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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS WITH HYPHENATED CHROMATOGRAPHIC TECHNIQUES FOR THE DETERMINATION OF NPS AND DRUGS OF ABUSE IN BIOLOGICAL MATRICES

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INTRODUCTION

All substances with psychoactive properties that are not under international drug control conventions of 1961 and of 1971, and that represent a risk to health similar to classical controlled drugs of abuse are defined "New Psychoactive Substances" (NPS).[1]

NPS are designed as analogs of well-known classes of drugs and can be of synthetic or natural origin such as synthetic cannabinoids, synthetic cathinones, fentanyl and benzodiazepines derivatives, phenethylamines, tryptamines, khat, kratom.

NPS emerged in the early 1990s to circumvent the low as alternatives to controlled substances. Since then, appeared new public health risks and problems due to different legislation concerning controlled substances in the world. In 1993, The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) was instituted as the agency responsible for collecting, analysing and sharing information on drugs and drugs addiction trend at European level. The EMCDDA works in cooperation with European Union Agency for Law Enforcement Cooperation (Europol), the European Medicines Agency (EMA), the European Commission and 30 national early warning systems to provide evidence-based information on illicit drugs and related harms.[2] In response to health risks caused by NPS consumption, has been initially created a "joint action concerning the information exchange, the risk assessment, and the control of new synthetic drugs" (97/396/JHA)[3] but after suggestions for the improvement of the system it was replaced by the European Council Decision 2005/387/JHA[4].[5] The developed three-step legal framework of early warning, risk assessment, and control measures has been revised and new regulations have been introduced to cope with the increasing spread of new psychoactive substances.[6] In fact, at the end of 2018 a new legislation, strengthening and speeding up the existing procedures of the three steps mechanism, came into effect replacing the European Council Decision 2005/387/JHA. It comprises The Regulation (EU) 2017/2101 of the European

Parliament and of the Council of 15 November 2017 and the Directive (EU) 2017/2103 of the European Parliament and of the Council of 15 November 2017.[7] According to the latest data reported in the European drug report 2021, 830 NPS have been monitored by the EMCDDA from 1997 to the end of 2020, 209 of which were synthetic cannabinoids and 156 were cathinones.[8] These new drugs sometimes are more potent and toxic than the earlier generations and can have different and unknown effects that are difficult to interpret and manage by medical professionals, researchers, scientists, law enforcement agencies. It is reported that in European Union opioids use (including heroin, morphine, fentanyl derivatives) caused 76% of fatal overdoses, but during the last years deaths associated to synthetic cannabinoids and classical drugs of abuse as amphetamines and cocaine were also registered.[8]

In 2009, the Department for Anti-Drug Policies (DPA) of the Presidency of the Council of Ministers established in Italy the National Focal Point for the National Early Warning System (SNAP) on NPS monitoring. The SNAP has the primary role of early detect and monitor NPS emerged in the market including adulterants, synthesis-related impurities and contaminants, secondly the dissemination between laboratories of cutting-edge analytical methodologies for the detection of these substances.[9]

At the international level, in 2013 The United Nations Office on Drugs and Crime (UNODC) launched its Early Warning Advisory on NPS (EWA) as an internet based platform for sharing information on NPS including chemical and pharmacological data, report trends on NPS, and support documentation on laboratory analysis.[10]

All these agencies at regional, national and international level cooperate to better understand NPS phenomenon worldwide, to address the challenges posed by the spread of synthetic drugs in the market, and to implement prevention actions and/or problem-solving interventions related to health impairments caused by drug use.

Chapter 1.

NEW PSYCHOACTIVE SUBSTANCES CLASSIFICATION

NPS can be categorized by their psychopharmacological effects (e.g. hallucinogens, stimulants, depressants, dissociatives) although there are other types of classifications based on different criteria such as legal situation, origin (e.i. synthetic, natural) and chemical structure.[11]

The EMCDDA and the UNODC adopt miscellaneous criteria based on effects, origin and/or chemical structure, and report the following classes: synthetic cannabinoids, synthetic cathinones, phenethylamines, aminoindanes, tryptamines, piperazines, phencyclidine-type substances, opioids, piperidines, pyrrolidines, plants and extracts, other substances .[12,13]

In this chapter are briefly described the different NPS families covered by this research project.

1.1 Synthetic Cannabinoids

Synthetic cannabinoids are an ever-expanding class of substances related to Δ9-tetrahydrocannabinol (Δ9-THC), the principal constituent of *Cannabis,* identified and isolated in 1964 by Gaoni and Mechoulam.[14] They act as Synthetic Cannabinoid Receptor Agonists (SCRA) in fact they bind the same cannabinoid receptors of Δ9-THC (CB1 and CB2).

Initially, they were designed for research purposes such as the study of the endocannabinoid system and the development of new medicines for their therapeutic benefits.[15–17]

Currently there are more than ten synthetic cannabinoids classifications based on their chemical structure: naphthoylindoles (e.g. JWH-018, JWH-210, JWH-122, JWH-073, JWH-398); naphthylmethylindoles (e.g. JWH-175); naphthoylpyrroles (e.g. JWH-307); naphthylmethylindenes (e.g. JWH-176); phenylacetylindoles (e.g. JWH-250); cyclohexylphenols (e.g. CP 47,497 and its homologues); adamantoylindoles (e.g. AB-00, AM 1248); aminoalkylindoles (e.g. WIN 55,212-2); benzoylindoles (e.g. AM694); dibenzopyrans (e.g. Δ9-THC, UH-210);

tetramethylcyclopropyl ketone indoles (e.g. UR-144); quinolinyl ester indoles (e.g. PB-22); indazole carboxamide (5F-APICA, ADB-PINACA).[18]

In the mid-2000s, they appeared in Europe in products called 'Spice' and as above mentioned the EMCDDA is now monitoring 209 synthetic cannabinoid with intense psychoactive effects and they continue to be widely available across Europe.[8] Synthetic cannabinoids due to a stronger binding affinity than Δ9-THC for CB1 and CB2 receptors have much more potent pharmacological effects. Cognitive impairment, sickness, sedation, nausea, vomiting, depressed breathing, hypertension, tachycardia, chest pain, muscle twitches, acute renal failure, mood and perception alterations, paranoia, anxiety drowsiness are some of adverse effects observed and reported by consumers.[19] Several fatal intoxication due to synthetic cannabinoids consumption have been reported demonstrating the health risks posed by these substances.[20]

1.2 Synthetic cathinones

Synthetic cathinones have become the second NPS largest group monitored by the European Union Early Warning System. They appeared on the black market in 2003 and in 2005 methylone was the first substance notified to the EMCDDA.[21] They are analogs of S-(-)-cathinone, the natural component of Catha edulis plant, and were designed as legal alternatives to controlled stimulants like MDMA.[22] They are structurally related to phenylethylamines and amphetamine but with a ketone group at the β carbon. Modifications of the α alkyl side chain and/or the alkyl amino group, of the aromatic ring (e.g. adding a methylenedioxy group or halogens) lead to a range of substances with different and intense psychoactive properties.[23]

Based on chemical structure they can be divided in:

- N-alkylated derivatives (sometimes also with the aromatic ring halogenated),
- 3,4-methylenedioxy-N-alkylated derivatives,
- 3,4-methylenedioxy-N-pyrrolidine derivatives,
- N-pyrrolidine derivatives,

-Naphthyl derivatives.[24]

Synthetic cathinones act with different potency, affinity and selectivity as inhibitors of the dopamine transporter (DAT), norepinephrine transporter (NET) and serotonin transporters (SERT) exhibiting sympathomimetic effect. Furthermore, they enhance the monoamine signaling among cells acting as releasing agents with an increase brain levels of noradrenaline (NE), serotonin (5-HT), and dopamine (DA). Euphoria, increased concentration, talkativeness, sexual desire are the recreational effects reported by consumers, but heavy toxicological effects include paranoia, agitation, hallucinations, extreme anxiety hyperthermia, tachycardia, hypertension, depression, confusion, violence, suicidal thought.[25–27]

Up to 2017 several deaths due to mephedrone, methylone, methylenedioxypyrovalerone, ethylone, buthylone, α-ethylaminopentiophenone, 3',4'-Methylenedioxy-α-pyrrolidinobutyrophenone, 3,4-dimethylmethcathinone, 4'-methyl-α-pyrrolidinohexiophenone, α-pyrrolidinovalerophenone have been reported in the literature and confirmed by postmortem toxicological analyses.[28,29]

In 2020, another extensive literature search was carried out, covering a period between 2017-2020, to identify relevant scientific publications reporting worldwide fatalities associated to synthetic cathinones consumption alone or in combination with other drugs.[30] From the research emerged that most abused synthetic cathinones were N-ethylpentylone, N-ethylhexedrone, and 4-chloromethcathinone, furthermore there was a trend of co-consumption with other stimulants to enhance their effects documented by the finding of multiple stimulants in victims' biological specimens.[30]

1.3 Opioids

The World Health Organization defines the generic term "Opioids" as the set of substances of natural origin (alkaloids from *Papaver somniferum*, called opiates) and their semi-synthetic analogs with analgesic properties and euphoric effects but inducing stupor, coma and respiratory depression in high doses.[31]

Some opiates (e.g. morphine, codeine), semi-synthetic analogs (e.g. hydrocodone, oxycodone, buprenorphine, heroin) and synthetic derivatives (e.g. methadone, fentanyl, pentazocine, tramadol) are used for pain management, in human and veterinary medicine and in anesthesia, but most of them are misused for non-medical consumption and are illegally produced.

In Europe, heroin is the first illicit opioid used followed by other synthetic opioids such as methadone, buprenorphine and fentanyl. During the mid-1970s and 1990s there were in Europe two waves of heroin addiction, affecting western and central/eastern countries, respectively. In 2017, opioids were the first cause for entering in specialized drug treatment programs demonstrating an increase in synthetic opioids use.[32]

Fentanyl was initially designed for the induction of anesthesia, then new medical formulation were introduced on the market for the management of pain (controlled-release patches, sublingual spray) but its clinical success has grown so much that it has entered the black market and has become illicitly trafficked as adulterants of heroin and other drugs and fake medicine.[33]

Fentanyls stimulate mu-, delta- and kappa- opioid receptors with a potency 50-100 times higher than morphine, users have reported that these substances are good substituents of heroin having a milder immediate physical response to intravenous injection and a longer sleepy, painless, feelings of relaxation and euphoria effects. Some of adverse effects are nausea, vomiting, constipation, dizziness, anxiety, hallucinations, respiratory and CNS depression and repeated use leads to tolerance and dependence with craving, anxiety, diarrhea, bone pain, abdominal cramps, sweating, shivers, trembling as withdrawal symptoms.[34–37]

In 2017 fentanyl was related to 333 fatalities in Europe while methoxyacetylfentanyl, cyclopropylfentanyl, carfentanil and acryloylfentanyl were formally riskassessed by the EMCDDA during 2017-2018 because involved in more than 200 overdose deaths.[38,39]

1.4 Tryptamines

Tryptamines are indolamines structurally related to the endogenous neurotransmitter 5-hydroxytryptamine (serotonin or 5-HT), molecules characterized by the presence of an indole group joined to an amino group via an ethyl sidechain. Many tryptamines have natural origin, in fact can be found in plants, fungi but also animals. Ayahuasca is a brew typical of the South America prepared by prolonged decoction of many plant including *Banisteropsis caapi and Mimosa hostilis* containing both considerable amount of N,N-dimethyltryptamine (DMT), and *Psychotria viridis* full of harmine, harmaline and tetrahydroharmine. *Psilocybe* spp. mushrooms contain psilocybin and psilocin two psychedelic indolamines. Lysergamide (LSA), is a natural analog of the LSD, found in seeds of plants such as *Argyreia nervosa*, *Ipomea violacea* and *Rivea corymbose*.[40]

Bufotenina and 5-methoxy-N,N-dimethyltryptamine 5-MeO-DMT are potent hallucinogens secret by the desert toad *Bufo alvarius*.[41]

Indolamines can be classified according to modifications of the simple DMT or psylocybin structure. The introduction of different functional groups on the nitrogen atom of the side chain, on the α position of the side chain and/or in the aromatic ring (at the 4-position or at the 5-position) leads to:

- α substituted derivatives: α-methyltryptamine, α-ethyltryptamine
- tryptamines 4-substituted: 4-hydroxy-N,N-diethyltryptamine (4-HO-DPT), 4 Acetoxy-diisopropyltryptamine (4-AcODIPT);
- tryptamines 5-substituted: 5-Methoxy-α-methyltryptamine (5-MeOAMT), 5-Methoxy-dipropyltryptamine (5-MeODPT), 5-Methoxy-N-methyl-N-isopropyltryptamine (5-MeO-MIPT).[42]

Tryptamines are involved in regulation and modulation of multiple processes within the central nervous system acting primarily as 5-HT_2 agonists receptors even if they bind with different affinities also a range of 5-HT_1 and 5-HT_2 receptor

subtypes such as 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{2A} and 5-HT_{2C} receptors. Confirmed cases of intoxications and fatalities are reported in the literatures, these substances are categorized as hallucinogens, namely that produce altered states of consciousness with diverse perceptual effects including auditory/visual/sensory hallucinations, distortions of the time and space, hypersensitivity and with side effects reported by consumers like nausea, vomiting, fear, paranoia, anxiety, tachycardia.[43,44] To date, 56 Tryptamines are monitored by the EMCDDA.[45]

2.1 Phenethylamines

Phenethylamines are a comprehensive group of natural and synthetic origin substances that share the phenethylamine backbone. Modifications on the core of the structure lead to a large variety of compounds with hallucinogenic, psychostimulants and empathogens effects.

Mescaline (3,4,5-trimethoxyphenethylamine) is the natural phenethylamine extracted from some members of Cactaceae plant family, which the most popular is *Lophophora williamsii* (commonly known as Peyote), it promotes the release and/or re-uptake of serotonin and is a full 5-HT_{2C} receptor agonist.[46]

Classic drugs of abuse such as amphetamine (α-methylphenethylamine), methamphetamine (α, N-dimethylphenethylamine), MDA (3,4-methylenedioxyamphetamine), MDMA (3,4-methylenedioxymethamphetamine) and their derivatives (e.g. paramethoxyamphetamine, PMA; paramethoxymethamphetamine, PMMA; 4-methylthioamphetamine, 4-MTA; 4-bromo-2,5-dimethoxyamphetamine, DOB and others) were the first synthetic phenethylamines listed in Schedules I or II of the 1971 United Nations Convention for their potential of abuse.[47]

Substitutions at different position of the aromatic ring, on the ethyl side chain and on the amino group were made to manufacture a wide range of phenethylamines. Other phenethylamine generations include the following classes:

- 2C-X derivatives (with methoxy group at 2 and 5 ring position),
- 2C-B derivatives (2,5-dimethoxy-4-bromophenethylamine)

- 2C-I derivatives (2,5-dimethoxy-4-iodophenethylamine)
- 2C-E derivatives (2,5-dimethoxy-4-ethylphenethylamine)
- NBOMe derivatives (N-benzylphenethylamine)
- benzodifurans derivatives (e.g. Bromodragonfly; 2C-B-Fly)
- MDA benzofuran analogues (e.g. 5-APB, 6-APB, 6-MAPB, 5-EAPB)

2C- phenethylamine series have hallucinogenic and sympathomimetic effects because bind with different affinities both 5-HT_2 and alpha-adrenergic receptors. Different substituents on the core of the structure determine the agonist or antagonist role in the modulation of brain neurotransmitters. Clinical effects correlate with the amount ingested, in fact, low doses induces stimulants effects while moderate doses cause hallucinations, at high quantities signs of intoxication appear with tachycardia, hypertension, hyperthermia, and even death can occur with a cardio-pulmonary arrest or multi-organ failure.[48]

NBOMe derivatives are highly selective agonists of serotonin 5-HT_{2A} and 5-HT_{2C} receptors thus have potent psychedelic/hallucinogenic effects, even low doses has been associated with severe adverse reactions, intoxications and in some cases deaths.[49–51]

MDA benzofuran analogs being structurally related to MDMA and MDA share the same mechanism of action, but with effects more intense of parent compound. At nanomolar concentrations they act as potent releasing agent and inhibitors of noradrenaline, serotonin and dopamine uptake acting on DAT, SERT, NET, furthermore they are 5-HT_{2A} and 5-HT_{2B} receptor agonists.[52,53]

Benzodifuran derivatives, conversely to benzofuran, display high affinity for 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors and approaches the potency of LSD with long-lasting hallucinogenic effects.[54]

Last phenethylamine generations represent a threat to life, since 1997 the EMCDDA has been monitoring about 105 phenethylamines and several intoxication/fatalities have been reported in the literature.[45,50,55–57]

Chapter 2.

Aims of the thesis

Every year new substances appear into the drug market thus analytical laboratories are challenged with the identification in biological matrices of new synthetic compounds. The detection and/or the quantification of NPS often is necessary in various contexts such as driving under the influence of drugs (DUID), overdoses death, non-fatal intoxication, or emergency department admissions. Unknown substances in case of intoxications make difficult the evaluation of clinical effects, thus the development of specific healthcare practices. In cases of fatal intoxications, postmortem drug analyses may support assumptions made by the medical examiner or even establish the cause of death when autopsy examinations are inconclusive.[19]

Most of new psychoactive substances are designer analogs of classical drugs so they are structurally or functionally similar to illicit substances and one of the main challenges in toxicological analysis is for example to distinguish structural isomers, i.e. molecules with the same molecular formulas, but different order arrangements of atoms. The separation and the correct identification of structural isomers is necessary for the assessment of the legal status of a specific molecule. Furthermore, isomers can exert different pharmacological effects. In this situation, can be extremely important to develop suitable drugs analysis methodologies which can provide possible toxic or fatal concentration ranges allowing to implement appropriate health and social responses.

The ability to detect new and highly potent drugs depends on the availability of sophisticated and high-performance instruments with up-to-date analytical methods.

Hyphenated chromatographic techniques (GC-MS, HPLC-MS/MS, HPLC-HRMS) have become powerful tools largely used in clinical and forensic toxicology. Since 1980, GC-MS has been the first hyphenated technique used for targeted and untargeted screening, and quantification in toxicological analyses, ten

years later thanks to the introduction of high-performance liquid chromatography atmospheric pressure electrospray ionization (ESI) sources was possible to couple mass spectrometry with liquid chromatography, therefore HPLC-MS has become the gold standard in drugs testing.[58,59]

Currently, high resolution mass spectrometry has become the most powerful technique used for untargeted screening, quantification, drug metabolism and metabolomics.[60,61]

Since the spread of NPS is an ever-changing phenomenon, more comprehensive and updated analytical methodologies are required to be developed and validated allowing to assess polydrug consumption of both classic psychotropic drugs and emerging new psychoactive substances. In this context, the aim of the project was the development and validation of new methods based on hyphenated chromatographic techniques for the determination of NPS and classical drugs of abuse in biological matrices. All methods were applied to authentic samples to prove and verify their applicability.

The following methodologies have been developed to keep up with emerging drug molecules:

- a comprehensive high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS-MS) screening method for the determination of 77 NPS, 24 classic drugs and 18 related metabolites in blood, urine and oral fluid;
- a gas chromatography-mass spectrometry (GC-MS) method for the determination of JWH-122, JWH-210, and UR-144 in oral fluid of consumers supplemented by an ultra-high performance liquid chromatography high resolution mass spectrometry (UHPLC-HRMS) confirmatory method for the quantification of both the parent compounds and their metabolites;
- a simultaneous targeted screening and quantification method for the determination of synthetic cathinones in hair by ultra-high performance liquid chromatography high resolution mass spectrometry (UHPLC-HRMS).

Chapter 3.

A comprehensive HPLC–MS-MS screening method

A rapid and simple screening method by high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS-MS) has been developed and validated for the determination of 36 synthetic cannabinoids, 12 fentanyl analogs, 16 synthetic cathinones, 7 tryptamines, and 6 phenethylamine, 24 classic illicit drugs and 18 available metabolites in a single chromatographic run, in whole blood, urine and oral fluid. This experimental procedure and the obtained results have been published in the literature.[62]

3.1 Materials and methods

LC-MS-grade water, acetonitrile and methanol were obtained from Sigma-Aldrich® (Milano, Italy). Ammonium formate buffer was prepared with ≥ 99% purity ammonium formate salt (Sigma-Aldrich®) dissolved in LCMS-grade water. M3® buffer was purchased from Comedical® s.r.l. (Trento, Italy). Working standards of 3,4-dimethylmethcathinone, 4-methylethcathinone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, 4-fluoromethcathinone, 4-acetoxy-N,N-diisopropyltryptamine, 4-hydroxy-N,N-diethyltryptamine, 5-APB, 6-APB, 5-chloro-AB PINACA, 5-EAPB, 5-fluoro-ADB, 5-fluoro-AKB48, 5-fluoro-NNEI-2, 5-methoxy-α-methyltryptamine, 5-methoxy-dipropytryptamine, 5-methoxy-N, N-monoisopropyltryptamine, 6-MAPB, 6-O-monoacetylmorphine (6-MAM), AB CHMINACA, AB FUBINACA, acetoxy-dimethyltryptamine, acetylnorfentanyl, alfentanyl, AM-2201, AM-2233, AM-694, JWH-302, amphetamine, APP FUBINACA, benzoylecgonine, buphedrone, buprenorphine, butylone, butyrylfentanyl, butyrylfentanyl-carboxy metabolite, butyrylnorfentanyl, carfentanyl, CB-13, cis-3-metylnorfentanyl, clobazam, cocaethylene, cocaine, codeine, CP47,497-CB, CUMYL-5-fluoro- PINACA, CUMYL-PEGACLONE, cyclopropylfentanyl, cyclopropylnorfentanyl, despropionyl-p-fluorofentanyl, diethylcathinone, dihydrocodeine, dimethylcathinone, ethcathinone, ethylone,

fentanyl, flunitrazepam, furanylethylfentanyl, furanylnorfentanyl, JWH 203, JWH 251, JWH-007, JWH-016, JWH-019, JWH-081, JWH-098, JWH-122, JWH-147, JWH-210, JWH-398, ketamine, lorazepam, 3,4-methylendioxyamphetamine (MDA), mephedrone, 3,4-methylendioxy-N-ethylamphetamine (MDEA), methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), methadone, methcathinone, methoxyacetylfentanyl, methoxyacetylnorfentanyl, methylenedioxypyrovalerone, methylone, MMB 2201, morphine, N,N-diallyl-5-methoxy-tryptamine, naphyrone, norbuprenorphine, nordiazepam, norfentanyl, norketamine, norsufentanyl, N-phenetyl-4-piperidinone, oxycodone, penthedrone, penthylone, phenazepam, phenylacetylfentanyl, pravadoline, PX-1, PX-2, ADB FUBINACA, RCS-4, RCS-8, JWH-018, sufentanyl, temazepam, THJ-018, tramadol, trans-3-metyl-norfentanyl, UR 144, valeryl-fentanyl carboxy metabolite, zolpidem, Δ9-tetrahydrocannabinol, β-hydroxyfentanyl, β-hydroxythiofentanyl and deuterated internal standards (IS; 6-MAM-d3, amphetamine-d6, benzoylecgonine-d3, buprenorphine-d4, cocaethylene-d3, cocaine-d3, codeine-d3, fentanyl-d5, ketamine-d4, MDA-d5, MDEA-d5, MDMAd5, methamphetamine-d5, methadone-d3, morphine-d3, norbuprenorphine-d3, Δ9-tetrahydrocannabinol-d3) were purchased from Cayman Chemical (Ann Arbor, MI, USA) while 4′-methyl-α-pyrrolidinohexiophenone (MPHP), acetylfentanyl, furanylfentanyl and ritalinic acid were obtained from LGC, (Queens Road, Teddington, Middlesex, UK). Purchased standards were available in different formulations (e.g. powder form, methanolic solution), chemico-physical properties of each analyte are reported in *Table 1* (Appendix 1) All analytes were stored at -20°C until use.

3.2 Calibrators and quality controls preparation

Methanolic stock standard solutions of each analyte and working solutions containing all 119 substances were prepared at 1 mg/mL and 1 µg/mL, respectively. IS methanolic working solution with benzoylecgonine-d3, morphine-d3, amphetamine-d6, MDA-d5, codeine-d3, methamphetamine-d5,

MDMA-d5, MDEA-d5, 6-O-monoacetylmorphine-d3, cocaine-d3, ketamine-d4, cocaethylene-d3, norbuprenorphine-d3, methadoned3, fentanyl-d5, buprenorphine-d4, and Δ9-tetrahydrocannabinol-d3 were prepared at 10 and 1 µg/mL. All calibrators were daily prepared by spiking a pool of blank specimens with proper volumes of standard and deuterated standard solutions. In Appendix 1, *Table 2* shows the five concentration levels along with quality controls prepared for all analytes and biological matrices.

First calibrators and low QC samples were prepared by spiking a pool of blank specimens with a proper volume of stock standard solutions of each analyte separately to obtain the concentration reported in the abovementioned *Table 2* (see Appendix 1).

3.3 Biological matrices

Blank human blood, urine and oral fluid were obtained from the laboratory storehouse of blank biological samples. Pools of blank samples were prepared using 58 different postmortem blood, urine or antemortem oral fluid samples from the Section of Legal Medicine (Università Politecnica delle Marche, Ancona, Italy). All samples used as blank were prescreened for the presence of any drug of abuse and pharmaceuticals. Authentic postmortem blood and urine specimens were donated, without any information about demographics, detection of other drugs and cause of death, as discarded material by the Institute of Forensic Medicine of Strasbourg (France), and the Department of Medical and Health Sciences, Division of Drug Research of Linköping University (Sweden). Antemortem oral fluid specimens from authentic cases collected by spitting were donated as discarded material by the Polytechnique University of Marche. In addition, biological samples of in-house cases were also analyzed.

3.4 Samples treatment

Blood samples were rapidly pretreated with the following procedure: 70 µL M3® and 500 µL acetone:acetonitrile (8:2, v/v) were added to 100 µL whole blood spiked with 1 µL IS working solution. Supernatant was evaporated to dryness

under a nitrogen at 45°C, after vortexing and centrifuging. Samples were reconstituted in 1 mL mobile phase (dilution ratio 1:10, v/v), centrifuged, and supernatants were transferred into screw top autosampler vials for the injection. The dilution ratio (1:10, v/v) allowed to minimize the interferences due to endogenous substances in the matrix, without compromising the sensitivity.

100 µL oral fluid and urine samples fortified with appropriate volumes of working standard solutions and spiked with 1 and 5 µL IS working solution, respectively, were added to 500 µL of M3® buffer solution, and 1 µL was injected into the chromatographic system.

3.5 Instrumentation

Analyses were performed using an UPLC Waters Acquity I Class (Waters, Milford, MA, USA) instrument coupled with a Waters XEVO TQ-S Micro (Waters) tandem quadrupole mass spectrometer. Chromatographic separation was carried out with a Waters Oasis HLB reversed-phase column (5 µm 4.6 × 20 mm). Gradient elution was performed with mobile phase A (ammonium formate solution pH 9.5) and B (acetonitrile) at 1 mL/min flow rate and at 50°C. Initial conditions were 75:25 (A:B). Phase A was gradually ramped down from 75% to 0% and phase B gradually ramped up from 25% to 100% in ten minutes. The injection volume was 1 µL for all the matrices. Mass spectrometric analysis was performed in positive ion multiple reaction monitoring mode (ES + MRM). Direct infusion of 1µL stock standard solutions in the MS permitted the selection of two best analytes transitions, and at least one transition for deuterated standards. Selected transitions of the analytes were then confirmed in spiked samples. All Mass Spectrometry parameters (e.g. cone voltage, MRM transitions, collision energies) and retention times are listed in *Table 3* placed in Appendix 1

3.6 Validation of the analytical method

Method validation was performed according international guidelines.[63,64] The method was fully validated in whole blood, urine and oral fluid following a 5-day validation protocol. Limits of detection (LODs) and limits of quantifcation (LOQs)

were determined with decreasing concentrations of drug fortified blank matrix samples and thereafter calculating the signal-to-noise ratio. LOD was defined as the lowest concentration with acceptable chromatography with a signal-to-noise ratio higher than 3 and LOQ the lowest concentration with a signal-to-noise ratio higher than 10.

Synthetic cathinones calibration ranges for blood, urine and oral fluid were 0.60-200 ng/mL, 0.20-1000.0 ng/mL, 0.45-200.0 ng/mL, respectively. Phenethylamines linearity in blood, urine and oral fluid was assessed over ranges of 0.40-200 ng/mL, 0.30-1000.0 ng/mL and 0.36-200.0 ng/mL, respectively. Calibration curves for Triptamines ranged between 0.50-200.0 ng/mL, 0.20-1000.0 ng/mL, 0.4-200 ng/mL in blood, urine and oral fluid, respectively

Fentanyls calibrators were equal in oral fluid and urine (5.00 ng/mL, 20.0 ng/mL, 100.0 ng/mL, 200.0 ng/mL) except for LOQs (0.07 ng/mL and 0.06 ng/mL, respectively. Synthetic cannabinoids linear ranges in blood, urine and oral fluid were 0.60-200.0 ng/mL, 0.30-1000.0 ng/mL, 0.50-200.0 ng/mL, respectively. Different linear ranges have been selected for the 24 classical drugs of abuse and are detailed in *Table 2*.

Accuracy, precision, selectivity, linearity, sensitivity and carryover were determined injecting five different daily replicates of calibration points and five replicates of QC samples along three subsequent working days. Acceptable precision and accuracy were < 20%. Carryover was assessed by injecting drug-free samples of each matrix after the highest point of the calibration curve.

Over-the-curve samples (drug free samples spiked with concentration of drugs 5 or 10 times higher than the highest calibration point) were properly diluted and were tested for calibration curve fitting, precision and accuracy within 20%.

Analytical recovery and matrix effect were calculated at low, medium, and high QC concentrations using the experimental design proposed by Matuszewski et al.[65]: set 1 was composed of 5 replicates of analytes diluted in the mobile phase; sets 2 and 3 were composed of 5 different lots of blank samples fortified with analytes after and before extraction, respectively. Matrix effect was calculated by

dividing mean peak areas of set 2 by set 1, and analytical recovery was calculated by dividing mean peak areas of set 3 by set 2.

3.7 Results

The developed method determined 119 substances in a 10 min run time after a rapid and simple treatment of a quite small sample volume. Furthermore, the validation parameters obtained for different biological matrices satisfied the established international criteria.[63] No additional peaks due to endogenous substances and carryover interfering with analytes and ISs were detected. The method was linear for all analytes under investigation with a determination coefficient (R^2) always better than 0.99. Linearity of the curves was statistically confirmed by performing residual plots test, through Minitab® software (Minitab LLC, State College, Pennsylvania, USA), that satisfied the Scientific Working Group of Forensic Toxicology.[66] The linear range included the most commonly detected quantity of NPS in all the matrices investigated and LODs ranged from 0.03 ng/mL to 0.35 ng/mL in blood and from 0.03 ng/mL to 0.25 ng/mL oral fluid, while they ranged from 0.02 ng/mL to 0.25 ng/mL in urine. LOQs ranged between 0.08 ng/mL and 1 ng/mL in blood, 0.07ng/mL and 0.8 ng/mL in oral fluid and between 0.06 ng/mL and 0.5 ng/mL in urine. Recovery of analytes under investigation and matrix effect were always higher than 85% whereas intra-assay and inter-assay precision and accuracy were always better than 19% in all matrices. All validation results can be found in Appendix 1, listed in the *Table 4.*

Representative chromatograms of all analytes at the second calibration point (5ng/mL) in blood, urine and oral fluid are showed in *Figure 1*, *Figure 2,* and *Figure 3* (see Appendix 1).

3.8 Real samples

Fifty-six authentic blood, urine and oral fluid human samples collected from different subjects have been analysed. In the Appendix 1, the *Tables 5*, *6* and *7* contain concentrations of target compounds revealed in real blood, urine and oral fluid samples, respectively. When target analytes concentrations were higher than

the highest point of the calibration curve, the analysis was repeated performing a dilution of the sample. Blood samples from 17 real cases tested positive for fentanyl analogues, 3 of which were positive also for at least one classic illicit drug (morphine, codeine, oxycodone, 4-ANPP (4-anilino-N-phenethyl-piperidine)). Eight blood samples resulted positive to cyclopropylfentanyl and its metabolite cyclopropylnorfentanyl with an average concentration ± standard deviation (SD) of 7.7 ± 7.0 ng/mL and 29 ± 18 ng/mL (n= 8), respectively. Methoxyacetylfentanyl and its metabolite methoxyacetylnorfentanyl were detected at an average concentration ± SD of 36 ± 38 ng/mL and 4.1 ± 2.3 ng/mL (n = 4), respectively. Acetylfentanyl and its metabolite acetylnorfentanyl were detected at an average concentration ± SD of 41 ± 40 ng/mL and 45 ± 22 ng/mL (n = 3), respectively. 4-ANPP was detected at an average concentration ± SD of 3.5 ± 2.3 ng/mL (n = 9). Moreover, one sample tested positive for multiple substances, i.e. furanylfentanyl and its metabolite furanylnorfentanyl, sufentanyl, morphine, codeine, and oxycodone.

Urine samples from twenty-three real cases were positive to classic illicit drugs and fentanyl analogues. Cyclopropylfentanyl and its metabolite cyclopropylnorfentanyl were found in eleven urine samples at an average concentration ± SD of 47 ± 39 ng/mL and 420 ± 300 ng/mL, respectively. Mean methoxyacetylfentanyl and methoxyacetylnorfentanyl concentrations ± SD were 790 ± 850 ng/mL and 750 ± 910 ng/mL, respectively. Acetylfentanyl and its metabolite acetylnorfentanyl were detected at an average concentration ± SD of 1900. ± 1700 ng/mL and 6600 ± 3200 ng/mL (n = 5), respectively. 4-ANPP had an average concentration ± SD in urine of 51 ± 52 ng/mL (n = 10). Fentanyl and its metabolite norfentanyl were detected at an average concentration ± SD of 180 ± 350 ng/mL and 430 ± 840 ng/mL (n = 4), respectively. Nordiazepam, morphine, codeine and 6-MAM were found at an average concentration ± SD of 580 ± 310 ng/mL, 180 ± 170 ng/mL, 85 ± 65 ng/mL, 67 ± 74 ng/mL, respectively. Furthermore, one sample was positive to furanylfentanyl and its metabolite, one to ritalinic acid and a third one to noroxycodone.

Classic drugs of abuse, fentanyl analogues and several NPS were quantified in 14 oral fluids samples. Cocaine was detected in 4 samples at an average concentration ± SD of 28 ± 54 ng/mL while its metabolite was quantified in only 2 samples (8.6 ± 12 ng/mL, average concentration ± SD). 6-MAM was quantified at an average concentration ± SD of 0.47 ± 0.45 ng/mL, but just in one case was quantified also as morphine metabolite. Thus, morphine was present in 2 samples with an average concentration ± SD of 0.5 ± 0.6 ng/mL. Acetyl-o-dimethyltryptamine was quantified in OF004 and OF 008 and the average concentration ± SD was 60 ± 84 ng/mL. The average concentration ± SD of THJ 018 detected in four samples was 25 ± 48 ng/mL. Butylone was founded in 3 samples at an average concentration ± SD of 40 ± 69 ng/mL and carfentanyl was detected only in one oral fluid sample.

3.9 Discussion

A comprehensive HPLC-MS/MS method for the detection and the quantification of 119 substances of abuse including some metabolites in three different matrices has been developed and fully validated.

Considering recent trends of drugs abuse, published scientific literature and commercial availability standards, this study includes target compounds belonging to the most representative classes of emerged substances.[32,33]

The range of linearity for every analyte includes the concentration expected to be detected in each matrices in acute intoxication or fatal cases, according to values reported in the literature.[67–69]

The most prevalent detected compounds in blood were fentanyl analogues and related metabolites, being 4-ANPP quantified in the larger number of samples (n=9). The opiates morphine and codeine were detected in combination of cyclopropylfentanyl and cyclopropylnorfentanyl in one case, while oxycodone was coassumed with cycloproprylfentanyl in another case. Fentanyls were the were the predominantly detected molecules with the highest concentrations in urine samples, although other substances were also quantified (benzodiazepine, phenetylamine and 4 different opiates). Multiple classes of substances were

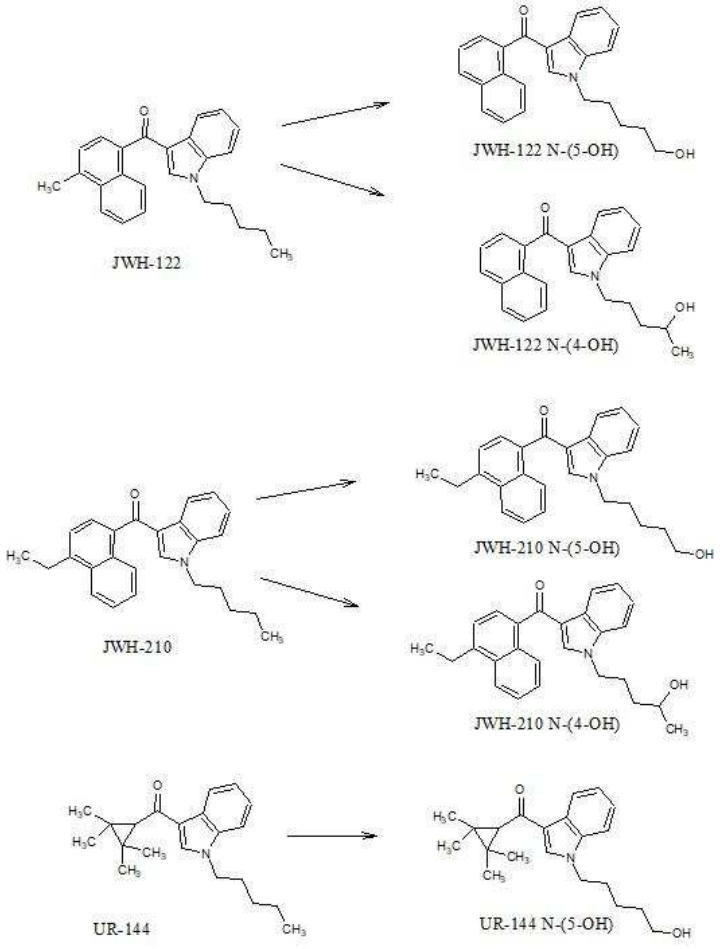
detected in 14 urine samples, while cyclopropylfentanyl and its metabolite and acetylfentanyl and its N-dealkylated metabolite acetylnorfentanyl were found in one single specimen. Finally, fentanyl analogues, opiates, a tryptamine, and a synthetic cannabinoid were revealed in oral fluid samples with polydrug detection occurred in 3 cases.

Chapter 4

Determination of synthetic cannabinoids and metabolites in oral fluid

Two analytical methods were developed and validated for the determination of JWH-122, JWH-210 and UR-144 (see Figure 4.) in oral fluid: a last generation gas chromatography–mass spectrometry (GC-MS) method and an ultra-high performance liquid chromatography high resolution mass spectrometry (UHPLC-HRMS) method for a complementary measurement of JWH-122, JWH-210, UR-144 and their metabolites time course in consumers oral fluid after a single smoking self-administration. All data reported in this chapter has been already published in the literature.[70]

Figure 4. *Chemical structures of the synthetic cannabinoids under investigation and related metabolites*



4.1 Study design and samples collection

In Spain, three subjects participated in a study evaluating the effects and pharmacokinetics of the synthetic cannabinoids JWH-122, JWH-210, and UR-144 in oral fluid. At the time of the study, these synthetic cannabinoids were legal in Spain and personal use was allowed in house and private clubs for cannabis smoking. All subjects with large experience in cannabis consumption (from weekly to monthly use) and, at least, two previous use of synthetic cannabinoids, psychostimulants and hallucinogens attended at a private cannabis-smoking club. Two females (ages 33 and 25 years old, weight 54 and 67 kg, height 1,50 and 1,71 m) and one male (39 years old, weight 60 kg and height 1.67 m) self-administered a cigarette containing synthetic cannabinoids mixed with tobacco and smoked it in 5 min. Each participant selected the synthetic cannabinoid to be consumed in the session. The first female chose JWH-122 (1 mg), the second female JWH-210 (3 mg) and the male UR-144 (1 mg). The doses were selected from previous experiences and in the range of those usually recommended in web fora and surveys.

These three substances were from an unknown source but were previously analysed for content by a Drug Checking Service performed by a Spanish non-governmental organization (Energy Control)[71].

The three synthetic cannabinoids consumers donated oral fluid (OF) samples for study research. Sample collection was authorized by the local Human Research Ethics Committee (CEI-HUGTiP ref. PI-18-267, Badalona, Spain). OF samples of the three consumers were collected at baseline, 10, 20, 40 minutes and at 1, 2, 3 and 4h after self-administration in Salivette tubes, that were then centrifuged, and supernatants stored at -20C until analysis.

4.2 Materials and Methods

(4-methyl-1-naphthalenyl)(1-pentyl-1H-indol-3-yl)-methanone (JWH-122), (4-ethyl-1-naphthalenyl)(1-pentyl-1H-indol-3-yl)-methanone (JWH-210) and (1-pentyl-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)-methanone (UR-144) and

five of their metabolites (1-(4-hydroxypentyl)-1H-indol-3-yl)(4-methylnaphthalen-1-yl)methanone (JWH-122 N-(4-OH), (1-(5-hydroxypentyl)-1H-indol-3-yl)(4-methylnaphthalen-1-yl)-methanone (JWH-122 N-(5-OH), (4-ethylnaphthalen-1-yl)(1-(4-hydroxypentyl)-1H-indol-3-yl)methanone (JWH-210 N-(4-OH), (4-ethylnaphthalen-1-yl)(1-(5-hydroxypentyl)-1H-indol-3-yl)methanone (JWH-210 N-(5-OH) and [1-(5-hydroxypentyl)-1H-indol-3-yl](2,2,3,3-tetramethylcyclopropyl)-methanone (UR-144 N-(5-OH) were purchased from Cayman Chemical (Cayman Chemical, MI, USA).

Deuterated standard Naphthalen-1-yl-(1-pentylindol-3-yl)- 1,1,2,2,3,3,4,4,5,5,5-D11-methanone (JWH-018-d11) used as internal standard was supplied by Lipomed (Milan, Italy).

The derivatizing agent N,OBis(trimethylsilyl)trifluoroacetamide (BSTFA + 1%TMS) was purchased from Restek Corporation (Bellefonte, Pennsylvania, USA). Ultrapure water, methanol, acetonitrile (all UHPLC–MS/MS grade) and all other reagents (HPLC grade) were obtained from Carlo Erba (Milan, Italy).

4.3 Calibrators and quality control preparation

Methanol stock solutions of each analyte were prepared at 0.01 µg/mL, 0.1 µg/mL, 1µg/mL and 10µg/mL. Internal Standard stock solutions containing JWH-018-d_{11} were prepared in methanol at 10 and 0.1 µg/mL for GC-MS and UHPLC-HRMS analyses, respectively.

Laboratory personnel donated drug-free oral fluid sample, that were individually analysed during method validation to exclude interferences and mixed to obtain a homogeneous pool of blank samples to be used for calibration standards and Quality Control (QC) samples.

GC-MS calibration points ranged from LOQ of each analyte, to 5 ng/mL, 10 ng/mL and 50 ng/mL.

UHPLC-HRMS calibrators ranged from LOQ of each analyte, 0.5 ng/mL, 1 ng/mL, 5 ng/mL, 10 ng/mL and 50 ng/mL. Calibration curves were prepared daily by adding the proper amounts of working solutions to 400 µL of pre-checked drug-

free OF pooled sample. QC samples were prepared fortifying drug-free OF samples with suitable amounts of methanol standards working solutions. QC concentrations at 40 ng/mL (high) and 20 (medium) ng/mL were used to establish intra-day and inter-day precision and accuracy for both GC-MS and UHPLC-HRMS assays while low QC were prepared at a concentration one and half times the calculated LOQ of each analyte for both analytical techniques.

4.4 Samples treatment

Four hundred µL OF samples were combined with 100 µL 0.1 M phosphate buffer pH 3.0 (acid pH was adjusted using drops of 1 N HCl), 20 µL of IS working solutions for UHPLC-HRMS analysis and 5 µL for GC-MS analysis. The samples were extracted twice with 3.0 mL of esane:ethylacetate mixture (9:1, v/v). The tubes were centrifuged at 4,000 rpm for 4 min. The organic phase was transferred to clear tube and dried under nitrogen stream.

The dried samples were derivatized with 50 µL mixture of BSTFA containing 1% TMCS and acetonitrile (1:1, v/v) at 70 °C for 30 min for GC-MS analysis.

The dried samples were resuspended with 100 µL mixture of mobile phase A (ammonium formate 2 mM in water, 0.1% formic acid) and B (ammonium formate 2 mM in methanol/acetonitrile 50/50, 0.1% formic acid) (50:50, v/v) for UHPLC-HRMS analysis.

4.5 GC-MS instrumentation

Analyses were performed with an Intuvo 9000 GC System coupled to a 5977 B MSD (Agilent Technologies, Palo Alto, CA, USA). A volume of 1 µL was injected in splitless mode at 260°C into the Ultra-Inert Intuvo GC column (HP-5MS UI, 30 m × 250 µm i.d, film thickness 0.25 µm; Agilent Technologies). Helium was used as carrier gas at a flow 1.2 mL/min. The oven ramp temperature was programmed as follows: initial temperature of 70 °C held for 2 min and increased at 30°C/min to 190°C, then increased to 290°C at 5°C/min for 10 min. Subsequently, the temperature was increased at 40°C/min to 340 °C to eliminate impurities from the

column. The transfer line temperature was set at 320°C. Mass spectrometer with positive ionization operated in selected ion monitoring mode (SIM).

The characteristic ions for the SIM mode were chosen using international libraries such as NIST Research Library (National Institute of Standards and Technology) and SWGDRUG MS Library (version 3.6).

In the Appendix 1 (*Table 8*) are reported GC-MS analytes retention time, quantitative and qualitative ions.

4.6 UHPLC-HRMS instrumentation

The UHPLC/ESI Q-Orbitrap system was used for OF analyses, it was equipped with an Ultimate 3000 LC pump and an Ultimate 3000 autosampler, coupled to a Thermo Scientific™ Q Exactive™ Focus Orbitrap Mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). The Q Exactive with a heated electrospray ionization (HESI) probe operated in positive ionization mode and was controlled by Xcalibur 2.2 software (ThermoFisher Scientific, Bremen, Germany). A Kinetex Biphenyl 100A (100 × 2.1 mm, 2.6 μm) (Phenomenex, Italy) set at 40°C was used for the chromatographic separation. The run time lasted 18 min with a mobile phase flow rate at 0.6 mL/min. Mobile phases were ammonium formate 2 mM in water with 0.1% formic acid (mobile phase A) and ammonium formate 2 mM in methanol/acetonitrile 50:50 (v/v) with 0.1% formic acid (mobile phase B). The UHPLC gradient started with an initial condition of 20% B, held for 2 min, increased to 81.4% B within 9 min, then increased to 100% B within 0.2 min, held for 4.3 min, returned to initial condition within 0.1min, and finally held for 2.4 min. LC flow was directed to waste for the first 4.5 min and after 13.5 min. MS parameters have been set as follows: ionization voltage 3.0 kV; sheath gas and auxiliary gas 35 and 15 arbitrary units, respectively; S-lens radio frequency RF level was 60; vaporizer temperature and capillary temperature were 320°C. Nitrogen was used for spray stabilization, for collision induced dissociation experiments in the higher-energy collisional dissociation (HCD) cell and as damping gas in the C-trap. Every week the instrument was tuned in positive and

negative modes. Data were acquired in full-scan data-dependent MS^2 (Dd-MS^2) mode with an inclusion list containing the exact masses of over 1400 compounds including parent compounds and their metabolites. The full MS scan range was 50-650 *m/z*, data were acquired with a mass resolution of 70,000. The precursor ions were fragmented with stepped normalized collision energy of 30, 35.0, 52.5 V and Orbitrap analyzer had a resolution of 17,500 and an isolation window of 2.0 *m/z*. Full scan data files were processed by Thermo Scientific XCalibur software 3.2 and mzCloud Mass Spectral Library was used as mass spectra international library for peak identification. This specific software uses a built-in database and mass spectral library with more than 1400 substances to identify drugs and metabolites by comparing retention times, isotope pattern matching and elemental composition determinations of compounds. UHPLC-HRMS analytes retention time, quantitative and qualitative ions are also reported in Appendix 1 (*Table 8*).

4.7 Method validation

The analytical methods, prior to real samples analysis, were completely validated in accordance to the internationally established criteria[72,73] for the assessment of linearity, LOD, LOQ, precision, accuracy, carry-over, matrix effect, ion suppression, recovery and stability. LOD was defined as the lowest analyte concentration that can be detected and identified. A signal-to-noise ratio higher than 3 was used for LOD measurement analysing different blank OF samples with decreasing concentrations of the spiked analyte. LOQ was the lowest concentration with a signal-to-noise ratio higher than 10. Method validation was performed along five subsequent working days and parameters were calculated using five different daily replicates of three QC samples (low, medium, and high). The effect of three freeze-thaw cycles (storage at –20°C) on the compounds stability in OF was evaluated by repeated analysis of five replicates of the three QC samples. Matrix effect and recovery were determined using the experimental plan proposed by Matuszewski et al. [74] Set 1 was five replicates of pure analytical standards QC solutions. Sets 2 and 3 were five different replicates of blank samples fortified with

QC solutions after and before extraction, respectively. Matrix effect was determined by dividing mean peak areas of set 2 by set 1 multiplied by 100, while recovery was determined by comparing the mean peak areas of compounds under investigation obtained in set 3 to those in set 2 multiplied by 100. Evaluation of carry-over was performed by injecting high calibrator followed by analysing blank OF samples.

4.8 GC-MS *and UHPLC-HRMS validation* results

Linear calibration curves obtained by GC-MS OF samples showed determination coefficients (R^2) equal or higher than 0.996. The GC-MS method linearity for JWH-122, JWH-210 and UR-144 was assessed from their limits of quantification (0.50 ng/mL, 1.00 ng/mL, 2.30 ng/mL, respectively) to 50 ng/mL. GC-MS method LODs were 0.70 ng/mL for both JWH-210 and UR-144, and 0.3 ng/mL for JWH-122. Mean analytical recoveries obtained for the three different quality control (QC) samples injected in GC-MS were always above 80% except for UR-144 whose recovery was about 70%. The intra- and inter-assay precision (measured as coefficient of variation, %CV) and accuracy (measured as % Error) determined in the three QC samples (n = 15) showed values within ± 12.3% in GC-MS.

UHPLC-HRMS method was linear for all analytes from limit of quantification (analytes LOQ range 0.07-0.25 ng/ml) to 50 ng/mL OF with determination coefficients (R^2) equal or higher than 0.991. UHPLC-HRMS method LODs had a range of 0.02-0.07 ng/mL. Measured %CV and % Error of QC samples were within ± 17.4% for the UHPLC-HRMS method. The mean analytical recoveries, like the GC-MS method, were always above 80% except for UR-144.

No endogenous interferences were found both in GC-MS and UHPLC-HRMS. No significant ion suppression/enhancement (less than 15%) occurred during GC-MS or UHPLC-HRMS runs. None of the analytes under investigation showed relevant degradation after three freeze/thaw cycles and concentrations were always within 10 % initial one. No carry-over was observed in blank OF samples after injecting highest curve calibrators. Quantitative results for parent synthetic cannabinoids in

OF obtained from the two assays were compared to assess the concordance between GC-MS and UHPLC-HRMS methods. A good and statistically significant correlation was obtained when comparing the results from the two assays (r^2 = 0.908 and Spearman's rs 0.936, $p < 0.0001$). A non-significant bias (-0.013) was found using Bland-Altman plot. All analytes validation data for both methodologies are listed in the *Tables 8*, *9* and *10* in the Appendix 1.

In *Figures 5* and *6* (see Appendix 1) are reported representative GC-MS and UHPLC-HRMS chromatograms obtained following the extraction of 400 µL blank OF samples spiked with 1 and 0.5 ng/mL analytes under investigation, respectively together with chromatograms of real samples of consumers at 20 minutes after starting Synthetic cannabinoids smoking.

4.9 Synthetic cannabinoids pharmacokinetics

Pharmacokinetics studies were performed to investigate the concentration-time course of these synthetic cannabinoids and their related metabolites in oral fluid samples of consumers. OF samples of the three consumers were collected before starting to smoke (baseline), at 10, 20, 40 minutes and at 1, 2, 3 and 4h after self-administration. In the OF sample from JWH-122 consumer, the parent drug presented a peak concentration (C_{max}) 4.00 ng/mL (GC-MS) and of 3.14 ng/mL (UHPLC-HRMS) at 20 min (T_{max}) after the start of smoking. The concentration decreased at 3 h and then raised again, in fact a second peak was observed both in GC-MS (2.00 ng/mL) and UHPLC-HRMS (1.60 ng/mL) analyses. JWH-122 N-(4-OH) was quantified by UHPLC-HRMS showing a concentration range of 0.29-0.36 ng/mL between 10 min and 3 h after the smoking while JWH-122 N-(5-OH) was only detected in traces under the LOQ.

The concentration-time curves of the JWH-210 in consumer OF displayed after 20 min a peak concentration of 8.10 ng/mL 7.30 ng/mL by GC-MS and UHPLC-HRMS, respectively. Then its concentration decreased becoming undetectable after 3h. JWH-210 metabolites were also measured by the UHPLC-HRMS method. JWH-210 N-(4-OH) and JWH-210 N-(5-OH) OF concentrations peaked

at 20 min yielding to a C_{max} of 0.29 ng/mL and 0.66 ng/mL, respectively and at 2 h following smoking (C_{max} :0.98 ng/mL and 0.61 ng/mL, respectively).

The time peak of UR-144 occurred at 20 min from smoking start with C_{max} of 7.40 ng/mL by GC-MS and 6.81 ng/mL by UHPLC-HRMS and then it decreased to undetectable values within the next 4 hours. UR-144 N-(5-OH) was only detected in traces during all the time-course and could never be quantified above the LOQ. These results are in agreement with the previous pharmacokinetic studies investigating the presence of JWH-210 and JWH-018 in oral fluid of consumers.[75,76]

In Appendix 1, *Figure 7* shows the concentrations time-curves of JWH-122, JWH-210, UR-144 and their metabolites.

Observational studies on pharmacological effects showed that after 20 min JWH-122 increases systolic blood pressure, diastolic blood pressure, and heart rate, while JWH-210 produces significant changes in subjective effects, similar to THC (intensity, high, good effects, and hunger).[77] Conversely, UR-144 exhibits cardiovascular effects like THC, but slightly higher than those induced by JWH-210, and less subjective effects such as intensity or high than THC.[78]

Chapter 5

Targeted screening and quantification of synthetic cathinones in hair by UHPLC-HRMS

A new targeted screening and quantitation method has been developed and validated for the determination of last generation synthetic cathinones in hair specimens. It has been successfully applied to real hair samples from NPS consumers.

5.1 Chemicals and reagents

Pentylone, Pentedrone, N-Ethylpentylone, Naphyrone, 1-Naphyrone, Methylone, Methedrone, Methcathinone, Mephedrone (4-MMC), Methylenedioxypyrovalerone (MDPV), 3,4-Methylenedioxy-α-pyrrolidinobutiophenone (MDPBP), Flephedrone (4-FMC), Euthylone, Ethylone, Ethcathinone, Dimethylcathinone, Butylone, Buphedrone, Benzedrone (4-MBC), α-Pyrrolidinovalerophenone (α-PVP), α-Pyrrolidinohexiophenone (α-PHP), α-Ethylaminopentiophenone (NEP), 4-methylethcathinone metabolite (4-MEC metab), 4-Methyl-α-pyrrolidinohexanophenone (MPHP), 4-Methylethcathinone (4-MEC), 4-fluoromethcathinone metabolite (4-FMC metab), 4-fluoro-α-Pyrrolidinohexanophenone (4-fluoro-alpha-PHP), 4-Ethylethcathinone (4-EEC), 4-bromomethcathinone (4-BMC), 3-Methylmetcathinone (3-MMC), 3-fluoro-α-Pyrrolidinovalerophenone (3-fluoro-α-PVP), 3-Clhoromethcathinone (3-CMC), 3,4-Dimethylmethcathinone (3,4-DMMC), 3,4-Methylenedioxy-α-Pyrrolidinohexanophenone (MDPHP), and internal standards (IStd) Mephedrone-d_3 were purchased from Cayman Chemical (Ann Arbor, MI, USA). Water, acetonitrile, methanol, dichloromethane, and formic acid (>95%) were obtained from Carlo Erba (Milano, Italy). All solvents were ultra high-performance liquid chromatography (UHPLC) grade and LC-MS grade. Ammonium formate buffer was acquired from Sigma-Aldrich® (Milan, Italy).

5.2 Instrumentation

UHPLC-HRMS analyses were performed on an UltiMate 3000 liquid chromatography coupled to a Thermo Scientific™ Q Exactive™ Focus Orbitrap Mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with a heated electrospray ionization (HESI II) source (Thermo Scientific, Waltham, MA, USA).

5.3 Calibrators and quality control solutions

Working standard solutions at 0.1 ng/µL and 1 ng/µL containing all synthetic cathinones were prepared by appropriate methanolic dilution using stock solutions. IStd solution was prepared separately at1 ng/µL diluting drug deuterium-labeled solution. Low-, medium-, and high-quality controls (QC) working solutions were daily prepared from different calibration stock solutions, when possible at 0.3, 5.0 and 8 ng/µL.

5.4 Hair sample preparation

Hair samples were decontaminated by three washing steps with dichloromethane and then were allowed to dry at room temperature. Hair samples were finely cut, 20 mg were placed into silanized glass vials and soaked in 250 µL of 2 mM ammonium formate, methanol and acetonitrile mixture (50/25/25, v/v/v) together with the appropriate volume of working solutions. An overnight incubation was performed at 40°C. After incubation, the samples were vortexed and centrifuged, and supernatant was evaporated to dryness under nitrogen stream. Samples were reconstituted with 100 µL of mobile phase mix (A:B, 80:20) and A 10 µL were injected into UHPLC-HRMS.

5.5 Liquid chromatography

Chromatographic separation was carried out using a Thermo Scientific™ Accucore™ Phenyl-Hexyl column (100 x 2.1 mm, 2.6 µm) maintained at 40 °C. Gradient elution was performed with mobile phase A (2mM Ammonium formate in water, 0.1% formic acid) and B (Ammonium formate 2mM in

MeOH/Acetonitrile 50/50, 0.1%formic acid) at 0.4 mL/min flow rate. The initial composition (1% B) was maintained for 0.5 min, increased from 1 to 10% B over 3.5 min, from 10 to 50% over 6 min, from 50 to 95% in 1 min, held at 95% for 1 min, and returned to initial conditions over 0.5 min. A 5.5 min equilibration followed, yielding a total run time of 18 min.

5.6 Mass Spectrometry

Thermo ScientificTM Q ExactiveTM Focus Orbitrap Mass spectrometer, equipped with a heated electrospray ionization (HESI) source (Thermo Scientific, Waltham, MA, USA) operating in the positive ionization mode, was used for the targeted screening and quantitation analysis of synthetic cathinones. The sheath gas (nitrogen) flow rate and the auxiliary gas (nitrogen) flow rate were set at 35, 15, respectively. Spray voltage was set at 3.0 kV for positive polarity. Capillary and source temperature were set at 320°C. Data were acquired in Full scan and targeted data-dependent MS/MS scan ($DdMS^2$, 17,500 resolution) using an inclusion list containing the exact mass, the polarity and retention times of targeted analytes. Full MS acquisition parameters were set as follow: 70,000 resolution, scan range 100 to 500 *m/z*, automatic Gain Control (AGC) target 1e6, maximum injection time (IT) was 200 ms. $DdMS^2$ acquisition parameters were: resolution 17,500, isolation window 2 m/z, AGC target 1e5; loop count 3. MS/MS spectrum were generated using stepped normalized collision energies of 17.5, 35.0, 52.5 %.

5.7 Software and Spectral Library identification

Stock solutions were injected with the above-described method for the acquisition of synthetic cathinones spectra and the creation of a compound database with the respective retention times. All acquired spectra were imported into the built-in library (mzVault). Data acquisition and processing were performed with TraceFinder (Version 4.1) and five parameters were set for positive identification criteria: exact mass of the parent ion, retention times, isotope pattern matching, ion fragment and mzVault library match. The matching threshold to establish Limit of

Identification (LOI) was set at 80% with <5 ppm parent ion exact mass deviation and 3 fragments match with <5 ppm deviation.

5.8 Method validation

International guidelines were used for the validation of this method evaluating the following parameters: selectivity, carry-over, linearity, accuracy, precision, limit of detection (LOD), Limit of identification, Lower Limit of Quantification (LLOQ), matrix effects, carry over, recovery and dilution integrity.[79–81]

Twenty different source of drug-free hair samples from laboratory personnel were analyzed to evaluate possible endogenous and exogenous interferences. Selectivity was assessed by adding a mixture of 20 pg/mg common drugs of abuse (cocaine, benzoylecgonine, amphetamine, MDMA, THC, morphine, codeine, diazepam) to hair while carryover was assessed by injecting drug-free samples after the highest concentration point of the calibration curve.

Accuracy, intra-run and inter-run precision were evaluated by analyzing low, medium and high QCs in triplicate over five days (n=15). Accuracy was calculated for each QC as 100×gran mean of observed concentration – known concentration/ known concentration (Bias %). One-way analysis of variation (ANOVA) approach was used for determination of intra-run and inter-run precision expressed as coefficient of variation (%CV). Bias values, intra-run and inter-run precision were considered acceptable when between ± 15 % and <15% CV, respectively.

LOD was determined with decreasing concentrations of drug-fortified pool of blank hair samples and with a signal to noise ratio of 3:1. LLOQ has been chosen as the lowest calibrator with a signal to noise ratio of 10:1.

Ion suppression/enhancement and dilution integrity for each analyte were measured at low and high QC concentrations.

Matrix effect was calculating with the following equation: ME = [(*B*/*A*)-1] x 100 where B is the peak area of the analytes in a blank sample fortified after extraction and A the peak area of the reference standards solution at the same concentration.[79–81]

Dilution integrity of samples with a concentration 5 and 10 times above the highest calibration point was evaluated verifying the curve fitting, bias and precision. The developed method was applied to a quality control hair sample provided by the Italian "NPS-LABVEQ" project. Furthermore, the applicability of the method has been proven by analyzing 8 authentic hair samples of new psychoactive substances consumers.

5.9 Results and discussion

A new UHPLC-HRMS method was developed and validated for the targeted screening and the quantification of 33 different synthetic cathinones.

Specifically, TraceFinder software provided the simultaneous screening and quantification of synthetic cathinones based on their full scan data in hair. The software permitted to identify synthetic cathinones reported in the *Table 10* (see Appendix 1) through exact mass, retention time, fragment ions and isotope patterns matching, and correspondence with a compound library. As showed in the *Table 10* (see Appendix 1) the measured exact mass error ranged between -3.1 ppm and 0.6 ppm demonstrating a good accuracy in the exact mass determination. Indeed, the majority of analytes under investigation have similar structure and some of them have also the same exact mass. The present method was able to chromatographically separate all isomers (3,4-DMMC, 4-MEC and pentedrone; ethylone and butylone; NEP and 4-EEC; naphyrone and 1-naphyrone; 3-MMC buphedrone, dimethylcathinone, ethcathinone; pentylone and euthylone; butylone and ethylone) with the exception of 4-MMC and 3-MMC. These latter two were only partially resolved under the established chromatographic conditions. Nevertheless, the system was able to give the right identification match, but it was not possible to simultaneously quantify them, for this reason 4-MMC has been separately validated.

LODs and LLOQs were 2 pg/mg and 5 pg/mg for all compounds, respectively. LOIs were determined according to the fitted parameters reported in section 5.7. No signal above LODs analytes was observed in blank matrix samples injected immediately after the highest concentration calibrator neither additional peak due

to the presence of other drugs interfered. Linearity was assessed from 5pg/mg (LLOQ) to 500 pg/mg by least squares regression with 1/x weighting yielding to determination coefficients (R^2) in the range 0.990-0.999. In the Appendix 1, *Table 11* lists regression equations, determination coefficients, LODs and LOI of all compounds investigated. Intra-day and inter-day precision and accuracy values were always acceptable (%CV < 15% and bias within ±15%). The majority of compounds showed matrix ion suppression within -15% except for 4-MEC with ion suppression at -18.60%, anyway it was within acceptable criteria (±25%). Eight substances showed matrix ion enhancement within 15%. Recovery was always higher than those indicated in the established criteria (>50%). In the Appendix 1, *Table 12* summarizes all results and *Figure 8* shows a representative chromatogram in overlay of all analytes under investigation.

The quality control hair sample provided by the Italian "NPS-LABVEQ" project was tested with the validated method and percent deviation was 0% and 7% for euthylone and ethylone.

Eight authentic samples were tested with the validated method. Two prescreened samples with the suspicion of benzedrone and naphyrone presence were not confirmed by the present method. Each analyzed samples had more than one synthetic cathinone except H04 sample which resulted positive only to methcathinone (10.8 pg/mg) and H08 specimen positive to 4-MEC (448 pg/mg) and its metabolite (4-MEC metab 123 pg/mg).

In Appendix 1, *Table 13* reports concentrations of analytes identified in hair samples. The most prevalent synthetic cathinones found were MDPHP with a concentration range of 6.0-1000.0 pg/mg, α-PHP, 4-MEC and 3-MMC. Three specimens were out of the calibration range thus the analysis was repeated performing a proper dilution of the sample.

H06 sample revealed the highest 3-MMC concentration (5000 pg/mg) along with 54.0 pg/mg α-PHP and 6.0 pg/mg MDPHP.

3-MMC was formally notificated to EMCDDA in 2012 and due to an increase in seizures and in related harms, it has been added to the list of new psychoactive substances under intensive monitoring.[82]

This method allowed to simultaneous screen and quantify several synthetic cathinones from last generation classes in hair samples consumers with a good analytical sensitivity. Furthermore, it permitted to discriminate among isomers with one limitation regarding 4-MMC and 3-MMC. The presented method is suitable for the inclusion of additional synthetic cathinones allowing to keep up with the evolution of the NPS phenomenon.

Chapter 6

Conclusions

The emergence of new psychoactive substances that are highly harmful to human health has highlighted the need to make extra efforts to develop analytical methodologies capable of detecting these new compounds. Due to the polydrug consumption, is necessary develop analytical methods that are suitable for the detection and quantification of a broad range of NPS together with classic illicit drugs in different biological matrices. In this research project were developed and validated cutting-edge analytical methods for forensic and clinical toxicology applications. The first methodology described in chapter 3 allowed to screen a broad panel of compounds belonging to different chemical classes in diverse biological matrices: blood, urine and oral fluid. The multi-analyte method advantages included good analytical sensitivity, simple pretreatment of small quantities of biological samples and rapid run time saving laboratory time and resources.[62]

As synthetic cannabinoids consumption has increased, it has become important to evaluate their pharmacokinetics to better understand the onset, magnitude, duration of pharmacodynamics effects and eventual toxic concentrations. In this context, two complementary analytical methods based on GC-MS and UHPLC-HRMS have been developed and validated for the determination of three specific cannabinoids (JWH-122, JWH-210 and UR-144) investigating their disposition in oral fluid of consumers. Oral fluid has become increasingly popular as alternative matrix to blood for non-invasively synthetic cannabinoids recent use monitoring. Low concentrations are expected to be found in oral fluid and GC-MS system allowed the target determination of the parent compounds but was not able to determine hydrophilic metabolites. UHPLC-HRMS confirmed the presence of parent compounds determined by GC-MS and allowed the identification and quantification of hydrophilic metabolites, undetectable for their physico-chemical characteristics by GC-MS. The advantage of UHPLC-HRMS was the metabolites

identification in absence of reference standards through the exact mass measurement with the help of a comprehensive in-house spectra library, demonstrating that also JWH-122, JWH-210 and UR-144 metabolites are excreted in oral fluid at low concentrations.[70]

Synthetic cathinones are the second largest group of NPS monitored by EMCDDA and recently 3-MMC and 3-CMC have been intensively monitoring.[82,83] In this regard, a new UHPLC-HRMS method has been developed and validated targeting last generation synthetic cathinones in hair. Hair testing is considered the most efficient tool for retrospective investigations on drugs consumption. The presented method reached a high sensitivity and specificity allowing the detection and quantification of synthetic cathinones at very low concentrations in hair. Furthermore, the method permitted not only the determination of parent compounds but also of some metabolites, proving that synthetic cathinones metabolites can be accumulated in hair.

All methodologies reported in this project were already applied to real samples confirming their reliability. In addition, these methodologies could be easily adapted by clinical and forensic laboratories allowing the inclusion of new emerging substances and streamlining the workflow of high throughput laboratories.

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Appendix 1

Table 1. *Chemico-physical properties of analytes under investigation.*

Substance	Methanolic solution (mg/mL)	Powder form (mg)	Salt*
Classic drugs			
Benzoylecgonine-d_3	1.0	–	hydrochloride
Benzoylecgonine	1.0	–	hydrochloride
Morphine	1.0	–	hydrochloride
Morphine-d_3	1.0	–	hydrochloride
Norfentanyl	1.0	–	–
Amphetamine-d_3	1.0	–	hydrochloride
Amphetamine	1.0	–	hydrochloride
Dihydrocodeine	1.0	–	–
Codeine	1.0	–	–
3,4-Methylenedioxyamphetamine (MDA)	1.0	–	hydrochloride
Metamphetamine	1.0	–	hydrochloride
MDA-d_5	1.0	–	hydrochloride
Codeine-d_3	**1.0**	**–**	**–**
Methamphetamine-d_5	**1.0**	**–**	**hydrochloride**
3,4-Methylenedioxymethamphetamine (MDMA)	1.0	–	hydrochloride
MDMA-d_5	1.0	–	hydrochloride
6-O-monoacetylmorphine (6-MAM)	1.0	–	–
3,4-methylenedioxy-N-ethylamphetamine-d_5 (MDEA-d_5)	1.0	–	hydrochloride
MDEA	1.0	–	hydrochloride
Norketamine	1.0	–	hydrochloride
Oxycodone	1.0	–	–
Tramadol	1.0	–	hydrochloride
Norsufentanyl	1.0	–	–
Zolpidem	1.0	–	–
Lorazepam	1.0	–	–
2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine	1.0	–	perchlorate
Cocaine	1.0	–	hydrochloride
Cocaine- d_3	1.0	–	hydrochloride
Ketamine-d_4	1.0	–	hydrochloride
Ketamine	1.0	–	hydrochloride
Cocaethylene	1.0	–	–
Norbuprenorphine-d_3	1.0	–	–
Norbuprenorphine	1.0	–	–
Nordiazepam	1.0	–	–
Flunitrazepam	1.0	–	–
Temazepam	1.0	–	–
Clobazam	1.0	–	–
Phenazepam	1.0	–	–
Methadone	1.0	–	hydrochloride

Methadone-d_3	1.0	–	hydrochloride
Fentanyl	–	5.0	–
Fentanyl-d_5	–	5.0	–
Sufentanyl	1.0	–	–
Buprenorphine-d_4	1.0	–	hydrochloride
Buprenorphine	1.0	–	hydrochloride
Δ_9-tetrahydrocannabinol-d_3 (Δ_9-THC-d_3)	1.0		
Δ_9-THC	1.0	–	–
Synthetic Cannabinoids			
PX-1	–	5.0	–
AB FUBINACA	–	1.0	–
5 CL AB PINACA	–	1.0	–
PX-2	–	5.0	–
ADB Fubinaca	–	1.0	–
AB CHMINACA	1.0	–	–
MMB 2201	–	1.0	–
Pravadoline	–	5.0	–
APP FUBINACA	–	1.0	–
5-F ADB	–	1.0	–
AM-2233	–	1.0	–
CUMYL 5F PINACA	–	1.0	–
AM-694	–	5.0	–
JWH 302	–	5.0	–
CUMYL PEGACLONE	–	5.0	–
RCS-4	–	1.0	–
JWH-251	–	5.0	–
AM-2201	–	1.0	–
UR 144	–	1.0	–
JWH-203	–	5.0	–
5F NNEI-2	–	1.0	–
5F-AKB48	1.0	–	–
RCS-8	–	1.0	–
JWH-018	–	1.0	–
CP47,497-C8	–	5.0	–
JWH-016	1.0	–	–
JWH-098	–	1.0	–
THJ 018	–	1.0	–
JWH-081	–	5.0	–
JWH-122	–	5.0	–
JWH-019	–	5.0	–
JWH-007	1.0	–	–
JWH-210	–	1.0	–
JWH-147	–	5.0	–
JWH-398	1.0	–	–
CB-13	–	5.0	–
Fentanyl analogues			
Methoxyacetyl norfentanyl		1.0	hydrochloride

Acetyl Norfentanyl-d_5	1.0	–	hydrochloride
Acetyl Norfentanyl	1.0	–	hydrochloride
Butyryl Fentanyl Carboxy metabolite	–	1.0	–
Valeryl Fentanyl Carboxy metabolite	–	1.0	–
Methoxyacetylfentanyl	–	1.0	hydrochloride
Furanyl Norfentanyl	–	1.0	hydrochloride
Cis-3-metyl Norfentanyl	–	1.0	–
Trans-3-metyl Norfentanyl	–	1.0	–
Butyryl Norfentanyl	–	1.0	hydrochloride
Cyclopropyl norfentanyl	–	1.0	hydrochloride
β-hydroxyfentanyl	–	1.0	hydrochloride
Alfentanyl	–	1.0	hydrochloride
Cyclopropylfentanyl	–	1.0	hydrochloride
β-hydroxythiofentanyl	–	–	hydrochloride
Furanylethyl Fentanyl	–	1.0	hydrochloride
Acetyl fentanyl	–	1.0	hydrochloride
Furanyl Fentanyl	–	1.0	hydrochloride
Carfentanyl	1.0	–	–
Butyryl Fentanyl	–	1.0	hydrochloride
Despropionyl-para-fluorofentanyl	–	1.0	–
4-ANPP	–	1.0	–
Phenyl Acetyl Fentanyl	–	1.0	hydrochloride
Synthetic Cathinones			
4-fluoromethcathinone	–	1.0	hydrochloride
Methcathinone	–	5.0	hydrochloride
Methylone	–	1.0	hydrochloride
Mephedrone	–	1.0	hydrochloride
Buphedrone	–	5.0	hydrochloride
Ethylone	–	1.0	hydrochloride
Buthylone	–	5.0	hydrochloride
Dimethylcathinone	–	5.0	hydrochloride
Diethylcathinone	–	5.0	hydrochloride
3,4-dimethylmethcathinone	–	5.0	hydrochloride
4-methylethcathinone	–	5.0	hydrochloride
Ethcathinone	–	5.0	hydrochloride
Penthedrone	1.0	–	hydrochloride
Penthylone	1.0	–	hydrochloride
Methylenedioxypyrovalerone (MDPV)	–	5.0	–
Naphyrone	–	1.0	hydrochloride
Tryptamines			
Acetyl-O-dimethyltryptamine (AcO-DMT)	–	5.0	hydrochloride
5-methoxy-α-methyltryptamine (5-MeO-AMT)	–	5.0	–
4-hydroxy-diethyltryptamine (4-OH-DET)	–	5.0	–
5-methoxy-N-methyl-N-isopropyltryptamine	–	5.0	–
4-acetoxy-diisopropyltryptamine (4-AcO-DIPT)	–	1.0	acetate
5-methoxy-dipropyltryptamine (5-MeO-DPT)	–	5.0	–
5-methoxy-diallyltryptamine (5-MeO-DALT)	–	5.0	–

Phenylethylamines			
Ritalinic acid	–	10.0	–
5-(2-aminopropyl)benzofuran (5-APB)	–	5.0	hydrochloride
6-(2-aminopropyl)benzofuran (6-APB)	–	5.0	hydrochloride
1-(benzofuran-6-yl)-N-methylpropan-2-amine	–	5.0	hydrochloride
1-(benzofuran-5-yl)-N-ethylpropan-2-amine	–	1.0	hydrochloride
4'-methyl-α-pyrrolidinohexiophenone (MPHP)	–	1.0	hydrochloride

* Substances whose salt was not reported have been obtained as free bases

Table 2. *Calibrators and quality control (QC) solutions: analytes concentrations for each calibration level and QC level in blood (B), urine (U) and oral fluid (OF).*

Analyte	Matrix	Lev 1 ng/mL	Low QC ng/mL	Lev 2 ng/mL	Lev 3 ng/mL	Mid QC ng/mL	Lev 4 ng/mL	High QC ng/mL	Lev 5 ng/mL
classic drugs									
Benzoylecgonine	**B**	0.40	1.20	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.30	1.00	5.0	20.0	80.0	100.0	160.0	200.0
Morphine	**B**	0.60	2.00	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Norfentanyl	**B**	0.10	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.08	0.30	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
Amphetamine	**B**	0.20	0.60	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.10	0.30	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.15	0.50	5.0	20.0	80.0	100.0	160.0	200.0
Dihydrocodeine	**B**	0.60	2.0	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.0	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Codeine	**B**	0.60	2.00	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	50.0	80.0	100.0	160.0	200.0
MDA	**B**	0.20	0.60	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.10	0.30	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.15	0.50	5.0	20.0	80.0	100.0	160.0	200.0
Metamphetamine	**B**	0.20	0.60	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.10	0.30	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.15	0.50	5.0	20.0	80.0	100.0	160.0	200.0
MDMA	**B**	0.20	0.60	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.10	0.30	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.15	0.50	5.0	20.0	80.0	100.0	160.0	200.0
6-MAM	**B**	0.60	2.00	5.0	20.0	80.0	100.0	160.0	200.0

	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
MDEA	**B**	0.20	0.60	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.10	0.30	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.15	0.50	5.0	20.0	80.0	100.0	160.0	200.0
Norketamine	**B**	0.30	1.00	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.10	0.30	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.20	0.60	5.0	20.0	80.0	100.0	160.0	200.0
Oxycodone	**B**	0.80	2.40	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.40	1.20	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.65	2.00	5.0	20.0	80.0	100.0	160.0	200.0
Tramadol	**B**	0.30	1.00	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.25	1.00	5.0	20.0	80.0	100.0	160.0	200.0
Norsufentanyl	**B**	0.10	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.08	0.30	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
Zolpidem	**B**	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.40	1.20	5.0	20.0	80.0	100.0	160.0	200.0
Lorazepam	**B**	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.45	1.50	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
EDDP	**B**	0.40	1.20	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.35	1.00	5.0	20.0	80.0	100.0	160.0	200.0
Cocaine	**B**	0.40	1.20	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.30	1.00	5.0	20.0	80.0	100.0	160.0	200.0
Ketamine	**B**	0.30	1.00	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.10	0.30	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.20	0.60	5.0	20.0	80.0	100.0	160.0	200.0
Cocaethylene	**B**	0.40	1.20	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.30	1.00	5.0	20.0	80.0	100.0	160.0	200.0
Norbuprenorphine	**B**	0.20	0.60	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.10	0.30	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.18	0.60	5.0	20.0	80.0	100.0	160.0	200.0
Nordiazepam	**B**	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.45	1.50	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Flunitrazepam	**B**	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.45	1.50	50.0	200.0	400.0	600.0	800.0	1000.0

	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Temazepam	**B**	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.45	1.50	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Clobazam	**B**	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.45	1.50	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Phenazepam	**B**	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.45	1.50	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Methadone	**B**	0.40	1.20	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.40	1.20	5.0	20.0	80.0	100.0	160.0	200.0
Fentanyl	**B**	0.10	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.08	0.30	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.10	0.30	5.0	20.0	80.0	100.0	160.0	200.0
Sufentanyl	**B**	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.06	0.20	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
Buprenorphine	**B**	0.20	0.60	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.10	0.30	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.18	0.60	5.0	20.0	80.0	100.0	160.0	200.0
Δ9-tetrahydrocannabinol (Δ9-THC)	**B**	1.00	3.00	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.50	1.50	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.80	2.40	5.0	20.0	80.0	100.0	160.0	200.0
Synthetic cannabinoids									
PX-1	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
AB-FUBINACA	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
5 Cl-AB-PINACA	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
PX-2	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
ADB-Fubinaca	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
AB CHMINACA	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0

	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
MMB 2201	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Pravadoline	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
APP-FUBINACA	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
5-F-ADB	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
AM-2233	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Cumyl-5-F-PINACA	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
AM-694	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
JWH 302	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Cumyl-PEGACLONE	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
RCS-4	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
JWH 251	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
AM-2201	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
UR 144	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
JWH 203	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0

5-F-NNEI-2	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
5-F-AKB48	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
RCS-8	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
JWH 018	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
CP47, 497-C8	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
JWH 016	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
JWH 098	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
THJ 018	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
JWH 081	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
JWH 122	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
JWH 019	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
JWH 007	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
JWH 210	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
JWH 147	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
JWH 398	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0

	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
CB-13	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Fentanyl analogues									
Methoxyacetyl norfentanyl	**B**	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.06	0.20	50.0	200.0	400.0	100.0	800.0	1000.0
	OF	0.07	0.20	5.0	20.0	80.0	100.0	160.0	200.0
Acetyl norfentanyl	**B**	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.06	0.20	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.07	0.20	5.0	20.0	80.0	100.0	160.0	200.0
Butyryl fentanyl carboxy metabolite	**B**	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.06	0.20	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.07	0.20	5.0	20.0	80.0	100.0	160.0	200.0
Valeryl fentanyl carboxy metabolite	**B**	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.06	0.20	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.07	0.20	5.0	20.0	80.0	100.0	160.0	200.0
Methoxyacetyl fentanyl	**B**	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.06	0.20	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.07	0.20	5.0	20.0	80.0	100.0	160.0	200.0
Furanyl norfentanyl	**B**	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.06	0.20	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.07	0.20	5.0	20.0	80.0	100.0	160.0	200.0
Cis-3-methyl norfentanyl	**B**	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.06	0.20	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.07	0.20	5.0	20.0	80.0	100.0	160.0	200.0
Trans-3-methyl norfentanyl	**B**	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.06	0.20	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.07	0.20	5.0	20.0	80.0	100.0	160.0	200.0
Butyryl norfentanyl	**B**	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.06	0.20	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.07	0.20	5.0	20.0	80.0	100.0	160.0	200.0
Cyclopropyl norfentanyl	**B**	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.06	0.20	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.07	0.20	5.0	20.0	80.0	100.0	160.0	200.0
β-hydroxyfentanyl	**B**	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.06	0.20	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.07	0.20	5.0	20.0	80.0	100.0	160.0	200.0
Alfentanyl	**B**	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.06	0.20	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.07	0.20	5.0	20.0	80.0	100.0	160.0	200.0
Cyclopropylfentanyl	**B**	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0

	U	0.06	0.20	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.07	0.20	5.0	20.0	80.0	100.0	160.0	200.0
β-hydroxythiofentanyl	**B**	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.06	0.20	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.07	0.20	5.0	20.0	80.0	100.0	160.0	200.0
Furanylethyl fentanyl	**B**	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.06	0.20	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.07	0.20	5.0	20.0	80.0	100.0	160.0	200.0
Acetyl fentanyl	**B**	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.06	0.20	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.07	0.20	5.0	20.0	80.0	100.0	160.0	200.0
Furanyl fentanyl	**B**	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.06	0.20	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.07	0.20	5.0	20.0	80.0	100.0	160.0	200.0
Carfentanyl	**B**	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.06	0.20	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.07	0.20	5.0	20.0	80.0	100.0	160.0	200.0
Butyryl fentanyl	**B**	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.06	0.20	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.07	0.20	5.0	20.0	80.0	100.0	160.0	200.0
Despropionyl-para-fluoro fentanyl	**B**	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.06	0.20	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.07	0.20	5.0	20.0	80.0	100.0	160.0	200.0
4-ANPP	**B**	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.06	0.20	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.07	0.20	5.0	20.0	80.0	100.0	160.0	200.0
Phenyl acetyl Fentanyl	**B**	0.08	0.30	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.06	0.20	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.07	0.20	5.0	20.0	80.0	100.0	160.0	200.0
Synthetic cathinones									
4-fluoromethcathinone	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Methcathinone	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Methylone	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Mephedrone	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Buphedrone	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0

	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Ethylone	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Buthylone	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Dimethylcathinone	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Diethylcathinone	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
3,4-DMMC	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
4-methyl ethcathinone	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Ethcathinone	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Penthedrone	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Penthylone	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Methylenedioxypyrovalerone (MDPV)	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Naphyrone	**B**	0.60	1.80	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.45	1.50	5.0	20.0	80.0	100.0	160.0	200.0
Tryptamine									
AcO-DMT	**B**	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	100.0	800.0	1000.0
	OF	0.40	1.20	5.0	20.0	80.0	100.0	160.0	200.0
5-MeO-AMT	**B**	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.40	1.20	5.0	20.0	80.0	100.0	160.0	200.0
4-OH-DET	**B**	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0

	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.40	1.20	5.0	20.0	80.0	100.0	160.0	200.0
5-MeO-MIPT	**B**	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.40	1.20	5.0	20.0	80.0	100.0	160.0	200.0
4-AcO-DIPT	**B**	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.40	1.20	5.0	20.0	80.0	100.0	160.0	200.0
5-MeO-DPT	**B**	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.40	1.20	5.0	20.0	80.0	100.0	160.0	200.0
5-MeO-DALT	**B**	0.50	1.50	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.20	0.60	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.40	1.20	5.0	20.0	80.0	100.0	160.0	200.0
Phenylethylamines									
Ritalinic acid	**B**	0.40	1.20	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.36	1.00	5.0	20.0	80.0	100.0	160.0	200.0
5-APB	**B**	0.40	1.20	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.36	1.00	5.0	20.0	80.0	100.0	160.0	200.0
6-APB	**B**	0.40	1.20	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.36	1.00	5.0	20.0	80.0	100.0	160.0	200.0
6-MAPB	**B**	0.40	1.20	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.36	1.00	5.0	20.0	80.0	100.0	160.0	200.0
5-EAPB	**B**	0.40	1.20	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.36	1.00	5.0	20.0	80.0	100.0	160.0	200.0
MPHP	**B**	0.40	1.20	5.0	20.0	80.0	100.0	160.0	200.0
	U	0.30	1.00	50.0	200.0	400.0	600.0	800.0	1000.0
	OF	0.36	1.00	5.0	20.0	80.0	100.0	160.0	200.0

Table 3. *Mass spectrometry parameters for analytes and internal standards.*

n.	Analyte	Retention time (min)	Cone Voltage (V)	Quantifier MRM transitions (m/z)	Collision energy (eV)	Qualifier MRM transition (m/z)	Collision energy (eV)
classic drugs							
1	**Benzoylecgonine-d_3**	0.38	30.00	293.10 > 171.10	20.00	-	-
2	**Benzoylecgonine**	0.38	30.00	290.10 > 168.10	20.00	290.1 > 105.1	33.00
3	**Morphine-d_3**	0.75	35.00	289.00 > 61.00	28.00	-	-
4	**Morphine**	0.76	35.00	286.00 > 165.10	40.00	286 > 153	40.00
5	**Norfentanyl**	0.93	25.00	233.10 > 84.20	20.00	233.10 > 55.30	34.00
6	**Amphetamine-d_6**	1.01	10.00	142.20 > 93.10	16.00	-	-
7	**Amphetamine**	1.01	15.00	136.00 > 119.10	8.00	136.00 > 91.10	15.00
8	**Dihydrocodeine**	1.03	35.00	302.10 > 199.10	34.00	302.10 > 201.10	30.00
9	**Codeine**	1.18	30.00	300.10 > 215.10	25.00	300.10 > 199.20	27.00
10	**3,4-Methylenedioxyamphetamine (MDA)**	1.20	20.00	180.00 > 133.10	18.00	180.00 > 163.10	10.00
11	**Methamphetamine**	1.21	20.00	150.10 > 91.10	12.00	150.10 > 119.10	10.00
12	**MDA-d_5**	1.23	20.00	184.71 > 137.20	18.00	184.71 > 109.70	10.00
13	**Codeine-d_3**	1.26	40.00	303.00 >215.10	25.00	303.00 > 199.10	30.00
14	**Methamphetamine-d_5**	1.29	20.00	154.80 > 91.80	12.00	154.80 > 119.10	10.00
15	**3,4-Methylenedioxymetamphetamine-d_5 (MDMA-d_5)**	1.29	20.00	199.10 > 116.51	12.00	199.10 > 135.25	20.00
16	**3,4-Methylenedioxymetamphetamine (MDMA)**	1.29	20.00	194.10 > 163.00	14.00	194.10 > 133.10	20.00
17	**6-O-monoacetylmorphine (6-MAM)**	1.46	30.00	328.10 > 165.10	40.00	328.10 > 181.20	40.00
18	**3,4-methylenedioxy-N-ethylamphetamine- d_5 (MDEA-d_5)**	1.51	20.00	213.10 > 163.10	14.00	213.10 > 105.10	26.00
19	**3,4-methylenedioxy-N-ethylamphetamine (MDEA)**	1.55	20.00	208.10 > 163.10	14.00	208.10 > 135.10	14.00
20	**Norketamine**	1.90	20.00	224.10 > 207.10	10.00	224.10 > 125.00	25.00
21	**Oxycodone**	1.99	25.00	316.10 > 241.20	30.00	316.10 > 256.10	30.00
22	**Tramadol**	2.02	25.00	264.10 > 58.10	15.00	-	-
23	**Norsufentanyl**	2.02	25.00	277.00 > 128.10	15.00	277.00 > 96.00	25.00
24	**6-O-monoacetylmorphine- d_3 (6-MAM-d_3)**	2.09	30.00	331.00 > 61.10	30.00	-	-
25	**Zolpidem**	2.13	45.00	308.10 > 235.20	34.00	208.10 > 263.10	28.00

26	**Lorazepam**	2.21	30.00	312.10 > 229.00	30.00	321.00 > 275.00	20.00
27	**2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)**	2.28	45.00	278.20 > 234.20	26.00	278.20 > 186.20	35.00
28	**Cocaine**	2.57	30.00	304.20 > 182.26	20.00	304.20 > 82.30	30.00
29	**Cocaine-d_3**	2.59	30.00	307.00 > 184.70	20.00	307.00 > 84.80	30.00
30	**Ketamine-d_4**	2.61	20.00	242.20 > 129.10	25.00	242.20 > 211.10	15.00
31	**Ketamine**	2.62	20.00	238.20 > 125.10	25.00	238.20 > 220.20	15.00
32	**Cocaethylene**	2.89	30.00	318.10 > 196.10	20.00	318.10 > 82.10	30.00
33	**Cocaethylene-d_3**	2.93	30.00	321.10 > 199.10	20.00	321.10 > 85.00	30.00
34	**Norbuprenorphine-d_3**	2.95	55.00	417.20 > 100.80	40.00	417.20 > 56.80	40.00
35	**Norbuprenorphine**	2.95	55.00	414.20 > 101.30	40.00	414.20 > 83.20	55.00
36	**Nordiazepam**	2.97	40.00	271.10 > 140.00	27.00	271.10 > 165.10	27.00
37	**Flunitrazepam**	3.02	52.00	314.10 > 239.10	36.00	314.10 > 268.10	26.00
38	**Temazepam**	3.04	34.00	301.00 >177.00	20.00	301.00 > 255.00	38.00
39	**Clobazam**	3.04	38.00	301.10 > 224.00	36.00	301.10 > 259.00	22.00
40	**Phenazepam**	3.08	25.00	350.70 > 104.70	45.00	350.70 > 206.00	35.00
41	**Methadone**	3.27	30.00	310.30 > 265.20	14.00	310.30 > 105.10	32.00
42	**Methadone-d_3**	3.30	38.00	313.20 > 105.10	28.00	-	-
43	**Fentanyl**	3.87	35.00	337.20 > 105.20	38.00	337.20 > 188.20	30.00
44	**Fentanyl-d_5**	3.89	35.00	342.20 > 105.20	38.00	342.20 > 188.20	30.00
45	**Sufentanyl**	4.41	16.00	387.20 > 238.10	38.00	387.20 > 111.00	18.00
46	**Buprenorphine-d_4**	5.65	55.00	472.20 > 59.20	80.00	-	-
47	**Buprenorphine**	5.65	55.00	468.20 > 55.20	80.00	468.20 > 84.20	40
48	**Δ9-tetrahydrocannabinol-d_3(Δ9-THC-d_3)**	5.79	45.00	318.00 > 123.00	34.00	-	-
49	**Δ9-tetrahydrocannabinol (Δ9-THC)**	6.19	45.00	315.20 > 193.10	34.00	315.20 > 123.00	22.00
synthetic cannabinoids							
50	**PX-1**	2.80	36.00	396.30 > 144.00	44.00	379.10 > 134.80	24.00
51	**AB-FUBINACA**	2.83	36.00	369.30 > 109.00	40.00	369.30 > 253.00	24.00
52	**5-Cl-AB-PINACA**	2.94	36.00	366.00 > 249.00	24.00	366.00 > 145.00	44.00
53	**PX-2**	2.97	26.00	397.30 > 145.00	46.00	397.30 > 233.00	22.00
54	**ADB-Fubinaca**	3.15	25.00	383.20 > 109.00	42.00	383.20 > 253.00	25.00
55	**AB CHMINACA**	3.46	38	357.40 > 241.20	28.00	357.40 > 145.00	46.00

56	**MMB 2201**	3.52	34.00	363.30 > 231.90	12.00	363.30 > 143.90	38.00
57	**Pravadoline**	3.77	45.00	379.10 > 113.90	32.00	379.10 > 134.80	24.00
58	**APP-FUBINACA**	3.9	20.00	417.30 > 109.00	40.00	417.30 > 253.00	24.00
59	**5-F-ADB**	4.1	45.00	378.30 > 105.00	24.00	378.30 > 318.00	14.00
60	**AM-2233**	4.75	45.00	459.00 > 111.90	22.00	459.00 > 97.80	34.00
61	**Cumyl-5-F-PINACA**	4.77	32.00	368.30 > 250.00	10.00	368.30 > 233.00	18.00
62	**AM-694**	4.99	45.00	436.00 > 202.70	40.00	436.00 > 230.70	28.00
63	**JWH 302**	5.16	45.00	336.10 > 121.10	22.00	322.00 > 134.80	26.00
64	**Cumyl-PEGACLONE**	5.26	30.00	373.30 > 255.00	10.00	373.30 > 119.00	24.00
65	**RCS-4**	5.33	45.00	322.00 > 106.80	40.00	322.00 > 134.80	24.00
66	**JWH 251**	5.51	45.00	319.80 > 105.90	22.00	319.80 > 214.20	15.00
67	**AM-2201**	5.59	45.00	360.20 > 126.90	40.00	360.20 > 154.90	28.00
68	**UR 144**	5.68	18.00	312.20 > 55.00	36.00	312.20 > 125.00	22.00
69	**JWH 203**	5.69	45.00	340.40 > 124.80	28.00	340.40 > 187.80	20.00
70	**5-F-NNEI-2**	5.76	22.00	375.30 > 232.00	20.00	375.30 > 144.00	42.00
71	**5-F-AKB48**	5.93	35.00	384.00 > 106.90	45.00	384.0 > 134.90	25.00
72	**RCS-8**	6.13	45.00	376.10 > 90.85	40.00	376.10 > 120.83	26.00
73	**JWH 018**	6.27	45.00	342.10>127.00	25.00	342.10 >155.00	34.00
74	**CP47, 497-C8**	6.32	45.00	386.70 > 104.80	22.00	386.70 > 120.80	24.00
75	**JWH 016**	6.33	45.00	341.70 > 127.10	44.00	341.70 > 155.10	24.00
76	**JWH 098**	6.33	45.00	385.80 > 157.20	42.00	385.80 > 185.10	26.00
77	**THJ 018**	6.36	25.00	377.20 > 248.90	16.00	377.20 > 212.90	24.00
78	**JWH 081**	6.38	45.00	371.80 > 157.09	40.00	371.80 > 185.08	26.00
79	**JWH 122**	6.48	45.00	356.10 > 140.90	40.00	356.10 > 168.80	26.00
80	**JWH 019**	6.52	45.00	356.10 > 255.07	26.00	356.10 > 228.10	26.00
81	**JWH 007**	6.56	45.00	355.80 > 127.09	48.00	355.80 > 155.09	26.00
82	**JWH 210**	6.69	45.00	369.80 > 183.10	26.00	369.80 > 214.20	24.00
83	**JWH 147**	6.70	45.00	382.10 > 127.09	48.00	382.10 > 155.06	22.00
84	**JWH 398**	6.90	45.00	376.06 > 161.07	48.00	376.06 > 189.06	26.00
85	**CB-13**	7.71	45.00	369.20 > 155.08	26.00	369.20 > 170.80	28.00
fentanyl analogues							
86	**Methoxyacetyl norfentanyl**	0.50	15.00	249.10 > 84.10	14.00	246.10 > 55.00	38.00

87	**Acetyl norfentanyl-d_5**	0.58	25.00	224.20 > 84.00	18.00	-	-
88	**Acetyl norfentanyl**	0.59	25.00	219.20 > 84.05	18.00	219.20 > 55.20	36.00
89	**Butyryl fentanyl carboxy metabolite**	0.76	25.00	381.20 > 105.00	42.00	381.20 > 188.10	30.00
90	**Valeryl fentanyl carboxy metabolite**	0.87	40.00	395.30 > 105.25	44.00	395.30 > 188.15	26.00
91	**Methoxyacetyl fentanyl**	1.1	30.00	353.30 > 188.00	20.00	249.10 > 84.10	18.00
92	**Furanyl norfentanyl**	1.15	16.00	271.00 >84.20	18.00	271.00 > 55.10	38.00
93	**Cis-3-metyl norfentanyl**	1.15	25.00	247.00 > 98.00	16.00	247.00 > 69.20	30.00
94	**Trans-3-metyl norfentanyl**	1.15	25.00	247.00 > 98.10	16.00	247.00 > 69.10	30.00
95	**Butyryl norfentanyl**	1.26	25.00	247.10 > 84.15	20.00	247.10 > 55.30	36.00
96	**Cyclopropyl norfentanyl**	1.33	25.00	245.20 > 177.10	18.00	245.20 > 84.10	20.00
97	**β-hydroxyfentanyl**	2.5	25.00	389.20 > 238.00	16.00	389.20 > 111.00	38.00
98	**Alfentanyl**	2.6	24.00	417.10 > 197.05	26.00	417.10 > 268.10	16.00
99	**Cyclopropylfentanyl**	3.06	25.00	349.20 > 105.00	30.00	349.20 > 188.10	25.00
100	**β-hydroxythiofentanyl**	3.14	25.00	359.20 > 192.00	22.00	359.20 > 111.00	38.00
101	**Furanylethyl fentanyl**	3.31	25.00	327.20 > 95.10	34.00	327.20 > 178.10	16.00
102	**Acetyl fentanyl**	3.33	25.00	322.20 > 105.00	36.00	322.20 > 188.00	20.00
103	**Furanyl fentanyl**	3.5	30.00	375.10 > 188.00	20.00	375.10 > 105.00	25.00
104	**Carfentanyl**	3.93	22.00	395.20 > 113.00	32.00	395.20 > 335.00	16.00
105	**Butyryl fentanyl**	4.22	30.00	351.20 > 105.00	40.00	351.20 > 188.10	22.00
106	**Despropionyl-para-fluoro fentanyl**	4.52	15.00	299.10 > 105.10	16.00	299.10 > 188.10	38.00
107	**Despropionyl fentanyl (4-ANPP)**	4.55	22.00	281.20 > 105.00	22.00	281.20 > 188.00	14.00
108	**Phenyl acetyl fentanyl**	4.96	46.00	399.30 > 188.05	24.00	399.30 > 105.05	44.00
synthetic cathinones							
109	**4-fluoromethcathinone**	0.83	35.00	205.00 > 102.80	28.00	205.00 > 148.70	26.00
110	**Methcathinone**	0.97	30.00	163.90 > 104.80	22.00	163.9 > 130.7	20.00
111	**Methylone**	1.15	30.00	208.1 > 159.9	16.00	208.1 > 131.9	26.00
112	**Mephedrone**	1.44	30.00	178.01 > 145	18.00	178.01 > 119	22.00
113	**Buphedrone**	1.45	30.00	178 > 130.3	26.00	178 > 91	32.00
114	**Ethylone**	1.63	30.00	222 > 174	20.00	222 > 146	26.00
115	**Buthylone**	1.63	25.00	222 > 173.9	20.00	222 > 145.9	26.00
116	**Dimethylcathinone**	1.80	30.00	177.70 > 72.10	22.00	177.7 > 105.30	20.00
117	**Diethylcathinone**	1.84	25.00	206.30 > 100.00	22.00	206.30 > 105.00	20.00

118	**3,4-dimethyl methcathinone**	1.85	30.00	192.00 > 143.90	28.00	192.00 > 158.80	22.00
119	**4-methyl ethcathinone**	1.89	35.00	192.00 > 145.30	18.00	192.00 > 91.00	34.00
120	**Ethcathinone**	1.94	30.00	177.7 > 72	16.00	177.7 > 105.20	22.00
121	**Penthedrone**	2.09	35.00	192.10 > 90.90	20.00	192.10 > 131.70	20.00
122	**Penthylone**	2.15	35.00	236.10 > 174.90	22.00	236.10 > 187.80	18.00
123	**Methylenedioxypyrovalerone (MDPV)**	3.42	30.00	276.1 > 126	26.00	276.1 > 134.8	24.00
124	**Naphyrone**	4.93	45.00	282.10 > 126.2	36.00	282.10 > 140.9	26.00
tryptamines							
125	**Acetyl-O-dimethyltryptamine (AcO-DMT)**	1.33	28.00	247 > 58.1	24.00	247 > 160	14.00
126	**5-methoxy-α-methyltryptamine (5-MeO-AMT)**	1.33	22.00	205.1 > 147	20.00	205.1 > 173	22.00
127	**4-hydroxy-diethyltryptamine (4-OH-DET)**	1.6	16.00	233.1 > 86.1	18.00	233.1 > 160	14.00
128	**5-methoxy-N-methyl-N-isopropyltryptamine (5-MeO-MIPT)**	1.8	10.00	247.1 > 86	14.00	247.1 > 174	16.00
129	**4-acetoxy-diisopropyltryptamine (4-AcO-DIPT)**	1.99	15.00	303.1 > 114	18.00	303.1 > 160	28.00
130	**5-methoxy-dipropyltryptamine (5-MeO-DPT)**	2.92	14.00	275.2 > 174	16.00	275.2 > 114	14.00
131	**5-methoxy-diallyltryptamine (5-MeO-DALT)**	3.78	24.00	271.2 > 110	14.00	271.2 > 174	18.00
phenylethylamines							
132	**Ritalinic Acid**	0.63	20.00	220.1 > 84.1	20.00	220.1 > 56	46.00
133	**5-(2-aminopropyl)benzofuran (5-APB)**	1.74	26.0	176.2 > 91	28.00	176.2 > 77	38.00
134	**6-(2-aminopropyl)benzofuran (6-APB)**	1.75	22.00	176.2 > 91	26.00	176.2 > 77	40.00
135	**1-(benzofuran-6-yl)-N-methylpropan-2-amine (6-MAPB)**	1.83	22.00	190.15 > 159	10.00	190.15 > 131	18.00
136	**1-(benzofuran-5-yl)-N-ethylpropan-2-amine (5-EAPB)**	2.11	24.00	204.15 > 131	20.00	204.15 > 91	30.00
137	**4'-methyl-α-pyrrolidinohexiophenone (MPHP)**	4.20	10.00	260.2 > 105	22.00	260.2 > 189	16.00

Table 4. *Validation parameters for analytes under investigation in whole blood (B), urine (U)and oral fluid (OF)quality control (QC) samples. Analytical recovery and matrix effect are displayed as the mean of low, medium and high QC concentration values. The concentration of each analyte in high, medium and low QC samples are reported in Table 2.*

Analyte	Matrix	Determination coefficient (R^2)	LOD (ng/ml)	LOQ (ng/ml)	Accuracy (%err)			Intra-assay precision (%CV)			Inter-assay precision (%CV)			Matrix effect (%)	Analytical Recovery (%)
					low QC	mid QC	high QC	low QC	mid QC	high QC	low QC	mid QC	high QC		
classic illicit drugs															
Benzoylecgonine	blood	0.9986	0.15	0.40	15.6	5.4	2.2	10.9	7.0	4.2	16.3	3.0	6.4	90.1	94.1
	urine	0.9992	0.08	0.20	15.3	10.3	4.3	11.6	5.0	3.4	15.5	10.5	4.3	95.0	96.0
	oral fluid	0.9986	0.10	0.30	16.8	6.7	3.5	11.9	9.4	5.5	13.9	8.7	6.4	89.1	92.0
Morphine	blood	0.9990	0.20	0.60	13.4	6.4	3.3	10.2	6.0	4.9	16.1	3.5	4.4	89.5	91.7
	urine	0.9984	0.10	0.30	15.2	11.0	4.0	12.5	4.3	3.7	15.2	9.4	7.0	91.1	95.6
	oral fluid	0.9991	0.15	0.45	15.5	7.8	4.3	11.0	9.5	4.1	15.0	9.4	5.2	89.2	93.1
Norfentanyl	blood	0.9984	0.04	0.10	17.3	4.9	2.4	12.3	7.9	4.6	15.9	2.8	7.0	88.9	93.9
	urine	0.9991	0.03	0.08	12.4	9.2	4.3	12.0	4.8	3.2	16.0	9.7	4.8	91.1	96.2
	oral fluid	0.9972	0.03	0.08	14.3	7.6	3.8	11.1	7.2	5.3	18.1	6.1	4.5	90.1	95.4
Amphetamine	blood	0.9978	0.06	0.20	13.5	4.8	3.7	10.1	6.8	4.1	15.8	3.1	6.8	90.1	90.2
	urine	0.9961	0.03	0.10	11.8	9.4	4.1	12.0	5.5	2.7	16.5	9.6	4.6	92.3	97.4
	oral fluid	0.9957	0.05	0.15	12.8	6.5	4.5	10.7	6.4	4.9	15.1	6.4	5.9	91.0	95.6
Dihydrocodeine	blood	0.9991	0.20	0.60	18.0	5.9	2.5	10.3	5.2	4.5	16.0	3.6	6.9	89.2	94.7
	urine	0.9985	0.10	0.30	16.0	8.6	2.8	13.2	12.9	3.2	15.5	9.2	4.6	94.0	94.4
	oral fluid	0.9976	0.15	0.45	16.1	7.4	3.1	11.4	11.6	3.5	16.2	5.4	8.0	88.2	93.8
Codeine	blood	0.9986	0.20	0.60	14.6	5.7	1.4	10.9	5.3	4.4	16.0	3.6	6.2	89.2	98.8
	urine	0.9978	0.10	0.30	16.4	6.5	2.9	11.3	8.5	2.9	15.4	10.5	5.5	94.8	96.9
	oral fluid	0.9992	0.15	0.45	15.3	6.9	3.0	10.8	7.4	3.9	15.1	7.9	6.9	90.2	95.9
MDA	blood	0.9963	0.06	0.20	16.0	7.4	3.7	10,7	6.1	4.6	16.0	3.6	6.9	90.0	94.9

	urine	0.9991	0.03	0.10	16.9	5.6	3.7	12.4	8.3	3.3	16.1	10.1	4.6	91.3	97.1
	oral fluid	0.9974	0.05	0.15	13.5	7.5	3.0	12.8	7.6	6.4	14.0	6.7	5.4	92.1	95.9
Metamphetamine	blood	0.9985	0.06	0.20	16.5	7.9	2.1	10.9	6.4	4.0	16.0	3.6	6.7	90.8	90.3
	urine	0.9997	0.03	0.10	15.1	6.2	2.4	12.5	8.6	3.1	16.3	10.4	4.7	91.7	96.0
	oral fluid	0.9958	0.05	0.15	15.7	7.7	2.7	11.9	6.9	3.6	15.9	7.9	5.3	92.0	93.9
MDMA	blood	0.9968	0.06	0.20	16.5	7.8	2.8	11.0	5.4	4.1	16.2	3.4	6.5	90.0	86.1
	urine	0.9980	0.03	0.10	15.9	7.0	2.3	12.4	8.0	3.2	15.8	9.9	4.7	92.4	91.1
	oral fluid	0.9961	0.05	0.15	11.6	8.2	4.1	10.9	6.8	5.9	14.4	7.9	5.1	91.5	92.1
6-MAM	blood	0.9987	0.20	0.60	15.4	6.9	2.0	11.4	5.7	4.2	15.3	2.9	6.6	89.2	87.1
	urine	0.9986	0.10	0.30	12.9	7.8	2.6	12.7	8.1	2.8	15.2	10.5	5.1	90.0	96.9
	oral fluid	0.9966	0.15	0.45	14.8	5.5	3.0	10.3	7.4	4.7	14.0	8.6	5.8	90.0	89.9
MDEA	blood	0.9983	0.07	0.20	15.3	7.6	2.5	11.9	5.8	4.6	16.7	2.7	6.2	90.0	85.7
	urine	0.9967	0.08	0.10	14.3	6.9	2.3	11.9	8.1	2.8	15.8	10.2	5.2	90.7	89.5
	oral fluid	0.9972	0.05	0.15	13.9	7.8	3.5	9.3	7.9	6.8	15.0	2.8	5.9	89.0	91.9
Norketamine	blood	0.9984	0.10	0.30	15.9	4.8	2.4	10.9	5.9	4.6	15.7	3.3	8.3	90.8	79.4
	urine	0.9990	0.03	0.10	16.8	4.7	1.8	12.4	8.6	3.2	16.4	10.1	6.8	92.5	85.0
	oral fluid	0.9964	0.07	0.20	13.9	5.7	3.5	13.3	7.5	5.3	15.6	7.9	5.9	90.0	89.0
Oxycodone	blood	0.9976	0.25	0.80	17.0	6.9	2.9	11.6	5.9	5.4	15.7	3.5	8.7	91.7	90.3
	urine	0.9987	0.15	0.40	13.7	7.5	2.7	11.1	7.3	2.6	16.5	10.5	7.9	92.3	94.9
	oral fluid	0.9965	0.25	0.65	12.8	6.8	3.1	12.7	6.0	3.9	13.7	6.9	5.8	93.1	94.2
Tramadol	blood	0.9979	0.10	0.30	16.1	5.6	1.6	11.6	5.5	4.9	16.7	3.6	8.9	88.1	90.8
	urine	0.9988	0.07	0.20	15.9	5.9	2.0	12.8	8.9	2.9	15.5	10.4	6.5	90.0	91.7
	oral fluid	0.9962	0.08	0.25	12.9	6.4	3.0	10.5	7.7	4.9	15.0	8.5	5.9	89.3	92.4
Norsufentanyl	blood	0.9981	0.04	0.10	16.3	4.8	2.1	10.7	5.4	4.7	15.9	2.9	7.9	90.2	88.4
	urine	0.9979	0.03	0.08	12.9	9.2	4.3	12.0	8.1	2.9	15.9	10.1	6.1	91.5	89.1

	oral fluid	0.9981	0.03	0.08	13.6	7.3	3.8	11.7	7.1	5.3	14.8	8.4	5.1	88.6	90.1
Zolpidem	blood	0.9973	0.18	0.50	14.5	5.8	2.6	11.3	6.0	4.8	15.2	2.6	8.9	88.8	85.6
	urine	0.9988	0.10	0.30	12.6	6.6	2.5	11.7	8.9	2.5	15.4	10.0	6.7	90.6	90.2
	oral fluid	0.9978	0.15	0.40	13.7	6.9	3.3	10.1	6.9	3.6	14.5	6.9	5.9	90.0	89.8
Lorazepam	blood	0.9983	0.18	0.50	16.0	6.6	1.9	10.3	5.9	4.6	15.6	3.0	8.0	91.0	87.0
	urine	0.9994	0.15	0.45	15.9	6.9	2.4	12.4	8.6	3.5	15.2	9.8	7.7	94.1	91.1
	oral fluid	0.9969	0.15	0.45	14.6	6.7	3.1	9.8	8,9	4.3	15.6	7.5	5.6	92.1	91.5
EDDP	blood	0.9976	0.15	0.40	15.4	6.9	1.9	11.5	5.9	4.0	15.7	3.4	6.3	89.4	88.9
	urine	0.9979	0.07	0.20	12.9	7.6	2.5	12.7	8.9	3.5	15.4	9.9	5.6	90.6	90.4
	oral fluid	0.9956	0.10	0.35	14.3	7.6	3.5	10.4	6.9	4.6	15.9	7.5	5.9	91.0	89.5
Cocaine	blood	0.9984	0.15	0.40	16.3	5.8	2.4	10.5	5.7	4.5	15.5	3.4	6.1	85.1	90.5
	urine	0.9979	0.07	0.20	15.6	10.0	4.1	11.4	8.6	2.5	15.2	10.0	4.3	92.3	93.7
	oral fluid	0.9992	0.10	0.30	15.6	7.9	4.5	11.1	7.2	3.9	14.9	7.3	7.9	91.5	91.7
Ketamine	blood	0.9969	0.10	0.30	15.8	4.9	2.4	9.9	5.9	3.9	15.9	2.5	6.0	89.2	85.0
	urine	0.9965	0.03	0.10	16.4	5.3	2.3	11.9	8.2	3.1	15.9	10.5	5.1	93.6	91.5
	oral fluid	0.9984	0.07	0.20	15.4	6.7	3.3	9.9	8.4	4.4	14.9	9.2	4.5	90.0	90.7
Cocaethylene	blood	0.9995	0.10	0.40	13.9	5.6	2.6	10.4	5.1	3.9	16.0	3.3	6.7	88.2	83.4
	urine	0.9979	0.07	0.20	15.1	9.8	3.6	11.0	8.6	2.4	16.4	11.1	4.6	91.0	93.1
	oral fluid	0.9983	0.10	0.30	15.3	8.5	3.5	9.0	6.6	5.4	15.0	8.8	5.6	90.6	90.4
Norbuprenorphine	blood	0.9971	0.07	0.20	16.1	5.2	2.6	11.0	5.9	4.3	15.6	2.4	6.1	92.0	78.8
	urine	0.9979	0.03	0.10	14.9	7.1	2.2	12.5	8.5	2.8	15.8	10.0	4.8	95.7	82.7
	oral fluid	0.9970	0.06	0.18	14.9	6.9	3.1	8.4	7.4	4.0	15.0	7.0	5.8	91.1	91.4
Nordiazepam	blood	0.9980	0.20	0.50	16.0	6.7	2.1	10.4	5.2	4.7	15.3	3.3	5.4	90.1	79.9
	urine	0.9979	0.15	0.45	15.9	6.7	2.4	12.0	8.9	2.9	16.0	9.8	4.4	91.0	90.2
	oral fluid	0.9981	0.15	0.45	13.9	5.9	2.9	11.3	7.5	3.2	16.5	10.5	4.9	90.2	90.0

Flunitrazepam	blood	0.9986	0.18	0.50	16.2	4.4	1.6	10.1	5.9	4.6	16.7	3.4	5.5	89.6	79.5
	urine	0.9995	0.15	0.45	15.7	6.7	2.4	11.4	8.9	3.4	15.6	10.7	5.2	92.0	87.9
	oral fluid	0.9984	0.15	0.45	16.1	7.7	2.6	9.9	6.6	4.9	14.9	7.7	3.5	87.4	79.0
Temazepam	blood	0.9979	0.18	0.50	15.9	6.8	2.6	10.7	5.7	4.9	15.8	3.2	6.5	91.0	89.9
	urine	0.9987	0.15	0.45	16.1	10.0	3.0	11.9	8.0	3.2	15.5	10.7	4.9	92.4	88.5
	oral fluid	0.9989	0.25	0.45	14.9	9.3	2.9	10.3	6.4	4.5	14.5	9.7	5.9	91.1	87.8
Clobazam	blood	0.9976	0.18	0.50	15.3	8.3	2.5	10.9	5.8	4.0	16.0	3.5	6.0	91.0	87.5
	urine	0.9969	0.15	0.45	14.8	6.9	2.3	11.6	9.4	2.6	15.8	11.1	4.7	95.0	89.9
	oral fluid	0.9951	0.15	0.45	16.1	7.8	2.7	10.1	7.7	3.5	15.6	8.6	5.1	88.3	79.8
Phenazepam	blood	0.9970	0.18	0.50	15.9	5.6	2.7	11.0	5.1	4.7	16.0	3.2	6.7	89.1	80.5
	urine	0.9983	0.15	0.45	14.9	8.3	2.4	11.0	8.1	3.9	15.1	9.1	5.6	90.0	93.0
	oral fluid	0.9969	0.15	0.45	15.8	7.9	3.0	11.1	6.9	3.4	15.7	6.9	5.0	90.1	90.5
Methadone	blood	0.9979	0.10	0.40	14.9	6.9	1.9	11.1	5.6	4.1	16.2	3.0	6.1	94.0	90.9
	urine	0.9953	0.07	0.20	13.8	7.9	2.8	12.0	8.9	2.1	16.6	9.9	5.3	95.2	89.8
	oral fluid	0.9973	0.10	0.40	14.5	6.6	3.4	10.5	8.9	5.5	16.2	7.5	6.0	89.1	91.0
Fentanyl	blood	0.9961	0.03	0.10	16.5	4.6	2.2	11.1	5.4	4.1	16.6	2.8	5.8	92.5	84.0
	urine	0.9987	0.03	0.08	12.9	8.7	3.9	12.0	8.4	2.4	15.7	10.9	4.4	95.2	92.5
	oral fluid	0.9949	0.03	0.10	15.0	5.9	4.8	11.2	7.9	3.5	16.1	7.7	4.6	91.6	85.9
Sufentanyl	blood	0.9995	0.03	0.08	17.0	4.8	2.5	10.4	5.3	4.3	16.0	2.7	5.1	92.8	88.6
	urine	0.9990	0.02	0.06	13.8	6.1	4.3	12.4	6.8	3.3	16.2	10.0	5.1	96.0	91.4
	oral fluid	0.9963	0.03	0.08	14.7	7.9	4.8	10.8	5.3	2.3	14.1	7.9	6.1	91.3	90.9
Buprenorphine	blood	0.9961	0.07	0.20	15.4	4.9	2.1	11.0	5.5	4.0	15.4	3.1	5.7	88.8	80.9
	urine	0.9985	0.03	0.10	14.8	7.5	2.8	12.0	8.3	3.4	16.0	9.2	4.6	90.0	81.4
	oral fluid	0.9978	0.06	0.18	15.5	6.8	2.5	10.3	7.5	3.9	15.4	7.6	5.6	90.1	83.6
Δ9-THC	blood	0.9991	0.35	1.00	14.7	5.7	2.1	10.2	8.5	4.9	15.6	2.4	6.6	90.1	83.9

	urine	0.9986	0.18	0.50	12.7	5.9	1.9	11.2	8.9	2.5	15.9	9.2	4.9	92.3	91.0
	oral fluid	0.9969	0.25	0.80	13.8	5.9	2.7	9.9	9.7	6.5	15.0	6.5	4.9	90.4	80.3
synthetic cannabinoids															
PX-1	blood	0.9948	0.20	0.60	12.8	10.2	3.9	15.8	7.1	6.9	15.3	12.1	6.9	87.1	89.7
	urine	0.9958	0.10	0.30	13.7	9.2	5.3	12.7	6.5	4.9	15.6	12.4	6.9	89.1	92.9
	oral fluid	0.9972	0.18	0.50	12.8	10.4	3.8	14.1	8.4	6.5	15.3	12.9	7.3	77.1	92.3
AB-FUBINACA	blood	0.9982	0.20	0.60	13.9	10.2	3.7	15.0	5.3	6.1	15.2	12.1	6.3	89.7	91.8
	urine	0.9944	0.10	0.30	14.7	9.6	4.5	12.7	4.9	5.6	15.1	12.5	6.5	92.1	92.9
	oral fluid	0.9965	0.18	0.50	12.8	8.9	2.2	18.1	7.2	6.4	12.2	12.8	5.9	88.2	92.9
5 Cl-AB-PINACA	blood	0.9959	0.20	0.60	12.5	11.0	3.6	15.3	5.8	4.5	13.0	12.5	6.9	88.1	89.7
	urine	0.9988	0.10	0.30	12.5	10.0	4.3	13.0	7.1	5.5	15.1	11.0	6.7	89.3	94.5
	oral fluid	0.9953	0.18	0.50	13.6	9.1	5.2	14.7	7.6	6.2	16.4	10.6	5.9	90.2	90.4
PX-2	blood	0.9963	0.20	0.60	11.8	10.2	4.1	14.8	6.7	6.3	15.1	12.1	6.9	91.2	91.5
	urine	0.9962	0.10	0.30	14.5	9.6	4.7	13.0	5.7	4.9	14.9	12.6	6.0	91.0	95.5
	oral fluid	0.9960	0.18	0.50	14.6	8.9	3.1	16.5	7.8	5.2	14.0	11.1	6.9	84.2	91.9
ADB-Fubinaca	blood	0.9974	0.20	0.60	13.8	9.9	3.2	15.1	5.9	6.0	14.8	11.7	6.5	89.1	90.2
	urine	0.9987	0.10	0.30	14.6	9.6	3.6	12.1	10.5	4.9	15.8	12.7	6.2	90.6	91.7
	oral fluid	0.9946	0.18	0.50	14.5	7.9	3.3	14.2	12.1	5.4	15.9	12.4	5.4	90.1	89.5
AB CHMINACA	blood	0.9969	0.20	0.60	13.6	10.5	4.1	15.2	13.0	6.1	14.9	12.0	6.7	91.0	90.1
	urine	0.9955	0.10	0.30	13.8	10.1	4.0	12.9	11.5	5.8	15.0	12.8	6.2	92.0	94.9
	oral fluid	0.9986	0.18	0.50	12.4	10.3	4.3	14.4	12.3	6.4	16.2	11.4	5.1	89.0	91.9
MMB 2201	blood	0.9969	0.20	0.60	13.7	11.0	4.0	14.5	12.1	6.0	15.1	12.5	6.7	89.1	94.0
	urine	0.9988	0.10	0.30	13.4	9.3	5.0	11.8	12.3	5.4	15.3	12.2	3.9	93.1	96.1
	oral fluid	0.9955	0.18	0.50	14.6	9.5	6.3	16.2	12.4	5.1	16.4	14.0	4,1	90.1	89.4
Pravadoline	blood	0.9968	0.20	0.60	13.7	10.3	4.1	15.1	13.6	6.1	15.3	13.1	2.4	91.0	91.7

	urine	0.9975	0.10	0.30	14.6	9.9	4.4	12.6	11.9	4.4	15.5	12.3	4.0	93.2	94.7
	oral fluid	0.9967	0.18	0.50	13.6	9.9	4.3	14.2	12.9	5.6	15.9	13.3	2.8	92.3	93.8
APP-FUBINACA	blood	0.9978	0.20	0.60	13.8	10.0	4.5	15.3	13.6	6.2	15.3	12.1	4.0	89.1	88.8
	urine	0.9988	0.10	0.30	14.5	9.3	4.2	13.7	12.7	4.1	15.6	13.6	2.9	92.2	94.3
	oral fluid	0.9968	0.18	0.50	14.6	10.0	4.2	16.2	12.5	6.2	15.3	13.3	4.0	90.0	88.7
5-F-ADB	blood	0.9975	0.20	0.60	12.9	10.0	4.0	15.0	12.4	6.7	14.9	12.9	3.0	90.2	90.7
	urine	0.9987	0.10	0.30	13.2	9.7	4.4	11.9	12.3	5.6	15.5	11.7	4.2	92.4	93.4
	oral fluid	0.9939	0.18	0.50	13.5	10.3	4.8	14.3	13.5	6.2	15.1	11.1	3.0	88.5	90.6
AM-2233	blood	0.9947	0.20	0.60	13.8	9.9	3.3	14.5	13.2	6.9	14.6	12.3	3.1	91.1	89.9
	urine	0.9965	0.10	0.30	14.0	10.1	4.2	13.2	12.1	4.0	15.3	11.4	2.2	91.1	92.7
	oral fluid	0.9977	0.18	0.50	13.9	10.2	4.2	14.2	13.4	5.5	15.7	13.5	4.6	91.0	91.7
Cumyl-5-F-PINACA	blood	0.9985	0.20	0.60	13.8	10.0	3.8	15.2	13.4	6.0	15.3	12.5	6.9	89.8	92.3
	urine	0.9969	0.10	0.30	14.4	11.0	4.3	12.6	12.7	5.2	14.4	11.1	6.9	92.2	94.4
	oral fluid	0.9979	0.18	0.50	14.3	10.2	3.2	11.9	13.9	4.1	14.3	12.4	5.8	86.3	90.6
AM-694	blood	0.9979	0.20	0.60	13.8	9.9	4.3	15.5	14.3	6.7	14.9	13.0	6.3	89.0	91.3
	urine	0.9988	0.10	0.30	14.1	9.6	4.4	13.0	12.0	4.9	14.9	11.1	7.6	92.4	92.5
	oral fluid	0.9955	0.18	0.50	14.1	11.0	5.8	12.4	12.3	5.9	14.6	12.0	6.2	90.0	89.7
JWH 302	blood	0.9964	0.20	0.60	12.9	9.2	4.0	15.2	11.7	6.2	15.9	12.6	7.1	91.2	88.4
	urine	0.9982	0.10	0.30	13.3	9.6	4.3	12.4	11.9	4.3	14.9	12.8	6.7	93.1	88.1
	oral fluid	0.9996	0.18	0.50	12.6	9.5	4.8	14.4	12.3	5.6	14.5	11.9	8.2	89.1	86.2
Cumyl-PEGACLONE	blood	0.9984	0.20	0.60	14.0	9.9	4.8	14.5	12.5	6.1	15.1	12.9	7.2	89.1	86.1
	urine	0.9948	0.10	0.30	14.3	8.8	4.4	12.3	12.1	4.9	16.3	12.7	6.9	91.2	87.9
	oral fluid	0.9968	0.18	0.50	13.9	9.9	5.7	14.8	11.1	6.1	15.3	12.8	6.2	91.0	92.0
RCS-4	blood	0.9926	0.20	0.60	13.6	10.1	4.1	15.1	13.8	6.1	14.8	11.9	7.6	89.5	89.5
	urine	0.9937	0.10	0.30	14.0	9.5	4.8	13.0	12.4	4.3	16.3	11.4	7.1	91.9	89.9

	oral fluid	0.9991	0.18	0.50	13.6	11.0	4.3	14.4	13.5	6.6	15.9	12.6	6.9	92.1	88.6
JWH 251	blood	0.9985	0.20	0.60	13.5	10.4	4.8	14.8	13.1	6.9	15.6	12.4	6.4	90.1	89.4
	urine	0.9949	0.10	0.30	13.8	10.0	5.4	12.4	12.7	4.3	16.1	12.9	6.6	93.0	94.0
	oral fluid	0.9965	0.18	0.50	14.2	11.0	6.9	15.6	13.8	6.5	14.4	12.9	7.1	89.1	86.1
AM-2201	blood	0.9987	0.20	0.60	13.8	9.8	3.8	15.3	13.9	6.1	15.1	12.4	6.3	89.1	86.2
	urine	0.9995	0.10	0.30	14.1	10.0	4.2	13.0	12.3	5.5	15.4	11.5	6.2	91.0	85.9
	oral fluid	0.9986	0.18	0.50	13.8	9.7	5.4	14.4	13.5	5.0	14.1	10.9	5.3	89.4	84.9
UR 144	blood	0.9976	0.20	0.60	13.6	10.2	5.0	15.4	14.1	6.9	14.9	12.8	6.9	91.1	88.1
	urine	0.9968	0.10	0.30	14.4	9.7	4.6	12.4	11.9	4.9	14.9	12.3	6.5	95.1	89.9
	oral fluid	0.9985	0.18	0.50	14.8	8.9	3.5	10.4	7.5	5.7	16.4	7.9	6.8	89.1	91.4
JWH 203	blood	0.9966	0.20	0.60	13.9	10.4	4.8	15.2	12.9	6.1	14.9	12.9	6.3	89.1	94.5
	urine	0.9946	0.10	0.30	13.8	11.0	5.5	13.1	12.4	4.9	15.3	12.4	6.7	92.3	91.1
	oral fluid	0.9989	0.18	0.50	13.2	10.3	5.8	14.1	12.8	5.9	12.8	13.0	5.9	90.1	85.6
5-F-NNEI-2	blood	0.997	0.20	0.60	13.5	7.7	4.1	15.2	13.3	6.9	15.5	13.9	6.9	90.4	91.7
	urine	0.9964	0.10	0.30	14.5	6.4	4.8	13.1	13.1	4.7	15.8	12.8	7.7	94.2	93.6
	oral fluid	0.9948	0.18	0.50	13.8	8.7	4.6	14.9	12.9	5.9	14.6	11.1	7.5	89.1	91.2
5-F-AKB48	blood	0.9983	0.20	0.60	13.2	5.8	4.5	15.7	13.2	6.6	15.5	12.1	6.3	89.9	89.9
	urine	0.9990	0.10	0.30	14.0	4.9	4.5	13.7	12.9	4.9	16.2	11.9	6.2	90.3	91.6
	oral fluid	0.9962	0.18	0.50	14.3	9.6	3.2	10.4	8.5	4.7	15.7	6.9	6.1	89.1	92.0
RCS-8	blood	0.9979	0.20	0.60	13.8	7.9	4.8	14.9	13.9	6.6	15.1	12.2	7.5	89.2	88.2
	urine	0.9983	0.10	0.30	14.0	4.9	5.5	13.0	11.7	4.8	16.5	11.1	7.5	92.1	88.5
	oral fluid	0.9955	0.18	0.50	13.3	7.8	5.2	14.3	12.9	5.5	15.7	11.9	6.1	91.2	87.1
JWH 018	blood	0.9964	0.20	0.60	13.5	6.9	4.8	15.3	14.5	6.7	15.9	11.9	7.4	87.1	85.9
	urine	0.9987	0.10	0.30	13.7	5.9	5.1	13.2	12.4	4.1	15.9	12.3	6.7	89.8	86.4
	oral fluid	0.9991	0.18	0.50	12.9	7.0	5.3	14.1	13.8	6.4	14.3	11.1	7.1	88.4	92.1

CP47, 497-C8	blood	0.9973	0.20	0.60	13.2	6.2	4.4	15.1	13.8	7.5	15.1	13.2	7.6	89.1	89.2
	urine	0.9937	0.10	0.30	14.6	10.1	4.4	13.0	12.1	4.5	15.7	12.5	6.7	90.9	94.2
	oral fluid	0.9932	0.18	0.50	15.1	12.0	5.4	15.1	14.0	5.1	14.9	12.9	7.8	90.1	89.8
JWH 016	blood	0.9982	0.20	0.60	13.3	10.4	4.2	15.2	13.9	7.1	16.2	12.3	6.8	90.1	89.0
	urine	0.9991	0.10	0.30	14.2	10.9	5.1	12.7	12.4	4.2	15.2	12.4	6.9	93.0	90.1
	oral fluid	0.9992	0.18	0.50	14.3	10.5	5.4	14.5	13.0	5.6	15.1	13.5	7.2	92.2	88.9
JWH 098	blood	0.9966	0.20	0.60	13.8	10.0	4.4	15.8	14.1	6.6	15.2	12.1	6.9	88.1	89.7
	urine	0.9976	0.10	0.30	13.7	10.5	4.8	12.9	12.4	5.6	16.3	12.8	6.9	92.0	91.1
	oral fluid	0.9989	0.18	0.50	15.8	9.9	5.6	13.9	12.8	7.4	15.1	12.9	7.3	90.2	90.4
THJ 018	blood	0.9991	0.20	0.60	13.4	10.2	4.7	15.5	14.1	6.1	15.9	12.4	7.5	89.1	89.9
	urine	0.9963	0.10	0.30	14.3	9.4	5.2	12.7	12.9	4.5	15.5	12.8	6.1	91.1	94.2
	oral fluid	0.9975	0.18	0.50	14.6	7.6	3.9	11.9	8.8	5.6	15.9	7.5	6.6	89.1	93.1
JWH 081	blood	0.9948	0.20	0.60	12.4	9.8	4.4	15.1	13.4	6.8	15.1	12.9	7.4	90.1	90.0
	urine	0.9943	0.10	0.30	13.3	8.9	4.1	11.9	12.5	4.9	14.6	12.4	6.9	91.0	91.2
	oral fluid	0.9982	0.18	0.50	14.5	9.8	4.8	14.4	12.8	5.1	15.5	12.3	6.2	90.1	90.2
JWH 122	blood	0.9981	0.20	0.60	13.4	10.4	4.1	15.0	14.2	6.7	15.4	13.4	6.3	89.1	88.4
	urine	0.9983	0.10	0.30	13.8	10.3	4.7	13.2	11.3	4.9	15.7	12.3	6.9	93.0	90.7
	oral fluid	0.9946	0.18	0.50	13.5	9.9	4.4	14.4	13.4	5.1	16.1	12.5	6.7	90.1	90.1
JWH 019	blood	0.9965	0.20	0.60	13.2	10.4	4.5	15.4	11.9	6.2	15.2	13.5	7.5	90.0	93.1
	urine	0.9985	0.10	0.30	14.0	10.1	4.4	13.4	11.6	4.7	16.5	12.4	6.9	93.1	95.9
	oral fluid	0.9968	0.18	0.50	13.8	11.0	4.2	15.0	13.0	5.2	15.8	12.2	6.8	92.1	92.1
JWH 007	blood	0.9945	0.20	0.60	13.4	10.6	4.7	15.7	13.1	7.1	15.1	13.9	7.3	89.1	91.9
	urine	0.9993	0.10	0.30	14.1	10.4	4.3	12.1	12.7	4.3	15.6	12.8	6.5	91.4	91.8
	oral fluid	0.9967	0.18	0.50	15.6	6.4	3.8	12.3	9.9	3.1	14.8	6.8	4.4	93.1	92.0
JWH 210	blood	0.9984	0.20	0.60	13.6	10.0	4.8	15.7	12.8	6.7	14.9	12.3	7.2	89.3	92.1

	urine	0.9976	0.10	0.30	14.0	9.8	4.4	12.1	12.5	4.2	15.8	11.2	6.5	91.0	95.5
	oral fluid	0.9945	0.18	0.50	14.4	11.3	5.4	14.9	14.5	6.0	13.9	10.1	6.4	89.4	90.4
JWH 147	blood	0.9952	0.20	0.60	13.6	9.3	4.8	15.1	13.2	6.4	15.1	13.3	7.4	88.1	91.2
	urine	0.9992	0.10	0.30	13.2	10.1	4.2	12.1	13.1	4.4	16.3	13.0	6.8	92.2	94.5
	oral fluid	0.9928	0.18	0.50	14.4	9.5	4.4	14.5	14.1	5.1	15.9	13.1	5.8	90.1	95.1
JWH 398	blood	0.9944	0.20	0.60	13.5	10.2	4.1	15.2	14.0	6.5	15.1	12.6	7.9	89.2	93.6
	urine	0.9961	0.10	0.30	14.1	10.2	4.6	12.5	12.1	4.9	15.2	13.1	6.9	90.1	97.8
	oral fluid	0.9946	0.18	0.50	13.7	10.9	4.5	14.2	13.4	5.2	15.1	13.2	6.7	92.1	90.5
CB-13	blood	0.9962	0.20	0.60	14.3	10.2	4.9	15.1	14.0	6.7	15.1	12.5	7.2	87.2	93.0
	urine	0.9980	0.10	0.30	14.5	10.9	5.1	12.3	13.1	4.5	16.1	12.5	6.3	90.1	95.1
	oral fluid	0.9945	0.18	0.50	14.4	9.8	4.8	12.5	12.6	6.1	15.8	12.8	6.7	91.1	93.5
fentanyl analogues															
Methoxyacetyl norfentanyl	blood	0.9989	0.03	0.08	11.9	12.0	2.6	11.5	9.9	5.7	12.1	11.0	9.2	90.2	81.5
	urine	0.9990	0.02	0.06	12.4	12.2	2.2	12.8	10.7	11.6	14.1	7.0	9.1	90.4	80.1
	oral fluid	0.9984	0.03	0.07	14.9	5.4	3.9	12.2	8.1	3.1	16.2	10.2	4.6	91.6	97.2
Acetyl norfentanyl	blood	0.9961	0.03	0.08	13.1	8.7	2.1	12.5	7.3	6.9	12.0	5.5	9.2	91.8	91.6
	urine	0.9941	0.02	0.06	13.2	9.0	2.4	11.3	6.5	6.3	12.5	10.5	9.3	95.3	92.2
	oral fluid	0.9965	0.03	0.07	14.5	11.9	3.1	16.7	11.6	6.3	16.3	12.3	6.7	90.2	88.1
Butyryl fentanyl carboxy metabolite	blood	0.9967	0.03	0.08	12.6	12.5	7.1	12.5	9.9	10.3	12.5	10.1	9.2	90.1	86.0
	urine	0.9988	0.02	0.06	11.9	10.8	8.5	11.9	12.3	11.6	11.9	7.2	8.1	91.2	87.3
	oral fluid	0.9982	0.03	0.07	15.7	6.8	3.2	10.2	9.2	3.5	16.2	7.6	6.2	90.0	95.7
Valeryl fentanyl carboxy metabolite	blood	0.9975	0.03	0.08	12.5	9.8	10.1	12.8	8.0	8.3	11.8	9.5	10.5	90.1	93.1
	urine	0.9986	0.02	0.06	13.5	11.2	8.2	11.5	11.0	9.7	11.9	8.1	7.4	97.2	91.0
	oral fluid	0.9969	0.03	0.07	13.8	11.0	4.8	14.6	13.1	5.2	15.3	12.8	6.5	92.6	91.0
Methoxyacetyl fentanyl	blood	0.9982	0.03	0.08	11.8	12.1	7.7	11.7	9.1	6.1	14.6	11.0	9.4	89.0	84.1

	urine	0.9976	0.02	0.06	11.9	12.5	7.4	11.7	10.2	5.3	12.3	10.2	9.1	90.7	80.2
	oral fluid	0.9990	0.03	0.07	13.3	6.6	5.2	10.7	6.9	4.8	15.5	6.1	5.8	91.6	95.0
Furanyl norfentanyl	blood	0.9937	0.03	0.08	12.5	8.8	5.5	13.8	11.9	3.8	12.5	11.9	9.5	90.3	94.0
	urine	0.9962	0.02	0.06	12.0	9.2	6.3	12.6	11.1	8.5	13.9	14.0	9.7	90.9	93.1
	oral fluid	0.9986	0.03	0.07	13.4	10.5	5.5	14.9	13.2	6.9	15.9	11.4	6.8	89.8	89.2
Cis-3-methyl norfentanyl	blood	0.9981	0.03	0.08	11.3	8.2	8.1	11.1	7.2	7.8	12.9	5.1	10.5	90.1	86.0
	urine	0.9942	0.02	0.06	10.8	8.2	7.3	11.6	9.6	9.5	11.5	8.2	7.6	90.1	89.3
	oral fluid	0.9932	0.03	0.07	14.6	5.5	3.8	12.4	8.1	3.3	15.9	10.1	4.6	91.6	87.5
Trans-3-methyl norfentanyl	blood	0.9963	0.03	0.08	11.3	8.3	7.1	11.1	11.8	10.6	9.9	8.5	7.1	90.0	83.0
	urine	0.9975	0.02	0.06	10.1	8.2	5.7	11.1	10.7	11.6	14.2	7.2	9.2	91.0	90.4
	oral fluid	0.9976	0.03	0.07	15.8	6.7	3.8	12.5	7.5	3.9	14.8	6.9	4.6	93.5	92.0
Butyryl norfentanyl	blood	0.9948	0.03	0.08	12.6	5.9	3.3	10.6	8.1	9.4	10.6	10.9	9.1	90.5	92.6
	urine	0.9966	0.02	0.06	10.1	8.7	4.9	10.8	12.3	8.9	12.2	10.9	8.3	91.5	88.9
	oral fluid	0.9980	0.03	0.07	13.1	10.6	5.3	14.6	14.1	6.8	15.7	11.5	6.9	89.9	89.5
Cyclopropyl norfentanyl	blood	0.9968	0.03	0.08	10.1	5.8	6.9	10.1	12.0	8.9	11.3	10.1	6.9	90.0	94.3
	urine	0.9987	0.02	0.06	10.4	5.2	9.9	11.5	11.9	7.8	12.9	9.5	8.6	90.7	95.9
	oral fluid	0.9982	0.03	0.07	14.7	11.9	4.8	16.7	13.3	6.4	15.9	12.7	6.9	90.5	87.7
β-hydroxyfentanyl	blood	0.9969	0.03	0.08	11.6	11.2	10.9	12.1	11.9	14.1	11.0	9.9	5.8	89.8	90.0
	urine	0.9982	0.02	0.06	10.6	7.9	9.2	12.5	8.3	11.5	10.9	8.9	7.6	90.8	88.5
	oral fluid	0.9980	0.03	0.07	15.9	6.9	4.5	12.3	6.8	3.9	14.9	6.7	4.9	93.8	92.2
Alfentanyl	blood	0.9962	0.03	0.08	10.7	7.9	8.4	11.5	7.7	10.9	10.4	11.9	10.1	90.0	87.0
	urine	0.9961	0.02	0.06	10.4	7.5	7.6	11.9	9.2	9.8	10.9	11.4	9.5	90.2	80.4
	oral fluid	0.9933	0.03	0.07	13.3	10.8	6.6	14.9	13.4	6.9	15.5	11.5	6.9	89.9	89.6
Cyclopropylfentanyl	blood	0.9945	0.03	0.08	13.8	9.7	9.0	11.1	8.3	8.3	12.1	10.3	9.8	90.1	93.5
	urine	0.9965	0.02	0.06	12.6	9.1	10.3	12.5	7.4	8.6	12.7	9.4	11.6	91.5	93.5

	oral fluid	0.9977	0.03	0.07	14.9	5.1	6.5	12.3	6.2	3.6	15.9	10.1	4.8	91.7	87.3
β-hydroxythiofentanyl	blood	0.9991	0.03	0.08	13.7	13.0	5.0	13.1	10.6	9.3	11.4	10.2	10.0	90.5	90.9
	urine	0.9946	0.02	0.06	11.4	12.2	7.0	13.4	11.2	7.1	11.7	5.2	5.7	90.3	85.5
	oral fluid	0.9979	0.03	0.07	17.0	6.3	3.2	12.1	6.2	5.1	14.5	8.3	5.6	91.1	93.2
Furanylethyl fentanyl	blood	0.9983	0.03	0.08	11.5	5.4	4.1	9.9	10.8	10.4	9.7	12.5	6.5	90.6	90.1
	urine	0.9991	0.02	0.06	12.3	5.9	6.8	12.5	10.2	11.5	10.6	8.3	7.3	90.5	89.9
	oral fluid	0.9969	0.03	0.07	15.7	6.9	3.3	10.5	7.9	3.5	16.2	7.4	6.1	90.2	95.9
Acetyl fentanyl	blood	0.9982	0.03	0.08	12.5	9.8	7.4	13.8	11.1	10.6	11.1	10.0	8.6	91.2	83.5
	urine	0.9992	0.02	0.06	10.4	11.4	9.6	10.2	8.9	10.6	12.9	9.3	7.5	90.5	86.5
	oral fluid	0.9973	0.03	0.07	13.9	11.5	4.8	14.9	13.1	5.1	15.6	12.2	6.5	92.9	91.2
Furanyl fentanyl	blood	0.9988	0.03	0.08	10.9	8.2	7.2	13.0	10.1	10.1	11.3	9.7	11.0	90.1	77.0
	urine	0.9977	0.02	0.06	10.9	8.7	8.2	11.6	10.7	9.8	12.9	10.1	10.4	91.5	74.9
	oral fluid	0.9982	0.03	0.07	13.4	10.7	5.7	14.9	13.6	6.9	15.6	11.7	6.9	89.9	89.2
Carfentanyl	blood	0.9969	0.03	0.08	13.2	8.6	5.5	11.0	12.0	10.1	11.9	10.2	9.9	90.0	90.9
	urine	0.9978	0.02	0.06	10.7	11.2	8.7	11.8	10.8	7.9	12.3	10.3	9.9	90.5	90.4
	oral fluid	0.9984	0.03	0.07	15.7	5.5	3.4	13.8	8.9	4.9	15.9	7.9	5.8	90.8	88.3
Butyryl fentanyl	blood	0.9986	0.03	0.08	12.6	5.9	3.2	10.1	9.8	9.1	10.1	9.9	10.0	90.6	91.7
	urine	0.9953	0.02	0.06	10.9	7.9	10.4	11.9	9.5	8.9	11.4	11.3	13.6	90.5	90.5
	oral fluid	0.9967	0.03	0.07	16.3	6.3	3.3	12.0	7.5	5.0	14.4	8.5	5.6	90.6	93.0
Despropionyl-para-fluoro fentanyl	blood	0.9984	0.03	0.08	11.6	8.6	6.1	11.8	5.1	6.0	13.5	8.0	7.0	91.1	84.9
	urine	0.9975	0.02	0.06	11.6	6.9	7.6	10.4	4.7	6.1	12.1	3.2	4.5	91.5	90.6
	oral fluid	0.9965	0.03	0.07	15.2	8.2	4.1	11.2	7.0	3.9	15.5	9.7	5.6	89.6	93.3
4-ANPP	blood	0.9978	0.03	0.08	10.7	7.9	7.1	11.1	10.8	9.1	12.4	9.7	9.9	91.0	75.1
	urine	0.9981	0.02	0.06	10.8	7.9	5.0	11.9	9.7	11.5	10.4	7.6	4.9	90.6	91.8
	oral fluid	0.9945	0.03	0.07	13.9	10.4	4.6	14.8	13.2	5.1	15.4	12.4	6.6	92.5	91.0

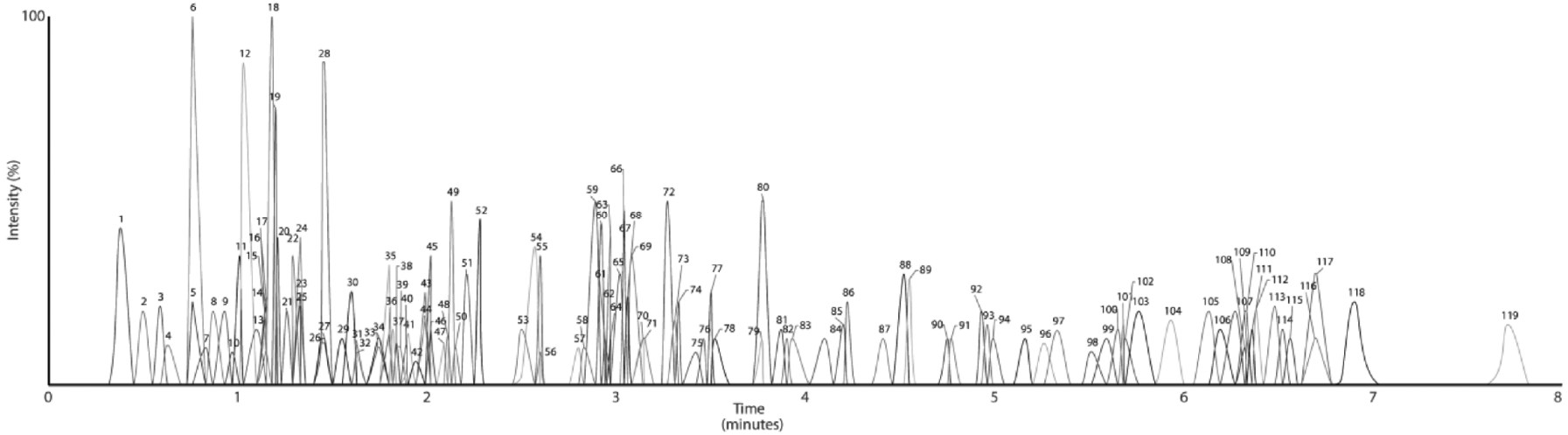
Phenyl acetyl Fentanyl	blood	0.9965	0.03	0.08	10.5	7.3	6.1	11.4	10.6	7.1	11.1	9.9	7.1	90.0	91.9
	urine	0.9980	0.02	0.06	10.5	8.1	9.1	12.9	7.2	11.9	10.8	8.5	9.4	90.8	91.8
	oral fluid	0.9991	0.03	0.07	13.4	9.9	5.4	14.9	12.3	6.9	15.6	11.3	6.8	89.9	89.2
synthetic cathinones															
4-fluoromethcathinone	blood	0.9923	0.20	0.60	12.4	6.5	5.1	9.3	5.0	3.1	17.5	4.2	3.1	88.2	73.6
	urine	0.9947	0.07	0.20	13.2	8.0	6.1	12.1	10.2	5.0	16.9	10.1	5.0	91.0	76.9
	oral fluid	0.996	0.15	0.45	17.0	6.5	4.9	12.0	8.8	5.7	12.9	8.8	7.5	90.0	91.0
Methcathinone	blood	0.9976	0.20	0.60	12.5	8.5	5.1	9.4	7.6	3.3	17.5	4.5	2.9	89.6	80.4
	urine	0.9982	0.07	0.20	12.9	7.8	6.6	11.8	12.2	7.1	15.8	6.6	4.9	90.3	86.9
	oral fluid	0.9948	0.15	0.45	15.6	8.8	5.0	14.6	12.9	6.9	15.4	9.6	6.8	91.1	89.0
Methylone	blood	0.9956	0.20	0.60	12.1	6.5	4.6	9.4	5.3	3.0	17.3	4.2	2.8	88.7	84.5
	urine	0.9972	0.07	0.20	12.5	8.1	3.7	13.4	9.0	7.1	12.9	8.0	2.3	91.0	87.4
	oral fluid	0.9948	0.15	0.45	14.4	7.5	6.6	14.3	12.8	8.8	13.6	11.9	6.1	90.0	90.4
Mephedrone	blood	0.9985	0.20	0.60	11.7	6.9	6.2	9.5	5.5	2.8	17.6	4.5	2.9	90.0	91.2
	urine	0.9976	0.07	0.20	12.3	8.7	4.9	12.9	13.9	7.6	15.9	6.2	4.5	90.0	95.0
	oral fluid	0.9963	0.15	0.45	15.8	6.9	4.1	12.3	10.1	6.9	15.1	9.5	3.2	90.1	92.9
Buphedrone	blood	0.9980	0.20	0.60	11.8	7.4	4.7	9.5	4.7	2.9	17.6	4.8	2.9	90.2	87.2
	urine	0.9987	0.07	0.20	12.5	7.8	3.0	11.2	11.2	7.7	18.1	5.0	7.6	91.0	94.0
	oral fluid	0.9978	0.15	0.45	13.2	6.3	4.0	10.3	6.9	4.0	17.4	12.9	7.8	90.0	98.6
Ethylone	blood	0.9985	0.20	0.60	12.7	6.2	5.7	8.8	5.3	2.9	17.7	4.6	3.2	89.9	90.2
	urine	0.9990	0.07	0.20	13.4	7.8	4.0	13.9	9.9	9.0	13.9	6.7	4.4	91.0	95.0
	oral fluid	0.9986	0.15	0.45	15.5	7.5	3.1	10.3	7.9	5.8	18.4	8.3	4.7	89.0	95.3
Buthylone	blood	0.9971	0.20	0.60	12.6	8.0	4.2	8.9	4.5	3.2	17.6	4.4	3.3	90.0	89.6
	urine	0.9980	0.07	0.20	13.1	7.8	6.0	15.0	14.3	8.9	15.8	6.9	2.9	91.0	90.0
	oral fluid	0.9962	0.15	0.45	13.0	10.2	5.5	11.8	11.6	8.5	14.4	10.6	7.8	89.1	89.0

Dimethylcathinone	blood	0.9995	0.20	0.60	11.9	6.6	5.0	8.9	5.5	3.2	17.4	4.0	3.2	89.8	78.1
	urine	0.9977	0.07	0.20	12.7	9.9	7.3	12.8	11.3	8.0	16.9	6.9	6.9	92.1	77.6
	oral fluid	0.9988	0.15	0.45	15.9	5.1	5.9	12.6	9.8	6.9	18.1	6.6	3.8	91.1	81.3
Diethylcathinone	blood	0.9980	0.20	0.60	11.9	6.8	5.1	9.2	5.6	3.1	17.7	4.2	2.8	90.8	81.6
	urine	0.9973	0.07	0.20	12.9	8.7	6.1	11.8	10.7	9.3	14.9	3.9	4.8	92.1	83.9
	oral fluid	0.9966	0.15	0.45	14.5	9.5	5.8	12.8	13.8	6.8	16.5	12.9	6.1	90.1	88.4
3,4-dimethylmethcathinone	blood	0.9976	0.20	0.60	12.0	6.5	5.2	9.1	5.8	2.9	17.5	4.2	2.9	90.4	90.3
	urine	0.9984	0.07	0.20	13.7	8.0	6.1	14.0	9.1	10.1	14.2	5.0	3.9	92.0	92.9
	oral fluid	0.9961	0.15	0.45	13.8	11.0	4.5	14.9	10.5	7.0	11.5	11.7	8.5	91.4	89.5
4-methyl ethcathinone	blood	0.9963	0.20	0.60	12.2	6.5	5.7	8.9	6.3	2.5	17.6	4.2	2.9	90.6	75.6
	urine	0.9969	0.07	0.20	13.3	8.2	3.1	13.8	9.0	8.6	14.6	5.4	3.3	90.0	77.2
	oral fluid	0.9960	0.15	0.45	15.4	5.7	5.1	12.6	9.9	3.8	14.9	7.6	6.7	90.1	85.0
Ethcathinone	blood	0.9976	0.20	0.60	12.4	6.6	5.3	9.0	6.3	3.3	17.5	4.3	3.3	89.8	87.6
	urine	0.9980	0.07	0.20	13.3	8.3	6.2	12.1	10.2	7.1	18.1	7.9	6.1	92.1	88.0
	oral fluid	0.9976	0.15	0.45	14.0	7.6	5.7	10.1	10.0	7.1	15.5	7.1	8.4	89.1	91.0
Penthedrone	blood	0.9989	0.20	0.60	11.7	6.3	5.0	9.0	5.8	3.0	16.4	4.2	2.7	91.3	91.3
	urine	0.9973	0.07	0.20	12.2	7.3	6.1	13.1	10.4	7.5	18.3	8.8	3.6	93.0	96.0
	oral fluid	0.9993	0.15	0.45	13.5	6.9	3.2	11.5	7.7	5.8	14.7	5.1	5.7	91.0	96.0
Penthylone	blood	0.9978	0.20	0.60	12.4	6.6	4.9	9.4	5.8	2.8	17.6	4.4	2.9	90.9	86.0
	urine	0.9985	0.07	0.20	13.8	6.9	5.5	12.9	9.6	9.2	15.8	5.4	2.9	94.0	84.8
	oral fluid	0.9950	0.15	0.45	15.4	7.8	7.3	11.3	7.9	3.9	17.1	10.7	4.9	89.0	90.2
MDPV	blood	0.9977	0.20	0.60	11.9	6.4	5.0	9.0	6.9	3.3	17.3	4.1	2.9	91.9	90.8
	urine	0.9983	0.07	0.20	12.6	7.8	5.8	12.1	10.7	9.0	12.7	8.1	4.4	91.4	89.0
	oral fluid	0.9939	0.15	0.45	15.9	5.9	6.6	12.3	9.6	6.9	15.0	11.6	4.1	91.0	88.1
Naphyrone	blood	0.9935	0.20	0.60	12.3	6.1	5.1	9.1	5.2	3.3	17.4	4.1	3.2	90.5	85.9

	urine	0.9942	0.07	0.20	13.8	7.9	5.3	12.0	10.5	9.2	14.8	5.7	6.0	90.0	89.1
	oral fluid	0.9965	0.15	0.45	15.9	5.6	3.9	13.0	8.1	6.8	17.1	10.8	4.8	90.0	85.9
Tryptamine															
AcO-DMT	blood	0.9978	0.18	0.50	14.5	14.3	5.8	12.6	12.9	8.7	12.5	12.8	4.8	90.0	81.6
	urine	0.9982	0.07	0.20	13.6	13.0	6.9	12.0	11.0	10.1	17.1	11.1	3.4	90.1	85.9
	oral fluid	0.9958	0.15	0.40	15.6	9.7	5.0	11.9	10.5	2.9	16.4	10.7	3.7	89.1	94.9
5-MeO-AMT	blood	0.9964	0.18	0.50	14.7	15.0	3.9	12.1	14.2	8.6	14.9	9.8	4.9	89.0	88.4
	urine	0.9976	0.07	0.20	13.1	12.2	5.6	13.2	13.7	9.3	14.9	11.0	4.9	91.0	91.8
	oral fluid	0.9966	0.15	0.40	13.4	7.9	5.3	14.3	9.8	3.6	16.0	6.8	2.0	91.0	96.0
4-OH-DET	blood	0.9982	0.18	0.50	13.9	15.0	6.5	13.6	11.8	9.8	13.1	13.1	2.9	90.0	78.9
	urine	0.9979	0.07	0.20	12.6	12.3	7.0	12.0	12.8	8.1	12.9	9.9	2.9	91.0	79.0
	oral fluid	0.9995	0.15	0.40	17.4	6.9	3.0	12.1	9.9	6.9	10.3	10.3	4.1	89.0	91.0
5-MeO-MIPT	blood	0.9948	0.18	0.50	13.9	14.1	6.9	14.1	12.4	6.0	12.6	12.1	2.1	90.0	88.0
	urine	0.9946	0.07	0.20	12.9	12.2	5.2	12.9	12.9	6.8	15.2	12.8	3.5	92.0	89.0
	oral fluid	0.9963	0.15	0.40	13.3	7.8	6.1	11.3	6.9	4.7	13.4	6.5	3.9	91.1	94.7
4-AcO-DIPT	blood	0.9987	0.18	0.50	14.7	13.2	5.3	14.1	14.2	6.1	11.9	11.8	2.1	90.3	79.0
	urine	0.9948	0.07	0.20	13.3	13.7	6.0	12.1	11.2	6.9	14.1	11.7	4.8	91.0	74.0
	oral fluid	0.9958	0.15	0.40	15.3	5.9	4.1	13.1	9.9	7.9	17.9	7.9	4.8	90.3	90.1
5-MeO-DPT	blood	0.9969	0.18	0.50	13.7	12.0	6.3	12.7	11.3	3.7	12.5	14.8	1.8	90.1	83.6
	urine	0.9986	0.07	0.20	13.8	14.0	6.1	12.1	13.0	6.1	15.4	11.4	4.9	91.5	83.2
	oral fluid	0.9954	0.15	0.40	14.1	7.7	4.8	10.2	9.6	3.5	13.5	5.8	2.9	89.0	89.5
5-MeO-DALT	blood	0.9949	0.18	0.50	14.0	14.2	7.1	12.8	13.4	3.4	13.7	12.9	3.3	90.0	84.6
	urine	0.9977	0.07	0.20	13.8	12.3	7.9	11.8	14.1	6.2	15.2	13.1	4.9	90.1	81.7
	oral fluid	0.9975	0.15	0.40	15.7	7.5	5.7	12.1	12.1	5.4	18.1	6.8	2.7	90.6	88.9
Phenylethylamines															

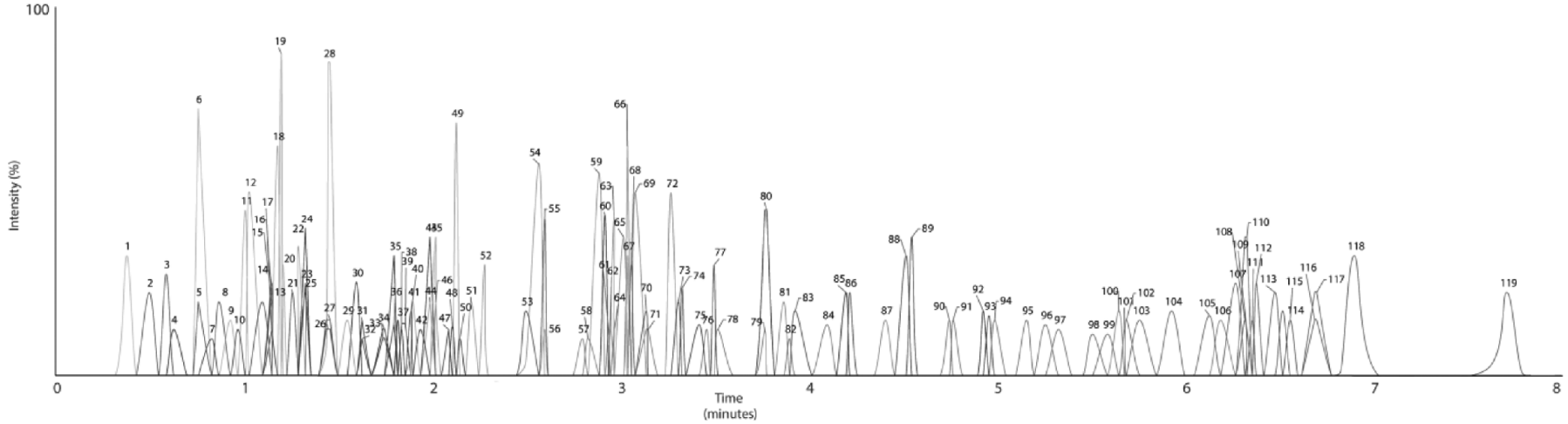
Ritalinic acid	blood	0.9968	0.15	0.40	16.0	7.8	2.9	9.0	12.1	4.0	17.9	6.3	5.7	90.5	85.0
	urine	0.9976	0.10	0.30	15.8	9.1	2.9	14.0	13.5	9.0	17.0	6.3	3.1	92.1	84.9
	oral fluid	0.9987	0.12	0.36	14.3	6.5	3.5	11.6	8.1	9.5	17.3	8.6	6.7	90.1	89.0
5-APB	blood	0.9959	0.15	0.40	16.0	7.9	2.9	10.3	6.0	5.3	16.6	6.3	4.9	91.2	90.0
	urine	0.9962	0.10	0.30	15.8	7.6	4.8	12.1	13.4	7.0	14.9	6.9	3.0	92.1	89.0
	oral fluid	0.9963	0.12	0.36	15.3	5.9	4.3	12.6	9.9	4.9	16.6	9.6	3.8	91.1	83.5
6-APB	blood	0.9974	0.15	0.40	16.0	7.8	3.4	9.9	5.9	3.3	17.9	7.6	5.0	89.0	95.4
	urine	0.9963	0.10	0.30	15.9	6.9	4.4	13.0	12.3	7.4	15.9	5.9	4.6	90.0	85.9
	oral fluid	0.9957	0.12	0.36	15.9	6.6	4.8	13.1	8.6	7.8	15.3	6.9	7.8	93.1	92.0
6-MAPB	blood	0.9988	0.15	0.40	16.6	7.8	2.5	9.3	4.0	2.4	17.7	4.6	5.3	90.0	85.6
	urine	0.9984	0.10	0.30	16.3	6.9	3.5	12.0	13.3	3.5	14.7	4.9	4.6	90.1	88.0
	oral fluid	0.9982	0.12	0.36	14.9	7.6	3.1	11.9	10.8	3.4	18.5	3.8	2.4	89.0	91.0
5-EAPB	blood	0.9976	0.15	0.40	16.0	7.8	2.9	10.1	8.5	3.8	18.6	5.2	2.9	91.0	88.0
	urine	0.9984	0.10	0.30	15.9	7.8	3.8	12.3	10.7	4.3	17.2	8.1	3.3	92.0	98.0
	oral fluid	0.9988	0.12	0.36	17.0	6.9	4.1	11.9	10.2	5.4	18.3	10.3	4.3	90.3	95.0
MPHP	blood	0.9976	0.15	0.40	16.0	7.7	3.5	10.1	7.3	4.9	16.5	7.3	3.9	90.0	90.2
	urine	0.9986	0.10	0.30	15.7	7.3	4.2	12.3	10.2	5.1	16.9	7.8	3.1	90.0	92.3
	oral fluid	0.9972	0.12	0.36	15.3	6.9	4.5	14.1	12.3	5.8	15.7	7.7	5.8	93.0	91.0

Figure 1. *HPLC–MS-MS chromatogram of whole blood spiked with all the target analytes at a concentration of 5 ng/mL.*



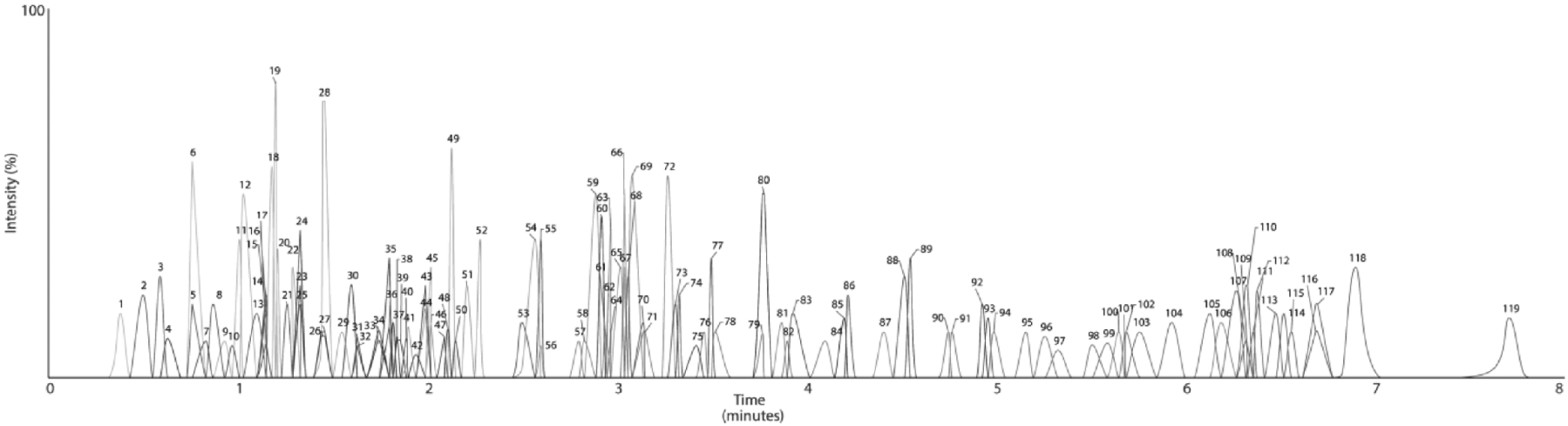
1: BZG; 2: Methoxyacetyl norfentanyl; 3: Acetyl Norfentanyl; 4: Ritalinic Acid; 5: Butyrylfentanyl Carboxy metabolite, 6: Morphine; 7: 4-fluoromethcathinone; 8: Valerylfentanyl Carboxy metabolite; 9: Norfentanyl; 10: Methcathinone; 11: Amphetamine;12: Dihydrocodeine; 13: Methoxyacetylfentanyl;14: Furanyl Norfentanyl; 15: Cis-3-Metyl Norfentanyl; 16: Trans-3-Metyl Norfentanyl; 17: Methylone; 18: Codeine; 19: MDA; 20:Methamphetamine; 21: Butyryl Norfentanyl; 22: MDMA; 23: 5-MeO-AMT; 24: AcO DMT; 25: Cyclopropyl Norfentanyl; 26: Mephedrone; 27: Buphedrone; 28: 6-MAM; 29: MDEA; 30: 4 OH DET; 31: Butylone; 32: Ethylone; 33: 5-APB; 34: 6-APB; 35: 5-MeO-MIPT; 36: Dimethylcathinone; 37: 6-MAPB; 38: Diethylcathinone; 39: 3,4-dimethylmethcathinone; 40: 4-methylethcathinone; 41: Norketamine; 42: Ethcathinone; 43: 4-AcO-DIPT; 44: Oxycodone; 45: Tramadol; 46: Norsufentanyl; 47: Penthedrone; 48: 5-EAPB; 49: Zolpidem; 50: Penthylone; 51: Lorazepam; 52: EDDP; 53: β-hydroxyfentanyl; 54: Cocaine; 55: Alfentanyl; 56: Ketamine; 57: PX-1; 58: AB FUBINACA; 59: Cocaethylene; 60: 5-MeO-DPT; 61: 5 CL AB PINACA; 62: Norbuprenorphine; 63: Nordiazepam; 64: PX-2; 65: Flunitrazepam; 66: Temazepam; 67: Clobazam; 68: Cyclopropylfentanyl; 69: Phenazepam; 70: β-hydroxythiofentanyl; 71: ADB Fubinaca; 72: Methadone; 73: Furanylethylfentanyl; 74: Acetylfentanyl; 75: MDPV; 76: AB CHMINACA; 77: Furanylfentanyl; 78: MMB 2201; 79: Pravadoline; 80: 5-MeO-DALT;81: Fentanyl; 82: APP FUBINACA; 83: Carfentanyl; 84: 5-F ADB; 85: MPHP; 86: Butyrylfentanyl; 87: Sufentanyl; 88: Despropionyl-para-fluorofentanyl; 89 4-ANPP; 90: AM-2233; 91: CUMYL 5F PINACA; 92: Naphyrone; 93: Phenylacetylfentanyl; 94: AM-694; 95: JWH 302; 96: CUMYL PEGACLONE; 97: RCS-4; 98: JWH 251; 99: AM-2201; 100: Buprenorphine; 101: UR 144; 102: JWH 203; 103: 5f NNEI-2; 104: 5F-AKB48; 105: RCS-8; 106: THC; 107: JWH 018; 108: CP47, 497-C8; 109: JWH 016; 110: JWH 098; 111: THJ 018; 112: JWH 081; 113: JWH 122; 114: JWH 019; 115: JWH 007; 116: JWH 210; 117: JWH 147; 118: JWH 398; 119: CB-13

Figure 2. *HPLC–MS-MS chromatogram of urine spiked with all the target analytes at a concentration of 50 ng/mL.*



1: BZG; 2: Methoxyacetyl norfentanyl; 3: Acetyl Norfentanyl; 4: Ritalinic Acid; 5: Butyrylfentanyl Carboxy metabolite, 6: Morphine; 7: 4-fluoromethcathinone; 8: Valerylfentanyl Carboxy metabolite; 9: Norfentanyl; 10: Methcathinone; 11: Amphetamine;12: Dihydrocodeine;13: Methoxyacetylfentanyl;14: Furanyl Norfentanyl; 15: Cis-3-Metyl Norfentanyl; 16: Trans-3-Metyl Norfentanyl; 17: Methylone; 18: Codeine; 19: MDA; 20: Methamphetamine; 21: Butyryl Norfentanyl; 22: MDMA; 23: 5-MeO-AMT; 24: AcO DMT; 25: Cyclopropyl Norfentanyl; 26: Mephedrone; 27: Buphedrone; 28: 6-MAM; 29: MDEA; 30: 4 OH DET; 31: Butylone; 32: Ethylone; 33: 5-APB; 34: 6-APB; 35: 5-MeO-MIPT; 36: Dimethylcathinone; 37: 6-MAPB; 38: Diethylcathinone; 39: 3,4-dimethylmethcathinone; 40: 4-methylethcathinone; 41: Norketamine; 42: Ethcathinone; 43: 4-AcO-DIPT; 44: Oxycodone; 45: Tramadol; 46: Norsufentanyl; 47: Penthedrone; 48: 5-EAPB; 49: Zolpidem; 50: Penthylone; 51: Lorazepam; 52: EDDP; 53: β-hydroxyfentanyl; 54: Cocaine; 55: Alfentanyl; 56: Ketamine; 57: PX-1; 58: AB FUBINACA; 59: Cocaethylene; 60: 5-MeO-DPT; 61: 5 CL AB PINACA; 62: Norbuprenorphine; 63: Nordiazepam; 64: PX-2; 65: Flunitrazepam; 66: Temazepam; 67: Clobazam; 68: Cyclopropylfentanyl; 69: Phenazepam; 70: β-hydroxythiofentanyl; 71: ADB Fubinaca; 72: Methadone; 73: Furanylethylfentanyl; 74: Acetylfentanyl; 75: MDPV; 76: AB CHMINACA; 77: Furanylfentanyl; 78: MMB 2201; 79: Pravadoline; 80: 5-MeO-DALT;81: Fentanyl; 82: APP FUBINACA; 83: Carfentanyl; 84: 5-F ADB; 85: MPHP; 86: Butyrylfentanyl; 87: Sufentanyl; 88: Despropionyl-para-fluorofentanyl; 89 4-ANPP; 90: AM-2233; 91: CUMYL 5F PINACA; 92: Naphyrone; 93: Phenylacetylfentanyl; 94: AM-694; 95: JWH 302; 96: CUMYL PEGACLONE; 97: RCS-4; 98: JWH 251; 99: AM-2201; 100: Buprenorphine; 101: UR 144; 102: JWH 203; 103: 5f NNEI-2; 104: 5F-AKB48; 105: RCS-8; 106: THC; 107: JWH 018; 108: CP47, 497-C8; 109: JWH 016; 110: JWH 098; 111: THJ 018; 112: JWH 081; 113: JWH 122; 114: JWH 019; 115: JWH 007; 116: JWH 210; 117: JWH 147; 118: JWH 398; 119: CB-13.

Figure 3. *HPLC–MS-MS chromatogram of oral fluid spiked with all the target analytes at a concentration of 5 ng/mL.*



1: BZG; 2: Methoxyacetyl norfentanyl; 3: Acetyl Norfentanyl; 4: Ritalinic Acid; 5: Butyrylfentanyl Carboxy metabolite, 6: Morphine; 7: 4-fluoromethcathinone; 8: Valerylfentanyl Carboxy metabolite; 9: Norfentanyl; 10: Methcathinone; 11: Amphetamine;12: Dihydrocodeine;13: Methoxyacetylfentanyl;14: Furanyl Norfentanyl; 15: Cis-3-Methyl Norfentanyl; 16: Trans-3-Methyl Norfentanyl; 17: Methylone; 18: Codeine; 19: MDA; 20: Methamphetamine; 21: Butyryl Norfentanyl; 22: MDMA; 23: 5-MeO-AMT; 24: AcO DMT; 25: Cyclopropyl Norfentanyl; 26: Mephedrone; 27: Buphedrone; 28: 6-MAM; 29: MDEA; 30: 4 OH DET; 31: Butylone; 32: Ethylone; 33: 5-APB; 34: 6-APB; 35: 5-MeO-MIPT; 36: Dimethylcathinone; 37: 6-MAPB; 38: Diethylcathinone; 39: 3,4-dimethylmethcathinone; 40: 4-methylethcathinone; 41: Norketamine; 42: Ethcathinone; 43: 4-AcO-DIPT; 44: Oxycodone; 45: Tramadol; 46: Norsufentanyl; 47: Penthedrone; 48: 5-EAPB; 49: Zolpidem; 50: Penthylone; 51: Lorazepam; 52: EDDP; 53: β-hydroxyfentanyl; 54: Cocaine; 55: Alfentanyl; 56: Ketamine; 57: PX-1; 58: AB FUBINACA; 59: Cocaethylene; 60: 5-MeO-DPT; 61: 5 CL AB PINACA; 62: Norbuprenorphine; 63: Nordiazepam; 64: PX-2; 65: Flunitrazepam; 66: Temazepam; 67: Clobazam; 68: Cyclopropylfentanyl; 69: Phenazepam; 70: β-hydroxythiofentanyl; 71: ADB Fubinaca; 72: Methadone; 73: Furanylethylfentanyl; 74: Acetylfentanyl; 75: MDPV; 76: AB CHMINACA; 77: Furanylfentanyl; 78: MMB 2201; 79: Pravadoline; 80: 5-MeO-DALT;81: Fentanyl; 82: APP FUBINACA; 83: Carfentanyl; 84: 5-F ADB; 85: MPHP; 86: Butyrylfentanyl; 87: Sufentanyl; 88: Despropionyl-para-fluorofentanyl; 89 4-ANPP; 90: AM-2233; 91: CUMYL 5F PINACA; 92: Naphyrone; 93: Phenylacetylfentanyl; 94: AM-694; 95: JWH 302; 96: CUMYL PEGACLONE; 97: RCS-4; 98: JWH 251; 99: AM-2201; 100: Buprenorphine; 101: UR 144; 102: JWH 203; 103: 5f NNEI-2; 104: 5F-AKB48; 105: RCS-8; 106: THC; 107: JWH 018; 108: CP47, 497-C8; 109: JWH 016; 110: JWH 098; 111: THJ 018; 112: JWH 081; 113: JWH 122; 114: JWH 019; 115: JWH 007; 116: JWH 210; 117: JWH 147; 118: JWH 398; 119: CB-13.

Table 5. *Concentration (ng/mL) of target analytes in real whole blood samples.*

	B002	B004	B005	B006	B007	B008	B010	B011	B012	B013	B014	B016	B017	B018	B019	B020	B022	B025
Cyclopropyl fentanyl	4.3	3.0	6.4	21	16	0.8	4.9	-	-	-	-	-	-	-	-	5.1	-	-
Cyclopropyl norfentanyl	5.0	54	9.9	42	46	22	36	-	-	-	-	-	-	-	-	21	-	-
Methoxyacetylfentanyl	-	-	-	-	-	-	3.5	-	-	26	23	91	-	-	-	-	-	-
Methoxyacetylnorfentanyl	-	-	-	-	-	-	1.1	-	-	4.4	4.1	6.7	-	-	-	-	-	-
Furanylfentanyl	-	-	-	-	-	-	-	3.6	-	-	-	-	-	-	-	-	-	-
Furanylnorfentanyl	-	-	-	-	-	-	-	0.9	-	-	-	-	-	-	-	-	-	-
Acetylfentanyl	-	-	-	-	-	-	-	-	-	-	-	-	87	16	20	-	-	-
Acetylnorfentanyl	-	-	-	-	-	-	-	-	-	-	-	-	70	26	40	-	-	-
4-ANPP	-	-	-	-	-	-	0.2	7.9	-	3.8	3.6	4.6	4.3	1.2	2.5	-	0.4	-
Sufentanyl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.7
Morphine	148	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Codeine	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxycodone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	241	-	-

4-ANPP, 4-anilino-N-phenethyl-piperidine

Table 6. *Concentration (ng/mL) of target analytes in real urine samples.*

	000	001	002	003	004	005	006	007	008	009	010	011	012	013	014	016	017	018	019	036	037	038	040
Cyclopropyl fentanyl	-	11	108	7.4	20	83	100	36	28	88	41	-	1.5	-	-	-	-	-	-	-	-	-	-
Cyclopropyl norfentanyl	-	120	240	350	380	130	910	400	830	530	720	-	16	-	-	-	-	-	-	-	-	-	-
Methoxyacetyl fentanyl	-	-	-	-	-	-	-	-	-	-	180	-	-	1900	1000	70	-	-	-	-	-	-	-
Methoxyacetyl norfentanyl	-	-	-	-	-	-	-	-	-	-	170	-	-	840	2000	4.2	-	-	-	-	-	-	-
Furanylfentanyl	-	-	-	-	-	-	-	-	-	-	-	85	-	-	-	-	-	-	-	-	-	-	-
Furanylnorfentanyl	-	-	-	-	-	-	-	-	-	-	-	22	-	-	-	-	-	-	-	-	-	-	-
Acetylfentanyl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2800	2900	62	3600	7.1	-	-
Acetyl norfentanyl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8900	8400	7800	7000	910	-	-
4-ANPP	-	-	-	-	-	-	-	-	-	-	5.6	130	-	39	27	1.6	120	68	9.4	110	-	0.2	-
Fentanyl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.7	0.9	-	-	-	710	12
Norfentanyl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11	12	-	-	-	1700	9.9
Ritalinic Acid	62	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nordiazepam	-	-	-	-	-	-	-	-	-	390	-	-	630	-	-	-	-	-	-	-	-	310	1000
Noroxycodone	-	-	-	-	-	-	-	-	-	-	-	-	12	-	-	-	-	-	-	-	-	-	-
Morphine	-	380	-	-	-	-	-	-	-	-	-	-	58	-	-	-	-	-	-	-	-	-	97
Codeine	150	130	-	-	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	45
6-MAM	-	120	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15

4-ANPP: 4-anilino-N-phenethyl-piperidine; 6-MAM: 6-O-Monoacetylmorphine

Table 7. *Concentration (ng/mL) of target analytes in real oral fluid samples.*

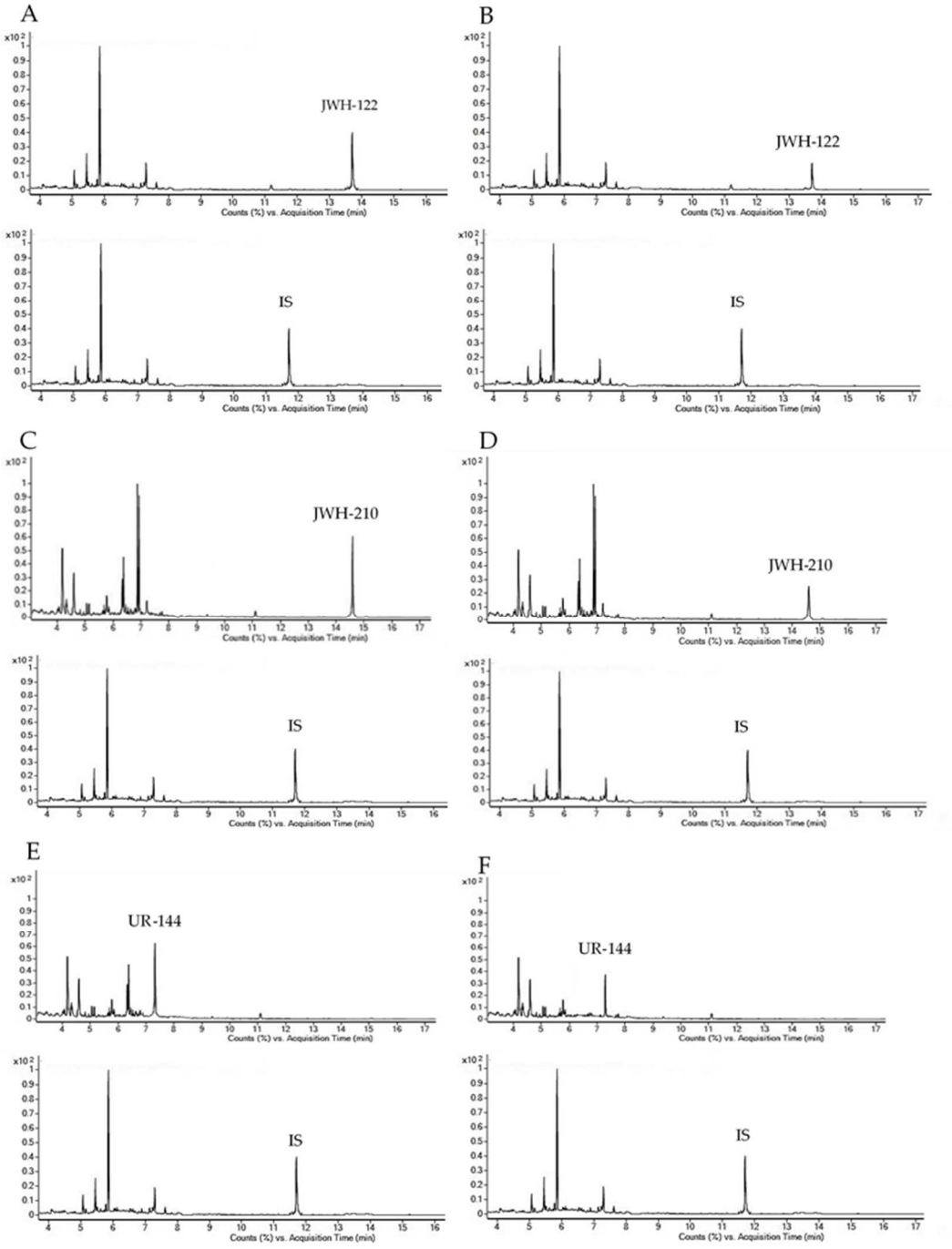
	OF000	OF001	OF002	OF003	OF004	OF005	OF006	OF007	OF008	OF009	OF010	OF011	OF012	OF013	OF014
Butylone	-	-	-	-	-	120	0.1	-	-	-	1.0	-	-	-	-
Carfentanyl	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AcO-DMT	-	-	-	-	0.6	-	-	-	120	-	-	-	-	-	-
BEG	-	-	-	-	-	-	-	-	-	17	-	0.3	-	-	-
Cocaine	-	0.2	-	-	-	-	0.4	-	-	110	-	1	-	-	-
Morphine	0.1	-	-	1.0	-	-	-	-	-	-	-	-	-	-	-
6-MAM	1.0	0.2	0.2	-	-	-	-	-	-	-	-	-	-	-	-
THJ 018	-	-	-	-	-	-	-	-	-	-	2.0	-	0.2	97	0.4

AcO DMT: Acetyl-o-dimethyltryptamine; BEG: Benzoylecgonine; 6-MAM: 6-O-Monoacetylmorphine

Table 8. *UHPLC-HRMS and GC-MS MS parameters and relative retention time (RRt) in targeted MS/MS and SIM mode, respectively.*

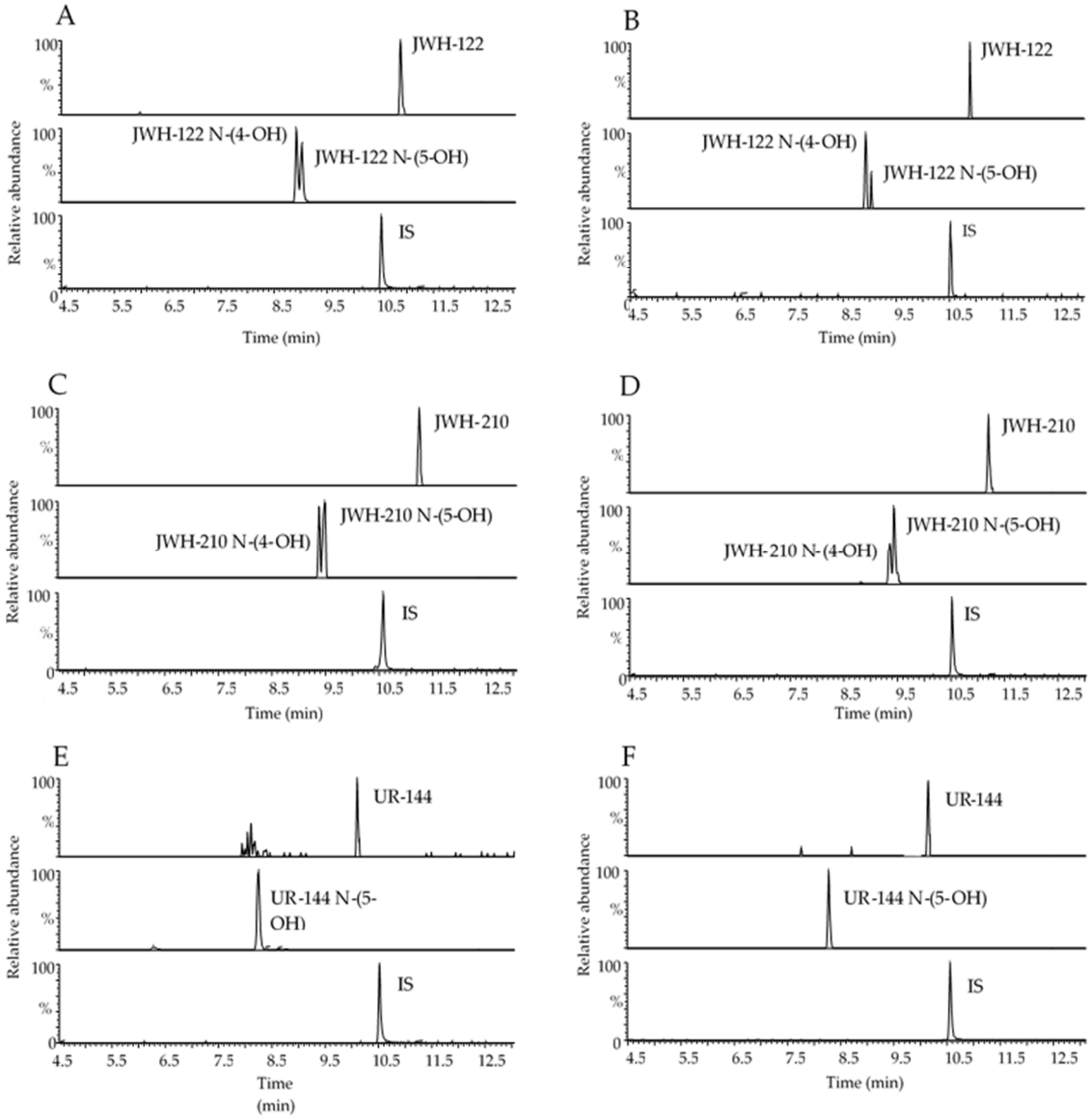
	UHPLC-HRMS				GC-MS		
Analytes	**Chemical Formula**	**RRt (min)**	**Quantifier (*m/z*) $[M + H]^+$**	**Qualifiers (*m/z*)**	**RT (min)**	**Quantifier (*m/z*)**	**Qualifiers (*m/z*)**
JWH-122	$C_{25}H_{25}NO$	1.03	356.2008	169.0646 214.1223	13.9	298	214 284
JWH-122 N-(4-OH)	$C_{25}H_{25}NO_2$	0.84	372.1949	141.0698 169.0649	-	-	-
JWH-122 N-(5-OH)	$C_{25}H_{25}NO_2$	0.85	372.1951	141.0698 169.0647	-	-	-
JWH-210	$C_{26}H_{27}NO$	1.06	370.2157	183.0803 214.1222	14.8	214	144 183
JWH-210 N-(4-OH)	$C_{26}H_{27}NO_2$	0.88	386.2105	144.0443 183.0806	-	-	-
JWH-210 N-(5-OH)	$C_{26}H_{27}NO_2$	0.89	386.2106	183.0803 230.1172	-	-	-
UR-144	$C_{21}H_{29}NO$	0.96	312.2325	125.0961 244.0963	7.1	214	144 296
UR-144 N-(5-OH)	$C_{21}H_{29}NO_2$	0.78	328.2271	125.0961 230.1172	-	-	-
JWH-018-d_{11}	$C_{24}H_{12}D_{11}NO$	1.00	353.2537	-	11.99	352	-

Figure 5. *Representative GC-MS single ion monitoring chromatograms of synthetic cannabinoids in oral fluid extract.*



Blank oral fluid fortified with 1.00 ng/mL JWH-122 (A), JWH-210 (C) and UR-144 (E). Oral fluid samples consumers containing 4.00 ng/mL JWH-122 (B); 8.10 ng/mL JWH-210 (D); 7.40 ng/mL UR-144 (F)

Figure 6. *Representative UHPLC-HRMS chromatograms of synthetic cannabinoids in oral fluid extract.*



A: OF extract spiked with 0.5 ng/mL JWH-122, JWH-122 N-(4-OH) and JWH-122 N-(5-OH);
C: OF extract spiked 0.5 ng/mL JWH-210, JWH-210 N-(4-OH) and JWH-210 N-(5-OH);
E: OF extract spiked 0.5 ng/mL UR-144 and UR-144 N-(5-OH);
Authentic OF samples containing 3.14 ng/mL JWH-122, 0.29 ng/mL JWH-122 N-(4-OH) and JWH-122 N-(5-OH) in traces under the LOQ (B); 7.30 ng/mL JWH-210, 0.29 ng/mL JWH-210 N-(4-OH) and 0.66 ng/mL JWH-210 N-(5-OH) (D); 6.81 ng/mL UR-144 (F).

Table 9. *GC-MS and UHPLC-HRMS calibration curve parameters, LODs, LOQs and recovery of SCs and their metabolites in OF.*

GC-MS						
Analytes	**Correlation Coefficient (R^2)[a]**	**LOD**	**LOQ**	**Mean Recovery (%)[b]**		
				QC samples		
				Low	**Medium**	**High**
JWH-122	0.997 ± 0.003	0.30	0.50	80.3	81.8	81.5
JWH-210	0.996 ± 0.001	0.70	1.00	83.7	83.9	84.4
UR-144	0.997 ± 0.003	0.70	2.30	71.5	78.0	71.1
UHPLC-HRMS						
Analytes	**Determination Coefficient (R^2)[a]**	**LOD**	**LOQ**	**Mean Recovery (%)[b]**		
				QC samples		
				Low	**Medium**	**High**
JWH-122	0.997 ± 0.003	0.07	0.25	84.4	93.5	98.6
JWH-122 N-(4-OH)	0.996 ± 0.001	0.03	0.10	97.4	84.5	97.8
JWH-122 N-(5-OH)	0.997 ± 0.003	0.03	0.10	85.3	87.6	88.8
JWH-210	0.997 ± 0.004	0.06	0.20	92.8	85.7	97.4
JWH-210 N-(4-OH)	0.996 ± 0.001	0.02	0.07	90.6	87.0	101.5
JWH-210 N-(5-OH)	0.996 ± 0.004	0.03	0.10	89.9	86.8	99.8
UR-144	0.991 ± 0.009	0.05	0.15	71.8	75.3	70.1
UR-144 N-(5-OH)	0.996 ± 0.003	0.03	0.10	97.9	102.1	101.2

[a] Mean of three replicates of calibration curves; [b] Mean of five replicates; LOD, limits of detection; LOQ, limits of quantification.

Table 10. *GC-MS and UHPLC-HRMS intra- and inter-day precision and accuracy values for synthetic cannabinoids and their metabolites.*

GC-MS									
Analytes	**Intra-day precision (CV%)**			**Inter-day precision (CV%)**			**Accuracy (% Error)**		
	QC samples[a]								
	Low	**Medium**	**High**	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
JWH-122	4.8	7.3	9.4	9.7	8.7	6.9	8.7	8.7	7.3
JWH-210	3.2	9.1	5.3	9.9	5.3	7.2	9.9	10.2	10.1
UR-144	5.7	9.2	8.3	10.1	12.3	11.2	9.1	8.5	9.9
UHPLC-HRMS									
Analytes	**Intra-day precision (CV%)**			**Inter-day precision (CV%)**			**Accuracy (% Error)**		
	QC samples[a]								
	Low	**Medium**	**High**	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
JWH-122	2.2	4.2	8.6	11.4	5.7	5.9	13.7	9.9	7.7
JWH-122 N-(4-OH)	4.1	6.9	3.1	10.1	8.3	7.4	10.6	9.6	3.7
JWH-122 N-(5-OH)	7.6	15.4	12.8	12.0	15.4	11.9	8.7	7.5	13.5
JWH-210	6.2	5.2	8.4	15.6	5.4	6.9	8.5	9.8	4.2
JWH-210 N-(4-OH)	4.8	7.1	6.2	12.6	7.1	5.2	11.4	8.7	10.3
JWH-210 N-(5-OH)	15.1	7.7	8.5	17.4	11.2	8.4	8.0	10.3	10.4
UR-144	7.6	15.4	12.8	12.0	15.4	11.9	8.7	7.5	10.8
UR-144 N-(5-OH)	4.1	6.9	3.8	10.1	8.0	7.0	10.6	9.6	3.7

[a] Mean of five replicates (n=15) along five subsequent working days

Figure 7. *Oral fluid concentration time-profiles of JWH-122 (A), JWH-210 (B), UR-144 (C) and their metabolites by GC-MS and UHPLC-HRMS.*

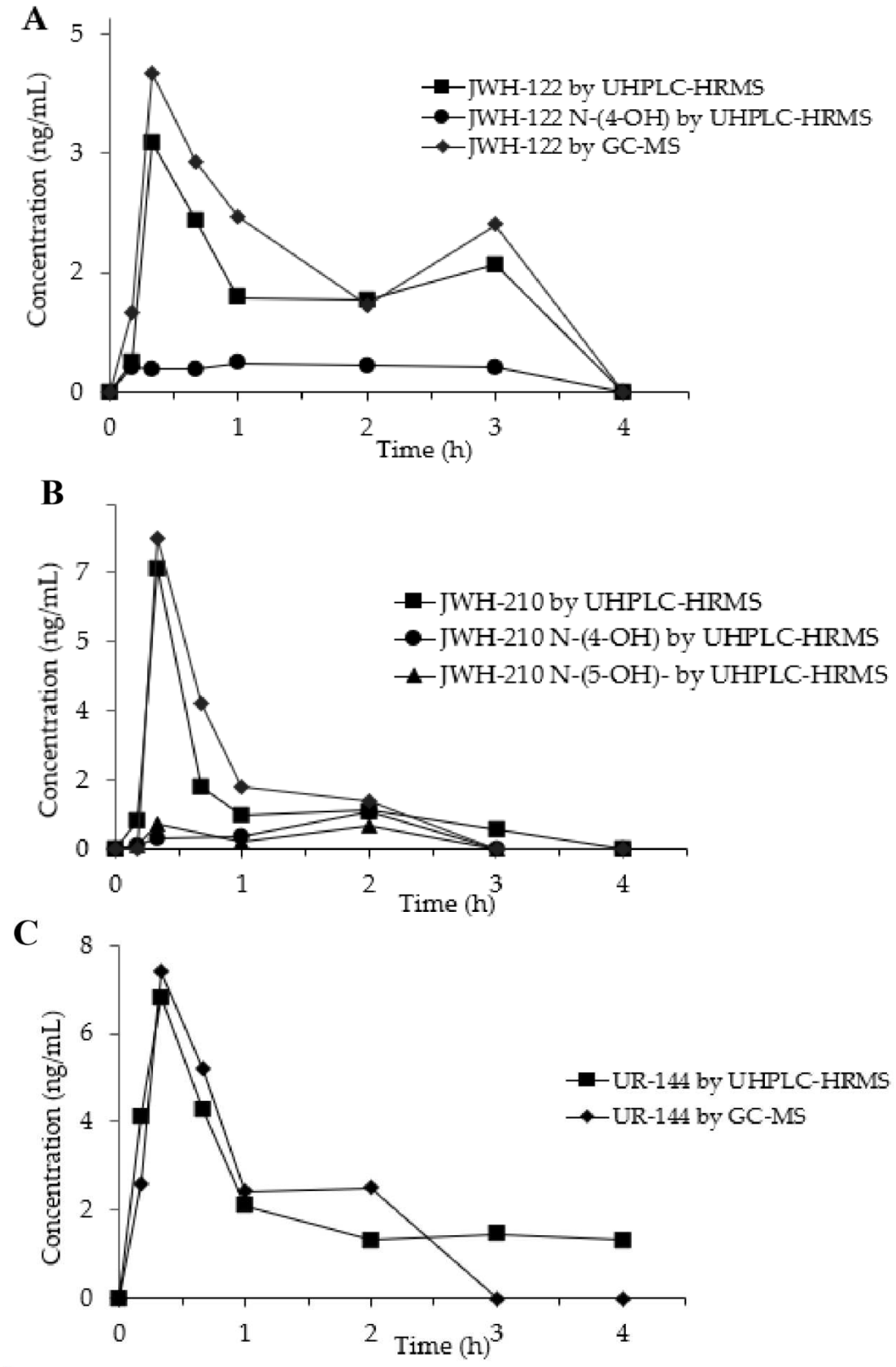


Table 11. *Molecular formula, exact mass of the protonated analyte, Δ m/z, retention time (RT) of synthetic cathinones under investigation.*

Analyte	Molecular Formula	*m/z* $[M-H]^+$	Δ *m/z* (ppm)	Retention time (min.)
1-Naphyrone	$C_{19}H_{23}NO$	282,1852	0.4	8.82
3,4-DMMC	$C_{12}H_{17}NO$	192,1387	-3.0	6.19
3-CMC	$C_{10}H_{12}NOCl$	198,0682	-3.0	4.58
3-fluoro-α-PVP	$C_{15}H_{20}FNO$	250,1603	-1.6	6.84
3-MMC	$C_{11}H_{15}NO$	178,1226	-0.6	4.64
4-BMC	$C_{10}H_{12}NOBr$	242,0177	-0.3	5.34
4-EEC	$C_{13}H_{19}NO$	206,1540	-0.8	6.74
4-fluoro-α-PHP	$C_{16}H_{22}FNO$	264,1757	-0.6	8.04
4-FMC	$C_{10}H_{12}FNO$	182,0978	-0.2	2.86
4-FMC metab	$C_{10}H_{14}FNO$	184,1133	-0.9	2.78
4-MEC	$C_{12}H_{17}NO$	192,1384	-1.7	5.40
4-MEC metab	$C_{12}H_{19}NO$	194,1545	-3.0	5.26
4-MMC	$C_{11}H_{15}NO$	178,1227	-1.2	4.61
Benzedrone	$C_{17}H_{19}NO$	254,1542	-3.00	8.37
Buphedrone	$C_{11}H_{15}NO$	178,1226	-0.6	4.24
Butylone	$C_{12}H_{15}NO_3$	222,1123	0.0	4.98
Dimethylcathinone	$C_{11}H_{15}NO$	178,1226	-0.4	3.25
Ethcathinone	$C_{11}H_{15}NO$	178,1221	0.6	3.48
Ethylone	$C_{12}H_{15}NO_3$	222,1127	-1.9	4.20
Euthylone	$C_{13}H_{17}NO_3$	236,1283	-1.7	5.53
MDPBP	$C_{15}H_{19}NO_3$	262,1437	-0.6	5.88
MDPHP	$C_{17}H_{23}NO_3$	290,1756	-3.0	8.06
MDPV	$C_{16}H_{21}NO_3$	276,1594	-0.5	6.94
Mephedrone d3	$C_{11}H_{12}D_3NO$	181,1420	-0.7	4.60
Methcathinone	$C_{10}H_{13}NO$	164,1069	0.3	2.77
Methedrone	$C_{11}H_{15}NO_2$	194,1175	-0.1	4.10
Methylone	$C_{11}H_{13}NO_3$	208,0968	-0.0	3.40
MPHP	$C_{17}H_{25}NO$	260,2012	-1.8	8.71
Naphyrone	$C_{19}H_{23}NO$	282,1852	-1.0	9.16
NEP	$C_{13}H_{19}NO$	206,1545	-3.0	6.33
N-Ethylpentylone	$C_{14}H_{19}NO_3$	250,1443	-3.1	6.68
Pentedrone	$C_{12}H_{17}NO$	192,1382	-0.4	5.94
Pentylone	$C_{13}H_{17}NO_3$	236,1281	-0.8	6.34
α-PHP	$C_{16}H_{23}NO$	246,1851	-1.9	7.86
α-PVP	$C_{15}H_{21}NO$	232,1698	-1.2	6.60

3,4-DMMC, 3,4-Dimethylmethcathinone; 3-CMC, chloromethcathinone; 3-fluoro-α-PVP, 3-fluoro-α-Pyrrolidinovalerophenone; 3-MMC, 3-Methylmetcathinone; 4-BMC, 4-bromomethcathinone; 4-EEC, 4-Ethylethcathinone; 4-fluoro-alpha-PHP, 4-fluoro-α-Pyrrolidinohexanophenone; 4-FMC, Flephedrone; 4-FMC metab, 4-fluoromethcathinone metabolite; 4-MEC, 4-Methylethcathinone; 4-MEC metab, 4-methylethcathinone metabolite, 4-MMC, Mephedrone; MDPBP, 3,4-Methylenedioxy-α-pyrrolidinobutiophenone; MDPHP, 3,4-Methylenedioxy-α-Pyrrolidinohexanophenone; MDPV; MPHP, 4-Methyl-α-pyrrolidinohexanophenone; NEP, α-Ethylaminopentiophenone); α-PHP, α-Pyrrolidinohexiophenone; α-PVP, α-Pyrrolidinovalerophenone

Table 12. *Regression equations, determination coefficients, LODs and LOIs.*

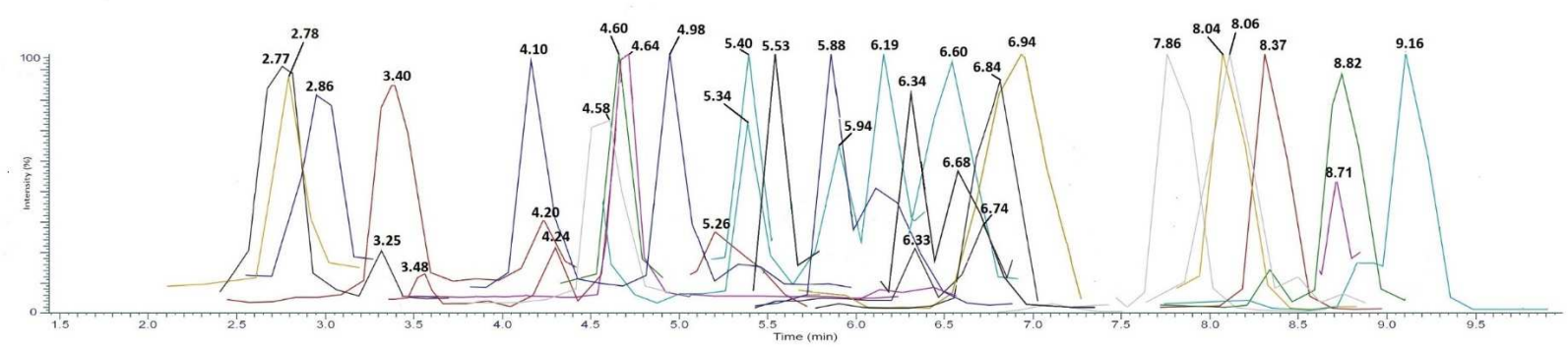
Analytes	Equation	R^2	LOD	LOI
1-Naphyrone	$y = 0.01789x + 0.0615$	0.998	2	4
3,4-DMMC	$y = 0.01656x + 0.0404$	0.996	2	3
3-CMC	$y = 0.01415x + 0.0070$	0.999	2	3
3-fluoro-α-PVP	$y = 0.01249x + 0.0213$	0.992	2	2
3-MMC	$y = 0.03250x + 0.0135$	0.999	2	2
4-MMC	$y = 0.03671x + 0.0174$	0.995	2	2
4-BMC	$y = 0.00823x + 0.0002$	0.989	2	4
4-EEC	$y = 0.01392x + 0.0552$	0.994	2	4
4-fluoro-α-PHP	$y = 0.06368x + 0.0223$	0.998	2	2
4-FMC	$y = 0.00983x + 0.0679$	0.998	2	2
4-FMC metab	$y = 0.02254x + 0.0254$	0.999	2	2
4-MEC	$y = 0.01199x + 0.0575$	0.999	2	4
4-MEC metab	$y = 0.00095x + 0.0197$	0.996	2	4
Benzedrone	$y = 0.02475x + 0.0229$	0.999	2	4
Buphedrone	$y = 0.01695x + 0.0682$	0.998	2	3
Butylone	$y = 0.01942x + 0.0670$	0.993	2	3
Dimethylcathinone	$y = 0.0128x - 0.00187$	0.995	2	3
Ethcathinone	$y = 0.0102x + 0.04894$	0.999	2	2
Ethylone	$y = 0.01842x + 0.0531$	0.999	2	3
Euthylone	$y = 0.02019x + 0.0865$	0.990	2	3
MDPBP	$y = 0.01466x + 0.0606$	0.992	2	3
MDPHP	$y = 0.03369x + 0.0716$	0.999	2	5
MDPV	$y = 0.06790x + 0.0324$	0.990	2	4
Methcathinone	$y = 0.01937x + 0.1255$	0.995	2	2
Methedrone	$y = 0.01803x + 0.1034$	0.992	2	3
Methylone	$y = 0.02206x + 0.1158$	0.999	2	2
MPHP	$y = 0.02002x + 0.0950$	0.999	2	4
Naphyrone	$y = 0.05886x + 0.0355$	0.990	2	3
NEP	$y = 0.01449x + 0.0662$	0.991	2	3
N-Ethylpentylone	$y = 0.01062x + 0.0286$	0.992	2	4
Pentedrone	$y = 0.01408x + 0.0498$	0.997	2	3
Pentylone	$y = 0.01264x + 0.0716$	0.993	2	2
α-PHP	$y = 0.00256x + 0.0346$	0.994	2	5
α-PVP	$y = 0.01408x + 0.0663$	0.993	2	2

***Table 13**. Intra-, inter-day precision, and accuracy values (N=15, %CV and bias%) in hair for 33 synthetic cathinones at low QC (15 pg/mg), medium QC (250 pg/mg), high QC (400 pg/mg). Matrix enhancement/suppression (ME) expressed as mean values of low and high QC*

Analytes	intra-day precision (%CV)			inter-day precision (%CV)			Accuracy (bias %)			ME (%)	
	Low QC	Med QC	High QC	Low QC	Med QC	High QC	Low QC	Med QC	High QC	Low QC	High QC
1-Naphyrone	2.37	2.19	1.18	3.48	9.87	2.15	4.96	-5.26	-12.51	8.00	12.00
3,4-DMMC	0.51	11.94	0.58	0.51	0.58	1.99	0.01	-0.47	4.78	-7.94	-4.01
3-CMC	6.29	1.40	1.58	5.44	1.24	3.46	-4.50	0.85	2.08	-11.84	-2.64
3-fluoro-α-PVP	2.05	1.43	0.75	5.17	1.65	5.05	-1.54	4.00	3.09	-2.04	-5.92
3-MMC	2.39	2.50	2.27	2.11	4.62	2.31	0.34	-2.00	1.12	-13.24	-11.71
4-MMC	3.32	1.09	1.84	2.74	1.44	1.60	0.99	2.17	0.21	-9.86	-13.82
4-BMC	8.41	6.35	0.81	7.72	6.35	3.04	6.27	3.09	4.56	9.08	14.10
4-EEC	2.77	0.18	0.77	5.93	2.46	6.27	0.12	-0.24	6.64	-12.62	-11.67
4-fluoro-α-PHP	0.83	2.18	2.44	2.00	8.07	2.73	1.49	-4.29	-13.93	8.25	2.71
4-FMC	5.29	1.19	0.64	5.04	3.41	0.76	2.84	1.18	-1.45	-5.00	8.00
4-FMC metab	5.17	1.31	4.85	4.80	2.77	3.93	6.24	2.14	-12.41	-11.00	12.00
4-MEC	1.21	1.41	3.13	0.88	1.91	2.81	0.69	1.06	1.78	-18.60	-4.32
4-MEC metab	3.51	0.74	5.78	4.56	0.96	4.60	0.47	1.71	1.93	-8.62	-11.45
Benzedrone	0.83	2.05	1.04	1.32	1.60	0.75	1.18	0.94	-8.83	1.00	5.00
Buphedrone	1.92	1.35	1.60	2.58	1.49	1.22	5.03	1.45	1.18	-3.59	5.09
Butylone	4.37	0.37	0.88	3.38	0.31	0.96	6.06	0.84	1.67	-13.67	-8.50
Dimethylcathinone	0.80	0.52	0.18	1.49	0.37	0.70	3.12	1.35	1.08	1.00	3.00
Ethcathinone	1.54	0.38	0.42	2.34	0.44	0.86	2.95	0.94	0.13	12.00	15.00
Ethylone	2.68	2.77	1.09	5.05	2.30	2.82	3.57	1.68	0.74	-12.00	-9.00
Euthylone	1.32	1.79	1.28	3.65	1.63	1.50	0.40	0.93	1.74	-10.35	-11.71
MDPBP	3.29	0.31	3.23	6.44	1.36	2.69	0.91	2.32	2.87	-13.31	-6.52
MDPHP	0.46	1.03	1.16	0.83	2.06	0.83	1.93	4.27	-11.02	-12.92	3.34
MDPV	1.70	0.52	0.26	3.74	1.81	2.37	1.48	0.88	-0.01	-6.56	-2.41
Methcathinone	0.84	0.23	2.36	3.84	0.87	2.87	6.19	2.00	-1.79	-14.00	15.00
Methedrone	3.14	0.70	0.54	9.02	0.60	0.76	7.74	2.24	0.95	-6.00	-4.61
Methylone	1.68	2.74	1.16	5.84	3.80	4.28	6.76	1.79	-4.94	-10.55	6.79
MPHP	1.31	2.25	0.51	6.12	1.91	0.56	7.49	1.10	-6.99	-2.80	10.76

Naphyrone	3.10	1.22	1.27	2.31	2.96	1.61	10.18	4.61	4.05	-1.69	-1.78
NEP	3.85	0.74	1.99	8.07	0.56	3.64	3.29	0.03	4.32	-6.85	-10.16
N-Ethylpentylone	3.58	0.33	0.21	3.89	0.78	1.20	1.06	1.54	3.04	-9.35	-2.04
Pentedrone	0.71	2.85	0.73	1.20	3.75	0.88	3.04	1.78	3.42	-3.18	0.95
Pentylone	0.95	1.84	0.85	0.72	1.37	1.41	3.93	0.60	2.22	-11.94	-8.91
α-PHP	1.95	2.04	6.14	4.72	9.34	10.48	-0.77	-6.65	3.20	-7.82	-5.42
α-PVP	3.84	0.63	0.69	3.28	1.23	5.18	0.42	0.74	12.43	-7.86	-8.57

Figure 8. *Overlay chromatograms of synthethic cathinones under investigation spiked at 50 pg/mg in blank hair pool*



methcathinone (2.77) , 4-FMC metab (2.78), 4-FMC (2.86), dimethylcathinone (3.25), methylone (3.40), ethcathinone (3.48), methedrone (4.10), ethylone (4.20), buphedrone (4.24), 3-CMC (4.58), mephedrone d3 (4.60), 3-MMC (4.64), butylone (4.98), 4-MEC metab (5.26), 4-BMC (5.34), 4-MEC (5.40), euthylone (5.53), MDPBP (5.88), pentedrone (5.94), 3,4-DMMC (6.19), NEP (6.33), pentylone (6.34), α-PVP (6.60), N-Ethylpentylone (6.68), 4-EEC (6.74), 3-fluoro-α-PVP (6.84), MDPV (6.94), α-PHP (7.86), 4-fluoro-α-PHP (8.04), MDPHP (8.06), benzedrone (8.37), MPHP (8.71), 1-Naphyrone (8.82), naphyrone (9.16)

Table 14. *Concentration (pg/mg) of target analytes in hair of NPS consumers*

	H01	H02	H03	H04	H05	H06	H07	H08
MDPHP	1000.0	-	14.5	-	50.0	6.0	-	-
3,4 DMMC	-	-	27.4	-	-	-	-	-
4-MEC	-	11.5	-	-	-	-	-	448.0
4-MEC metab	-	-	-	-	-	-	-	123.0
α-PHP	-	-	-	-	554	54.0	-	-
Butylone	-	-	-	-	-	-	176.0	-
4-MMC	91.3	-	-	-	-	-	-	-
Euthylone	-	-	-	-	-	-	-	-
Ethylone	-	-	-	-	-	-	-	-
Methcathinone	-	15.7	-	10.8	-	-	-	-
3-MMC	-	-	-	-	556.0	5000.0	-	-
Methylone	-	-	-	-	-	-	140.0	-