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Comprehensive Analysis of Synthetic Cannabinoids and Metabolites in Oral Fluid by Liquid Chromatography Triple Quadrupole Mass Spectrometry

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

COMPREHENSIVE ANALYSIS OF SYNTHETIC CANNABINOIDS AND
METABOLITES IN ORAL FLUID BY LIQUID CHROMATOGRAPHY TRIPLE
QUADRUPOLE MASS SPECTROMETRY

A thesis submitted in partial fulfillment of

the requirements for the degree of

MASTER OF SCIENCE

in

FORENSIC SCIENCE

by

Carmen T. Mulet

2019

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

This thesis, written by Carmen T. Mulet, and entitled Comprehensive Analysis of Synthetic Cannabinoids and Metabolites in Oral Fluid by Liquid Chromatography Triple Quadrupole Mass Spectrometry, having been approved in respect to style and intellectual content, is referred to you for judgment.

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Florida International University, 2019

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DEDICATION

I dedicate this work to my parents for their tremendous support, love and encouragement.

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I wish to thank my friends, A.M., A.T., J.R., B.J., L.A., and staff, Pupi a.k.a. ‘my FIU Mom,’ from the FATF department and MSFS program who have shared kind words of advice and motivation throughout these two years. Their experiences and insight not only helped me achieve the completion of my degree, but it instilled in me a positive outlook towards my future involvement in forensics. Furthermore, I would like to extend my gratitude towards my committee members, Dr. McCord, Dr. Almirall, and especially Dr. DeCaprio. Their lessons, mentorship and candor have taught and inspired me to strive for success, despite hardships, and to remain humble when doing so. Honestly, a big ‘thank you’ to you all.

ABSTRACT OF THE THESIS

COMPREHENSIVE ANALYSIS OF SYNTHETIC CANNABINOIDS AND
METABOLITES IN ORAL FLUID BY LIQUID CHROMATOGRAPHY TRIPLE
QUADRUPOLE MASS SPECTROMETRY

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Carmen T. Mulet

Florida International University, 2019

Miami, Florida

Professor Anthony P. DeCaprio, Major Professor

According to NIDA, synthetic cannabinoids (SC) include man-made, mind-altering chemicals. Their typically rapid metabolism and instability in biological matrices requires fast analytical methods for detection and confirmation; therefore, the use of oral fluid (OF) matrix is advantageous because collection is fast and non-invasive. The use of liquid chromatography triple quadrupole mass spectrometry (LC-QqQ-MS) and online solid phase extraction (SPE) for sample pretreatment minimizes bias and facilitates low level detection.

This study demonstrates the first use of online SPE for sample pretreatment in the comprehensive analysis of 100 SC and metabolites in OF by LC-QqQ-MS. The method was validated according the ASB Standard 036, First Edition 2018. Results show that the quantitative method is able to meet the required criteria. Bias and precision values were ≤+20% for all compounds. LOD's and LOQ's ranged from 0.4-3.8 ng/mL and 1.1-11.6 ng/mL, respectively. Recovery, matrix effects, process efficiency, carryover and stability

were also examined and validated. Furthermore, the validated method was able to correctly identify SC spiked into authentic OF samples at relevant levels for forensic toxicological analysis, indicating that it can serve as a valuable tool in intoxication cases.

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LIST OF ABBREVIATIONS AND SYMBOLS

Δ^9 -THC	delta-9-tetrahydrocannabinol
[A]	Aqueous mobile phase
[B]	Organic mobile phase
°C	Degrees Celsius
→	To
2-AG	2-arachidonylglycerol
AAPCC	American Association of Poison Control Centers'
ACN	Acetonitrile
AJS	Agilent Jet Stream Technology
ASB	Academy Standards Board
CAV	Cell accelerator voltage
CE	Collision energy (V)
CNS	Central nervous system
CV	Coefficient of variance
DEA	Drug Enforcement Agency
DI	Deionized water
dMRM	Dynamic MRM
DMSO	Dimethyl sulfoxide
DUID	Driving under the influence of drugs
ELISA	Enzyme-linked immunosorbent assay
EM	Electron multiplier
ESI	Electrospray ionization

FA	Formic acid
GC/MS	Gas chromatography mass spectrometry
H ₂ O	Water
HPLC	High-performance liquid chromatography
hr(s)	Hour(s)
IR	Infrared
ISTD	Internal standard
K ₂	Synthetic cannabinoid
K _i	Inhibitory constant
LC-QqQ-MS	Liquid chromatography triple quadrupole mass spectrometry
LC/MS	Liquid chromatography mass spectrometry
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantitation
ME	Matrix effects
MeOH	Methanol
mg/mL	Milligram per milliliter
min	Minutes
MRM	Multiple reaction monitoring
ms	Milliseconds
MS	Mass spectrometry
MTBE	<i>tert</i> -methyl butyl ether
MW	Molecular weight (g/mol)

ng/mL	Nanogram per milliliter (ppb)
NFLIS	National Forensic Laboratory Information System
N ₂	Nitrogen (g)
NIDA	National Institute on Drug Abuse
NMR	Nuclear magnetic resonance
NPDS	National Poison Data System
NPS	Novel psychoactive substance(s)
OF	Oral fluid
PE	Process efficiency
ppb	Parts per billion
ppm	Parts per million
q	Collision cell
QC	Quality control
QqQ	Triple quadrupole
RE	Recovery
RSD	Relative standard deviation
RT	Room temperature (23°C); Retention time (min)
s	Seconds
SC	Synthetic cannabinoid(s)
SLE+	Supported liquid extraction
SPE	Solid phase extraction
SWGTOX	Scientific Working Group of Forensic Toxicologists
TIC	Total ion chromatogram

TLC	Thin layer chromatography
V	Volts
$\mu\text{g/mL}$	Microgram per milliliter (ppm)

PART ONE: INTRODUCTION

1.1. Synthetic Cannabinoids

“K2, Spice, and Black Mamba” are just some of the commonly referred to street names for the emerging class of NPS known as synthetic cannabinoids (1). According to NIDA, a synthetic cannabinoid is any man-made, mind-altering chemical that is sprayed on dry, shredded plant material and can either be smoked or sold as liquids to be vaporized in e-cigarettes (2).

A 2018 NFLIS mid-year report revealed 10,598 total SC reports within the United States with 67% of reported cases identified as 5F-ADB and FUB-AMB (3). The attraction for these drugs, oftentimes by teenagers and young adults, is that they are marketed as ‘synthetic marijuana’ and not detected by traditional drug tests (4). However, they do not have identical structure and pharmacology as Δ^9 -THC, the main active ingredient in marijuana, therefore, they do not share the same effects on the CB₁ cannabinoid receptor. SC act as full agonists at CB₁ and can be more potent and dangerous than Δ^9 -THC (5). The 2017 AAPCC NPDS annual report identified a total of 1,959 SC intoxication cases with 1,295 of these being single exposure (6).

Potential health risks caused by the consumption of SC include but are not limited to hyperthermia, seizures, hallucinations and acute kidney injury (7). Hydroxylated synthetic cannabinoid metabolites can in some cases be as toxic as parent drugs, where they remain full agonists at nanomolar concentrations, as in the case of JWH 018 (8). Due to the rapid metabolism of SC and their instability in blood, urine, and other matrices, fast analytical methods are needed for the detection and confirmation of parent drugs, as well as metabolites, at very low levels.

Current analytical methods for the detection of synthetic cannabinoids include ELISA (9), GC/MS and LC/MS. A consequence of having established methods is that when novel SC with slightly differing structures emerge their detection using the same technique becomes difficult. Structure modification is one way that clandestine chemists circumvent laws and are able to keep creating SC that may not meet the criteria of a ‘cannabimimetic’ agent (10).

1.2. Purpose of Research

The rapid production of novel SC in the underground market imposes a health risk to the general public, especially towards young adults seeking a new ‘high’ and willing to experiment with stronger yet affordable drugs that are not illegal to possess due to their retail label ‘not for human consumption.’ Unfortunately, the use of these drugs creates a stronger dependence at the CB₁ cannabinoid receptor. Their binding affinity can be much stronger than that of components of classical marijuana (Figure 1), oftentimes causing serious health effects such as tremors, muscle cramps, increased heart rate, seizures and even death. Therefore, detection and confirmation of SC are paramount in the field of clinical toxicology, for example, during routine drug screens or when dealing with intoxication cases.

Figure 1: Comparison of (a) Δ^9 -THC, $K_i = 15.29$ nM, and (b) (1-pentyl-1H-indol-3-yl)-1-naphthalenyl-methanone (JWH-018), $K_i = 1.22$ nM, a SC that emerged in 2008 and was later placed under Schedule I of the Controlled Substances Act.

Due to the typically rapid metabolism of SC and instability in biological matrices (11) blood and urine may not be ideal specimens for the detection and confirmation of parent drugs, as well as metabolites, at very low levels. Collection in OF has been suggested as a suitable, non-traditional matrix because it is rapid, easy to collect, requires less sample processing compared to urine or blood, and runs little risk of adulteration (12).

We hypothesize that a comprehensive analysis of SC and metabolites in OF by LC-QqQ-MS using online SPE for sample pretreatment will be able to detect and quantify current novel parent drugs, as well as metabolites, in a non-invasive matrix using a relatively swift analytical technique sensitive enough to be able to detect low levels of pharmacologically different synthetic cannabinoids.

1.3. Goals of the Project

The analysis of SC has been extensively studied in serum (13) and urine (14) matrices, and to a lesser extent in or OF (15). Hess et al (16) provides an LC/MS/MS method using LLE for a set of 93 SC in serum. Using MRM for analysis, the method had LODs as low as < 1 ng/mL for 81 standards and was able to detect and quantify the presence of SC for 64 authentic serum samples at concentrations as low as 0.1–5 ng/mL.

Traditionally, metabolites of SC have been analyzed in urine or blood matrices (17-19). However, Amaratunga et al (20) reported the first quantitative analysis of XLR-11 and UR-144 and their metabolites, as well as pyrolysis products, in OF by LC/MS/MS while using classical (off-line) SPE for sample cleanup. Their results demonstrated the detection and quantification of the 4-hydroxy-UR-144 metabolite in OF, but not that of XLR-11. In general, few reports exist on the analysis of SC metabolites in OF.

OF is a viscous matrix composed of water, ions, and high molecular weight molecules, including proteins and enzymes (21). A sample pretreatment step is, therefore, required prior to sample analysis, typically by LLE or SPE. Unfortunately, both procedures are time-consuming and involve large amounts of solvents for cleanup. Conversely, online-SPE can be used to purify samples prior to analysis, as demonstrated by Heuett et al (22), who used this technique prior to illicit drug detection in wastewater. However, methods for the analysis of a wide class of SC and metabolites in OF using online SPE are not currently available in the literature.

1.3.1. Specific Aim 1. Creation of an online SPE LC-QqQ-MS method

An LC-QqQ-MS based dynamic MRM method for 100 SC compounds, either Schedule I, temporarily Schedule I or recently emerged parent drugs (not scheduled), will be created and optimized in MeOH (Appendix 1). Online SPE parameters for sample pretreatment will then be incorporated and tested using a subset of 20 SC compounds consisting of eight parent SC and metabolites of PB-22 (23), JWH 018 (24) and AB-PINACA (25) in MeOH. The use of metabolites is to infer the presence of parent drug in cases where parent drugs have fully metabolized. This subset will be used to optimize: 1) solvent composition, 2) valve time switching, 3) online SPE cartridge selection and 4) matrix compatibility.

1.3.2. Specific Aim 2. Validation of an online SPE LC-QqQ-MS method

The online SPE LC-QqQ-MS method will be validated using ASB Standard 036, First Edition 2018, and others (26-31). The following parameters will be assessed: calibration curve model, LOD, LOQ, intra- and inter-day bias and precision, ME, PE, RE, freeze-and-thaw stability, carryover and selectivity. Method validation criteria requires

bias and precision to be $\leq 20\%$ (26). Results obtained should demonstrate the online-SPE LC-QqQ-MS method to be fast, sensitive and effective for the detection and confirmation of 100 SC compounds in OF.

1.3.3. Specific Aim 3. Authentic sample screen

To fully validate the comprehensive method, a screen of authentic samples in OF will be attempted, pending the collection of relevant specimens. Results obtained should confirm the method to be applicable for the detection of either parent drug or metabolite in OF collected from SC abusers.

1.4. Project Limitations

Pyrolysis products generated in the case of smoking can lead to oral cavity contamination hindering SC quantitation and further interpretation. It is recommended in these cases to take samples 1-2 h after smoking but perhaps leading to loss of detection due to the rapid metabolism of SC.

Additionally, in the case that authentic samples cannot be obtained by the laboratory, a blind study will be carried out by spiking into OF representative samples, as substitute, and treated as authentic. The samples will be prepared and analyzed accordingly to test the applicability of the online SPE LC-QqQ-MS method.

PART TWO: LITERATURE REVIEW

2.1. Introduction to cannabinoids

The term ‘cannabinoid’ refers to drugs that bind to CB₁ and CB₂ receptors in the brain and elsewhere but can also be used to refer to drugs that have similar pharmacological properties to that of Δ^9 -tetrahydrocannabinol (THC) (32). Three types of cannabinoids are recognized in the literature, namely phytocannabinoids, endocannabinoids and synthetic cannabinoids. Phytocannabinoids are terpenophenols that are naturally found in the marijuana plant, while endocannabinoids are ligands produced within the CNS and include compounds such as 2-AG and anandamide. Additional compounds which do not fall in either of the two aforementioned categories are considered SC, or rather laboratory-derived drugs (33).

2.2. Emergence of synthetic cannabinoids

SC were originally investigated in academic laboratories, (*e.g.*, the HU series by Hebrew University and the JWH series by Clemson University), and in pharmaceutical companies (*e.g.*, the CP series by Pfizer and tetramethylcyclopropyl derivatives by Abbott Laboratories) for possible therapeutic use. The intention had been to enable the study of the endocannabinoid system by observing how certain drugs interacted with the cannabinoid receptors CB₁ and CB₂, in the hope of discovering ligands that would favor CB₂ activation over that of CB₁ (34). However, the emergence of SC for illicit use first became apparent in Germany in 2008 and from there spread to different parts of Europe, China, the United States, and other parts of the world where they were abused for their psychoactive properties (35). Smoking of SC-laced potpourri known as ‘K2’ and ‘Spice’ became notably popular among young individuals as a way to evade a positive drug

screen as these compounds did not show up on traditional tests (36). Growing concern among public law officials who received an increase in calls from poison control centers prompted action from the federal government (37).

2.3. DEA Scheduling

The Controlled Substances Act was added onto the Comprehensive Drug Abuse Prevention and Control Act of 1970 in order to regulate how certain drugs are manufactured, used and distributed. Each drug is listed as being either Schedule I-V, with those listed as Schedule I being the most regulated. According to the CSA, a number of SC (*e.g.*, JWH 018, CP-47,497, JWH 073, AM2201, etc.) have been determined as Schedule I; possessing no currently accepted medical use, a high potential for abuse, and lacking safety under medical supervision (38). With the creation of the Synthetic Drug Abuse Prevention Act of 2012, temporary scheduling of emergent SC considered to be very hazardous was made possible, which facilitated the addition of the drug as Schedule I for one to two years (10). Most recently, the DEA ordered temporarily placement of 5F-EDMB-PINACA, 5F-MDMB-PICA, FUB-AKB48, 5F-CUMYL-PINACA and FUB-144 into Schedule I (39).

2.4. Classification

Since their creation, hundreds of SC have been modified to include a variety of benzoyl, naphthoyl, phenylacetyl, carboxylate, thiazolyl, and carboxamide derivative structural groups (40). Nonetheless, a SC is composed of four key elements as illustrated in Figure 2: 1) a core, typically an indole or indazole, 2) a linker, notably a ketone or amide, 3) a ring, such as a phenyl, cyclopropyl or naphthyl, and 4) a tail consisting of a hydrophobic alkyl group (32). The addition of a halogen or other functional group onto

the tail, ring or core is commonly seen with newer SC, which often creates an increase in binding affinities, K_i , and potency (41). Shevyrin et al. proposed the following classification for currently existing SC: 1) classical, 2) non-classical, 3) hybrid, 4) 3-carbonylindoles, 5) 3-carbonylindazoles, 6) eicosanoids, and 7) miscellaneous. The largest group noted as number four, formerly known as aminoalkylindoles, now includes naphthoylindoles, phenylacetylindoles, benzoylindoles, cycloalkanecarbonylindoles, indole-3-carboxamides, indole-3-carboxylates and other 3-carbonylindoles. The classification mentioned above is not exhaustive and has implemented modifications with emerging SC (42).

Figure 2: Illustrates that while a SC is mainly composed of a core, linker, ring and tail there are a variety of options for each demonstrating its complexity in detection (43).

2.5. Pharmacology and Toxicology

The mechanism of action for most SC involves binding to CB₁ cannabinoid receptors as full agonists in the CNS. This allows activation of G_{i/o}-proteins that inhibit the activity of downstream intracellular effector adenylyl cyclase (AC) which reduce cAMP levels. Measurements of these values give an indication of SC potency and efficacy. While many SC are potent at nanomolar concentrations, the pharmacological effects they possess cannot be definitively predicted. For example, as determined in rat models, while JWH 015 and JWH 073 impair visual, acoustic, and tactile sensorimotor systems, JWH 018 and AM2201 increase CNS excitability; all four compounds belonging to the aminoalkylindole class yet having different effects (41, 44).

2.6. Salivary glands and Oral fluid matrix

Oral fluid is generated by three major salivary glands in the oral cavity, namely the parotid, sublingual, and submandibular (21). The movement of drugs excreted by salivary glands into the oral cavity is dependent on factors of pH, pKa, lipophilicity, and amount of unbound drug circulating in the bloodstream (45-46). Small polar drugs are transferred from blood to OF by paracellular transport, while electrolytes and some proteins are assisted by active transport. In contrast, highly lipophilic drugs such as SC are able to freely cross by passive transcellular diffusion (10). Kneisel et al. demonstrated the transfer of SC from blood to OF and concluded that this process can occur at very low nanomolar levels (47). Coulter et al. was only able to detect 0.5 ng/mL of JWH 018 in OF from one subject 12 h after smoking and hardly any drug from a second subject at 5 h (48). However, disadvantages of this matrix include a short detection window, especially in the case of cannabis, and low volume and drug collection

(49). Few reports exist on the analysis of SC and their metabolites in OF. In one study, Amaratunga et al. (20) reported quantitative analysis of XLR-11 and UR-144 and their metabolites, as well as pyrolysis products, in OF by LC/MS/MS. Hence, the use of LC-QqQ-MS is the method of choice for the detection of SC in OF because it is a sensitive technique capable of rapid analysis at low concentrations through the use of chromatographic and mass spectral techniques.

2.7. Sample purification

Traditional matrices for the detection of SC include blood and serum, as well as urine for the confirmation of metabolites. Oftentimes these matrices require lengthy purification techniques in order to extract the DOA, which can be expensive and require large volumes of solvent when dealing with methods such as LLE or SPE (50-51). For example, Mazzarino et al. (50) proposed an analytical method for the detection of aminoalkylindole SC analogues and their metabolites in urine, blood, hair and OF. Sample purification was carried out using LLE with 5-7 mL of MTBE for *in vitro*, urine, serum/plasma and OF samples. Wohlfarth et al. purified two authentic samples using SLE+ cartridges for the analysis of AB-PINACA and 5F-AB-PINACA metabolites in urine eluting twice with 3 mL ethyl acetate (25). Similarly, analysis of JWH 073 and its metabolites in blood and urine by Ozturk et al. (51) used OASIS HLB SPE cartridges for matrix clean-up and 3 mL ethyl acetate for elution. In the case of OF, it is possible to reduce the effect of the matrix by simply diluting with solvents prior to analysis, as demonstrated by Malaca et al. (52) who were able to detect a range of drugs in OF by diluting 1:3 with water and injecting into an UHPLC-MS/MS. Online SPE for the detection of SC is limited, but the technique has been used for the analysis of 42

therapeutic drugs and drugs of abuse in urine (53) and for the analysis of seven amphetamines in blood and urine (54).

2.8. Detection techniques

Confirmatory techniques for the identification of SC and metabolites typically utilizes mass spectrometry for fragmentation confirmation (55). A review by Znalezionna et al. (19) on identification and separation techniques also mentions the use of NMR and IR spectroscopy for analysis of herbal mixes containing pharmacologically different compounds, immunoassays carried out for the analysis of over 2,000 urine samples, TLC for the separation of 21 SC in herbal blends, and more. Coulter et al. (48) published one of the first analytical methods with OF for the detection of JWH 018, JWH 073, JWH 200, JWH 250, HU-210, CP-47,497 and CP-47,497 C8 homologue using LC/MS/MS. The study was able to detect JWH 018 as low as 3 ng/mL 20 min after smoking from one of their subjects.

PART THREE: INSTRUMENTATION

3.1. Overview

In liquid chromatography, solvents of different polarities are passed through a column creating a mobile and stationary phase, respectively. The sample, or analyte, is flushed through the column allowing for interactions to take place. By varying factors such as polarity and solvent pH the interactions between the sample and column strengthen or weaken depending on analyte pKa. The sample continues to interact and move along the column until it elutes from the analytical column. The retention time represents how long it takes for this process to occur.

The sample is then ionized prior to reaching the MS. Ions are generated through ESI, which is a soft ionization technique, by drying and converting mobile phase droplets into smaller units. Superheated gas is used to evaporate the mobile phase until the Rayleigh limit is reached and a coulombic explosion occurs (Figure 3). The ions are then filtered prior to entering the MS by varying capillary voltage.

Figure 3: (Left to right) ESI technique: representation of heated droplet reaching its Rayleigh limit and creating a coulombic explosion of ions in the gas phase.

Once inside the spectrometer, the ions are filtered according to their mass-to-charge (m/z) ratio. A triple quadrupole (QqQ) MS consists of three main chambers that are used

for filtering. The first chamber consists of a quadrupole (Q_1) that filters only ions of a target mass and are called precursor ions ($[M+H]^+$ in positive mode ESI). The second chamber is a hexapole collision cell (q) where the precursor ions are hit at high speeds by an inert gas (*e.g.*, N_2) to generate fragment ions. The third chamber has a second quadrupole (Q_3) which is used to filter through the fragments and select product ions for compound identification. Control and detection of ions is accomplished by varying voltages and selecting different modes on the instrument to scan either in full scan, MRM, dynamic MRM, or triggered MRM mode. Full scan acquires all generated precursor ions from a sample, while MRM scans for compound transitions (precursor $\rightarrow$ product ion) multiple times throughout the analytical run. Similarly, dynamic and triggered MRM scan transitions but are limited by a time-frame; however, primary transitions must reach a certain threshold prior to collecting data for triggered MRM.

The detector consists of a conversion dynode which sends the generated signal through to the EM for quantitation. Each signal generates a chromatogram on the software. The proposed method in this thesis involves the use of dynamic MRM mode, as described above.

3.2. Hardware

Analysis was performed using an Agilent 1290 Infinity II autosampler and binary LC (Agilent Technologies, Santa Clara, CA) coupled to an Agilent 6470 triple quadrupole MS (Figure 4) equipped with electrospray ionization (ESI) and Agilent Jet Stream (AJS) Technology (Figure 5). Online SPE was performed using an Agilent 1290 Infinity II Online SPE system consisting of a 1290 Infinity Flexible Cube (FlexCube) and

two valves operated in 'Direct Inject' or 'Online SPE' mode.

Figure 4: Agilent 6470 QqQ-MS Model.

Figure 5: (Left to right) Agilent Jet Stream Technology and ESI source.

3.3. LC Conditions

Gradient elution was performed using 5 mM ammonium formate + 0.1% FA for aqueous [A] phase and MeOH + 0.1% FA for organic [B] phase. Solvent flow was 0.4 mL/min set to deliver 10% B (0 min), 10% B (1.50 min), 80% B (2.50 min), 90% B (8.00 min), 100% B (10.00 min) and 5% B (10.01 min). Total run time was 11.00 min with a post-column equilibration time of 1.00 min. A 1 μ L sample was injected from the temperature-controlled autosampler (23°C) to the online SPE system and through a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column (Agilent, Santa Clara, CA) maintained at 40°C and connected to an in-line filter (0.3 μ m). A 30 s needle wash consisting of ACN:MeOH:DI-H₂O (25:25:50, v/v/v) was used to minimize carryover effects.

3.4. Flexible Cube Setup

The online SPE FlexCube instrumentation, consisting of a flush pump and solvent selection valve with three solvent lines (A1A, A2A and B2B), was operated in the ‘Online SPE’ mode and maintained at a flow of 0.5 mL/min for delivery alternating through a pair of Agilent PLRP-s SPE cartridges on the second valve. Each cartridge was loaded/reconditioned (A2A) and washed (A2A, B2B and A1A) with (90:10, v:v) 0.1% FA in water:MeOH (A2A), 100% ACN (B2B) and 100% MeOH (A1A). At the start of an injection, the flush pump was set to load a sample onto cartridge 1 using A2A for 170 s while the binary pump simultaneously delivered solvent through cartridge 2 following the LC gradient parameters. The flow path of the flush pump ended in ‘waste’ and that of the binary pump carried on towards the column. At 2.84 min, the online SPE system was set to immediately switch the flow path and back-flush the compounds from cartridge 1

onto the analytical column using the binary pump; meanwhile, cartridge 2 was cleaned by the flush pump with A2A for 60 s, B2B for 60 s, A1A for 120 s, and then reconditioned with A2A for 120 s. The next sample was loaded onto cartridge 2 and the process repeated vice versa. Details of the FlexCube parameters utilized in the online SPE protocol are shown in Figure 6 (56).

Figure 6: Agilent 1290 Infinity II Online SPE system depicting (a) binary (0.4 mL/min) and (b) flush pump (0.5 mL/min) solvent flow and sample loading (arrows) through Agilent PLRP-s (15 μ m) SPE cartridge 1: sample was delivered onto the cartridge with 0.1% formic acid in water (A2A) for 170 s and then the flow path was switched at 2.84 min to elute the compounds onto the analytical column using the binary pump; meanwhile, cartridge 2 was cleaned by the flush pump with 0.1% formic acid in water (A2A) for 60 s, 100% ACN (B2B) for 60 s, 100% MeOH (A1A) for 120 s, and then reconditioned with A2A for 120 s. MassHunter qualitative software profile showing binary (red line) and flush pump (green intervals) solvent deliveries depicted above.

3.5. MS Parameters

The mass spectrometer was operated in positive ESI mode using dynamic MRM with a cycle time of 800 ms, three MRM repeats and a cell accelerator voltage (CAV) of 4 V for each compound as detailed in Table 1. The drying and sheath gas were set to 300°C (5 L/min) and 250°C (11 L/min), respectively, while the nebulizer operated at 45 psi. The capillary voltage was fixed at +3500 V. Agilent MassHunter Software (Version B.08.02) was used for data acquisition and analysis.

Table 1. Compound data, dynamic MRM QqQ-MS transitions, optimized MS parameters, internal standards, and Mix assignments for all analytes.

ID #	Compound	Molecular Formula	Transitions ^a (m/z)			Frag. (V)	CE (V)	RT (min)	Internal Standard	Mix #
1	(+/-) JWH 018 N-(2-hydroxypentyl) metabolite	C ₂₄ H ₂₃ NO ₂	358.2	→	155.1	120	28	5.33	(+/-) JWH 018 N-(4-hydroxypentyl) metabolite- <i>d</i> ₅	M-1
			358.2	→	127.1	120	56			
2	(+/-) JWH 018 N-(3-hydroxypentyl) metabolite	C ₂₄ H ₂₃ NO ₂	358.2	→	155.1	120	24	5.20	(+/-) JWH 018 N-(4-hydroxypentyl) metabolite- <i>d</i> ₅	M-2
			358.2	→	127.1	120	56			
3	(+/-) JWH 018 N-(4-hydroxypentyl) metabolite	C ₂₄ H ₂₃ NO ₂	358.2	→	155.1	120	20	4.94	(+/-) JWH 018 N-(4-hydroxypentyl) metabolite- <i>d</i> ₅	M-2
			358.2	→	127.1	120	56			
4	(+/-) WIN 55,212 (mesylate)	C ₂₇ H ₂₆ N ₂ O ₃ • CH ₃ SO ₃ H	427.2	→	155.0	120	24	5.41	JWH 200- <i>d</i> ₅	M-1
			427.2	→	127.0	120	60			
5	5-chloro AB-PINACA	C ₁₈ H ₂₅ ClN ₄ O ₂	365.2	→	348.1	96	4	4.91	AB-PINACA- <i>d</i> ₉	M-1
			365.2	→	320.1	96	12			
6	5-fluoro ABICA	C ₁₉ H ₂₆ FN ₃ O ₂	348.2	→	331.1	84	4	4.52	ADBICA- <i>d</i> ₉	M-2
			348.2	→	232.1	84	20			
7	5-fluoro AB-PINACA	C ₁₈ H ₂₅ FN ₄ O ₂	349.2	→	304.1	108	12	4.56	AB-PINACA- <i>d</i> ₉	M-2
			349.2	→	233.0	108	24			
8	5-fluoro ADBICA	C ₂₀ H ₂₈ FN ₃ O ₂	362.2	→	345.2	96	8	4.78	ADBICA- <i>d</i> ₉	M-2
			362.2	→	232.1	96	20			
9	5-fluoro ADB-PINACA	C ₁₉ H ₂₇ FN ₄ O ₂	363.2	→	346.2	96	4	4.86	ADB-PINACA- <i>d</i> ₉	M-2
			363.2	→	318.2	96	12			
10	5-fluoro AMB	C ₁₉ H ₂₆ FN ₃ O ₃	364.2	→	304.1	108	12	5.20	AB-PINACA- <i>d</i> ₉	M-2
			364.2	→	233.0	108	20			
11	5-fluoro PB-22	C ₂₃ H ₂₁ FN ₂ O ₂	377.2	→	232.1	96	12	5.29	PB-22- <i>d</i> ₉	M-2
			377.2	→	144.0	96	44			
12	5-fluoro PB-22 3-carboxyindole metabolite	C ₁₄ H ₁₆ FNO ₂	250.1	→	206.1	120	12	4.44	PB-22- <i>d</i> ₉	M-2
			250.1	→	118.0	120	20			

13	AB-CHMINACA	$C_{20}H_{28}N_4O_2$	357.2 357.2	→ →	340.1 241.0	96 96	4 28	5.79	AB-FUBINACA- d_4	M-0 M-1
14	AB-FUBINACA	$C_{20}H_{21}FN_4O_2$	369.2 369.2	→ →	324.1 109.0	96 96	12 48	4.67	AB-FUBINACA- d_4	M-0 M-1
15	AB-PINACA	$C_{18}H_{26}N_4O_2$	331.2 331.2	→ →	286.1 215.0	96 96	12 28	5.26	AB-PINACA- d_9	M-0 M-1
16	AB-PINACA N-(4-hydroxypentyl) metabolite	$C_{18}H_{26}N_4O_3$	347.2 347.2	→ →	330.1 302.1	108 108	4 12	4.18	AB-PINACA- d_9	M-0 M-1
17	AB-PINACA N-(5-hydroxypentyl) metabolite	$C_{18}H_{26}N_4O_3$	347.2 347.2	→ →	330.1 302.1	96 96	4 12	4.18	AB-PINACA- d_9	M-0 M-2
18	AB-PINACA pentanoic acid metabolite	$C_{18}H_{24}N_4O_4$	361.2 361.2	→ →	344.1 316.1	96 96	4 12	4.13	AB-PINACA- d_9	M-0 M-1
19	ADB-FUBINACA	$C_{21}H_{23}FN_4O_2$	383.2 383.2	→ →	366.1 338.1	108 108	4 12	4.97	AB-FUBINACA- d_4	M-2
20	ADBICA	$C_{20}H_{29}N_3O_2$	344.2 344.2	→ →	214.1 144.0	96 96	20 44	5.66	ADBICA- d_9	M-1
21	ADB-PINACA	$C_{19}H_{28}N_4O_2$	345.2 345.2	→ →	300.1 215.0	96 96	12 24	5.68	ADB-PINACA- d_9	M-2
22	AM1220	$C_{26}H_{26}N_2O$	383.2 383.2	→ →	112.1 98.1	120 120	20 40	3.98	AM2201- d_5	M-1
23	AM2201	$C_{24}H_{22}FNO$	360.2 360.2	→ →	155.0 127.0	120 120	24 56	5.75	AM2201- d_5	M-1
24	AM2201 2-hydroxyindole metabolite	$C_{24}H_{22}FNO_2$	376.2 376.2	→ →	270.1 252.1	120 120	24 44	7.76	AM2201- d_5	M-2
25	AM2201 5-hydroxyindole metabolite	$C_{24}H_{22}FNO_2$	376.2 376.2	→ →	155.0 127.0	120 120	28 56	4.83	AM2201- d_5	M-2
26	AM2233	$C_{22}H_{23}IN_2O$	459.1 459.1	→ →	112.1 98.1	120 120	24 40	3.96	AM2201- d_5	M-1

27	AM694	C ₂₀ H ₁₉ FINO	436.1 436.1	→ →	230.9 202.9	120 120	28 56	5.20	AM2201- <i>d</i> ₅	M-2
28	HU-211	C ₂₅ H ₃₈ O ₃	387.3 387.3	→ →	243.1 71.1	108 108	16 24	8.98	JWH 018- <i>d</i> ₉	M-2
29	JWH 007	C ₂₅ H ₂₅ NO	356.2 356.2	→ →	155.0 127.0	120 120	24 56	7.50	JWH 007- <i>d</i> ₉	M-2
30	JWH 015	C ₂₃ H ₂₁ NO	328.2 328.2	→ →	155.0 127.0	120 120	20 48	6.04	JWH 015- <i>d</i> ₇	M-2
31	JWH 018	C ₂₄ H ₂₃ NO	342.2 342.2	→ →	214.1 127.1	95 95	24 56	7.13	JWH 018- <i>d</i> ₉	M-0 M-1
32	JWH 018 2-hydroxyindole metabolite	C ₂₄ H ₂₃ NO ₂	358.2 358.2	→ →	340.2 270.1	108 108	16 24	9.82	JWH 018- <i>d</i> ₉	M-1
33	JWH 018 4-hydroxyindole metabolite	C ₂₄ H ₂₃ NO ₂	358.2 358.2	→ →	155.1 127.1	120 120	28 60	7.19	JWH 018 4-hydroxyindole metabolite- <i>d</i> ₉	M-0 M-1
34	JWH 018 5-hydroxyindole metabolite	C ₂₄ H ₂₃ NO ₂	358.2 358.2	→ →	155.0 127.0	120 120	24 56	5.77	JWH 018 N-pentanoic acid metabolite- <i>d</i> ₅	M-1
35	JWH 018 6-hydroxyindole metabolite	C ₂₄ H ₂₃ NO ₂	358.2 358.2	→ →	155.0 127.0	120 120	24 60	5.60	JWH 018 6-hydroxyindole metabolite- <i>d</i> ₉	M-2
36	JWH 018 7-hydroxyindole metabolite	C ₂₄ H ₂₃ NO ₂	358.2 358.2	→ →	155.0 127.0	120 120	24 56	6.19	JWH 018 7-hydroxyindole metabolite- <i>d</i> ₉	M-0 M-1
37	JWH 018 adamantyl analog	C ₂₄ H ₃₁ NO	350.2 350.2	→ →	135.1 93.1	120 120	32 56	9.55	JWH 018- <i>d</i> ₉	M-2
38	JWH 018 N-(4-oxo-pentyl) metabolite	C ₂₄ H ₂₁ NO ₂	356.2 356.2	→ →	155.0 127.0	120 120	20 56	4.97	JWH 018 N-pentanoic acid metabolite- <i>d</i> ₅	M-0 M-1
39	JWH 018 N-(5-hydroxypentyl) metabolite	C ₂₄ H ₂₃ NO ₂	358.2 358.2	→ →	155.0 127.0	120 120	20 52	4.99	JWH 018 N-(5-hydroxypentyl) metabolite- <i>d</i> ₅	M-0 M-1
40	JWH 018 N-propanoic acid metabolite	C ₂₂ H ₁₇ NO ₃	344.1 344.1	→ →	155.0 127.0	108 108	24 56	4.68	JWH 018 N-pentanoic acid metabolite- <i>d</i> ₄	M-0 M-1

41	JWH 019	C ₂₅ H ₂₅ NO	356.2 356.2	→ →	155.0 127.0	120 120	24 56	7.98	JWH 018- <i>d</i> ₉	M-2
42	JWH 020	C ₂₆ H ₂₇ NO	370.2 370.2	→ →	155.0 127.0	120 120	28 60	8.81	JWH 018- <i>d</i> ₉	M-1
43	JWH 073	C ₂₃ H ₁₄ D ₇ NO	328.2 328.2	→ →	155.0 127.0	120 120	24 48	6.48	JWH 073- <i>d</i> ₇	M-2
44	JWH 081	C ₂₅ H ₂₅ NO ₂	372.2 372.2	→ →	185.0 157.0	120 120	28 48	7.68	JWH 081- <i>d</i> ₉	M-2
45	JWH 098	C ₂₆ H ₂₇ NO ₂	386.2 386.2	→ →	185.0 157.0	120 120	24 48	7.99	JWH 018- <i>d</i> ₉	M-1
46	JWH 122	C ₂₅ H ₂₅ NO	356.2 356.2	→ →	169.0 141.0	120 120	24 48	8.09	JWH 122- <i>d</i> ₉	M-0 M-1
47	JWH 182	C ₂₇ H ₂₉ NO	384.2 384.2	→ →	197.1 141.0	120 120	28 52	9.29	JWH 018- <i>d</i> ₉	M-0 M-1
48	JWH 200	C ₂₅ H ₂₄ N ₂ O ₂	385.2 385.2	→ →	155.0 114.1	120 120	20 28	4.70	JWH 200- <i>d</i> ₅	M-2
49	JWH 203	C ₂₁ H ₂₂ ClNO	340.1 340.1	→ →	144.0 125.0	113 113	44 32	6.80	JWH 018- <i>d</i> ₉	M-2
50	JWH 210	C ₂₆ H ₂₇ NO	370.2 370.2	→ →	183.1 155.1	120 120	28 44	8.59	JWH 210- <i>d</i> ₉	M-2
51	JWH 249	C ₂₁ H ₂₂ BrNO	384.1 384.1	→ →	168.9 90.1	120 120	28 60	6.98	JWH 018- <i>d</i> ₉	M-2
52	JWH 251	C ₂₂ H ₂₅ NO	320.2 320.2	→ →	214.1 105.1	120 120	24 24	6.97	JWH 250- <i>d</i> ₅	M-2
53	JWH 307	C ₂₆ H ₂₄ FNO	386.2 386.2	→ →	155.0 127.0	120 120	20 56	7.66	JWH 018- <i>d</i> ₉	M-2
54	JWH 307 5'-isomer	C ₂₆ H ₂₄ FNO	386.2 386.2	→ →	155.1 127.1	120 120	20 60	6.90	JWH 018- <i>d</i> ₉	M-1

55	JWH 387	C ₂₄ H ₂₂ BrNO	420.1 420.1	→ →	233.0 205.0	120 120	28 52	8.83	JWH 018- <i>d</i> ₉	M-2
56	JWH 398	C ₂₄ H ₂₂ ClNO	376.2 376.2	→ →	189.0 161.0	120 120	24 52	8.62	JWH 398- <i>d</i> ₉	M-2
57	JWH 412	C ₂₄ H ₂₂ FNO	360.2 360.2	→ →	173.0 145.0	108 108	24 56	7.74	JWH 018- <i>d</i> ₉	M-2
58	MAM2201 N-(3-fluoropentyl) isomer	C ₂₅ H ₂₄ FNO	374.2 374.2	→ →	169.1 141.1	120 120	28 52	6.82	MAM2201- <i>d</i> ₅	M-1
59	MAM2201 N-(5-chloropentyl) analog	C ₂₅ H ₂₄ ClNO	390.2 390.2	→ →	169.1 141.1	108 108	28 56	6.95	MAM2201- <i>d</i> ₅	M-2
60	PB-22	C ₂₃ H ₂₂ N ₂ O ₂	359.2 359.2	→ →	214.1 144.0	60 60	8 40	6.27	PB-22- <i>d</i> ₉	M-0 M-1
61	PB-22 3-carboxyindole metabolite	C ₁₄ H ₁₇ NO ₂	232.1 232.1	→ →	132.0 118.0	120 120	16 20	5.11	PB-22- <i>d</i> ₉	M-0 M-1
62	PB-22 N-(4-hydroxypentyl) metabolite	C ₂₃ H ₂₂ N ₂ O ₃	375.2 375.2	→ →	230.1 144.0	84 84	12 36	4.66	PB-22- <i>d</i> ₉	M-0 M-1
63	PB-22 N-(5-hydroxypentyl) metabolite	C ₂₃ H ₂₂ N ₂ O ₃	375.2 375.2	→ →	230.1 144.0	96 96	8 44	4.62	PB-22- <i>d</i> ₉	M-0 M-2
64	PB-22 N-pentanoic acid metabolite	C ₂₃ H ₂₀ N ₂ O ₄	389.2 389.2	→ →	244.1 144.0	84 84	12 40	4.58	PB-22- <i>d</i> ₉	M-0 M-1
65	RCS-4	C ₂₁ H ₂₃ NO ₂	322.2 322.2	→ →	135.0 77.1	120 120	24 60	6.47	RCS-4- <i>d</i> ₉	M-2
66	RCS-4-C4 homolog	C ₂₀ H ₂₁ NO ₂	308.2 308.2	→ →	135.0 77.0	103 103	20 48	5.85	RCS-4- <i>d</i> ₉	M-1
67	RCS-8	C ₂₅ H ₂₉ NO ₂	376.2 376.2	→ →	121.0 91.1	120 120	24 60	8.18	RCS-4- <i>d</i> ₉	M-2
68	THJ 018	C ₂₃ H ₂₂ N ₂ O	343.2 343.2	→ →	215.0 145.0	108 108	16 16	8.10	JWH 018- <i>d</i> ₉	M-1

69	THJ 2201	C ₂₃ H ₂₁ FN ₂ O	361.2 361.2	→ →	233.0 145.0	120 120	16 40	6.43	JWH 018- <i>d</i> ₉	M-2
70	UR-144	C ₂₁ H ₂₉ NO	312.2 312.2	→ →	125.1 55.1	120 120	20 40	8.10	UR-144- <i>d</i> ₅	M-1
71	XLR11	C ₂₁ H ₂₈ FNO	330.2 330.2	→ →	125.1 55.1	120 120	24 48	6.44	XLR11- <i>d</i> ₅	M-0 M-1
72	XLR11 6-hydroxyindole metabolite	C ₂₁ H ₂₈ FNO ₂	346.2 346.2	→ →	125.1 55.1	120 120	24 48	5.28	XLR11- <i>d</i> ₅	M-2
73	XLR11 N-(4-hydroxypentyl) metabolite	C ₂₁ H ₂₈ FNO ₂	346.2 346.2	→ →	248.1 144.0	96 96	20 36	5.00	XLR11- <i>d</i> ₅	M-2
74	(+/-) JWH 018 N-(4-hydroxypentyl) metabolite- <i>d</i> ₅	C ₂₄ H ₁₈ D ₅ NO ₂	363.2 363.2	→ →	156.1 155.0	120 120	20 20	4.95	-	IS
75	AB-FUBINACA- <i>d</i> ₄	C ₂₀ H ₁₇ D ₄ FN ₄ O ₂	373.2 373.2	→ →	328.2 109.0	96 96	12 48	4.73	-	IS
76	AB-PINACA- <i>d</i> ₉	C ₁₈ H ₁₇ D ₉ N ₄ O ₂	340.3 340.3	→ →	295.2 224.1	96 96	12 24	5.27	-	IS
77	ADBICA- <i>d</i> ₉	C ₂₀ H ₂₀ D ₉ N ₃ O ₂	353.3 353.3	→ →	336.3 223.1	108 108	4 20	5.66	-	IS
78	ADB-PINACA- <i>d</i> ₉	C ₁₉ H ₁₉ D ₉ N ₄ O ₂	354.3 354.3	→ →	337.2 224.1	96 96	4 28	5.73	-	IS
79	AM2201- <i>d</i> ₅	C ₂₄ H ₁₇ D ₅ FNO	365.2 365.2	→ →	155.0 127.0	96 96	28 60	5.78	-	IS
80	JWH 007- <i>d</i> ₉	C ₂₅ H ₁₆ D ₉ NO	365.3 365.3	→ →	155.0 127.0	120 120	28 56	7.62	-	IS
81	JWH 015- <i>d</i> ₇	C ₂₃ H ₁₄ D ₇ NO	335.2 335.2	→ →	155.0 127.0	120 120	24 60	6.01	-	IS
82	JWH 018 4-hydroxyindole metabolite- <i>d</i> ₉	C ₂₄ H ₁₄ D ₉ NO ₂	367.2 367.2	→ →	155.0 127.0	120 120	28 52	7.26	-	IS

83	JWH 018 6-hydroxyindole metabolite- <i>d</i> ₉	C ₂₄ H ₁₄ D ₉ NO ₂	367.2 367.2	→ →	155.0 127.0	120 120	24 56	5.62	-	IS
84	JWH 018 7-hydroxyindole metabolite- <i>d</i> ₉	C ₂₄ H ₁₄ D ₉ NO ₂	367.2 367.2	→ →	155.0 127.0	120 120	28 56	6.18	-	IS
85	JWH 018 N-(5-hydroxypentyl) metabolite- <i>d</i> ₅	C ₂₄ H ₁₈ D ₅ NO ₂	363.2 363.2	→ →	155.0 127.0	120 120	20 56	4.91	-	IS
86	JWH 018 N-pentanoic acid metabolite- <i>d</i> ₄	C ₂₄ H ₁₇ D ₄ NO ₃	376.2 376.2	→ →	155.0 127.0	120 120	24 60	4.82	-	IS
87	JWH 018 N-pentanoic acid metabolite- <i>d</i> ₅	C ₂₄ H ₁₆ D ₅ NO ₃	377.2 377.2	→ →	155.0 127.0	120 120	24 60	4.81	-	IS
88	JWH 018- <i>d</i> ₉	C ₂₄ H ₁₄ D ₉ NO	351.2 351.2	→ →	155.0 127.0	120 120	24 56	7.25	-	IS
89	JWH 073- <i>d</i> ₇	C ₂₃ H ₁₄ D ₇ NO	335.2 335.2	→ →	155.0 127.0	120 120	24 56	6.49	-	IS
90	JWH 081- <i>d</i> ₉	C ₂₅ H ₁₆ D ₉ NO ₂	381.3 381.3	→ →	185.1 157.1	120 120	28 48	7.63	-	IS
91	JWH 122- <i>d</i> ₉	C ₂₅ H ₁₆ D ₉ NO	365.3 365.3	→ →	169.1 141.1	108 108	28 52	8.06	-	IS
92	JWH 200- <i>d</i> ₅	C ₂₅ H ₁₉ D ₅ N ₂ O ₂	390.2 390.2	→ →	155.0 114.1	120 120	20 28	4.65	-	IS
93	JWH 210- <i>d</i> ₉	C ₂₆ H ₁₈ D ₉ NO	379.3 379.3	→ →	183.1 155.1	120 120	28 44	8.63	-	IS
94	JWH 250- <i>d</i> ₅	C ₂₂ H ₂₀ D ₅ NO ₂	341.2 341.2	→ →	121.0 91.1	108 108	20 56	6.47	-	IS
95	JWH 398- <i>d</i> ₉	C ₂₄ H ₁₃ D ₉ ClNO	385.2 385.2	→ →	189.0 161.0	120 120	28 56	8.66	-	IS
96	MAM2201- <i>d</i> ₅	C ₂₅ H ₁₉ D ₅ FNO	379.2 379.2	→ →	169.1 141.1	120 120	28 56	6.31	-	IS

97	PB-22- <i>d</i> ₉	C ₂₃ H ₁₃ D ₉ N ₂ O ₂	368.2 368.2	→ →	223.1 145.0	96 96	12 44	6.39	-	IS
98	RCS-4- <i>d</i> ₉	C ₂₁ H ₁₄ D ₉ NO ₂	331.2 331.2	→ →	135.0 77.1	120 120	28 60	6.40	-	IS
99	UR-144- <i>d</i> ₅	C ₂₁ H ₂₄ D ₅ NO	317.3 317.3	→ →	125.1 55.2	120 120	24 44	8.18	-	IS
100	XLR11- <i>d</i> ₅	C ₂₁ H ₂₃ D ₅ FNO	335.3 335.3	→ →	125.1 55.1	120 120	24 52	6.55	-	IS

^aTransition consists of precursor ion (1) → product ion (2); transition used for quantitation in bold.

^bFrag - fragmentor voltage, CE - collision energy, RT - retention time.

PART FOUR: METHOD DEVELOPMENT

4.1. Overview

Method optimization was carried out according to the parameters outlined in Figure

7. A subset of 20 SC compounds (M-0) was created for preliminary use as indicated (Table 1).

Figure 7: Flow chart depicting method optimization (gray) and validation (black) outline for the online SPE LC-QqQ-MS quantitative method consisting of four main steps: 1) Flow injection analysis; 2) Optimizer; 3) Dynamic MRM and 4) Online SPE.

4.2. Flow Injection Analysis

A 1.0 µg/mL in MeOH standard was created for each SC compound and injected through a void column (spacer) using an isocratic 20:80 solvent delivery consisting of [A] 5 mM ammonium formate + 0.1% FA and [B] MeOH + 0.1% FA. Total run time was 1.0 min. This was done in order to confirm the presence of a SC compound in the standard.

4.3. Optimizer

Each 1.0 µg/mL in MeOH standard was analyzed by MassHunter MS Optimizer software (Version B.08.02) where fragmentor voltage and collision cell energy was varied for each compound. Product ion abundance was tabulated per SC compound and greatest values (max: 2 transitions) used for the method.

4.4. Dynamic MRM

4.4.1. LC gradient

The preliminary mobile phase gradient included [A] 5 mM ammonium formate + 0.1% FA and [B] MeOH + 0.1% FA run at 0.0 min (10% B), 5.0 min (60% B), 8.0 min (100% B) and stop at 10.0 min (1 µL injection). Most of the SC tested were seen to elute with a high organic content (>80% B) indicating strong hydrophobicity (Figure 8). The gradient was adjusted by creating a shallower slope during the organic delivery in order to improve the chromatography.

Figure 8: The chromatograms shown above represent M-0 analyzed by direct injection mode on the LC-QqQ-MS system using a not-yet optimized binary gradient of (a) 0.0 min (10% B), 5.0 min (60% B), 8.0 min (100% B) and stop at 10.0 min. (b) Below, it is demonstrated that most of the SC compounds are hydrophobic in nature and the gradient can be adjusted by creating a shallower slope to improve the chromatography.

4.4.2. Retention time acquisition

Each 1.0 µg/mL in MeOH standard (n=100) was analyzed in full scan mode using the optimized LC gradient. Compound elution times were extracted from TIC profiles by searching for precursor ions $[M+H]^+$, which were calculated based on MW (Appendix 2).

4.4.3. Mobile phase optimization

Retention times were added to their respective MRM transitions to finalize the dynamic MRM method. Binary pump solvent selection was further tested by varying the aqueous phase between 5 and 20 mM ammonium formate with 0.1% FA, while the organic phase was varied using 100% MeOH, 100% ACN and (50:50, v:v) MeOH:ACN with 0.1% FA. Higher recoveries were shown when using 100% MeOH for organic modifier as compared to 100% ACN or (50:50, v:v) MeOH:ACN. No change was observed when varying pH for the aqueous phase. It was therefore decided to remain using 5 mM ammonium formate + 0.1% FA for mobile phase A and MeOH + 0.1% FA for mobile phase B.

4.4.4. Co-eluting compounds

Isomeric compounds, those having identical retention times and precursor and/or product ions, were separated into different mixes to avoid a false positive confirmation by the analytical method. However, in the case of an authentic sample screen the method would be unable to differentiate between isomers and an accurate reporting would have to be determined through additional methods.

4.5. Online SPE

Preliminary tests of the on-line system were administered to optimize: 1) solvent composition, 2) valve time switching, 3) online SPE cartridge selection and 4) matrix

compatibility. Analyte recovery was assessed for each compound using absolute peak areas from injection of a subset of 20 SC and metabolites (M-0) in MeOH ranging in elution time.

4.5.1. Flush pump solvent selection

For the Flexcube system, 0.1% FA in water and 0.1% FA in water:MeOH (75:25, v:v) were tested for loading/reconditioning and 100% MeOH (single wash) or 100% ACN followed by 100% MeOH (double wash) for washing. Loading/reconditioning with only 0.1% FA in water favored the more hydrophilic compounds and an increase in ionization was observed for hydrophobic SC when loading/reconditioning with 0.1% FA in water:MeOH (75:25, v:v). However, using 0.1% FA in water:MeOH (75:25, v:v) also created significant peak tailing for the hydrophilic compounds; therefore, a compromise was made by using 0.1% FA in water:MeOH (90:10, v:v), resulting in minimal tailing and improved recoveries (Figure 9).

Wash performance was monitored by injection of a sample through one cartridge followed by a blank through the other cartridge and observing for peaks from the latter. Clean cartridges were used prior to injecting and wash flow-through was collected and analyzed by direct injection to monitor sample loss. No blank peaks or sample loss was observed when using two washes. An additional 0.1% FA in water:MeOH step was later added prior to this double wash for better instrument compatibility.

Figure 9: Flexible cube flush pump solvent optimization: a) Chromatogram represents sample loading/reconditioning using 0.1% formic acid in water; b) Chromatogram represents sample loading/reconditioning using (75:25, v:v) 0.1% formic acid in water:MeOH. Adding organic solvent to the loading/reconditioning phase increased the signal for very hydrophobic compounds (rectangle), however also created peak tailing (circle).

4.5.2. Valve time switching

Valve time switching was experimentally adjusted by injection with a void/spacer to determine the time (t_s) at which the compounds reached the head of the column and then switching with the column replaced at various intervals prior to t_s (57). Highest ion intensities were observed when loading for 170 s and switching at 2.84 min.

4.5.3. SPE cartridge selection

Cartridge stationary phase evaluation was performed by injection of M-0 through an Evolute® Express ABN cartridge and a Bond Elut PLRP-s cartridge. Similar ion intensities were found for hydrophilic compounds, but a slightly greater ion intensity when using the PLRP-s for hydrophobic compounds favored the use of this cartridge (Figure 10).

Figure 10: (a) The chromatogram above represents M-0 injected through the online SPE system using a (purple) PLRP-s cartridge and a (blue) Biotage cartridge for sample clean-up. The signal was found to be similar, however an increase in ionization for very hydrophobic compounds (circle) favored the PLRP-s cartridge. (b) Depicts the flexible cube flush pump solvent system that was used prior to optimization.

4.5.4. OF matrix compatibility

Comparison of method compatibility for OraFlx synthetic OF (Dyna-Tek Industries, Lenexa, KS) and OF from pooled human donors (Lee Biosolutions, Maryland Heights, MO) was performed by spiking with a mix of 20 SC (*i.e.*, M-0) and analyzing through online SPE in order to see if synthetic OF matrix could be used to facilitate full validation studies. A paired *t*-test was used to compare the ratios of analyte area counts to IS ($p < 0.05$). A significant difference was found for more than half the compounds. In addition, studies were conducted to compare ME, RE, and PE in spiked authentic OF and synthetic OF. This study indicated that, for the majority of SC tested, results for these parameters were improved for authentic as compared to synthetic OF (Table 2). Consequently, it was concluded that synthetic OF was an unsuitable matrix for full validation studies and further work utilized authentic blank human OF (Figure 11).

Table 2. Comparison of pooled human OF and synthetic OF when spiking with M-0.

No.	Compound	Pooled RE	Synthetic RE	Pooled ME	Synthetic ME	PE	<i>p</i>
1	AB-CHMINACA	89%	68%	97%	74%	92%	0.0041
2	AB-FUBINACA	90%	9%	95%	9%	94%	0.0405
3	AB-PINACA	91%	43%	97%	46%	94%	0.3821
4	AB-PINACA N-(4-hydroxypentyl) metabolite	92%	1%	97%	1%	95%	0.0002
5	AB-PINACA N-(5-hydroxypentyl) metabolite	93%	1%	96%	1%	97%	0.0003
6	AB-PINACA pentanoic acid metabolite	96%	2%	100%	2%	96%	0.0008
7	JWH 018	9%	16%	20%	38%	43%	0.0749
8	JWH 018 4-hydroxyindole metabolite	2%	4%	10%	21%	19%	0.5524
9	JWH 018 7-hydroxyindole metabolite	12%	19%	26%	40%	48%	0.1885
10	JWH 018 N-(4-oxo-pentyl) metabolite	59%	16%	82%	22%	72%	0.0055
11	JWH 018 N-(5-hydroxypentyl) metabolite	38%	11%	85%	25%	45%	0.0358
12	JWH 018 N-propanoic acid metabolite	78%	6%	94%	7%	83%	0.0007
13	JWH 122	4%	10%	11%	27%	35%	0.0378
14	JWH 182	3%	3%	15%	14%	18%	0.0196
15	PB-22	26%	30%	40%	47%	64%	0.2486
16	PB-22 3-carboxyindole metabolite	86%	33%	97%	37%	89%	0.0031
17	PB-22 N-(4-hydroxypentyl) metabolite	77%	9%	84%	10%	92%	0.0004
18	PB-22 N-(5-hydroxypentyl) metabolite	79%	10%	84%	10%	94%	0.0005
19	PB-22 N-pentanoic acid metabolite	79%	4%	91%	4%	87%	0.0002
20	XLR11	54%	57%	77%	81%	70%	0.8672

RE Recovery, ME Matrix Effects, PE Process Efficiency, *p* p-value (0.05).

^aRecovery = [response* from online SPE in OF / response* from direct injection in MeOH] x 100%.

^bMatrix Effects = [response* from online SPE in OF / response* from online SPE in MeOH] x 100%.

^cProcess Efficiency = [response* from online SPE in MeOH / response* from direct injection in MeOH] x 100%.

*Response is considered to be the average peak areas of three separately prepared samples.

Figure 11: (a) Online SPE delivery of M-0 using pooled human OF (green) and synthetic OF (blue). Greater ionization was seen when spiking in pooled human OF and so further method validation studies were completed with this matrix. (b) Depicts the flexible cube flush pump solvent system that was used prior to optimization.

4.6. Online SPE LC-QqQ-MS Method

4.6.1. Chemicals and Reagents

All reference standards listed in Table 1 were acquired as either 1 mg/mL solutions in MeOH or 1 mg neat solid from Cayman Chemical (Ann Arbor, MI). An Evolute® Express ABN On-line SPE cartridge (30 x 2.1 mm, 20 µm avg. particle size) was purchased from Biotage® (Charlotte, NC), while Bond Elut Online SPE PLRP-s cartridges (2.1 x 12.5 mm, 15 µm particle size) were from Agilent Technologies (Santa Clara, CA). Oral fluid (OF) from pooled human donors (2 female, 1 male) was purchased from Lee Biosolutions, Inc. (Maryland Heights, MO) and OraFlx synthetic OF was obtained from Dyna-Tek (Lenexa, KS). HPLC grade solvents (*i.e.*, water with 0.1% formic acid (FA), acetonitrile (ACN) and MeOH), ammonium formate (99%) and formic acid (99%) Optima™ LC/MS were all obtained from Thermo Fisher Scientific (Waltham, MA).

4.6.2. Standard stock preparation

A 10 mg/mL in MeOH or dimethyl sulfoxide (DMSO) stock solution was prepared per compound and kept at -21°C for long-term storage. Two mixes, M-1 (200-500 ng/mL in MeOH) and M-2 (100-500 ng/mL in MeOH), of parent and metabolite synthetic cannabinoids were created from these stocks, as well as an internal standard (IS) mix (250 ng/mL in MeOH), for daily use when creating calibrators (L), quality controls (QC) or spiking samples and stored at -21°C.

4.6.3. Calibrators and QC standards

Six-point calibration curves (L1-L6) were prepared by spiking with either M-1 or M-2 into a mix of pooled human OF and Quantisal™ buffer (1:3, v:v) for final

concentrations within 0.1-100 ng/mL. QCs were prepared similarly at low (QC1), medium (QC2) and high (QC3) concentrations ranging from 2.0-10 ng/mL, 6.0-30 ng/mL and 10-50 ng/mL, respectively. All standards were spiked with IS mix for a final concentration of 25 ng/mL (Figure 12). Appendix 2 demonstrates a typical calibration curve, including QCs, and precursor and product ion for each SC parent and metabolite.

4.6.4. Matrix blank and Instrument blank

Blanks were created in ACN and in pooled human OF matrix to account for the instrument and matrix blank, respectively. This was done to account for any interfering peaks that could have arisen from the instrument or matrix affecting the analyte response.

4.6.5. Possible sources of error

It was observed during the analytical runs that improper solvent delivery from the flush pump could negatively impact the recovery of SC. Thorough monitoring of the pump during method validation was paramount in order to reduce the need of having to rerun samples. Additionally, in order to minimize the possibility of carryover in the case of improper solvent delivery, blanks were always run in between calibrators, QCs and samples, accordingly (Figure 13).

Figure 12: Online SPE dynamic MRM profile for Mix 1 (a) and Mix 2 (b) in oral fluid and buffer (1:3, v:v) matrix obtained using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column.

Figure 13. The chromatograms above represent improper solvent delivery from the flush pump (profiles also shown) and its effect on samples: (a) When the pump is delivering solvent properly the sample adheres as expected; (b) When there is no reconditioning the sample does not adhere to the cartridge; (c) In the case of inadequate reconditioning there is evidence of partial adherence and obvious peak tailing of the sample.

4.7. Guidelines

4.7.1. ASB Standard 036, First Edition 2018

The method was validated according to ASB Standard 036, First Edition 2018, formerly known as the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines, and others (26-31).

4.8. Validation Parameters

4.8.1. Carryover and Selectivity

The possibility of carryover was observed by injecting blank samples immediately after the highest CAL level onto: 1) alternating SPE cartridges and 2) the same cartridge, and then searching for peaks. Injecting a blank onto alternating cartridges was used to test carryover after a wash step, while injecting a blank on the same cartridge examined how much analyte remained regardless of a wash. Instrument and matrix blanks were created in ACN and in pooled human OF:buffer (1:3, v:v) matrix, respectively, and were scanned for endogenous peaks. Additionally, method selectivity was tested by spiking a matrix blank with a variety of different classes of drugs that might commonly be detected in SC users (58), including Δ^9 -THC, Δ^8 -THC, $\pm$ 11-nor-9-carboxy- Δ^9 -THC, cannabidiol, nicotine, cocaine, 3,4-methylenedioxymethamphetamine, $\square$ -pyrrolidinovalerophenone, methylenedioxypyrovalerone, methylphenidate, oxycodone, pentobarbital and alprazolam and looking for interfering peaks at standard retention times.

4.8.2. Linearity, LOD and LOQ

Calibration models, consisting of six levels with five replicates each run on separate days (n=30), were finalized through analysis of outliers using Grubbs' test on JMP software, examination of residual plots confirmed by F-test on Excel, and Goodness-of-

Fit test on MassHunter ($p < 0.05$) (59-63). The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using the formulas $LOD = (3.3s_y)/avg_m$ and $LOQ = (10s_y)/avg_m$, where s_y represents the standard deviation of the y-intercept and avg_m the average slope from linear calibration curves.

4.8.3. Bias and Precision

Intra- and inter-day bias and precision were determined at each QC level by triplicate analysis on five separate days. Bias was calculated by subtracting the mean and nominal concentrations and dividing by the nominal, while precision was calculated by dividing standard deviation and mean concentration values. Both were expressed as percentages with acceptable values being within $\pm 20\%$ (26).

4.8.4. Matrix Effects, Process Efficiency and Recovery

Numerous protocols have relied on Matuszewski et al. (28) for the assessment of ME, RE and PE by using a pre- and post-extraction spiking procedure of analyte and IS. However, calculating these values using those approaches is not feasible for an online SPE system because of the addition of the standards post-extraction. Once the system valve switches and begins to back-flush the cartridge, in theory, a 1 μ L injection of SC and IS mix would then need to be added to the flow path almost instantaneously to reach the head of the analytical column at the same time as a typical pre-extraction run. Instead, the usual formulas for ME, RE and PE were modified in order to be applicable to this study, with data calculated from five day QC triplicates to determine the parameters. The modified equations are as follows:

$$\text{Equation 1: } ME (\%) = \frac{a}{b} = \frac{[Online\ SPE\ in\ OF]}{[Online\ SPE\ in\ MeOH]} \times 100, \text{ if } \left[\frac{\text{analyte area}}{IS\ area}\right]$$

$$\text{Equation 2: } PE (\%) = \frac{b}{c} = \frac{[Online\ SPE\ in\ MeOH]}{[Direct\ injection\ in\ MeOH]} \times 100, \text{ if } \left[\frac{\text{analyte area}}{IS\ area}\right]$$

$$\text{Equation 3: } RE (\%) = \frac{a}{c} = \frac{[Online\ SPE\ in\ OF]}{[Direct\ injection\ in\ MeOH]} \times 100, \text{ if } \left[\frac{\text{analyte area}}{IS\ area}\right]$$

where a - c are area ratios of an analyte and its IS, with a determined in OF:buffer (1:3, v:v) matrix processed by online SPE, b determined in MeOH analyzed by online SPE and c determined in MeOH quantified by direct injection into the analytical column.

Matuszewski et al. proposed that when examining the potential for ME, the analyte response post-extraction should be compared to the response in a neat solvent. Since the sample clean-up proposed for the present method consists of online SPE, it is reasonable for Equation 1 to compare the signal of the analytes in OF matrix to that of analytes in MeOH processed through online SPE. For ME, a result greater than 100% was reported as ion enhancement while less than 100% represented ion suppression. When formulating Equations 2 and 3, notice was placed on the common understanding that RE should not be affected by the matrix, while PE should. This is arguable for online SPE because PE should reflect how well the system is able to trap the analyte without being affected by the matrix. Consequently, Equation 2 for PE as used in this study compares the signal from online SPE with analytes in MeOH to that of a process that would not include interaction with the PLRP-s cartridge, *i.e.*, with direct injection of analytes in

MeOH. RE evaluates the analyte response obtained from the proposed method in OF compared to that of a signal not affected by matrix or process, as seen in Equation 3.

4.8.5. Freeze-and-Thaw Studies

Analyte stability was recorded for three freeze-and-thaw cycles using three sets of triplicates at low (L1-3) and high (H1-3) concentration levels. On the first day of analysis all standards were prepared fresh and tested against a calibration curve ($t_i = 0$ h). Following analysis, the sets were placed in the freezer at -20°C for $t = 24$ h (first cycle). The samples were then removed from their storage, allowed to thaw at RT without assistance (~ 30 min.), and a single set tested against a fresh calibration curve. The remaining sets were then placed back into the freezer and the process was repeated at $t = 48$ h (second cycle) and 72 h (third cycle). A fourth set (L4 and H4) prepared at the start of analysis was similarly tested but kept in the autosampler (23°C) for the entirety of the freeze-and-thaw analysis in order to monitor autosampler stability. A sample was considered stable if its response bias was $<20\%$ when compared to its original response at $t_i = 0$ h.

4.9. Blind Study

As routine screening of OF for SC and metabolites is currently not widely used, authentic OF samples with known positive SC detection were not available for this study. As an alternative, a study using blank human OF samples spiked with SC analytes was performed in order to test the applicability of the method. These specimens were prepared “blind” (*i.e.*, without the knowledge of the analyst) by adding the appropriate amount of SC and MeOH to $160\ \mu\text{L}$ of OF:buffer (1:3, v:v) plus $20\ \mu\text{L}$ of IS mix to yield nominal concentrations ranging from $5 - 40\ \text{ng/mL}$ in a final volume of $200\ \mu\text{L}$. Samples

were then processed using the online SPE LC-QqQ-MS method described above (Figure 14).

Agilent Mass Hunter Quantitative and Qualitative software (Version B.08.02) were used for data analysis. Criteria for the confirmation (signal-to-noise, $S/N \geq 3$) and quantitation ($S/N \geq 10$) of a SC parent compound or metabolite included identified presence using both PLRP-s cartridges, with quantitation data derived from the cartridge used to create the calibration curve.

Figure 14: The proposed sequence shown above represents the a) collection, b) clean-up and c) quantitation of a sample by online SPE LC-QqQ-MS: a) Approximately 1 mL of oral fluid containing SC is collected using a Quantisal™ collection device with 3 mL buffer for analyte extraction and stabilization. A 90 µL aliquot is transferred to an amber glass vial and spiked with 10 µL internal standard; b) A 1 µL injection is loaded onto cartridge 1 using the FlexCube pump and then eluted onto the analytical column using the binary pump (green); meanwhile cartridge 2 is cleaned/reconditioned for loading of the next sample (blue); c) The sample is then qualitatively assessed (chromatogram) prior to plotting against a calibration curve for quantitation.

PART FIVE: RESULTS

5.1. Validation of the online SPE LC-QqQ-MS Method

5.1.1. Matrix blank and Instrument blank

No endogenous peaks were detected in either type of blank at analyte retention times suggesting that acquired abundances came from SC compounds.

5.1.2. Carryover and Selectivity

In this method, when a sample is back-flushed from one cartridge using the binary pump, the other cartridge is simultaneously being washed and then reconditioned by the flush pump for the next load. Injection of a sample followed by a blank run through the washed/reconditioned cartridge did not show any peaks, indicating that the wash was sufficient in cleaning the PLRP-s phase. Injection of a sample followed by a blank run through the same unwashed, reconditioned cartridge also did not show any peaks, demonstrating full elution of the SC compounds. No endogenous peaks were detected in either type of blank at analyte retention times, suggesting that acquired abundances came exclusively from SC compounds. Interference from an injection of a mix of compounds commonly abused by SC users was not detected by the analytical method.

5.1.3. Linearity, LOD and LOQ

Peak area ratios of analyte to internal standard were used to examine four parameters for each calibration model with values of $p < 0.05$ regarded as statically significant. Outliers were detected for six compounds and were removed prior to further analysis. Visual inspection of residual plots supported a linear heteroscedastic pattern for most compounds as there was a random distribution about zero and an increase in standard deviation at higher concentrations. An F-test for two sample variances at the highest and

lowest calibration level confirmed the difference as statistically significant, and, consequently, a weighted $1/x$ linear regression was applied to each calibration curve to minimize the sum of the relative error for the residuals at higher concentrations. Goodness-of-fit was confirmed using MassHunter software. Careful consideration was used when fitting this type of regression to compounds with favorable non-linear heteroscedastic behavior (Table 3). Quality control accuracy aided in determining the fit of the data as suitable (61). After confirming the calibration model, LODs and LOQs were calculated and reported as 0.4-3.8 ng/mL and 1.1-11.6 ng/mL, respectively (Table 4).

Table 3. Calibration model determination.

No.	Compound	G statistic ¹	Grubb's test ² (– or +)	Residual Plot ³ (A, B, C)	F statistic ⁴	F-test ⁵	Goodness-of-Fit test
1	(+/-) JWH 018 N-(2-hydroxypentyl) metabolite	2.3	–	B	1665.0	Reject	Linear, 1/x weight
2	(+/-) JWH 018 N-(3-hydroxypentyl) metabolite	2.4	–	B	97.9	Reject	Linear, 1/x weight
3	(+/-) JWH 018 N-(4-hydroxypentyl) metabolite	2.3	–	B	68.5	Reject	Linear, 1/x weight
4	(+/-) WIN 55,212 (mesylate)	2.5	–	B	551.8	Reject	Linear, 1/x weight
5	5-chloro AB-PINACA	2.0	–	C	45.4	Reject	Linear, 1/x weight
6	5-fluoro AB-PINACA	2.2	–	B	409.8	Reject	Linear, 1/x weight
7	5-fluoro ABICA	2.4	–	B	384.5	Reject	Linear, 1/x weight
8	5-fluoro ADB-PINACA	2.2	–	B	130.4	Reject	Linear, 1/x weight
9	5-fluoro ADBICA	2.2	–	B	158.0	Reject	Linear, 1/x weight
10	5-fluoro AMB	2.4	–	B	500.8	Reject	Linear, 1/x weight
11	5-fluoro PB-22	2.1	–	B	15.2	Reject	Linear, 1/x weight
12	5-fluoro PB-22 3-carboxyindole metabolite	4.3	+	B	1.6	Do not reject	Linear, 1/x weight
13	AB-CHMINACA	2.3	–	B	328.6	Reject	Linear, 1/x weight
14	AB-FUBINACA	2.1	–	B	32.1	Reject	Linear, 1/x weight
15	AB-PINACA	2.2	–	B	90.3	Reject	Linear, 1/x weight

16	AB-PINACA N-(4-hydroxypentyl) metabolite	2.3	–	B	151.2	Reject	Linear, 1/x weight
17	AB-PINACA N-(5-hydroxypentyl) metabolite	2.0	–	C	1.2	Do not reject	Linear, 1/x weight
18	AB-PINACA pentanoic acid metabolite	2.5	–	B	381.6	Reject	Linear, 1/x weight
19	ADB-FUBINACA	2.2	–	B	99.8	Reject	Linear, 1/x weight
20	ADB-PINACA	2.1	–	B	23.0	Reject	Linear, 1/x weight
21	ADBICA	2.1	–	B	113.2	Reject	Linear, 1/x weight
22	AM1220	2.7	–	B	7551.8	Reject	Linear, 1/x weight
23	AM2201	2.1	–	B	185.9	Reject	Linear, 1/x weight
24	AM2201 2-hydroxyindole metabolite	3.3	+	C	15.6	Reject	Linear, 1/x weight
25	AM2201 5-hydroxyindole metabolite	3.7	+	C	26.8	Reject	Linear, 1/x weight
26	AM2233	2.4	–	B	267.4	Reject	Linear, 1/x weight
27	AM694	2.1	–	B	60.8	Reject	Linear, 1/x weight
28	HU-211	2.8	–	B	172.3	Reject	Linear, 1/x weight
29	JWH 007	2.1	–	B	14.9	Reject	Linear, 1/x weight
30	JWH 015	2.1	–	B	13.7	Reject	Linear, 1/x weight
31	JWH 018	2.2	–	B	181.7	Reject	Linear, 1/x weight
32	JWH 018 2-hydroxyindole metabolite	2.7	–	B	407.0	Reject	Linear, 1/x weight
33	JWH 018 4-hydroxyindole metabolite	2.2	–	B	214.4	Reject	Linear, 1/x weight

34	JWH 018 5-hydroxyindole metabolite	2.4	–	B	454.4	Reject	Linear, 1/x weight
35	JWH 018 6-hydroxyindole metabolite	2.1	–	B	91.9	Reject	Linear, 1/x weight
36	JWH 018 7-hydroxyindole metabolite	2.4	–	B	791.7	Reject	Linear, 1/x weight
37	JWH 018 adamantyl analog	2.7	–	B	123.6	Reject	Linear, 1/x weight
38	JWH 018 N-(4-oxo-pentyl) metabolite	3.4	+	B	53.7	Reject	Linear, 1/x weight
39	JWH 018 N-(5-hydroxypentyl) metabolite	2.3	–	B	745.3	Reject	Linear, 1/x weight
40	JWH 018 N-propanoic acid metabolite	2.3	–	B	204.5	Reject	Linear, 1/x weight
41	JWH 019	2.4	–	B	351.2	Reject	Linear, 1/x weight
42	JWH 020	2.1	–	C	165.7	Reject	Linear, 1/x weight
43	JWH 073	2.2	–	B	56.9	Reject	Linear, 1/x weight
44	JWH 081	2.2	–	B	85.8	Reject	Linear, 1/x weight
45	JWH 098	2.1	–	C	220.3	Reject	Linear, 1/x weight
46	JWH 122	2.1	–	B	81.1	Reject	Linear, 1/x weight
47	JWH 182	2.2	–	C	82.7	Reject	Linear, 1/x weight
48	JWH 200	2.1	–	B	18.8	Reject	Linear, 1/x weight
49	JWH 203	2.1	–	B	89.3	Reject	Linear, 1/x weight
50	JWH 210	2.1	–	B	27.6	Reject	Linear, 1/x weight
51	JWH 249	2.1	–	B	58.0	Reject	Linear, 1/x weight

52	JWH 251	2.2	–	B	45.0	Reject	Linear, 1/x weight
53	JWH 307	2.2	–	C	74.6	Reject	Linear, 1/x weight
54	JWH 307 5'-isomer	2.2	–	B	140.9	Reject	Linear, 1/x weight
55	JWH 387	2.5	–	B	82.0	Reject	Linear, 1/x weight
56	JWH 398	2.2	–	B	99.4	Reject	Linear, 1/x weight
57	JWH 412	2.1	–	B	17.9	Reject	Linear, 1/x weight
58	MAM2201 N-(3-fluoropentyl) isomer	2.3	–	B	220.2	Reject	Linear, 1/x weight
59	MAM2201 N-(5-chloropentyl) analog	2.3	–	B	160.7	Reject	Linear, 1/x weight
60	PB-22	2.3	–	B	222.8	Reject	Linear, 1/x weight
61	PB-22 3-carboxyindole metabolite	2.4	–	B	548.4	Reject	Linear, 1/x weight
62	PB-22 N-(4-hydroxypentyl) metabolite	2.3	–	B	189.3	Reject	Linear, 1/x weight
63	PB-22 N-(5-hydroxypentyl) metabolite	2.3	–	B	407.1	Reject	Linear, 1/x weight
64	PB-22 N-pentanoic acid metabolite	2.4	–	B	545.6	Reject	Linear, 1/x weight
65	RCS-4	2.2	–	B	63.3	Reject	Linear, 1/x weight
66	RCS-4-C4 homolog	2.3	–	B	188.0	Reject	Linear, 1/x weight
67	RCS-8	2.4	–	B	299.5	Reject	Linear, 1/x weight
68	THJ 018	2.4	–	B	396.9	Reject	Linear, 1/x weight
69	THJ 2201	2.6	–	B	120.4	Reject	Linear, 1/x weight

70	UR-144	2.1	–	B	144.3	Reject	Linear, 1/x weight
71	XLR11	2.1	–	B	43.7	Reject	Linear, 1/x weight
72	XLR11 6-hydroxyindole metabolite	4.4	+	C	14.8	Reject	Linear, 1/x weight
73	XLR11 N-(4-hydroxypentyl) metabolite	3.4	+	B	1.5	Do not reject	Linear, 1/x weight

LOD Limit of detection, *LOQ* Limit of quantitation.

¹G(0.05)=2.9.

²(–) No outlier detected, (+) 1 or more outliers detected.

³A Linear, homoscedastic; B Linear, heteroscedastic; C Nonlinear, heteroscedastic

⁴F(0.05)=6.4.

⁵H₀=variances are equal.

Table 4. LOD and LOQ values for SC in OF:buffer (1:3, v:v) matrix for all analytes (n=5).

ID #	Compound	LOD ^a (ng/mL)	LOQ ^b (ng/mL)
1	(+/-) JWH 018 N-(2-hydroxypentyl) metabolite	2.4	7.4
2	(+/-) JWH 018 N-(3-hydroxypentyl) metabolite	0.5	1.4
3	(+/-) JWH 018 N-(4-hydroxypentyl) metabolite	0.7	2.0
4	(+/-) WIN 55,212 (mesylate)	1.3	3.8
5	5-chloro AB-PINACA	1.4	4.4
6	5-fluoro ABICA	2.2	6.5
7	5-fluoro AB-PINACA	1.2	3.7
8	5-fluoro ADBICA	1.6	4.9
9	5-fluoro ADB-PINACA	0.9	2.7
10	5-fluoro AMB	1.8	5.4
11	5-fluoro PB-22	1.5	4.7
12	5-fluoro PB-22 3-carboxyindole metabolite	1.4	4.2
13	AB-CHMINACA	2.3	7.1
14	AB-FUBINACA	1.8	5.3
15	AB-PINACA	1.1	3.4
16	AB-PINACA N-(4-hydroxypentyl) metabolite	1.7	5.2
17	AB-PINACA N-(5-hydroxypentyl) metabolite	3.1	9.3
18	AB-PINACA pentanoic acid metabolite	2.2	6.8
19	ADB-FUBINACA	2.4	7.2
20	ADBICA	1.2	3.5
21	ADB-PINACA	1.0	2.9
22	AM1220	1.6	4.8
23	AM2201	1.4	4.3
24	AM2201 2-hydroxyindole metabolite	1.6	4.8
25	AM2201 5-hydroxyindole metabolite	2.8	8.4
26	AM2233	1.7	5.0

27	AM694	2.0	6.1
28	HU-211	3.7	11.3
29	JWH 007	1.0	3.1
30	JWH 015	0.4	1.1
31	JWH 018	1.2	3.6
32	JWH 018 2-hydroxyindole metabolite	0.9	2.7
33	JWH 018 4-hydroxyindole metabolite	1.9	5.6
34	JWH 018 5-hydroxyindole metabolite	0.8	2.5
35	JWH 018 6-hydroxyindole metabolite	2.2	6.7
36	JWH 018 7-hydroxyindole metabolite	2.3	6.9
37	JWH 018 adamantyl analog	1.7	5.2
38	JWH 018 N-(4-oxo-pentyl) metabolite	2.3	6.8
39	JWH 018 N-(5-hydroxypentyl) metabolite	1.8	5.5
40	JWH 018 N-propanoic acid metabolite	1.8	5.5
41	JWH 019	3.7	11.2
42	JWH 020	3.8	11.6
43	JWH 073	2.0	6.0
44	JWH 081	1.4	4.1
45	JWH 098	3.1	9.3
46	JWH 122	1.2	3.7
47	JWH 182	1.6	4.9
48	JWH 200	0.5	1.5
49	JWH 203	1.6	4.9
50	JWH 210	0.8	2.3
51	JWH 249	1.2	3.8
52	JWH 251	1.7	5.3
53	JWH 307	0.9	2.6
54	JWH 307 5'-isomer	1.4	4.3

55	JWH 387	3.5	10.5
56	JWH 398	2.3	7.0
57	JWH 412	1.5	4.6
58	MAM2201 N-(3-fluoropentyl) isomer	1.6	4.9
59	MAM2201 N-(5-chloropentyl) analog	2.3	7.1
60	PB-22	1.2	3.7
61	PB-22 3-carboxyindole metabolite	1.2	3.6
62	PB-22 N-(4-hydroxypentyl) metabolite	2.1	6.3
63	PB-22 N-(5-hydroxypentyl) metabolite	3.3	9.9
64	PB-22 N-pentanoic acid metabolite	2.4	7.3
65	RCS-4	1.5	4.6
66	RCS-4-C4 homolog	1.8	5.5
67	RCS-8	1.5	4.4
68	THJ 018	2.1	6.4
69	THJ 2201	1.5	4.5
70	UR-144	1.5	4.7
71	XLR11	1.1	3.4
72	XLR11 6-hydroxyindole metabolite	2.1	6.4
73	XLR11 N-(4-hydroxypentyl) metabolite	0.4	1.2

^aLOD = $(3.3s_y)/avg_m$, where s_y is the standard deviation of the y-intercept and avg_m the average slope of linear calibration curves.

^bLOQ = $(10s_y)/avg_m$, where s_y is the standard deviation of the y-intercept and avg_m the average slope of linear calibration curves.

¹n=5.

5.1.4. Bias and Precision

Bias and precision data are reported in Table 5. Mix 1 and Mix 2 intra- and inter-day bias and precision for QC samples were all $< \pm 20\%$, thus meeting the desired criteria (26). Bias was no greater than $\pm 12\%$ for all concentrations; however, a positive bias was evident at higher concentrations. Imprecision was similar for all QC samples with intra-day precision reported as 3-19% and inter-day ranging from 2-14%.

Table 5. Bias and intra- and inter-day precision values for SC in OF:buffer (1:3, v:v) matrix for all analytes (n=5).

ID #	Compound	Bias (%)			Intra-day precision (%)			Inter-day precision (%)		
		QC1 ^a	QC2	QC3	QC1	QC2	QC3	QC1	QC2	QC3
1	(+/-) JWH 018 N-(2-hydroxypentyl) metabolite	-2	-3	1	16	12	9	10	9	7
2	(+/-) JWH 018 N-(3-hydroxypentyl) metabolite	-8	-4	-1	11	8	7	10	6	5
3	(+/-) JWH 018 N-(4-hydroxypentyl) metabolite	-8	-3	-1	14	6	6	9	5	5
4	(+/-) WIN 55,212 (mesylate)	-4	-5	0	9	9	6	11	8	5
5	5-chloro AB-PINACA	-1	0	3	10	6	6	11	5	5
6	5-fluoro ABICA	-6	-1	0	16	10	8	9	8	6
7	5-fluoro AB-PINACA	-6	2	-1	15	9	6	9	6	4
8	5-fluoro ADBICA	-9	-2	-1	18	6	5	9	6	4
9	5-fluoro ADB-PINACA	-7	0	0	15	8	5	9	5	3
10	5-fluoro AMB	-9	-3	-1	13	8	9	8	5	6
11	5-fluoro PB-22	-6	-4	0	12	7	7	9	5	5
12	5-fluoro PB-22 3-carboxyindole metabolite	-3	1	0	17	18	6	10	10	5
13	AB-CHMINACA	-2	-1	0	13	9	9	10	6	5
14	AB-FUBINACA	0	-1	2	10	10	9	12	5	6
15	AB-PINACA	0	-1	1	10	5	9	11	4	5
16	AB-PINACA N-(4-hydroxypentyl) metabolite	0	-6	-2	10	5	9	10	3	6
17	AB-PINACA N-(5-hydroxypentyl) metabolite	-4	-4	-3	15	6	5	10	4	4
18	AB-PINACA pentanoic acid metabolite	-4	-4	-2	7	4	8	8	3	5
19	ADB-FUBINACA	-9	-2	5	13	3	7	11	4	5
20	ADBICA	-9	-3	-1	11	5	9	11	5	6

21	ADB-PINACA	-1	-1	2	13	5	5	9	3	3
22	AM1220	0	-6	0	14	8	14	11	7	8
23	AM2201	1	-2	1	15	11	6	12	7	5
24	AM2201 2-hydroxyindole metabolite	-9	-6	-7	15	7	9	13	5	7
25	AM2201 5-hydroxyindole metabolite	-3	0	0	13	13	17	12	8	12
26	AM2233	-1	-3	0	12	10	8	10	6	7
27	AM694	-7	-2	1	13	5	6	9	4	4
28	HU-211	-8	-5	-2	13	9	7	12	7	7
29	JWH 007	-9	-2	1	11	5	10	8	4	6
30	JWH 015	-8	-1	0	17	4	3	9	3	3
31	JWH 018	0	-2	1	14	7	10	11	6	8
32	JWH 018 2-hydroxyindole metabolite	-3	-6	-10	14	19	13	14	11	9
33	JWH 018 4-hydroxyindole metabolite	0	-1	2	14	8	12	11	5	6
34	JWH 018 5-hydroxyindole metabolite	-4	-3	-3	16	7	8	12	5	6
35	JWH 018 6-hydroxyindole metabolite	-2	3	3	11	14	10	13	9	6
36	JWH 018 7-hydroxyindole metabolite	-2	0	1	12	7	6	12	7	7
37	JWH 018 adamantyl analog	-8	0	2	11	4	8	7	3	5
38	JWH 018 N-(4-oxo-pentyl) metabolite	-1	-3	-1	12	6	6	12	5	5
39	JWH 018 N-(5-hydroxypentyl) metabolite	-2	-2	2	16	6	9	12	6	7
40	JWH 018 N-propanoic acid metabolite	-2	0	0	15	9	8	10	5	5
41	JWH 019	-7	-2	1	13	12	11	11	7	9
42	JWH 020	-2	11	10	13	10	5	13	8	5
43	JWH 073	-8	-1	3	16	6	6	12	7	5

44	JWH 081	-8	-2	1	11	5	8	8	4	5
45	JWH 098	-1	5	3	12	7	6	11	6	5
46	JWH 122	-2	-2	1	11	8	10	11	5	6
47	JWH 182	1	11	12	15	13	9	12	10	7
48	JWH 200	-5	-2	0	10	4	3	8	4	2
49	JWH 203	-7	-2	1	11	7	7	9	5	4
50	JWH 210	-8	-2	-1	17	4	10	9	4	6
51	JWH 249	-8	-2	1	11	5	6	9	4	5
52	JWH 251	-8	0	1	8	8	6	8	5	5
53	JWH 307	-9	4	4	13	6	8	9	6	6
54	JWH 307 5'-isomer	-3	-3	1	10	6	7	11	6	7
55	JWH 387	-4	10	6	15	8	5	11	5	4
56	JWH 398	-8	-3	1	13	7	5	9	4	3
57	JWH 412	-7	1	2	11	6	6	9	4	5
58	MAM2201 N-(3-fluoropentyl) isomer	-1	-3	2	12	7	11	12	6	8
59	MAM2201 N-(5-chloropentyl) analog	-6	1	3	9	6	9	10	5	6
60	PB-22	0	-1	1	13	9	6	12	6	4
61	PB-22 3-carboxyindole metabolite	3	2	6	13	6	11	11	6	7
62	PB-22 N-(4-hydroxypentyl) metabolite	2	0	3	10	9	6	11	6	4
63	PB-22 N-(5-hydroxypentyl) metabolite	-2	-1	4	13	4	6	8	5	5
64	PB-22 N-pentanoic acid metabolite	3	2	4	9	7	6	9	5	5
65	RCS-4	-7	-1	0	10	6	6	9	6	3
66	RCS-4-C4 homolog	1	-1	1	13	8	8	12	6	6

67	RCS-8	-9	4	3	9	7	7	8	6	5
68	THJ 018	-1	0	2	11	6	6	11	5	6
69	THJ 2201	-7	-2	1	11	6	6	8	5	5
70	UR-144	-1	-1	2	13	7	11	11	6	9
71	XLR11	0	-1	2	10	6	11	12	5	7
72	XLR11 6-hydroxyindole metabolite	-9	-3	0	14	6	6	9	4	3
73	XLR11 N-(4-hydroxypentyl) metabolite	-4	-1	2	14	8	4	8	6	3

^aQC1, 2-10 ng/mL; QC2, 6-30 ng/mL; QC3, 10-50 ng/mL.

5.1.5. Recovery, Matrix Effects and Process Efficiency

RE, ME, and PE values are reported in Table 6. The majority of the compounds had recoveries between 80-120%. Exceptions included compounds 3 ((+/-) JWH 018 N-(4-hydroxypentyl) metabolite), 6 (5-fluoro ABICA), 12 (5-fluoro PB-22 3-carboxyindole metabolite), and 61 (PB-22 3-carboxyindole metabolite), which had one or more QC values with RE >140%. In addition, compounds 16 (AB-PINACA N-(4-hydroxypentyl) metabolite), 17 (AB-PINACA N-(5-hydroxypentyl) metabolite), 22 (AM1220), 24 (AM2201 2-hydroxyindole metabolite), 32 (JWH 018 2-hydroxyindole metabolite), 42 (JWH 020), 47 (JWH 182), and 67 (RCS-8) had one or more QCs with RE <60%. For matrix effects, most SC had values in the 80 - 120% range at two or more QC levels. Analytes with ME data indicative of ion enhancement included compound 6 (5-fluoro ABICA), 12 (5-fluoro PB-22 3-carboxyindole metabolite), and 55 (JWH 387). Analytes that exhibited pronounced ion suppression included compounds 16 (AB-PINACA N-(4-hydroxypentyl) metabolite), 17 (AB-PINACA N-(5-hydroxypentyl) metabolite), 22 (AM1220), and 32 (JWH 018 2-hydroxyindole metabolite). Process efficiency was >90% for most of the compounds. Fewer than one-fourth of the compounds were found to have more than one QC with PE values not within 80-120%. Examples included compounds 47 (JWH 182) and 55 (JWH 387), which had PE values of <55% for QC1-3.

Table 6. Recovery (RE), matrix effects (ME) and process efficiency (PE) data for SC in OF:buffer (1:3, v:v) for all analytes (n=5).

ID #	Compound	Recovery (%) ^a			Matrix effects (%) ^b			Process efficiency (%) ^c		
		QC1 ^d	QC2	QC3	QC1	QC2	QC3	QC1	QC2	QC3
1	(+/-) JWH 018 N-(2-hydroxypentyl) metabolite	93	96	100	84	90	95	110	107	105
2	(+/-) JWH 018 N-(3-hydroxypentyl) metabolite	82	88	91	89	94	97	92	94	94
3	(+/-) JWH 018 N-(4-hydroxypentyl) metabolite	135	146	150	98	106	109	138	137	138
4	(+/-) WIN 55,212 (mesylate)	82	91	96	86	89	97	95	102	99
5	5-chloro AB-PINACA	96	104	109	94	102	106	102	102	103
6	5-fluoro ABICA	136	153	148	134	151	148	102	102	100
7	5-fluoro AB-PINACA	114	122	125	115	123	126	99	100	99
8	5-fluoro ADBICA	108	118	118	109	119	119	99	99	99
9	5-fluoro ADB-PINACA	98	108	109	98	109	109	100	99	100
10	5-fluoro AMB	82	92	94	91	102	104	91	90	91
11	5-fluoro PB-22	95	104	109	99	102	106	96	102	102
12	5-fluoro PB-22 3-carboxyindole metabolite	169	186	182	134	148	145	127	126	126
13	AB-CHMINACA	90	100	104	89	99	102	101	101	101
14	AB-FUBINACA	96	103	107	90	97	100	106	106	106
15	AB-PINACA	94	100	105	90	97	100	104	103	105
16	AB-PINACA N-(4-hydroxypentyl) metabolite	59	63	69	63	69	73	93	91	94
17	AB-PINACA N-(5-hydroxypentyl) metabolite	41	47	48	42	47	49	98	100	99
18	AB-PINACA pentanoic acid metabolite	72	77	82	65	73	74	110	105	110
19	ADB-FUBINACA	90	97	104	92	98	104	98	99	100
20	ADBICA	95	102	106	90	97	101	105	104	105

21	ADB-PINACA	91	98	101	90	99	101	101	99	100
22	AM1220	33	37	41	28	31	35	119	118	116
23	AM2201	94	100	104	90	99	106	104	101	99
24	AM2201 2-hydroxyindole metabolite	49	56	55	68	70	75	72	79	73
25	AM2201 5-hydroxyindole metabolite	114	119	124	100	105	111	114	113	111
26	AM2233	72	81	84	62	70	74	116	116	113
27	AM694	105	116	120	93	103	108	112	112	111
28	HU-211	121	127	130	99	105	109	123	121	119
29	JWH 007	87	100	103	90	103	107	97	97	97
30	JWH 015	91	104	105	90	102	103	101	102	102
31	JWH 018	93	101	106	89	96	101	104	105	104
32	JWH 018 2-hydroxyindole metabolite	36	35	34	48	44	43	75	79	78
33	JWH 018 4-hydroxyindole metabolite	90	98	103	88	97	101	103	101	102
34	JWH 018 5-hydroxyindole metabolite	97	104	107	79	85	88	123	123	122
35	JWH 018 6-hydroxyindole metabolite	89	98	98	93	101	100	95	97	98
36	JWH 018 7-hydroxyindole metabolite	83	90	91	90	99	101	93	91	90
37	JWH 018 adamantyl analog	92	100	102	100	108	110	92	93	93
38	JWH 018 N-(4-oxo-pentyl) metabolite	87	93	97	101	107	113	86	87	85
39	JWH 018 N-(5-hydroxypentyl) metabolite	98	109	114	85	96	99	115	113	115
40	JWH 018 N-propanoic acid metabolite	92	104	106	84	92	92	110	112	115
41	JWH 019	83	89	94	104	111	117	79	81	80
42	JWH 020	48	52	50	79	83	81	61	62	62
43	JWH 073	118	127	131	89	99	103	132	128	128

44	JWH 081	90	102	107	93	105	109	97	98	98
45	JWH 098	65	71	70	75	82	81	87	87	86
46	JWH 122	93	101	106	90	98	104	103	102	102
47	JWH 182	46	48	48	87	87	87	53	55	55
48	JWH 200	93	102	103	96	102	104	97	100	99
49	JWH 203	99	112	119	90	99	104	110	113	114
50	JWH 210	86	99	101	92	103	104	94	96	96
51	JWH 249	89	102	107	88	98	100	101	105	107
52	JWH 251	86	96	97	91	99	104	94	97	93
53	JWH 307	60	68	67	99	105	113	61	65	60
54	JWH 307 5'-isomer	103	113	119	95	104	109	108	109	109
55	JWH 387	60	65	61	129	134	137	47	48	45
56	JWH 398	89	99	103	91	103	106	97	96	98
57	JWH 412	81	91	93	100	109	113	81	84	83
58	MAM2201 N-(3-fluoropentyl) isomer	90	96	102	87	92	97	103	105	105
59	MAM2201 N-(5-chloropentyl) analog	66	73	75	88	95	102	75	77	74
60	PB-22	92	101	104	89	96	101	103	105	103
61	PB-22 3-carboxyindole metabolite	124	135	144	95	102	109	131	132	132
62	PB-22 N-(4-hydroxypentyl) metabolite	116	126	133	99	106	113	117	119	118
63	PB-22 N-(5-hydroxypentyl) metabolite	116	125	133	98	105	110	118	119	121
64	PB-22 N-pentanoic acid metabolite	116	127	133	95	103	109	122	124	122
65	RCS-4	90	102	105	92	103	106	98	99	99
66	RCS-4-C4 homolog	102	112	116	96	105	109	106	106	106

67	RCS-8	58	67	66	84	93	95	69	72	70
68	THJ 018	86	95	98	79	87	90	109	109	108
69	THJ 2201	105	119	124	95	107	108	111	112	115
70	UR-144	101	111	117	88	97	100	114	115	116
71	XLR11	94	101	105	91	99	101	103	103	104
72	XLR11 6-hydroxyindole metabolite	92	101	105	84	94	99	109	107	106
73	XLR11 N-(4-hydroxypentyl) metabolite	118	122	126	106	111	114	111	110	111

^aRecovery = [response ratio* from online SPE in OF/response ratio* from direct injection in MeOH] x 100%.

^bMatrix Effects = [response ratio* from online SPE in OF/response ratio* from online SPE in MeOH] x 100%.

^cProcess Efficiency = [response ratio* from online SPE in MeOH/response ratio* from direct injection in MeOH] x 100%.

^dQC1, 2-10 ng/mL; QC2, 6-30 ng/mL; QC3, 10-50 ng/mL.

*Response ratio = analyte response/ISTD response.

5.1.6. Freeze-and-Thaw Studies

Stability data are reported in Table 7. Most of the SC compounds were shown to be stable for three freeze-and-thaw cycles, with a calculated bias of <25%. Similar stability for most analytes was also noted at RT in the autosampler for up to 72 h. A few SC did exhibit substantially lower stability as a function of time and/or storage temperature. For example, Compound 55 (JWH 387) and 61 (PB-22 3-carboxyindole metabolite) showed loss of stability at 48 h of reanalysis at low concentrations in either temperature.

Compounds 8 (5-fluoro ADB-PINACA) and 12 (5-fluoro PB-22 3-carboxyindole metabolite) were reported to be more unstable at RT than in the freezer, especially at lower concentration levels. Compound 28 (HU-211) appeared to be slightly more stable in the autosampler than in the freezer. Finally, a few compounds, including 24 (AM2201 2-hydroxyindole metabolite) and 32 (JWH 018 2-hydroxyindole metabolite) appeared to have poor stability regardless of temperature.

Table 7. Freeze-and-thaw stability data for SC in OF:buffer (1:3, v:v) matrix for all analytes (n=3).

ID #	Compound	LOW spike (RT) ^{a, b}			HIGH spike (RT)			LOW spike (-20°C)			HIGH spike (-20°C)		
		24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
1	(+/-) JWH 018 N-(2-hydroxypentyl) metabolite	1	6	7	3	3	2	1	4	14	2	6	3
2	(+/-) JWH 018 N-(3-hydroxypentyl) metabolite	2	4	1	4	3	5	2	4	36	2	6	37
3	(+/-) JWH 018 N-(4-hydroxypentyl) metabolite	4	8	10	7	12	6	4	7	25	3	6	26
4	(+/-) WIN 55,212 (mesylate)	6	1	9	5	3	4	2	6	9	2	1	0
5	5-chloro AB-PINACA	1	14	5	1	0	1	2	1	1	2	4	2
6	5-fluoro ABICA	7	13	13	12	1	15	3	14	34	10	2	45
7	5-fluoro AB-PINACA	7	9	8	7	0	6	4	3	16	11	2	25
8	5-fluoro ADBICA	8	15	12	11	3	4	9	0	27	8	9	30
9	5-fluoro ADB-PINACA	11	11	12	13	2	6	1	2	16	7	4	21
10	5-fluoro AMB	18	0	0	13	0	1	8	7	6	15	2	1
11	5-fluoro PB-22	3	0	1	0	1	0	4	5	7	1	2	4
12	5-fluoro PB-22 3-carboxyindole metabolite	17	15	18	14	6	36	1	8	39	19	2	46
13	AB-CHMINACA	2	7	3	4	1	2	0	8	4	2	14	1
14	AB-FUBINACA	5	5	5	2	1	3	1	3	4	1	3	3
15	AB-PINACA	3	1	0	0	1	1	2	1	7	0	2	1
16	AB-PINACA N-(4-hydroxypentyl) metabolite	1	20	1	7	1	7	3	4	10	3	0	4
17	AB-PINACA N-(5-hydroxypentyl) metabolite	4	12	3	4	4	3	10	2	5	10	9	3
18	AB-PINACA pentanoic acid metabolite	2	18	19	3	1	0	2	6	18	9	2	7
19	ADB-FUBINACA	2	9	2	0	1	6	1	23	33	6	7	48
20	ADBICA	2	1	2	3	1	1	1	1	4	1	0	1

21	ADB-PINACA	1	1	1	1	0	1	3	4	0	0	1	0
22	AM1220	4	15	1	1	0	3	7	9	1	0	7	4
23	AM2201	2	0	4	3	2	2	2	1	1	4	0	1
24	AM2201 2-hydroxyindole metabolite	37	7	42	58	50	56	3	15	20	30	35	47
25	AM2201 5-hydroxyindole metabolite	16	4	9	13	3	12	7	1	0	17	14	6
26	AM2233	0	26	19	2	11	15	3	6	8	1	3	6
27	AM694	3	6	0	2	7	5	0	5	11	1	6	9
28	HU-211	14	14	18	19	20	20	12	22	22	21	9	34
29	JWH 007	2	10	2	2	0	2	7	4	1	5	1	1
30	JWH 015	1	4	3	2	1	1	2	3	0	0	1	0
31	JWH 018	1	5	5	4	5	6	3	1	2	1	1	0
32	JWH 018 2-hydroxyindole metabolite	59	12	39	59	64	60	50	32	11	70	35	48
33	JWH 018 4-hydroxyindole metabolite	1	6	6	0	1	2	1	6	5	0	4	3
34	JWH 018 5-hydroxyindole metabolite	8	0	3	8	11	17	7	3	9	5	2	1
35	JWH 018 6-hydroxyindole metabolite	0	9	3	3	4	3	4	2	7	2	1	1
36	JWH 018 7-hydroxyindole metabolite	4	4	5	0	6	2	13	2	10	4	0	5
37	JWH 018 adamantyl analog	12	5	9	12	6	1	0	0	5	16	0	5
38	JWH 018 N-(4-oxo-pentyl) metabolite	3	6	3	2	7	9	1	8	3	6	1	4
39	JWH 018 N-(5-hydroxypentyl) metabolite	4	13	9	1	4	1	1	1	5	3	0	7
40	JWH 018 N-propanoic acid metabolite	0	17	20	9	2	4	5	17	4	5	10	4
41	JWH 019	2	8	1	1	3	0	4	10	12	3	1	11
42	JWH 020	7	2	16	3	1	2	5	4	1	21	5	1
43	JWH 073	4	2	4	2	10	7	1	1	2	4	4	3

44	JWH 081	3	1	4	1	3	1	5	7	8	0	1	0
45	JWH 098	1	4	5	2	5	11	10	9	5	6	7	2
46	JWH 122	1	3	6	3	3	0	1	2	2	4	2	0
47	JWH 182	6	1	23	4	2	6	15	2	10	23	4	9
48	JWH 200	1	0	1	0	2	1	2	1	3	1	2	2
49	JWH 203	1	7	3	6	3	9	3	5	6	4	3	13
50	JWH 210	1	0	2	8	1	3	8	10	0	3	4	6
51	JWH 249	0	0	2	2	4	1	0	1	0	1	5	7
52	JWH 251	1	2	3	3	2	2	1	2	2	6	1	2
53	JWH 307	1	25	9	0	15	7	16	12	6	4	0	1
54	JWH 307 5'-isomer	4	3	5	6	2	0	6	4	9	5	3	5
55	JWH 387	14	24	22	13	7	11	5	20	28	12	8	25
56	JWH 398	1	2	1	6	7	5	2	7	5	3	8	0
57	JWH 412	3	11	8	4	10	8	14	8	5	2	5	2
58	MAM2201 N-(3-fluoropentyl) isomer	1	1	1	1	0	4	0	5	4	8	4	4
59	MAM2201 N-(5-chloropentyl) analog	4	24	11	2	9	7	4	15	8	6	8	0
60	PB-22	3	4	3	2	2	2	3	3	3	3	0	1
61	PB-22 3-carboxyindole metabolite	2	22	32	2	18	31	5	20	21	11	9	9
62	PB-22 N-(4-hydroxypentyl) metabolite	5	14	14	3	11	14	1	5	3	0	7	6
63	PB-22 N-(5-hydroxypentyl) metabolite	6	4	0	2	2	4	6	4	20	3	9	26
64	PB-22 N-pentanoic acid metabolite	2	16	22	1	18	17	3	10	4	4	12	8
65	RCS-4	5	1	5	5	1	4	5	1	4	1	5	4
66	RCS-4-C4 homolog	3	1	1	1	4	10	9	1	1	3	4	5

67	RCS-8	6	27	3	1	12	1	12	10	4	0	2	9
68	THJ 018	3	5	7	2	7	4	0	11	3	5	3	7
69	THJ 2201	7	15	9	13	4	3	7	8	19	10	4	21
70	UR-144	4	3	2	3	8	6	4	2	1	9	4	5
71	XLR11	4	2	7	2	3	3	3	0	4	1	1	0
72	XLR11 6-hydroxyindole metabolite	21	3	5	27	1	5	8	2	27	23	5	43
73	XLR11 N-(4-hydroxypentyl) metabolite	9	4	8	14	3	8	0	0	9	11	2	7

^aRT - room temperature (23°C).

^bLOW, 2-10 ng/mL; HIGH, 10-50 ng/mL.

5.1.7. Blind Study

A blind study was carried out with 15 blank authentic human OF samples spiked with 0-3 individual SC to further test applicability of the method (Table 8). The online SPE LC-QqQ-MS protocol was able to correctly identify virtually all SC parents and metabolites at nominal levels of 5-40 ng/mL in OF. There were two false positives noted; PB-22 N-(5-hydroxypentyl) metabolite detected in addition to the correct analyte PB-22 N-(4-hydroxypentyl) metabolite in Sample 5 and JWH 018 5-hydroxyindole metabolite detected in addition to the correct analyte JWH 018 6-hydroxyindole metabolite in Sample 9. As these compounds have the same transitions and only very small differences in retention time as compared to the correct isomer (Table 1), this result was not unexpected. In addition, one compound not included in the dynamic MRM method, JWH 250 (present in Sample 6), was not detected. Differences between nominal and measured concentrations were generally <30%. Higher values for some compounds may be attributed to the use of IS not chemically identical to every analyte, due to differences in relative ionization or other factors.

Table 8. Blind spike study for SC samples in OF:buffer (1:3, v:v) matrix (n=2).

Sample	Compound Spiked	Compound Detected	Conc. Nominal (ng/mL)	Conc. Detected (ng/mL)
1	JWH 018 4-hydroxyindole metabolite	JWH 018 4-hydroxyindole metabolite	15	13
	JWH 018 7-hydroxyindole metabolite	JWH 018 7-hydroxyindole metabolite	15	17
2	blank	<i>nd</i> ^a	-	-
3	AB-CHMINACA	AB-CHMINACA	15	24
4	UR-144	UR-144	15	30
	XLR-11	XLR-11	15	19
	PB-22	PB-22	15	14
5	PB-22 N-(4-hydroxypentyl) metabolite	PB-22 N-(4-hydroxypentyl) metabolite	15	14
		PB-22 N-(5-hydroxypentyl) metabolite	-	26
6	XLR-11 6-hydroxyindole metabolite	XLR-11 6-hydroxyindole metabolite	15	9
	XLR-11 N-(4-hydroxypentyl) metabolite	XLR-11 N-(4-hydroxypentyl) metabolite	15	13
	JWH 250	<i>nd</i>	15	n.d.
7	ADB-FUBINACA	ADB-FUBINACA	15	14
8	blank	<i>nd</i>	-	-
9	JWH 018 6-hydroxyindole metabolite	JWH 018 6-hydroxyindole metabolite	15	20
		JWH 018 5-hydroxyindole metabolite	-	32
10	PB-22 3-carboxyindole metabolite	PB-22 3-carboxyindole metabolite	5	15
	PB-22 N-pentanoic acid metabolite	PB-22 N-pentanoic acid metabolite	40	40
11	blank	<i>nd</i>	-	-
12	5-fluoro AB-PINACA	5-fluoro AB-PINACA	5	10
13	AM2201	AM2201	5	10
14	AM2201 2-hydroxyindole metabolite	AM2201 2-hydroxyindole metabolite	15	11
	AM2201 5-hydroxyindole metabolite	AM2201 5-hydroxyindole metabolite	15	25
15	JWH 200	JWH 200	40	17

^a*nd* - No detection.

PART SIX: DISCUSSION

The detection of synthetic cannabinoids and metabolites in traditional matrices such as blood, serum, and urine share commonalities, specifically the invasive nature of collecting a sample and the time-consuming process that comes from extraction and clean-up (16, 64-65). Ambroziak et al (64) was able to develop a screening method for 72 SC in whole blood by LC-QqQ-MS and used protein precipitation for sample processing. These authors reported sample processing and analysis time of at least 26 min. In another report, Hess et al (16) developed a method to detect 93 SC in human serum by LC-MS/MS. They used 4 mL of a 99:1 mix of n-hexane:ethyl acetate for LLE, dry down, and reconstitution in 100 μ L MeOH:H₂O:IPA (50:35:15, v:v). Their analytical run time was reported as 18.0 min. Scheidweiler and Huestis (65) quantified 20 SC and 33 metabolites in urine using supported liquid extraction (SLE+) and analysis by LC-QTrap-MS. However, this approach also employed a hydrolysis step with an additional 2 h of processing time. These protocols are indicative of the typically lengthy required purification steps and analysis time for SC in traditional sample matrices.

Limited work has also been reported on the analysis of SC in OF (15, 47-48, 52, 66-68). For example, Malaca et al. (52) were able to detect a range of drugs (including the SC 5F-AKB48) in OF using a simple dilute-and-shoot protocol consisting of diluting OF samples 1:3 with water and analyzing by UHPLC-MS/MS. Williams et al. (66) and Kneisel et al. (15) reported crash-and-shoot treatment prior to LC-MS-MS for analysis of 19 and 28 SC in OF, respectively. While such methods avoid a clean-up step and therefore can facilitate rapid analysis they can also suffer from issues with matrix effects and greater need for routine instrument maintenance and care. Other workers have

employed additional sample processing steps for SC in OF. In two early reports, Coulter et al. (48) utilized off-line SPE for purification of five SC in OF, while Kneisel et al. (15) employed LLE of 30 SC in OF prior to LC-MS/MS analysis. Rocchi et al. (67) reported on a multiclass LC-MS/MS method for a variety of drugs in OF, including 17 SC and metabolites that employed microextraction by packed sorbent (MEPS) clean-up. Blandino et al. (68) utilized SLE clean-up prior to UHPLC-MS/MS analysis to confirm 11 SC in OF. While clearly effective for confirmatory analysis and quantitation of SC in OF, off-line clean-up procedures inevitably increase the cost and time required for such analysis.

Online SPE approaches have also been employed for sample processing of drugs and other xenobiotics in various sample matrices. However, there are currently no available reports of analysis of SC in OF using this technique. A major advantage of online SPE coupled to LC-MS analysis over conventional processing approaches is that the system fully loads, cleans, and elutes analytes from the sample without having to utilize manual processing prior to injection. This advantage holds promise for developing reliable methods for rapid analysis of SC in OF.

In the present study, we report for the first time a method incorporating the analysis of 73 SC and metabolites in OF by LC-QqQ-MS using online SPE for sample pretreatment. Overall, the online SPE system significantly reduced the amount of time required to process and analyze OF samples. Optimization of the method revealed that, in the case of lipophilic compounds such as SC, the use of the PLRP-s online system was highly favorable, likely because styrene-divinyl-benzene copolymers are reported as having high efficiency towards non-polar and medium polarity molecules and better

recoveries for phenolic compounds (69-70). No major interferences or sample loss was revealed, indicating the method to be specific for the compounds of interest while efficiently trapping them onto the online SPE cartridge. Quality control assessment for bias and precision showing values <20% support acceptable repeatability and accuracy when working with online SPE in OF matrix for SC analysis.

The method LOD and LOQ values calculated here were approximately twice those previously reported for urine and ~10-fold higher than those reported for serum (13, 16-17, 65). It should be noted that, because some LOQ values were calculated to be higher than their respective QC1 level, additional studies performed by injecting QC1 10 times through the online SPE system revealed $S/N \geq 10$ for all compounds at this level. In any case, LOQ values in the low ppb as demonstrated in this study are expected to be satisfactory for routine antemortem screening of OF for SC (41).

Examination of the ME, RE, and PE data indicated acceptable results for most SC and metabolites, although there were some exceptions as described previously. In freeze-thaw studies, the majority of SC and metabolites showed good stability when stored at -20°C or in the autosampler at 23°C for up to 72 h. However, in view of anomalous stability results for certain SC as noted in the present study and in previous reports (71-73), processing of OF samples as soon as possible is recommended. Long-term stability was not examined in the present study. Finally, studies with spiked authentic OF indicated excellent qualitative identification and acceptable quantitation for a number of SC and metabolites.

PART SEVEN: CONCLUSION

The present work demonstrates that online SPE coupled to LC-QqQ-MS/MS analysis is a rapid, sensitive, and robust method for the detection and confirmation of a large number of SC compounds and metabolites in OF. This is the first report demonstrating online SPE as a practical approach to sample clean-up for a range of structurally diverse parent and metabolite SC in OF. Furthermore, the validated method was able to correctly identify SC spiked into authentic OF samples at relevant levels for forensic toxicological analysis, indicating that it can serve as a valuable tool in intoxication cases. For example, analysis of 29 SC in 3,078 blood samples from DUID cases by Kronstrand et al. (74) found 862 to be positive for one or more SC compound with mean concentrations of <0.53 ng/mL. In the case of isobaric compounds, it is recommended that additional sophisticated MS techniques, such as 2D-LC-MS/MS, are used in order to correctly identify these compounds (75).

For future applications, it is expected that newer SC and metabolites can be incorporated into the online SPE method with relative ease, a process facilitated by the ability of the PLRP-s cartridge to provide acceptable recoveries for a wide range of structurally diverse SC. Furthermore, investigations involving the analysis of SC samples in blood or urine while using the online SPE protocol would help extend the applicability of the method in clinical and forensic toxicology casework.

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Appendix 1. Synthetic Cannabinoid Common Name, Synonym or Abbreviation, Drug Enforcement Administration (DEA) Scheduling and Chemical Name

No.	Common Name, Synonym or Abbreviation	Schedule ¹	Chemical Name
1	(+/-)-JWH 018 N-(2-hydroxypentyl) metabolite		[1-(2-hydroxypentyl)-1H-indol-3-yl]-1-naphthalenyl-methanone
2	(+/-)-JWH 018 N-(3-hydroxypentyl) metabolite		(1-(3-hydroxypentyl)-1H-indol-3-yl)(naphthalen-1-yl)methanone
3	(+/-)-JWH 018 N-(4-hydroxypentyl) metabolite		(1-(4-hydroxypentyl)-1H-indol-3-yl)(naphthalen-1-yl)-methanone
4	(+/-)-WIN 55,212 (mesylate)		[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenyl-methanone, methanesulfonate
5	5-chloro AB-PINACA, 5-chloro ABP		N-[(1S)-1-(aminocarbonyl)-2-methylpropyl]-1-(5-fluoropentyl)-1H-indazole-3-carboxamide
6	5-fluoro ABICA		N-[(1S)-1-(aminocarbonyl)-2-methylpropyl]-1-(5-fluoropentyl)-1H-indole-3-carboxamide
7	5-fluoro AB-PINACA, AB-PINACA 5-fluoro analog		N-[(1S)-1-(aminocarbonyl)-2-methylpropyl]-1-(5-fluoropentyl)-1H-indazole-3-carboxamide
8	5-fluoro ADBICA, 5F-ADBICA		N-[1-(aminocarbonyl)-2,2-dimethylpropyl]-1-(5-fluoropentyl)-1H-indole-3-carboxamide
9	5-fluoro ADB-PINACA		N-[1-(aminocarbonyl)-2,2-dimethylpropyl]-1-(5-fluoropentyl)-1H-indazole-3-carboxamide
10	5-fluoro AMB, 5-fluoro AMP, 5-fluoro MMB-PINACA		N-[[1-(5-fluoropentyl)-1H-indazol-3-yl]carbonyl]-L-valine, methyl ester
11	5-fluoro PB-22, 5-fluoro QUPIC	I	1-(5-fluoropentyl)-8-quinolinyl ester-1H-indole-3-carboxylic acid
12	5-fluoro PB-22 3-carboxyindole metabolite		1-(5-fluoropentyl)-1H-indole-3-carboxylic acid
13	AB-CHMINACA	I	N-[(1S)-1-(aminocarbonyl)-2-methylpropyl]-1-(cyclohexylmethyl)-1H-indazole-3-carboxamide
14	AB-FUBINACA	I	N-[(1S)-1-(aminocarbonyl)-2-methylpropyl]-1-[(4-fluorophenyl)methyl]-1H-indazole-3-carboxamide
15	AB-PINACA	I	(S)-N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-pentyl-1H-indazole-3-carboxamide
16	AB-PINACA N-(4-hydroxypentyl) metabolite		N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(4-hydroxypentyl)-1H-indazole-3-carboxamide

17	AB-PINACA N-(5-hydroxypentyl) metabolite		N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(5-hydroxypentyl)-1H-indazole-3-carboxamide
18	AB-PINACA pentanoic acid metabolite		3-[[[1-(aminocarbonyl)-2-methylpropyl]amino]carbonyl]-1H-indazole-1-pentanoic acid
19	ADB-FUBINACA	I	N-[1-(aminocarbonyl)-2,2-dimethylpropyl]-1-[(4-fluorophenyl)methyl]-1H-indazole-3-carboxamide
20	ADBICA		N-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-pentyl-1H-indole-3-carboxamide
21	ADB-PINACA	I	N-[1-(aminocarbonyl)-2,2-dimethylpropyl]-1-pentyl-1H-indazole-3-carboxamide
22	AM1220		[1-[(1-methyl-2-piperidinyl)methyl]-1H-indol-3-yl]-1-naphthalenyl-methanone
23	AM2201	I	[1-(5-fluoropentyl)-1H-indol-3-yl]-1-naphthalenyl-methanone
24	AM2201 2-hydroxyindole metabolite		(1-(5-fluoropentyl)-2-hydroxy-1H-indol-3-yl)(naphthalen-1-yl)methanone
25	AM2201 5-hydroxyindole metabolite		(1-(5-fluoropentyl)-5-hydroxy-1H-indol-3-yl)(naphthalen-1-yl)methanone
26	AM2233		(2-iodophenyl)[1-[(1-methyl-2-piperidinyl)methyl]-1H-indol-3-yl]-methanone
27	AM694	I	[1-(5-fluoropentyl)-1H-indol-3-yl](2-iodophenyl)-methanone
28	HU-211, Dexanabinol		3-(1,1-dimethylheptyl)-6aS,7,10,10aS-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol
29	JWH 007		(2-methyl-1-pentyl-1H-indol-3-yl)-1-naphthalenyl-methanone
30	JWH 015		(2-methyl-1-propyl-1H-indol-3-yl)-1-naphthalenyl-methanone
31	JWH 018, AM678	I	(1-pentyl-1H-indol-3-yl)-1-naphthalenyl-methanone
32	JWH 018 2-hydroxyindole metabolite		(2-hydroxy-1-pentyl-1H-indol-3-yl)(naphthalen-1-yl)-methanone
33	JWH 018 4-hydroxyindole metabolite		(4-hydroxy-1-pentyl-1H-indol-3-yl)(naphthalen-1-yl)-methanone
34	JWH 018 5-hydroxyindole metabolite		(5-hydroxy-1-pentyl-1H-indol-3-yl)(naphthalen-1-yl)-methanone
35	JWH 018 6-hydroxyindole metabolite		(6-hydroxy-1-pentyl-1H-indol-3-yl)(naphthalen-1-yl)-methanone

36	JWH 018 7-hydroxyindole metabolite		(7-hydroxy-1-pentyl-1H-indol-3-yl)naphthalen-1-yl)-methanone
37	JWH 018 adamantyl analog, AB-001		(1s,3s)-adamantan-1-yl(1-pentyl-1H-indol-3-yl)methanone
38	JWH 018 N-(4-oxo-pentyl) metabolite		5-(3-(1-naphthoyl)-1H-indol-1-yl)pentan-2-one
39	JWH 018 N-(5-hydroxypentyl) metabolite		(1-(5-hydroxypentyl)-1H-indol-3-yl)(naphthalen-1-yl)methanone
40	JWH 018 N-propanoic acid metabolite		3-(1-naphthalenylcarbonyl)-1H-indole-1-propanoic acid
41	JWH 019	I	(1-hexyl-1H-indol-3-yl)-1-naphthalenyl-methanone
42	JWH 020		(1-heptyl-1H-indol-3-yl)-1-naphthalenyl-methanone
43	JWH 073	I	(1-butyl-1H-indol-3-yl)-1-naphthalenyl-methanone
44	JWH 081	I	(4-methoxy-1-naphthalenyl)(1-pentyl-1H-indol-3-yl)-methanone
45	JWH 098		(4-methoxy-1-naphthalenyl)(2-methyl-1-pentyl-1H-indol-3-yl)-methanone
46	JWH 122	I	(4-methyl-1-naphthalenyl)(1-pentyl-1H-indol-3-yl)-methanone
47	JWH 182		(1-pentyl-1H-indol-3-yl)(4-propyl-1-naphthalenyl)-methanone
48	JWH 200	I	[1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl]-1-naphthalenyl-methanone
49	JWH 203	I	2-(2-chlorophenyl)-1-(1-pentyl-1H-indol-3-yl)-ethanone
50	JWH 210		(4-ethyl-1-naphthalenyl)(1-pentyl-1H-indol-3-yl)-methanone
51	JWH 249		2-(2-bromophenyl)-1-(1-pentyl-1H-indol-3-yl)-ethanone
52	JWH 251		2-(2-methylphenyl)-1-(1-pentyl-1H-indol-3-yl)-ethanone
53	JWH 307		[5-(2-fluorophenyl)-1-pentyl-1H-pyrrol-3-yl]-1-naphthalenyl-methanone
54	JWH 307 5'-isomer		(2-(2-Fluorophenyl)-1-pentyl-1H-pyrrol-3-yl)(naphthalen-2-yl)methanone
55	JWH 387		(4-bromo-1-naphthalenyl)(1-pentyl-1H-indol-3-yl)-methanone
56	JWH 398	I	(4-chloro-1-naphthalenyl)(1-pentyl-1H-indol-3-yl)-methanone

57	JWH 412		(4-fluoro-1-naphthalenyl)(1-pentyl-1H-indol-3-yl)-methanone
58	MAM2201 N-(3-fluoropentyl) isomer		(1-(3-fluoropentyl)-1H-indol-3-yl)(4-methylnaphthalen-1-yl)methanone
59	MAM2201 N-(5-chloropentyl) analog, JWH 122 N-(5-chloropentyl) analog		(1-(5-chloropentyl)-1H-indol-3-yl)(4-methylnaphthalen-1-yl)methanone
60	PB-22, QUPIC	I	1-pentyl-8-quinolinyl ester-1H-indole-3-carboxylic acid
61	PB-22 3-carboxyindole metabolite		1-pentyl-1H-indole-3-carboxylic acid
62	PB-22 N-(4-hydroxypentyl) metabolite		quinolin-8-yl 1-(4-hydroxypentyl)-1H-indole-3-carboxylate
63	PB-22 N-(5-hydroxypentyl) metabolite		quinolin-8-yl 1-(5-hydroxypentyl)-1H-indole-3-carboxylate
64	PB-22 N-pentanoic acid metabolite		5-(3-((quinolin-8-yloxy)carbonyl)-1H-indol-1-yl)pentanoic acid
65	RCS-4, BTM-4, E-4, OBT-199, SR-19	I	(4-methoxyphenyl)(1-pentyl-1H-indol-3-yl)methanone
66	RCS-4-C4 homolog		(4-methoxyphenyl)(1-butyl-1H-indol-3-yl)-methanone
67	RCS-8, BTM-8, SR-18	I	1-(1-(2-cyclohexylethyl)-1H-indol-3-yl)-2-(2-methoxyphenyl)ethanone
68	THJ 018, JWH 018 indazole analog		1-naphthalenyl(1-pentyl-1H-indazol-3-yl)-methanone
69	THJ 2201, AM2201 indazole analog, 5-fluoropentyl JWH018 indazole analog, 5-fluoro THJ 018	I	[1-(5-fluoropentyl)-1H-indazol-3-yl]-1-naphthalenyl-methanone
70	UR-144, KM-X1	I	(1-pentyl-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)-methanone
71	XLR11, 5-fluoro-UR-144, 5-FUR-144	I	(1-(5-fluoropentyl)-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone
72	XLR11 6-hydroxyindole metabolite		[1-(5-fluoropentyl)-6-hydroxy-1H-indol-3-yl](2,2,3,3-tetramethylcyclopropyl)-methanone
73	XLR11 N-(4-hydroxypentyl) metabolite		[1-(5-fluoro-4-hydroxypentyl)-1H-indol-3-yl](2,2,3,3-tetramethylcyclopropyl)-methanone
74	(+/-)-JWH 018 N-(4-hydroxypentyl) metabolite- <i>d</i> ₅		(1-(4-hydroxypentyl)-2,5,6,7,8-d ₅ -1H-indol-3-yl)(naphthalen-1-yl)-methanone
75	AB-FUBINACA- <i>d</i> ₄		N-[(1S)-1-(aminocarbonyl)-2-methylpropyl]-1-[(4-fluorophenyl)methyl]-4,5,6,7-d ₄ -1H-indazole-3-carboxamide
76	AB-PINACA- <i>d</i> ₉		(S)-N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(pentyl-2,2',3,3',4,4',5,5,5-d ₉)-1H-indazole-3-carboxamide

77	ADBICA- <i>d</i> ₉		N-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(pentyl-2,2',3,3',4,4',5,5,5-d ₉)-1H-indole-3-carboxamide
78	ADB-PINACA- <i>d</i> ₉		N-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(pentyl-2,2',3,3',4,4',5,5,5-d ₉)-1H-indazole-3-carboxamide
79	AM2201- <i>d</i> ₅		[1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl-2,4,5,6,7-d ₅]-1-naphthalenyl-methanone
80	JWH 007- <i>d</i> ₉		(2-methyl-1-pentyl-1H-indol-3-yl)-1-naphthalenyl-2,2,3,3,4,4,5,5,5-d ₉ -methanone
81	JWH 015- <i>d</i> ₇		(2-methyl-1-propyl-(1,1,2,2,3,3,3-d ₇)-1H-indol-3-yl)-1-naphthalenyl-methanone
82	JWH 018 4-hydroxyindole metabolite- <i>d</i> ₉		(4-hydroxy-1-pentyl-2,2,3,3,4,4,5,5,5-d ₉ -1H-indol-3-yl)(naphthalen-1-yl)-methanone
83	JWH 018 6-hydroxyindole metabolite- <i>d</i> ₉		(6-hydroxy-1-pentyl-2,2,3,3,4,4,5,5,5-d ₉ -1H-indol-3-yl)(naphthalen-1-yl)-methanone
84	JWH 018 7-hydroxyindole metabolite- <i>d</i> ₉		(7-hydroxy-1-pentyl-1H-indol-3-yl)naphthalen-1-yl)-methanone-2,2',3,3',4,4',5,5,5-d ₉
85	JWH 018 N-(5-hydroxypentyl) metabolite- <i>d</i> ₅		(1-(5-hydroxypentyl)-1H-indol-3-yl)-methanone-2',4',5',6',7'-d ₅
86	JWH 018 N-pentanoic acid metabolite- <i>d</i> ₄		5-(3-(1-naphthoyl)-1H-indol-1-yl)-pentanoic-3,3,4,4-d ₄ acid
87	JWH 018 N-pentanoic acid metabolite- <i>d</i> ₅		5-(3-(1-naphthoyl)-1H-indol-1-yl)-pentanoic-2,4,5,6,7-d ₅ -acid
88	JWH 018- <i>d</i> ₉		1-naphthalenyl[1-(pentyl-2,2,3,3,4,4,5,5,5-d ₉)-1H-indol-3-yl]-methanone
89	JWH 073- <i>d</i> ₇		[1-(butyl-2,2,3,3,4,4,4-d ₇)-1H-indol-3-yl]-1-naphthalenyl-methanone
90	JWH 081- <i>d</i> ₉		(4-methoxy-1-naphthalenyl)(1-pentyl-1H-indol-3-yl)-methanone-2,2',3,3',4,4',5,5,5-d ₉
91	JWH 122- <i>d</i> ₉		(4-methyl-1-naphthalenyl)(1-pentyl-1H-indol-3-yl)-methanone-2,2',3,3',4,4',5,5,5-d ₉
92	JWH 200- <i>d</i> ₅		[1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl-2',4',5',6',7'-d ₅]-1-naphthalenyl-methanone
93	JWH 210- <i>d</i> ₉		(4-ethyl-1-naphthalenyl)(1-pentyl-1H-indol-3-yl)-methanone-d ₉
94	JWH 250- <i>d</i> ₅		2-(2-methoxyphenyl)-1-(1-pentyl-1H-indol-3-yl-2,4,5,6,7-d ₅)-ethanone

95	JWH 398- <i>d</i> ₉		(4-chloronaphthalen-1-yl) (1-pentylindolin-3-yl)-methanone-, 2', 3, 3', 4, 4', 5, 5, 5-d ₉
96	MAM2201- <i>d</i> ₅		[1-(5-fluoropentyl)-1H-indol-3-yl-d ₅](4-methyl-1-naphthalenyl)-methanone
97	PB-22- <i>d</i> ₉		1-pentyl-2,2',3,3',4,4',5,5,5-d ₉ -8-quinolinyl ester-1H-indole-3-carboxylic acid
98	RCS-4- <i>d</i> ₉		(4-methoxyphenyl)(1-pentyl-d ₉ -1H-indol-3-yl)methanone
99	UR-144- <i>d</i> ₅		(1-pentyl-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)-methanone-2,4,5,6,7-d ₅
100	XLR11- <i>d</i> ₅		(1-(5-fluoropentyl)-1H-indol-3-yl-2,4,5,6,7-d ₅)(2,2,3,3-tetramethylcyclopropyl)methanone

¹Controlled Substances Act (21 U.S.C. §801 et seq.) (CSA) schedule for the basic or parent chemical as of October 2018 according to the Drug Enforcement Administration (DEA).

Appendix 2. Data Acquisition and Quantitation

Compound: (+/-) JWH 018 N-(2-hydroxypentyl) metabolite

Figure 1. Retention time determination for (+/-) JWH 018 N-(2-hydroxypentyl) metabolite using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for (+/-) JWH 018 N-(2-hydroxypentyl) metabolite (QCs shown as triangles) (a); quantifier peak (358.2 $\rightarrow$ 155.1) (b) and (+/-) JWH 018 N-(4-hydroxypentyl) metabolite- d_5 as internal standard (c) also indicated.

Compound: (+/-) JWH 018 N-(3-hydroxypentyl) metabolite

Figure 1. Retention time determination for (+/-) JWH 018 N-(3-hydroxypentyl) metabolite using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for (+/-) JWH 018 N-(3-hydroxypentyl) metabolite (QCs shown as triangles) (a); quantifier peak (358.2 $\rightarrow$ 155.1) (b) and (+/-) JWH 018 N-(4-hydroxypentyl) metabolite-*d*₅ as internal standard (c) also indicated.

Compound: (+/-) JWH 018 N-(4-hydroxypentyl) metabolite

Figure 1. Retention time determination for (+/-) JWH 018 N-(4-hydroxypentyl) metabolite using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for (+/-) JWH 018 N-(4-hydroxypentyl) metabolite (QCs shown as triangles) (a); quantifier peak (358.2 $\rightarrow$ 155.1) (b) and (+/-) JWH 018 N-(4-hydroxypentyl) metabolite- d_5 as internal standard (c) also indicated.

Compound: (+/-) WIN 55,212 (mesylate)

Figure 1. Retention time determination for (+/-) WIN 55,212 (mesylate) using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μm) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for (+/-) WIN 55,212 (mesylate) (QCs shown as triangles) (a); quantifier peak (427.2 $\rightarrow$ 155) (b) and JWH 200- d_5 as internal standard (c) also indicated.

Compound: 5-chloro AB-PINACA

Figure 1. Retention time determination for 5-chloro AB-PINACA using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for 5-chloro AB-PINACA (QCs shown as triangles) (a); quantifier peak (365.2 $\rightarrow$ 320.1) (b) and AB-PINACA- d_9 as internal standard (c) also indicated.

Compound: 5-fluoro ABICA

Figure 1. Retention time determination for 5-fluoro ABICA using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for 5-fluoro ABICA (QCs shown as triangles) (a); quantifier peak (348.2 $\rightarrow$ 232.1) (b) and ADBICA-d₉ as internal standard (c) also indicated.

Compound: 5-fluoro AB-PINACA

Figure 1. Retention time determination for 5-fluoro AB-PINACA using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for 5-fluoro AB-PINACA (QCs shown as triangles) (a); quantifier peak (349.2 $\rightarrow$ 233) (b) and AB-PINACA- d_9 as internal standard (c) also indicated.

Compound: 5-fluoro ADBICA

Figure 1. Retention time determination for 5-fluoro ADBICA using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for 5-fluoro ADBICA (QCs shown as triangles) (a); quantifier peak (362.2 $\rightarrow$ 232.1) (b) and ADBICA-d₉ as internal standard (c) also indicated.

Compound: 5-fluoro ADB-PINACA

Figure 1. Retention time determination for 5-fluoro ADB-PINACA using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for 5-fluoro ADB-PINACA (QCs shown as triangles) (a); quantifier peak (363.2 $\rightarrow$ 318.2) (b) and ADB-PINACA- d_9 as internal standard (c) also indicated.

Compound: 5-fluoro AMB

Figure 1. Retention time determination for 5-fluoro AMB using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for 5-fluoro AMB (QCs shown as triangles) (a); quantifier peak (364.2 $\rightarrow$ 233) (b) and AB-PINACA-d₉ as internal standard (c) also indicated.

Compound: 5-fluoro PB-22

Figure 1. Retention time determination for 5-fluoro PB-22 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for 5-fluoro PB-22 (QCs shown as triangles) (a); quantifier peak (377.2 $\rightarrow$ 232.1) (b) and PB-22- d_9 as internal standard (c) also indicated.

Compound: 5-fluoro PB-22 3-carboxyindole metabolite

Figure 1. Retention time determination for 5-fluoro PB-22 3-carboxyindole metabolite using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for 5-fluoro PB-22 3-carboxyindole metabolite (QCs shown as triangles) (a); quantifier peak (250.1 $\rightarrow$ 206.1) (b) and PB-22-d₉ as internal standard (c) also indicated.

Compound: AB-CHMINACA

Figure 1. Retention time determination for AB-CHMINACA using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for AB-CHMINACA (QCs shown as triangles) (a); quantifier peak (357.2 $\rightarrow$ 241) (b) and AB-FUBINACA- d_4 as internal standard (c) also indicated.

Compound: AB-FUBINACA

Figure 1. Retention time determination for AB-FUBINACA using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for AB-FUBINACA (QCs shown as triangles) (a); quantifier peak (369.2 $\rightarrow$ 324.1) (b) and AB-FUBINACA- d_4 as internal standard (c) also indicated.

Compound: AB-PINACA

Figure 1. Retention time determination for AB-PINACA using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for AB-PINACA (QCs shown as triangles) (a); quantifier peak (331.2 $\rightarrow$ 215) (b) and AB-PINACA- d_9 as internal standard (c) also indicated.

Compound: AB-PINACA N-(4-hydroxypentyl) metabolite

Figure 1. Retention time determination for AB-PINACA N-(4-hydroxypentyl) metabolite using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (3-60 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for AB-PINACA N-(4-hydroxypentyl) metabolite (QCs shown as triangles) (a); quantifier peak (347.2 $\rightarrow$ 302.1) (b) and AB-PINACA- d_9 as internal standard (c) also indicated.

Compound: AB-PINACA N-(5-hydroxypentyl) metabolite

Figure 1. Retention time determination for AB-PINACA N-(5-hydroxypentyl) metabolite using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for AB-PINACA N-(5-hydroxypentyl) metabolite (QCs shown as triangles) (a); quantifier peak (347.2 $\rightarrow$ 302.1) (b) and AB-PINACA-*d*₉ as internal standard (c) also indicated.

Compound: AB-PINACA pentanoic acid metabolite

Figure 1. Retention time determination for AB-PINACA pentanoic acid metabolite using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (5-100 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for AB-PINACA pentanoic acid metabolite (QCs shown as triangles) (a); quantifier peak (361.2 $\rightarrow$ 316.1) (b) and AB-PINACA- d_9 as internal standard (c) also indicated.

Compound: ADB-FUBINACA

Figure 1. Retention time determination for ADB-FUBINACA using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for ADB-FUBINACA (QCs shown as triangles) (a); quantifier peak (383.2 $\rightarrow$ 338.1) (b) and AB-FUBINACA-d₄ as internal standard (c) also indicated.

Compound: ADBICA

Figure 1. Retention time determination for ADBICA using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for ADBICA (QCs shown as triangles) (a); quantifier peak (344.2 $\rightarrow$ 214.1) (b) and ADBICA- d_9 as internal standard (c) also indicated.

Compound: ADB-PINACA

Figure 1. Retention time determination for ADB-PINACA using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for ADB-PINACA (QCs shown as triangles) (a); quantifier peak (345.2 $\rightarrow$ 215) (b) and ADB-PINACA-d₉ as internal standard (c) also indicated.

Compound: AM1220

Figure 1. Retention time determination for AM1220 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (3-60 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for AM1220 (QCs shown as triangles) (a); quantifier peak (383.2 $\rightarrow$ 98.1) (b) and AM2201- d_5 as internal standard (c) also indicated.

Compound: AM2201

Figure 1. Retention time determination for AM2201 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2-40 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for AM2201 (QCs shown as triangles) (a); quantifier peak (360.2 $\rightarrow$ 127) (b) and AM2201-d₅ as internal standard (c) also indicated.

Compound: AM2201 2-hydroxyindole metabolite

Figure 1. Retention time determination for AM2201 2-hydroxyindole metabolite using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (right) and precursor ion (left) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for AM2201 2-hydroxyindole metabolite (QCs shown as triangles) (a); quantifier peak (376.2 $\rightarrow$ 270.1) (b) and AM2201- d_5 as internal standard (c) also indicated.

Compound: AM2201 5-hydroxyindole metabolite

Figure 1. Retention time determination for AM2201 5-hydroxyindole metabolite using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for AM2201 5-hydroxyindole metabolite (QCs shown as triangles) (a); quantifier peak (376.2 $\rightarrow$ 155) (b) and AM2201- d_5 as internal standard (c) also indicated.

Compound: AM2233

Figure 1. Retention time determination for AM2233 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for AM2233 (QCs shown as triangles) (a); quantifier peak (459.1 $\rightarrow$ 98.1) (b) and AM2201- d_5 as internal standard (c) also indicated.

Compound: AM694

Figure 1. Retention time determination for AM694 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for AM694 (QCs shown as triangles) (a); quantifier peak (436.1 $\rightarrow$ 230.9) (b) and AM2201- d_5 as internal standard (c) also indicated.

Compound: HU-211

Figure 1. Retention time determination for HU-211 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (right) and precursor ion (left) also shown.

Figure 2. Typical calibration curve (4-80 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for HU-211 (QCs shown as triangles) (a); quantifier peak (387.3 $\rightarrow$ 71.1) (b) and JWH 018-*d*₉ as internal standard (c) also indicated.

Compound: JWH 007

Figure 1. Retention time determination for JWH 007 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2-40 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 007 (QCs shown as triangles) (a); quantifier peak (356.2 $\rightarrow$ 127) (b) and JWH 007-d₉ as internal standard (c) also indicated.

Compound: JWH 015

Figure 1. Retention time determination for JWH 015 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (1-20 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 015 (QCs shown as triangles) (a); quantifier peak (328.2 $\rightarrow$ 127) (b) and JWH 015- d_7 as internal standard (c) also indicated.

Compound: JWH 018

Figure 1. Retention time determination for JWH 018 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2-40 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 018 (QCs shown as triangles) (a); quantifier peak (342.2 $\rightarrow$ 127) (b) and JWH 018- d_9 as internal standard (c) also indicated.

Compound: JWH 018 2-hydroxyindole metabolite

Figure 1. Retention time determination for JWH 018 2-hydroxyindole metabolite using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (right) and precursor ion (left) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 018 2-hydroxyindole metabolite (QCs shown as triangles) (a); quantifier peak (358.2 $\rightarrow$ 270.1) (b) and JWH 018- d_9 as internal standard (c) also indicated.

Compound: JWH 018 4-hydroxyindole metabolite

Figure 1. Retention time determination for JWH 018 4-hydroxyindole metabolite using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 018 4-hydroxyindole metabolite (QCs shown as triangles) (a); quantifier peak (358.2 $\rightarrow$ 127.1) (b) and JWH 018 4-hydroxyindole metabolite- d_9 as internal standard (c) also indicated.

Compound: JWH 018 5-hydroxyindole metabolite

Figure 1. Retention time determination for JWH 018 5-hydroxyindole metabolite using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 018 5-hydroxyindole metabolite (QCs shown as triangles) (a); quantifier peak (358.2 $\rightarrow$ 155) (b) and JWH 018 N-pentanoic acid metabolite- d_5 as internal standard (c) also indicated.

Compound: JWH 018 6-hydroxyindole metabolite

Figure 1. Retention time determination for JWH 018 6-hydroxyindole metabolite using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 018 6-hydroxyindole metabolite (QCs shown as triangles) (a); quantifier peak (358.2 $\rightarrow$ 155) (b) and JWH 018 6-hydroxyindole metabolite- d_9 as internal standard (c) also indicated.

Compound: JWH 018 7-hydroxyindole metabolite

Figure 1. Retention time determination for JWH 018 7-hydroxyindole metabolite using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 018 7-hydroxyindole metabolite (QCs shown as triangles) (a); quantifier peak (358.2 $\rightarrow$ 155) (b) and JWH 018 7-hydroxyindole metabolite- d_9 as internal standard (c) also indicated.

Compound: JWH 018 adamantyl analog

Figure 1. Retention time determination for JWH 018 adamantyl analog using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (right) and precursor ion (left) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 018 adamantyl analog (QCs shown as triangles) (a); quantifier peak (350.2 $\rightarrow$ 135.1) (b) and JWH 018- d_9 as internal standard (c) also indicated.

Compound: JWH 018 N-(4-oxo-pentyl) metabolite

Figure 1. Retention time determination for JWH 018 N-(4-oxo-pentyl) metabolite using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 018 N-(4-oxo-pentyl) metabolite (QCs shown as triangles) (a); quantifier peak (356.2 $\rightarrow$ 155) (b) and JWH 018 N-pentanoic acid metabolite- d_5 as internal standard (c) also indicated.

Compound: JWH 018 N-(5-hydroxypentyl) metabolite

Figure 1. Retention time determination for JWH 018 N-(5-hydroxypentyl) metabolite using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 018 N-(5-hydroxypentyl) metabolite (QCs shown as triangles) (a); quantifier peak (358.2 $\rightarrow$ 155) (b) and JWH 018 N-(5-hydroxypentyl) metabolite- d_5 as internal standard (c) also indicated.

Compound: JWH 018 N-propanoic acid metabolite

Figure 1. Retention time determination for JWH 018 N-propanoic acid metabolite using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 018 N-propanoic acid metabolite (QCs shown as triangles) (a); quantifier peak (344.1 $\rightarrow$ 155) (b) and JWH 018 N-pentanoic acid metabolite- d_4 as internal standard (c) also indicated.

Compound: JWH 019

Figure 1. Retention time determination for JWH 019 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 019 (QCs shown as triangles) (a); quantifier peak (356.2 $\rightarrow$ 127) (b) and JWH 018-*d*₉ as internal standard (c) also indicated.

Compound: JWH 020

Figure 1. Retention time determination for JWH 020 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2-40 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 020 (QCs shown as triangles) (a); quantifier peak (370.2 $\rightarrow$ 155) (b) and JWH 018-*d*₉ as internal standard (c) also indicated.

Compound: JWH 073

Figure 1. Retention time determination for JWH 073 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 073 (QCs shown as triangles) (a); quantifier peak (328.2 $\rightarrow$ 127) (b) and JWH 073- d_7 as internal standard (c) also indicated.

Compound: JWH 081

Figure 1. Retention time determination for JWH 081 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2-40 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 081 (QCs shown as triangles) (a); quantifier peak (372.2 $\rightarrow$ 185) (b) and JWH 081- d_9 as internal standard (c) also indicated.

Compound: JWH 098

Figure 1. Retention time determination for JWH 098 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (right) and precursor ion (left) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 098 (QCs shown as triangles) (a); quantifier peak (386.2 $\rightarrow$ 185) (b) and JWH 018-*d*₉ as internal standard (c) also indicated.

Compound: JWH 122

Figure 1. Retention time determination for JWH 122 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (right) and precursor ion (left) also shown.

Figure 2. Typical calibration curve (2-40 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 122 (QCs shown as triangles) (a); quantifier peak (356.2 $\rightarrow$ 169) (b) and JWH 122- d_9 as internal standard (c) also indicated.

Compound: JWH 182

Figure 1. Retention time determination for JWH 182 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 µm) column. Structure (right) and precursor ion (left) also shown.

Figure 2. Typical calibration curve (2-40 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 182 (QCs shown as triangles) (a); quantifier peak (384.2 → 197.1) (b) and JWH 018-*d*₉ as internal standard (c) also indicated.

Compound: JWH 200

Figure 1. Retention time determination for JWH 200 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (1-20 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 200 (QCs shown as triangles) (a); quantifier peak (385.2 $\rightarrow$ 155) (b) and JWH 200- d_5 as internal standard (c) also indicated.

Compound: JWH 203

Figure 1. Retention time determination for JWH 203 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 203 (QCs shown as triangles) (a); quantifier peak (340.1 $\rightarrow$ 125) (b) and JWH 018-*d*₉ as internal standard (c) also indicated.

Compound: JWH 210

Figure 1. Retention time determination for JWH 210 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (right) and precursor ion (left) also shown.

Figure 2. Typical calibration curve (2-40 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 210 (QCs shown as triangles) (a); quantifier peak (370.2 $\rightarrow$ 183.1) (b) and JWH 210- d_9 as internal standard (c) also indicated.

Compound: JWH 249

Figure 1. Retention time determination for JWH 249 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 249 (QCs shown as triangles) (a); quantifier peak (381.4 $\rightarrow$ 168.9) (b) and JWH 018-*d*₉ as internal standard (c) also indicated.

Compound: JWH 251

Figure 1. Retention time determination for JWH 251 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 251 (QCs shown as triangles) (a); quantifier peak (320.2 $\rightarrow$ 105.1) (b) and JWH 250- d_5 as internal standard (c) also indicated.

Compound: JWH 307

Figure 1. Retention time determination for JWH 307 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (1-20 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 307 (QCs shown as triangles) (a); quantifier peak (386.2 $\rightarrow$ 155) (b) and JWH 018-*d*₉ as internal standard (c) also indicated.

Compound: JWH 307 5'-isomer

Figure 1. Retention time determination for JWH 307 5'-isomer using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2-40 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 307 5'-isomer (QCs shown as triangles) (a); quantifier peak (386.2 $\rightarrow$ 155.1) (b) and JWH 018-*d*₉ as internal standard (c) also indicated.

Compound: JWH 387

Figure 1. Retention time determination for JWH 387 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (right) and precursor ion (left) also shown.

Figure 2. Typical calibration curve (3-60 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 387 (QCs shown as triangles) (a); quantifier peak (420.1 $\rightarrow$ 233) (b) and JWH 018-*d*₉ as internal standard (c) also indicated.

Compound: JWH 398

Figure 1. Retention time determination for JWH 398 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (right) and precursor ion (left) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 398 (QCs shown as triangles) (a); quantifier peak (376.2 $\rightarrow$ 189) (b) and JWH 398- d_9 as internal standard (c) also indicated.

Compound: JWH 412

Figure 1. Retention time determination for JWH 412 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for JWH 412 (QCs shown as triangles) (a); quantifier peak (360.2 $\rightarrow$ 173) (b) and JWH 018-*d*₉ as internal standard (c) also indicated.

Compound: MAM2201 N-(3-fluoropentyl) isomer

Figure 1. Retention time determination for MAM2201 N-(3-fluoropentyl) isomer using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2-40 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for MAM2201 N-(3-fluoropentyl) isomer (QCs shown as triangles) (a); quantifier peak (374.2 $\rightarrow$ 169.1) (b) and MAM2201-d₅ as internal standard (c) also indicated.

Compound: MAM2201 N-(5-chloropentyl) analog

Figure 1. Retention time determination for MAM2201 N-(5-chloropentyl) analog using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (3-60 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for MAM2201 N-(5-chloropentyl) analog (QCs shown as triangles) (a); quantifier peak (390.2 $\rightarrow$ 169.1) (b) and MAM2201- d_5 as internal standard (c) also indicated.

Compound: PB-22

Figure 1. Retention time determination for PB-22 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for PB-22 (QCs shown as triangles) (a); quantifier peak (359.2 $\rightarrow$ 214.1) (b) and PB-22- d_9 as internal standard (c) also indicated.

Compound: PB-22 3-carboxyindole metabolite

Figure 1. Retention time determination for PB-22 3-carboxyindole metabolite using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (3-60 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for PB-22 3-carboxyindole metabolite (QCs shown as triangles) (a); quantifier peak (232.1 $\rightarrow$ 132) (b) and PB-22-d₉ as internal standard (c) also indicated.

Compound: PB-22 N-(4-hydroxypentyl) metabolite

Figure 1. Retention time determination for PB-22 N-(4-hydroxypentyl) metabolite using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for PB-22 N-(4-hydroxypentyl) metabolite (QCs shown as triangles) (a); quantifier peak (375.2 $\rightarrow$ 230.1) (b) and PB-22-d₉ as internal standard (c) also indicated.

Compound: PB-22 N-(5-hydroxypentyl) metabolite

Figure 1. Retention time determination for PB-22 N-(5-hydroxypentyl) metabolite using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (5-100 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for PB-22 N-(5-hydroxypentyl) metabolite (QCs shown as triangles) (a); quantifier peak (375.2 $\rightarrow$ 230.1) (b) and PB-22-d₉ as internal standard (c) also indicated.

Compound: PB-22 N-pentanoic acid metabolite

Figure 1. Retention time determination for PB-22 N-pentanoic acid metabolite using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for PB-22 N-pentanoic acid metabolite (QCs shown as triangles) (a); quantifier peak (389.2 $\rightarrow$ 244.1) (b) and PB-22-d₉ as internal standard (c) also indicated.

Compound: RCS-4

Figure 1. Retention time determination for RCS-4 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2-40 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for RCS-4 (QCs shown as triangles) (a); quantifier peak (322.2 $\rightarrow$ 135) (b) and RCS-4-d₉ as internal standard (c) also indicated.

Compound: RCS-4-C4 homolog

Figure 1. Retention time determination for RCS-4-C4 homolog using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for RCS-4-C4 homolog (QCs shown as triangles) (a); quantifier peak (308.2 $\rightarrow$ 135) (b) and RCS-4-d₉ as internal standard (c) also indicated.

Compound: RCS-8

Figure 1. Retention time determination for RCS-8 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (right) and precursor ion (left) also shown.

Figure 2. Typical calibration curve (2-40 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for RCS-8 (QCs shown as triangles) (a); quantifier peak (376.2 $\rightarrow$ 121) (b) and RCS-4- d_9 as internal standard (c) also indicated.

Compound: THJ 018

Figure 1. Retention time determination for THJ 018 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (right) and precursor ion (left) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for THJ 018 (QCs shown as triangles) (a); quantifier peak (343.2 $\rightarrow$ 215) (b) and JWH 018- d_9 as internal standard (c) also indicated.

Compound: THJ 2201

Figure 1. Retention time determination for THJ 2201 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for THJ 2201 (QCs shown as triangles) (a); quantifier peak (361.2 $\rightarrow$ 233) (b) and JWH 018-*d*₉ as internal standard (c) also indicated.

Compound: UR-144

Figure 1. Retention time determination for UR-144 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (right) and precursor ion (left) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for UR-144 (QCs shown as triangles) (a); quantifier peak (312.2 $\rightarrow$ 125.1) (b) and UR-144-*d*₅ as internal standard (c) also indicated.

Compound: XLR11

Figure 1. Retention time determination for XLR11 using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for XLR11 (QCs shown as triangles) (a); quantifier peak (330.2 $\rightarrow$ 125.1) (b) and XLR11- d_5 as internal standard (c) also indicated.

Compound: XLR11 6-hydroxyindole metabolite

Figure 1. Retention time determination for XLR11 6-hydroxyindole metabolite using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for XLR11 6-hydroxyindole metabolite (QCs shown as triangles) (a); quantifier peak (346.2 $\rightarrow$ 125.1) (b) and XLR11-d₅ as internal standard (c) also indicated.

Compound: XLR11 N-(4-hydroxypentyl) metabolite

Figure 1. Retention time determination for XLR11 N-(4-hydroxypentyl) metabolite using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Figure 2. Typical calibration curve (2.5-50 ng/mL) in oral fluid and buffer (1:3, v:v) matrix for XLR11 N-(4-hydroxypentyl) metabolite (QCs shown as triangles) (a); quantifier peak (346.2 $\rightarrow$ 248.1) (b) and XLR11- d_5 as internal standard (c) also indicated.

Compound: (+/-) JWH 018 N-(4-hydroxypentyl) metabolite-*d*₅

Figure 1. Retention time determination for (+/-) JWH 018 N-(4-hydroxypentyl) metabolite-*d*₅ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Compound: AB-FUBINACA-*d*₄

Figure 1. Retention time determination for AB-FUBINACA-*d*₄ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Compound: AB-PINACA-*d*₉

Figure 1. Retention time determination for AB-PINACA-*d*₉ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μm) column. Structure (left) and precursor ion (right) also shown.

Compound: ADBICA-*d*₉

Figure 1. Retention time determination for ADBICA-*d*₉ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Compound: ADB-PINACA-*d*₉

Figure 1. Retention time determination for ADB-PINACA-*d*₉ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Compound: AM2201-*d*₅

Figure 1. Retention time determination for AM2201-*d*₅ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μm) column. Structure (left) and precursor ion (right) also shown.

Compound: JWH 007-*d*₉

Figure 1. Retention time determination for JWH 007-*d*₉ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μm) column. Structure (left) and precursor ion (right) also shown.

Compound: JWH 015-*d*₇

Figure 1. Retention time determination for JWH 015-*d*₇ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Compound: JWH 018 4-hydroxyindole metabolite-*d*₉

Figure 1. Retention time determination for JWH 018 4-hydroxyindole metabolite-*d*₉ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Compound: JWH 018 6-hydroxyindole metabolite-*d*₉

Figure 1. Retention time determination for JWH 018 6-hydroxyindole metabolite-*d*₉ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Compound: JWH 018 7-hydroxyindole metabolite-*d*₉

Figure 1. Retention time determination for JWH 018 7-hydroxyindole metabolite-*d*₉ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Compound: JWH 018 N-(5-hydroxypentyl) metabolite-*d*₅

Figure 1. Retention time determination for JWH 018 N-(5-hydroxypentyl) metabolite-*d*₅ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Compound: JWH 018 N-pentanoic acid metabolite-*d*₄

Figure 1. Retention time determination for JWH 018 N-pentanoic acid metabolite-*d*₄ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Compound: JWH 018 N-pentanoic acid metabolite-*d*₅

Figure 1. Retention time determination for JWH 018 N-pentanoic acid metabolite-*d*₅ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Compound: JWH 018-*d*₉

Figure 1. Retention time determination for JWH 018-*d*₉ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Compound: JWH 073-*d*₇

Figure 1. Retention time determination for JWH 073-*d*₇ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Compound: JWH 081-*d*₉

Figure 1. Retention time determination for JWH 081-*d*₉ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μm) column. Structure (left) and precursor ion (right) also shown.

Compound: JWH 122-*d*₉

Figure 1. Retention time determination for JWH 122-*d*₉ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 µm) column. Structure (right) and precursor ion (left) also shown.

Compound: JWH 200-*d*₅

Figure 1. Retention time determination for JWH 200-*d*₅ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Compound: JWH 210-*d*₉

Figure 1. Retention time determination for JWH 210-*d*₉ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (right) and precursor ion (left) also shown.

Compound: JWH 250-*d*₅

Figure 1. Retention time determination for JWH 250-*d*₅ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Compound: JWH 398-*d*₉

Figure 1. Retention time determination for JWH 398-*d*₉ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (right) and precursor ion (left) also shown.

Compound: MAM2201-*d*₅

Figure 1. Retention time determination for MAM2201-*d*₅ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Compound: PB-22-*d*₉

Figure 1. Retention time determination for PB-22-*d*₉ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Compound: RCS-4-*d*₉

Figure 1. Retention time determination for RCS-4-*d*₉ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.

Compound: UR-144-*d*₅

Figure 1. Retention time determination for UR-144-*d*₅ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (right) and precursor ion (left) also shown.

Compound: XLR11-*d*₅

Figure 1. Retention time determination for XLR11-*d*₅ using a Zorbax Eclipse Plus C18 Rapid Resolution HD (3.0 x 100 mm x 1.8 μ m) column. Structure (left) and precursor ion (right) also shown.