acid metabolite. Oleamide is an agonist of cannabinoid 1 (CB_1) receptors and AKB48 N-pentanoic acid metabolite is a potential urinary metabolite of the synthetic cannabinoid AKB48.

4.3.5 Matrix Effects

Matrix effects occur when compounds that naturally occur in the sample matrix co-elute with analytes of interest and cause either suppression or enhancement of the ionization of the target molecule. Suppression or enhancement of analyte ions can affect the values for LOD, LOQ, and bias in quantitative methods and therefore, values should not exceed ±25%. The equation used to calculate matrix effects is shown in Equation 8. However, if the calculated value for matrix effects is outside of the ±25% range, the laboratory conducting the validation must demonstrate that the matrix effects do not cause significant adverse effects to the critical validation parameters.

$$M.E.(\%) = \left(\frac{\bar{X}area\ of\ Set\ 2}{\bar{X}area\ of\ Set\ 1} - 1\right) x\ 100 \qquad (8)$$

where $\bar{X}$ is the average area of each set, with Set 1 representing standards prepared in water and Set 2 representing standards prepared in urine.

Samples for each set were prepared at low and high concentrations of 5 and 100 ng/mL, respectively. Six replicates of each sample at each concentration were analyzed with the peak areas of each analyte measured and recorded using the Qualitative Analysis software. Calculated ion suppression and enhancement values for Mixes 1-3 are shown in Table 16, Table 17, and Table 18, respectively.

Nearly half of the compounds in Mix 1 did not have significant ion suppression or enhancement due to matrix effects. However, there were several compounds that did exhibit significant matrix effects. More than half of the compounds in Mix 2 displayed significant ion suppression, as indicated by ionization differences between the neat samples prepared in water and the samples prepared in urine, particularly at the high concentration of 100 ng/mL. Several compounds in Mix 3 also displayed significant ion suppression at both the low and high concentrations. However, the critical validation parameters (*e.g.,* LOD, LOQ, and bias) of the compounds of both mixes prepared in urine are still well within acceptable ranges.

The matrix effects exhibited were recorded but do not prevent validation of the method for these compounds. The results of the matrix effect studies indicate that more thorough sample preparation for urine specimens containing NPS may be necessary prior to analysis. Possible cleanup steps may include further dilution (1:10) or SPE, either on-line or off-line. There was no clear trend as to which compounds exhibited the most significant matrix effects, however, it was noted that several of the tryptamine and metabolites of the synthetic cannabinoid classes were among those that exhibited the most ion suppression or enhancement.

4.3.6 Carryover

Analytical methods that include a chromatographic separation step may be subject to issues of carryover, which occurs when analytes from a previous sample appear in the next sample of a batch, usually as a result of incomplete elution from the separation column. Carryover may lead to inaccurate qualitative or quantitative results thus negatively affecting the reliability of the method. In order to assess carryover, blank urine samples were analyzed immediately after the highest concentration calibrator, which was 120 ng/mL. The analysis of the blank urine samples was conducted in triplicate for Mixes 1-3. Using both the Qualitative and Quantitative Analysis software, it was determined that there was no significant carryover of any compounds in any of the mixes. An example of the lack of carryover from the method is shown in Figure 7 through the overlay of the chromatograms generated for oleamide at 5 ng/mL and in the blank urine that was analyzed immediately following a 120 ng/mL sample. Oleamide was one of the few analytes that exhibited carryover effects and had the largest peak area of those generated. However, when compared with the peak area of oleamide demonstrated at a low concentration of 5 ng/mL, it is clearly shown that the degree of carryover is minimal.

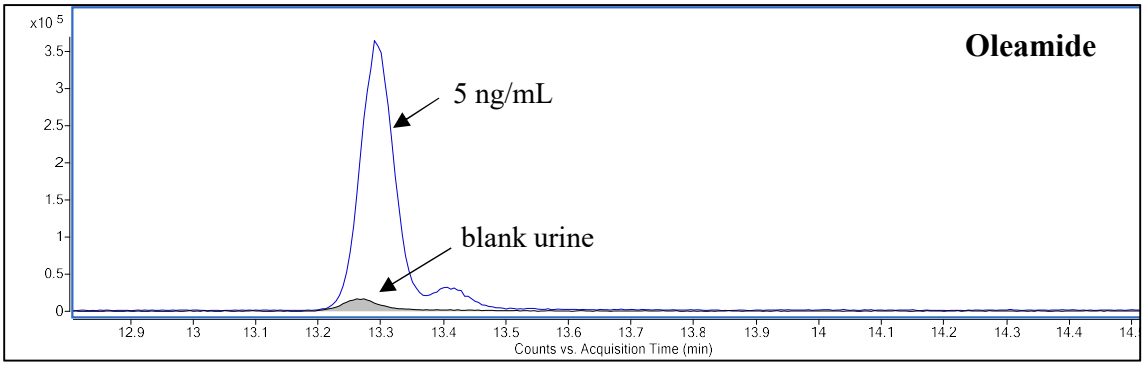

Figure 7. Carryover study for oleamide in Mix 3 comparing analyte concentration present in a 5 ng/mL sample with that in a blank urine sample that was analyzed immediately following a 120 ng/mL sample.

4.3.7 Analysis of Blind-Spiked Specimens

Analysis of the blind-spiked urine specimens was conducted using the validated LC-QTOF-MS screening method. Chromatographic separation of the urine specimens was conducted using the Zorbax Eclipse Plus C_{18} RRHD column and an injection volume of 10 µL. The ESI source was operated in positive mode.

Two acquisition methods were used with the QTOF-MS; Full Scan MS and Auto MS/MS. The same LC, ESI source, and scan parameters were used for each acquisition method. Full Scan MS collected all ion data and did not subject the ions to any collision energy. MS range was 100-1000 *m/z* and the acquisition rate was 1.5 spectra/s. The Auto MS/MS used an MS range of 100-1000 *m/z* with an acquisition rate of 3 spectra/s, while the MS/MS mass range was 50-1000 *m/z* with an acquisition rate of 3 spectra/s. MS/MS fragmentation was performed at three collision energies of 10, 20, and 40 eV. The quadrupole used a narrow isolation width of 1.3 *m/z*. Reference mass correction was also enabled to ensure proper instrumental calibration throughout the analysis, using masses 121.0509 *m/z* and 922.0098 *m/z*.

Agilent MassHunter Qualitative Analysis software was used to perform library and database searches for data collected using both Full Scan MS and Auto MS/MS modes. The compound database and library previously created was used for searching. For Full Scan MS data analysis, searching was conducted using the "Find Compounds by Formula" (FBF) followed by the "Search Library" and "Search Database" commands. Analysis of the data collected using Auto MS/MS mode was very similar except that the "Find Compounds by Auto MS/MS" command was used instead of the FBF. The potential compound list generated after this command usually included over 250

compounds per sample. This list was then subjected to a search using the accurate mass library which employed both forward (matching peaks in the sample against those in the library) and reverse (matching peaks in the library against those in the sample) searching. Search parameters were set to include precursor ion expansion of ±10 ppm + 2 mDa and product ion expansion of ±20 ppm + 2 mDa. The library search parameters were set to a minimum of 70 for both forward and reverse scores.

Compounds identified by the library search were then subjected to a database search which compared the mass of a compound in the sample with the corresponding molecular formula in the database. The database search had a mass tolerance set to ±10 ppm. Positive identifications required a library score >80 and a database score >70. Mass error was also required to be <10 ppm.

The scores generated through database and library searching are calculated using an algorithm that is proprietary information of the MassHunter Qualitative Analysis software. However, some factors that contribute to this score may include isotope abundance, and the presence and intensity of specific ion fragments.

Two sets of 20 blind-spiked specimens were prepared in diluted urine (1:5) by a laboratory member that was not conducting the analysis, hence the term "blind." The specimens contained 0-1 NPS and were identical for both sets. The concentration of compounds in Set 1 was either 2 or 20 ng/mL with all specimens in Set 2 prepared at a concentration of 200 ng/mL.

Screening of Set 1 resulted in positive identification of the NPS or blank specimen for 13/20 specimens (Table 19). Screening of the higher level Set 2 samples resulted in more positive identifications, with 15/20 correctly identified (Table 20).

Three of the specimens in Set 2 (specimens 4, 8, and 9) were not originally identified correctly, however, the true identity of the NPS in the specimen was included on a "hit list" or list of possible identifications. Closer analysis of the ion fragmentation patterns for the analyzed specimens, as well as consideration of the retention times included as part of the in-house database, ultimately resulted in the correct identification.

Specimen 1 from both sets contained mephedrone but was identified as 3-methylmethcathinone (3-MMC) using the compound database. Both NPS have the chemical formula $C_{11}H_{15}NO$ with an identical accurate mass of 177.1154 Da. This result demonstrates the importance of MS/MS spectral data in the identification of NPS and the determination between two very different compounds that share the same chemical formula. The MS/MS spectral data for mephedrone were not included in the HRMS library; inclusion of MS/MS data for mephedrone would have resulted in the correct identification of the compound in specimen 1.

Although not all of the compounds in the blind-spiked specimens were correctly identified, the preliminary qualitative screening results indicate the utility of the compound database and HRMS spectral library for identification of NPS in human urine specimens. It is important to note that the HRMS library used in the above study was created in-house and had not been curated to remove background ions as curation of the library was not included in the scope of the present research. The method used for screening did not include any extraction or preconcentration procedures prior to analysis, and instead utilized a "dilute-and-shoot" approach in which samples in urine were simply diluted using water at a ratio of 1:5. Dilute-and-shoot is a simple method for screening of urine specimens, but it does not remove endogenous and possibly interfering compounds,

which could contribute to the misidentification or nonidentification of some samples. Use of a more thorough sample preparation may have resulted in improved results for the screening of the blind-spiked samples.

4.4 Conclusion

Validation of a method for screening and confirmation of NPS and related compounds was completed following guidelines established by the Toxicology Subcommittee of OSAC. Studies were conducted on NPS in urine to assess linearity, accuracy (bias), precision, LOD, LOQ, freeze-thaw stability, ion suppression or enhancement due to matrix effects, and carryover. While not all compounds were able to be fully validated using the method, a significant majority of the compounds in each mixture were successfully validated. It was expected that the positive-mode method would not be applicable to all NPS of interest given their wide variety of physiochemical properties, however, the results demonstrate that a mixture approach is a viable technique for validation of a screening and confirmation method for a significantly large number of NPS and related compounds.

The validated method was also used in conjunction with the compound database and HRMS spectral library that was developed (see Chapter 3) to qualitatively screen two series of 20 blind-spiked specimens in human urine. The qualitative screening was conducted as a preliminary test of applicability to real-world specimens. Positive identification was achieved for the majority of the compounds present in the specimens, indicating potential for real-world applicability. It is believed that identification could be further improved through curation of the MS/MS spectral data in the HRMS library to remove ions from compounds present in the background. However, as a preliminary test,

applicability of the database and HRMS spectral library used with the validated method for screening and confirmation was confirmed.

Table 11. LOD and LOQ values for compounds in Mix 1.

Compound Name	LOD (ng/mL)	LOQ (ng/mL)
α-Pyrrolidinopentiophenone metabolite 1	0.6	1.8
25I-NBMD	0.2	0.6
3-Methylbuphedrone	0.5	1.6
4-APDB	2.5	7.5
4-fluoro-α-Pyrrolidinobutiophenone	0.4	1.2
4'-fluoro-α-Pyrrolidinopropiophenone	0.5	1.4
4-hydroxy MET	0.8	2.4
4-methoxy PV8	0.7	2.2
4-methoxy-α-Pyrrolidinopentiophenone	0.7	2.1
4-Methyl-α-ethylaminobutiophenone	0.2	0.6
5-fluoro SDB-006	0.7	2.2
5-Fluoropentylindole	0.6	1.9
5-MAPB	0.7	2.0
AB-005	0.3	0.9
AM-2233 azepane isomer	0.5	1.5
AM694 N-(5-hydroxypentyl) metabolite	0.6	1.8
AMT	2.9	8.8
Benocyclidine	1.4	4.2
Flubromazepam	0.3	0.8
JWH 031 2'-isomer	0.7	2.1
JWH 081 N-(5-hydroxypentyl) metabolite	1.0	3.0
JWH 200 5-hydroxyindole metabolite	0.7	2.2
JWH 203	1.0	3.1
N-Methyltryptamine	2.0	6.1
NPB-22	0.5	1.5
PB-22 6-hydroxyisoquinoline isomer	0.5	1.5
PCMPA	0.5	1.5
THCA-A	1.4	4.1
UR-144 N-heptyl analog	0.8	2.3

Table 12. LOD and LOQ values for compounds in Mix 2.

Compound Name	LOD (ng/mL)	LOQ (ng/mL)
2,3-methylenedoxy pyrovalerone	0.7	2.1
25I-NBF	0.3	0.9
2C-T-2	0.5	1.4
2C-T-4	0.5	1.5
2-fluoromethcathinone	2.2	6.8
3,4-dimethoxy- α-pyrrolidinopentiophenone	1.7	4.4
3-methyl-α-pyrrolidinopropiophenone	0.8	2.4
4'-methyl-N-methylhexanophenone	0.7	2.1
4-ethyl-N,N-dimethylcathinone	1.2	3.6
4-hydroxy MiPT	0.2	0.7
4-MMC	1.0	3.1
A-796260	0.4	1.3
AB-005 azepine isomer	0.4	1.1
AB-FUBINACA 3-fluorobenzyl isomer	0.8	2.9
ADB-PINACA isomer 1	0.4	1.3
AKB48 N-(5-fluoropentyl) analog	0.4	1.1
Clencyclohexerol	0.2	0.7
EG-018	0.3	1.0
JWH 018 N-(5-hydroxypentyl) metabolite	0.5	1.6
JWH 018 N-propanoic acid metabolite	0.4	1.1
KM 233	0.3	1.0
Loperamide	0.5	1.5
MAM2201 N-pentanoic acid metabolite	0.3	0.9
N-Ethylbuphedrone	0.8	2.4
PCPr	0.4	1.1
RCS-4 2-methoxy isomer	0.9	2.8
SER-601	0.1	0.3
UR-144 Degradant	0.4	1.2
XLR11 N-(2-fluoropentyl) isomer	0.4	1.3
Δ8-THC	0.8	2.5

Table 13. LOD and LOQ values for compounds in Mix 3.

Compound Name	LOD (ng/mL)	LOQ (ng/mL)
(R)-(−)-MT-45	0.5	1.4
2,3-Dichlorophenylpiperazine	0.6	1.7
25H-NBOMe	0.3	0.8
2C-T	1.3	3.9
2-Methoxyamphetamine	1.0	2.9
3,4-Dimethylethcathinone	0.3	0.8
3C-P	0.8	2.5
4-Methoxyamphetamine	0.8	2.3
5-fluoro NNEI	0.2	0.6
9-octadecenamide/oleamide	1.6	4.7
AB-CHMINACA	0.8	2.3
AKB48 N-pentanoic acid metabolite	0.3	0.8
Cathine	1.2	3.8
Diclofensine	0.2	0.7
FUB-PB-22	0.3	0.9
HMA	1.3	3.9
MBZP	0.6	1.9
Mephedrone	0.4	1.2
Methylenedioxy Pyrovalerone metabolite 2	0.7	2.0
N-methyl-2-AI	0.4	1.1
NRG-3	1.0	2.9
PB-22 N-(5-hydroxypentyl) metabolite	0.3	1.0
RCS-4 N-(4-hydroxypentyl) metabolite	1.0	3.0
UR-144 N-(2-chloropentyl) analog	0.5	1.6

Table 14. Stability data for Mix 2 compounds after three freeze-thaw cycles.

	Bias (%)	
Compound Name	**Low (5 ng/mL)**	**High (120 ng/mL)**
2,3-methylenedoxy pyrovalerone	9.3	4.6
25I-NBF	9.6	7.9
2C-T-2	14.3	6.9
2C-T-4	9.8	5.0
2-fluoromethcathinone	25.9	5.1
3,4-dimethoxy- α-pyrrolidinopentiophenone	4.8	5.6
3-methyl-α-pyrrolidinopropiophenone	11.5	6.5
4'-methyl-N-methylhexanophenone	13.8	7.2
4-ethyl-N,N-dimethylcathinone	14.3	9.6
4-hydroxy MiPT	16.8	16.3
4-MMC	4.9	4.0
A-796260	7.0	19.5
AB-005 azepine isomer	20.7	7.6
AB-FUBINACA 3-fluorobenzyl isomer	4.7	14.0
ADB-PINACA isomer 1	9.9	5.7
AKB48 N-(5-fluoropentyl) analog	38.5	9.4
Clencyclohexerol	19.4	17.7
EG-018	84.1	0.2
JWH 018 N-(5-hydroxypentyl) metabolite	18.3	9.1
JWH 018 N-propanoic acid metabolite	16.7	19.9
KM 233	73.6	12.8
Loperamide	3.9	4.9
MAM2201 N-pentanoic acid metabolite	12.1	4.8
N-Ethylbuphedrone	17.9	11.2
PCPr	8.5	10.9
RCS-4 2-methoxy isomer	18.4	5.1
SER-601	82.7	3.2
UR-144 Degradant	52.3	7.5
XLR11 N-(2-fluoropentyl) isomer	47.5	3.6
Δ8-THC	67.0	19.1

Table 15. Stability data for Mix 3 compounds after three freeze-thaw cycles.

	Bias (%)	
Compound Name	**Low (5 ng/mL)**	**High (120 ng/mL)**
(R)-(−)-MT-45	4.1	5.5
2,3-Dichlorophenylpiperazine	7.1	8.0
25H-NBOMe	2.5	8.0
2C-T	3.1	4.6
2-Methoxyamphetamine	8.5	3.9
3,4-Dimethylethcathinone	10.6	10.9
3C-P	1.6	5.4
4-Methoxyamphetamine	5.3	18.8
5-fluoro NNEI	5.9	12.9
9-octadecenamide/oleamide	32.8	11.8
AB-CHMINACA	17.7	10.0
AKB48 N-pentanoic acid metabolite	29.2	14.6
Cathine	16.1	14.5
Diclofensine	5.6	3.3
FUB-PB-22	9.7	12.5
HMA	8.3	6.3
MBZP	1.9	9.1
Mephedrone	7.9	11.7
Methylenedioxy Pyrovalerone metabolite 2	6.1	15.7
N-methyl-2-AI	12.5	13.8
NRG-3	5.5	15.1
PB-22 N-(5-hydroxypentyl) metabolite	10.5	9.5
RCS-4 N-(4-hydroxypentyl) metabolite	10.6	13.3
UR-144 N-(2-chloropentyl) analog	18.3	5.5

Table 16. Ion suppression and enhancement (matrix effects) values for compounds in Mix 1.

	M.E. (%)	
Compound Name	**Low (5 ng/mL)**	**High (100 ng/mL)**
25I-NBMD	8.04	-27.14
3-Methylbuphedrone	-18.57	-21.87
4-APDB	-52.74	-47.14
4-fluoro-α-Pyrrolidinobutiophenone	-29.12	-32.58
4'-fluoro-α-Pyrrolidinopropiophenone	-22.64	-28.71
4-hydroxy MET	-38.35	-45.23
4-methoxy PV8	4.16	-30.44
4-methoxy-α-Pyrrolidinopentiophenone	-7.17	-22.65
4-Methyl-α-ethylaminobutiophenone	-22.95	-39.56
5-fluoro SDB-006	-20.65	-13.49
5-Fluoropentylindole	-7.85	-6.14
5-MAPB	-11.10	-26.86
AB-005	113.44	-22.24
AM-2233 azepane isomer	-10.89	-40.69
AM694 N-(5-hydroxypentyl) metabolite	-5.16	-2.61
AMT	-30.27	-34.55
Benocyclidine	21.52	-25.53
Flubromazepam	-55.79	-27.25
JWH 031 2'-isomer	-19.97	13.60
JWH 081 N-(5-hydroxypentyl) metabolite	1.70	11.84
JWH 200 5-hydroxyindole metabolite	-24.72	-12.64
JWH 203	1.14	-3.39
N-Methyltryptamine	-92.05	-54.53
NPB-22	49.11	-3.00
PB-22 6-hydroxyisoquinoline isomer	53.07	-2.49
PCMPA	-3.68	-22.44
THCA-A	-28.26	-3.48
UR-144 N-heptyl analog	-46.81	-2.99
α-Pyrrolidinopentiophenone metabolite 1	-20.50	-28.36

Table 17. Ion suppression and enhancement (matrix effects) values for compounds in Mix 2.

	M.E. (%)	
Compound Name	**Low (5 ng/mL)**	**High (100 ng/mL)**
2,3-methylenedoxy pyrovalerone	-15.0	-40.3
25I-NBF	-52.5	-60.7
2C-T-2	-62.7	-63.0
2C-T-4	-76.0	-73.1
2-fluoromethcathinone	-16.9	-41.4
3,4-dimethoxy- α-pyrrolidinopentiophenone	19.2	-43.2
3-methyl-α-pyrrolidinopropiophenone	3.1	-48.0
4'-methyl-N-methylhexanophenone	-75.1	-79.3
4-ethyl-N,N-dimethylcathinone	-41.2	-66.0
4-hydroxy MiPT	-8.2	-15.8
4-MMC	-68.4	-74.1
A-796260	-7.4	-41.3
AB-005 azepine isomer	-54.7	-59.9
AB-FUBINACA 3-fluorobenzyl isomer	-19.2	-38.3
ADB-PINACA isomer 1	9.1	-16.7
AKB48 N-(5-fluoropentyl) analog	-8.3	-5.1
Clencyclohexerol	-71.3	-52.1
EG-018	-16.9	-8.0
JWH 018 N-(5-hydroxypentyl) metabolite	-1.5	-37.2
JWH 018 N-propanoic acid metabolite	-19.0	-32.2
KM 233	-18.8	-18.6
Loperamide	-2.1	-34.6
MAM2201 N-pentanoic acid metabolite	-3.4	-18.2
N-Ethylbuphedrone	-43.6	-30.2
PCPr	-32.9	-65.6
RCS-4 2-methoxy isomer	16.3	-8.4
SER-601	-3.1	-5.2
UR-144 Degradant	19.4	-5.0
XLR11 N-(2-fluoropentyl) isomer	18.0	-5.5
Δ8-THC	-16.6	-23.4

Table 18. Ion suppression and enhancement (matrix effects) values for compounds in Mix 3.

	M.E. (%)	
Compound Name	**Low (5 ng/mL)**	**High (100 ng/mL)**
(R)-(−)-MT-45	32.5	-30.5
2,3-Dichlorophenylpiperazine	-74.3	-76.4
25H-NBOMe	21.6	-29.7
2C-T	-3.7	-45.6
2-Methoxyamphetamine	-45.9	-52.8
3,4-Dimethylethcathinone	-38.5	-48.5
3C-P	64.3	-26.8
4-Methoxyamphetamine	-67.6	-58.8
5-fluoro NNEI	-30.6	-18.0
9-octadecenamide/oleamide	-3.1	-6.4
AB-CHMINACA	-3.0	-4.9
AKB48 N-pentanoic acid metabolite	-4.2	-5.5
Cathine	-70.5	-19.9
Diclofensine	-24.4	-52.6
FUB-PB-22	-20.6	-11.2
HMA	-36.6	8.7
MBZP	-6.8	-32.2
Mephedrone	-73.3	-80.1
Methylenedioxy Pyrovalerone metabolite 2	9.9	-49.4
N-methyl-2-AI	-64.4	-67.0
NRG-3	-44.2	-57.0
PB-22 N-(5-hydroxypentyl) metabolite	2.3	-32.7
RCS-4 N-(4-hydroxypentyl) metabolite	-20.1	-16.1
UR-144 N-(2-chloropentyl) analog	-40.7	-3.6

Table 19. Results of blind-spiked urine specimen screening for Set 1. Specimens were prepared at concentrations of 2 or 20 ng/mL.

Sample	True ID	Correct
1	Mephedrone	No
2	PB-22 N-(5-hydroxypentyl) metabolite	Yes
3	MDPV metabolite 2	Yes
4	BB-22	Yes
5	AKB48 N-pentanoic acid	No
6	Blank	Yes
7	PB-22 4-hydroxyisoquinoline isomer	Yes
8	PB-22 5-hydroxyisoquinoline isomer	Yes
9	AB-FUBINACA	Yes
10	MDPV	Yes
11	25H-NBOMe	No
12	MDPV	No
13	MDPV metabolite 1	Yes
14	2C-T	No
15	PCEEA	Yes
16	Blank	Yes
17	5-fluoro PB-22 3-carboxyindole metabolite	No
18	25H-NBOMe	Yes
19	PB-22 N-(5-hydroxypentyl) metabolite	Yes
20	2C-T	No

Table 20. Results of blind-spiked urine specimen screening for Set 2. Specimens were prepared at concentrations of 200 ng/mL.

Sample	True ID	Correct
1	Mephedrone	No
2	PB-22 N-(5-hydroxypentyl) metabolite	Yes
3	MDPV metabolite 2	Yes
4	BB-22	Yes
5	AKB48 N-pentanoic acid	No
6	Blank	Yes
7	PB-22 4-hydroxyisoquinoline isomer	Yes
8	PB-22 5-hydroxyisoquinoline isomer	Yes
9	AB-FUBINACA	Yes
10	MDPV	Yes
11	25H-NBOMe	No
12	MDPV	Yes
13	MDPV metabolite 1	Yes
14	2C-T	No
15	PCEEA	Yes
16	Blank	Yes
17	5-fluoro PB-22 3-carboxyindole metabolite	Yes
18	25H-NBOMe	No
19	PB-22 N-(5-hydroxypentyl) metabolite	Yes
20	2C-T	Yes

5. DEVELOPMENT OF 2D-LC METHOD

5.1 Introduction

Novel psychoactive substances (NPS), also known as "designer drugs" and "legal highs," refer to emerging drugs of abuse that are variations of existing compounds intended to cause a CNS psychotropic effect. Major categories of NPS include synthetic cannabinoids, cathinones, piperazines, tryptamines, and phenethylamines.[40,69] As more and more NPS are synthesized and appear on the market, analytical complications arise, due to the need to separate and identify compounds with minor or novel structural differences. These changes may include the alteration of a single functional group or the shifting of a functional group on the molecule to create isomers. Such alterations may render some NPS so comparable in structure and physicochemical properties that they cannot be separated using traditional techniques such as gas chromatography (GC) or liquid chromatography (LC) and therefore will not be detected as individual compounds.[70-72] NPS of interest that are in complex matrices may also provide an analytical challenge, as a multitude of components can interfere with the detection of the analyte of interest.[98]

Synthetic cannabinoids (SC) were originally developed for research purposes to study the pharmacology of compounds that interacted with CB1 and/or CB2 receptors.[25] SC in particular pose a significant challenge to chromatographic separations due to the large number of compounds and metabolites that exist, and even more so due to the presence of numerous isomers for many SC.[155-157] For example, the naphthoylindole derivatives have increased in street popularity and include compounds such as JWH 018, JWH 019, JWH 080, and JWH 250, all of which share a structure with variation only on

the indole alkyl side chain.[77,158] Isomers of SC may also have similar chromatographic retention times, making baseline separation difficult. Such co-elution can be a major analytical challenge during traditional one-dimensional (1D) chromatographic analyses of large numbers of NPS, including SC. This can be problematic for identification purposes if the coeluting compounds are unknown or previously unreported, particularly if they are indistinguishable using accurate mass data (*e.g.,* in the case of isomeric derivatives).[25,143]

Currently, analytical methods for detecting large numbers of SC in a single analysis are not as common as methods intended for a small group of select SC.[26,156,157,159,160] One such approach was applied to a mixture containing 54 SC and 21 NPS from other classes.[26] However, this screening method relied on the use of known retention times and accurate masses of precursor and qualifier ions. Such is generally the case for other methods requiring the use of libraries and databases which depend on known accurate masses and retention times.[26,143,157,161] This reliance on library data becomes problematic when screening for unknown or previously unreported SC, since there exist many isomers and related compounds with similar accurate masses, fragmentation patterns, and/or chromatographic retention times. In these instances, it is important to have an improved initial separation, so that compounds may be analyzed individually once they reach the detector.[45,77]

Two-dimensional liquid chromatography (2D-LC) has been proposed as a method to improve separation and resolution of complex mixtures prior to further mass spectral analysis.[73,74] This method has been shown to be effective in the separation of pharmaceuticals and small molecules such as methamphetamine, anti-retroviral drugs, traditional Chinese medicinal preparations, and antiretroviral drugs from complex

samples.[71,72,75,88-90,92,105] In the case of NPS such as SC, the improved resolving power that 2D-LC can provide may be useful for chromatographically resolving these compounds prior to characterization by mass spectrometry.

To date, there are no reports on the use of 2D-LC specifically for separation of co-eluting or isomeric NPS, although the demonstrated utility of 2D-LC for other classes of xenobiotics indicates that such a method could be effective.[81,89,94,162] In the present study, the separation of both isomeric and non-isobaric mixtures of SC was examined using a 2D-LC separation system coupled with high resolution mass spectrometry (HRMS). To perform the separation, two orthogonal LC column systems, or dimensions, were joined to improve the resolving power of the overall separation by combining the power of each dimension.[79,80,86] This work does not present a validated method, but rather serves as a proof-of-concept investigation. Results of this study confirm the potential utility of comprehensive 2D-LC combined with HRMS for the separation and identification of co-eluting, non-isobaric and isomeric SC.

5.2 Materials and Methods

5.2.1 Chemicals

Methanol, acetonitrile, and HPLC water (all Optima LC/MS grade) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Trifluoroacetic acid (TFA) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

5.2.2 Standards

The synthetic cannabinoids JWH 073 2'-naphthyl-N-(1,1-dimethylethyl) isomer, JWH 080, JWH 203, FUB-144, PB-22, MAM 2201 N-(2-fluoropentyl) isomer, and XLR-12, in addition to five isomers of the synthetic cannabinoid JWH 019 (*i.e.*, N-(2-fluorohexyl),

N-(3-fluorohexyl), N-(4-fluorohexyl), N-(5-fluorohexyl), and N-(6-fluorohexyl) isomers) were obtained as neat solids from Cayman Chemical (Ann Arbor, MI, USA). Individual stock solutions of the standards were prepared at concentrations of 1 μg/mL in methanol.

Stock solutions of the SC were used to create individual component working solutions as well as three test mixes, each containing five individual components. In previous 1D-LC work, very close retention times were observed for the individual components of each mix using a Zorbax Eclipse Plus RRHD C_{18} column (3.0 x 100 mm; 1.8 μm; Agilent Technologies, Santa Clara, CA). Retention times were collected over 20 min using a gradient that reached 90% B at 9.5 min, then held for the final 6.5 min. These retention times are given in Table 21. In other words, the separation of all the constituents in the mixtures described here was not achievable by conventional means. Co-elution (CE) Mix 1 contained five JWH 019 isomers, while CE Mixes 2 and 3 each contained five non-isobaric (but co-eluting) synthetic cannabinoids. The composition of each mix is shown in Table 21 and the structures of all compounds examined are shown in Figure 8. The individual working solutions and mixes were prepared in MeOH:H_2O (50:50 *v/v*) with each component present at a concentration of 1 μg/mL.

Table 21. Synthetic cannabinoids present in each co-elution (CE) mix.

	Compound Name	[a]Peak #	Molecular Formula	Exact Mass (Da)	[b]RT (min)
CE Mix 1	JWH 019 N-(2-fluorohexyl) isomer	1	$C_{25}H_{24}FNO$	373.1842	12.16
	JWH 019 N-(3-fluorohexyl) isomer	2	$C_{25}H_{24}FNO$	373.1842	12.25
	JWH 019 N-(4-fluorohexyl) isomer	3	$C_{25}H_{24}FNO$	373.1842	11.90
	JWH 019 N-(5-fluorohexyl) isomer	4	$C_{25}H_{24}FNO$	373.1842	11.75
	JWH 019 N-(6-fluorohexyl) isomer	5	$C_{25}H_{24}FNO$	373.1842	11.76
CE Mix 2	JWH 080	6	$C_{24}H_{23}NO_2$	357.1729	12.16
	JWH 203	7	$C_{21}H_{22}ClNO$	339.1390	12.22
	PB-22	8	$C_{23}H_{22}N_2O_2$	346.1681	11.88
	MAM 2201 N-(2-fluoropentyl) isomer	9	$C_{25}H_{24}FNO$	373.1842	12.20
	XLR12	10	$C_{20}H_{24}F_3NO$	351.1810	12.01
CE Mix 3	JWH 073 2'-naphthyl-N-(1,1-dimethylethyl) isomer	11	$C_{23}H_{21}NO$	327.1623	12.20
	JWH 019 N-(2-fluorohexyl) isomer	1	$C_{25}H_{24}FNO$	373.1842	12.16
	JWH 080	6	$C_{24}H_{23}NO_2$	357.1729	12.16
	JWH 203	7	$C_{21}H_{22}ClNO$	339.1390	12.22
	FUB-144	12	$C_{23}H_{24}FNO$	349.1842	12.26

[a]Peak number as indicated in Figures 13, 14, and 15.
[b]Retention time observed in 1D-LC separation performed in Chapter 4.

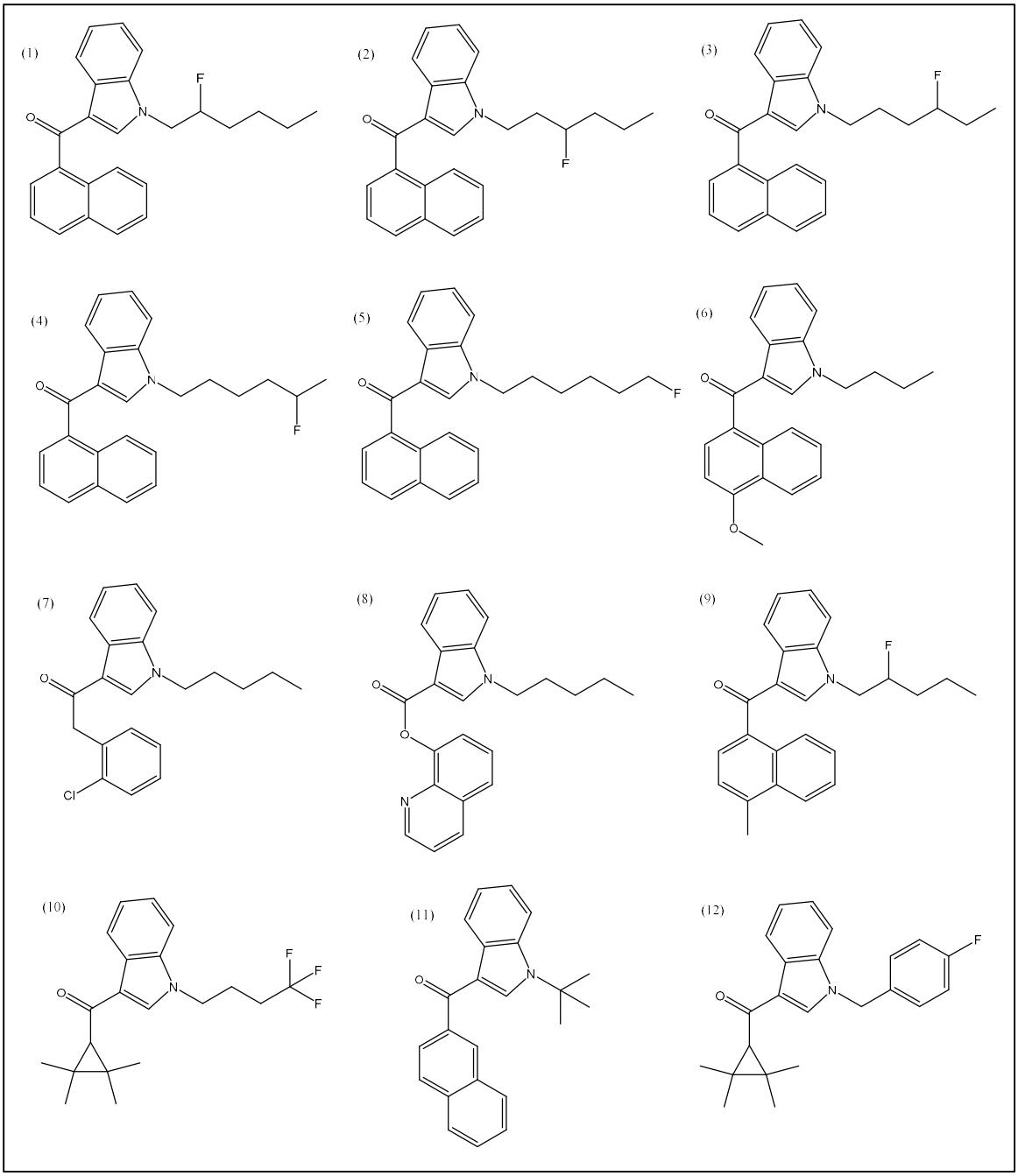

Figure 8. Structures of NPS in CE mixes (see Table 21 for mixture compositions): (1) JWH 019 N-(2-fluorohexyl) isomer, (2) JWH 019 N-(3-fluorohexyl) isomer, (3) JWH 019 N-(4-fluorohexyl) isomer, (4) JWH 019 N-(5-fluorohexyl) isomer, (5) JWH 019 N-(6-fluorohexyl) isomer, (6) JWH 080, (7) JWH 203, (8) PB-22, (9) MAM2201 N-(2-fluoropentyl) isomer, (10) XLR12, (11) JWH 073 2'-naphthyl-N-(1,1-dimethylethylisomer), and (12) FUB-144.

.

5.2.3 Instrumentation

Comprehensive, on-line 2D-LC analysis was performed with an Agilent Infinity 1290 2D-LC solution system (Agilent Technologies, Santa Clara, CA, USA) composed of two G4220A binary pumps, a G4226A temperature controlled autosampler, a G1316C thermostatted column compartment, and a G1170A Infinity Valve Drive with a two-position eight-port switching valve (pressure limit 1200 bar; p/n 5067-4214) and two 20-µL sampling loops. The configuration of the 2D-LC instruments is shown in Figure 9. Schematic of the two-position, four-port duo valve (right) used in conjunction with ta two-position, six-port valve (left) to connect the two dimensions..

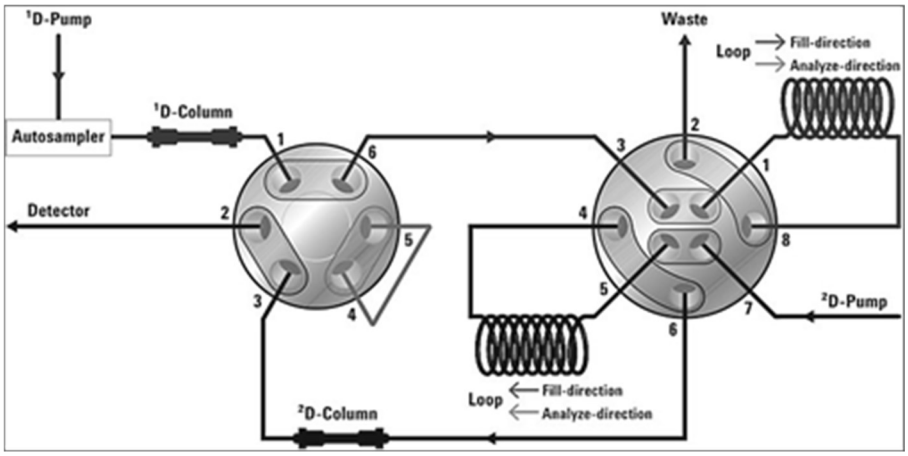

Figure 9. Schematic of the two-position, four-port duo valve (right) used in conjunction with ta two-position, six-port valve (left) to connect the two dimensions.

For initial investigation of the columns chosen for each dimension, two Agilent 1290 diode array detectors were used, one after the ^{1}D separation and another following the ^{2}D separation. Once the initial investigations were completed, the 2D-LC system utilized the same Agilent 1290 diode array detector placed directly after the first dimension. In addition, an Agilent 6530 Accurate-Mass Quadrupole Time-of-Flight

(QTOF) mass spectrometer with Jetstream ESI source was placed directly after the second dimension and used as the ^{2}D detector (Figure 10). Positive mode ESI source settings were as follows: gas temperature, 300 ºC; drying gas flow rate, 8 L/min; nebulizer pressure, 35 psi; sheath gas temperature, 350 ºC; sheath gas flow rate, 11 L/min; capillary voltage, 3500 V; and nozzle voltage, 1000 V. The QTOF fragmentor and skimmer voltages were 175 and 65 V, respectively. Mass spectral data were collected in Full Scan mode with a mass range of 100 – 1700 *m/z,* and an acquisition rate of 1 spectrum/s. No collision energy was employed during the MS data collection.

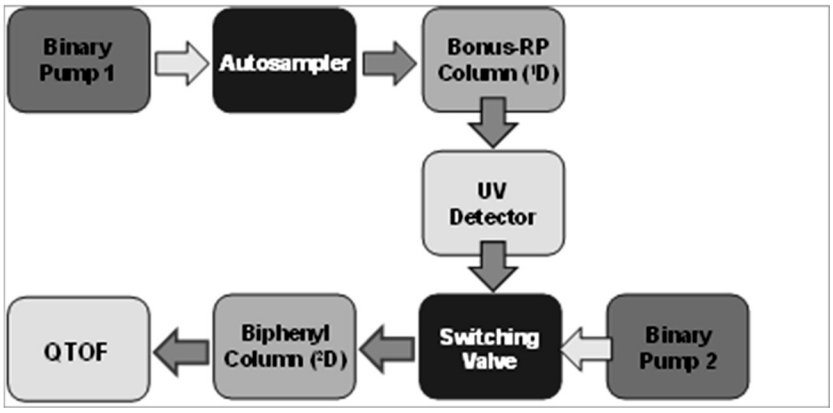

Figure 10. Schematic for the 2D-LC setup used in final system.

The software used to acquire, view, and analyze the analytical data included MassHunter Data Acquisition (version B.06.01), MassHunter Qualitative Analysis (version B.07.00), Open Lab Control Panel, and ChemStation (version C.01.07), all from Agilent Technologies (Santa Clara, CA, USA). Compounds were identified based on accurate mass with a mass tolerance of ±5 ppm using the Qualitative Analysis software.

5.2.4 Analytical Columns and Separation

Separation of the components of each mix was initially examined in 1D-LC mode on each of the three columns listed below. These separations involved a 20-min gradient with (A) water + 0.1% formic acid and (B) MeOH + 0.1% formic acid at a flow rate of 0.3 mL/min. The gradient ran 5% to 95% B from 0.5 to 9.5 min, where it was then held at 95% for the remainder of the analysis.

Both the ^{1}D and ^{2}D separations employed reversed-phase LC conditions. Three columns were tested; a Zorbax Eclipse Plus RRHD column (3.0 x 100 mm; 1.8 μm; Agilent Technologies, Santa Clara, CA), a Poroshell 120 Bonus-RP column (2.1 x 150mm, 2.7 μm; Agilent Technologies, Santa Clara, CA), and an Ascentis Express biphenyl column (2.1 mm x 100 mm, 2.7 μm; Supelco, Bellefonte, PA). In the final 2D method, the Poroshell 120 Bonus-RP column was chosen for ^{1}D and the Ascentis Express biphenyl column was chosen for ^{2}D. Parameters for each dimension were optimized separately in 1D-LC separations before optimizing the complete 2D-LC separation system. Both dimensions used HPLC water with 0.1% TFA for the aqueous (A) solvent. For the ^{1}D separation, the (B) solvent was a mixture of acetonitrile (ACN) and water (95:5 *v/v*), and in the second dimension it was a mixture of methanol (MeOH) and water (95:5 *v/v*). Columns were maintained at 40 °C in both dimensions. The temperature controlled autosampler was maintained at a temperature of 20 °C. In the first dimension, the flow rate was 0.1 mL/min, and the following solvent gradient was used for elution from the column: 5-5-80-80-95-95-100-10-5-5% B from 0-0.5-19-20-30-37-42-42.01-45 min, respectively. The semi-shifted gradient used in the second dimension is shown in Figure 11 and the ^{2}D flow rate was 0.55 mL/min. The ^{2}D gradient stop time was 0.95

min and the modulation time was 1.15 min.

For each injection, UV spectra were collected with the diode array detector after the ^{1}D column at 210, 220, 230, 240, and 250 nm. This was done to monitor any potential component separation that occurred solely in the first dimension. Working solutions of each compound were first run individually through the 2D-LC system to collect UV spectra and chromatograms for each compound. CE mixes were run under the same conditions and their data compared to those collected for the individual compounds. The volume of sample injected into the ^{1}D column was 2 µL.

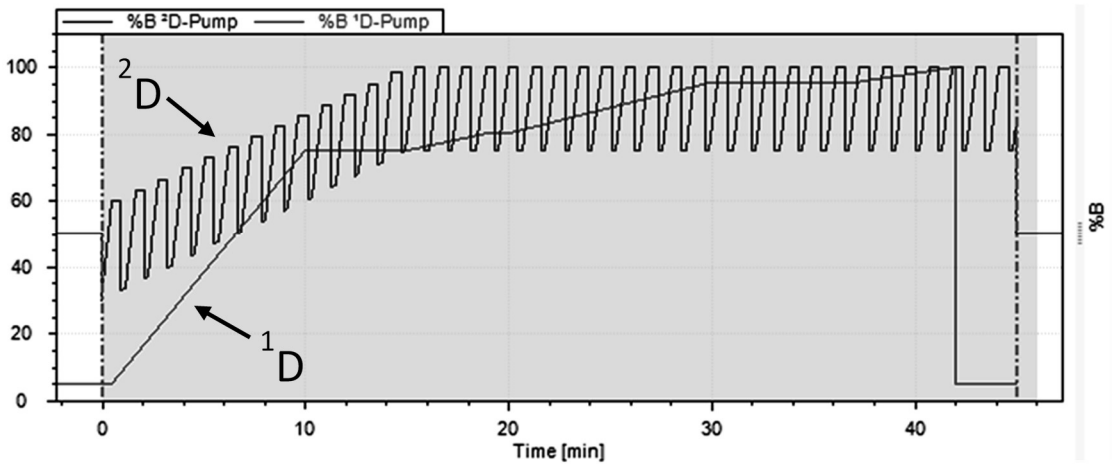

Figure 11. Solvent gradients used in the first and second dimensions of the 2D-LC separation.

5.3 Results and Discussion

Tentative determination of unknown compounds in samples can be conducted using a method known as suspect screening through the use of predicted or characteristic properties of compounds (*i.e.,* accurate mass, isotope pattern, product ion spectrum).[63] This method, though, is most effective when sample data are compared with previously collected data in a library or database. In the case of truly unknown and unreported

compounds, it could be difficult to determine if the sample data represents a single analyte or a possible mixture of analytes or endogenous compounds, thus highlighting the need for adequate separation.[46]

The use of 2D-LC has several benefits for analytical separation of both small and large molecules. These include increased peak capacities, greater resolving power, separation of isomers and isobars, and better separation of compounds and metabolites, particularly in complex mixtures.[70,72,76,86,88,118] The increased separation power of 2D-LC derives from the combined resolution and peak capacities of the two orthogonal dimensions. There are two general approaches to 2D-LC; comprehensive and heart-cutting separation. The present research focused on the use of comprehensive 2D-LC, which involves the complete transfer of all effluent from ^{1}D to ^{2}D. This approach enables full automation of the chromatographic analysis, is preferred for use in non-targeted analyses, and minimizes analyte loss. Low analyte loss makes comprehensive analysis the preferred 2D method for analytes present at low concentrations in complex matrices.[70,71,85,86,163] Despite these advantages, development of a working on-line, comprehensive 2D-LC method is time-consuming, due to the need to optimize all aspects of each individual dimension, including column type, mobile phase selection and compatibility, analysis time, gradient, and flow rate, prior to development of the complete 2D system.[73,98,108]

The improved separation power of 2D-LC itself does not directly lead to identification, but can provide additional, orthogonal information to assist in proper identification of compounds. This is particularly evident when identification based on MS-data might be challenging based on interfering compounds or in instances of

previously unreported substances. Less lengthy methods with orthogonal properties exist, but they may have their own limitations. Techniques combining ion mobility spectrometry (IMS) with HRMS to collect orthogonal identification information represent are examples of such methods. In IMS, substances are characterized based on the speed at which analyte ions move through an applied electric field and gas atmosphere before reaching the detector. Minute differences in this speed, or drift time, combined with the orthogonal resolving power of HRMS might be able to provide identification of isomeric and chemically related compounds.[164] However, increased performance with IMS methods, particularly for applications with drugs of abuse, is often a result of improved sample preparation steps such as solid-phase extraction (SPE) or paper spray.[165,166] These steps require more time and effort for sample preparation prior to analysis and could cause potential sample loss, contamination, or even decrease of ion intensities, which can be avoided through the use of a comprehensive 2D-LC method of separation. Overlapping drift time peaks may also still occur in some instances. These overlaps could be corrected by using a different drift gas, however, when working with unknown compounds, it is not always apparent that such overlap has occurred and that differing IMS conditions are needed during the analysis prior to MS-detection.[165]

In the present study, an RP x RP separation was developed involving an Agilent Poroshell 120 Bonus-RP column in the first dimension and an Ascentis Express biphenyl column in the second dimension. The latter column was chosen due to its ability to separate aromatic compounds, including synthetic cannabinoids, as a result of the π-π interactions and the influence of analyte shape on interactions between the compound ring structures and the biphenyl moieties of the column stationary phase.[167,168]

Resolution of the components of each mix was initially examined on each column separately. The initial 1D-LC separation of CE Mixes 1 and 2 on the Zorbax Eclipse Plus C_{18} column demonstrated that there was co-elution of multiple compounds in each mix (Figure 12a and 12b). The Bonus-RP column was then investigated for its selectivity toward the compounds in CE Mixes 1 and 2. Figure 13a and 13b show the chromatograms for these two mixes, respectively. This column demonstrated improved separation of the mixture components, indicating that it would be a good selection for the final 2D-LC method.

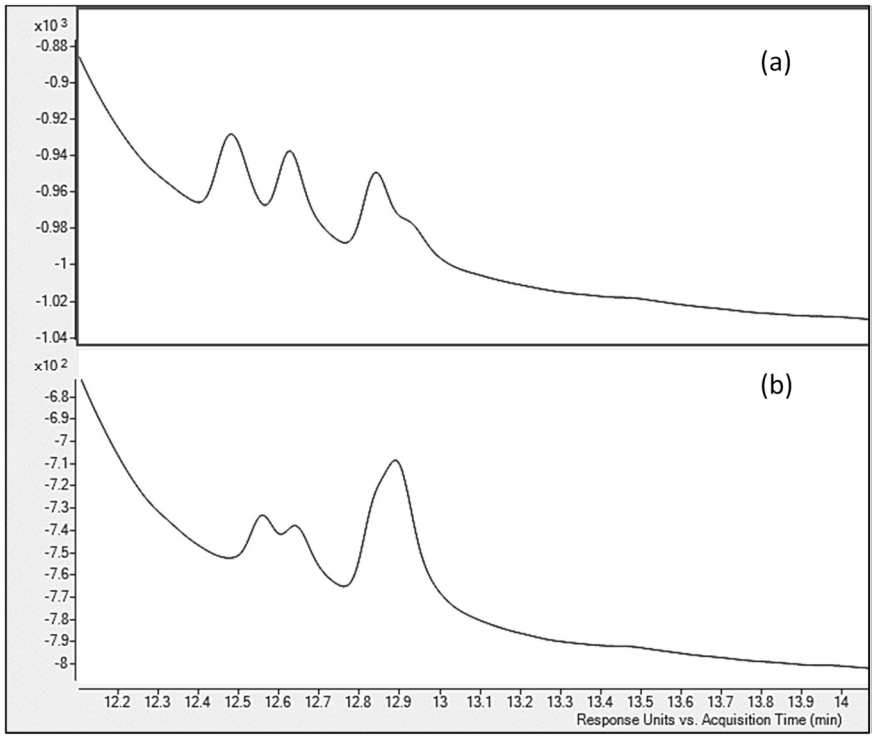

Figure 12. UV chromatograms (λ=220 nm) for 1D separations of (a) CE Mix 1, and (b) CE Mix 2 with the Zorbax Eclipse Plus C_{18} column.

Use of the biphenyl column in 1D-LC separation mode also demonstrated some initial separation of the isomeric compounds in CE Mix 1 (Figure 14a) and the non-isobaric components of CE Mix 2 (Figure 14b). These data suggested that the biphenyl column could be a good choice for use in the final 2D-system in order to achieve full separation and resolution of all mixture components. The Bonus-RP and biphenyl columns were then combined in a 2D-system and tested for separation of CE mixes using diode array and QTOF-MS for detection in the first and second dimensions, respectively. Optimization of the final 2D-LC method based on the results of the 1D-LC experiments was performed to increase its applicability to the wider range of compounds included in the CE mixes, as well as to facilitate coupling of the two dimensions. While the mechanics of a 2D-LC system are only briefly discussed here, further information can be found in recently published works discussing general function of 2D-LC systems.[74,102]

The resulting 2D-LC method was then tested on CE Mixes 1 and 2 (*i.e.,* isomeric and non-isobaric component mixtures, respectively) with diode array detection following the first dimension and QTOF-MS identification following the second. In addition, as a further test, a new non-isobaric component mixture (*i.e.,* CE Mix 3) was formulated. This contained two components from CE Mix 2 (JWH 080 and JWH 203) in addition to three other SC for which very close retention times were also observed in 1D-LC analyses (JWH 073 2'-naphthyl-N-(1,1-dimethylethyl) isomer, JWH 019 N-(2-fluorohexyl) isomer, and FUB-144). CE Mixes 1 and 2 were used for initial testing of the 2D-LC method. CE Mix 3 was later created containing components from CE Mixes 1 and 2, in addition to other non-isobaric compounds, as a final test of the method. 2D-LC of CE Mix 1 resulted in excellent separation of the JWH-019 2-, 5-, and 6-fluorohexyl

isomers with slight co-elution of the 3- and 4-fluorohexyl isomers (Figure 15a). 2D-LC of CE Mix 2 resulted in complete separation of PB-22, JWH 203, and XLR-12, with slight co-elution of MAM-2201 N-(2-fluoropentyl) isomer and JWH 080 (Figure 15b). Finally, 2D-LC separation of CE Mix 3 yielded complete resolution of all five cannabinoid components (Figure 15c).

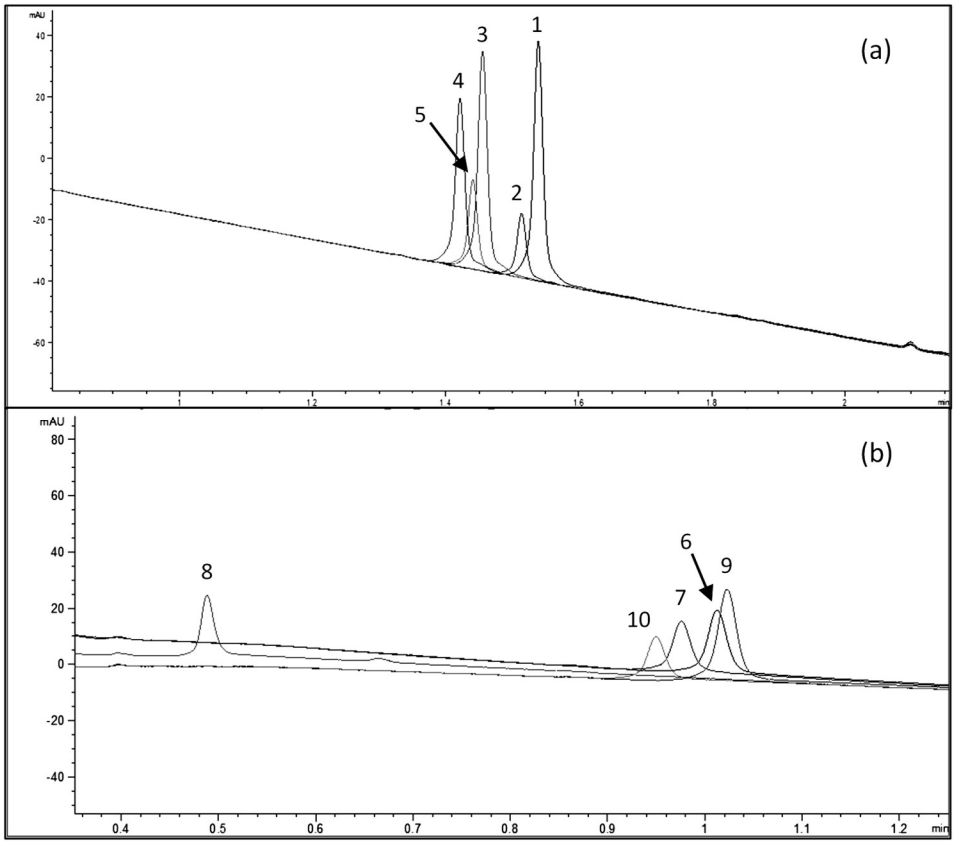

Figure 13. UV chromatograms (λ=240 nm) for 1D separations of (a) CE Mix 1, and (b) CE Mix 2 using the Bonus-RP column over 3 min at a flow rate of 0.5 mL/min with a gradient of 50-95% B; A: water + 0.1% TFA, and B: acetonitrile. Peak numbers correspond to compounds listed in Table 21.

The present research focused on the initial development of an on-line, comprehensive 2D-LC method for the separation of synthetic cannabinoids using a reversed-phase (RP) column in each dimension. 2D-LC involving RP columns has been employed with pharmaceuticals and other small molecules, suggesting their potential

utility for separation of NPS including SC,[85,94,97,108,124] Two detectors were used for method development; a diode array detector after the first dimension and a quadrupole time-of-flight (QTOF) mass spectrometer after the second dimension. In a final method, the first detector would be unnecessary, as the ^{2}D MS detector provides accurate mass data of the separated compounds for identification within ±5 ppm.

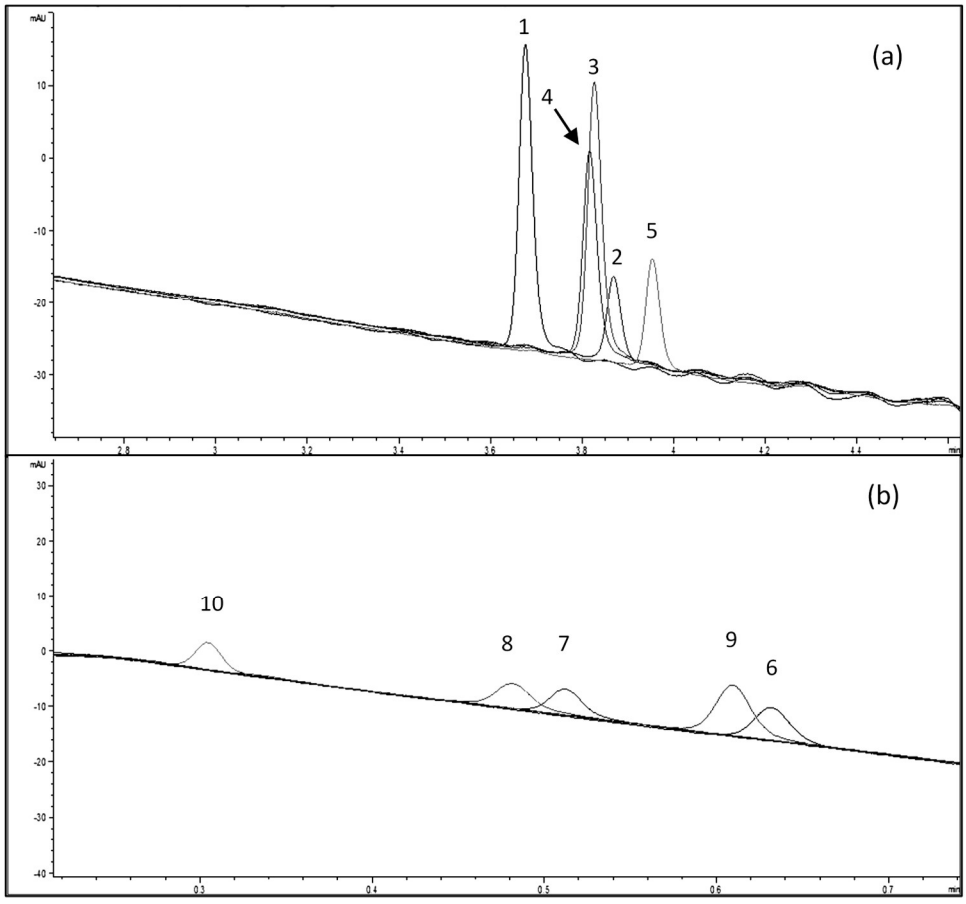

Figure 14. UV chromatograms (λ=240 nm) for 1D separation of (a) CE Mix 1 over 5 min, and (b) CE Mix 2 over 3 min using the biphenyl column at a flow rate of 0.5 mL/min with a gradient of 70-95% B; A: water + 0.1% TFA, and B: MeOH. Peak numbers correspond to compounds listed in Table 21.

The Bonus-RP column was ultimately selected for use in ^{1}D in part due to its column packing which has a polar amide group in the long alkyl chain, making it a good choice for use with basic analytes, generally resulting in good peak shape, as well as the

different separation selectivities it demonstrated as compared to standard C_{18} columns, including the one initially tested in the present study.[169,170] The stationary phase in the biphenyl column used in the second dimension is quite different, with a biphenyl moiety bonded to the silica particle. This chemistry provides selectivity that is complementary to typical C_{18} RP columns, which is useful in the context of a 2D-LC method. Consequently, a biphenyl column can be an ideal choice for use with SC, due to the greater affinity for the aromatic groups and alkyl chains present in many of these compounds.[167,168,171]

5.4 Conclusion

The present report is the first to describe a comprehensive, on-line two-dimensional liquid chromatography (RP x RP) method that would be a suitable technique for the separation of NPS. Results of the study serve as a proof-of-concept for the application of 2D-LC to the separation of isomeric and structurally related SC. We believe that with further investigation, optimization, and validation, 2D-LC will be a viable tool for more reliable separation of complex mixtures of SC compared to what can currently be achieved using conventional 1D-LC.

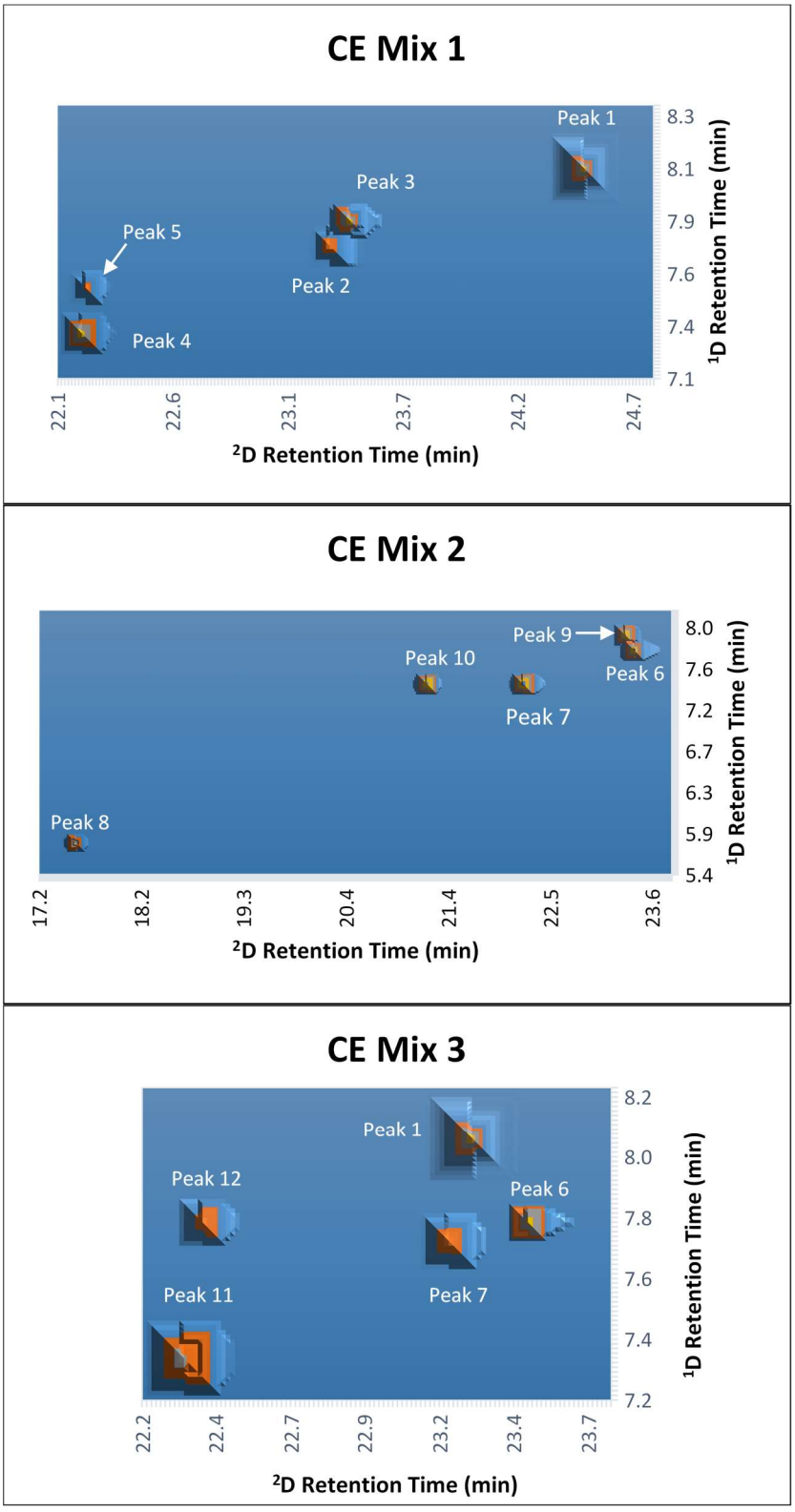

Figure 15. Contour plots demonstrating the 2D separation of CE mixes 1, 2, and 3. Peak number correspond to those listed in Table 21.

6. SUMMARY AND PROSPECT

The constant emergence of novel psychoactive substances will continue to present new analytical challenges to both forensic and clinical toxicology laboratories. Current screening methods typically rely on screening methods designed for specific compounds or compound classes which have demonstrated inconsistent, and often lacking, applicability to specimens containing NPS. Other methods of screening, such as the use of accurate mass databases and MS/MS libraries, present another option for more comprehensive, general screening and confirmation of compounds in human specimens. However, these tools are lacking in the presence of many NPS and remedying such deficiencies requires resources to which some laboratories do not have access (*i.e.,* poor availability of reference standards). The ability to identify these substances in forensic and clinical human specimens is important for conducting comprehensive toxicological screening and confirmation. It was this project's aim to create a large high resolution MS/MS spectral library and compound database for several hundreds of novel psychoactive substances and related compounds in order to aid the forensic and clinical toxicological communities in detecting and identifying such substances in human specimens. A method for comprehensive screening and confirmation was also validated for use with the HRMS spectral library and compound database using a mixture approach.

Mass spectral techniques have gained favor in toxicological laboratories for screening purposes due to their capability of collecting data with high degrees of selectivity and sensitivity which greatly aid in identification substances in toxicological samples. These techniques also have an advantage over other methods of screening, such

as immunoassays, in that they are not designed to detect a specific compound or compound class, but rather can be applied to a much broader range of potential analytes.

Spectral libraries and compound databases are used in conjunction with mass spectrometry techniques and can be extremely useful for screening purposes. In cases where a reference standard is not available to a laboratory, these resources can be searched and can present a tentative identification based on information contained within the database. When data are collected using high resolution mass spectrometry techniques, such as the LC-QTOF-MS used in this research, the selectivity is greatly increased thus improving confidence in correct identification. This high resolution and high mass accuracy enables differentiation between compounds with very similar accurate masses. The characteristic MS/MS spectral data collected using HRMS techniques also increases confidence in identification based on fragmentation patterns of each analyte. HRMS data collection can include information for all ions present in the sample which can then be retrospectively searched when new NPS are reported without the need for sample reanalysis. The use of the compound database and MS/MS spectral library created by this work can greatly assist forensic and clinical toxicological laboratories in saving valuable time and resources when attempting to identify novel psychoactive substances in toxicological specimens.

Following completion of the compound database and MS/MS library, a comprehensive method for screening and confirmation was validated following standard guidelines from the Toxicology Subcommittee of the Organization of Scientific Area Committees. Proof of concept was achieved for the use of a mixture approach for validation as opposed to validating the method for a single compound or class of

compounds one at a time. This technique saved valuable time and resources, and also demonstrated that the method could be applied comprehensively to a broad range of NPS from a variety of compound classes. The mixture approach has shown that it can be used in the future by other laboratories when validating toxicological screening and confirmation methods for a large group of NPS. The method was successfully validated for detection and identification of NPS at low ng/mL concentrations. Applicability of the method with the database and HRMS spectral library was also demonstrated through the qualitative screening of blind-spiked human urine specimens.

Finally, throughout the creation of the compound database and MS/MS spectral library, and during the subsequent method validation, it became apparent that some compounds could not be differentiated based on MS/MS data or chromatographically. This was especially apparent with isomeric compounds and those with related chemical structures. Typically, HRMS methods may be used to determine compounds with highly similar accurate masses, however, in instances of unreported and truly unknown NPS, it would be difficult to determine if a collected mass spectrum was representative of a single analyte or of multiple ones. In the case of isomers which have the same or similar MS/MS spectra, chromatographic information can be used. However, if adequate separation of the compounds cannot be achieved through traditional chromatographic methods, a different approach must be used. Two-dimensional liquid-chromatography (2D-LC) was investigated as part of this project to determine if it could be a viable option for separation of co-eluting compounds; mainly isomers and structurally similar compounds.

Mixtures of synthetic cannabinoids—both isomeric and non-isobaric—were created for the 2D-LC investigations. An on-line, comprehensive method using a Bonus-RP column in the first dimension and a biphenyl column in the second dimension was developed as a proof-of-concept for the application of 2D-LC to separation of such mixtures. Separation was successfully achieved for all compounds present in each mixture, but further development will be required in order to broaden applicability of the technique to NPS from other compound classes, as separation parameters can be compound specific.

A large compound database and MS/MS spectral library was successfully created and a corresponding method for screening and confirmation was fully validated using a mixture approach. These resources present valuable tools for forensic and clinical toxicology laboratories to use when screening for a wide variety of NPS. A two-dimensional liquid chromatographic method for separation of synthetic cannabinoids was also developed and demonstrated successful separation of isomeric and non-isobaric compounds from that class.

Future work will be required, however, to update and expand the compound database and MS/MS spectral library as more NPS are reported and as appropriate reference standards become commercially available. HRMS techniques should continue to gain favor for toxicological applications as they demonstrate improved selectivity and sensitivity over other screening approaches. Further investigation of two-dimensional liquid chromatography for improved separation of isomeric and structurally related NPS should also be conducted, as complete separation using traditional methods is likely to be challenged as more compounds emerge on the market.

REFERENCES

1. Behonick, G.; Shanks, K. G.; Firchau, D. J.; Mathur, G.; Lynch, C. F.; Nashelsky, M. Four postmertem case reports with quantitative detection of the synthetic cannabinoid, 5F-PB-22. *Journal of Analytical Toxicology* **2014,** *38*, 559-562.

2. deRoux, S. J.; Dunn, W. A. "Bath salts" the New York City Medical Examiner experience: A 3-year retrospective review. *Journal of Forensic Sciences* **2017,** *62*, 695-699.

3. 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*, 602-606.

4. Lee, D.; Chronister, C. W.; Broussard, W. A.; Utley-Bobak, S. R.; Schultz, D. L.; Vega, R. S. Illicit fentanyl-related fatalities in Florida: toxicological findings. *Journal of Analytical Toxicology* **2016,** *40*, 588-594.

5. Henderson, G. L. Designer drugs: past history and future prospects. *Journal of Forensic Sciences* **1988,** *33*, 569-575.

6. 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*, 716-727.

7. Broecker, S.; Herre, S.; Wüst, B.; Zweigenbaum, J.; Pragst, F. Development and practical application of a library of CID accurate mass specra of more than 2,500 toxic compounds for systematic toxicological analysis by LC-QTOF-MS with data-dependant acquisition. *Analytical and Bioanalytical Chemistry* **2011,** *400*, 101-117.

8. Peters, F. T. Recent advances of liquid chromatography-(tandem) mass spectrometry in clinical and forensic toxicology. *Clinical Biochemistry* **2011,** *44*, 54-65.

9. Rosano, T. G.; Wood, M.; Ihenetu, K.; Swift, T. A. Drug screening in medical examiner casework by high-resolution mass spectrometry (UPLC-MS^E-TOF). *Journal of Analytical Toxicology* **2013,** *37*, 593.

10. Logan, B. K.; Mohr, A. L. A.; Krotulski, A. J.; Papsun, D. M.; Kacinko, S. L.; Ropero-Miller, J. D.; Huestis, M. A. Reports of adverse events associated with use of novel psychoactive substances, 2013-2016: A review. *Journal of Analytical Toxicology* **2017,** *41*, 573-610.

11. Lua, I. A.; Lin, S.-L.; Lin, H. R.; Lua, A. C. Replacing immunoassays for mephedrone, ketamines, and six amphetamine-type stimulants with flow injection

analysis tandem mass spectrometry. *Journal of Analytical Toxicology* **2012,** *36*, 575-581.

12. Nakanishi, K.; Miki, A.; Zaitsu, 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*, 174-181.

13. DEA Diversion Control Division . Controlled Substance Schedules. 2018.

14. Title 21 United States Code (U.S.C.) Controlled Substances Act. *U. S. Department of Justice, Drug Enforcement Administration Diversion Control Division* **2018**.

15. Carroll, F. I.; Lewin, A. H.; Mascarella, S. W.; Seltzman, H. H.; Reddy, P. A. Designer drugs: a medicinal chemistry perspective. *Annals of the New York Academy of Sciences* **2012,** *1248*, 18-38.

16. Boumba, V. A.; Di Rago, M.; Peka, M.; Drummer, O. H.; Gerostamoulos, D. The analysis of 132 novel psychoactive substances in human hair using a single extraction by tandem LC/MS. *Forensic Science International* **2017,** *279*, 192-202.

17. Hill, S. L.; Thomas, S. H. L. Clinical toxicology of newer recreational drugs. *Clinical Toxicology* **2011,** *49*, 705-719.

18. Grabenauer, M.; Krol, W. L.; Wiley, J. L.; Thomas, B. F. Analysis of synthetic cannabinoids using high-resolution mass spectrometry and mass defect filtering: implications for nontargeted screening of designer drugs. *Analytical Chemistry* **2012,** *84*, 5574-5581.

19. Griffiths, P.; Sedefov, R.; Gallegos, A.; Lopez, D. How globalization and market innovation challenge how we think and respond to drug use: 'Spice" a case study. *Addiction* **2010,** *105*, 951-953.

20. Donohue, K. M.; Steiner, R. R. JWH-018 and JWH-022 as Combustion Products of AM2201. *Microgram Journal* **2012,** *9*, 52-56.

21. Finley, S. A. Two Additional Synthetic Drug Traffickers Plead Guilty in Curious Goods Case. The United States Department of Justice. 2012.

22. DEA Diversion Control Division *4-Methylmethcathinone (Mephedrone)*; 13.

23. Drug Enforcement Administration . DEA Scheduling Actions. 2014. 11-9-2014.

24. Nieddu, M.; Burrai, L.; Baralla, E.; Pasciu, V.; Varoni, M. V.; Briguglio, I.; Demontis, M. P.; Boatto, G. ELISA detection of 30 new amphetamine designer

drugs in whole blood, urine and oral fluid using Neogen® "Amphetamine" and "Methamphetamine/MDMA" kits. *Journal of Analytical Toxicology* **2016,** *40*, 492-497.

25. Scheidweiler, K. B.; Jarvis, M. J. Y.; Huestis, M. A. Nontargeted SWATH acquisition for identifying 47 synthetic cannabinoid metabolites in human urine by liquid chromatography-high-resolution tandem mass spectrometry. *Analytical and Bioanalytical Chemistry* **2015,** *407*, 883-897.

26. Sundström, M.; Pelander, A.; Angerer, V.; Hutter, M.; Kneisel, S.; Ojanperä, I. A high-sensitivity ultra-high performance liquid chromatography/high-resolution time-of-flight mass spectrometry (UHPLC-HR-TOFMS) method for screening synthetic cannabinoids and other drugs of abuse in urine. *Analytical and Bioanalytical Chemistry* **2013,** *405*, 8463-8474.

27. Ojanpera, I.; Pelander, A.; Laks, S.; Gergov, M.; Vuori, E.; Witt, M. Application of Accurate Mass Measurement to Urine Drug Screening. *Journal of Analytical Toxicology* **2005,** *29*, 34-40.

28. Ojanperä, I.; Pelander, A.; Ojanperä, S. Comprehensive toxicological and forensic drug screening by LC/QTOF-MS. Ferrer, I., Thurman, E. M., Eds.; 2009; pp 173-195.

29. Andersson, M.; Gustavsson, E.; Stephanson, N.; Beck, O. Direct injection LC-MS/MS method for identification and quantification of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine in urine drug testing. *Journal of Chromatography B* **2008,** *861*, 22-28.

30. Svensson, J.-O.; Andersson, M.; Gustavsson, E.; Beck, O. Electrospray LC-MS method with solid-phase extraction for accurate determination of morphine-, codeine-, and ehtylmorphine-glucuronides and 6-acetylmorphine in urine. *Journal of Analytical Toxicology* **2007,** *31*, 81-86.

31. Gustavsson, E.; Andersson, M.; Stephanson, N.; Beck, O. Validation of direct injection electrospray LC-MS/MS for confirmation of opiates in urine drug testing. *Journal of Mass Spectrometry* **2007,** *42*, 881-889.

32. Politi, L.; Morini, L.; Polettini, A. A direct screening procedure for diuretics in human urine by liquid chromatography-tandem mass spectrometry with information dependent acquisition. *Clinica Chimica Acta* **2007,** *386*, 46-52.

33. 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*, 133-137.

34. Mueller, C. A.; Weinmann, W.; Dresen, S.; Schreiber, A.; Gergov, M. Development of a multi-target screening analysis for 301 drugs using QTrap liquid chromatography/tandem mass spectrometry system and automated library searching. *Rapid Communications in Mass Spectrometry* **2005,** *19*, 1332-1338.

35. Substance Abuse and Mental Health Services Administration . Drug Testing. 2015.

36. Decaestecker, T. N.; Vande Casteele, S. R.; Wallemacq, P. E.; Van Peteghem, C. H.; Defore, D. L.; Van Bocxlaer, J. F. Information-dependent acquisition-mediated LC-MS/MS screening procedure with semiquantitative potential. *Analytical Chemistry* **2004,** *76*, 6365-6373.

37. Concheiro, M.; Castaneto, M.; Kronstrand, R.; Huestis, M. A. Simultaneous determination of 40 novel psychoactive substances in urine by liquid chromatography-high resolution mass spectrometry and library matching. *Journal of Chromatography A* **2015,** *1397*, 32-42.

38. Dresen, S.; Ferreirós, N.; Gnann, H.; Zimmermann, R.; Weinmann, W. Detection and identification of 700 drugs by multi-target screening with a 3200 Q TRAP® LC-MS/MS system and library searching. *Analytical and Bioanalytical Chemistry* **2010,** *396*, 2425-2434.

39. 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*, 444-451.

40. Meyer, M. R.; Maurer Hans H. Review: 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.

41. Petrie, M.; Lynch, K. L.; Ekins, S.; Chang, J. S.; Goetz, R. J.; Wu, A. H. B.; Krasowski, M. D. Cross-reactivity studies and predicitve modeling of "Bath Salts" and other amphetamine-type stimulants with amphetamine screening immunoassays. *Clinical Toxicology* **2013,** *51*, 83-91.

42. Stein, S. Mass spectral reference libraries: an ever-expanding resource for chemical identification. *Analytical Chemistry* **2012,** *84*, 7274-7284.

43. Stein, S. E. Chemical structure identification by mass spectral library searching. *Journal of the American Society for Mass Spectrometry* **1995,** *6*, 644-655.

44. Moorthy, A. S.; Wallace, W. E.; Kearsley, A. J.; Tchekhovskoi, D. V.; Stein, S. E. Combining fragment-ion and neutral-loss matching during mass spectral library searching: A new general purpose algorithm applicable to illicit drug identification. *Analytical Chemistry* **2017,** *89*, 13261-13268.

45. Alechaga, É.; Moyano, E.; Galceran, M. T. Wide-range screening of psychoactive substances by FIA-HRMS: identification strategies. *Analytical and Bioanalytical Chemistry* **2015,** *407*, 4567-4580.

46. 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,** *42*, 220-231.

47. Pavlic, M.; Libiseller, K.; Oberacher, H. Combined use of ESI-QqTOF-MS and ESI-QqTOF-MS/MS with mass-spectral library search for qualitative analysis of drugs. *Analytical and Bioanalytical Chemistry* **2006,** *386*, 69-82.

48. Smith, R. M. *Understanding Mass Spectra: A Basic Approach;* John Wiley & Sons, Inc.: New York, NY, 1999.

49. NIST 17 Mass Spectral Library & Search Software Version 2017 Brochure. 2017.

50. Lee, H. K.; Ho, C. S.; Iu, Y. P. H.; Lai, P. S. J.; Shek, C. C.; Lo, Y.-C.; Klinke, H. B.; Wood, M. Development of a broad toxicological screening technique for urine using ultra-performance liquid chromatography and time-of-flight mass spectrometry. *Analytica Chimica Acta* **2009,** *649*, 80-90.

51. Lynch, K. L.; Breaud, A. R.; Vandenberghe, H.; Wu, A. H. B.; Clarke, W. Performance evaluation of three liquid chromatography mass spectrometry methods for broad spectrum drug screening. *Clinica Chimica Acta* **2010,** *411*, 1474-1481.

52. de Hoffmann, E.; Stroobant, V. *Mass Spectrometry: Principles and Applications;* 3rd ed.; John Wiley & Sons, Ltd.: 2007.

53. 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*, 2521-2526.

54. Namera, A.; Kawamura, M.; Nakamoto, A.; Saito, T.; Nagao, M. Comprehensive review of the detection methods for synthetic cannabinoids and cathinones. *Forensic Toxicology* **2015,** *33*, 175-194.

55. Yanes, E. G.; Lovett, D. P. High-throughput bioanalytical method for analysis of synthetic cannabinoid metabolites in urine using salting-out sample preparation and LC-MS/MS. *Journal of Chromatography B* **2012,** *909*, 42-50.

56. 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*, 86-91.

57. Weinmann, W.; Gergov, M.; Goerner, G. MS/MS-libraries with triple quadrupole-tandem mass spectrometers for drug identification and drug screening. *Analusis* **2000,** *28*, 941.

58. Oberacher, H.; Pavlic, M.; Libiseller, K.; Schubert, B.; Sulyok, M.; Schuhmacher, R.; Csaszar, E.; Köfeler, H. C. On the inter-instrument and the inter-laboratory transferability of a tandem mass spectral reference library: 1. Results of an Austrian multicenter study. *Journal of Mass Spectrometry* **2009,** *44*, 485-493.

59. Broecker, S.; Herre, S.; Pragst, F. General unknown screening in hair by liquid chromatography-hybrid quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). *Forensic Science International* **2012,** *218*, 68-81.

60. Polettini, A.; Gottardo, R.; Pascali, J. P.; Tagliaro, F. Implementation and performance evaluation of a database of chemical formulas for the screening of pharmaco/toxicologically relevant compounds in biological samples using electrospray ionization-time-of-flight mass spectrometry. *Analytical Chemistry* **2008,** *80*, 3050-3057.

61. Ojanperä, S.; Pelander, A.; Pelzing, M.; Krebs, I.; Vuori, E.; Ojanperä, I. Isotopic pattern and accurate mass determination in urine drug screening by liquid chromatography/time-of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry* **2006,** *20*, 1161-1167.

62. Pelander, A.; Tyrkkö, E.; Ojanperä, I. *In silico* methods for predicting metabolism and mass fragmentation applied to quetiapine in liquid chromatography/time-of-flight mass spectrometry in urine drug screening. *Rapid Communications in Mass Spectrometry* **2009,** *23*, 506-514.

63. Colby, J. M.; Thoren, K. L.; Lynch, K. L. Suspect screening using LC-QqTOF is a useful tool for detecting drugs in biological specimens. *Journal of Analytical Toxicology* **2018,** *42*, 207-213.

64. Kinyua, J.; Negreira, N.; Ibáñez, M.; Bijlsma, L.; Hernández, F.; Covaci, A.; van Nuijs, A. L. N. A data-independent acquisition workflow for qualitative screening of new psychoactive substances in biological samples. *Analytical and Bioanalytical Chemistry* **2015,** *407*, 8773-8785.

65. Choi, B. K.; Hercules, D. M.; Zhang, T.; Gusev, A. I. Comparison of quadrupole, time-of-flight, and Fourier transform mass analyzers for LC-MS applications. *Current Trends in Mass Spectrometry* **2003,** *18*, 24-31.

66. 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*, 1203-1220.

67. Broecker, S.; Pragst, F.; Bakdash, A.; Herre, S.; Tsokos, M. Combined use of liquid-chromatography-hybrid quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) and high performance liquid chromatography with photodiode array detector (HPLC-DAD) in systematic toxicological analysis. *Forensic Science International* **2011,** *212*, 215-226.

68. Muñoz-Muñoz, A. C.; Pekol, T.; Schubring, D.; Johnson, C.; Andrade, L. Identification of novel opioid interferences using high-resolution mass spectrometry. *Journal of Analytical Toxicology* **2018,** *42*, 6-16.

69. Ibáñez, M.; Sancho, J. V.; Bijlsma, L.; van Nuijs, A. L. N.; Covaci, A.; Hernández, F. Comprehensive analytical strategies based on high-resolution time-of-flight mass spectrometry to identify new psychoactive substances. *Trends in Analytical Chemistry* **2014,** *57*, 107-117.

70. Fairchild, J. N.; Horváth, K.; Guiochon, G. Approaches to comprehensive multidimensional liquid chromatography systems. *Journal of Chromatography A* **2009,** *1216*, 1363-1371.

71. Fairchild, J. N.; Horváth, K.; Guiochon, G. Theoretical advantages and drawbacks of on-line, multidimensional liquid chromatography using multiple columns operated in parallel. *Journal of Chromatography A* **2009,** *1216*, 6210-6217.

72. Jayamanne, M.; Granelli, I.; Tjernberg, A.; Edlund, P.-O. Development of a two-dimensional liquid chromatography system for isolation of drug metabolites. *Journal of Pharmaceutical and Biomedical Analysis* **2010,** *51*, 649-657.

73. Pirok, B. W. J.; Gargano, A. F. G.; Schoenmakers, P. J. Optimizing separations in online comprehensive two-dimensional liquid chromatography. *Journal of Separation Science* **2018,** *41*, 68-98.

74. Stoll, D. R.; Carr, P. W. Two-dimensional liquid chromatography: a state of the art tutorial. *Analytical Chemistry* **2017,** *89*, 519-531.

75. Guiochon, G.; Marchetti, N.; Mriziq, K.; Shalliker, R. A. Implementations of two-dimensional liquid chromatography. *Journal of Chromatography A* **2008,** *1189*, 109-168.

76. Rao, R. N.; Kumar, K. N.; Shinde, D. D. Determination of rat plasma levels of sertraline enantiomers using direct injection with achiral-chiral column switching by LC-ESI/MS/MS. *Journal of Pharmaceutical and Biomedical Analysis* **2010,** *52*, 398-405.

77. Hudson, S.; Ramsey, J. The emergence and analysis of synthetic cannabinoids. *Drug Testing and Analysis* **2011,** *3*, 466-478.

78. Stoll, D. R. Guidelines for bioanalytical 2D chromatography method development and implementation. *Bioanalysis* **2010,** *2*, 105-122.

79. Cassiano, N.; Barreiro, J.; Oliveira, R.; Cass, Q. Direct bioanalytical sample injection with 2D LC-MS. *Bioanalysis* **2012,** *4*, 2737-2756.

80. Donato, P.; Cacciola, F.; Quinto Tranchida, P.; Dugo, P.; Mondello, L. Mass spectrometry detection in comprehensive liquid chromatography: basic concepts, instrumental aspects, applications and trends. *Mass Spectrometry Reviews* **2012,** *31*, 523-559.

81. Mondello, L.; Dugo, P.; Kumm, T.; Cacciola, F.; Dugo, G. Two-dimensional comprehensive liquid chromatography. In *Handbook of HPLC*, 2 ed.; Corradini, D., Phillips, T. M., Eds.; CRC Press: 2010.

82. Andrighetto, L. M.; Burns, N. K.; Stevenson, P. G.; Pearson, J. R.; Henderson, L. C.; Bowen, C. J.; Conlan, X. A. In-silico optimisation of two-dimensional high performance liquid chromatography for the determination of Australian methamphetamine seizure samples. *Forensic Science International* **2016,** *266*, 511-516.

83. Opiteck, G. J.; Lewis, K. C.; Jorgenson, J. W.; Anderegg, R. J. Comprehensive on-line LC/LC/MS of proteins. *Analytical Chemistry* **1997,** *69*, 1518-1524.

84. Opiteck, G. J.; Ramirez, S. M.; Jorgenson, J. W.; Moseley, M. A. Comprehensive two-dimensional high performance liquid chromatography for the isolation of overexpressed proteins and proteome mapping. *Analytical Biochemistry* **1998,** *258*, 349-361.

85. Malerod, H.; Lundanes, E.; Greibrokk, T. Recent advances in on-line multidimensional liquid chromatography. *Analytical Methods* **2010,** *2*, 110-122.

86. Agilent Technologies, I. *Agilent 1290 Infinity 2D-LC-Solution: User Guide;* 2012.

87. Bedani, F.; Schoenmakers, P. J.; Janssen, H.-G. Theories to support method development in comprehensive two-dimensional liquid chromatography - A review. *Journal of Separation Science* **2012,** *35*, 1697-1711.

88. Fairchild, J. N.; Horváth, K.; Gooding, J. R.; Campagna, S. R.; Guiochon, G. Two-dimensional liquid chromatography/mass spectrometry/mass spectrometry separation of water-soluble metabolites. *Journal of Chromatography A* **2010,** *1217*, 8161-8166.

89. Rao, R. N.; Shinde, D. D. Two-dimensional LC-MS/MS determination of antiretroviral drugs in rat serum and urine. *Journal of Pharmaceutical and Biomedical Analysis* **2009,** *50*, 994-999.

90. Jourdil, J.-F.; Tonini, J.; Stanke-Labesque, F. Simultaneous quantitation of azole antifungals, antibiotics, imatinib, and raltegravir in human plasma by two-dimensional high-performance liquic chromatography-tandem mass spectrometry. *Journal of Chromatography B* **2013,** *919-920*, 1-9.

91. Mella, M.; Schweitzer, B.; Mallet, C.; Moore, T.; Botch-Jones, S. Detection of cocaine and metabolites in bone following decomposition using 2D LC-MS-MS. *Journal of Analytical Toxicology* **2018,** *42*, 265-275.

92. Mallet, C.; Botch-Jones, S. Illicit drug analysis using two-dimension liquid chromatography/tandem mass spectrometry. *Journal of Analytical Toxicology* **2016,** *40*, 617-627.

93. Ing-Lorenzini, K. R.; Desmeaules, J. A.; Besson, M.; Veuthey, J.-L.; Dayer, P.; Daali, Y. Two-dimensional liquid-chromatography-ion trap mass spectrometry for the simultaneous determination of ketorolac enantiomers and paracetamol in human plasma: application to a pharmacokinetic study. *Journal of Chromatography A* **2009,** *1216*, 3851-3856.

94. Li, Y.; Gu, C.; Gruenhagen, J.; Zhang, K.; Yehl, P.; Chetwyn, N. P.; Medley, C. D. A size exclusion-reveresed phase two dimensional-liquid chromatography methodology for stability and small molecule related species in antibody drug conjugates. *Journal of Chromatography A* **2015,** *1393*, 81-88.

95. Pascoe, R.; Foley, J. P.; Gusev, A. I. Reduction in matrix-related signal suppression effects in electrospray ionization mass spectrometry using on-line two-dimensional liquid chromatography. *Analytical Chemistry* **2001,** *73*, 6014-6023.

96. Joseph, S.; Subramanian, M.; Khera, S. Simultaneous and stereospecific analysis of warfarin oxidative metabolism using 2D LC/Q-TOF. *Bioanalysis* **2015,** *7*, 2297-2309.

97. Sandra, P.; Vanhoenacker, G.; Steenbeke, M.; David, F.; Sandra, K.; Brunelli, C.; Szucs, R. On-line two-dimensional liquid chromatography (2D-LC) for the analysis of pharmaceuticals. *LC-GC North America* **2016,** *29*, 610-617.

98. Stoll, D. Recent advances in 2D-LC for bioanalysis. *Bioanalysis* **2015,** *7*, 3125-3142.

99. Pandohee, J.; Holland, B. J.; Li, B.; Tsuzuki, T.; Stevenson, P. G.; Barnett, N. W.; Pearson, J. R.; Jones, O. A. H.; Conlan, X. A. Screening of cannabinoids in industrial-grade hemp using two-dimensional liquid chromatography coupled with acidic potassium permanganate chemiluminescence detection. *Journal of Separation Science* **2015,** *38*, 2024-2032.

100. Agilent Technologies, I. Performance evaluation of the Agilent 1290 Infinity 2D-LC Solution for comprehensive two-dimensional liquid chromatography: Technical Overview. 2012.

101. Regalado, E. L.; Schariter, J. A.; Welch, C. J. Investigation of two-dimensional high performance liquid chromatography approaches for reversed phase resolution of warfarin and hydroxywarfarin isomers. *Journal of Chromatography A* **2014,** *1363*, 200-206.

102. Stoll, D. Introduction to Two-Dimensional Liquid Chromatography - Theory and Practice. In *Handbook of Advanced Chromatography/Mass Spectrometry Techniques*, Holcapek, M., Byrdwell, W. C., Eds.; Elsevier: London, 2017; pp 227-286.

103. Stoll, D. R.; Li, X.; Wang, X.; Carr, P. W.; Porter, S. E. G.; Rutan, S. C. Fast, comprehensive two-dimensional liquid chromatography. *Journal of Chromatography A* **2007,** *1168*, 3-43.

104. Bedani, F.; Kok, W. Th.; Janssen, H.-G. Optimal gradient operation in comprehensive liquid chromatography x liquid chromatography systems with limited orthogonality. *Analytica Chimica Acta* **2009,** *654*, 77-84.

105. Krieger, S. Authentication of traditional Chinese prescriptions using comprehensive 2D-LC. *LC-GC North America* **2015,** *33*, 18.

106. François, I.; Sandra, K.; Sandra, P. Comprehensive liquid chromatography: fundamental aspects and practical considerations--a review. *Analytica Chimica Acta* **2009,** *641*, 14-31.

107. Chen, X.; Kong, L.; Su, X.; Fu, H.; Ni, J.; Zhao, R.; Zou, H. Separation and identification of compounds in *Rhizoma chuanxiong* by comprehensive two-dimensional liquid chromatography coupled to mass spectrometry. *Journal of Chromatography A* **2004,** *1040*, 169-178.

108. Li, D.; Jakob, C.; Schmitz, O. Practical considerations in comprehensive two-dimensional liquid chromatography systems (LCxLC) with reversed-phase in both dimensions. *Analytical and Bioanalytical Chemistry* **2015,** *407*, 153-167.

109. Zhang, J.; Tao, D.; Duan, J.; Liang, Z.; Zhang, W.; Zhang, L.; Huo, Y.; Zhang, Y. Separation and identification of compounds in *Adinandra nitida* by comprehensive two-dimensional liquid chromatography coupled to atmospheric pressure chemical ionization source ion trap tandem mass spectrometry. *Analytical and Bioanalytical Chemistry* **2006,** *386*, 586-593.

110. Holcapek, M.; Ovcacíková, M.; Lísa, M.; Cífková, E.; Hájek, T. Continuous comprehensive two-dimensional liquid chromatography-electrospray ionization

mass spectrometry of complex lipidomic samples. *Analytical and Bioanalytical Chemistry* **2015,** *407*, 5033-5043.

111. Yang, P.; Pursch, M. Recent advances in two-dimensional liquid chromatography. *Chromatography Today* **2017,** 24-29.

112. Gu; Haiwei; Huang, Y.; Carr, P. W. Peak capacity optimization in comprehensive two dimensional liquid chromatography: A practical approach. *Journal of Chromatography A* **2011,** *1218*, 64-73.

113. Dugo, P.; Cacciola, F.; Kumm, T.; Dugo, G.; Mondello, L. Comprehensive multidimensional liquid chromatography: Theory and applications. *Journal of Chromatography A* **2008,** *1184*, 353-368.

114. Cao, J.-L.; Wei, J.-C.; Chen, M.-W.; Su, H.-X.; Wan, J.-B.; Wang, Y.-T.; Li, P. Application of two-dimensional chromatography in the analysis of Chinese herbal medicines. *Journal of Chromatography A* **2014,** *1371*, 1-14.

115. Hu, L.; Chen, X.; Kong, L.; Su, X.; Ye, M.; Zou, H. Improved performance of comprehensive two-dimensional HPLC separation of traditional Chinese medicines by using a silica monolithic column and normalization of peak heights. *Journal of Chromatography A* **2005,** *1092*, 191-198.

116. Krieger, S. Comprehensive 2D-LC analysis of Chinese herbal medicine. *LCGC Europe* **2014,** *27*, 677.

117. Horváth, K.; Fairchild, J.; Guiochon, G. Optimization strategies for off-line two-dimensional liquid chromatography. *Journal of Chromatography A* **2009,** *1216*, 2511-2518.

118. Potts, L.; Stoll, D.; Li, X.; Carr, P. The impact of sampling time on peak capacity and analysis speed in on-line comprehensive two-dimensional liquid chromatography. *Journal of Chromatography A* **2010,** *1217*, 5700-5709.

119. Sarrut, M.; Szabolcs, F.; Janin-Bussat, M.-C.; Colas, O.; Guillarme, D.; Beck, A.; Heinisch, S. Analysis of antibody-drug conjugates by comprehensive on-line two-dimensional hydrophobic interaction chromatography x reversed phase liquid chromatography hyphenated to high resolution mass spectrometry. II-Identification of sub-units for the characterization of even and odd load drug species. *Journal of Chromatography B* **2016,** *1032*, 91-102.

120. Sarrut, M.; Szabolcs, F.; Janin-Bussat, M.-C.; Colas, O.; Guillarme, D.; Beck, A.; Heinisch, S. Analysis of antibody-drug conjugates by comprehensive on-line two-dimensional hydrophobic interaction chromatography x reversed phase liquid chromatography hyphenated to high resolution mass spectrometry. I - Optimization of separation conditions. *Journal of Chromatography B* **2016,** *1032*, 103-111.

121. Vanhoenacker, G.; Vandenheede, I.; David, F.; Sandra, P.; Sandra, K. Comprehensive two-dimensional liquid chromatography of therapeutic monoclonal antibody digests. *Analytical and Bioanalytical Chemistry* **2015,** *407*, 355-366.

122. Ikegami, T.; Hara, T.; Kimura, H.; Kobayashi, H.; Hosoya, K.; Cabrera, K.; Tanaka, N. Two-dimensional reversed-phase liquid chromatography using two monolithic silica C18 columns and diferent mobile phase modifiers in the two dimensions. *Journal of Chromatography A* **2006,** *1106*, 112-117.

123. Wang, J.; Xu, Y.; Wen, C.; Wang, Z. Application of a trap-free two-dimensional liquid chromatography combined with ion trap/time-of-flight mass spectrometry for separation and characterization of impurities and isomers in cefpiramide. *Analytica Chimica Acta* **2017,** *992*, 42-54.

124. Pellett, J.; Lukulay, P.; Mao, Y.; Bowen, W.; Reed, R.; Ma, M.; Munger, R. C.; Dolan, J. W.; Wrisley, L.; Medwid, K.; Toltl, N. P.; Chan, C. C.; Skibic, M.; Biswas, K.; Wells, K. A.; Snyder, L. R. "Orthogonal" separations for reversed-phase liquid chromatography. *Journal of Chromatography A* **2006,** *1101*, 122-135.

125. Jandera, P.; Fischer, J.; Lahovská, H.; Novotná, K.; Cesla, P.; Kolárová, L. Two-dimensional liquid chromatography normal-phase and reversed-phase separation of (co)oligomers. *Journal of Chromatography A* **2005,** *1119*, 3-10.

126. Gray, M. J.; Sweeney, A. P.; Dennis, G. R.; Slonecker, P. J.; Shalliker, R. A. Comprehensive coupled reversed-phase reversed-phase separations of a complex isomeric mixture. *Analyst* **2003,** *128*, 598-604.

127. Gray, M. J.; Dennis, G. R.; Slonecker, P. J.; Shalliker, R. A. Evaluation of the two-dimensional reversed-phase-reversed-phase separations of low-molecular mass polystyrenes. *Journal of Chromatography A* **2003,** *1015*, 89-98.

128. Stoll, D. R.; Cohen, J. D.; Carr, P. W. Fast, comprehensive online two-dimensional high performance liquid chromatography through the use of high temperature ultra-fast gradient elution reversed-phase liquid chromatography. *Journal of Chromatography A* **2006,** *1122*, 123-137.

129. Gray, M. J.; Dennis, G. R.; Slonecker, P. J.; Shalliker, R. A. Comprehensive two-dimensional separations of complex mixtures using reversed-phase reversed-phase liquid chromatography. *Journal of Chromatography A* **2004,** *1041*, 101-110.

130. Gilar, M.; Olivova, P.; Daly, A. E.; Gebler, J. C. Two-dimensional separation of peptides using RP-RP-HPLC system with different pH in first and second separation dimensions. *Journal of Separation Science* **2005,** *28*, 1694-1703.

131. Gilar, M.; Olivova, P.; Daly, A. E.; Gebler, J. C. Orthogonality of separation in two-dimensional liquid chromatography. *Analytical Chemistry* **2005,** *77*, 6426-6434.

132. Opiteck, G. J.; Jorgenson, J. W. Two-dimensional SEC/RPLC coupled to mass spectrometry for the analysis of peptides. *Analytical Chemistry* **1997,** *69*, 2283-2291.

133. D'Attoma, A.; Grivel, C.; Heinisch, S. On-line comprehensive two-dimensional separations of charged compounds using reversed-phase high performance liquid chromatography and hydrophilic interaction chromatography. Part I: Orthogonality and practical peak capacity considerations. *Journal of Chromatography A* **2012,** *1262*, 148-159.

134. François, I.; de Villiers, A.; Sandra, P. Considerations on the possibilities and limitations of comprehensive normal phase-reversed phase liquid chromatography (NPLC x RPLC). *Journal of Separation Science* **2006,** *29*, 492-498.

135. Blahová, E.; Jandera, P.; Cacciola, F.; Mondello, L. Two-dimensional and serial column reversed-phase separation of phenolic antioxidants on octadecyl-, polyethyleneglycol-, and pentafluorophenylpropyl-silica columns. *Journal of Separation Science* **2006,** *29*, 555-566.

136. Kalili, K. M.; de Villiers, A. Off-line comprehensive two-dimensional hydrophilic interaction x reversed phase liquid chromatographic analysis of green tea phenolics. *Journal of Separation Science* **2010,** *33*, 853-863.

137. François, I.; de Villiers, A.; Tienpont, B.; David, F.; Sandra, P. Comprehensive two-dimensional liquid chromatography applying two parallel columns in the second dimension. *Journal of Chromatography A* **2008,** *1178*, 33-42.

138. Dugo, P.; Kumm, T.; Crupi, M. L.; Cotroneo, A.; Mondello, L. Comprehensive two-dimensional liquid chromatography combined with mass spectrometric detection in the analyses of triacylglycerols in natural lipidic matrixes. *Journal of Chromatography A* **2006,** *1112*, 269-275.

139. Jones, D. R.; Boysen, G.; Miller, G. Novel multi-mode ultra performance liquid chromatography-tandem mass spectrometry assay for profiling enantiomeric hydroxywarfarins and warfarin in human plasma. *Journal of Chromatography B* **2011,** *879*, 1056-1062.

140. 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*, 1383-1397.

141. 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.

142. Allen, K. R. Screening for drugs of abuse: which matrix, oral fluid or urine? *Annals of Clinical Biochemistry* **2011,** *48*, 531-541.

143. Wohlfarth, A.; Scheidweilder, K. B.; Chen, X.; Liu, H.; 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*, 3730-3738.

144. 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*, 519-528.

145. Seither, J. Z.; Arroyo-Mora, L. E.; Hindle, R.; DeCaprio, A. P. Development of a compound database and high resolution MS/MS spectral library for the identification of designer drugs by LC-QTOF-MS. Unpublished Work, 2014.

146. Gaudette, F.; Benito, J.; Steagall, P.; Beaudry, F. Assessment of tandem mass spectrometry and high-resolution mass spectrometry for the analysis of bupivacaine in plasma. *Biomedical Chromatography* **2015,** *29*, 1724-1730.

147. 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.

148. Peters, F. T.; Drummer, O. H.; Musshoff, F. Validation of new methods. *Forensic Science International* **2007,** *165*, 216-224.

149. Scientific Working Group for Forensic Toxicology (SWGTOX) *Standard Practices for Method Validation in Forensic Toxicology*; 13.

150. Toxicology Subcommittee of the Organization of Scientific Area Committees *Standard Practices for Method Validation in Forensic Toxicology*; 17.

151. Shanks, K. G.; Dahn, T.; Terrell, A. R. Detection of JWH-018 and JWH-073 by UPLC-MS-MS in postmortem whole blood casework. *Journal of Analytical Toxicology* **2012,** *31*, 333-337.

152. Sasaki, C.; Saito, T.; Shinozuka, T.; Irie, W.; Murakami, C.; Maeda, K.; Nakamura, N.; Oishi, M.; Nakamura, S.; Kurihara, K. A case of death caused by abuse of a synthetic cannaibinoid *N*-1-naphthalenyl-1-pentyl-1*H*-indole-3-carboxamide. *Forensic Toxicology* **2015,** *33*, 165-169.

153. Hasegawa, K.; Wurita, A.; Suzuku, O. Postmortem distribution of AB-CHMINACA, 5-fluoro-AMB, and diphenidine in body fluids and solid tissues in a fatal poisoning case: usefulness of adipose tissue for detection of the drugs in unchanged forms. *Forensic Toxicology* **2015,** *33*, 45-53.

154. Kronstrand, R.; Brinkhagen, L.; Birath-Karlsson, C.; Roman, M.; Josefsson, M. LC-QTOF-MS as a superior strategy to immunoassay for the comprehensive analysis of synthetic cannabinoids in urine. *Analytical and Bioanalytical Chemistry* **2014,** *406*, 3599-3609.

155. Castaneto, M. S.; Wohlfarth, A.; Desrosiers, N. A.; Hartman, R. L.; Gorelick, D. A.; Huestis, M. A. Synthetic cannabinoids pharmacokinetics and detection methods in biological matrices. *Drug Metabolism Reviews* **2015,** *47*, 124-174.

156. Kneisel, S.; Auwärter, V. Analysis of 30 synthetic cannabinoids in serum by liquid chromatography-electrospray ionization tandem mass spectrometry after liquid-liquid extraction. *Journal of Mass Spectrometry* **2012,** *47*, 825-835.

157. Kneisel, S.; Speck, M.; Moosmann, B.; Corneillie, T. M.; Butlin, N. G.; Auwärter, V. LC/ESI-MS/MS method for quantification of 28 synthetic cannabinoids in neat oral fluid and its application to preliminary studies on their detection windows. *Analytical and Bioanalytical Chemistry* **2013,** *405*, 4691-4706.

158. Logan, B. K.; Kacinko, S. L.; McMullin, M. M.; Xu, A. *Technical Bulletin: NMS Labs test for JWH-018, JWH-019, JWH-073, JWH-250 and AM-2201 Primary Monohydroxy Metabolite in Human Urine*; 11.

159. Kneisel, S.; Auwärter, V.; Kempf, J. Analysis of 30 synthetic cannabinoids in oral fluid using liquid chromatography-electrospray ionization tandem mass spectrometry. *Drug Testing and Analysis* **2012,** *5*, 657-669.

160. Scheidweilder, K. B.; Huestis, M. A. Simultaneous quantification of 20 synthetic cannabinoids and 21 metabolites, and semi-quantification of 12 alkyl hydroxy metabolites in human urine by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A* **2014,** *1327*, 105-117.

161. Salomone, A.; Gerace, E.; D'Urso, F.; Di Corcia, D.; Vincenti, M. Simultaneous analysis of several sythetic cannabinoids, THC, CBD and CBN, in hair by ultra-high performance liquid chromatography tandem mass spectrometry. Method validation and application to real samples. *Journal of Mass Spectrometry* **2012,** *47*, 604-610.

162. Li, X.; Uboh, C. E.; Soma, L. R.; Liu, Y.; Guan, F.; Aurand, C. R.; Bell, D. S.; You, Y.; Chen, J.; Maylin, G. A. Sensitive hydrophilic interaction liquid chromatography/tandem mass spectrometry method for rapid detection, quantification and confirmation of cathinone-derived designer drugs for doping

control in equine plasma. *Rapid Communications in Mass Spectrometry* **2014,** *28*, 217-229.

163. Frank, M. Agilent 1290 Infinity 2D-LC Solution: A new flexible and user-friendly 2D-LC solution for the most complex samples. 2012.

164. Eiceman, G. A.; Karpas, Z.; Hill Jr., H. H. Introduction to Ion Mobility Spectrometry. In *Ion Mobility Spectrometry*, 3rd ed.; Taylor & Francis: Boca Raton, 2014.

165. Eiceman, G. A.; Karpas, Z.; Hill Jr., H. H. Drugs of Abuse. In *Ion Mobility Spectrometry*, 3rd ed.; Taylor & Francis: Boca Raton, 2014.

166. Manicke, N. E.; Belford, M. Separation of opiate isomers using electrospray ionization and paper spray coupled to high-field asymmetric waveform ion mobility spectrometry. *Journal of the American Society for Mass Spectrometry* **2015,** *26*, 701-705.

167. Bell, D. S.; Shollenberger, D.; Cramer, H. Evaluation of retention and selectivity using biphenyl stationary phases. *LC-GC North America* **2017,** *35*, 360-365.

168. Euerby, M. R.; Petersson, P.; Campbell, W.; Roe, W. Chromatographic classification and comparison of commercially available reversed-phase liquid chromatographic columns containing phenyl moieties using principal component analysis. *Journal of Chromatography A* **2007,** *1154*, 138-151.

169. Euerby, M. R.; Petersson, P. Chromatographic classification and comparison of commercially available reversed-phase liquid chromatographic columns containing polar embedded groups/amino endcappings using principal component analysis. *Journal of Chromatography A* **2004,** *1088*, 1-15.

170. Layne, J. Characterization and comparison of the chromatographic performance of conventional, polar-embedded, and polar-endcapped reversed-phase liquid chromatography stationary phases. *Journal of Chromatography A* **2002,** *957*, 149-164.

171. Marchand, D. H.; Croes, K.; Dolan, J. W.; Snyder, L. R.; Henry, R. A.; Kallury, K. M. R.; Waite, S.; Carr, P. W. Column selectivity in reversed-phase liquid chromatography: VIII. Phenylalkyl and fluoro-substituted columns. *Journal of Chromatography A* **2005,** *1062*, 65-78.

APPENDICES

Appendix 1. Compounds included in the NPS database with their unique in-house identifying number.

Compound Name	Formula	Accurate Mass	FIU Number
(±)-Ethylphenidate	C15H21NO2	247.1572	FIU 0279
2,4,6-Trimethoxyamphetamine	C12H19NO3	225.1365	FIU 0280
2-Bromoamphetamine	C9H12BrN	213.0153	FIU 0281
2-Chloroamphetamine	C9H12ClN	169.0658	FIU 0282
2-methoxy Ketamine	C14H19NO2	233.1416	FIU 0283
2-Methoxyamphetamine	C10H15NO	165.1154	FIU 0284
3,4-EDMC	C12H15NO3	221.1052	FIU 0285
3-Bromoamphetamine	C9H12BrN	213.0153	FIU 0286
3-Chloroamphetamine	C9H12ClN	169.0658	FIU 0287
3-Iodoamphetamine	C9H12IN	261.0014	FIU 0288
3-Methoxyamphetamine	C10H15NO	165.1154	FIU 0289
4-Bromoamphetamine	C9H12BrN	213.0153	FIU 0290
4-Chloroamphetamine	C9H12ClN	169.0658	FIU 0291
4-Hydroxyamphetamine	C9H13NO	151.0997	FIU 0292
4-Methoxyamphetamine	C10H15NO	165.1154	FIU 0293
4-MTA	C10H15NS	181.0925	FIU 0294
4-MTA (hydrochloride preparation)	C10H15NS	181.0925	FIU 0295
D-Amphetamine	C9H13N	135.1048	FIU 0296
deschloro-N-ethyl-Ketamine	C14H19NO	217.1467	FIU 0297
Diclofensine	C17H17Cl2NO	321.0687	FIU 0298
DOI	C11H16INO2	321.0226	FIU 0299
HMA	C10H15NO2	181.1103	FIU 0300
Lisdexamfetamine	C15H25N3O	263.1998	FIU 0301
Propylhexedrine	C10H21N	155.1674	FIU 0302
CMP	C10H17N	151.1361	FIU 0303
2,5-DMMA	C12H19NO2	209.1416	FIU 0304
3,4-DHMA	C10H15NO2	181.1103	FIU 0305
4-bromo-2,5-DMMA	C12H18BrNO2	287.0521	FIU 0306
para-Methoxymethamphetamine	C11H17NO	179.1310	FIU 0307
Methiopropamine	C8H13NS	155.0769	FIU 0308
N,N-DMA	C11H17N	163.1361	FIU 0309
2C-G	C12H19NO2	209.1416	FIU 0310
2C-T	C11H17NO2S	227.0980	FIU 0311
2C-T-7	C13H21NO2S	255.1293	FIU 0312
2C-TFM	C11H14F3NO2	249.0977	FIU 0313
bk-2C-B	C10H12BrNO3	273.0001	FIU 0314

3C-B-fly	C13H16BrNO2	297.0364	FIU 0315
3C-P	C14H23NO3	253.1678	FIU 0316
N-methyl-2-AI	C10H13N	147.1048	FIU 0317
Escaline	C12H19NO3	225.1365	FIU 0318
Mescaline	C11H17NO3	211.1208	FIU 0319
2-Amino-1-phenylbutane	C10H15N	149.1204	FIU 0320
2-Ethylamino-1-phenylbutane	C12H19N	177.1517	FIU 0321
4-CAB	C10H14ClN	183.0815	FIU 0322
Cathine	C9H13NO	151.0997	FIU 0323
(R)-(−)-MT-45	C24H32N2	348.2565	FIU 0324
(S)-(+)-MT-45	C24H32N2	348.2565	FIU 0325
2,3-Dichlorophenylpiperazine	C10H12Cl2N2	230.0378	FIU 0326
MBZP	C12H18N2	190.1470	FIU 0327
MT-45	C24H32N2	348.2565	FIU 0328
Mepirapim	C19H27N3O	313.2154	FIU 0329
2-Fluoroisocathinone	C9H10FNO	167.0746	FIU 0330
3,4-Dimethylethcathinone	C13H19NO	205.1467	FIU 0331
4-methoxy-N,N-Dimethylcathinone	C12H17NO2	207.1259	FIU 0332
4-Methylethcathinone metabolite ((±)-Ephedrine stereochemistry)	C12H19NO	193.1467	FIU 0333
4-Methylethcathinone metabolite ((±)-Pseudoephedrine stereochemistry)	C12H19NO	193.1467	FIU 0334
N-ethyl-N-Methylcathinone	C12H17NO	191.1310	FIU 0335
Isopentedrone	C12H17NO	191.1310	FIU 0336
Mephedrone	C11H15NO	177.1154	FIU 0337
Mephedrone metabolite ((±)-Ephedrine stereochemistry)	C11H17NO	179.1310	FIU 0338
Mephedrone metabolite ((±)-Pseudoephedrine stereochemistry)	C11H17NO	179.1310	FIU 0339
NRG-3	C16H19NO	241.1467	FIU 0340
Pentedrone metabolite ((±)-Ephedrine stereochemistry)	C12H19NO	193.1467	FIU 0341
Pentedrone metabolite ((±)-Pseudoephedrine stereochemistry)	C12H19NO	193.1467	FIU 0342
Benzedrone	C17H19NO	253.1467	FIU 0343
(−)-(S)-Cathinone	C9H11NO	149.0841	FIU 0344

2,3-Dimethylethcathinone	C13H19NO	205.1467	FIU 0345
2,4-Dimethylethcathinone	C13H19NO	205.1467	FIU 0346
Diethylcathinone	C13H19NO	205.1467	FIU 0347
2,3-Dimethylmethcathinone	C12H17NO	191.1310	FIU 0348
2,4-Dimethylmethcathinone	C12H17NO	191.1310	FIU 0349
3,4-Dimethylmethcathinone metabolite ((±)-Ephedrine stereochemistry)	C12H19NO	193.1467	FIU 0350
3,4-Dimethylmethcathinone metabolite ((±)-Pseudoephedrine stereochemistry)	C12H19NO	193.1467	FIU 0351
3-Bromomethcathinone	C10H12BrNO	241.0102	FIU 0352
4-Bromomethcathinone	C10H12BrNO	241.0102	FIU 0353
4-Fluoromethcathinone metabolite ((±)-Ephedrine stereochemistry)	C10H14FNO	183.1059	FIU 0354
4-Fluoromethcathinone metabolite ((±)-Pseudoephedrine stereochemistry)	C10H14FNO	183.1059	FIU 0355
(−)-3,4-Methylenedioxy Pyrovalerone	C16H21NO3	275.1521	FIU 0356
(+)-3,4-Methylenedioxy Pyrovalerone	C16H21NO3	275.1521	FIU 0357
2,3-MDA	C10H13NO2	179.0946	FIU 0358
2,3-MDMA	C11H15NO2	193.1103	FIU 0359
3,4-MDMA	C11H15NO2	193.1103	FIU 0360
3,4-Methylenedioxy-5-methylethcathinone	C13H17NO3	235.1208	FIU 0361
3,4-Methylenedioxy-N-benzylcathinone	C17H17NO3	283.1208	FIU 0362
Methylenedioxy Pyrovalerone	C16H21NO3	275.1521	FIU 0363
Methylenedioxy Pyrovalerone Metabolite 1	C16H23NO3	277.1678	FIU 0364
Methylenedioxy Pyrovalerone metabolite 2	C15H21NO3	263.1521	FIU 0365
N-acetyl-3,4-Methylenedioxymethcathinone	C13H15NO4	249.1001	FIU 0366
N-hydroxy MDA	C10H13NO3	195.0895	FIU 0367
Myristicin	C11H12O3	192.0786	FIU 0368
Piperonyl methyl ketone	C10H10O3	178.0630	FIU 0369
Safrole	C10H10O2	162.0681	FIU 0370

25B-NBOMe	C18H22BrNO3	379.0783	FIU 0371
25C-NBOMe	C18H22ClNO3	335.1288	FIU 0372
25D-NBOMe	C19H25NO3	315.1834	FIU 0373
25E-NBOMe	C20H27NO3	329.1991	FIU 0374
25G-NBOMe	C20H27NO3	329.1991	FIU 0375
25H-NBOMe	C18H23NO3	301.1678	FIU 0376
25H-NBOMe imine analog	C18H21NO3	299.1521	FIU 0377
25I-NBF	C17H19FINO2	415.0445	FIU 0378
25I-NBMD	C18H20INO4	441.0437	FIU 0379
25I-NBOMe 3-methoxy isomer	C18H22INO3	427.0644	FIU 0380
25I-NBOMe 4-methoxy isomer	C18H22INO3	427.0644	FIU 0381
25I-NBOMe imine analog	C18H20INO3	425.0488	FIU 0382
25N-NBOMe	C18H22N2O5	346.1529	FIU 0383
25T2-NBOMe	C19H25NO3S	347.1555	FIU 0384
30C-NBOMe	C20H26ClNO5	395.1500	FIU 0385
3-methoxy PCP	C18H27NO	273.2093	FIU 0386
4-methoxy PCP	C18H27NO	273.2093	FIU 0387
PCEEA	C16H25NO	247.1936	FIU 0388
PCMPA	C16H25NO	247.1936	FIU 0389
PCPr	C15H23N	217.1830	FIU 0390
Benocyclidine	C19H25NS	299.1708	FIU 0391
2-methyl-α-Pyrrolidinopropiophenone	C14H19NO	217.1467	FIU 0392
3,4-dimethoxy-α-Pyrrolidinopentiophenone	C17H25NO3	291.1834	FIU 0393
3'-fluoro-α-Pyrrolidinopropiophenone	C13H16FNO	221.1216	FIU 0394
3-methyl-α-Pyrrolidinopropiophenone	C14H19NO	217.1467	FIU 0395
4’-Methyl-N-methylhexanophenone	C14H21NO	219.1623	FIU 0396
4-fluoro-α-Pyrrolidinobutiophenone	C14H18FNO	235.1372	FIU 0397
4-fluoro-α-Pyrrolidinopentiophenone	C15H20FNO	249.1529	FIU 0398
4'-fluoro-α-Pyrrolidinopropiophenone	C13H16FNO	221.1216	FIU 0399
4-methoxy-α-Pyrrolidinobutiophenone	C15H21NO2	247.1572	FIU 0400
4-methoxy-α-Pyrrolidinopentiophenone	C16H23NO2	261.1729	FIU 0401

4-Methyl-α-ethylaminobutiophenone	C13H19NO	205.1467	FIU 0402
4-Methyl-α-ethylaminopentiophenone	C14H21NO	219.1623	FIU 0403
α-Ethylaminopentiophenone	C13H19NO	205.1467	FIU 0404
α-Pyrrolidinobutiophenone	C14H19NO	217.1467	FIU 0405
α-Pyrrolidinobutiothiophenone	C12H17NOS	223.1031	FIU 0406
α-Pyrrolidinopentiophenone metabolite 1	C15H23NO	233.1780	FIU 0407
α-Pyrrolidinopentiothiophenone	C13H19NOS	237.1187	FIU 0408
4-fluoro PV8	C17H24FNO	277.1842	FIU 0409
4-fluoro PV9	C18H26FNO	291.1998	FIU 0410
4-methoxy PV8	C18H27NO2	289.2042	FIU 0411
4-methoxy PV9	C19H29NO2	303.2198	FIU 0412
PV8	C17H25NO	259.1936	FIU 0413
PV9	C18H27NO	273.2093	FIU 0414
Pyrovalerone	C16H23NO	245.1780	FIU 0415
4-APB	C11H13NO	175.0997	FIU 0416
4-APDB	C11H15NO	177.1154	FIU 0417
5-APDB	C11H15NO	177.1154	FIU 0418
5-EAPB	C13H17NO	203.1310	FIU 0419
5-MAPB	C12H15NO	189.1154	FIU 0420
5-MAPDB	C12H17NO	191.1310	FIU 0421
6-APB	C11H13NO	175.0997	FIU 0422
6-APDB	C11H15NO	177.1154	FIU 0423
7-APB	C11H13NO	175.0997	FIU 0424
3-Methylbuphedrone	C12H17NO	191.1310	FIU 0425
4-Fluorobuphedrone	C11H14FNO	195.1059	FIU 0426
4-Methylbuphedrone	C12H17NO	191.1310	FIU 0427
4-methyl-N-Methylbuphedrone	C13H19NO	205.1467	FIU 0428
Buphedrone metabolite ((±)-Ephedrine stereochemistry)	C11H17NO	179.1310	FIU 0429
Buphedrone metabolite ((±)-Pseudoephedrine stereochemistry)	C11H17NO	179.1310	FIU 0430
N-Ethylbuphedrone	C12H17NO	191.1310	FIU 0431
Dimethocaine	C16H26N2O2	278.1994	FIU 0432
Nitracaine	C16H24N2O4	308.1736	FIU 0433
(−)-11-nor-9-carboxy-Δ9-THC	C21H28O4	344.1988	FIU 0434
(±)-Cannabichromene	C21H30O2	314.2246	FIU 0435

(±)-ORG 28611	C23H33N3O2	383.2573	FIU 0436
5-fluoro NNEI	C24H23FN2O	374.1794	FIU 0437
5-fluoro NNEI 2'-naphthyl isomer	C24H23FN2O	374.1794	FIU 0438
5-fluoro SDB-005	C23H21FN2O2	376.1587	FIU 0439
5-fluoro SDB-006	C21H23FN2O	338.1794	FIU 0440
A-796260	C22H30N2O2	354.2307	FIU 0441
A-836339	C16H26N2O2S	310.1715	FIU 0442
JW 618	C17H14F6N2O2	392.0959	FIU 0443
JW 642	C21H20F6N2O3	462.1378	FIU 0444
MN-25	C26H37N3O3	439.2835	FIU 0445
MN-25-2-methyl derivative	C27H39N3O3	453.2991	FIU 0446
NNEI	C24H24N2O	356.1889	FIU 0447
NNEI 2'-naphthyl isomer	C24H24N2O	356.1889	FIU 0448
Salvinorin A	C23H28O8	432.1784	FIU 0449
Salvinorin B	C21H26O7	390.1679	FIU 0450
SDB-005	C23H22N2O2	358.1681	FIU 0451
SDB-006	C21H24N2O	320.1889	FIU 0452
THCA-A	C22H30O4	358.2144	FIU 0453
Δ8-THC	C21H30O2	314.2246	FIU 0454
Δ9-THC	C21H30O2	314.2246	FIU 0455
AM1248 azepane isomer	C26H34N2O	390.2671	FIU 0456
AM2201 benzimidazole analog	C23H21FN2O	360.1638	FIU 0457
JZL 184	C27H24N2O9	520.1482	FIU 0458
KM 233	C25H30O2	362.2246	FIU 0459
KML29	C24H21F6NO7	549.1222	FIU 0460
LY2183240	C17H17N5O	307.1433	FIU 0461
LY2183240 2’-isomer	C17H17N5O	307.1433	FIU 0462
SER-601	C28H38N2O2	434.2933	FIU 0463
Tetrahydrocannabivarin	C19H26O2	286.1933	FIU 0464
Yangonin	C15H14O4	258.0892	FIU 0465
Cannabidiolic Acid	C22H30O4	358.2144	FIU 0466
Cannabigerol	C21H32O2	316.2402	FIU 0467
EG-018	C28H25NO	391.1936	FIU 0468
(±)-JWH 018 N-(2-hydroxypentyl) metabolite	C24H23NO2	357.1729	FIU 0469
(±)-JWH 018 N-(3-hydroxypentyl) metabolite	C24H23NO2	357.1729	FIU 0470
(±)-JWH 018 N-(4-hydroxypentyl) metabolite	C24H23NO2	357.1729	FIU 0471

(R)-(−)-JWH 018 N-(4-hydroxypentyl) metabolite	C24H23NO2	357.1729	FIU 0472
(S)-(+)-JWH 018 N-(4-hydroxypentyl) metabolite	C24H23NO2	357.1729	FIU 0473
5-fluoro JWH 018 adamantyl analog	C24H30FNO	367.2311	FIU 0474
JWH 018 2-hydroxyindole metabolite	C24H23NO2	357.1729	FIU 0475
JWH 018 4-hydroxyindole metabolite	C24H23NO2	357.1729	FIU 0476
JWH 018 5-hydroxyindole metabolite	C24H23NO2	357.1729	FIU 0477
JWH 018 6-hydroxyindole metabolite	C24H23NO2	357.1729	FIU 0478
JWH 018 7-hydroxyindole metabolite	C24H23NO2	357.1729	FIU 0479
JWH 018 8-quinolinyl carboxamide	C23H23N3O	357.1841	FIU 0480
JWH 018 benzimidazole analog	C23H22N2O	342.1732	FIU 0481
JWH 018 N-(1-ethylpropyl) isomer	C24H23NO	341.1780	FIU 0482
JWH 018 N-(4-oxo-pentyl) metabolite	C24H21NO2	355.1572	FIU 0483
JWH 018 N-(5-hydroxypentyl) metabolite	C24H23NO2	357.1729	FIU 0484
JWH 018 N-(5-hydroxypentyl) β-D-Glucuronide	C30H31NO8	533.2050	FIU 0485
JWH 018 N-pentanoic acid β-D-Glucuronide	C30H29NO9	547.1842	FIU 0486
JWH 018 N-propanoic acid metabolite	C22H17NO3	343.1208	FIU 0487
JWH 019 5-hydroxyindole metabolite	C25H25NO2	371.1885	FIU 0488
JWH 019 N-(2-fluorohexyl) isomer	C25H24FNO	373.1842	FIU 0489
JWH 019 N-(3-fluorohexyl) isomer	C25H24FNO	373.1842	FIU 0490
JWH 019 N-(4-fluorohexyl) isomer	C25H24FNO	373.1842	FIU 0491
JWH 019 N-(5-fluorohexyl) isomer	C25H24FNO	373.1842	FIU 0492
JWH 019 N-(5-hydroxyhexyl) metabolite	C25H25NO2	371.1885	FIU 0493

JWH 019 N-(6-fluorohexyl) isomer	C25H24FNO	373.1842	FIU 0494
JWH 019 N-(6-hydroxyhexyl) metabolite	C25H25NO2	371.1885	FIU 0495
JWH 019 N-(6-hydroxyhexyl) β-D-Glucuronide	C31H33NO8	547.2206	FIU 0496
JWH 030 2-naphthoyl isomer	C20H21NO	291.1623	FIU 0497
JWH 031 2'-isomer	C21H23NO	305.1780	FIU 0498
JWH 071	C21H17NO	299.1310	FIU 0499
JWH 073 2-hydroxyindole metabolite	C23H21NO2	343.1572	FIU 0500
JWH 073 4-hydroxyindole metabolite	C23H21NO2	343.1572	FIU 0501
JWH 073 5-hydroxyindole metabolite	C23H21NO2	343.1572	FIU 0502
JWH 073 6-hydroxyindole metabolite	C23H21NO2	343.1572	FIU 0503
JWH 073 6-methoxyindole analog	C24H23NO2	357.1729	FIU 0504
JWH 073 7-hydroxyindole metabolite	C23H21NO2	343.1572	FIU 0505
JWH 073 N-(2-hydroxybutyl) metabolite	C23H21NO2	343.1572	FIU 0506
JWH 073 N-(4-hydroxybutyl) metabolite	C23H21NO2	343.1572	FIU 0507
JWH 073 N-(4-hydroxybutyl) β-D-Glucuronide	C29H29NO8	519.1893	FIU 0508
JWH 073 N-butanoic acid metabolite	C23H19NO3	357.1365	FIU 0509
(±)-JWH 073 N-(3-hydroxybutyl) metabolite	C23H21NO2	343.1572	FIU 0510
(R)-(−)-JWH 073 N-(3-hydroxybutyl) metabolite	C23H21NO2	343.1572	FIU 0511
(S)-(+)-JWH 073 N-(3-hydroxybutyl) metabolite	C23H21NO2	343.1572	FIU 0512
JWH 080	C24H23NO2	357.1729	FIU 0513
JWH 081 4-hydroxynaphthyl metabolite	C24H23NO2	357.1729	FIU 0514
JWH 081 N-(4-hydroxypentyl) metabolite	C25H25NO3	387.1834	FIU 0515
JWH 081 N-(5-hydroxypentyl) metabolite	C25H25NO3	387.1834	FIU 0516
JWH 081 N-pentanoic acid metabolite	C25H23NO4	401.1627	FIU 0517

JWH 081-N-(cyclohexylmethyl) analog	C27H27NO2	397.2042	FIU 0518
JWH 116	C26H27NO	369.2093	FIU 0519
JWH 122 N-(4-hydroxypentyl) metabolite	C25H25NO2	371.1885	FIU 0520
JWH 122 N-(5-hydroxypentyl) metabolite	C25H25NO2	371.1885	FIU 0521
JWH 133	C22H32O	312.2453	FIU 0522
JWH 145 2-phenyl isomer	C26H25NO	367.1936	FIU 0523
JWH 146	C28H29NO	395.2249	FIU 0524
JWH 149	C26H27NO	369.2093	FIU 0525
JWH 167	C21H23NO	305.1780	FIU 0526
JWH 176	C25H24	324.1878	FIU 0527
JWH 193	C26H26N2O2	398.1994	FIU 0528
JWH 198	C26H26N2O3	414.1943	FIU 0529
JWH 200 4-hydroxyindole metabolite	C25H24N2O3	400.1787	FIU 0530
JWH 200 5-hydroxyindole metabolite	C25H24N2O3	400.1787	FIU 0531
JWH 200 6-hydroxyindole metabolite	C25H24N2O3	400.1787	FIU 0532
JWH 200 7-hydroxyindole metabolite	C25H24N2O3	400.1787	FIU 0533
JWH 203	C21H22ClNO	339.1390	FIU 0534
JWH 203 N-(4-hydroxypentyl) metabolite	C21H22ClNO2	355.1339	FIU 0535
JWH 203 N-(5-hydroxypentyl) metabolite	C21H22ClNO2	355.1339	FIU 0536
JWH 203 N-pentanoic acid metabolite	C21H20ClNO3	369.1132	FIU 0537
JWH 210 2-ethylnaphthyl isomer	C26H27NO	369.2093	FIU 0538
JWH 210 5-hydroxyindole metabolite	C26H27NO2	385.2042	FIU 0539
JWH 210 N-(4-hydroxypentyl) metabolite	C26H27NO2	385.2042	FIU 0540
JWH 210 N-(5-hydroxypentyl) metabolite	C26H27NO2	385.2042	FIU 0541
JWH 210 N-pentanoic acid metabolite	C26H25NO3	399.1834	FIU 0542
JWH 213	C27H29NO	383.2249	FIU 0543
JWH 250 5-hydroxyindole metabolite	C22H25NO3	351.1834	FIU 0544

JWH 250 N-(4-hydroxypentyl) metabolite	C22H25NO3	351.1834	FIU 0545
JWH 250 N-(5-hydroxypentyl) metabolite	C22H25NO3	351.1834	FIU 0546
JWH 250 N-pentanoic acid metabolite	C22H23NO4	365.1627	FIU 0547
JWH 307 5'-isomer	C25H24FNO	373.1842	FIU 0548
JWH 309 5'-isomer	C30H27NO	417.2093	FIU 0549
JWH 387	C24H22BrNO	419.0885	FIU 0550
JWH 398 N-(4-hydroxypentyl) metabolite	C24H22ClNO2	391.1339	FIU 0551
JWH 398 N-(5-hydroxypentyl) metabolite	C24H22ClNO2	391.1339	FIU 0552
JWH 398 N-pentanoic acid metabolite	C24H20ClNO3	405.1132	FIU 0553
JWH 412	C24H22FNO	359.1685	FIU 0554
(−)-CP 47,497	C21H34O2	318.2559	FIU 0555
(−)-CP 55,940	C24H40O3	376.2977	FIU 0556
(+)-CP 47,497	C21H34O2	318.2559	FIU 0557
(+)-CP 55,940	C24H40O3	376.2977	FIU 0558
(±)3-epi CP 47,497-C8-homolog	C22H36O2	332.2715	FIU 0559
(±)5-epi CP 55,940	C24H40O3	376.2977	FIU 0560
(±)-CP 47,497	C21H34O2	318.2559	FIU 0561
(±)-CP 47,497-C7-hydroxy metabolite	C21H34O3	334.2508	FIU 0562
(±)-CP 47,497-C8-homolog	C22H36O2	332.2715	FIU 0563
(±)-CP 55,940	C24H40O3	376.2977	FIU 0564
(±)-epi CP 47,497	C21H34O2	318.2559	FIU 0565
CP 47,497-C6-homolog	C20H32O2	304.2402	FIU 0566
CP 47,497-C8-homolog C-8-hydroxy metabolite	C22H36O3	348.2664	FIU 0567
CP 47,497-C9-homolog	C23H38O2	346.2872	FIU 0568
CP 47,497-para-quinone analog	C21H32O3	332.2351	FIU 0569
RCS-4 4-hydroxyphenyl metabolite	C20H21NO2	307.1572	FIU 0570
RCS-4 M10 metabolite	C20H21NO3	323.1521	FIU 0571
RCS-4 M11 metabolite	C20H19NO3	321.1365	FIU 0572
RCS-4 M9 metabolite	C20H21NO3	323.1521	FIU 0573
RCS-4 N-(4-hydroxypentyl) metabolite	C21H23NO3	337.1678	FIU 0574

RCS-4 N-(5-carboxypentyl) metabolite	C21H21NO4	351.1471	FIU 0575
RCS-4 N-(5-hydroxypentyl) metabolite	C21H23NO3	337.1678	FIU 0576
5-fluoro NPB-22	C22H20FN3O2	377.1540	FIU 0577
5-fluoro PB-22	C23H21FN2O2	376.1587	FIU 0578
5-fluoro PB-22 3-carboxyindole metabolite	C14H16FNO2	249.1165	FIU 0579
5-fluoro PB-22 3-hydroxyquinoline isomer	C21H21FN2O2	352.1587	FIU 0580
5-fluoro PB-22 4-hydroxyisoquinoline isomer	C23H21FN2O2	376.1587	FIU 0581
5-fluoro PB-22 4-hydroxyquinoline isomer	C23H21FN2O2	376.1587	FIU 0582
5-fluoro PB-22 5-hydroxyisoquinoline isomer	C23H21FN2O2	376.1587	FIU 0583
5-fluoro PB-22 5-hydroxyquinoline isomer	C23H21FN2O2	376.1587	FIU 0584
5-fluoro PB-22 6-hydroxyisoquinoline isomer	C23H21FN2O2	376.1587	FIU 0585
5-fluoro PB-22 6-hydroxyquinoline isomer	C23H21FN2O2	376.1587	FIU 0586
5-fluoro PB-22 7-hydroxyisoquinoline isomer	C23H21FN2O2	376.1587	FIU 0587
5-fluoro PB-22 7-hydroxyquinoline isomer	C23H21FN2O2	376.1587	FIU 0588
5-fluoro PB-22 8-hydroxyisoquinoline isomer	C23H21FN2O2	376.1587	FIU 0589
5-fluoro PB-22 N-(2-fluoropentyl) isomer	C23H21FN2O2	376.1587	FIU 0590
5-fluoro PB-22 N-(3-fluoropentyl) isomer	C23H21FN2O2	376.1587	FIU 0591
5-fluoro PB-22 N-(4-fluoropentyl) isomer	C23H21FN2O2	376.1587	FIU 0592
FDU-PB-22	C26H18FNO2	395.1322	FIU 0593
FUB-PB-22	C25H17FN2O2	396.1274	FIU 0594
NPB-22	C22H21N3O2	359.1634	FIU 0595
PB-22	C23H22N2O2	358.1681	FIU 0596
PB-22 3-carboxyindole metabolite	C14H17NO2	231.1259	FIU 0597
PB-22 3-hydroxyquinoline isomer	C23H22N2O2	358.1681	FIU 0598
PB-22 4-hydroxyisoquinoline isomer	C23H22N2O2	358.1681	FIU 0599

PB-22 4-hydroxyquinoline isomer	C23H22N2O2	358.1681	FIU 0600
PB-22 5-hydroxyisoquinoline isomer	C23H22N2O2	358.1681	FIU 0601
PB-22 5-hydroxyquinoline isomer	C23H22N2O2	358.1681	FIU 0602
PB-22 6-hydroxyisoquinoline isomer	C23H22N2O2	358.1681	FIU 0603
PB-22 6-hydroxyquinoline isomer	C23H22N2O2	358.1681	FIU 0604
PB-22 7-hydroxyisoquinoline isomer	C23H22N2O2	358.1681	FIU 0605
PB-22 7-hydroxyquinoline isomer	C23H22N2O2	358.1681	FIU 0606
PB-22 8-hydroxyisoquinoline isomer	C23H22N2O2	358.1681	FIU 0607
PB-22 N-(4-hydroxypentyl) metabolite	C23H22N2O3	374.1630	FIU 0608
PB-22 N-(4-hydroxypentyl)-3-carboxyindole metabolite	C14H17NO3	347.1208	FIU 0609
PB-22 N-(5-hydroxypentyl) metabolite	C23H22N2O3	374.1630	FIU 0610
PB-22 N-(5-hydroxypentyl)-3-carboxyindole metabolite	C14H17NO3	247.1208	FIU 0611
PB-22 N-pentanoic acid metabolite	C23H20N2O4	388.1423	FIU 0612
PB-22 N-pentanoic acid-3-carboxyindole metabolite	C14H15NO4	261.1001	FIU 0613
AM2201 8-quinolinyl carboxamide	C23H22FN3O	375.1747	FIU 0614
BB-22	C25H24N2O2	384.1838	FIU 0615
BB-22 3-carboxyindole metabolite	C16H19NO2	257.1416	FIU 0616
BB-22 3-hydroxyquinoline isomer	C25H24N2O2	384.1838	FIU 0617
BB-22 4-hydroxyisoquinoline isomer	C25H24N2O2	384.1838	FIU 0618
BB-22 4-hydroxyquinoline isomer	C25H24N2O2	384.1838	FIU 0619
BB-22 5-hydroxyisoquinoline isomer	C25H24N2O2	384.1838	FIU 0620
BB-22 5-hydroxyquinoline isomer	C25H24N2O2	384.1838	FIU 0621

BB-22 6-hydroxyisoquinoline isomer	C25H24N2O2	384.1838	FIU 0622
BB-22 6-hydroxyquinoline isomer	C25H24N2O2	384.1838	FIU 0623
BB-22 7-hydroxyisoquinoline isomer	C25H24N2O2	384.1838	FIU 0624
BB-22 7-hydroxyquinoline isomer	C25H24N2O2	384.1838	FIU 0625
BB-22 8-hydroxyisoquinoline isomer	C25H24N2O2	384.1838	FIU 0626
AM1220 azepane isomer	C26H26N2O	382.2045	FIU 0627
AM2201 2-hydroxyindole metabolite	C24H22FNO2	375.1635	FIU 0628
AM2201 5-hydroxyindole metabolite	C24H22FNO2	375.1635	FIU 0629
AM2201 6-hydroxyindole metabolite	C24H22FNO2	375.1635	FIU 0630
AM2201 7-hydroxyindole metabolite	C24H22FNO2	375.1635	FIU 0631
AM2201 N-(4-hydroxypentyl) metabolite	C24H22FNO2	375.1635	FIU 0632
AM2233 azepane isomer	C22H23IN2O	458.0855	FIU 0633
EAM2201	C26H26FNO	387.1998	FIU 0634
NM2201	C24H22FNO2	375.1635	FIU 0635
AM2201 N-(3-chloropentyl) isomer	C24H22ClNO	375.1390	FIU 0636
MAM2201 N-(2-fluoropentyl) isomer	C25H24FNO	373.1842	FIU 0637
MAM2201 N-(3-fluoropentyl) isomer	C25H24FNO	373.1842	FIU 0638
MAM2201 N-(4-fluoropentyl) isomer	C25H24FNO	373.1842	FIU 0639
MAM2201 N-(4-hydroxypentyl) metabolite	C25H24FNO2	389.1791	FIU 0640
MAM2201 N-(5-chloropentyl) analog	C25H24ClNO	389.1546	FIU 0641
MAM2201 N-(5-chloropentyl) analog-d5	C25H19D5ClNO	394.1860	FIU 0642
MAM2201 N-pentanoic acid metabolite	C25H23NO3	385.1678	FIU 0643
(±)-UR-144 N-(4-hydroxypentyl) metabolite	C21H29NO2	327.2198	FIU 0644
UR-144 Degradant	C21H29NO	311.2249	FIU 0645

UR-144 Degradant N-pentanoic acid metabolite	C21H27NO3	341.1991	FIU 0646
UR-144 N-(2-chloropentyl) analog	C21H28ClNO	345.1859	FIU 0647
UR-144 N-(2-hydroxypentyl) metabolite	C21H29NO2	327.2198	FIU 0648
UR-144 N-(3-chloropentyl) analog	C21H28ClNO	345.1859	FIU 0649
UR-144 N-(4-chloropentyl) analog	C21H28ClNO	345.1859	FIU 0650
UR-144 N-(5-bromopentyl) analog	C21H28BrNO	389.1354	FIU 0651
UR-144 N-(5-chloropentyl) analog	C21H28ClNO	345.1859	FIU 0652
UR-144 N-(5-hydroxypentyl) metabolite	C21H29NO2	327.2198	FIU 0653
UR-144 N-(5-hydroxypentyl) β-D-Glucuronide	C27H37NO8	503.2519	FIU 0654
UR-144 N-pentanoic acid metabolite	C21H27NO3	341.1991	FIU 0655
UR-144 N-(5-methylhexyl) analog	C23H33NO	339.2562	FIU 0656
UR-144 N-heptyl analog	C23H33NO	339.2562	FIU 0657
FUB-144	C23H24FNO	349.1842	FIU 0658
XLR11 6-hydroxyindole metabolite	C21H28FNO2	345.2104	FIU 0659
XLR11 Degradant	C21H28FNO	329.2155	FIU 0660
XLR11 N-(2-fluoropentyl) isomer	C21H28FNO	329.2155	FIU 0661
XLR11 N-(3-fluoropentyl) isomer	C21H28FNO	329.2155	FIU 0662
XLR11 N-(4-fluoropentyl) isomer	C21H28FNO	329.2155	FIU 0663
XLR11 N-(4-hydroxypentyl) metabolite	C21H28FNO2	345.2104	FIU 0664
XLR11 N-(4-pentenyl) analog	C21H27NO	309.2093	FIU 0665
XLR12	C20H24F3NO	351.1810	FIU 0666
Acetyl fentanyl	C21H26N2O	322.2045	FIU 0667
Acetyl norfentanyl	C13H18N2O	218.1419	FIU 0668
Butyryl fentanyl	C23H30N2O	350.2358	FIU 0669
para-Fluorofentanyl	C22H27FN2O	354.2107	FIU 0670
AH 7921	C16H22Cl2N2O	328.1109	FIU 0671
ATM4 4-acetoxy analog	C23H25NO5	395.1733	FIU 0672

3-hydroxy Phenazepam	C15H10BrClN2O2	363.9614	FIU 0673
Bromazepam	C14H10BrN3O	315.0007	FIU 0674
Delorazepam	C15H10Cl2N2O	304.0170	FIU 0675
Diclazepam	C16H12Cl2N2O	318.0327	FIU 0676
Etizolam	C17H15ClN4S	342.0706	FIU 0677
Flubromazepam	C15H10BrFN2O	331.9961	FIU 0678
MCOPPB	C26H40N4	408.3253	FIU 0679
Pyrazolam	C16H12BrN5	353.0276	FIU 0680
1'-naphthoyl-2-methylindole	C20H15NO	285.1154	FIU 0681
2-Fluoropentylindole	C13H16FN	205.1267	FIU 0682
3-Fluoropentylindole	C13H16FN	205.1267	FIU 0683
4-Fluoropentylindole	C13H16FN	205.1267	FIU 0684
5-fluoropentyl-3-pyridinoylindole	C19H19FN2O	310.1481	FIU 0685
5-Fluoropentylindole	C13H16FN	205.1267	FIU 0686
5-IT	C11H14N2	174.1157	FIU 0687
6-IT	C11H14N2	174.1157	FIU 0688
A-834735	C22H29NO2	339.2198	FIU 0689
A-834735 degredant	C22H29NO2	339.2198	FIU 0690
methyl-1-(5-fluoropentyl)-1H-indole-3-Carboxylate	C15H18FNO2	263.1322	FIU 0691
methyl-1-(cyclohexylmethyl)-1H-indole-3-carboxylate	C17H21NO2	271.1572	FIU 0692
methyl-1-pentyl-1H-indole-3-Carboxylate	C15H19NO2	245.1416	FIU 0693
ADBICA	C20H29N3O2	343.2260	FIU 0694
ADBICA N-(4-hydroxypentyl) metabolite	C20H29N3O3	359.2209	FIU 0695
ADBICA N-(5-hydroxypentyl) metabolite	C20H29N3O3	359.2209	FIU 0696
ADBICA N-pentanoic acid metabolite	C20H27N3O4	373.2002	FIU 0697
AM694 N-(5-hydroxypentyl) metabolite	C20H20INO2	433.0539	FIU 0698
AM694 N-pentanoic acid metabolite	C20H18INO3	447.0331	FIU 0699
Harmine	C13H12N2O	212.0950	FIU 0700
tetrahydro-Harmine	C13H16N2O	216.1263	FIU 0701
5-chloro AB-PINACA	C18H25ClN4O2	364.1666	FIU 0702
5-fluoro ABICA	C19H26FN3O2	347.2009	FIU 0703
5-fluoro AB-PINACA	C18H25FN4O2	348.1962	FIU 0704

5-fluoro AB-PINACA N-(4-hydroxypentyl) metabolite	C18H25FN4O3	364.1911	FIU 0705
5-fluoro ADBICA	C20H28FN3O2	361.2166	FIU 0706
5-fluoro ADB-PINACA	C19H27FN4O2	362.2118	FIU 0707
5-fluoro AMB	C19H26FN3O3	363.1958	FIU 0708
5-fluoro MN-18	C23H22FN3O	375.1747	FIU 0709
5-fluoro-AKB48 N-(4-hydroxypentyl) metabolite	C23H30FN3O2	399.2322	FIU 0710
5-fluoro-THJ	C22H21FN4O	376.1699	FIU 0711
AB-005	C23H32N2O	352.2515	FIU 0712
AB-005 azepane isomer	C23H32N2O	352.2515	FIU 0713
AB-CHMINACA	C20H28N4O2	356.2212	FIU 0714
AB-FUBINACA	C20H21FN4O2	368.1649	FIU 0715
AB-FUBINACA 2-fluorobenzyl isomer	C20H21FN4O2	368.1649	FIU 0716
AB-FUBINACA 3-fluorobenzyl isomer	C20H21FN4O2	368.1649	FIU 0717
AB-FUBINACA isomer 1	C20H21FN4O2	368.1649	FIU 0718
AB-FUBINACA isomer 2	C20H21FN4O2	368.1649	FIU 0719
AB-FUBINACA isomer 5	C20H21FN4O2	368.1649	FIU 0720
AB-PINACA	C18H26N4O2	330.2056	FIU 0721
AB-PINACA N-(2-fluoropentyl) isomer	C18H25FN4O2	348.1963	FIU 0722
AB-PINACA N-(4-fluoropentyl) isomer	C18H25FN4O2	348.1963	FIU 0723
AB-PINACA N-(4-hydroxypentyl) metabolite	C18H26N4O3	346.2005	FIU 0724
AB-PINACA N-(5-hydroxypentyl) metabolite	C18H26N4O3	346.2005	FIU 0725
AB-PINACA pentanoic acid metabolite	C18H24N4O4	360.1798	FIU 0726
AKB48 N-(5-fluoropentyl) analog	C23H30FN3O	383.2373	FIU 0727
MN-18	C23H23N3O	357.1841	FIU 0728
THJ	C22H22N4O	358.1794	FIU 0729
THJ 018	C23H22N2O	342.1732	FIU 0730
THJ 2201	C23H21FN2O	360.1638	FIU 0731
ADB-FUBINACA	C21H23FN4O2	382.1805	FIU 0732
ADB-PINACA	C19H28N4O2	344.2212	FIU 0733
ADB-PINACA isomer 1	C19H28N4O2	344.2212	FIU 0734
ADB-PINACA isomer 2	C19H28N4O2	344.2212	FIU 0735
ADB-PINACA isomer 3	C19H28N4O2	344.2212	FIU 0736

ADB-PINACA N-(4-hydroxypentyl) metabolite	C19H28N4O3	360.2161	FIU 0737
ADB-PINACA N-(5-hydroxypentyl) metabolite	C19H28N4O3	360.2161	FIU 0738
ADB-PINACA pentanoic acid metabolite	C19H26N4O4	374.1954	FIU 0739
AKB48 N-(4-fluorobenzyl) analog	C25H26FN3O	403.2060	FIU 0740
AKB48 N-(4-hydroxypentyl) metabolite	C23H31N3O2	381.2416	FIU 0741
AKB48 N-(5-hydroxypentyl) metabolite	C23H31N3O2	381.2416	FIU 0742
AKB48 N-pentanoic acid metabolite	C23H29N3O3	395.2209	FIU 0743
AMB	C19H27N3O3	345.2052	FIU 0744
4-acetoxy DiPT	C18H26N2O2	302.1994	FIU 0745
4-acetoxy DMT	C14H18N2O2	246.1368	FIU 0746
4-hydroxy DET	C14H20N2O	232.1576	FIU 0747
4-hydroxy DiPT	C16H24N2O	260.1889	FIU 0748
4-hydroxy MET	C13H18N2O	218.1419	FIU 0749
4-hydroxy MiPT	C14H20N2O	232.1576	FIU 0750
4-methyl-α-Ethyltryptamine	C13H18N2	202.1470	FIU 0751
5-methoxy-α-Ethyltryptamine	C13H18N2O	218.1419	FIU 0752
DiPT	C16H24N2	244.1939	FIU 0753
DOET	C13H21NO2	223.1572	FIU 0754
DPT	C16H24N2	244.1939	FIU 0755
N-Methyltryptamine	C11H14N2	174.1157	FIU 0756
AMT	C5H10N2S	130.0565	FIU 0757
Methylphenidate	C14H19NO2	233.1416	FIU 0758
MMAI	C11H15NO	177.1154	FIU 0759
Etaqualone	C17H16N2O	264.1263	FIU 0760
Hydroxy Bupropion	C13H18ClNO2	255.1026	FIU 0761
Lagochiline	C20H36O5	356.2563	FIU 0762
Levamisole	C11H12N2S	204.0721	FIU 0763
Loperamide	C29H33ClN2O2	476.2231	FIU 0764
N-Phenylacetyl-L-prolylglycine ethyl ester	C17H22N2O4	318.1580	FIU 0765
Phenylpiracetam	C12H14N2O2	218.1055	FIU 0766
PRE-084	C19H27NO3	317.1991	FIU 0767
Sildenafil	C22H30N6O4S	474.2049	FIU 0768
Sildenafil Citrate	C22H30N6O4S	474.2049	FIU 0769
Thiosildenafil	C22H30N6O3S2	490.1821	FIU 0770

Acetildenafil	C25H34N6O3	466.2692	FIU 0771
Benzydamine	C19H23N3O	309.1841	FIU 0772
Boldenone Cypionate	C27H38O3	410.2821	FIU 0773
Caffeine	C8H10N4O2	194.0804	FIU 0774
Carisoprodol	C12H24N2O4	260.1736	FIU 0775
Cl-2201	C24H21ClFNO	393.1296	FIU 0776
Clencyclohexerol	C14H20Cl2N2O2	318.0902	FIU 0777

Appendix 2. Compounds included in the internal standard database with their unique in-house identifying number.

Compound Name	Formula	Accurate Mass	FIU Number
JWH 007-d_9	C22H16D9NO	328.2501	FIU 0778
JWH 015-d_7	C23H14D7NO	334.2063	FIU 0779
JWH 018-d_9	C24H14D9NO	350.2345	FIU 0780
JWH 018 2-hydroxyindole metabolite-d_9	C24H14D9NO2	366.2294	FIU 0781
JWH 018 4-hydroxyindole metabolite-d_9	C24H14D9NO2	366.2294	FIU 0782
JWH 018 5-hydroxyindole metabolite-d_9	C24H14D9NO2	366.2294	FIU 0783
JWH 018 6-hydroxyindole metabolite-d_9	C24H14D9NO2	366.2294	FIU 0784
JWH 018 7-hydroxyindole metabolite-d_9	C24H14D9NO2	366.2294	FIU 0785
(±)-JWH 018 N-(4-hydroxypentyl) metabolite-d_5	C24H18D5NO2	362.2043	FIU 0786
JWH 018 N-(5-hydroxypentyl) metabolite-d_5	C24H18D5NO2	362.2043	FIU 0787
JWH 018 N-(5-hydroxypentyl) β-D-Glucuronide-d_5	C30H26D5NO8	538.2364	FIU 0788
JWH 018 N-pentanoic acid metabolite-d_5	C24H16D5NO3	376.1865	FIU 0789
JWH 018 N-pentanoic acid metabolite-d_4	C24H17D4NO3	375.1773	FIU 0790
JWH 019 N-(5-hydroxyhexyl) metabolite-d_5	C25H20D5NO2	376.2199	FIU 0791
JWH 073-d_7	C23H14D7NO	334.2063	FIU 0792
(±)-JWH 073 N-(3-hydroxybutyl)metabolite-d_5	C23H16D5NO2	348.1886	FIU 0793
JWH 073 N-(4-hydroxybutyl) metabolite-d_5	C23H16D5NO2	348.1886	FIU 0794
JWH 073 2-hydroxyindole metabolite-d_7	C23H14D7NO2	350.2012	FIU 0795
JWH 073 4-hydroxyindole metabolite-d_7	C23H14D7NO2	350.2012	FIU 0796

JWH 073 5-hydroxyindole metabolite-d_7	C23H14D7NO2	350.2012	FIU 0797
JWH 073 6-hydroxyindole metabolite-d_7	C23H14D7NO2	350.2012	FIU 0798
JWH 073 7-hydroxyindole metabolite-d_7	C23H14D7NO2	350.2012	FIU 0799
JWH 073 N-butanoic acid metabolite-d_5	C23H14D5NO3	362.1679	FIU 0800
JWH 081-d_9	C25H16D9NO2	380.2450	FIU 0801
JWH 081 N-(4-hydroxypentyl) metabolite-d_5	C25H20D5NO3	392.2148	FIU 0802
JWH 081 N-(5-hydroxypentyl) metabolite-d_5	C25H20D5NO3	392.2148	FIU 0803
JWH 081 N-pentanoic acid metabolite-d_5	C25H18D5NO4	406.1941	FIU 0804
JWH 122-d_9	C25H16D9NO	364.2501	FIU 0805
JWH 122 N-(5-hydroxypentyl) metabolite-d_5	C25H20D5NO2	376.2199	FIU 0806
JWH 200-d_5	C25H19D5N2O2	389.2152	FIU 0807
JWH 203 N-(4-hydroxypentyl) metabolite-d_5	C21H17D5ClNO2	360.1653	FIU 0808
JWH 203 N-(5-hydroxypentyl) metabolite-d_5	C21H17D5ClNO2	360.1653	FIU 0809
JWH 203 N-pentanoic acid metabolite-d_5	C21H15D5ClNO3	360.1653	FIU 0810
JWH 210-d_9	C26H10D9NO	370.2032	FIU 0811
JWH 250-d_5	C22H20D5NO2	340.2199	FIU 0812
JWH 250 N-(4-hydroxypentyl) metabolite-d_5	C22H20D5NO3	356.2148	FIU 0813
JWH 250 N-(5-hydroxypentyl) metabolite-d_5	C22H20D5NO3	356.2148	FIU 0814
JWH 398-d_9	C24H13D9ClNO	384.1955	FIU 0815
(±)-JWH 398 N-(4-hydroxypentyl) metabolite-d_5	C24H17ClD5NO2	396.1653	FIU 0816

JWH 398 N-(5-hydroxypentyl) metabolite d_5	C24H17ClD5NO2	396.1653	FIU 0817
JWH 398 N-pentanoic acid metabolite-d_5	C24H15ClD5NO3	410.1446	FIU 0818
(-)-11-nor-9-carboxy-Δ9-THC-d_3	C21H25D3O4	347.2176	FIU 0819
(±)-CP 47,497-d_{11}	C21H23D11O2	329.3249	FIU 0820
(±)-CP 47,497-C8-homolog-d_7	C22H29D7O2	339.3155	FIU 0821
(±)-CP 55,940-d_{11}	C24H29D11O3	387.3668	FIU 0822
AM694 N-pentanoic acid metabolite-d_5	C20H13D5INO3	452.0645	FIU 0823
AM2201-d_5	C24H17D5FNO	364.1999	FIU 0824
AM2201 N-(4-hydroxypentyl) metabolite-d_5	C24H17D5FNO2	380.1948	FIU 0825
MAM2201-d_5	C25H19D5FNO	378.2156	FIU 0826
MAM2201 N-pentanoic acid metabolite-d_5	C25H18D5NO3	390.1992	FIU 0827
PB-22-d_9	C23H13D9N2O2	367.2246	FIU 0828
UR-144-d_5	C21H24D5NO	316.2563	FIU 0829
UR-144 N-(4-hydroxypentyl) metabolite-d_5	C21H24D5NO2	332.2512	FIU 0830
UR-144 N-(5-hydroxypentyl) metabolite-d_5	C21H24D5NO2	332.2512	FIU 0831
UR-144 N-pentanoic acid metabolite-d_5	C21H22D5NO3	346.2305	FIU 0832
XLR11-d_5	C21H23D5FNO	334.2469	FIU 0833
XLR11 N-(4-hydroxypentyl) metabolite-d_5	C21H23D5FNO2	350.2418	FIU 0834
RCS-4-d_9	C21H14D9NO2	330.2294	FIU 0835
RCS-4 N-(4-hydroxypentyl) metabolite-d_5	C21H18D5NO3	342.1992	FIU 0836
RCS-4 N-(5-hydroxypentyl) metabolite-d_5	C21H18D5NO3	342.1992	FIU 0837
RCS-4 N-pentanoic acid metabolite-d_5	C21H16D5NO4	356.1784	FIU 0838

25I-NBOMe-d_3	C18H19D3INO3	430.0833	FIU 0839
Benocyclidine-d_{10}	C19H15D10NS	309.2335	FIU 0840
3,4-Methylenedioxy Pyrovalerone-d_8	C16H13D8NO3	283.2024	FIU 0841
AB-PINACA-d_9	C18H17D9N4O2	339.2621	FIU 0842
ADB-PINACA-d_9	C19H19D9N4O2	353.2777	FIU 0843
ADBICA-d_9	C20H20D9N3O2	352.2825	FIU 0844
AB-FUBINACA-d_4	C20H17D4FN4O2	372.1900	FIU 0845
AKB48-d_9	C23H22D9N3O	374.3032	FIU 0846
Acetyl fentanyl-d_5	C21H22D5ClN2O	363.2126	FIU 0847
Acetyl norfentanyl-d_5	C13H13D5N2O	223.1733	FIU 0848
Norsufentanil-d_3	C16H21D3N2O2	279.2026	FIU 0849
Butylone-d_3	C12H12D3NO3	224.1240	FIU 0850
cis-Tramadol-d_6	C16H19D6NO2	269.2262	FIU 0851
Meconin-d_3	C10H7D3O4	197.0767	FIU 0852

Appendix 3. The novel psychoactive substances added to the HRMS library along with ions collected at different collision energies that had relative abundances greater than 10% in the MS/MS spectra.

Compound Name	CE (eV)	Ion (Relative Abundance %)
(±)-Ethylphenidate	10	248.16451 (100); 84.08077 (56)
	20	84.08077 (100)
	40	84.08077 (100); 56.04948 (17)
2-Bromoamphetamine	10	168.96474 (100); 196.99603 (57); 214.02258 (21)
	20	168.96474 (100)
	40	90.04640 (100); 168.96474 (87); 89.03857 (43); 117.06988 (24); 91.05423 (11)
2-Chloroamphetamine	10	125.01525 (100); 153.04655 (33); 170.07310 (11)
	20	125.01525 (100)
	40	170.01525 (100); 89.03857 (75); 98.99960 (40); 90.04640 (30); 63.02293 (10)
2-Methoxy ketamine	10	203.10666 (100); 175.11174 (79); 234.14885 (33)
	20	121.06479 (100); 175.11174 (93); 203.10666 (54); 67.05423 (14)
	40	91.05423 (100); 121.06479 (43); 67.05423 (12)
3,4-Ethylenedioxymethcathinone	10	204.10190 (100); 222.11247 (46)
	20	204.10190 (100); 189.07843 (83); 148.07568 (34); 163.07536 (16); 58.06512 (16); 133.05222 (12)
	40	133.05222 (100); 91.05423 (47); 148.07568 (40); 120.08077 (20); 105.05730 (20); 58.06512 (18); 189.07843 (14); 77.03857 (13); 65.03857 (10)
3-Bromoamphetamine	10	196.99603 (100); 168.96474 (96); 214.02258 (13); 118.07771 (12)
	20	168.96474 (100); 118.07771 (23); 117.06988 (13)
	40	117.06988 (100); 90.04640 (81); 168.96474 (75); 89.03857 (28); 91.05423 (21); 115.05423 (14)
3-Chloroamphetamine	10	125.01525 (100); 153.04655 (54)
	20	125.01525 (100)

	40	125.01525 (100); 89.03857 (65); 98.99960 (34); 90.04640 (24)
3-Iodoamphetamine	10	244.98216 (100); 216.95087 (97); 262.00873 (43)
	20	216.95087 (100); 118.07771 (13)
	40	90.04640 (100); 117.06988 (74); 216.95087 (51); 89.03857 (23)
3-Methoxyamphetamine	10	121.06398 (100); 149.09555 (51)
	20	121.06398 (100); 91.05381 (20)
	40	91.05383 (100); 78.04608 (73); 65.03838 (57); 77.03832 (47); 121.06432 (26)
4-Bromoamphetamine	10	196.99603 (100); 168.96474 (95); 214.02258 (13); 118.07771 (12)
	20	168.96474 (100); 118.07771 (23); 117.06988 (13)
	40	117.06988 (100); 90.04640 (81); 168.96474 (75); 89.03857 (28); 91.05423 (21); 115.05423 (14)
4-Chloroamphetamine	10	125.01525 (100); 153.04655 (54)
	20	125.01525 (100)
	40	125.01525 (100); 89.03857 (65); 98.99960 (34); 90.04640 (24)
4-Hydroxyamphetamine	10	107.04914 (100); 135.08044 (96)
	20	107.04914 (100)
	40	77.03857 (100); 107.04914 (42); 79.05423 (14); 51.02293 (12); 91.05423 (11)
4-Methylthioamphetamine	10	165.07324 (100); 117.06988 (32); 137.04195 (25)
	20	137.04195 (100); 117.06988 (99); 118.07771 (27); 115.05423 (24); 165.07324 (20); 150.04977 (20); 135.02629 (10)
	40	117.06988 (100); 91.05423 (73); 115.05423 (70); 122.01847 (48); 121.01065 (25); 135.02629 (16); 137.04195 (16); 78.04640 (16)
4-Methylthioamphetamine (hydrochloride)	10	165.07324 (100); 117.06988 (32); 137.04195 (24)
	20	117.06988 (100); 137.04195 (97); 118.07771 (26); 115.05423 (23);

		165.07324 (18); 150.04977 (17); 135.02629 (10)
	40	117.06988 (100); 91.05423 (74); 115.05423 (67); 122.01847 (48); 121.01065 (26); 135.02629 (18); 78.04640 (17); 137.04195 (16)
d-Amphetamine	10	91.05423 (100); 119.08553 (25)
	20	91.05423 (100)
	40	91.05423 (100); 65.03857 (87); 39.02293 (10)
Deschloro-N-ethyl-Ketamine	10	173.09608 (100); 218.15395 (62); 145.10118 (37); 46.06512 (27); 91.05423 (11)
	20	91.05423 (100); 145.10118 (91); 173.09608 (31); 46.06512 (19); 67.05423 (18); 129.06987 (14)
	40	91.05423 (100)
Diclofensine	10	322.07599 (100)
	20	322.07599 (100); 121.06479 (24); 279.03378 (21); 44.04948 (11); 291.03378 (10)
	40	44.04948 (100); 121.06479 (86); 91.05423 (33); 158.97629 (24); 209.09608 (23); 241.04147 (23); 221.09608 (19); 244.06494 (16); 165.03398 (15); 256.06494 (15); 213.04655 (13); 208.08827 (12); 77.03857 (11); 182.97629 (10)
DOI	10	305.00330 (100); 322.02985 (17); 276.97198 (10)
	20	276.97198 (100); 305.00330 (80); 178.09883 (72); 289.97983 (45); 163.07536 (20); 150.06754 (10)
	40	135.08044 (100); 274.95636 (55); 105.06988 (55); 163.07536 (40); 105.03349 (38); 246.96144 (37); 120.05697 (35); 91.05423 (31); 77.03857 (24); 90.04640 (21); 103.05423 (19); 148.05188 (18); 79.05423 (17); 289.97983 (14); 122.07262 (13); 276.97198 (12); 178.09883 (11)

HMA	10	165.09100 (100); 137.05971 (27); 133.06479 (22); 105.06988 (13)
	20	100.06988 (100); 137.05971 (95); 133.06479 (62); 165.09100 (18); 79.05423 (17)
	40	77.03857 (100); 79.05423 (52); 94.04131 (43); 103.05423 (32); 105.06988 (17); 65.03857 (17); 66.04640 (16); 122.03623 (16); 51.02293 (10)
Lisdexamfetamine	10	264.20703 (100); 247.18050 (38); 84.08077 (29); 129.10223 (22)
	20	84.08077 (100); 247.18050 (14); 119.08553 (13); 136.11208 (10)
	40	84.08077 (100); 91.05423 (37); 56.04948 (16)
Propylhexedrine	10	156.17468 (100); 69.06988 (43); 83.08553 (17)
	20	69.06988 (100); 55.05423 (46); 83.08553 (35); 41.03858 (21); 57.06988 (11)
	40	55.05423 (100); 41.03858 (90); 69.06988 (14); 39.02293 (14)
CMP	10	58.06512 (100); 79.05423 (44); 93.06988 (23); 121.10117 (12)
	20	79.05423 (100); 58.06512 (63); 77.03857 (30); 93.06988 (21);
	40	77.03857 (100); 79.05423 (19); 51.02293 (18); 58.06512 (16)
2,5-DMMA	10	179.10666 (100); 151.07536 (42); 210.14885 (37); 164.08318 (13)
	20	151.07536 (100); 164.08318 (61); 121.06479 (32); 179.10666 (22); 149.05971 (10); 123.08044 (10)
	40	91.05423 (100); 121.06479 (73); 149.05971 (59); 77.03857 (55); 78.04640 (23); 65.03857 (16); 107.04914 (15); 93.06988 (13); 103.05423 (10);
3,4-DHMA	10	151.07536 (100), 123.04406 (41), 182.11756 (16)
	20	123.04406(100), 105.06988(22), 151.07536(14), 133.06479(10)

	40	77.03858(100), 123.04406(43), 51.02293(28), 79.05423(28), 103.05423(16), 105.06988(13), 105.03349(10), 65.03858(10)
4-bromo-2,5-DMMA	10	257.01717(100), 288.05937(76), 228.98587(14)
	20	228.98587(100), 257.01717(68), 178.09883(63), 241.99369(31)
	40	135.08044(100), 226.97022(63), 198.9753(58), 163.07536(52), 105.06988(46), 105.03349(34), 91.05423(29), 120.05697(28), 168.96474(23), 77.03858(23), 178.09883(17), 122.07262(16), 90.0464(15), 228.98587(15), 92.06205(15), 241.99369(15), 103.05423(14), 148.05188(13), 79.05423(11)
para-Methoxymethamphetamine	10	149.09609(100), 121.06479(38)
	20	121.06479(100), 149.09609(15), 91.05423(11)
	40	91.05423(100), 121.06479(72), 77.03858(51), 78.0464(47), 65.03858(25)
N,N-DMA	10	91.05423(100), 164.14338(65), 119.08553(27)
	20	91.05423(100)
	40	91.05423(100), 65.03858(32)
2C-G	10	178.09883(100), 193.12231(43), 210.14886(17)
	20	178.09883(100), 163.07536(41)
	40	163.07536(100), 91.05423(76), 105.06988(40), 135.08044(30), 79.05423(28), 107.08553(23), 115.05423(23), 117.06988(20), 77.03858(14), 133.06479(10)
2C-T	10	211.07873(100)
	20	211.07873(100), 196.05525(60), 134.07262(33), 166.04469(28), 181.03178(23), 164.08318(22)

	40	91.05423(100), 119.04914(45), 121.06479(37), 151.02121(28), 181.03178(26), 134.07262(22), 123.0263(21), 77.03858(21), 147.04406(20), 105.06988(18), 103.05423(18), 44.97935(18), 79.05423(16), 104.06205(15), 136.05188(14), 78.0464(13), 135.03148(13), 137.05971(12), 165.03686(12), 133.06479(10), 149.05971(10)
2C-T-7	10	239.11003(100)
	20	239.11003(100), 197.06308(34), 224.08655(24), 164.08318(17), 167.05251(16), 182.0396(15)
	40	167.01613(100), 91.05423(68), 134.07262(54), 121.06479(36), 119.04914(32), 149.05971(28), 182.0396(27), 152.02904(20), 125.00556(15), 151.02121(12), 139.02121(11), 135.03148(11), 77.03858(11), 164.08318(10)
2C-TFM	10	233.07839(100), 250.10494(29), 218.05492(21)
	20	218.05492(100), 233.07839(54), 203.03144(13)
	40	203.03144(100), 127.03538(34), 113.03972(26), 133.0448(24), 218.05492(20), 115.05423(13), 91.05423(12), 147.04161(11), 151.03538(10)
bk-2C-B	10	178.06245(100), 177.07843(96), 274.00733(45), 256.98078(40), 228.98587(23), 162.05495(17)
	20	162.05495(100), 178.06245(93), 177.07843(85), 228.98587(37), 163.03897(31), 198.9753(24), 224.97838(14)
	40	162.05495(100), 163.03897(50), 134.06004(33), 119.03657(22), 77.03858(21), 105.03349(20), 91.05423(16), 181.95999(12), 209.9549(12), 90.0464(12), 147.03148(11)
3C-B-fly	10	

	20	281.01717(100), 202.09883(71), 252.98587(26)
	40	187.07536(100), 173.05971(54), 202.09883(36), 159.08044(32), 174.06753(28), 145.06479(26), 159.04406(18), 131.08553(14), 131.04914(13), 252.98587(12), 201.09101(10)
3C-P	10	195.10157(100), 237.14852(52)
	20	195.10157(100), 107.04914(55), 163.07536(47), 167.07027(45), 135.08044(23)
	40	107.04914(100), 91.05423(37), 77.03858(37), 79.05423(35), 103.05423(34), 105.06988(22), 115.05423(22), 167.07027(16), 123.04406(12), 147.04406(10)
N-methyl-2-AI	10	117.06988(100), 148.11208(42), 115.05423(11)
	20	117.06988(100), 115.05423(54), 91.05423(23)
	40	91.05423(100), 115.05423(97), 65.03858(29)
Escaline	10	209.11722(100), 181.08592(91)
	20	181.08592(100), 121.06479(34), 91.05423(24), 166.06245(22), 93.06988(20), 149.05971(16), 103.05423(13), 77.03858(11)
	40	91.05423(100), 77.03858(77), 103.05423(23), 65.03858(20), 133.02841(20), 78.0464(15), 123.04406(14), 121.06479(12), 105.03349(10)
Mescaline	10	195.10157(100), 180.0781(16)
	20	195.10157(100), 180.0781(90), 165.05462(85), 164.08318(31), 149.05971(21),133.02841(21), 150.06753(17), 133.06479(13), 121.06479(12), 137.05971(11), 105.06988(10)
	40	
2-Amino-1-phenylbutane	10	91.05423(100), 133.10118(10)
	20	91.05423(100)
	40	91.05423(100), 65.03858(81)

2-Ethylamino-1-phenylbutane	10	91.05423(100), 178.15903(39), 133.10118(10)
	20	91.05423(100)
	40	91.05423(100), 65.03858(34)
4-CAB	10	125.01525(100), 167.0622(12)
	20	125.01525(100)
	40	125.01525(100), 89.03858(57), 98.9996(32), 90.0464(22)
Cathine	10	134.09643(100), 117.06988(46), 115.05423(16)
	20	115.05423(100), 117.06988(85), 91.05423(53), 134.09643(37), 56.04948(14)
	40	91.05423(100), 115.05423(66), 65.03858(37), 118.06513(16), 77.03858(11)
(R)-(−)-MT-45	10	349.26383(100), 181.10118(11)
	20	181.10118(100), 169.16993(34), 349.26383(26)
	40	166.0777(100), 181.10118(79), 103.05423(67), 165.06988(43), 179.08553(22), 87.09167(19), 153.06988(11)
(S)-(+)-MT-45	10	349.26383(100), 181.10118(11)
	20	181.10118(100), 169.16993(34), 349.26383(25)
	40	166.0777(100), 181.10118(78), 103.05423(69), 165.06988(44), 179.08553(23), 87.09167(20), 153.06988(11)
2,3-Dichlorophenylpiperazine	10	231.04503(100), 188.00283(11)
	20	188.00283(100), 231.04503(81), 153.03398(42), 44.04948(15), 152.02615(15)
	40	152.02615(100), 117.0573(59), 153.03398(43), 118.06513(39), 44.04948(18), 91.05423(15), 188.00283(14)
MBZP	10	191.15428(100), 91.05423(43), 99.09167(15)
	20	91.05423(100), 99.09167(12), 191.15428(12)
	40	91.05423(100), 65.03858(27), 58.06513(10)

MT-45	10	349.26383(100), 181.10118(11)
	20	181.10118(100), 169.16993(35), 349.26383(25)
	40	166.0777(100), 181.10118(77), 103.05423(68), 165.06988(44), 179.08553(23), 87.09167(19), 153.06988(10)
Mepirapim	10	214.12264(100), 314.22269(11)
	20	214.12264(100)
	40	144.04439(100), 214.12264(35), 43.05423(19), 116.04948(12)
2-Fluoroisocathinone	10	123.06045(100), 151.05537(76), 103.05423(30), 168.08192(23)
	20	103.05423(100), 123.06045(77), 77.03858(25)
	40	77.03858(100), 103.05423(25), 51.02293(17)
3,4-Dimethylethcathinone	10	188.14338(100), 206.15394(59), 159.10425(16), 173.1199(14), 160.11208(12)
	20	188.14338(100), 159.10425(95), 173.1199(90), 158.09643(62), 160.11208(45), 133.10118(28), 145.0886(25), 144.08078(17), 105.06988(10)
	40	158.09643(100), 144.08078(47), 105.06988(22), 91.05423(21), 115.05423(18), 143.07295(18), 145.0886(11), 117.06988(11)
4-methoxy-N,N-Dimethylcathinone	10	208.13321(100), 72.08078(47), 163.07536(29), 135.08044(19)
	20	72.08078(100), 135.08044(76), 163.07536(28), 105.06988(14)
	40	72.08078(100), 77.03858(42), 79.05423(32), 105.06988(31), 103.05423(29), 91.05423(15), 135.08044(12)
4-Methylethcathinone metabolite ((±)-Ephedrine stereochemistry)	10	176.14338(100), 194.15394(16)
	20	176.14338(100), 131.08553(43), 91.05423(28), 147.10425(22), 161.1199(21), 146.09643(15), 105.06988(13), 148.11208(12)

	40	91.05423(100), 115.05423(45), 116.06205(36), 131.07295(26), 146.09643(20), 105.06988(19), 130.06513(19), 129.06988(18), 144.08078(16), 43.01784(12), 42.03383(10), 128.06205(10)
N-ethyl-N-Methylcathinone	10	192.13829(100), 133.06479(20), 105.06988(19), 86.09643(11)
	20	105.06988(100), 86.09643(62), 133.06479(29), 192.13829(26), 58.06513(12)
	40	77.03858(100), 105.06988(49), 58.06513(49), 86.09643(47), 79.05423(39), 103.05423(26), 105.03349(12), 44.04948(12), 130.06513(10)
Isopentedrone	10	91.05423(100), 192.13829(70), 161.09609(62), 174.12773(45), 132.08078(13), 119.04914(12)
	20	91.05423(100)
	40	91.05423(100), 65.03858(17)
Mephedrone metabolite ((±)-Ephedrine stereochemistry)	10	149.09609(100), 121.06479(38)
	20	121.06479(100), 149.09609(15), 91.05423(11)
	40	91.05423(100), 121.06479(72), 77.03858(51), 78.0464(47), 65.03858(25)
Mephedrone metabolite ((±)-Pseudoephedrine stereochemistry)	10	162.12773(100)
	20	162.12773(100), 147.10425(72), 91.05423(39), 131.08553(37), 105.06988(20), 116.06205(16), 70.06513(13), 146.09643(12), 129.06988(12), 56.04948(11), 132.08078(11)
	40	91.05423(100), 115.05423(67), 105.06988(63), 116.06205(34), 77.03858(28), 146.09643(25), 56.04948(22), 131.07295(21), 132.08078(20), 79.05423(19), 103.05423(15), 42.03383(14), 65.03858(14), 128.06205(13), 129.06988(12), 130.06513(12)

NRG-3	10	224.14338(100), 242.15394(84), 211.11174(30), 182.09643(26), 141.06988(16), 181.0886(12)
	20	182.09643(100), 181.0886(84), 141.06988(60), 224.14338(57), 167.07295(16), 211.11174(15), 180.08078(13), 194.09643(10)
	40	180.08078(100), 181.0886(80), 141.06988(76), 127.05423(43), 167.07295(42), 194.09643(40), 115.05423(20), 166.06513(13)
Pentedrone metabolite ((±)-Ephedrine stereochemistry)	10	176.14338(100), 194.15394(20)
	20	176.14338(100), 133.0886(61), 91.05423(38), 117.06988(18), 134.09643(17), 145.10118(14), 132.08078(14), 120.08078(12)
	40	91.05423(100), 132.08078(33), 77.03858(20), 56.04948(19), 115.05423(18), 79.05423(17), 104.06205(16), 118.06513(16), 43.05423(14), 133.0886(13), 103.05423(12), 42.03383(12), 65.03858(10)
Pentedrone metabolite ((±)-Pseudoephedrine stereochemistry)	10	176.14338(100)
	20	176.14338(100), 133.0886(68), 91.05423(35), 134.09643(19), 117.06988(17), 132.08078(16), 145.10118(14), 120.08078(12), 119.07295(12)
	40	91.05423(100), 132.08078(40), 56.04948(21), 104.06205(20), 118.06513(20), 117.0573(16), 133.0886(16), 115.05423(16), 42.03383(13), 103.05423(12), 130.06513(12), 119.07295(11)
Benzedrone	10	254.15394(100), 91.05423(69), 236.14338(20)
	20	91.05423(100)
	40	91.05423(100)
(−)-(S)-Cathinone	10	132.08078(100), 133.06479(20), 105.06988(20), 117.0573(18), 150.09134(12)
	20	117.0573(100), 105.06988(46), 132.08078(44)

	40	77.03858(100), 90.0464(81), 117.0573(80), 89.03858(53), 51.02293(27), 79.05423(22), 103.05423(14), 130.06513(11)
2,3-Dimethylethcathinone	10	188.14338(100), 206.15394(46), 159.10425(11), 173.1199(10), 160.11208(10)
	20	173.1199(100), 159.10425(99), 188.14338(71), 158.09643(53), 160.11208(46), 145.0886(23), 133.10118(21), 144.08078(14)
	40	158.09643(100), 144.08078(51), 143.07295(18), 105.06988(17), 91.05423(15), 115.05423(12), 145.0886(10)
2,4-Dimethylethcathinone	10	188.14338(100), 206.15394(37)
	20	173.1199(100), 159.10425(99), 188.14338(79), 158.09643(48), 160.11208(45), 145.0886(22), 133.10118(22), 72.08078(20), 144.08078(12)
	40	158.09643(100), 144.08078(45), 105.06988(17), 143.07295(16), 91.05423(16), 115.05423(13), 128.06205(10)
Diethylcathinone	10	188.14338(100), 206.15394(59), 159.10425(16), 173.1199(14), 160.11208(12)
	20	188.14338(100), 159.10425(95), 173.1199(90), 158.09643(62), 160.11208(45), 133.10118(28), 145.0886(25), 144.08078(17), 105.06988(10)
	40	158.09643(100), 144.08078(47), 105.06988(22), 91.05423(21), 115.05423(18), 143.07295(18), 145.0886(11), 117.06988(11)
2,3-Dimethylmethcathinone	10	161.09609(100), 133.06479(15)
	20	133.06479(100), 161.09609(50)
	40	133.06479(100), 105.06988(69), 77.03858(67), 79.05423(62), 103.05423(47), 91.05423(40), 117.06988(23), 115.05423(20), 128.06205(17), 146.07262(14),

		55.01784(11), 131.04914(11), 120.05697(10)
2,4-Dimethylmethcathinone	10	174.12773(100), 192.13829(26), 161.09609(21), 145.0886(14), 146.09643(13)
	20	145.0886(100), 105.06988(45), 174.12773(35), 146.09643(35), 144.08078(35), 159.10425(21)
	40	144.08078(100), 105.06988(18), 91.05423(18)
3,4-Dimethylmethcathinone metabolite ((±)-Ephedrine stereochemistry)	10	176.14338(100)
	20	176.14338(100), 161.1199(49), 145.10118(28), 130.0777(15), 105.06988(14), 56.04948(13), 70.06513(13)
	40	105.06988(100), 129.06988(84), 115.05423(78), 56.04948(66), 91.05423(57), 119.08553(57), 146.09643(47), 131.07295(46), 130.0777(44), 128.06205(41), 160.11208(35), 117.06988(29), 77.03858(25), 79.05423(25), 145.0886(24), 42.03383(23), 103.05423(17), 161.1199(14), 43.01784(14), 144.08078(12), 127.05423(12)
3,4-Dimethylmethcathinone metabolite ((±)-Pseudoephedrine stereochemistry)	10	176.14338(100)
	20	176.14338(100), 161.1199(51), 145.10118(25), 70.06513(15), 130.0777(14), 56.04948(13), 105.06988(12)
	40	
3-Bromomethcathinone	10	145.0886(100), 242.0175(74)
	20	145.0886(100), 144.08078(23)
	40	144.08078(100)
4-Bromomethcathinone	10	145.0886(100), 242.0175(74)
	20	145.0886(100), 144.08078(23)
	40	144.08078(100)
	10	166.10265(100)

4-Fluoromethcathinone metabolite ((±)-Ephedrine stereochemistry)	20	166.10265(100), 151.07918(70), 135.06045(55), 115.05423(29), 109.0448(19), 133.0448(12), 70.06513(11)
	40	109.0448(100), 133.0448(52), 115.05423(46), 150.07135(16), 122.05263(15), 83.02915(14), 43.01784(10)
4-Fluoromethcathinone metabolite ((±)-Pseudoephedrine stereochemistry)	10	166.10265(100)
	20	166.10265(100), 151.07918(70), 135.06045(55), 115.05423(29), 109.0448(19), 133.0448(12), 70.06513(11)
	40	109.0448(100), 133.0448(52), 115.05423(46), 150.07135(16), 122.05263(15), 83.02915(14), 43.01784(10)
(−)-3,4-Methylenedioxy Pyrovalerone	10	276.15942(100)
	20	276.15942(100), 175.07536(63), 205.08592(62), 126.12773(62), 135.04406(49), 149.02332(26)
	40	126.12773(100), 135.04406(82), 149.02332(70), 84.08078(34), 121.02841(31), 65.03858(15), 175.07536(14), 133.02841(10)
(+)-3,4-Methylenedioxy Pyrovalerone	10	276.15942(100)
	20	276.15942(100), 205.08592(61), 126.12773(60), 175.07536(60), 135.04406(49), 149.02332(25)
	40	126.12773(100), 135.04406(81), 149.02332(70), 84.08078(33), 121.02841(32), 65.03858(15), 175.07536(15), 133.02841(11)
2,3-MDA	10	163.07536(100), 135.04406(71), 133.06479(16), 105.06988(15), 180.10191(11)
	20	135.04406(100), 105.06988(69), 133.06479(20), 79.05423(12)
	40	77.03858(100), 79.05423(40), 105.06988(21), 51.02293(19), 103.05423(19)
2,3-MDMA	10	163.07536(100), 194.11756(53), 135.04406(52), 133.06479(11)

	20	135.04406(100), 105.06988(48), 133.06479(20), 163.07536(15)
	40	77.03858(100), 79.05423(54), 105.06988(39), 103.05423(24), 135.04406(24), 51.02293(14), 105.03349(11)
3,4-MDMA	10	163.07536(100), 194.11756(19), 135.04406(12), 133.06479(11)
	20	105.06988(100), 135.04406(91), 133.06479(77), 163.07536(58), 58.06513(12), 79.05423(10)
	40	77.03858(100), 79.05423(62), 105.06988(43), 103.05423(36), 135.04406(26), 51.02293(11)
3,4-Methylenedioxy-5-methylethcathinone	10	236.12812(100), 188.10699(69), 218.11756(68), 72.08078(18)
	20	188.10699(100), 189.07843(15), 218.11756(15), 160.11208(13), 72.08078(13)
	40	105.06988(100), 145.0886(95), 160.1094(77), 173.08352(72), 188.0706(68),130.06513(63), 144.08078(59), 132.08078(56), 115.05423(55), 79.05423(54), 133.06479(48),72.08078(41), 77.03858(39), 154.06513(39), 103.05423(39), 91.05423(37), 131.07295(35), 155.07295(22), 128.06205(22), 44.04948(21), 189.07843(17), 172.07569(14), 163.07536(11), 149.05971(11), 161.05971(10), 158.09375(10)
3,4-Methylenedioxy-N-benzylcathinone	10	284.12812(100), 91.05423(26), 266.11756(15)
	20	91.05423(100)
	40	91.05423(100)
Methylenedioxy Pyrovalerone	10	276.15942(100)
	20	276.15942(100), 126.12773(65), 175.07536(65), 205.08592(64), 135.04406(53), 149.02332(26)
	40	126.12773(100), 135.04406(81), 149.02332(69), 84.08078(32), 121.02841(32), 65.03858(14), 175.07536(14), 133.02841(10)

Methylenedioxy Pyrovalerone Metabolite 1	10	278.17507(100)
	20	175.07536(100), 278.17507(95), 126.12773(83), 72.08078(38), 207.10157(30), 137.05971(30), 151.03897(30)
	40	
Methylenedioxy Pyrovalerone metabolite 2	10	264.15942(100)
	20	264.15942(100), 72.08078(97), 126.12773(69), 123.04406(69), 193.08592(42), 137.02332(30), 175.07536(29)
	40	123.04406(100), 126.12773(93), 137.02332(67), 72.08078(41), 84.08078(39), 109.02841(36), 81.03349(17), 97.0886(10)
N-acetyl-3,4-Methylenedioxymethcathinone	10	208.09682(100), 250.10738(49), 190.08626(13), 160.07569(12)
	20	160.07569(100), 190.08626(61), 208.09682(48), 58.06513(21)
	40	132.08078(100), 160.07569(82), 58.06513(21), 117.0573(19), 91.05423(13)
N-hydroxy MDA	10	163.07536(100), 135.04406(16), 133.06479(14)
	20	105.06988(100), 135.04406(87), 133.06479(78), 163.07536(43), 79.05423(15)
	40	77.03858(100), 79.05423(72), 103.05423(41), 105.06988(32), 135.04406(22), 51.02293(17)
25C-NBOMe	10	336.1361(100), 121.06479(51)
	20	121.06479(100), 91.05423(11)
	40	91.05423(100), 121.06479(23), 93.06988(12)
25D-NBOMe	10	316.19072(100), 121.06479(26)
	20	121.06479(100), 91.05423(12)
	40	91.05423(100), 121.06479(19), 93.06988(11)
25E-NBOMe	10	330.20637(100), 121.06479(20)
	20	121.06479(100), 193.12231(12), 91.05423(10)
	40	91.05423(100), 121.06479(25), 93.06988(13)

25G-NBOMe	10	330.20637(100), 121.06479(20)
	20	121.06479(100), 193.12231(12), 91.05423(10)
	40	91.05423(100), 121.06479(25), 93.06988(13)
25H-NBOMe	10	302.17507(100), 121.06479(45)
	20	121.06479(100), 91.05423(14)
	40	91.05423(100), 121.06479(15)
25I-NBF	10	416.05173(100)
	20	290.98765(100), 275.96417(26), 416.05173(23)
	40	275.96417(100), 260.9407(54), 109.0448(53), 149.05971(30), 134.07262(26), 164.08318(19), 290.98765(19), 104.06205(15), 121.06479(13), 91.05423(10)
25I-NBOMe 3-methoxy isomer	10	121.06479(100), 428.07171(32)
	20	121.06479(100)
	40	121.06479(100)
25I-NBOMe 4-methoxy isomer	10	428.07171(100), 290.98765(11)
	20	121.06479(100), 290.98765(72), 272.1407(47), 428.07171(44), 275.96417(16)
	40	121.06479(100), 275.96417(23), 91.05423(22)
25I-NBOMe imine analog	10	426.05606(100)
	20	426.05606(100), 290.98765(65), 275.96417(21)
	40	275.96417(100), 260.9407(46), 149.05971(28), 290.98765(24), 134.07262(24), 164.08318(20), 121.06479(12)
25T2-NBOMe	10	348.16279(100), 121.06479(21)
	20	121.06479(100), 211.07536(13)
	40	91.05423(100), 121.06479(33), 93.06988(15)
30C-NBOMe	10	181.08592(100)
	20	181.08592(100)
	40	181.08592(100), 148.05188(29)
3-methoxy PCP	10	86.09643(100), 189.12739(60)
	20	86.09643(100), 121.06479(80), 189.12739(36), 81.06988(19)

	40	121.06479(100), 86.09643(57), 91.05423(24), 81.06988(16)
4-methoxy PCP	10	189.12739(100), 86.09643(23), 121.06479(12)
	20	121.06479(100), 189.12739(62), 86.09643(23)
	40	121.06479(100)
PCEEA	10	159.11683(100), 90.09134(84), 91.05423(20)
	20	91.05423(100), 159.11683(32), 90.09134(23), 44.04948(19), 81.06988(19)
	40	91.05423(100), 44.04948(10)
PCMPA	10	90.09134(100), 159.11683(99), 91.05423(17), 248.20089(11)
	20	91.05423(100), 159.11683(45), 90.09134(42), 81.06988(19), 58.06513(17)
	40	91.05423(100)
PCPr	10	159.11683(100), 60.08078(44), 91.05423(32), 81.06988(11)
	20	91.05423(100), 60.08078(18), 159.11683(14), 81.06988(13)
	40	91.05423(100)
Benocyclidine	10	215.0889(100), 86.09643(39)
	20	215.0889(100), 147.0263(86), 86.09643(49), 81.06988(11)
	40	147.0263(100), 86.09643(17)
2-methyl-α-Pyrrolidinopropiophenone	10	218.15394(100)
	20	119.08553(100), 218.15394(83), 147.08044(65), 98.09643(56), 70.06513(19)
	40	98.09643(100), 91.05423(99), 119.08553(55), 117.06988(36), 56.04948(27), 70.06513(17), 84.08078(15), 103.05423(14), 55.05423(13), 77.03858(13), 104.06205(10)
3,4-dimethoxy-α-Pyrrolidinopentiophenone	10	292.19072(100)
	20	221.11722(100), 151.07536(87), 292.19072(83), 126.12773(82), 165.05462(25), 193.12231(12)

	40	151.07536(100), 126.12773(80), 165.05462(32), 84.08078(23), 137.05971(12)
3'-fluoro-α-Pyrrolidinopropiophenone	10	222.12887(100)
	20	222.12887(100), 98.09643(69), 123.06045(57), 70.06513(27), 151.05537(26)
	40	98.09643(100), 103.05423(68), 77.03858(28), 70.06513(27), 95.02915(27), 56.04948(24), 123.06045(21), 84.08078(17), 123.02407(14), 55.05423(11)
3-methyl-α-Pyrrolidinopropiophenone	10	218.15394(100)
	20	119.08553(100), 218.15394(83), 147.08044(65), 98.09643(56), 70.06513(19)
	40	98.09643(100), 91.05423(99), 119.08553(55), 117.06988(36), 56.04948(27), 70.06513(17), 84.08078(15), 103.05423(14), 55.05423(13), 77.03858(13), 104.06205(10)
4'-Methyl-N-methylhexanophenone	10	202.15903(100), 220.16959(54), 189.12739(29), 146.09643(18), 105.06988(13)
	20	146.09643(100), 105.06988(63), 145.0886(53), 202.15903(49), 131.07295(16), 144.08078(14), 119.04914(13), 158.09643(13), 159.10425(12)
	40	144.08078(100), 105.06988(50), 158.09643(45), 91.05423(38), 145.0886(31), 131.07295(30), 130.06513(18), 77.03858(11), 79.05423(11)
4-fluoro-α-Pyrrolidinobutiophenone	10	236.14452(100)
	20	109.0448(100), 236.14452(85), 165.07102(56), 112.11208(51), 137.0761(40), 123.02407(26), 70.06513(20)
	40	109.0448(100), 112.11208(60), 95.02915(46), 123.02407(45), 84.08078(27), 70.06513(17)
	10	250.16017(100)

4-fluoro-α-Pyrrolidinopentiophenone	20	109.0448(100), 250.16017(79), 126.12773(40), 179.08667(39), 123.02407(23), 70.06513(14)
	40	109.0448(100), 126.12773(57), 123.02407(53), 95.02915(48), 84.08078(33), 70.06513(11)
4'-fluoro-α-Pyrrolidinopropiophenone	10	222.12887(100)
	20	222.12887(100), 123.06045(85), 98.09643(82), 151.05537(64), 70.06513(19)
	40	98.09643(100), 103.05423(85), 123.06045(34), 77.03858(30), 56.04948(27), 70.06513(18), 95.02915(17), 123.02293(14), 84.08078(14), 55.05423(11)
4-Methyl-α-ethylaminobutiophenone	10	188.14338(100), 206.15394(72), 161.09609(20), 160.11208(16), 159.10425(13)
	20	159.10425(100), 105.06988(68), 144.08078(62), 188.14338(55), 160.11208(54), 132.08078(24), 131.07295(11), 158.09643(10)
	40	144.08078(100), 105.06988(40), 91.05423(33), 130.06513(18), 143.07295(12), 158.09643(11)
4-Methyl-α-ethylaminopentiophenone	10	202.15903(100), 220.16959(79), 175.11174(27), 160.11208(12), 105.06988(12)
	20	105.06988(100), 202.15903(81), 160.11208(81), 159.10425(69), 144.08078(58), 132.08078(50), 173.1199(33), 174.12773(27), 119.04914(20), 131.07295(14), 175.11174(14), 158.09643(14), 145.0886(11)
	40	144.08078(100), 105.06988(39), 91.05423(31), 130.06513(14), 158.09643(12), 117.0573(10)
α-Ethylaminopentiophenone	10	188.14338(100), 206.15394(99), 146.09643(21), 161.09609(18), 91.05423(15)

	20	91.05423(100), 146.09643(83), 118.06513(67), 188.14338(49), 130.06513(48), 145.0886(38), 159.10425(24), 105.03349(23), 160.11208(21), 117.0573(12), 131.07295(11)
	40	130.06513(100), 91.05423(82), 77.03858(53), 117.0573(21), 118.06513(17), 105.03349(11)
α-Pyrrolidinobutiothiophenone	10	224.11036(100), 112.11208(20)
	20	112.11208(100), 125.04195(33), 224.11036(19), 153.03686(16), 97.01065(10)
	40	112.11208(100), 97.01065(45), 110.98991(42), 70.06513(22), 84.08078(16), 55.05423(15)
α-Pyrrolidinopentiophenone metabolite 1	10	234.18524(100), 216.17468(23), 72.08078(14)
	20	72.08078(100), 216.17468(76), 173.1199(26), 234.18524(19), 91.05423(16), 145.10118(15)
	40	72.08078(100), 91.05423(69), 79.05423(43), 43.05423(38), 172.11208(20), 103.05423(17), 104.06205(14), 117.06988(13), 77.03858(13), 41.03858(12), 105.06988(11)
α-Pyrrolidinopentiothiophenone	10	238.12601(100), 126.12773(17)
	20	126.12773(100), 97.01065(43), 238.12601(22), 167.05251(12)
	40	126.12773(100), 97.01065(60), 110.98991(41), 84.08078(33), 97.0886(20), 55.05423(10)
4-fluoro PV8	10	278.19147(100)
	20	278.19147(100), 109.0448(69), 154.15903(24), 207.11683(16), 123.02407(12), 70.06513(11)
	40	109.0448(100), 154.15903(48), 123.02407(47), 84.08078(29), 95.02915(25)
4-fluoro PV9	10	292.20712(100)
	20	292.20712(100), 109.0448(49), 168.17468(16)

	40	109.0448(100), 168.17468(49), 123.02407(43), 84.08078(26), 95.02915(17)
4-methoxy PV8	10	290.21146(100)
	20	121.06479(100), 219.13796(98), 290.21146(96), 154.15903(76), 135.04406(25)
	40	121.06479(100), 154.15903(56), 135.04406(40), 84.08078(24), 77.03858(15)
4-methoxy PV9	10	304.22711(100)
	20	304.22711(100), 233.15361(73), 121.06479(71), 168.17468(54), 135.04406(17)
	40	121.06479(100), 168.17468(59), 135.04406(41), 84.08078(22), 77.03858(11)
PV8	10	260.20089(100)
	20	260.20089(100), 91.05423(75), 154.15903(21), 70.06513(17), 189.12739(15), 119.04914(14), 105.03349(14)
	40	91.05423(100), 77.03858(37), 105.03349(35), 154.15903(35), 84.08078(28)
PV9	10	274.21654(100)
	20	274.21654(100), 91.05423(50), 168.17468(14), 70.06513(12)
	40	91.05423(100), 105.03349(37), 168.17468(35), 77.03858(28), 84.08078(25), 70.06513(10)
4-APB	10	159.08044(100), 131.04914(73)
	20	131.04914(100), 91.05423(20)
	40	91.05423(100), 77.03858(99), 131.04914(80), 115.05423(42), 103.05423(23), 116.06205(20), 65.03858(19), 128.06205(11)
4-APDB	10	161.09609(100), 133.06479(61)
	20	133.06479(100), 161.09609(14), 120.05697(11)
	40	77.03858(100), 91.05423(64), 79.05423(56), 103.05423(44), 105.06988(38), 133.06479(33),

		115.05423(20), 117.06988(14), 128.06205(11)
5-APDB	10	161.09616(100), 178.12288(10)
	20	161.09609(100), 105.06997(55), 146.07258(42), 131.07957(18), 91.05413(14)
	40	103.05423(100), 131.04918(51), 91.05425(41), 77.03853(40), 115.05446(36)
5-EAPB	10	159.08044(100), 131.04914(35), 204.13829(29)
	20	131.04914(100), 159.08044(19)
	40	131.04914(100), 91.05423(60), 77.03858(29), 116.06205(17), 115.05423(15), 103.05423(15)
5-MAPB	10	159.08044(100), 131.04914(45), 190.12264(19)
	20	131.04914(100), 159.08044(13), 91.05423(11)
	40	131.04914(100), 91.05423(77), 77.03858(51), 115.05423(25), 116.06205(20), 103.05423(20)
5-MAPDB	10	161.09609(100), 133.06479(15)
	20	133.06479(100), 161.09609(50)
	40	133.06479(100), 105.06988(69), 77.03858(67), 79.05423(62), 103.05423(47), 91.05423(40), 117.06988(23), 115.05423(20), 128.06205(17), 146.07262(14), 55.01784(11), 131.04914(11), 120.05697(10)
6-APB	10	159.08044(100), 131.04914(68)
	20	131.04914(100), 91.05423(19)
	40	91.05423(100), 77.03858(87), 131.04914(78), 115.05423(48), 103.05423(23), 116.06205(23), 65.03858(16)
6-APDB	10	161.09609(100), 133.06479(56)
	20	133.06479(100), 161.09609(16)

	40	77.03858(100), 79.05423(64), 91.05423(52), 103.05423(52), 105.06988(50), 133.06479(48), 115.05423(21), 117.06988(18), 128.06205(15), 55.01784(10)
7-APB	10	131.04914(100), 159.08044(75)
	20	131.04914(100)
	40	77.03858(100), 131.04914(70), 91.05423(53), 115.05423(27), 103.05423(25), 116.06205(12)
3-Methylbuphedrone	10	174.12773(100), 192.13829(42), 161.09609(20), 145.0886(15), 146.09643(14), 105.06988(10)
	20	145.0886(100), 105.06988(46), 146.09643(36), 144.08078(35), 174.12773(33), 159.10425(22), 131.07295(10)
	40	144.08078(100), 91.05423(20), 105.06988(20)
4-Fluorobuphedrone	10	178.10265(100), 196.11322(37), 165.07102(19), 150.07135(18), 149.06353(13)
	20	149.06353(100), 150.07135(51), 109.0448(49), 178.10265(37), 148.0557(15), 163.07918(12)
	40	148.0557(100), 109.0448(65), 149.06353(37), 95.02915(33), 108.03698(16), 135.04788(15), 162.07135(12), 83.02915(11)
4-Methylbuphedrone	10	174.12773(100), 192.13829(26), 161.09609(21), 145.0886(14), 146.09643(13)
	20	145.0886(100), 105.06988(45), 174.12773(35), 146.09643(35), 144.08078(35), 159.10425(21)
	40	144.08078(100), 105.06988(18), 91.05423(18)
4-methyl-N-Methylbuphedrone	10	206.15394(100), 161.09609(56), 105.06988(17), 133.10118(11)
	20	105.06988(100), 86.09643(37), 119.04914(35), 161.09609(27), 133.10118(17)

	40	91.05423(100), 105.06988(78), 86.09643(53), 71.07295(26), 119.04914(21), 65.03858(16), 79.05423(14), 77.03858(12)
N-Ethylbuphedrone	10	174.12773(100), 192.13829(60), 146.09643(25), 147.08044(16), 91.05423(14), 145.0886(13)
	20	145.0886(100), 91.05423(99), 130.06513(82), 146.09643(76), 174.12773(53), 118.06513(50), 105.03349(17), 117.0573(15)
	40	130.06513(100), 91.05423(72), 77.03858(45), 117.0573(19)
Dimethocaine	10	279.2067(100), 120.04439(12), 142.15903(11)
	20	120.04439(100), 142.15903(47), 86.09643(31), 279.2067(14)
	40	120.04439(100), 86.09643(53), 92.04948(36)
(±)-Cannabichromene	10	315.23186(100), 193.12231(55), 259.16926(44), 81.06988(32), 233.15361(30), 135.11683(14), 231.13796(10), 219.13796(10)
	20	
	40	
(±)-ORG 28611	10	270.14886(100), 384.26455(37)
	20	270.14886(100)
	40	174.05495(100), 270.14886(33), 55.05423(19)
5-fluoro NNEI	10	375.18672(100), 232.11322(23)
	20	232.11322(100)
	40	232.11208(100), 144.04439(86)
5-fluoro NNEI 2'-naphthyl isomer	10	375.18672(100), 232.11322(31)
	20	232.11322(100)
	40	232.11322(100), 144.04439(86)
5-fluoro SDB-005	10	233.10847(100)
	20	233.10847(100), 213.10224(26)
	40	145.03964(100), 213.10224(24), 69.06988(18), 177.04587(16)
5-fluoro SDB-006	10	339.18672(100)
	20	232.11322(100), 339.18672(89), 206.13395(71), 91.05423(40)

	40	91.05423(100), 144.04439(25), 232.11322(16), 118.06513(15)
A-796260	10	355.238(100), 125.09609(14)
	20	125.09609(100), 114.09134(32), 355.238(32)
	40	114.09134(100), 125.09609(51), 55.05423(39), 97.10118(26), 70.06513(21), 57.06988(19), 69.06988(12)
A-836339	10	311.17878(100), 187.08996(63)
	20	187.08996(100), 125.09609(18)
	40	187.08996(100), 59.04914(71), 55.05423(56), 129.0481(42), 125.09609(37), 57.06988(32), 155.06375(32), 97.10118(23), 69.06988(14)
JW 618	10	393.10322(100)
	20	393.10322(100)
	40	169.0886(100), 393.10322(79), 197.10732(26)
JW 642	10	463.14509(100), 183.08044(24)
	20	183.08044(100), 463.14509(30)
	40	183.08044(100), 155.08553(15), 165.06988(12), 168.05697(11)
MN-25	10	440.29077(100)
	20	440.29077(100), 261.15975(34), 114.09134(14)
	40	114.09134(100), 81.06988(26), 176.10699(12), 261.15975(10)
MN-25-2-methyl derivative	10	454.30642(100), 275.1754(12)
	20	275.1754(100), 454.30642(96), 114.09134(47)
	40	114.09134(100)
NNEI	10	357.19614(100), 214.12264(29)
	20	214.12264(100)
	40	144.04439(100), 214.12264(86), 43.05423(14)
NNEI 2'-naphthyl isomer	10	357.19614(100), 214.12264(38)
	20	214.12264(100)
	40	144.04439(100), 214.12264(82), 43.05423(13)
SDB-005	10	215.11789(100)

	20	215.11789(100)
	40	145.03964(100), 215.11789(17)
SDB-006	10	321.19614(100)
	20	214.12264(100), 188.14338(63), 321.19614(51), 91.05423(43), 132.08078(16)
	40	91.05423(100), 144.04439(24), 118.06513(13), 132.08078(10)
Δ8-THC	10	315.23186(100)
	20	315.23186(100), 193.12231(72), 259.16926(44), 135.11683(40), 93.06988(31), 233.15361(18), 181.12231(15), 235.16926(13), 231.13796(13), 247.16926(13), 207.13796(11), 107.08553(10), 109.10118(10)
	40	
Δ9-THC	10	315.23186(100)
	20	315.23186(100), 193.12231(90), 259.16926(47), 135.11683(42), 93.06988(30), 221.15361(21), 235.16926(21), 233.15361(18), 81.06988(17), 181.12231(16), 109.10118(12), 123.04406(11), 107.08553(11), 207.13796(11)
	40	
AM1248 azepane isomer	10	391.27439(100)
	20	391.27439(100), 112.11208(58), 135.11683(23)
	40	112.11208(100), 135.11683(51), 58.06513(13)
AM2201 benzimidazole analog	10	361.17107(100)
	20	361.17107(100), 233.10847(24), 177.04587(17), 155.04914(15), 273.10224(13)
	40	155.04914(100), 127.05423(65), 177.04587(27), 145.03964(25), 129.04472(14)
KM 233	10	363.23186(100), 119.08553(37)
	20	119.08553(100), 363.23186(10)
	40	91.05423(100), 119.08553(84)
LY2183240	10	280.14444(100), 72.04439(48), 167.08553(29), 87.05529(20)

	20	72.04439(100), 167.08553(84), 87.05529(13)
	40	72.04439(100), 167.08553(37)
LY2183240 2'-isomer	10	280.14444(100), 72.04439(54), 167.08553(32), 87.05529(19)
	20	72.04439(100), 167.08553(84), 87.05529(16)
	40	72.04439(100), 167.08553(39)
SER-601	10	435.3006(100)
	20	435.3006(100), 135.11683(70), 284.16451(25)
	40	135.11683(100), 284.16451(20)
Tetrahydrocannabivarin	10	287.20056(100)
	20	
	40	
Yangonin	10	259.09649(100), 231.10157(14)
	20	161.05971(100), 231.10157(53), 259.09649(40), 216.0781(31),199.07536(31), 171.08044(28), 209.05971(24), 198.06753(15), 203.10666(13), 185.05971(13), 133.06479(10), 213.09101(10)
	40	133.06479(100), 128.06205(67), 68.99711(48), 118.04132(33), 151.05423(31), 161.05971(29), 139.05423(28), 115.05423(24), 140.06205(23), 103.05423(22), 171.08044(22), 79.05423(22), 127.05423(21), 77.03858(20), 141.06988(20), 155.04914(18), 152.06205(18), 90.0464(18), 156.05697(17), 157.06479(16), 145.06479(15), 184.05188(13), 129.06988(13), 144.05697(12), 173.05971(12), 142.04132(12), 183.04406(12), 168.05697(11)
Cannabidiolic Acid	10	341.21112(100)
	20	341.21112(100)
	40	
Cannabigerol	10	193.12231(100), 317.24751(15)
	20	193.12231(100)
	40	

EG-018	10	392.20089(100), 155.04914(11)
	20	155.04914(100), 392.20089(55), 264.13829(12)
	40	155.04914(100), 127.05423(85)
(±)-JWH 018 N-(2-hydroxypentyl) metabolite	10	358.18041(100)
	20	358.17982(100), 155.04864(92), 230.11689(20)
	40	127.05377(100), 155.04848(86), 160.03941(16)
(±)-JWH 018 N-(3-hydroxypentyl) metabolite	10	358.18016(100), 155.04914(20)
	20	155.04914(100), 358.18016(18)
	40	127.05423(100), 155.04914(89)
(±)-JWH 018 N-(4-hydroxypentyl) metabolite	10	358.1799(100), 155.04864(10)
	20	155.0487(100), 358.18016(46)
	40	127.05391(100), 155.04858(83)
(R)-(−)-JWH 018 N-(4-hydroxypentyl) metabolite	10	358.18016(100), 155.04914(20)
	20	155.04914(100), 358.18016(18)
	40	127.05423(100), 155.04914(89)
(S)-(+)-JWH 018 N-(4-hydroxypentyl) metabolite	10	358.18016(100), 155.04914(20)
	20	155.04914(100), 358.18016(18)
	40	127.05423(100), 155.04914(89)
5-fluoro JWH 018 adamantyl analog	10	368.23842(100)
	20	368.23842(100), 135.11683(22)
	40	135.11683(100), 93.06988(13), 107.08553(11)
JWH 018 2-hydroxyindole metabolite	10	358.18041(100)
	20	358.17982(100), 155.04864(92), 230.11689(20)
	40	
JWH 018 4-hydroxyindole metabolite	10	358.18016(100)
	20	358.18016(100), 155.04914(43), 230.11756(26)
	40	127.05423(100), 155.04914(86), 160.0393(42), 230.11756(24)
JWH 018 5-hydroxyindole metabolite	10	358.18041(100)
	20	358.17982(100), 155.04864(92), 230.11689(20)
	40	127.05377(100), 155.04848(86), 160.03941(16)
JWH 018 6-hydroxyindole metabolite	10	358.17987(100)
	20	155.04841(100), 358.17954(66)
	40	127.05384(100), 155.04844(85)

JWH 018 7-hydroxyindole metabolite	10	358.18031(100), 155.04876(31)
	20	155.04881(100), 358.18008(14)
	40	127.05397(100), 155.0488(91), 144.04422(12)
JWH 018 8-quinolinyl carboxamide	10	214.1224(100), 358.19139(25)
	20	214.12235(100)
	40	144.04403(100), 214.12243(75), 43.05417(16)
JWH 018 benzimidazole analog	10	343.18056(100)
	20	343.18089(100), 215.11756(46), 273.10216(30), 155.04888(18)
	40	155.04889(100), 127.05411(70), 145.03937(30), 131.05994(16)
JWH 018 N-(1-ethylpropyl) isomer	10	342.18495(100)
	20	155.04897(100), 342.18524(99), 214.12262(12), 144.04442(11)
	40	127.05428(100), 155.04894(91), 144.04412(48)
JWH 018 N-(4-oxo-pentyl) metabolite	10	356.16468(100), 155.04894(24)
	20	155.04899(100), 356.16464(17)
	40	127.05414(100), 155.04895(83), 85.06484(13), 43.01812(12)
JWH 018 N-(5-hydroxypentyl) metabolite	10	358.18031(100), 155.04876(31)
	20	155.04881(100), 358.18008(14)
	40	127.05397(100), 155.0488(91), 144.04422(12)
JWH 018 N-(5-hydroxypentyl) β-D-Glucuronide	10	358.17854(100), 534.21023(75), 155.04867(21)
	20	358.17916(100), 155.04793(59)
	40	155.04839(100), 127.05485(12)
JWH 018 N-pentanoic acid β-D-Glucuronide	10	372.1595(100), 548.18965(26)
	20	372.15923(100), 155.04822(30)
	40	155.04922(100), 372.15997(16), 127.05253(12)
JWH 018 N-propanoic acid metabolite	10	344.12759(100)
	20	155.04863(100), 344.12812(61), 216.0651(25)
	40	127.05395(100), 155.04838(58), 216.06493(18)
JWH 019 5-hydroxyindole metabolite	10	372.19623(100)
	20	372.19547(100), 155.04901(70), 244.13285(17)

	40	155.04867(100), 127.05409(99), 160.03885(14)
JWH 019 N-(2-fluorohexyl) isomer	10	374.19147(100)
	20	374.19151(100), 155.04866(75), 246.12832(21)
	40	155.04884(100), 127.05404(97), 246.12835(21), 144.04371(13)
JWH 019 N-(3-fluorohexyl) isomer	10	374.19142(100)
	20	374.19107(100), 155.04886(70), 246.12872(19)
	40	155.04842(100), 127.05395(90), 246.12956(16), 144.04406(11)
JWH 019 N-(4-fluorohexyl) isomer	10	374.19147(100)
	20	374.19129(100), 155.04898(78), 246.12871(14)
	40	155.04915(100), 127.05429(82), 144.0434(13)
JWH 019 N-(5-fluorohexyl) isomer	10	374.19129(100)
	20	374.19135(100), 155.0488(89), 354.18542(17), 246.1283(14)
	40	155.04852(100), 127.05384(86), 144.04367(12)
JWH 019 N-(5-hydroxyhexyl) metabolite	10	372.1955(100), 155.04848(47)
	20	155.04868(100)
	40	155.0487(100), 127.05403(86)
JWH 019 N-(6-fluorohexyl) isomer	10	374.19162(100)
	20	374.19113(100), 155.04903(61), 246.12808(15)
	40	155.04871(100), 127.05394(87), 144.04347(19), 246.12794(11)
JWH 019 N-(6-hydroxyhexyl) metabolite	10	372.19583(100), 155.04883(46)
	20	155.04886(100)
	40	155.04866(100), 127.0541(83)
JWH 019 N-(6-hydroxyhexyl) β-D-Glucuronide	10	372.19554(100), 548.22603(54), 155.04802(22)
	20	372.19577(100), 155.04842(69)
	40	155.04837(100)
JWH 030 2-naphthoyl isomer	10	292.16976(100), 155.04879(47)
	20	155.0489(100), 164.10682(14)
	40	127.05413(100), 155.04861(24)
JWH 031 2'-isomer	10	306.18494(100), 155.0487(35)
	20	155.0488(100), 178.12241(12)

	40	127.05391(100), 155.04869(34)
JWH 071	10	300.13787(100), 155.04898(11)
	20	155.04872(100), 300.13813(30), 172.07525(25)
	40	127.05391(100), 155.04876(26), 172.07504(11)
JWH 073 4-hydroxyindole metabolite	10	344.16411(100)
	20	155.04832(100), 344.16347(87), 216.10097(21)
	40	127.05398(100), 155.04852(67), 160.03883(17)
JWH 073 5-hydroxyindole metabolite	10	344.16411(100)
	20	155.04832(100), 344.16347(87), 216.10097(21)
	40	127.05398(100), 155.04852(67), 160.03883(17)
JWH 073 6-hydroxyindole metabolite	10	344.16452(100)
	20	155.04885(100), 344.16474(48)
	40	127.05409(100), 155.04854(64)
JWH 073 6-methoxyindole analog	10	358.1799(100), 155.04864(10)
	20	155.0487(100), 358.18016(46)
	40	127.05391(100), 155.04858(83)
JWH 073 7-hydroxyindole metabolite	10	344.16439(100)
	20	155.04853(100), 344.16436(94), 216.1013(24)
	40	127.0539(100), 155.04866(67), 160.03896(24)
JWH 073 N-(2-hydroxybutyl) metabolite	10	344.16451(100)
	20	155.04887(100), 344.16454(81), 216.10148(16)
	40	127.05405(100), 155.04869(72), 144.04405(18)
JWH 073 N-(4-hydroxybutyl) metabolite	10	344.1641(100), 155.04872(19)
	20	155.04864(100), 344.16374(24)
	40	127.05388(100), 155.0486(74), 144.04396(13)
JWH 073 N-(4-hydroxybutyl) β-D-Glucuronide	10	520.19659(100), 344.16451(83), 155.04914(23)
	20	344.16451(100), 155.04914(53), 520.19659(10)
	40	155.04914(100), 127.05423(13)
JWH 073 N-butanoic acid metabolite	10	358.14334(100), 155.04805(13)
	20	155.04888(100), 358.14371(44)

	40	127.05405(100), 155.04903(79), 144.04354(13)
(±)-JWH 073 N-(3-hydroxybutyl) metabolite	10	344.16438(100), 155.04816(14)
	20	155.04863(100), 344.16415(37)
	40	127.05398(100), 155.04881(74)
(R)-(−)-JWH 073 N-(3-hydroxybutyl) metabolite	10	344.16432(100), 155.04874(14)
	20	155.04872(100), 344.16417(37)
	40	127.05389(100), 155.04855(73)
(S)-(+)-JWH 073 N-(3-hydroxybutyl) metabolite	10	344.16432(100), 155.04874(14)
	20	155.04872(100), 344.16417(37)
	40	127.05389(100), 155.04855(73)
JWH 080	10	358.18025(100)
	20	185.05942(100), 358.18007(76), 200.10638(33)
	40	185.0594(100), 157.06449(59), 144.04404(29), 200.10645(20), 127.05422(13)
JWH 081 4-hydroxynaphthyl metabolite	10	358.17969(100)
	20	171.04385(100), 358.17981(90), 214.12166(23)
	40	171.04365(100), 143.04898(43), 115.05384(17), 144.04501(17)
JWH 081 N-(4-hydroxypentyl) metabolite	10	388.19072(100), 185.05924(19)
	20	185.05945(100), 388.19091(35)
	40	185.05943(100), 157.06412(36), 144.04417(12)
JWH 081 N-(5-hydroxypentyl) metabolite	10	
	20	185.05941(100), 388.19067(28)
	40	185.05947(100), 157.06447(37), 144.04444(15)
JWH 081 N-pentanoic acid metabolite	10	402.17045(100), 185.05877(13)
	20	185.05911(100), 402.17062(70), 244.09684(15)
	40	185.05907(100), 157.06425(34), 144.04445(15)
JWH 081-N-(cyclohexylmethyl) analog	10	398.21153(100)
	20	398.21162(100), 185.05946(46), 240.13795(14)
	40	185.05949(100), 157.06436(25), 144.04405(18)
JWH 116	10	370.21647(100)

	20	370.21645(100), 183.08009(55), 214.12216(42)
	40	144.04422(100), 155.08497(84), 183.07978(81), 214.12217(64), 141.06957(51)
JWH 122 N-(4-hydroxypentyl) metabolite	10	372.1955(100), 155.04848(47)
	20	155.04868(100)
	40	155.0487(100), 127.05403(86)
JWH 122 N-(5-hydroxypentyl) metabolite	10	372.19587(100), 169.06461(27)
	20	169.06454(100), 372.19622(22)
	40	169.06471(100), 141.06976(83), 144.04373(17)
JWH 145 2-phenyl isomer	10	368.20084(100), 155.04879(28)
	20	155.04883(100), 368.20129(11)
	40	127.05407(100), 155.04899(90)
JWH 146	10	396.23189(100), 155.04867(40)
	20	155.04889(100)
	40	155.04887(100), 127.05399(80)
JWH 149	10	370.21591(100)
	20	370.21622(100), 169.06418(97), 228.13783(22)
	40	169.06417(100), 141.06956(87), 158.05945(19), 228.13735(14)
JWH 167	10	306.18524(100)
	20	91.05412(100), 306.18506(95), 214.12257(64), 188.14263(26)
	40	91.05425(100), 144.04423(50), 214.12246(19)
JWH 176	10	255.11683(100), 325.19508(86), 141.06988(11), 324.18725(11)
	20	255.11683(100), 141.06988(24), 253.10118(18), 254.109(13)
	40	
JWH 193	10	399.20638(100), 169.0642(18)
	20	169.06439(100), 399.2067(38), 114.09134(31)
	40	169.0643(100), 114.09129(75), 141.06936(52), 70.06525(12)
JWH 198	10	415.20157(100), 185.05911(19)
	20	185.05938(100), 415.20162(41), 114.09124(25)

	40	185.05946(100), 114.09142(55), 157.06473(23)
JWH 200 4-hydroxyindole metabolite	10	401.1854(100)
	20	155.04869(100), 401.18597(90), 114.09131(74)
	40	114.09126(100), 155.04888(64), 127.05435(34), 70.06478(15)
JWH 200 5-hydroxyindole metabolite	10	401.18602(100), 155.04887(15)
	20	155.04907(100), 114.09171(30), 401.18662(29)
	40	155.04905(100), 114.09131(94), 127.05448(58), 70.06499(16)
JWH 200 6-hydroxyindole metabolite	10	401.18588(100), 155.04891(24)
	20	155.04921(100), 401.18635(24), 114.09095(21)
	40	155.04876(100), 127.05407(70), 114.09171(50), 70.06396(11)
JWH 200 7-hydroxyindole metabolite	10	401.18597(100)
	20	155.04842(100), 401.18571(90), 114.0911(77)
	40	114.09121(100), 155.04868(66), 127.05338(34), 70.06431(17)
JWH 203	10	340.14619(100)
	20	340.14596(100), 125.01508(90), 188.14249(23), 214.12275(11)
	40	125.01519(100), 144.04449(10)
JWH 203 N-(4-hydroxypentyl) metabolite	10	356.14118(100), 186.12737(26)
	20	125.01534(100), 186.12747(84), 356.14075(19), 130.06503(16), 282.0681(10)
	40	125.01529(100)
JWH 203 N-(5-hydroxypentyl) metabolite	10	356.14058(100), 186.12701(11)
	20	125.01503(100), 186.12714(36), 204.13768(30), 356.1395(18)
	40	125.01507(100)
JWH 203 N-pentanoic acid metabolite	10	370.12015(100), 200.10631(12)
	20	125.01478(100), 200.10624(54), 370.12045(37), 218.11713(24)
	40	125.01491(100)
JWH 210 2-ethylnaphthyl isomer	10	370.21647(100)
	20	370.21645(100), 183.08009(55), 214.12216(42)

	40	144.04422(100), 155.08497(84), 183.07978(81), 214.12217(64), 141.06957(51)
JWH 210 5-hydroxyindole metabolite	10	386.21146(100)
	20	386.21164(100), 183.0799(62), 230.11634(17)
	40	183.08055(100), 155.08506(50), 230.11714(24), 160.03803(23), 153.06916(20)
JWH 210 N-(4-hydroxypentyl) metabolite	10	386.21146(100), 183.07986(16)
	20	183.07995(100), 386.21091(39)
	40	183.07987(100), 155.08505(45), 153.06853(18), 144.0433(15)
JWH 210 N-(5-hydroxypentyl) metabolite	10	386.21122(100), 183.08002(23)
	20	183.08012(100), 386.21097(28)
	40	183.0799(100), 155.08475(48), 153.06952(19), 144.04371(17)
JWH 210 N-pentanoic acid metabolite	10	400.19072(100), 183.08025(12)
	20	183.08044(100), 400.1908(73), 244.09647(13)
	40	183.07985(100), 155.0847(43), 144.04433(16), 153.06926(16)
JWH 213	10	384.23191(100)
	20	384.23188(100), 183.08006(79), 228.13773(21)
	40	183.07984(100), 155.08492(47), 153.06982(20), 158.05948(19), 228.13742(19)
JWH 250 5-hydroxyindole metabolite	10	
	20	121.06479(100), 352.19072(18)
	40	91.05423(100), 121.06479(82), 93.06988(24), 146.06004(14), 160.07569(14), 131.04914(11)
JWH 250 N-(4-hydroxypentyl) metabolite	10	352.19072(100), 121.06479(14), 186.12773(12)
	20	121.06479(100), 186.12773(18), 352.19072(14)
	40	91.05423(100), 121.06479(89), 93.06988(26), 130.06513(20), 69.06988(12)
JWH 250 N-(5-hydroxypentyl) metabolite	10	352.19072(100), 121.06479(20)
	20	121.06479(100)

	40	91.05423(100), 121.06479(87), 93.06988(25), 130.06513(20)
JWH 250 N-pentanoic acid metabolite	10	366.16998(100), 121.06479(17)
	20	121.06479(100), 366.16998(18)
	40	121.06479(100), 91.05423(92), 93.06988(24), 130.06513(18), 55.05423(12)
JWH 309 5'-isomer	10	418.21654(100), 155.04914(23)
	20	155.04914(100), 418.21654(27)
	40	155.04914(100), 127.05423(64)
JWH 387	10	420.09575(100)
	20	420.09575(100), 232.95965(90), 214.12264(10)
	40	232.95965(100), 204.96474(69), 126.0464(12)
JWH 398 N-(4-hydroxypentyl) metabolite	10	392.14118(100), 189.01017(24)
	20	189.01017(100), 392.14118(22)
	40	189.01017(100), 161.01525(58)
JWH 398 N-(5-hydroxypentyl) metabolite	10	392.14118(100), 189.01017(36)
	20	189.01017(100), 392.14118(15)
	40	189.01017(100), 161.01525(55)
JWH 398 N-pentanoic acid metabolite	10	406.12045(100), 189.01017(18)
	20	189.01017(100), 406.12045(30)
	40	189.01017(100), 161.01525(51)
JWH 412	10	360.17582(100)
	20	173.03972(100), 360.17582(85)
	40	173.03972(100), 145.0448(67)
CP 47,497-C8-homolog C-8-hydroxy metabolite	10	175.11174(100), 331.26316, (70), 83.08553(37), 157.15869(25), 81.06988(11), 251.20056(11), 97.10118(11)
	20	
	40	
RCS-4 4-hydroxyphenyl metabolite	10	308.16451(100), 121.02841(12)
	20	121.02841(100), 308.16451(30)
	40	121.02841(100), 93.03349(37), 65.03858(17)
RCS-4 M10 metabolite	10	324.15942(100), 121.02841(54)
	20	121.02841(100)
	40	121.02841(100), 93.03349(30), 65.03858(14)
RCS-4 M11 metabolite	10	322.14377(100), 121.02841(47)

	20	121.02841(100)
	40	121.02841(100), 93.03349(34), 65.03858(13)
RCS-4 M9 metabolite	10	324.15942(100), 121.02841(36), 186.12773(11)
	20	121.02841(100)
	40	121.02841(100), 93.03349(29), 65.03858(12)
RCS-4 N-(4-hydroxypentyl) metabolite	10	338.17507(100), 135.04406(36)
	20	135.04406(100)
	40	135.04406(100), 77.03858(23), 107.04914(17)
RCS-4 N-(5-carboxypentyl) metabolite	10	352.15433(100), 135.04406(22)
	20	135.04406(100), 352.15433(21)
	40	135.04406(100), 77.03858(18), 107.04914(16)
RCS-4 N-(5-hydroxypentyl) metabolite	10	338.17507(100), 135.04406(55)
	20	
	40	
5-fluoro NPB-22	10	233.10836(100), 378.1612(80)
	20	233.10814(100), 213.10145(21)
	40	145.03926(100), 213.10242(33), 177.04546(21), 69.06945(20), 41.03849(10)
5-fluoro PB-22	10	232.11323(100)
	20	232.11315(100)
	40	144.04429(100), 232.11317(90)
5-fluoro PB-22 3-carboxyindole metabolite	10	250.12378(100), 206.13395(40), 118.06513(11)
	20	118.06513(100), 132.08078(59), 206.13395(57), 174.05495(27),130.06513(24), 232.11322(18), 69.06988(13), 41.03858(11)
	40	
5-fluoro PB-22 3-hydroxyquinoline isomer	10	377.16596(100), 232.11309(34)
	20	232.11315(100)
	40	232.11304(100), 144.04431(90)
5-fluoro PB-22 4-hydroxyisoquinoline isomer	10	377.16711(100), 232.11374(67)
	20	232.11318(100)
	40	232.1138(100), 144.04445(95)
	10	232.11243(100)

5-fluoro PB-22 4-hydroxyquinoline isomer	20	232.11232(100)
	40	144.04412(100), 232.11246(76)
5-fluoro PB-22 5-hydroxyquinoline isomer	10	377.16595(100), 232.1125(11)
	20	232.11321(100), 377.16592(43)
	40	232.11289(100), 144.04419(85)
5-fluoro PB-22 6-hydroxyisoquinoline isomer	10	377.16711(100), 232.11374(67)
	20	232.11318(100)
	40	232.1138(100), 144.04445(95)
5-fluoro PB-22 6-hydroxyquinoline isomer	10	377.16595(100), 232.1125(11)
	20	232.11321(100), 377.16592(43)
	40	232.11289(100), 144.04419(85)
5-fluoro PB-22 7-hydroxyisoquinoline isomer	10	377.16698(100), 232.11358(51)
	20	232.11358(100)
	40	232.11372(100), 144.0445(86)
5-fluoro PB-22 7-hydroxyquinoline isomer	10	232.11329(100)
	20	232.11319(100)
	40	144.04415(100), 232.11325(96), 212.10664(33), 69.06999(11)
5-fluoro PB-22 8-hydroxyisoquinoline isomer	10	377.16698(100), 232.11358(51)
	20	232.11358(100)
	40	232.11372(100), 144.0445(86)
5-fluoro PB-22 N-(2-fluoropentyl) isomer	10	232.11352(100)
	20	232.11323(100)
	40	232.1137(100), 144.04443(47), 212.10724(14)
5-fluoro PB-22 N-(3-fluoropentyl) isomer	10	232.11355(100)
	20	232.1136(100)
	40	232.11376(100), 144.04443(51), 212.10733(25), 69.06988(14)
5-fluoro PB-22 N-(4-fluoropentyl) isomer	10	232.11329(100)
	20	232.11319(100)
	40	144.04415(100), 232.11325(96), 212.10664(33), 69.06999(11)
FDU-PB-22	10	252.08152(100)
	20	252.08145(100), 109.04474(30)
	40	109.04453(100)
FUB-PB-22	10	252.08213(100)
	20	252.08184(100), 109.04477(28)
	40	109.0448(100)
NPB-22	10	215.11752(100), 360.17002(38)

	20	215.11747(100)
	40	145.03919(100), 215.11746(21)
PB-22	10	359.17519(100), 214.12217(12)
	20	214.12233(100), 359.17484(29)
	40	144.04408(100), 214.12205(96), 43.05453(13)
PB-22 3-carboxyindole metabolite	10	232.13321(100), 188.14338(53), 132.08078(28), 118.06513(20), 231.12538(11)
	20	118.06513(100), 132.08078(81), 43.05423(37), 174.05495(29), 188.14338(25), 214.12264(16), 130.06513(13), 144.04439(13)
	40	
PB-22 3-hydroxyquinoline isomer	10	359.17547(100), 214.12239(36)
	20	214.12241(100)
	40	144.04418(100), 214.1223(80), 43.0542(14)
PB-22 4-hydroxyisoquinoline isomer	10	214.1223(100), 359.17495(81)
	20	214.12248(100)
	40	144.04412(100), 214.1222(73), 43.05459(14)
PB-22 5-hydroxyisoquinoline isomer	10	359.17519(100), 214.12217(12)
	20	214.12233(100), 359.17484(29)
	40	144.04408(100), 214.12205(96), 43.05453(13)
PB-22 5-hydroxyquinoline isomer	10	359.1754(100), 214.12227(61)
	20	214.12233(100)
	40	144.04407(100), 214.12229(77), 43.05435(15)
PB-22 6-hydroxyisoquinoline isomer	10	359.17556(100), 214.12238(85)
	20	214.12205(100)
	40	144.04423(100), 214.12237(77), 43.05439(16)
PB-22 6-hydroxyquinoline isomer	10	359.17514(100), 214.12194(12)
	20	214.12224(100), 359.17481(30)
	40	144.04403(100), 214.12223(90), 43.05439(12)
PB-22 7-hydroxyisoquinoline isomer	10	359.17523(100), 214.12192(10)
	20	214.12241(100), 359.17512(39)
	40	144.04412(100), 214.12234(90), 43.05456(13)

PB-22 7-hydroxyquinoline isomer	10	359.17532(100), 214.12257(97)
	20	214.12234(100)
	40	144.04429(100), 214.12219(79), 43.05455(14)
PB-22 8-hydroxyisoquinoline isomer	10	359.17532(100), 214.12257(97)
	20	214.12234(100)
	40	144.04429(100), 214.12219(79), 43.05455(14)
PB-22 N-(4-hydroxypentyl) metabolite	10	230.1175(100)
	20	230.11738(100), 144.04415(12)
	40	144.0441(100), 69.06993(47), 230.11744(15)
PB-22 N-(5-hydroxypentyl) metabolite	10	230.11732(100)
	20	230.11733(100)
	40	144.04419(100), 230.11707(52), 69.06979(21)
PB-22 N-pentanoic acid metabolite	10	244.09659(100)
	20	244.09686(100)
	40	144.04411(100), 244.09588(40), 55.05422(38), 101.05968(19), 83.04914(17)
PB-22 N-pentanoic acid-3-carboxyindole metabolite	10	244.09682(100), 200.10699(17)
	20	244.09682(100), 144.04439(47), 200.10699(34), 172.11208(32), 156.08078(26), 55.05423(21), 101.05971(21), 83.04914(14), 118.06513(13)
	40	
AM2201 8-quinolinyl carboxamide	10	232.11281(100), 376.18197(34)
	20	232.11268(100)
	40	232.11298(100), 144.04378(97)
BB-22	10	240.13795(100)
	20	240.13811(100)
	40	144.04399(100), 240.13784(68), 55.05441(33), 97.10104(11)
BB-22 3-carboxyindole metabolite	10	258.1487(100), 214.15839(12)
	20	118.06476(100), 55.05426(74), 132.08022(51), 176.07002(47), 214.1601(32)
	40	55.05452(100), 118.06426(41), 91.0538(19)
	10	385.19081(100), 240.13766(23)

BB-22 3-hydroxyquinoline isomer	20	240.13806(100), 385.19072(15)
	40	
BB-22 4-hydroxyisoquinoline isomer	10	385.19061(100), 240.13759(71)
	20	240.13787(100)
	40	144.0439(100), 240.13754(83), 55.05427(33), 97.10078(13)
BB-22 5-hydroxyisoquinoline isomer	10	385.19022(100)
	20	240.13758(100), 385.19022(73)
	40	144.04375(100), 240.13759(95), 55.05409(30), 97.10075(11)
BB-22 5-hydroxyquinoline isomer	10	385.19058(100), 240.13757(57)
	20	240.13766(100)
	40	144.04389(100), 240.13771(84), 55.05412(33), 97.10106(11)
BB-22 6-hydroxyisoquinoline isomer	10	385.19059(100), 240.13772(47)
	20	240.13712(100)
	40	144.04395(100), 240.13749(84), 55.05407(31), 97.10096(11)
BB-22 6-hydroxyquinoline isomer	10	385.1904(100)
	20	385.19046(100), 240.13764(99)
	40	144.04374(100), 240.13785(97), 55.05416(26), 97.10072(11)
BB-22 7-hydroxyisoquinoline isomer	10	385.1904(100)
	20	385.19046(100), 240.13764(99)
	40	144.04374(100), 240.13785(97), 55.05416(26), 97.10072(11)
BB-22 7-hydroxyquinoline isomer	10	385.19058(100), 240.13757(57)
	20	240.13766(100)
	40	144.04389(100), 240.13771(84), 55.05412(33), 97.10106(11)
BB-22 8-hydroxyisoquinoline isomer	10	385.1909(100), 240.13799(54)
	20	240.13781(100)
	40	144.04402(100), 240.138(84), 55.05416(31), 97.10108(13)
AM2201 2-hydroxyindole metabolite	10	376.17073(100)
	20	270.09134(100), 358.16017(98), 376.17073(94), 155.04914(26), 282.09134(17), 252.08078(16), 338.15508(12), 172.0393(11)

	40	252.08078(100), 270.09134(59), 155.04914(13), 172.0393(12), 127.05423(10), 41.03858(10)
AM2201 5-hydroxyindole metabolite	10	376.17011(100)
	20	376.17073(100), 155.0486(70), 248.10677(16)
	40	155.04842(100), 127.05373(95), 160.03877(17), 248.10863(14)
AM2201 6-hydroxyindole metabolite	10	376.16995(100)
	20	155.0484(100), 376.17001(86)
	40	155.04842(100), 127.05364(98)
AM2201 7-hydroxyindole metabolite	10	232.11279(100)
	20	232.11267(100)
	40	144.04382(100), 232.11275(71)
AM2201 N-(4-hydroxypentyl) metabolite	10	376.17047(100), 155.04854(11)
	20	155.0488(100), 376.16991(68)
	40	155.04874(100), 127.05393(86), 144.04407(16)
EAM2201	10	388.20657(100)
	20	388.20703(100), 183.07974(53), 232.11256(21)
	40	183.07995(100), 155.08479(48), 232.11249(30), 144.04395(29), 153.06945(21)
NM2201	10	232.11279(100)
	20	232.11267(100)
	40	144.04382(100), 232.11275(71)
AM2201 N-(3-chloropentyl) isomer	10	376.14584(100)
	20	376.14585(100), 155.04856(73), 248.08291(18)
	40	155.04848(100), 127.05385(94), 144.04371(11), 212.10635(10)
MAM2201 N-(2-fluoropentyl) isomer	10	374.19092(100)
	20	374.19136(100), 169.06436(74), 232.11316(32)
	40	169.06435(100), 141.06934(93), 232.11267(32), 144.04394(17)
MAM2201 N-(3-fluoropentyl) isomer	10	374.19058(100)
	20	374.19113(100), 169.06408(73), 232.11293(25)
	40	169.06415(100), 141.06949(86), 232.11261(25), 144.04376(15)

MAM2201 N-(4-hydroxypentyl) metabolite	10	390.18588(100)
	20	169.06401(100), 390.18626(92), 248.10786(14)
	40	169.06397(100), 141.06913(71), 144.04338(23)
MAM2201 N-(5-chloropentyl) analog	10	390.16182(100)
	20	390.16184(100), 169.0643(57), 248.08311(21)
	40	169.0643(100), 141.06948(77), 248.08329(21), 144.04415(20)
MAM2201 N-(5-chloropentyl) analog-d5	10	395.19306(100)
	20	395.19318(100), 169.06417(42), 253.11452(12), 170.07067(11)
	40	169.0642(100), 141.0695(69), 170.07058(26), 253.11489(16), 142.07587(15)
MAM2201 N-pentanoic acid metabolite	10	386.17497(100), 169.06486(12)
	20	169.06409(100), 386.17501(59), 244.09626(10)
	40	169.06401(100), 141.06942(74), 144.04331(15)
(±)-UR-144 N-(4-hydroxypentyl) metabolite	10	328.22697(100), 125.09588(18)
	20	125.09583(100), 328.22643(24)
	40	55.05414(100), 125.09539(66), 57.06983(52), 69.0697(48), 97.10102(43)
UR-144 Degradant	10	312.23111(100), 214.12199(21)
	20	214.12167(100), 312.23172(19)
	40	144.044(100), 214.12215(47), 43.05436(18), 116.04933(11)
UR-144 Degradant N-pentanoic acid metabolite	10	342.20616(100), 244.09637(15)
	20	244.09646(100), 342.20644(29)
	40	144.04413(100), 55.05408(52), 244.09673(36), 101.05909(20), 83.04897(19)
UR-144 N-(2-chloropentyl) analog	10	346.19287(100)
	20	346.19308(100), 125.0959(45), 248.08341(19), 328.18257(11)
	40	55.05415(100), 144.04412(69), 125.09582(63), 97.10111(42), 57.06971(40)
	10	328.22705(100)

UR-144 N-(2-hydroxypentyl) metabolite	20	328.22711(100), 125.09568(62), 230.11697(17), 310.21609(12)
	40	55.05424(100), 144.04439(53), 125.09598(51), 57.06973(41), 97.10065(39)
UR-144 N-(3-chloropentyl) analog	10	346.19293(100)
	20	346.19274(100), 125.09581(43), 248.08341(16)
	40	55.05419(100), 125.09577(73), 69.06952(52), 97.10066(46), 57.07002(43)
UR-144 N-(4-chloropentyl) analog	10	346.19319(100)
	20	346.19326(100), 125.09576(43), 248.08309(14)
	40	55.05427(100), 125.09559(75), 69.06957(59), 97.101(46), 57.06979(41)
UR-144 N-(5-bromopentyl) analog	10	390.14256(100)
	20	390.14259(100), 125.09586(31), 292.03295(13)
	40	55.05432(100), 125.09593(88), 69.06954(52), 97.10085(50), 292.03236(44)
UR-144 N-(5-chloropentyl) analog	10	346.19251(100)
	20	346.19311(100), 125.09577(42), 248.0839(16)
	40	55.05428(100), 125.09563(65), 144.04417(44), 97.1009(44), 57.06973(42)
UR-144 N-(5-hydroxypentyl) metabolite	10	328.22691(100), 125.09596(27)
	20	125.09581(100), 328.22682(17)
	40	55.05412(100), 125.09583(69), 57.06974(48), 97.10075(41), 69.06929(39)
UR-144 N-(5-hydroxypentyl) β-D-Glucuronide	10	504.25802(100), 328.22683(31), 125.09491(12)
	20	
	40	
UR-144 N-pentanoic acid metabolite	10	342.2062(100), 125.09622(11)
	20	125.09588(100), 342.20637(55), 244.09565(13)
	40	55.05408(100), 125.09563(66), 97.10091(39), 57.06983(33), 144.04386(32)

UR-144 N-(5-methylhexyl) analog	10	340.26263(100)
	20	340.26329(100), 125.09567(36), 242.15289(13)
	40	55.0542(100), 125.09579(79), 57.06972(61), 144.04445(50), 97.10105(48)
UR-144 N-heptyl analog	10	340.26232(100)
	20	340.26306(100), 125.0958(42), 242.15301(15)
	40	55.05411(100), 125.0956(73), 57.06961(61), 144.04446(48), 97.10088(43)
FUB-144	10	350.19059(100)
	20	350.19098(100), 125.09571(51), 109.04444(17), 252.08119(16)
	40	109.04452(100), 55.05414(20), 125.0953(13)
XLR11 6-hydroxyindole metabolite	10	346.21725(100)
	20	346.21741(100), 125.09575(85), 248.10762(11)
	40	55.05391(100), 125.09544(68), 97.10079(44), 57.06956(39), 69.06901(28)
XLR11 Degradant	10	330.22234(100)
	20	330.22269(100), 125.09581(55), 232.11312(26), 312.21194(13)
	40	55.05432(100), 232.1134(55), 125.09591(50), 57.06977(43), 97.10095(35)
XLR11 N-(2-fluoropentyl) isomer	10	330.22234(100)
	20	330.22269(100), 125.09581(55), 232.11312(26) ,312.21194(13)
	40	55.05432(100), 232.1134(55), 125.09591(50), 57.06977(43), 97.10095(35)
XLR11 N-(3-fluoropentyl) isomer	10	330.2216(100)
	20	330.2227(100), 125.09588(52), 232.11311(22), 312.21146(11)
	40	55.05416(100), 125.09562(54), 232.11309(45), 57.06985(44), 97.1008(37)
	10	330.22193(100)

XLR11 N-(4-fluoropentyl) isomer	20	330.22271(100), 125.09573(55), 232.11296(19)
	40	55.05408(100), 125.09551(59), 144.04421(47), 57.06976(45), 97.10081(39)
XLR11 N-(4-hydroxypentyl) metabolite	10	346.21768(100), 248.10834(12)
	20	248.10778(100), 346.21761(41)
	40	144.04379(100), 248.10792(30), 67.05413(19), 87.06026(12)
XLR11 N-(4-pentenyl) analog	10	310.21594(100)
	20	310.21638(100), 125.09583(98), 212.10656(35), 292.20574(14), 97.10107(12)
	40	55.05428(100), 57.07(45), 125.09567(34),144.04478(34), 97.10083(31)
XLR12	10	352.18872(100)
	20	352.18845(100), 125.09588(34), 254.07835(19), 334.17718(12)
	40	254.07881(100), 55.05415(90), 125.09597(61), 144.04426(45), 97.10076(40)
Acetyl fentanyl	10	323.21099(100)
	20	188.14277(100), 323.21137(84), 105.06997(11)
	40	105.06983(100), 188.14296(18)
Acetyl norfentanyl	10	219.1482(100), 84.08043(44)
	20	84.08044(100), 56.04947(20), 55.05437(11)
	40	55.05443(100), 56.04949(55), 84.08061(25), 42.03397(15), 94.06508(14)
Butyryl fentanyl	10	351.24227(100)
	20	351.24284(100), 188.14289(95)
	40	105.06976(100), 188.14324(33)
para-Fluorofentanyl	10	355.21752(100)
	20	355.21766(100), 188.14321(78)
	40	105.06974(100), 188.14301(32)
AH 7921	10	329.118(100), 284.06034(23)
	20	284.06043(100), 172.95501(34), 46.06524(26), 95.08563(18), 189.98181(18)

	40	172.95528(100), 95.0855(47), 46.06533(24), 144.96023(21), 67.05401(18)
ATM4 4-acetoxy analog	10	396.18034(100), 378.16981(33), 354.17022(27), 305.11737(12)
	20	281.11728(100), 354.16972(76), 305.11693(58), 249.09069(32), 323.12785(19)
	40	221.09551(100), 249.09063(40), 217.06478(19), 234.06732(17), 266.09372(15)
3-hydroxy Phenazepam	10	364.96869(100)
	20	
	40	
Bromazepam	10	316.00793(100)
	20	316.008(100), 288.01261(27), 209.09459(18), 261.00239(13), 182.083(11)
	40	182.08334(100), 208.08578(40), 209.09353(38), 259.99373(30), 183.97511(19)
Delorazepam	10	305.02429(100), 304.28364(10)
	20	305.02423(100)
	40	140.02671(100), 206.08231(41), 165.02068(29), 241.05169(26), 228.0446(26)
Diclazepam	10	319.03997(100)
	20	319.03975(100)
	40	227.04904(100), 154.04173(68), 125.01521(28), 275.01344(28), 220.09816(26)
Etizolam	10	343.07775(100)
	20	343.07742(100), 314.03804(26)
	40	314.03875(100), 259.02108(47), 138.03513(25), 295.07326(23), 224.05003(23)
Flubromazepam	10	333.00333(100)
	20	333.00333(100)
	40	226.09008(100), 183.97564(86), 206.08385(47), 105.0573(35), 179.07295(26), 208.97089(23), 109.0448(17), 257.99129(17), 333.00333(16), 104.04948(15),

		225.08225(15) ,198.07135(15), 205.07602(13), 130.05255(12), 211.07918(10)
Pyrazolam	10	354.03416(100), 230.13916(38)
	20	354.03429(100), 230.13921(41)
	40	
1'-naphthoyl-2-methylindole	10	286.12244(100), 155.04888(18)
	20	155.0491(100), 158.05984(36), 286.12264(18), 127.05433(10)
	40	127.05423(100), 158.05972(19), 155.04895(17)
5-IT	10	158.09626(100), 130.065(22)
	20	130.0651(100), 117.05753(47), 158.09621(36), 143.07208(12)
	40	130.06497(100), 117.05727(81), 77.03861(48), 103.05426(40), 115.05401(31)
6-IT	10	158.09626(100), 130.065(22)
	20	130.0651(100), 117.05753(47), 158.09621(36), 143.07208(12)
	40	130.06497(100), 117.05727(81), 77.03861(48), 103.05426(40), 115.05401(31)
A-834735	10	340.22671(100)
	20	125.09588(100), 340.22724(89)
	40	55.05402(100), 125.09563(94), 97.10079(50), 69.06952(47), 57.06966(41)
A-834735 degredant	10	340.22761(100), 242.11781(11)
	20	242.1176(100), 340.22727(54)
	40	99.08037(100), 242.1172(72), 69.06995(64), 144.04399(36), 81.0696(36)
methyl-1-(5-fluoropentyl)-1H-indole-3-Carboxylate	10	264.13943(100), 232.11383(27)
	20	232.1138(100), 144.04486(21), 132.08066(20), 264.13996(12)
	40	144.0446(100), 130.06485(99), 117.05733(68), 116.04948(55), 129.05739(31)
	10	272.16343(100)

methyl-1-(cyclohexylmethyl)-1H-indole-3-carboxylate	20	240.13756(100), 144.04427(71), 272.16492(64), 190.08472(57), 176.06989(39)
	40	55.05439(100), 144.04452(49), 117.05779(26), 116.04914(25), 130.06373(22)
methyl-1-pentyl-1H-indole-3-Carboxylate	10	246.14886(100), 214.12264(38)
	20	214.12264(100), 144.04439(36), 132.08078(22), 43.05423(11)
	40	
ADBICA	10	327.2067(100), 214.12264(69)
	20	214.12264(100)
	40	214.12264(100), 144.04439(85), 43.05423(13)
ADBICA N-(4-hydroxypentyl) metabolite	10	343.20122(100), 230.11742(67), 360.32389(53)
	20	230.11747(100)
	40	144.04396(100), 69.06932(39), 230.11599(35)
ADBICA N-(5-hydroxypentyl) metabolite	10	343.20168(100), 230.11757(56), 360.32318(42)
	20	230.11703(100)
	40	144.04409(100), 230.11737(93), 69.0699(21)
ADBICA N-pentanoic acid metabolite	10	357.18088(100), 244.09682(53)
	20	244.09682(100)
	40	144.04439(100), 244.09682(95), 55.05423(33), 101.05971(23), 83.04914(16)
AM694 N-(5-hydroxypentyl) metabolite	10	434.0607(100), 230.92931(54)
	20	230.92991(100), 434.06091(12)
	40	230.93002(100), 202.93537(47)
AM694 N-pentanoic acid metabolite	10	448.03976(100), 230.9297(35)
	20	230.92933(100), 448.03941(25)
	40	230.92981(100), 202.93487(39)
tetrahydro-Harmine	10	188.10729(100), 200.10736(72), 217.13204(10)
	20	188.10712(100), 200.10748(38), 173.08421(23),185.08318(12)
	40	158.06068(100), 145.08847(95), 130.06534(94), 173.0838(84), 156.081(54)

5-chloro AB-PINACA	10	320.152(100), 348.1475(59), 249.07871(10)
	20	249.07813(100), 320.15247(90)
	40	213.10183(100), 145.0396(67), 249.07836(46), 69.06954(16), 193.01531(10)
5-fluoro ABICA	10	232.11377(100), 331.18298(91)
	20	232.11365(100)
	40	144.044(100), 232.11323(99)
5-fluoro AB-PINACA	10	304.18197(100), 332.17688(66)
	20	233.10847(100), 304.18197(74)
	40	145.03964(100), 213.10224(77), 233.10847(56), 177.04587(31), 69.06988(30), 41.03858(10)
5-fluoro AB-PINACA N-(4-hydroxypentyl) metabolite	10	320.1788(100), 348.17199(71)
	20	249.10329(100), 320.17775(69)
	40	
5-fluoro ADBICA	10	345.19866(100), 232.11447(55)
	20	232.11392(100)
	40	232.11437(100), 144.0446(56)
5-fluoro ADB-PINACA	10	318.19782(100), 346.19342(82)
	20	233.10885(100), 318.19848(94)
	40	213.10263(100), 233.10929(99), 145.03971(85), 69.06982(35), 177.04638(34)
5-fluoro AMB	10	364.20455(100), 304.18299(73), 233.10906(27), 332.17759(15)
	20	233.10919(100), 304.18314(41), 213.10265(12)
	40	145.03985(100), 213.10293(49), 233.10884(26), 69.06996(24), 177.04607(23)
5-fluoro MN-18	10	233.10904(100), 376.18315(86)
	20	233.1089(100), 213.10218(19)
	40	145.03998(100), 213.10267(29), 69.06989(19), 177.04602(19), 41.03896(11)
5-fluoro-AKB48 N-(4-hydroxypentyl) metabolite	10	400.24064(100), 135.11715(46)
	20	135.11713(100)
	40	135.11701(100)
5-fluoro-THJ	10	377.1785(100)

	20	233.10886(100), 377.17824(39), 359.16801(34), 213.10224(16)
	40	145.03996(100), 213.10207(41), 69.06995(20), 177.04614(20), 359.16849(19)
AB-005	10	353.25935(100)
	20	112.11227(100), 353.25947(84), 125.09627(56), 98.09656(54), 256.16996(36)
	40	98.09672(100), 112.11252(49), 125.09617(15), 58.0654(14), 55.05447(11)
AB-005 azepane isomer	10	353.25874(100), 112.11208(27)
	20	112.11208(100)
	40	112.11208(100), 58.06513(27)
AB-CHMINACA	10	340.20319(100), 312.20749(92), 241.13421(11)
	20	241.13399(100), 312.20783(83)
	40	241.13399(100), 145.03959(97), 55.05414(12)
AB-FUBINACA	10	324.15153(100), 352.14816(50), 253.07882(12)
	20	253.07806(100), 324.1513(69)
	40	109.04533(100), 253.07734(26)
AB-FUBINACA 2-fluorobenzyl isomer	10	324.15178(100), 352.14623(69), 253.07786(14)
	20	253.0776(100), 324.15151(47)
	40	109.04479(100), 253.07769(25)
AB-FUBINACA 3-fluorobenzyl isomer	10	324.15178(100), 352.14623(69), 253.07786(14)
	20	253.0776(100), 324.15151(47)
	40	109.04479(100), 253.07769(25)
AB-FUBINACA isomer 1	10	324.15185(100), 352.14689(48), 253.07617(18)
	20	253.07734(100), 324.15149(33)
	40	109.04494(100), 253.07802(18)
AB-FUBINACA isomer 2	10	324.15178(100), 352.14623(69), 253.07786(14)
	20	253.0776(100), 324.15151(47)
	40	109.04479(100), 253.07769(25)
AB-FUBINACA isomer 5	10	352.14558(100), 324.15067(29), 253.07717(15)
	20	253.07717(100), 324.15067(26)

	40	109.0448(100), 253.07717(21)
AB-PINACA	10	286.19185(100), 314.187(46), 215.11826(16)
	20	215.11781(100), 286.19223(37)
	40	145.03994(100), 215.1182(76)
AB-PINACA N-(2-fluoropentyl) isomer	10	304.18241(100), 332.17836(49), 233.10847(10)
	20	233.10894(100), 304.18257(65)
	40	233.10887(100), 145.03961(39)
AB-PINACA N-(4-fluoropentyl) isomer	10	304.18228(100), 332.17763(56)
	20	233.1088(100), 304.1823(57)
	40	145.03964(100), 213.10229(90), 233.10886(80), 69.06993(47), 177.0455(34)
AB-PINACA N-(4-hydroxypentyl) metabolite	10	302.18699(100), 330.18267(86)
	20	302.18674(100), 231.11332(72), 213.10217(57)
	40	213.1022(100), 145.04073(65), 69.06983(25), 175.04903(18)
AB-PINACA N-(5-hydroxypentyl) metabolite	10	302.18699(100), 330.18267(86)
	20	302.18674(100), 231.11332(72), 213.10217(57)
	40	213.1022(100), 145.04073(65), 69.06983(25), 175.04903(18)
AB-PINACA pentanoic acid metabolite	10	316.16624(100), 344.16098(71), 360.32446(66)
	20	316.16593(100), 245.09243(81), 298.15558(69), 217.0968(38), 227.07955(21)
	40	217.09748(100), 227.08192(88), 199.08861(19), 145.03973(18), 175.05113(12)
AKB48 N-(5-fluoropentyl) analog	10	384.24534(100), 135.11694(53)
	20	135.11696(100)
	40	135.11701(100), 93.06999(12)
MN-18	10	215.11827(100), 358.19236(52)
	20	215.11816(100)
	40	145.03972(100), 215.11867(23)
THJ	10	359.18772(100), 215.1181(15)
	20	215.11811(100), 341.17679(23), 359.18734(18)

	40	145.03971(100), 215.1183(33), 341.17646(11)
THJ 018	10	215.11829(100), 343.18154(87)
	20	215.11774(100)
	40	145.03956(100), 215.11782(11)
THJ 2201	10	361.17225(100), 233.10866(78)
	20	233.10873(100), 213.10228(29)
	40	145.03963(100), 213.10312(17), 69.07009(14), 177.04602(13), 41.03897(12)
ADB-FUBINACA	10	338.16723(100), 366.1627(65), 253.07688(11)
	20	253.07754(100), 338.16796(90)
	40	109.04492(100), 253.07694(35)
ADB-PINACA	10	300.20752(100), 328.20255(67), 215.11832(11)
	20	215.11821(100), 300.20782(56)
	40	215.11819(100), 145.03969(92)
ADB-PINACA isomer 1	10	300.20778(100), 328.20252(82), 215.11795(11)
	20	215.11785(100), 300.20765(58)
	40	215.11816(100), 145.03973(89)
ADB-PINACA isomer 2	10	300.20751(100), 328.20283(45), 215.11827(10)
	20	215.11813(100), 300.20756(45)
	40	145.03922(100), 215.11823(90)
ADB-PINACA isomer 3	10	300.20769(100), 328.20257(48), 215.11754(17)
	20	215.11823(100), 300.20699(23)
	40	145.03974(100), 215.11786(82)
ADB-PINACA N-(4-hydroxypentyl) metabolite	10	344.19823(100), 316.20279(99)
	20	316.20225(100), 231.11382(90), 213.10295(33)
	40	213.10198(100), 145.03946(64), 69.06984(38), 175.0488(12)
ADB-PINACA N-(5-hydroxypentyl) metabolite	10	344.19704(100), 316.2023(83)
	20	316.20226(100), 231.11287(73), 213.10263(41)
	40	213.10193(100), 145.03951(37), 69.07013(16), 175.05033(13)
	10	330.1825(100), 358.17585(87)

ADB-PINACA pentanoic acid metabolite	20	330.18171(100), 245.09227(54), 217.09701(18)
	40	217.09694(100), 227.08119(77), 145.03884(14), 199.08577(13)
AKB48 N-(4-fluorobenzyl) analog	10	404.21327(100), 135.11683(90)
	20	135.11663(100)
	40	135.11683(100), 93.06988(11)
AKB48 N-(4-hydroxypentyl) metabolite	10	382.25027(100), 135.11709(39)
	20	135.11698(100)
	40	135.11713(100), 93.07001(12)
AKB48 N-(5-hydroxypentyl) metabolite	10	382.25008(100), 135.11694(33)
	20	135.1169(100), 382.25017(12)
	40	135.117(100)
AKB48 N-pentanoic acid metabolite	10	396.22964(100), 135.11735(39)
	20	135.11701(100), 396.22989(10)
	40	135.11694(100), 93.06995(11)
AMB	10	346.21373(100), 286.19223(87), 215.11817(48), 314.18695(19)
	20	215.11811(100), 286.1922(19)
	40	145.0399(100), 215.11807(44)
4-acetoxy DiPT (hydrochloride)	10	303.2067(100), 114.12773(24), 202.08626(10)
	20	114.12773(100), 160.07569(63), 202.08626(52), 102.12773(10)
	40	160.07569(100), 132.08078(17), 72.08078(16), 115.05423(13), 114.12773(12)
4-acetoxy DMT (hydrochloride)	10	247.14467(100), 58.0653(97), 202.08648(32), 160.07558(10)
	20	58.0653(100), 160.07575(53), 202.08604(12)
	40	58.0653(100), 160.07562(35), 115.05413(27), 132.08089(14), 117.05807(11)
4-hydroxy DET	10	86.09633(100), 233.16548(40), 160.07596(25)
	20	86.09633(100), 160.07553(63)
	40	115.05453(100), 86.09669(80), 160.0759(30), 58.0653(29), 117.05774(29)
4-hydroxy DiPT (hydrochloride)	10	261.19643(100), 114.12793(74), 160.07606(34)

	20	160.07544(100), 114.1279(71)
	40	115.05463(100), 160.07583(86), 132.08089(37), 117.05785(30), 72.08096(20)
4-hydroxy MET	10	72.08056(100), 219.14957(30), 160.07585(26)
	20	72.08054(100), 160.07577(54)
	40	115.05454(100), 72.08087(86), 44.04976(37), 117.0576(27), 160.07585(17)
4-hydroxy MiPT	10	86.09639(100), 233.16535(55), 160.0758(29)
	20	86.09634(100), 160.07562(77), 44.04969(12)
	40	115.05444(100), 44.04969(57), 160.0758(32), 117.05757(28), 132.08095(22)
4-methyl-α-Ethyltryptamine	10	186.12803(100), 144.08101(82), 146.09652(13)
	20	144.08104(100)
	40	144.08101(100), 143.07364(35), 115.05462(21), 91.05421(16), 142.06532(14)
5-methoxy-α-Ethyltryptamine	10	202.12275(100), 160.07585(68)
	20	160.07585(100)
	40	117.05757(100), 145.05235(88), 160.07578(42), 130.06483(16)
DiPT	10	114.12776(100), 245.20164(80), 144.08122(49)
	20	144.08069(100), 114.12788(59)
	40	144.08077(100), 117.06917(36), 143.07304(34), 127.05445(21), 115.05447(18)
DPT (hydrochloride)	10	114.1274(100), 245.20177(41), 144.0808(24)
	20	114.12757(100), 144.08075(80), 86.09646(12)
	40	144.08086(100), 117.06898(39), 143.0728(36), 86.09649(28), 127.05426(23)
N-Methyltryptamine	10	144.08056(100), 132.08092(21)
	20	144.08053(100), 132.08102(17), 117.06839(11)

	40	115.05437(100), 117.06115(79), 143.07301(69), 91.05434(65), 77.03856(32)
AMT (hydrochloride)	10	131.06375(100)
	20	55.0544(100), 131.06379(89), 59.99032(26), 89.01698(21), 77.01687(18)
	40	59.99027(100), 55.05438(25), 71.99048(22)
Methylphenidate (hydrochloride)	10	234.14846(100), 84.08066(62)
	20	84.08044(100)
	40	84.08049(100), 56.04966(21)
MMAI (hydrochloride)	10	161.09616(100), 178.12288(10)
	20	161.09609(100), 105.06997(55), 146.07258(42), 131.07957(18), 91.05413(14)
	40	103.05423(100), 131.04918(51), 91.05425(41), 77.03853(40), 115.05446(35)
Etaqualone	10	265.13323(100)
	20	265.13338(100), 146.09647(11)
	40	131.07315(100), 146.09651(97), 118.06529(44), 130.06553(41), 105.07006(33)
Hydroxy Bupropion	10	238.09923(100)
	20	238.09962(100), 167.04886(28), 139.03111(20), 166.04196(19), 131.07325(14)
	40	131.07319(100), 103.05449(96), 130.06542(83), 139.03103(78), 166.04195(47)
Levamisole	10	205.07886(100)
	20	205.07919(100), 178.06849(61)
	40	91.05434(100), 123.02655(29), 128.06108(18), 117.06299(17), 77.03874(16)
Loperamide	10	477.23075(100), 266.15462(34)
	20	266.15383(100), 477.23181(21)
	40	266.1543(100), 210.12803(36)
N-Phenylacetyl-L-prolylglycine ethyl ester	10	188.10741(100), 216.10233(82), 70.06521(34)
	20	70.06517(100), 188.10711(23)
	40	70.06517(100), 91.0545(22)

Phenylpiracetam	10	174.09098(100), 202.08589(24)
	20	174.09129(100), 145.06486(61), 129.06994(46), 117.06928(13)
	40	117.06935(100), 127.05441(83), 91.0543(62), 115.05414(46), 129.07019(45)
Sildenafil	10	475.21351(100)
	20	475.2122(100)
	40	58.06502(100), 283.11827(28), 100.09952(22), 99.09134(20.00593)
Sildenafil Citrate	10	475.2122(100)
	20	475.2122(100)
	40	58.06513(100), 283.11895(22), 100.0995(20), 99.09167(20)
Thiosildenafil	10	491.19018(100)
	20	491.1908(100)
	40	58.06521(100), 299.09722(47), 99.09142(29), 100.09958(24), 341.14216(14)
Acetildenafil	10	467.27705(100)
	20	467.27718(100)
	40	111.092(100), 127.12303(77), 297.13534(69), 84.08075(66), 72.08079(57)
Benzydamine	10	310.19154(100), 86.09635(48)
	20	86.09617(100)
	40	86.09651(100), 58.06544(76)

VITA

MELANIE NICOLE ECKBERG

2009-2013	B.S., Forensic Science Virginia Commonwealth University Richmond, Virginia
	B.S., Chemistry Virginia Commonwealth University Richmond, Virginia
2012	Undergraduate Researcher Poklis Research Group Virginia Commonwealth University Richmond, Virginia
2012	Intern United States Postal Inspection Service National Forensic Laboratory Services Dulles, Virginia
2013-2014	Teaching Assistant Florida International University Miami, Florida
2013-2016	M.S., Forensic Science Florida International University Miami, Florida
2013-2018	Doctoral Student Florida International University Miami, Florida
2017-2018	Teaching Assistant Florida International University Miami, Florida
2012-present	Society of Forensic Toxicologists, Associate Member
2015-present	American Academy of Forensic Scientists, Associate Member

PUBLICATIONS AND PRESENTATIONS

Eckberg, M.N., Arroyo-Mora, L.E., DeCaprio, A.P. (2015) *Expanded Compound Database and High Resolution MS/MS Spectral Library for the Detection of Designer Drugs by LC-QTOF-MS*. International Forensic Research Institute Symposium, March 2015, Miami, FL.

Eckberg, M.N., Arroyo-Mora, L.E., DeCaprio, A.P. (2015) *Expanded Compound Database and High Resolution MS/MS Spectral Library for the Detection of Designer Drugs by LC-QTOF-MS*. Society of Forensic Toxicologists Annual Meeting, October 2015, Atlanta, GA.

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

Eckberg, M.N., Arroyo-Mora, L.E., Stoll, D.R., DeCaprio, A.P. (2017) *The Separation of Chemically Similar and Isobaric Novel Psychoactive Substances (NPS) Using Two-Dimensional Liquid Chromatography (2D-LC).* American Academy of Forensic Sciences Annual Meeting, February 2017, New Orleans, LA.

Eckberg, M.N., Arroyo-Mora, L.E., Stoll, D.R., DeCaprio, A.P. (2017) *The Separation of Chemically Similar and Isobaric Novel Psychoactive Substances (NPS) Using Two-Dimensional Liquid Chromatography (2D-LC).* International Forensic Research Institute Symposium, March 2017, Miami, FL.

Eckberg, M.N., Arroyo-Mora, L.E., DeCaprio, A.P. (2018) *Development of a High Resolution Mass Spectrometry (HRMS) Library and Method Validation for Screening and Confirmation of 800+ Novel Psychoactive Substances (NPS) by Liquid Chromatography/Quadrupole Time-of-Flight/Mass Spectrometry (LC/QTOF/MS).* American Academy of Forensic Sciences Annual Meeting, February 2018. Seattle, WA.

Eckberg, M.N., Arroyo-Mora, L.E., DeCaprio, A.P. (2018) *Development of a High Resolution Mass Spectrometry (HRMS) Library and Method Validation for Screening and Confirmation of 800+ Novel Psychoactive Substances (NPS) by Liquid Chromatography/Quadrupole Time-of-Flight/Mass Spectrometry (LC/QTOF/MS).* International Forensic Research Institute Symposium, May 2018, Miami, Florida.

Eckberg, M.N., Arroyo-Mora, L.E., Stoll, D.R., DeCaprio, A.P.: *Separation and Identification of Isomeric and Structurally Related Synthetic Cannabinoids Using Two-Dimensional Liquid Chromatography and High Resolution Mass Spectrometry*. (In print).