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RESEARCH ARTICLE

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Development and validation of a rapid LC-MS/MS method for the detection of 182 novel psychoactive substances in whole blood

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Abstract

Introduction: The analysis of novel psychoactive substances (NPS) represents a challenge in forensic toxicology, due to the high number of compounds characterized by different structures and physicochemical properties both among different subclasses and within a single subclass of NPS. The aim of the present work is the development and validation of a targeted liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the detection of NPS in whole blood.

Materials and methods: A protein-precipitation based LC-MS/MS method for the detection of more than 180 NPS was developed and validated by assessing the following parameters: selectivity, linearity, accuracy, precision, limit of detection (LOD) and of quantification (LOQ) recovery, and matrix effect. Then, the method was applied to real forensic samples.

Results: The method allowed the identification of 132 synthetic cannabinoids, 22 synthetic opioids, and 28 substances among synthetic cathinones, stimulants, and other drugs. Validation was successfully achieved for most of the compounds. Linearity was in the range of 0.25–10 ng/ml for synthetic cannabinoids and 0.25–25 ng/ml for other drugs. Accuracy and precision were acceptable according to international guidelines. Three cases tested positive for fentanyl and ketamine, in the setting of emergency room administration.

Conclusions: The present methodology represents a fast, not expensive, wide-panel method for the analysis of more than 180 NPS by LC-MS/MS, which can be profitably applied both in a clinical context and in postmortem toxicology.

KEYWORDS

forensic toxicology, mass spectrometry, novel psychoactive substances, screening method, validation

1 | INTRODUCTION

The term *Novel Psychoactive Substances* (NPS) encompasses a high number of molecules with very different chemical characteristics, originally defined for not being covered by the United Nations International Drug Conventions 1961–1971.[1] Since 1997, the European Monitoring Centre for Drug and Drug Addiction (EMCDDA) has been monitoring 820 NPS at the end of 2020, including synthetic cannabinoids (SCs), synthetic opioids (SOs), synthetic cathinones (SCAs), designer benzodiazepines (dBZDs), phenethylamines, and tryptamines.[2] The peak of new compounds per year in the drug market has been reported around 2014–2015 and, even if the prevalence is still high, has then decreased, with lower diversity in the consumed substances,[1,2] partially reflecting national and international legislations, such as the German act on NPS and the Chinese regulations, which appear to have a high impact on the European market.[1–3] SCs were first detected around 2006[4,5]; they represent the largest group of NPS and have so far dominated the market, accounting together with SCAs for 62% of NPS seizures in 2018, while in recent years, SOs and dBZDs are growing in numbers.[2] NPS have been claimed by the suppliers as *safe* and *legal* alternatives to common drugs of abuse, and sold under codes like *research chemicals*, *smart drugs*, *legal highs*, *dietary supplements*, or *bath salts*, often declared to be *not for human consumption*. They are increasingly encountering the favor of online and physical consumers, despite their toxicity is often greater than that of the corresponding classical illicit drug.[2,6–8]

One of the reasons for the rapid spreading of NPS across the public is the poor detectability at screening tests performed on biological fluids.[8] Indeed, the analysis of NPS cannot be based on common immunoenzymatic methods of screening and usually requires either liquid or gas chromatography (LC or GC) coupled to mass spectrometry (MS) for both screening and confirmatory analysis.[8–11]

In forensic toxicology, the bioanalysis of NPS is particularly challenging and is required when a suspicion of intoxication is coupled to a negative toxicology for classical drugs of abuse, especially when a strong hint arises from circumstantial data.[12–15] In fact, in post-mortem investigations, there is a lack of macroscopically pathognomonic signs at autopsy which could point towards the intake of NPS of a certain class, while in the living subject, the signs of intoxication do not significantly differ from those caused by classical illicit drugs.

Due to constantly evolving novel compounds, laboratories are forced to choose between applying high sensitivity and specificity target methods for a small set of molecules, which is time and material-consuming, or to perform broad range screening methods including many substances, which have to be confirmed by for further (quantitative) target analyses. Screening methodology is complicated by the fact that chemical diversity usually requires adapted sample preparation, mobile phases, and chromatographic and MS/MS conditions in order to achieve good identification power and usually only allows a qualitative or semi-quantitative approach when covering a high number of compounds.[9,11,16]

Moreover, since they only work with a pre-defined set of substances, it is necessary to constantly update the methods in order to include the substances newly synthesized and introduced on the market.[9,11]

The aim of the present work is the development and validation of a target LC-MS/MS method for the detection of more than 180 NPS in whole blood and its application to forensic cases.

2 | MATERIAL AND METHODS

2.1 | Chemicals and reagents

Standard solutions of 132 SCs, 22 SOs, and 28 among SCAs, stimulants, and other drugs were provided by the National Health Institute within the National Early Warning System[17] (Panels 1 and 2). The panel of SC was kindly integrated by 98 standard solutions of SCs provided by the Forensic Toxicology Department of the Institute of Forensic Medicine, Medical Center – University of Freiburg (Panel 3). Composition of Panels 1–3 was the following (semi-systematic names).

2.1.1 | Panel 1

Standards of 3,4-dimethylmethcathinone (3,4-DMMC), 4-fluoromethcathinone (4-FMC), 4-methylethcathinone (4-MEC), AM-2201, AM-2233, AM-694, buphedrone, butylone, ethcathinone, ethylone, JWH-007, JWH-016, JWH-019, JWH-081, JWH-098, JWH-122, JWH-203, JWH-210, JWH-251, JWH-302, JWH-398, ketamine, MDPV, methcathinone (MCAT), methedrone (4-Methoxy MCAT), methylone, nordiazepam, pentylone, RCS-4, RCS-8 and WIN 48,098 (pravadoline) were provided by Comedical s.r.l. (Italy, Trento) at 0.1 mg/ml.

2.1.2 | Panel 2

Standards of (±)-*cis*-3-methyl norfentanyl, (±)-*trans*-3-methyl norfentanyl, αET, β-hydroxy fentanyl, β-hydroxythiofentanyl, β-phenyl fentanyl, 4-Acetoxy-DiPT (4-AcO-DiPT), 4-ANPP, 5-APB/6-APB, 5-Cl-THJ 018, 5-EAPB, 5F-ADB, 5F-APP-PICA (PX-1), 5F-APP-PINACA (PX-2), 5F-CumylPINACA, 5F-NNEI 2'-Naphthyl Isomer, 5-MAPB/6-MAPB, 5-methoxy-AMT (5-MeO-AMT), 5-methoxy-DALT (5-MeO-DALT), 5-Methoxy-DMT (5-MeO-DMT), 5-Methoxy-DPT (5-MeO-DPT), 5-Methoxy-MiPT (5-MeO-MiPT), AB-CHMINACA, AB-FUBINACA, acetyl fentanyl, acetyl norfentanyl, ADB-FUBINACA, alfentanyl, APP-FUBINACA, butyryl fentanyl, butyryl fentanyl carboxy metabolite, butyryl norfentanyl, carfentanyl, Cumyl-PEGACLONE (SGT-151), cyclopropylfentanyl, despropionyl *para*-fluorofentanyl, ethylphenidate, fentanyl, furanyl norfentanyl, JWH-018, JWH-200, JWH-250, MDMB-CHMICA, mephedrone (4-Methyl MCAT, 4-MMC), methoxyacetyl norfentanyl, MMB-2201 (5F-AMB-PICA), N,N-dimethylcathinone, N,N-dimethyltryptamine (DMT), norfentanyl, phenylfentanyl, phenylacetyl fentanyl, ritalinic acid and valeryl

fentanyl carboxy metabolite were provided by Comedical s.r.l. (Italy, Trento) at 0.05 mg/ml.

2.1.3 | Panel 3

4-HTMPIPO, 4F-MDMB-BINACA, 5F-AB-001, 5F-AB-PICA (5F-ABICA), 5F-AB-PINACA, 5F-ADB-PICA (5F-ADBICA), 5F-ADB-PINACA, 5F-AMB-PINACA, 5F-EMB-PINACA, 5F-JWH-412, 5F-MDMB-P7AICA, 5F-MDMB-PICA, 5F-MDMB-PINACA (5F-ADB), 5F-PCN (5F-MN-21), 5F-PY-PICA, A-796,260, A-834,735, AB-001, AB-005, AB-005 azepane, AB-BICA, AB-CHMICA, AB-FUB7AICA (AB-7-FUBAICA), AB-FUBICA, AB-FUBINACA 2/3-fluorobenzyl isomers, AB-PICA, AB-PINACA, ADB-BICA, ADB-BINACA, ADB-CHMICA, ADB-FUBICA, ADB-PICA, ADB-PINACA, AKB-48 (APINACA), AM-1220 azepane, AM-1235, AM-1241, AM-1248, AM-1248 azepane, AM-2201 indazole carboxamide, AM-2232, AM-2233 azepane, AM-630, AM-679, AMB-CHMICA, AMB-CHMINACA, AMB-FUBICA, AMB-FUBINACA, AMB-PICA, AMB-PINACA, PB-22, Cumyl-4CN-BINACA, Cumyl-BICA, Cumyl-PICA, Cumyl-THPINACA, EG-018, EG-2201, FUB-JWH-018, FUB-NPB-22, FUB-PB-22, JWH-011, JWH-015, JWH-020, JWH-022, JWH-030, JWH-031, JWH-073, JWH-080, JWH-122 N-(4-pentenyl) analog, JWH-145, JWH-147, JWH-182, JWH-213, JWH-249, JWH-307, JWH-309, JWH-370, JWH-387, JWH-412, JWH-424, M-144, MDMB-4en-PINACA, MDMB-CHMCZCA, MDMB-CHMINACA, MDMB-FUBICA, MDMB-FUBINACA, MDMB-PICA, MDMB-PINACA, MEPIRAPIM, MMB-022 (MMB-4en-PICA), MN-25, N-Phenyl-SDB-006, NE-CHMIMO, SDB-005, THJ-2201, WIN 55,212–2, XLR-11, XLR-12 were purchased from Cayman Chemical (Ann Arbor, Michigan, USA) and kindly provided by the Forensic Toxicology Department of the Institute of Forensic Medicine, Medical Center – University of Freiburg. Compounds were diluted in methanol starting with a concentration of 0.01 mg/ml.

Internal standards (IS), nordiazepam-D5 and ketamine-D4, were obtained from Sigma Aldrich (Steinheim, Germany).

Water was obtained through a Millipore Milli-Q®. Formic acid, methanol, IPA, ACN were purchased by Merck (Germany, Darmstadt). All reagents and solvents were of LC/MS grade.

2.2 | Preparation of working solution and mobile phases

Individual methanolic solutions were used to prepare 7 working mixtures of standards.

- Panel 1, mix 1 at a concentration of 1,000 ng/ml;
- Panel 2, mix 2 at a concentration of 500 ng/ml;
- Panel 3, mixes 3–7, at a concentration of 500 ng/ml.

Internal standard mixture containing nordiazepam-D5 and ketamine-D4 was also prepared at a concentration of 0.01 mg/ml. Standards, stocks, and working solutions were stored at −20°C until their use.

Mobile phase A, 0.1% formic acid in water, and mobile phase B, 0.1% formic acid in acetonitrile were freshly prepared before the analysis. Seal wash was prepared as water/methanol 50:50 v/v. Strong wash was prepared as 0.2% formic acid in 2-propanol/acetonitrile/water/methanol (25:25:25:25 v/v/v/v). As a weak wash, mobile phase A was used.

2.3 | Sample preparation

Two samples of 500 μl of whole blood, one for SCs of Panel 3 and one for all other substances, for a total amount of 1 ml, were spiked with 10 μl of deuterated IS (final concentration: 200 ng/ml) and with a variable amount of the working solutions. After precipitation with 1.5 ml of cold acetonitrile, samples were vortexed and centrifuged (MPW Med. instruments, MPW 223e, Poland, Warsaw) at 3,000 rpm for 15 min. All the organic solvent was transferred into a 5 ml vial and evaporated under gentle nitrogen stream at 40°C. Reconstitution was performed with 150 μl of mobile phase B for SCs of Panels 1–3 and with mobile phase A/B: (80:20, v/v) for all other substances. Injection volume was 10 μl.

2.4 | UPLC–MS/MS

LC-MS/MS analysis was performed with a Waters Acquity (Ultra High-Performance Liquid Chromatography) UHPLC® (Milford, MA), coupled to a quadrupole mass detector Waters Xevo TQD, equipped with an electrospray ion source (ESI) operating in positive mode. Chromatographic separation was achieved on an Acquity UPLC® HSS C18 column (1.8 μm, 2.1 × 150 mm from Waters, Italy, Milan).

Gradient elution was as follows: Mobile phase B starting concentration was 10%, linearly increased to 40% at 8.0 min, further increased to 95% at 13.0 min, kept constant for 1.5 min, decreased to the starting conditions in 0.5 min, and kept at 10% for 2 min for equilibration. Total run time was 17 min. Flow rate was set at 0.4 ml/min. The autosampler was cooled down to 10°C. The column temperature was set to 40°C.

The MS was operated with positive ionization in Multiple Reaction Monitoring (MRM) mode. Specific MRM transitions and collision energies were determined by literature search, on substances tuned with the same MS-device, and a series of experiments performed on individual standards at a concentration of 1,000 ng/ml. Two characteristic transitions were chosen for each analyte. Due to the high number of analytes, two different MS methods were developed, one for substances included in Panels 1 and 2 and one for substances included in Panel 3. A total of three injections were done: extracts containing substances from Panels 1–2, reconstituted in mobile phase B and mobile phase A/B (80:20, v/v), run with the same MS method (first and second injections), followed by a third injection for substances of Panel 3 with the dedicated MS method. Extracts (containing substances of Panels 1 and 2) reconstituted with mobile phases B and A/B: (80:20, v/v) were analyzed with one MS methods,

while extracts containing substances from Panel 3 only ran with the dedicated MS method. Each method was composed of multiple detection windows containing approximately 10 compounds each, with a time ± 0.5 min from the retention time of the respective substance.

Optimized MS parameters were as follows: capillary voltage 3.50 kV, desolvation gas temperature 400°C, source gas flow (nitrogen) desolvation rate 800 L/h, cone 20 L/h, gas in collision argon, dwell time 0.01 s.

2.5 | Method validation

The method was validated according to the guidelines of the German Society of Toxicological and Forensic Chemistry (GTFCh),[18] evaluating for all analytes the following analytical parameters: selectivity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), recovery, and matrix effect.

Selectivity was assessed by analyzing six blank blood samples from different individuals, with six blank post-mortem blood samples and with two blood samples spiked with common illicit and therapeutic drugs, by checking for interfering peaks.

Linearity was assessed using a 6-point calibration curve for the substances of Panel 3 and a 7-point calibration curve for substances included in Panels 1 and 2, by spiking appropriate amounts of each mixture (or of intermediate dilutions of the mixtures) to blank blood, resulting in the following final concentrations: 0.25, 0.5, 1.25, 2.5, 5, and 10 ng/ml for Panel 3 and 0.25, 0.5, 1.25, 2.5, 5, 10, and 25 ng/ml for Panels 1 and 2.

Six calibration batches, all including a blank whole blood sample spiked with IS only (zero sample), were analyzed on six consecutive days. Calibration curves were constructed through linear regression by plotting the area ratio of each substance with its internal standard versus the concentration of the analytes. GraphPad Prism 8.2.1 was used for this task.

For the assessment of accuracy and precision, quality control (QC) samples were analyzed in two replicates for each concentration per day (intra-day precision) and on six consecutive days (inter-day precision) by spiking pooled whole blood samples to obtain the following final concentrations:

- 1 ng/ml for Panel 3 mix: QC low;
- 4 ng/ml for Panel 3 mix: QC high;
- 2 ng/ml for Panels 1 and 2 mixes: QC low;
- 12.5 ng/ml for Panels 1 and 2 mixes: QC high.

For all analytes which fulfilled identification criteria (retention time and ion ratio) at the first point of the calibration curve, LOD and LOQ were determined with an additional five-point curve, at the final concentrations of 0.06, 0.1, 0.125, 0.15, and 0.2 ng/ml, through the software Valistat 2.0 software (Arvecon GmbH, Walldorf, Germany), in accordance with the guidelines of the GTFCh.[18] When the points were judged too few by Valistat, or when the detection of the compound was not possible at the first point of the calibration curve, LOQ was defined by the lowest concentration detectable with a signal-to-noise ratio of at least 10, accuracy ± 20% and precision ± 10%.[18] For these substances, the LOD was assumed as 1/3 of the LOQ.

Accuracy and precision were obtained by bias calculation and relative standard errors, through Valistat software. Recovery and matrix effect were evaluated for all analytes at 2 and 4 ng/ml, by comparing absolute peak areas or the ratio between them and the IS, and by analyzing three sets of samples in duplicates. For recovery, each analyte in the QC samples (A) was compared with blood samples processed as a blank and spiked after the extraction step at the same concentration level (B). In order to assess matrix effect, that is, potential ion suppression/enhancement due to the sample matrix, B samples were compared to pure standards in a mixtures of mobile phases A and B (80:20, v/v) for all substances except for SCs, which were tested in mobile phase B (C).

2.6 | Application to real forensic cases

The validated method was applied to 10 samples of blood collected during forensic autopsies of both drug users and non-drug users and to 15 samples of blood collected in the frame of driving under the influence of drugs (DUID). Samples were stored at −20°C until analysis.

3 | RESULTS

3.1 | Method optimization

A target LC-MS/MS method was developed for the selective identification in whole blood of 182 NPS including 132 SCs, 22 SOs, and 28 among SCAs stimulants and other drugs. In Tables 1 and 2, substances, together with the IUPAC name, retention time, detection window, quantifier and qualifier ions, cone voltage, and collision energies, are shown. The total preparation of samples is achieved in approximately 30 min by protein precipitation, followed by three LC-MS runs of 17 min each, for a total of 51 min for each sample. Mobile phases were chosen on the basis of previous studies performed on psychoactive drugs,[19] and the addition of formic acid resulted in a slight enhancement of the signal for all the analytes. On the basis of preliminary analyses, reconstitution was performed with mobile phase B for SCs, while for all the other analytes, a mixture of mobile phase A and B (80/20, [v/v]) was chosen. Even if this was not identical to the starting LC conditions, no retention or carry over effect was seen.

The chromatographic conditions were optimized in order to achieve a separation of analytes with the same nominal mass and fragment ions, for example, cis- vs trans-methyl-norfentanyl, JWH-007 vs 019, JWH-015 vs JWH-073, JWH-018 vs JWH-016, FUB-NPB-22 vs MDMB-FUBINACA, FUB-PB-22 vs MDMB-FUBICA, or butylone vs ethylone. Indeed, the chromatographic method allowed separating all isomers and analytes with the same mass by retention time, except

TABLE 1 Analytes of interest (semi-systematic and IUPAC names) of panels 1 and 2, together with retention time (RT), cone voltage (CV), quantifier (*) and qualifier ion transitions, and collision energies (Ce)

N	Analyte	RT (min)	Detection window (min)	Precursor ion (m/z)	Product ions (m/z)	CV (V)	Ce (V)
Synthetic cannabinoids (SCs)							
1	5-Cl-AB-PINACA N-[(2S)-1-amino-3-methyl-1-oxobutan-2-yl]-1-(5chloropentyl)indazole-3-carboxamide	11.0	9.5–13.0	366	249 145*	25	24 44
2	5-Cl-THJ-018 1-(5-Chloropentyl)-1H-indazol-3-yl](1-naphthyl) methanone	13.3	11.5–15	377	249* 213	25	16 24
3	5F-ADB methyl (2R)-2-[[1-(5-fluoropentyl)indazole-3-carbonyl] amino]-3,3-dimethylbutanoate	12.3	10.5–15	378	233* 318	20	20 10
4	5F-AKB-48 N-(adamantan-1-yl)-1-(5-fluoropentyl)-1H-indazole-3-carboxamide	13.6	12–15.5	384	135* 107	20	50 24
5	5F-NNEI 2′-naphthyl isomer 1-(5-Fluoropentyl)-N-(naphthalen-2-yl)-1H-indole-3-carboxamide	12.4	10.5–15	375	232* 144	22	20 42
6	AB-CHMINACA N-[(2S)-1-amino-3-methyl-1-oxobutan-2-yl]-1-(cyclohexylmethyl)-1H-indazole-3-carboxamide	11.6	9.5–13.0	357	145* 241	20	46 28
7	AB-FUBINACA N-[(2S)-1-amino-3-methyl-1-oxobutan-2-yl]-1-[(4-fluorophenyl)methyl]-1H-indazole-3-carboxamide	10.4	7.5–12	369	253* 109	20	20 40
8	ADB-FUBINACA N-[(2S)-1-amino-3,3-dimethyl-1-oxobutan-2-yl]-1-[(4-fluorophenyl)methyl]-1H-indazole-3-carboxamide	10.9	9.5–13.0	383	253* 109	25	25 42
9	AM-2201 [1-(5-fluoropentyl)indol-3-yl]-naphthalen-1-ylmethanone	12.5	10.5–15	360	127* 155	20	46 42
10	AM-2233 (2-iodophenyl)-[1-[(1-methylpiperidin-2-yl)methyl] indol-3-yl]methanone	7.9	6–8.8	459	98* 112	45	50 50
11	AM-694 [1-(5-fluoropentyl)indol-3-yl]-(2-iodophenyl) methanone	12.2	10.5–15	436	231* 203	20	36 40
12	APP-FUBINACA N-[(2S)-1-amino-1-oxo-3-phenylpropan-2-yl]-1-[(4-fluorophenyl)methyl]indazole-3-carboxamide	10.8	9.5–13.0	417	109* 253	20	40 24
13	Cumyl -PEGACLONE 5-pentyl-2-(2-phenylpropan-2-yl)-2,5-dihydro-1H-pyrido[4,3-b]indol-1-one	13	11.5–15	373	255* 119	30	24 10
14	JWH-007 (2-methyl-1-pentylindol-3-yl)-naphthalen-1-ylmethanone	13.6	11.7–15	356	127* 155	20	40 34
15	JWH-016 (1-butyl-2-methylindol-3-yl)-naphthalen-1-ylmethanone	13.2	11.5–15	342	127* 155	20	44 34
16	JWH-018 (1-pentyl-1H-indol-3-yl)-1-naphthalenyl-methanone	13.4	11.7–15	342	127* 155	20	44 34
17	JWH-019 (1-hexyl-1H-indol-3-yl)(naphthalen-1-yl)methanone	13.7	12–15.5	356	127* 228	20	38 30
18	JWH-081 (4-Methoxynaphthalen-1-yl)(1-pentyl-1H-indol-3-yl) methanone	13.5	11.7–15	372	185* 157	20	26 40

TABLE 1 (Continued)

N	Analyte	RT (min)	Detection window (min)	Precursor ion (m/z)	Product ions (m/z)	CV (V)	Ce (V)
19	JWH-098 (4-methoxynaphthalen-1-yl)-(2-methyl-1-pentylindol-3-yl)methanone	13.6		386	185* 127	20	26 34
20	JWH-122 (4-Methylnaphthalen-1-yl)(1-pentyl-1H-indol-3-yl) methanone	13.7	11.7–15	356	169* 141	20	24 44
21	JWH-200 [1-(2-morpholin-4-ylethyl)indol-3-yl]-naphthalen-1-ylmethanone	8.2	6–8.8	385	114* 155	20	46 42
22	JWH-203 2-(2-chlorophenyl)-1-(2-methyl-1-pentyl-1H-indol-3-yl)ethanone	13.2	11.5–15	340	125* 214	20	34 22
23	JWH-210 (4-ethyl-1-naphthalenyl)(1-pentyl-1H-indol-3-yl)-methanone	14.0	12–15.5	370	183* 214	20	24 26
24	JWH-250 2-(2-methoxyphenyl)-1-(1-pentyl-1H-indol-3-yl) ethanone	12.9	10.5–15	336	121* 91	20	50 32
25	JWH-251 2-(3-methylphenyl)-1-(1-pentyl-1H-indol-3-yl) ethanone	13.2	11.5–15	320	105* 214	20	22 20
26	JWH-302 2-(3-methoxyphenyl)-1-(1-pentylindol-3-yl)ethenone	12.7	10.5–15	336	214* 144	20	30 44
27	MDMB-CHMICA methyl (2S)-2-{[1-(cyclohexylmethyl)-1H-indole-3-carbonyl]amino}3,3-dimethylbutanoate	13.0	11.5–15	385	240* 144	20	24 46
28	MMB-2201 methyl (2S)-2-{[1-(5-fluoropentyl)-1H-indole-3-carbonyl]amino}-3-methylbutanoate	11.4	9.5–13.0	363	232* 144	34	12 38
29	RCS-4 (4-methoxyphenyl)(1-pentyl-1H-indol-3-yl)methanone	12.7	10.5–15	322	135* 107	20	20 20
30	RCS-8 1-[1-(2-cyclohexylethyl)-1H-indol-3-yl]-2-(2-methoxyphenyl)ethenone	13.7	12–15.5	376	121* 91	20	24 48
31	WIN 48.098 (4-methoxyphenyl)-[2-methyl-1-(2-morpholin-4-ylethyl)indol-3-yl]methanone	7.1	6–8.8	379	135* 114	45	24 32
Synthetic opioids (SOs)							
32	(±)-cis-3-methyl norfentanyl N-[(3R,4S)-3-methylpiperidin-4-yl]-N-phenylpropanamide	4.2	3.0–5.5	247	69 98*	25	29 18
33	(±)-trans-3-methyl norfentanyl N-[(3R,4R)-3-methylpiperidin-4-yl]-N-phenylpropanamide	4.0	3.0–5.5	247	69 98*	25	29 18
34	β-Hydroxy fentanyl N-[1-(2-hydroxy-2-phenylethyl)piperidin-4-yl]-N-phenylpropanamide	5.8	4.5–7.2	353	204 335*	35	38 16
35	β-Hydroxythiofentanyl N-[1-(2-hydroxy-2-thiophen-2-ylethyl)piperidin-4-yl]-N-phenylpropanamide	5.4	4.0–6.2	359	192* 111	35	22 38
36	β-Phenyl fentanyl N-(1-phenethylpiperidin-4-yl)-N,3-diphenylpropanamide	9.4	7.5–12	413	105* 188	35	44 26
37	4-ANPP N-phenyl-1-(2-phenylethyl)piperidin-4-amine	6.4	4.5–7.2	281	105* 188	42	30 16

(Continues)

TABLE 1 (Continued)

N	Analyte	RT (min)	Detection window (min)	Precursor ion (m/z)	Product ions (m/z)	CV (V)	Ce (V)
38	Acetyl fentanyl N-Phenyl-N-[1-(2-phenylethyl)-4-piperidinyl]-acetamide	5.5	4.0–6.2	323	105* 188	25	36 20
39	Acetyl norfentanyl N-phenyl-N-piperidin-4-ylacetamide	2.3	1.0–3.0	219	55 84*	25	36 18
40	Alfentanyl N-[1-[2-(4-ethyl-5-oxotetrazol-1-yl)ethyl]-4-(methoxymethyl)piperidin-4-yl]-N-phenylpropanamide	6.4	4.5–7.2	417	197* 268	24	26 16
41	Butyryl fentanyl N-phenyl-N-[1-(2-phenylethyl)piperidin-4-yl] butanamide	7.6	6–8.8	351	105* 188	30	45 40
42	Butyryl fentanyl carboxy metabolite 4-oxo-4-(N-[1-(2-phenylethyl)piperidin-4-yl]anilino) butanoic acid	5.3	4.0–6.2	381	105* 188	25	45 42
43	Butyryl norfentanyl N-phenyl-N-4-piperidinyl-butanamide	4.6	3.0–5.5	247	55 84*	25	36 10
44	Carfentanyl methyl 1-(2-phenylethyl)-4-(N-propanoylanilino) piperidine-4-carboxylate	7.4	6–8.8	395	113* 105	22	32 52
45	Cyclopropylfentanyl N-phenyl-N-[1-(2-phenylethyl)piperidin-4-yl] cyclopropanecarboxamide	7.1	6–8.8	349	105* 188	25	36 20
46	Despropionyl para-fluorofentanyl N-(4-fluorophenyl)-1-phenethylpiperidin-4-amine	6.7	4.5–7.2	299	105* 188	25	38 16
47	Fentanyl N-phenyl-N-[1-(2-phenylethyl)piperidin-4-yl] propenamide	5.5	4.5–7.2	377	105* 188	40	30 38
48	Furanyl norfentanyl N-phenyl-N-piperidin-4-ylfuran-2-carboxamide	3.6	2.0–4.6	271	55* 84	16	38 18
49	Methoxyacetyl norfentanyl 2-methoxy-N-phenyl-N-piperidin-1-ium-4-ylacetamide	2.2	1.0–3.0	249	55 84*	15	38 14
50	Norfentanyl N-phenyl-N-piperidin-4-ylpropanamide	3.5	2.0–4.6	233	55 84*	25	34 20
51	Phenylfentanyl N-phenyl-N-[1-(2-phenylethyl)-4-piperidyl]benzamide	7.9	6–8.8	385	105* 188	40	46 24
52	Phenylacetyl fentanyl N-phenyl-N-[1-(2-phenylethyl)-4-piperidyl]benzamide	8.8	7.5–12	399	105 188*	46	46 24
53	Valeryl fentanyl carboxy metabolite 5-oxo-5-(N-[1-(2-phenylethyl)piperidin-4-yl]anilino) pentanoic acid	5.5		395	105 188*	40	44 26
Synthetic cathinones (SCAs) stimulants and others							
54	αET 1-(1H-indol-3-yl)butan-2-amine	4.0		189	58 130*	26	16 16
55	3,4-DMMC, 3,4-dimethylmethcathinone 1-(3,4-Dimethylphenyl)-2-(methylamino)propan-1-one	4.4	3.0–5.5	192	159 174*	20	15 13
56	4-FMC, 4-Fluoromethcathinone 1-(4-fluorophenyl)-2-(methylamino)propan-1-one	2.3	1.0–3.0	182	149 164	20	15 10
57	4-MEC, 4-Methylethcathinone 2-(Ethylamino)-1-(4-methylphenyl)propan-1-one	3.6	2.0–4.6	192	145* 174	13	17 13

TABLE 1 (Continued)

N	Analyte	RT (min)	Detection window (min)	Precursor ion (m/z)	Product ions (m/z)	CV (V)	Ce (V)
58	5-APB/6-APB 1-(Benzofuran-5-yl)-propan-2-amine 1-(Benzofuran-6-yl)propan-2-amine	3.6	2.0–4.6	176	77 91*	22	40 26
59	5-EAPB 1-(1-Benzofuran-5-yl)-N-ethylpropan-2-amine	4.4	3.0–5.5	204	91 131*	24	30 20
60	5-MAPB/6-MAPB 1-(1-Benzofuran-5-yl)-N-methylpropan-2-amine 1-(1-Benzofuran-6-yl)-N-methylpropan-2-amine	3.1	2.0–4.6	190	131 159*	20	18 10
61	5-MeO-AMT 1-(5-methoxy-1H-indol-3-yl)propan-2-amine	3.1	1.7–4.0	205	147 173*	22	20 22
62	5-MeO-DALT N-[2-(5-methoxy-1H-indol-3-yl)ethyl]-N-prop-2-enylprop-2-en-1-amine	5.1	4.0–6.2	271	110* 174	24	18 14
63	5-MeO-DMT 2-(5-methoxy-1H-indol-3-yl)-N,N-dimethylethanamine	3.0	1.7–4.0	219	58* 130	20	46 12
64	5-MeO-DPT N-[2-(5-methoxy-1H-indol-3-yl)ethyl]-N-propylpropan-1-amine	5.8	4.5–7.2	275	114* 174	14	16 14
65	5-MeO-MiPT N-[2-(5-methoxy-1H-indol-3-yl)ethyl]-N-methylpropan-2-amine	4.0	2.0–4.6	247	86* 174	10	14 16
66	Buphedrone 2-(methylamino)-1-phenylbutan-1-one	3.2	1.5–3.6	178	91 160*	20	26 10
67	Butylone 1-(1,3-benzodioxol-5-yl)-2-(methylamino)butan-1-one	3.0	1.7–4.0	222	174 204*	27	19 13
68	Ethcathinone 2-(ethylamino)-1-phenylpropan-1-one	2.2	1.0–3.0	178	72 105*	30	22 16
69	Ethylone 1-(1,3-benzodioxol-5-yl)-2-(ethylamino)propan-1-one	2.6	1.5–3.6	222	174* 204	27	19 13
70	Ethylphenidate ethyl 2-phenyl-2-piperidin-2-ylacetate	5.3	4.0–6.2	248	56 84*	50	24 14
71	Ketamine	3.20	1.7–4.0	238.2	125.1* 220.2	30	26 15
72	MDPV 1-(1,3-benzodioxol-5-yl)-2-(pyrrolidin-1-yl)pentan-1-one	4.7	3.0–5.5	276	126* 135	30	25 23
73	Mephedrone 2-(Methylamino)-1-(4-methylphenyl)propan-1-one		1.7–4.0	178	145 160*	20	18 12
74	Methcatinone 2-(methylamino)-1-phenylpropan-1-one	2.0	1.0–3.0	164	131* 146	13	6 6
75	Methedrone 1-(4-methoxyphenyl)-2-(methylamino)propan-1-one	2.7	1.5–3.6	194	161 176*	20	13 8
76	Methylone 1-(1,3-benzodioxol-5-yl)-2-(methylamino)propan-1-one	2.2	1.0–3.0	208	132 160*	27	27 15
77	N,N-Dimethylcathinone 2-(Dimethylamino)-1-phenylpropan-1-on	2.2	1.0–3.0	178	72* 77	20	20 40
78	N,N-DMT, N,N-Dimethyltryptamine 2-(1H-indol-3-yl)-N,N-dimethylethanamine	2.9	1.5–3.6	189	58* 117	20	34 12

(Continues)

TABLE 1 (Continued)

N	Analyte	RT (min)	Detection window (min)	Precursor ion (m/z)	Product ions (m/z)	CV (V)	Ce (V)
79	Nordiazepam	9.0	7.5–12	271.1	140*	50	35
					165.1		25
80	Pentylone 1-(1,3-benzodioxol-5-yl)-2-(methylamino)pentan-1-one	4.0	3.0–5.5	236	188*	27	12
					218		8
81	Ritalinic acid 2-phenyl-2-piperidin-2-ylacetic acid	3.3	2.0–4.6	220	56	20	46
					84*		20
	Nordiazepam-D5	8.9	7.5–12	276	165*	50	28
					213		28
	Ketamine-D4	3.19	1.7–4.0	242.2	129.1	35	30
					242		10

Abbreviation: *N*, number.

for 5- and 6-APB, 5- and 6-MAPB, and the couples 5F-MDMB-PINACA/5F-ADB and 5F-EMB-PINACA/5F-AEB.

MRM transitions included in the MS/MS method were monitored in several detection windows (at least ± 0.5 min from the expected retention time of the analytes included in the respective window), which allowed to achieve a sufficient number of points to define the chromatographic peak. Analyte identification was performed by targeted MS/MS on the basis of mass of the precursor ion, two diagnostic fragments, retention time (± 0.2 min), and area ratio of quantifier and qualifier ions (±20%), fulfilling the EU Commission Decision 2002/657/EC confirmation criteria.

3.2 | Method validation

Successful validation was achieved for the vast majority of the compounds. Validation parameters and particularly linearity (R^2), accuracy, precision, LOD, and LOQ are shown in Table 3. No interfering peaks due to endogenous substances were detected, except for a minimal interference in the case of 5F-EMB-PINACA, with an area 0.90% with respect to the maximum concentrations of the calibration curve.

The method produced linear calibration functions for all the analytes of interest in the tested range, with R^2 always better than 0.99 except for 5F-MDMB-P7AICA, AB-CHMINACA, AM-1235, MDMB-PICA, 4-FMC, ethcathinone, methcathinone, and N,N-dimethylcathinone (R^2 was 0.94–0.98 with no need for a weighing factor; see the supporting information). All the analytes of interest, except for 13 SCs (5F-AKB-48, AB-001, AB-CHMINACA, ADB-CHMICA, ADB-PINACA, AKB-48, AM-1235, EG-018, JWH-016, JWH-203, JWH-210, MDMB-PICA, and MDMB-PINACA, and three stimulants (ethcathinone, methcatinone, and N,N-dimethylcathinone), showed accuracies and precisions within the requirements reported in the guidelines of the GTFCh.[18] Particularly, 5F-AKB-48 and AM-1235 did not meet the requirement for a full validation at the lower QC but showed acceptable accuracy and precision at the higher concentration. AB-001, conversely, shows better parameters at 1 ng/ml.

LOQs were in the range 0.04–0.97 ng/ml for all substances, and mostly <0.50 ng/ml, except for 5-Cl-AB-PINACA (1.25 ng/ml), 5F-AKB-48 (1.25 ng/ml), AM-1235 (1.25 ng/ml), 4-FMC (2.5 ng/ml), and mephedrone (1.25 ng/ml). Generally, higher sensitivities were achieved for SCs.

With the chosen extraction procedure, recovery and matrix effect of analytes under investigation were always higher than 75% and lower than 125% for all SOs. For SCAs, stimulants and other drugs, recovery, and matrix effect were also acceptable (>70% and <130%), with respect to the limit imposed by the GTFCh guidelines,[18] except the following six compounds: 4-FMC, 5-MeO-DALT, butylone, ethcathinone, MDPV, and methcathinone. Within SCs, the number of compounds not meeting the criteria for recovery and matrix effect was higher and included the following: 5F-ADB, 5F-AKB-48, 5F-AMB-PINACA, 5F-JWH-412, 5F-PCN, A-834,735, AB-005, AB-005 azepane, AB-FUBINACA, ADB-FUBICA, AKB-48, AM-1220 azepane, AM-1235, AM-1248 azepane, AM-2201 indazole carboxamide, AM-2232, AM-2233-azepane, AM-630, AMB-CHMICA, AMB-CHMINACA, AMB-FUBINACA, AMB-PINACA, Cumyl-BICA, Cumyl-PICA, EG-018, FUB-JWH-018, FUB-NPB-22, FUB-PB-22, JWH-015, JWH-020, JWH-030, JWH-122, JWH-145, JWH-147, JWH-182, JWH-213, JWH-250, JWH-302, JWH-370, JWH-387, JWH-412, MDMB-4en-PINACA, MDMB-CHMCZCA, MDMB-CHMINACA, MDMB-FUBINACA, MDMB-PICA, MN-25, N-phenyl-SDB-006, NE-CHMIMO, THJ-2201 XLR-11, XLR-11 isomer. Matrix effect and recovery for all analytes are shown in the supporting information.

3.3 | Application to real forensic cases

All the samples collected during forensic autopsies, with a post-mortem interval ranging from 2 to 10 days after death, tested negative for NPS, while several fatal drug intoxications were detected by applying previously validated methods for classical drugs of abuse. Among DUID samples, three tested positive for fentanyl and

TABLE 2 Analytes of interest (semi-systematic and IUPAC names) of panel 3, together with retention time (RT), cone voltage (CV), quantifier (*) and qualifier ion transitions and collision energies (Ce)

N	Analyte	RT (min)	Detection window (min)	Precursor ion (m/z)	Product ions (m/z)	CV (V)	Ce (V)
Synthetic cannabinoids (SCs)							
82	4-HTMPIPO 4-hydroxy-3,3,4-trimethyl-1-(1-pentyl-1H-indol- 3-yl) pentan-1-one	10.7	10–11.7	330	144 214*	20	45 30
83	4F-MDMB-BINACA methyl (S)-2-(1-[4-fluorobutyl]-1*H*-indazole-3-carboxamido)-3,3-dimethylbutanoate	11.9	11.5–12.8	364	219* 304	36	24 18
84	5F-AB-001 (adamantan-1-yl)[1-(5-fluoropentyl)-1H-indol-3-yl] methanone	13.5	12.6–14	368	79 135*	36	40 34
85	5F-AB-PICA N-[(2S)-1-Amino-3-methyl-1-oxobutan-2-yl]-1-(5-fluoropentyl)-1H-indole-3-carboxamide	9.8	9.1–11	348	232* 331	36	20 10
86	5F-AB-PINACA N-[(2S)-1-Amino-3-methyl-1-oxobutan-2-yl]-1-(5-fluoropentyl)-1H-indazole-3-carboxamide	10.0	9.1–11	349	145 233*	36	40 20
87	5F-ADB-PICA N-[(2S)-1-Amino-3,3-dimethyl-1-oxobutan-2-yl]-1-(5-fluoropentyl)-1H-indole-3-carboxamide	10.3	9.1–11	362	144 232*	34	40 20
88	5F-ADB-PINACA N-[(2S)-1-Amino-3,3-dimethyl-1-oxobutan-2- yl]-1-(5-fluoropentyl)-1H-indazole-3- carboxamide	10.6	10–11.7	363	233 318*	35	29 14
89	5F-AMB-PINACA Methyl (2S)-2-{[1-(5-fluoropentyl)-1H-indazole-3-carbonyl]amino}-3-methylbutanoate	12.0	10.8–12.5	364	233 304*	36	20 18
90	5F-APP-PICA N-(1-amino-1-oxo-3-phenylpropan-2-yl)-1-(5-fluoropentyl)indole-3-carboxamide	10.4	7.5–12	396	232* 144	26	26 44
91	5F-APP-PINACA N-(1-amino-1-oxo-3-phenylpropan-2-yl)-1-(5-fluoropentyl)-1H-indazole-3-carboxamide	10.6	7.5–12	397	233* 145	20	22 46
92	5F-Cumyl-PINACA 1-(5-fluoropentyl)-N-(2-phenylpropan-2-yl)indazole-3-carboxamide	12.5	10.5–15	368	233* 250	20	18 18
93	5F-EMB-PINACA/5F-MDMB-PINACA (isomers) Ethyl 2-[[1-(5-fluoropentyl)indazole-3-carbonyl] amino]-3-methyl-butanoate Methyl (2S)-2-{[1-(5-fluoropentyl)-1H-indazole-3-carbonyl]amino}-3,3-dimethylbutanoate	12.3	11.5–12.8	378	145 233*	36	40 24
94	5F-JWH-412 (4-Fluoro-1-naphthalenyl)[1-(5-fluoropentyl)-1H-indol-3-yl]methanone	12.7	11.9–13.4	378	145 173*	45	40 30
95	5F-MDMB-P7AICA methyl (S)-2-(1-(5-fluoropentyl)-1H-pyrrolo[2,3-b] pyridine-3-carboxamido)-3,3-dimethylbutanoate	11.1	10–11.7	378	145* 233	45	40 24
96	5F-MDMB-PICA methyl (2S)-2-{[1-(5-fluoropentyl)-1H-indole-3-carbonyl]amino}-3,3-dimethylbutanoate	11.8	10.8–12.5	377	144 232*	36	40 20
97	5F-PCN 1-(5-Fluoropentyl)-N-(naphthalen-1-yl)-1H-pyrrolo [3,2-c]pyridine-3-carboxamide	12.8	12.2–13.5	376	145 233*	40	40 20
98	5F-PY-PICA (1-(5-fluoropentyl)-1H-indol-3-yl)(pyrrolidin-1-yl) methanone	10.6	10–11.7	303	144 232*	35	30 20

(Continues)

TABLE 2 (Continued)

N	Analyte	RT (min)	Detection window (min)	Precursor ion (m/z)	Product ions (m/z)	CV (V)	Ce (V)
99	A-796,260 [1-(2-morpholin-4-ylethyl)-1H-indol-3-yl]-(2,2,3,3-tetramethylcyclopropyl) methanone	9.0	6.5–10	355	114 125*	36	32 24
100	A-834,735 [1-(oxan-4-ylmethyl)indol-3-yl]-(2,2,3,3-tetramethylcyclopropyl)methanone	12.6	11.9–13.4	340	125 242*	45	35 22
101	AB-001 (adamantan-1-yl)(1-pentyl-1H-indol-3-yl)methanone	14.3	13.5–15	350	79 135*	36	45 40
102	AB-005 {1-[(1-Methylpiperidin-2-yl)methyl]-1H-indol-3-yl}(2,2,3,3-tetramethylcyclo propyl)methanone	9.5	6.5–10	353	112* 125	36	45 38
103	AB-005 azepane (1-[1-methylazepan-3-yl]-1H-indol-3-yl)(2,2,3,3-tetramethylcyclo propyl)methanone	9.6	6.5–10	353	112* 125	36	45 38
104	AB-BICA N-[(2S)-1-amino-3-methyl-1-oxobutan-2-yl]-1-benzylindole-3-carboxamide	10.0	9.1–11	350	234* 333	20	20 15
105	AB-CHMICA N-[(2S)-1-amino-3-methyl-1-oxobutan-2-yl]-1-(cyclohexylmethyl)indazole-3-carboxamide	11.2	10–11.7	356	240* 339	35	20 15
106	AB-FUB7AICA N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamide	9.3	6.5–10	369	109* 253	36	40 20
107	AB-FUBICA N-[(2S)-1-amino-3-methyl-1-oxobutan-2-yl]-1-[(4-fluorophenyl)methyl]-1H-indole-3- carboxamide	10.2	9.1–11	368	109 252*	36	25 15
108	AB-FUBINACA 2/3-fluorobenzyl isomers N-[(1S)-1-(aminocarbonyl)-2-methylpropyl]-1-[(2-fluorophenyl)methyl]-1H-indazole-3-carboxamide	10.5	9.1–11	369	109* 253	36	40 20
109	AB-PICA N-[(2S)-1-amino-3-methyl-1-oxobutan-2-yl]-1-pentyl-1H-indole-3-carboxamide	10.7	10–11.7	330	144 214*	35	40 20
110	AB-PINACA N-[(2S)-1-amino-3-methyl-1-oxobutan-2-yl]-1-pentyl-1H-indazole-3-carboxamide	11.0	10–11.7	331	215* 286	20	24 20
111	ADB-BICA N-[(2S)-1-amino-3,3-dimethyl-1-oxobutan-2-yl]-1-benzyl-1H-indole-3-carboxamide	10.5	9.1–11	364	234* 347	20	24 20
112	ADB-BINACA N-[(2S)-1-Amino-3,3-dimethyl-1-oxobutan-2-yl]-1-benzyl-1H-indazole-3-carboxamide	10.8	10–11.7	365	91* 320	35	40 14
113	ADB-CHMICA N-[1-(aminocarbonyl)-2,2-dimethylpropyl]-1-(cyclohexylmethyl)-1H-indole-3-carboxamide	11.9	10.8–12.5	370	240 353	36	20 15
114	ADB-FUBICA N-[(2S)-1-amino-3,3-dimethyl-1-oxobutan-2-yl]-1-[(4-fluorophenyl)methyl]-1H-indole-3-carboxamide	10.6	10–11.7	382	252* 365	20	30 20
115	ADB-PICA/ADBICA N-[(2S)-1-Amino-3,3-dimethyl-1-oxobutan-2-yl]-1-pentyl-1H-indole-3-carboxamide	11.2	10.8–12.5	344	144 214*	20	40 20
116	ADB-PINACA N-(1-amino-3,3-dimethyl-1-oxo-2-butanyl)-1-pentyl-1H-indazole-3-carboxamide]	11.6	10.8–12.5	345	145 215*	20	40 20

TABLE 2 (Continued)

N	Analyte	RT (min)	Detection window (min)	Precursor ion (m/z)	Product ions (m/z)	CV (V)	Ce (V)
117	AKB-48 N-(adamantan-1-yl)-1-pentyl-1H-indazole-3-carboxamide	14.6	13.5–15	366	93* 135*	36	40 35
118	AM-1220 azepane (1-([1-methylazepan-3-yl]methyl)-1H-indol-3-yl)(naphthalen-1-yl)methanone	8.8	6.5–10	383	98* 155	45	50 40
119	AM-1235 1-[(5-fluoropentyl)-6-nitro-1H-indol-3-yl]-(naphthalen-1-yl)methanone	12.7	11.9–13.4	405	155* 277	45	35 20
120	AM-1241 (2-Iodo-5-nitrophenyl){1-[(1-methylpiperidin-2-yl)methyl]-1H-indol-3-yl}methanone	8.5	6.5–10	504	98* 112	45	35 20
121	AM-1248 [1-(1-methylpiperidin-2-yl)-1H-indol-3-yl](adamant-1-yl)methanone	10.0	9.1–11	391	112 135*	45	40 40
122	AM-1248 azepane adamantan-1-yl(1-[1-methylazepan-3-yl]-1H-indol-3-yl)methanone	10.1	9.1–11	391	112* 135	45	40 40
123	AM-2201 indazole carboxamide N-(naphthalen-1-yl)-1-(5-fluoropentyl)-1H-indazole-3-carboxamide	12.8	12.2–13.5	376	213 233*	45	24 24
124	AM-2232 [1-(4-cyanobutyl)-1H-indol-3-yl](naphthalen-1-yl)methanone	11.6	10.8–12.5	353	127* 155	45	38 45
125	AM-2233 azepane (2-iodophenyl)-[1-(1-methylazepan-3-yl)indol-3-yl]methanone	8.0	6.5–10	459	112* 231	45	50 20
126	AM-630 [6-iodo-2-methyl-1-(2-morpholin-4-ylethyl)indol-3-yl]-(4-methoxyphenyl)methanone	9.3	6.5–10	505	114 135*	45	40 35
127	AM-679 (2-iodophenyl)(1-pentyl-1H-indol-3-yl)methanone	13.0	12.2–13.5	418	203 231*	45	35 35
128	AMB-CHMICA methyl 2-{[1-(cyclohexylmethyl)-1H-indol-3-yl]formamido}-3-methylbutanoate	12.6	11.9–13.4	371	144 240*	20	24 15
129	AMB-CHMINACA methyl (1-(cyclohexylmethyl)-1H-indazole-3-carbonyl)-valinate	13.3	12.6–14	372	241* 312	36	20 15
130	AMB-FUBICA methyl (2S)-2-({1-[(4-fluorophenyl)methyl]-1H-indole-3-carbonyl}amino)-3-methylbutanoate	11.6	10.8–12.5	383	109* 252	36	35 24
131	AMB-FUBINACA methyl (2S)-2-({1-[(4-fluorophenyl)methyl]-1H-indazole-3-carbonyl}amino)-3-methylbutanoate	12.1	11.5–12.8	384	253* 324	45	24 18
132	AMB-PICA methyl (2S)-2-[(1-pentyl-1H-indole-3-carbonyl)amino]-3-methylbutanoate	12.2	11.5–12.8	345	144 214*	30	38 12
133	AMB-PINACA methyl (2S)-2-[(1-pentyl-1H-indazole-3- carbonyl)amino]-3-methylbutanoate	12.8	12.2–13.5	346	215* 286	36	28 20
134	BB-22 1-pentyl-1H-indole-3-carboxylic acid 8-quinolinyl ester	13.2	12.6–14	385	144 240*	36	40 20
135	Cumyl-4CN-BINACA 1-(4-cyanobutyl)-N-(2-phenylpropan-2-yl)-1H-indazole-3-carboxamide	11.5	10.8–12.5	361	226* 243	36	22 20

(Continues)

TABLE 2 (Continued)

N	Analyte	RT (min)	Detection window (min)	Precursor ion (m/z)	Product ions (m/z)	CV (V)	Ce (V)
136	Cumyl -BICA 1-Butyl-N-(2-phenylpropan-2-yl)-1H-indole-3-carboxamide	12.3	11.5–12.8	335	174 217*	30	40 20
137	Cumyl -PICA 1-Pentyl-N-(2-phenylpropan-2-yl)-1H-indole-3-carboxamide	12.7	11.9–13.4	349	188 231*	36	36 20
138	Cumyl -THPINACA N-(1-methyl-1-phenylethyl)-1-[(tetrahydro-2H-pyran-4-yl)methyl]-1H-indazole-3-carboxamide	11.8	10.8–12.5	378	243* 260	36	22 20
139	EG-018 (naphthalen-1-yl)(9-pentyl-9H-carbazol-3-yl) methanone	14.4	13.5–15	392	127* 155	45	45 38
140	EG-2201 [9-(5-fluoropentyl)-9H-carbazol-3-yl] (naphthalen-1-yl)methanone	13.5	13–14.5	410	127* 155	45	45 38
141	FUB-JWH-018 (1-(4-fluorobenzyl)-1H-indol-3-yl)(naphthalen-1-yl) methanone	12.7	11.9–13.4	380	109* 155	45	45 35
142	FUB-NPB-22 quinolin-8-yl 1-(4-fluorobenzyl)-1H-indazole-3-carboxylate	12.00	11.5–12.8	398	109* 253	45	45 20
143	FUB-PB-22 naphthalen-1-yl 1-[(4-fluorophenyl)methyl]-1H-indole-3-carboxylate	12.1	11.5–12.8	397	109* 252	45	45 24
144	JWH-011 (1-heptan-2-yl-2-methylindol-3-yl)-naphthalen-1-ylmethanone	14.0	13.5–15	384	127* 155	45	45 40
145	JWH-015 2-methyl-1-propyl-1H-indol-3-yl (naphthalen-1-yl) methanone	12.8	12.2–13.5	328	127* 155	45	45 22
146	JWH-020 (1-heptyl-1H-indol-3-yl)(naphthalen-1-yl)methanone	14.0	13.5–15	370	127* 155	45	45 35
147	JWH-022 naphthalen-1-yl[1-(pent-4-en-1-yl)-1H-indol-3-yl] methanone	13.0	12.2–13.5	340	127* 155	45	45 35
148	JWH-030 (1-hexylpyrrol-3-yl)-naphthalen-1-ylmethanone	12.7	11.9–13.4	292	127* 155	30	44 20
149	JWH-031 (1-hexyl-1H-pyrrol-3-yl)(naphthalen-1-yl)methanone	13.1	12.6–14	306	127* 155	45	44 20
150	JWH-073 (1-butyl-1H-indol-3-yl)(naphthalen-1-yl)methanone	13.0	12.2–13.5	328	127* 155	45	40 24
151	JWH-080 (1-butyl-1H-indol-3-yl)(4-methoxy-1-naphthalenyl)-methanone	13.1	12.6–14	358	185* 200	45	30 28
152	JWH-122 N-(4-pentenyl) (4-methylnaphthalen-1-yl)(1-(pent-4-en-1-yl)-1H-indol-3-yl)methanone	13.3	12.6–14	354	141 169*	45	40 30
153	JWH-145 naphthalen-1-yl(1-pentyl-5-phenyl-1H-pyrrol-3-yl) methanone	13.8	13–14.5	368	127 155*	45	28 30
154	JWH-147 (1-hexyl-5-phenyl-1H-pyrrol-3-yl)-naphthalen-1-ylmethanone	14.0	13.5–15	382	127* 155	45	45 40

TABLE 2 (Continued)

N	Analyte	RT (min)	Detection window (min)	Precursor ion (m/z)	Product ions (m/z)	CV (V)	Ce (V)
155	JWH-182 (1-pentyl-1H-indol-3-yl)(4-propylnaphthalen-1-yl) methanone	14.2	13.5–15	384	141 197*	45	45 20
156	JWH-213 (4-ethylnaphthalen-1-yl)(2-methyl-1-pentyl-1H-indol-3-yl)methanone	14.1	13.5–15	384	155 183*	45	40 20
157	JWH-249 2-(2-bromophenyl)-1-(1-pentyl-1H-indol-3-yl)-ethanone	13.3	12.6–14	384	144 169*	45	35 20
158	JWH-307 [5-(2-fluorophenyl)-1-pentyl-1H-pyrrol-3-yl] (naphthalene-1-yl)methanone	13.6	13–14.5	386	127* 155	45	45 35
159	JWH-309 1-naphthalenyl[5-(1-naphthalenyl)-1-pentyl-1H-pyrrol-3-yl]-methanone	14.2	13.5–15	418	127* 155	45	45 35
160	JWH-370 5-(2-methylphenyl)-1-pentyl-1H-pyrrol-3-yl (naphthalen-1-yl)methanone	14.0	13–14.5	382	127* 155	45	45 35
161	JWH-387 4-bromonaphthalen-1-yl(1-pentyl-1H-indol-3-yl) methanone	14.0	13.5–15	420	205 233*	45	30 25
162	JWH-412 (4-fluoronaphthalen-1-yl)(1-pentyl-1H-indol-3-yl) methanone	13.6	13–14.5	360	145 173*	45	40 30
163	JWH-424 (8-bromonaphthalen-1-yl)(1-pentyl-1H-indol-3-yl) methanone	13.2	12.6–14	420	205 233*	45	35 30
164	M-144 (1-(5-fluoropentyl)-2-methyl-1H-indol-3-yl) (2,2,3,3-tetramethylcyclopropyl)methanone	13.6	13–14.5	344	158 246*	36	34 20
165	MDMB-4en-PINACA methyl (S)-3,3-dimethyl-2-(1-(pent-4-en-1-yl)-1H-indazole-3-carboxamido)butanoate	12.8	12.2–13.5	358	145 213*	36	40 20
166	MDMB-CHMCZCA methyl (2S)-2-{[9-(cyclohexylmethyl)-9H-carbazole-3-carbonyl]amino}-3,3- dimethylbutanoate	13.7	13–14.5	435	290* 194	45	25 45
167	MDMB-CHMINACA methyl (2S)-2-{[1-(cyclohexylmethyl)-1H-indazole-3-carbonyl]amino}-3,3- dimethylbutanoate	13.71	13–14.5	386	241* 326	36	24 18
168	MDMB-FUBICA methyl (2S)-2-({1-[(4-fluorophenyl)methyl]-1H-indole-3-carbonyl}amino)-3,3-dimethylbutanoate	12.0	11.5–12.8	397	109* 252	45	40 20
169	MDMB-FUBINACA methyl (2S)-2-({1-[(4-fluorophenyl)methyl]-1H-indazole-3-carbonyl}amino)-3,3-dimethylbutanoate	12.5	11.9–13.4	398	253* 338	45	24 18
170	MDMB-PICA methyl (2S)-3,3-dimethyl-2-[(1-pentyl-1H-indole-3-carbonyl)amino]butanoate	13.1	11.9–13.4	359	144* 233	36	40 30
171	MDMB-PINACA methyl (2S)-3,3-dimethyl-2-[(1-pentyl-1H-indazole-3-carbonyl)amino]butanoate	13.2	12.6–14	360	145 215*	36	40 25
172	MEPIRAPIM (4-methylpiperazin-1-yl)(1-pentyl-1H-indol-3-yl) methanone	7.5	6.5–10	314	144 214*	36	40 20

(Continues)

TABLE 2 (Continued)

N	Analyte	RT (min)	Detection window (min)	Precursor ion (m/z)	Product ions (m/z)	CV (V)	Ce (V)
173	MMB-022 methyl (1-(pent-4-en-1-yl)-1H-indole-3-carbonyl)-L-valinate	11.8	10.8–12.5	343	144 212*	36	38 20
174	MN-25 7-methoxy-1-[2-(morpholin-4-yl)ethyl]-N- [(1S,2S,4R)-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl]-1H-indole-3-carboxamide	9.8	6.5–10	440	261* 353	45	25 18
175	N-Phenyl-SDB-006 1-pentyl-N-phenyl-1H-indole-3-carboxamide	12.4	11.5–12.8	307	144 214*	30	34 20
176	NE-CHMIMO [1-(cyclohexylmethyl)-1H-indol-3-yl]-1-naphthalenyl-methanone	13.8	13–14.5	368	127 155*	45	28 20
177	SDB-005 naphthalen-1-yl 1-pentyl-1H-indazole-3-carboxylate	13.7	13–14.5	359	145* 215	36	40 20
178	THJ-2201 [1-(5-fluoropentyl)-1H-indazol-3-yl] (naphthalen-1-yl) methanone	12.9	12.2–13.5	361	213* 233*	45	24 20
179	WIN 55.212–2 (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1- naphthalenylmethanone	10.9	10–11.7	427	127 155*	45	42 38
180	XLR-11 [1-(5-fluoropentyl)-1H-indol-3-yl] (2,2,3,3-tetramethylcyclopropyl)methanone	13.1	12.2–13.5	330	125* 330	36	32 10
181	XLR-11 isomer	12.7	11.9–13.4	330	125* 330	36	32 10
182	XLR-12 (2,2,3,3-tetramethylcyclopropyl) [1-(4,4,4-trifluorobutyl)-1H-indol-3-yl]methanone	13.1	12.6–14	352	125* 254	36	32 20

Abbreviation: *N*, number.

ketamine, administered in the emergency room. Concentrations of fentanyl and ketamine were in the range 0.65–1.67 and 570–1,000 ng/ml, respectively.

4 | DISCUSSION

The major challenge in the analysis of NPS resides in the diversity of structures and physicochemical properties among different NPS classes and within a single NPS class.[11,16] Several methods are already available in the literature to detect and/or quantify NPS in the main biological matrices[20–26] and particularly in whole blood,[27–31] though methods including a high number of compounds pertaining to different classes are still scarce.[22] In the present work, a LC-MS/MS screening method for the rapid determination of 182 NPS in blood, including a wide-panel of SCs and very recently emerged compounds, for example, 4F-MDMB-BINACA,[32,33] as well as multiple drug classes has been developed. Protein precipitation was chosen as an easy procedure for sample preparation. As reported in the literature, SCs tend to be better extracted by liquid–liquid extraction,[20,25,34,35] while both liquid–liquid and solid-phase extraction have been shown applicable for the extraction of fentanyl and its analogues,[21,36] as well as for amphetamines and tryptamines.[26] However, previous studies have also shown that protein precipitation could be used for SCs, SCAs, ketamine and stimulants with good efficiency.[11,22,37] This type of sample preparation strongly simplifies the laboratory routine in terms of easiness and time saving, only requiring a few minutes. Moreover, it is less expensive than other extraction procedures.[11,22] The use of different mobile phases for reconstitution (mobile phase B for SCs and mobile phase A/B, (80/20), (v/v) - for all other analytes) did not necessitate different chromatographic conditions, but only a total number of 3 injections per sample, with a run time of 17 minutes each.

An additional analytical challenge arises from the type of matrix to be analyzed. Serum and whole blood are certainly the preferable matrices to analyze NPS in fatal and non-fatal intoxications. Compared to serum or plasma, whole blood often requires additional steps in sample preparation, and some substances might show different concentrations in plasma or serum when compared to whole blood. However, the latter is often the only available matrix in postmortem toxicology. In fact, postmortem blood is characterized by a variable

TABLE 3 Precision (relative standard deviation or RSD), accuracy (bias), linearity (R^2: Regression coefficient), and limit of detection (LOD) and of quantification (LOQ) of the analytes

	QC low			QC high				
Analyte	Intraday (RSD %)	Interday (RSD %)	Accuracy (bias %)	Intraday (RSD %)	Interday (RSD %)	Accuracy (bias %)	R^2	LOD – LOQ
Synthetic cannabinoids (SCs)								
4-HTMPIPO	6.01	7.13	6.8	4.20	5.94	−0.86	0.990	0.06–0.15
4F-MDMB-BINACA	2.96	4.36	8.0	4.70	5.12	0.96	0.997	0.04–0.09
5-Cl-AB-PINACA	10.82	10.82	1.8	5.40	6.87	−6.19	0.997	0.42–1.25
5-Cl-THJ-018	14.98	14.98	−1.1	3.60	6.26	−5.46	0.995	0.17–0.5
5F-AB-001	3.28	9.36	1.10	5.60	5.62	−4.60	0.991	0.14–0.94
5F-AB-PICA	10.51	10.79	−2.9	5.40	6.19	−5.09	0.994	0.09–0.39
5F-AB-PINACA	3.97	7.42	6.6	6.00	6.26	−1.57	0.996	0.17–0.
5F-ADB	7.42	7.42	3.2	6.50	6.53	−4.67	0.999	0.14–0.97
5F-ADB-PICA	2.08	7.61	2.5	6.00	6.37	−4.16	0.993	0.13–0.85
5F-ADB-PINACA	4.05	8.94	1.8	6.00	6.22	−6.46	0.995	0.05–0.14
5F-AMB-PINACA	5.35	5.39	7.8	4.90	5.74	−4.13	0.999	0.10–0.51
5F-APP-PICA	11.41	11.41	−1.1	4.40	4.39	0.09	0.997	0.08–0.25
5F-APP-PINACA	10.29	10.29	−2.3	7.80	8.47	−3.04	0.998	0.08–0.25
5F-Cumyl-PINACA	11.85	12.25	−2.3	4.40	5.40	−0.57	0.998	0.01–0.04
5F-EMB-PINACA/5F-MDMB-PINACA (isomers)	4.72	7.35	9.0	2.20	5.40	−1.55	0.994	0.03–0.08
5F-JWH-412	8.23	8.23	5.8	2.60	4.70	0.31	0.997	0.08–0.25
5F-MDMB-P7AICA	8.88	9.63	5.4	7.16	7.16	−5.1	0.984	0.03–0.08
5F-MDMB-PICA	3.01	4.68	9.9	2.20	4.98	−3.0	0.993	0.04–0.10
5F-NNEI 2′-naphtyl isomer	13.59	13.59	−2.8	8.70	10.23	−0.76	0.997	0.04–0.11
5F-PCN	12.09	12.09	3.0	6.50	6.5	3.94	0.996	0.17–0.5
5F-PY-PICA	5.5	5.5	9.8	4.80	8.42	2.53	0.999	0.04–0.10
A-796,260	5.23	6.05	6.2	6.40	6.81	−0.51	0.994	0.05–0.13
A-834,735	8.92	8.92	4.6	9.30	9.33	−2.80	0.996	0.17–0.5
AB-005	4.93	4.93	9.4	4.90	6.24	2.44	0.990	0.08–0.26
AB-005 azepane	6.30	8.95	7.1	6.80	7.31	−1.34	0.991	0.07–0.20
AB-BICA	8.54	8.54	0.6	6.20	8.01	−4.67	0.990	0.13–0.86
AB-CHMICA	6.84	7.48	5.1	9.60	9.56	2.84	0.993	0.09–0.40
AB-FUB7AICA	7.37	9.76	0.0	6.70	6.90	−1.44	0.992	0.17–0.5
AB-FUBICA	6.80	8.48	6.2	9.30	9.33	−1.86	0.993	0.02–0.07
AB-FUBINACA	5.50	6.98	−1.8	6.50	9.77	−8.72	0.996	0.08–0.25
AB-FUBINACA 2 fluorobenzyl/3 fluoro	9.34	9.34	6.6	7.60	7.58	−1.99	0.995	0.04–0.11
AB-PICA	9.94	9.94	4.4	5.40	8.00	−3.63	0.990	0.05–0.12
AB-PINACA	3.79	7.32	3.0	5.00	5.36	−1.23	0.995	0.03–0.07
ADB-BICA	7.22	7.22	0.5	4.10	6.72	−2.92	0.998	0.05–0.15
ADB-BINACA	12.01	12–01	−0.6	1.90	3.38	−2.74	0.992	0.04–0.11
ADB-FUBICA	10.17	10.17	5.5	2.80	5.94	−4.21	0.998	0.06–0.19
ADB-FUBINACA	14.48	14.48	−0.5	8.40	8.42	−4.08	0.999	0.08–0.25
ADB-PICA/ADBICA	8.60	8.60	5.7	4.90	5.42	−3.41	0.991	0.02–0.05
AM-1220-azepane	6.99	8.33	1.3	5.00	7.78	−4.10	0.996	0.17–0.5
AM-1241	6.83	6.83	7.3	2.30	6.79	−0.37	0.995	0.07–0.21
AM-1248	3.19	3.69	5.7	4.70	8.68	−2.83	0.995	0.06–0.19
AM-1248 azepane	5.05	5.30	9.2	5.00	5.20	−0.92	0.992	0.04–0.10

(Continues)

TABLE 3 (Continued)

Analyte	QC low			QC high				
	Intraday (RSD %)	Interday (RSD %)	Accuracy (bias %)	Intraday (RSD %)	Interday (RSD %)	Accuracy (bias %)	R^2	LOD – LOQ
AM-2201	6.25	6.76	0.4	5.30	7.09	2.97	0.999	0.17–0.5
AM-2201 indazol carboxamide	9.80	9.80	−1.6	2.90	3.64	−5.39	0.994	0.17–0.5
AM-2232	7.34	7.34	7.6	5.50	6.27	−2.92	0.991	0.05–0.13
AM-2233	8.98	9.45	−3.6	2.50	2.92	0.67	0.999	0.06–0.19
AM-2233 azepane	9.38	10.33	4.5	4.40	4.44	−2.47	0.996	0.05–0.14
AM-630	6.32	7.47	2.5	7.60	7.85	−3.40	0.992	0.07–0.23
AM-679	8.52	8.52	4.1	6.00	6.04	−7.76	0.993	0.06–0.15
AM-694	12.29	12.29	−5.5	3.90	6.48	2.62	0.999	0.17–0.5
AMB-CHMICA	4.75	4.85	7.9	5.40	5.36	−1.23	0.998	0.13–0.78
AMB-CHMINACA	11.04	11.04	4.4	6.20	6.78	−4.13	0.993	0.17–0.5
AMB-FUBICA	6.42	6.42	9.6	3.80	6.84	−1.27	0.993	0.06–0.17
AMB-FUBINACA	4.44	6.78	6.2	5.90	5.92	−2.59	0.998	0.07–0.24
AMB-PICA	6.96	8.90	5.3	3.50	7.42	−2.16	0.994	0.09–0.33
AMB-PINACA	6.28	7.13	0.1	5.00	5.31	−2.63	0.996	0.17–0.5
APP-FUBINACA	11.34	13.49	0.0	13.60	14.54	−2.64	0.997	0.02–0.05
BB-22	6.77	7.10	4.9	4.90	8.26	−0.18	0.990	0.05–0.13
Cumyl-4CN-BINACA	6–75	7.18	5.1	3.60	4.79	1.4	0.998	0.08–0.28
Cumyl-BICA	6.72	7.99	0.8	3.10	5.26	2.64	0.999	0.10–0.48
Cumyl-PEGACLONE	9.12	9.12	5.4-	2.90	7.45-	−1.11	0.996	0.10–0.48
Cumyl-PICA	6.67	8.01	6.3	4.30	4.86	5.24	0.998	0.08–0.33
Cumyl-THPINACA	6.46	6.46	8.8	6.50	7.63	−0.65	0.999	0.11–0.57
EG-2201	4.94	9.55	3.4	7.60	7.63	−4.09	0.999	0.10–0.43
FUB-JWH-018	5.28	6.13	7.8	7.80	7.84	−3.27	0.994	0.04–0.10
FUB-NPB-22	3.33	3.91	9.6	3.80	4.31	1.63	0.999	0.08–0.32
FUB-PB-22	11.34	11.34	3.4	4.20	5.16	3.86	0.999	0.08–0.25
JWH-007	6.82	8.39	−2.6	5.00	4.98	−3.63	0.999	0.17–0.5
JWH-011	9.35	9.35	−3.7	6.80	8.87	−3.10	0.991	0.04–0.09
JWH-015	4.51	6.65	9.5	4.00	4.69	3.22	0.998	0.09–0.38
JWH-018	9.93	9.93	−2.6	9.00	8.98	−2.76	0.994	0.17–0.5
JWH-019	5.27	11.96	2.0	10.16	10.17	−0.38	0.999	0.03–0.09
JWH-020	14.91	14.91	0.2	3.70	4.45	3.52	0.994	0.06–0.18
JWH-022	6.47	6.47	9.3	4.80	4.84	−4.90	0.992	0.05–0.12
JWH-030	11.33	11.33	4.8	6.71	8.24	−1,81	0.999	0.06–0.16
JWH-031	8.21	8.21	7.9	5.40	5.38	−3.05	0.993	0.17–0.5
JWH-073	5.41	3.40	9.6	5.30	5.53	−3.09	0.995	0.03–0.09
JWH-080	2.89	9.78	2.4	2.50	6.03	−4.43	0.992	0.17–0.5
JWH-081	5.85	10.27	0.2	5.80	6.07	−3.87	0.999	0.13–0.79
JWH-098	6.42	6.42	−3.6	8.10	8.12	−2.89	0.999	0.17–0.5
JWH-122	10.42	10.42	−5.3	4.20	4.21	−4.00	0.999	0.03–0.09
JWH-122 N-(4-pentenyl)	10.25	10.25	4.8	4.10	6.12	0.70	0.991	0.03–0.07
JWH-145	5.10	9.27	2.5	5.70	5.67	−3.72	0.993	0.03–0.08
JWH-147	11.19	11.19	−1.9	8.70	9.19	−0.89	0.993	0.07–0.23
JWH-182	9.63	9.69	−0.4	3.10	5.60	−1.40	0.990	0.08–0.25
JWH-200	9.17	9.17	−2.8	1.70	4.24	−5.37	0.994	0.17–0.5

TABLE 3 (Continued)

Analyte	QC low			QC high				
	Intraday (RSD %)	Interday (RSD %)	Accuracy (bias %)	Intraday (RSD %)	Interday (RSD %)	Accuracy (bias %)	R^2	LOD – LOQ
JWH-213	9.79	9.79	1.5	2.20	2.21	0.69	0.991	0.06–0.18
JWH-249	7.41	8.92	2.2	4.10	4.11	−1.28	0.992	0.05–0.12
JWH-250	10.67	10.67	−2.4	3.10	7.13	−5.55	0.995	0.11–0.53
JWH-251	5.95	5.95	0.6	5.30	5.31	−6.23	0.998	0.17–0.5
JWH-302	0.83	0.86	−9.0	4.40	5.76	−4.41	0.999	0.04–0.10
JWH-307	14.08	14.08	−0.1	7.90	7.92	−1.18	0.992	0.02–0.07
JWH-309	12.53	12.27	−2.5	2.40	2.44	−7.50	0.992	0.10–0.48
JWH-370	14.37	14.37	−1.1	3.20	3.24	−3.20	0.993	0.10–0.49
JWH-387	10.21	10.21	−7.9	4.60	7.03	−2.14	0.991	0.13–0.82
JWH-412	13.71	13.71	−4.3	3.40	3.43	−0.31	0.994	0.07–0.20
JWH-424	11.49	11.49	−1.0	6.00	6.04	−5.65	0.993	0.08–0.32
M-144	6.45	8.65	3.0	5.10	5.12	−4.01	0.994	0.17–0.5
MDMB-4en-PINACA	6.42	7.52	1.7	5.20	5.22	−4.04	0.999	0.17–0.5
MDMB-CHMCZCA	7.05	7.05	−4.0	11.09	11.10	−3.33	0.999	0.07–0.22
MDMB-CHMICA	6.09	6.68	−8.9	3.80	4.88	−7.19	0.998	0.17–0.5
MDMB-CHMINACA	7.64	10.77	2.1	4.60	4.62	−4.32	0.998	0.17–0.5
MDMB-FUBICA	2.65	6.86	8.1	4.30	4.94	−0.91	0.999	0.11–0.54
MDMB-FUBINACA	7.88	7.88	3.3	9.40	9.38	−3.15	0.997	0.09–0.36
MEPIRAPIM	6.74	6.74	2.9	4.00	3.99	1.47	0.996	0.07–0.23
MMB-022	6.94	7.72	1.7	5.10	5.34	1.23	0.999	0.17–0.5
MMB-2201	4.97	4.97	−5.2	7.00	8.88	−2.43	0.998	0.17–0.5
MN-25	7.93	7.93	4.1	2.50	5.13	1.62	0.996	0.05–0.12
N-phenyl-SDB-006-	4.82	5.38	7.2	4.00	6.14	−0.95	0.992	0.06–0.17
NE-CHMIMO	5.27	5.27	2.3	8.40	8.37	0.30	0.995	0.08–0.26
RCS-4	11.11	11.11	−2.0	4.10	5.38	−2.95	0.997	0.12–0.67
RCS-8	4.97	9.91	−5.4	5.80	5.83	−4.19	0.998	0.07–0.19
SDB-005	3.32	6.95	7.4	5.10	5.78	−1.07	0.991	0.08–0.25
THJ-2201	7.08	7.08	0.8	4.60	4.56	−3.34	0.999	0.17–0.5
WIN 48.098	10.73	10.73	−4.7	2.90	5.32	−6.07	0.996	0.07–0.20
WIN 55.212–2	5.08	5.21	5.9	3.20	5.08	0.05	0.995	0.08–0.27
XLR-11	4.04	4.04	5.9	2.70	2.67	−3.28	0.999	0.08–0.27
XLR-11 isomer	4.08	4.08	9.4	5.50	5.58	−1.06	0.996	0.08–0.27
XLR-12	7.68	7.68	6.1	5.00	5.73	−1.12	0.995	0.09–0.33
Synthetic opioids (Sos)								
(±)-cis-3-methyl norfentanyl	3.31	9.56	2.7	8.00	8.54	−4.03	0.998	0.09–0.33
(±)-trans-3-methyl norfentanyl	6.02	8.46	7.0	3.70	4.94	−4.16	0.996	0.05–0.13
β-Hydroxy fentanyl	5.40	5.66	5.2	4.00	3.95	−0.72	0.995	0.04–0.10
β-Hydroxythiofentanyl	3.87	4.97	8.9	4.10	4.70	−3.81	0.996	0.03–0.08
β-Phenyl fentanyl	5.34	5.85	6.8	2.40	4.04	−0.58	0.993	0.07–0.22
4-ANPP	9.24	10.21	4.7	6.10	7.47	−1.61	0.992	0.17–0.5
Acetyl fentanyl	0.36	6.12	1.4	4.20	6.50	−5.05	0.997	0.03–0.08
Acetyl norfentanyl	0.84	7.22	3.1s	2.90	6.40	−5.50	0.996	0.08–0.30
Alfentanyl	2.08	9.45	4.7	4.20	4.23	−6.16	0.994	0.08–0.31
Butyryl fentanyl	7.40	7.40	9.6	3.40	6.24	−4.54	0.993	0.04–0.10

(Continues)

TABLE 3 (Continued)

Analyte	QC low			QC high				
	Intraday (RSD %)	Interday (RSD %)	Accuracy (bias %)	Intraday (RSD %)	Interday (RSD %)	Accuracy (bias %)	R^2	LOD – LOQ
Butyryl fentanyl carboxy metabolite	2.89	2.89	13.28	8.20	8.17	−4.64	0.996	0.04–0.10
Butyryl norfentanyl	4.88	7.67	4.7	6.60	6.61	−4.22	0.995	0.04–0.11
Carfentanyl	8.68	8.68	8.1	4.80	5.98	−2.05	0.996	0.07–0.25
Cyclopropylfentanyl	6.20	6.21	6.6	4.10	7.41	−3.85	0.993	0.09–0.33
Despropionyl para-fluorofentanyl	6.86	6.86	7.5	2.80	7.04	−3.02	0.996	0.08–0.25
Fentanyl	1.22	6.25	7.4	5.60	5.74	−3.52	0.996	0.07–0.23
Furanyl norfentanyl	9.02	9.02	4.5	4.20	7.22	−3.54	0.996	0.02–0.07
Methoxyacetyl norfentanyl	2.63	5.45	6.7	5.50	6.95	−5.76	0.997	0.11–0.53
Norfentanyl	2.98	2.98	11.35	4.80	5.36	−7.99	0.998	0.08–0.27
Phenylfentanyl	7.80	7.80	6.3	4.90	6.67	−4.94	0.995	0.09–0.40
Phenylacetyl fentanyl	7.83	7.83	3.6	5.70	7.37	−4.65	0.997	0.11–0.58
Valeryl fentanyl carboxy metabolite	6.09	8.14	6.8	6.90	6.94	−4.01	0.998	0.09–0.38
Synthetic cathinones (SCAs) stimulants and others								
αET	3.65	5.77	7.5	2,40	5.62	−5.48	0.998	0.17–0.5
3,4-DMMC	12.12	12.12	3.4	5.90	5.88	2.98	0.991	0.09–0.33
4-FMC	7.17	2.97	−8.3	7.10	7.11	−5.70	0.982	0.83–2.5
4-MEC	011.78	11.78	1.4	8.80	10.0	9.97	0.990	0.04–0.11
5-APB/6-APB	6.78	9.28	5.9	4.80	4.96	−0.44	0.993	0.11–0.59
5-EAPB	4.50	4.57	7.8	8.20	8.21	−5.74	0.994	0.06–0.16
5-MAPB/6-MAPB	8.53	8.53	8.5	3.00	6.66	−4.63	0.994	0.05–0.14
5-MeO-AMT	9.19	9.19	6.3	5.40	7.22	−4.25	0.996	0.13–0.84
5-MeO-DALT	7.76	8.17	5.7	3.50	5.42	−6.78	0.995	0.10–0.48
5-MeO-DMT	1.98	4.28	1.5	3.50	6.62	−4.88	0.993	0.07–0.21
5-MeO-DPT	10.59	10.59	5.1	6.20	7.54	−1.85	0.991	0.07–0.26
5-MeO-MiPT	1.03	7.77	4.9	3.90	4.67	0.96	0.996	0.12–0.74
Buphedrone	9.09	9.09	4.4	7.50	7.47	−0.84	0.994	0.04–0.11
Butylone	5.73	5.85	4.4	6.20	6.58	−4.15	0.998	0.04–0.11
Ethylone	4.51	6.04	5.9	7.10	9.60	−0.91	0.997	0.01–0.02
Ethylphenidate	6.75	6.75	7.9	6.90	6.93	−4.54	0.992	0.10–0.51
Ketamine	5.84	0.8	7.27	2.8	0.5	−15.31	0.998	0.17–0.5
MDPV	10.00	10.26	5.9	4.20	6.29	−2.09	0.997	0.07–0.24
Mephedrone	4.00	4.80	9.4	6.50	6.66	−5.76	0.998	0.42–1.25
Methedrone	9.18	9.18	7.1	4.10	7.99	−2.32	0.998	0.04–0.11
Methylone	5.55	5.55	6.5	7.20	7.17	−1.29	0.998	0.13–0.85
N.N-DMT	8.32	8.34	7.0	5.20	6.98	−5.17	0.995	0.12–0.66
Nordiazepam	16.81	11.9	−14.72	1.16	4.22	−12.04	0.998	0.17–0.5
Pentylone	9.86	9.86	6.6	9.20	9.2	−2.97	0.997	0.02–0.07
Ritalinic acid	11.05	11.05	2.5	6.30	6.96	−4.97	0.996	0.02–0.05

Note: Only validated compounds are shown.
Abbreviation: QC, quality control.

grade of hemolysis,[38] preventing serum or plasma separation. In the living subject, when an intoxication is suspected or in cases of suspected driving under the influence (DUI), blood and/or urine samples are often collected in hospitals. When the separation of serum is not performed directly in the hospital where the blood is taken, the vials are sometimes frozen and sent to a forensic laboratory, where separation of the hemolyzed material is no more achievable.[39]

As for the amount of whole blood, Adamowicz and Tokarczyk.[11] used 0.2 ml of blood, though the method was only a qualitative

screening with LODs ranging from 0.01 to 3.09 ng/ml. Other studies using the same amount of blood or serum showed higher sensitivity, though only analyzing a limited number of compounds.[22,37] In the method here presented, the use of a higher volume, similarly to previous studies,[20,23,26] provided a high sensitivity despite the high number of included substances. Nevertheless, future studies to reduce the needed volume of whole blood are encouraged.

Since the legislation on NPS is based on a substance-by substance (individual listing) basis or on generic or analogue control, rather than on define biological concentrations,[40] literature data on previous NPS analytical methods and on intoxications were used to establish the linearity ranges of the present study and to verify whether the sensitivity was acceptable. According to the literature, SCAs and stimulants in blood tend to be quantified mostly at few dozen/hundred nanograms per milliliter after recreational use and even higher levels are to be expected in cases of acute toxicity.[11,12,22,41,42] Tryptamines, fentanyl and SOs are also typically characterized by high concentrations in post-mortem or intoxication samples,[13,24,43,44] while expected concentrations of SCs in blood are generally lower.[13,14,44–46] Indeed, in the method of Kneisel and Auwärter,[20] the calibration points were in the range 0.01–2.0 ng/ml and the LODs in the range 0.001–0.1 ng/ml. However, concentrations up to 190 ng/ml have been reported.[47] Therefore, the LODs obtained with presented method are satisfactory for the purpose and provided sufficient sensitivity for all NPS classes.

Accuracy and precision were studied for all selected analytes at different concentrations and the criteria required for validation were met by 165 substances, which can be considered validated for quantitative purposes. The presented approach provides a very useful tool for the combined targeted analysis and broad screening of NPS in whole blood. Moreover, the method can be easily extended to include novel compounds, allowing for a quick adaption to the dynamic development of the NPS market.

The major limit of the present method resides in the recovery and, particularly, in the matrix effect for some molecules. As already shown in previous studies,[22] 4-FMC might be particularly problematic with regard to matrix effect. For SCs, in the study of Kneisel and Auwärter,[20] conducted on serum samples, most analytes were affected by remarkable matrix effects, and recovery was in the range 5.7–56%. Similarly, significant matrix effects were highlighted by methods involving protein precipitation, since this has been described to lead to large amounts of endogenous compounds in the injected sample, enhancing or reducing the signals.[11,32] Indeed, whole blood is a complex matrix, and it is very likely that the type of sample, as well as the employment of precipitation provoked matrix effects. However, the influence of such parameters, whenever linearity, accuracy and precision remain acceptable, is a matter of debate. Taking into account solely the analytes which showed acceptable recoveries and matrix effects, the method can be considered as a fully validated tool for 138 analytes of interest.

The difficulties related to ion suppression/enhancement have recently been shown in cases of analysis of whole blood samples with a method validated for serum.[48] Keeping in mind that the matrix effect could be severe, a standard addition method was suggested by the authors to provide a more precise quantification.

Another acknowledged limitation is represented by the use of only two internal standards. Though nordiazepam-D5 and ketamine-D4, which are widely available in most forensic laboratories, have proven satisfactory for the evaluation of accuracy and precision. Nevertheless, better results could be expected by using specific standards with more chemical similarity to the various NPS subclasses. On the other hand, the use of a limited number of broadly available internal standards can be seen as a strength of the method, in terms of costs and applicability in many forensic laboratories. On the basis of the chosen internal standard and due to its relevance as metabolite or co-consumed drug in NPS intoxications, nordiazepam was also included in the present method.

Finally, the presented method has so far only been applied to a very limited set of real-case samples. Despite the limitedness of the case study and the absence of positive findings regarding NPS intended "in a strict sense," the application of the method allowed the detection and quantification of ketamine and fentanyl. Online surveys have so far demonstrated a limited use of NPS in Italy in comparison to traditional drugs, with a prevalent consumption of phenethylamines and cathinones once/twice in lifetime.[49] Ketamine is one of the most cited NPS substances in the Italian mass media and its use was reported in online surveys by 66.7% of respondents,[49] while fentanyl is largely used in the emergency setting. In the literature, methods for NPS detection are usually applied only to a limited number of real-cases, due to difficulties in retrieving a wider casuistry[22,50] and the absence of broad-panel methods has so far hampered a thorough knowledge of the NPS prevalence in Italy. Even though the limited sample is certainly a drawback of the study, an extensive application of the method was beyond the scope of our research and future applications on a wider scale would be desirable to provide more comprehensive epidemiological data regarding NPS consumption.

5 | CONCLUSIONS

In the highly dynamic world of novel psychoactive substances (NPS), characterized by the ongoing emergence of multiple and chemically diverse compounds on the market, several challenges arise for the analysis of NPS. Since methods to simultaneously detect different classes of NPS are still scarce, the present methodology represents an easy, low cost, wide-panel method for the detection of more than 180 novel psychoactive substances, including 132 synthetic cannabinoids, 22 synthetic opioids, 28 among synthetic cathinones, stimulants and other drugs.

The developed method can be profitably applied both in a clinical context, with 17 × 3 min run time and a broad screening for multiple compounds, and in postmortem toxicology, where the multi-analyte method is advantageous by reducing time and costs of analysis. When considering real forensic cases and a quantitative analysis is requested, the matrix effect should be taken into consideration, and a multidisciplinary case-by-case evaluation, including an assessment of circumstantial, clinical, post-mortem, and toxicological data, is necessary.

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REFERENCES

1. EMCDDA. European drug report. Trends and developments. Luxembourg, 2020. https://www.emcdda.europa.eu/system/files/publications/13236/TDAT20001ENN_web.pdf. Accessed 28 July, 2021.
2. EMCDDA. New psychoactive substances: global markets, local threats and the COVID-19 pandemic —an update from the EU Early Warning System. Lisbon, 2020. https://www.emcdda.europa.eu/system/files/publications/13464/20205648_TD0320796ENN_PDF_rev.pdf. Accessed 28 July, 2021.
3. Halter S, Haschimi B, Mogler L, Auwärter V. Impact of legislation on NPS markets in Germany—the rise and fall of 5F-ADB. *Drug Test Anal*. 2020;12(6):853-856. https://doi.org/10.1002/dta.2786
4. Auwärter V, Dresen S, Weinmann W, Müller M, Pütz M, Ferreirós N. 'Spice' and other herbal blends: harmless incense or cannabinoid designer drugs? *J Mass Spectrom*. 2009;44(5):832-837. https://doi.org/10.1002/jms.1558
5. Uchiyama N, Kikura-Hanajiri R, Kawahara N, Haishima Y, Goda Y. Identification of a cannabinoid analog as a new type of designer drug in a herbal product. *Chem Pharm Bull(Tokyo)*. 2009;57(4):439-441. https://doi.org/10.1248/cpb.57.439
6. Schifano F, Orsolini L, Duccio Papanti G, Corkery JM. Novel psychoactive substances of interest for psychiatry. *World Psychiatry*. 2015;14(1):15-26. https://doi.org/10.1002/wps.20174
7. Baumann MH, Volkow ND. Abuse of New Psychoactive Substances: threats and solutions. *Neuropsychopharmacology*. 2016;41(3):663-665. https://doi.org/10.1038/npp.2015.260
8. Maurer HH, Brandt S. *New Psychoactive Substances*. Homburg (Saar): Springer; 2018.
9. Meyer MR, Maurer HH. Review: LC coupled to low- and high-resolution mass spectrometry for new psychoactive substance screening in biological matrices - Where do we stand today? *Anal Chim Acta*. 2016;927:13-20. https://doi.org/10.1016/j.aca.2016.04.046
10. Kong TY, Kim JH, Kim JY, et al. Rapid analysis of drugs of abuse and their metabolites in human urine using dilute and shoot liquid chromatography–tandem mass spectrometry. *Arch Pharm Res*. 2017;40(2):180-196. https://doi.org/10.1007/s12272-016-0862-1
11. Adamowicz P, Tokarczyk B. Simple and rapid screening procedure for 143 new psychoactive substances by liquid chromatography-tandem mass spectrometry. *Drug Test Anal*. 2016;8(7):652-667. https://doi.org/10.1002/dta.1815
12. Zaami S, Giorgetti R, Pichini S, Pantano F, Marinelli E, Busardò FP. Synthetic cathinones related fatalities: an update. *Eur Rev Med Pharmacol Sci*. 2018;22(1):268-274. https://doi.org/10.26355/eurrev_201801_14129
13. Kraemer M, Boehmer A, Madea B, Maas A. Death cases involving certain new psychoactive substances: a review of the literature. *Forensic Sci Int*. 2019;298:186-267. https://doi.org/10.1016/j.forsciint.2019.02.021
14. Giorgetti A, Busardò FP, Tittarelli R, Auwärter V, Giorgetti R. Postmortem toxicology: a systematic review of death cases involving synthetic cannabinoid receptor agonists. *Front Psych*. 2020;11(464):1-22. https://doi.org/10.3389/fpsyt.2020.00464
15. Giorgetti A, Centola C, Giorgetti R. Fentanyl novel derivative-related deaths. *Hum Psychopharmacol*. 2017;32(3):e2605. https://doi.org/10.1002/hup.2605
16. Kimble AN, DeCaprio AP. Systematic analysis of novel psychoactive substances. II. Development of a screening/confirmatory LC-QqQ-MS/MS method for 800+ compounds and metabolites in urine. *Forensic Chem*. 2019;6:100189. https://doi.org/10.1016/j.forc.2019.100189
17. National Early Warning System. 2021 Sistema Nazionale di Allerta Precoce. http://www.politicheantidroga.gov.it/it/attivita/sistema-nazionale-allerta-precoce/sistema-nazionale-di-allerta-precoce/. Accessed 28 July, 2021.
18. GTFCh. Requirements for the validation of analytical methods. 2009. https://www.gtfch.org/cms/images/stories/files/Appendix%20B%20GTFCh%2020090601.pdf. Accessed 28 July, 2021.
19. Pelletti G, Verstraete AG, Reyns T, et al. Prevalence of therapeutic drugs in blood of drivers involved in traffic crashes in the area of Bologna, Italy. *Forensic Sci Int*. 2019;302:109914. https://doi.org/10.1016/j.forsciint.2019.109914
20. Kneisel S, Auwärter V. Analysis of 30 synthetic cannabinoids in serum by liquid chromatography-electrospray ionization tandem mass spectrometry after liquid-liquid extraction. *J Mass Spectrom*. 2012;47(7):825-835. https://doi.org/10.1002/jms.3020
21. Fogarty MF, Papsun DM, Logan BK. Analysis of fentanyl and 18 novel fentanyl analogs and metabolites by LC-MS-MS, and report of fatalities associated with methoxyacetylfentanyl and cyclopropylfentanyl. *J Anal Toxicol*. 2018;42(9):592-604. https://doi.org/10.1093/jat/bky035
22. Vaiano F, Busardò FP, Palumbo D, et al. A novel screening method for 64 new psychoactive substances and 5 amphetamines in blood by LC-MS/MS and application to real cases. *J Pharm Biomed Anal*. 2016;129:441-449. https://doi.org/10.1016/j.jpba.2016.07.009
23. Fagiola M, Hahn T, Avella J. Screening of Novel Psychoactive Substances in postmortem matrices by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS-MS). *J Anal Toxicol*. 2018;42(8):562-569. https://doi.org/10.1093/jat/bky050
24. Lehmann S, Kieliba T, Beike J, Thevis M, Mercer-Chalmers-Bender K. Determination of 74 new psychoactive substances in serum using automated in-line solid-phase extraction-liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2017;1064:124-138. https://doi.org/10.1016/j.jchromb.2017.09.003
25. Montenarh D, Hopf M, Warth S, Maurer HH, Schmidt P, Ewald AH. A simple extraction and LC-MS/MS approach for the screening and identification of over 100 analytes in eight different matrices. *Drug Test Anal*. 2015;7(3):214-240. https://doi.org/10.1002/dta.1657
26. Wohlfarth A, Weinmann W, Dresen S. LC-MS/MS screening method for designer amphetamines, tryptamines, and piperazines in serum. *Anal Bioanal Chem*. 2010;396(7):2403-2414. https://doi.org/10.1007/s00216-009-3394-4
27. Odoardi S, Fisichella M, Romolo FS, Strano-Rossi S. High-throughput screening for new psychoactive substances (NPS) in whole blood by DLLME extraction and UHPLC-MS/MS analysis. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2015;1000:57-68. https://doi.org/10.1016/j.jchromb.2015.07.007
28. Ong RS, Kappatos DC, Russell SGG, et al. Simultaneous analysis of 29 synthetic cannabinoids and metabolites, amphetamines, and cannabinoids in human whole blood by liquid chromatography-tandem mass spectrometry—a New Zealand perspective of use in 2018. *Drug Test Anal*. 2020;12(2):195-214. https://doi.org/10.1002/dta.2697
29. Trana AD, Mannocchi G, Pirani F, et al. A comprehensive HPLC-MS-MS screening method for 77 new psychoactive substances, 24 classic

drugs and 18 related metabolites in blood, urine and oral fluid. *J Anal Toxicol*. 2020;44(8):769-783. https://doi.org/10.1093/jat/bkaa103
30. Ambach L, Redondo AH, König S, Angerer V, Schürch S, Weinmann W. Detection and quantification of 56 new psychoactive substances in whole blood and urine by LC-MS/MS. *Bioanalysis*. 2015;7(9):1119-1136. https://doi.org/10.4155/bio.15.48
31. Cláudia M, Pedro A, Tiago R, Francisco CR, Eugenia G. Determination of new psychoactive substances in whole blood using microwave fast derivatization and gas chromatography/mass spectrometry. *J Anal Toxicol*. 2020;44(1):92-102. https://doi.org/10.1093/jat/bkz053
32. Haschimi B, Mogler L, Halter S, et al. Detection of the recently emerged synthetic cannabinoid 4F-MDMB-BINACA in "legal high" products and human urine specimens. *Drug Test Anal*. 2019;11(9):1377-1386. https://doi.org/10.1002/dta.2666
33. Krotulski AJ, Mohr ALA, Kacinko SL, et al. 4F-MDMB-BINACA: a new synthetic cannabinoid widely implicated in forensic casework. *J Forensic Sci*. 2019;64(5):1451-1461. https://doi.org/10.1111/1556-4029.14101
34. Namera A, Kawamura M, Nakamoto A, Saito T, Nagao M. Comprehensive review of the detection methods for synthetic cannabinoids and cathinones. *Forensic Toxicol*. 2015;33(2):175-194. https://doi.org/10.1007/s11419-015-0270-0
35. Castaneto MS, Wohlfarth A, Desrosiers NA, Hartman RL, Gorelick DA, Huestis MA. Synthetic cannabinoids pharmacokinetics and detection methods in biological matrices. *Drug Metab Rev*. 2015;47(2):124-174. https://doi.org/10.3109/03602532.2015.1029635
36. Kahl JH, Gonyea J, Humphrey SM, Hime GW, Boland DM. Quantitative analysis of fentanyl and six fentanyl analogs in postmortem specimens by UHPLC-MS-MS. *J Anal Toxicol*. 2018;42(8):570-580. https://doi.org/10.1093/jat/bky054
37. Ambroziak K, Adamowicz P. Simple screening procedure for 72 synthetic cannabinoids in whole blood by liquid chromatography-tandem mass spectrometry. *Forensic Toxicol*. 2018;36(2):280-290. https://doi.org/10.1007/s11419-017-0401-x
38. Woydt L, Bernhard M, Kirsten H, et al. Intra-individual alterations of serum markers routinely used in forensic pathology depending on increasing post-mortem interval. *Sci Rep*. 2018;8(1):12811. https://doi.org/10.1038/s41598-018-31252-5
39. Pelletti G, Garagnani M, Rossi F, Roffi R, Barone R, Pelotti S. Optimization of cloned enzyme donor immunoassay cut-offs for drugs of abuse in whole blood of drivers involved in road accidents. *Forensic Sci Int*. 2019;294:27-33. https://doi.org/10.1016/j.forsciint.2018.10.023
40. UNODC Commission on Narcotic Drugs. New psychoactive substances: overview of trends, challenges and legal approaches. Vienna, 2016. https://www.unodc.org/documents/commissions/CND/CND_Sessions/CND_59/ECN72016_CRP2_V1601405_reissued.pdf. Accessed 13 September, 2021.
41. Paillet-Loilier M, Cesbron A, Le Boisselier R, Bourgine J, Debruyne D. Emerging drugs of abuse: current perspectives on substituted cathinones. *Subst Abuse Rehabil*. 2014;5:37-52. https://doi.org/10.2147/SAR.S37257
42. Prosser JM, Nelson LS. The toxicology of bath salts: a review of synthetic cathinones. *J Med Toxicol*. 2012;8(1):33-42. https://doi.org/10.1007/s13181-011-0193-z
43. Tittarelli R, Mannocchi G, Pantano F, Romolo FS. Recreational use, analysis and toxicity of tryptamines. *Curr Neuropharmacol*. 2015;13(1):26-46. https://doi.org/10.2174/1570159X13666141210222409
44. Elliott SP, Brandt SD, Smith C. The first reported fatality associated with the synthetic opioid 3,4-dichloro-N-[2-(dimethylamino) cyclohexyl]-N-methylbenzamide (U-47700) and implications for forensic analysis. *Drug Test Anal*. 2016;8(8):875-879. https://doi.org/10.1002/dta.1984
45. Adamowicz P. Fatal intoxication with synthetic cannabinoid MDMB-CHMICA. *Forensic Sci Int*. 2016;261:e5-e10. https://doi.org/10.1016/j.forsciint.2016.02.024
46. Gurney SM, Scott KS, Kacinko SL, Presley BC, Logan BK. Pharmacology, toxicology, and adverse effects of synthetic cannabinoid drugs. *Forensic Sci Rev*. 2014;26(1):53-78.
47. Hermanns-Clausen M, Kneisel S, Szabo B, Auwärter V. Acute toxicity due to the confirmed consumption of synthetic cannabinoids: clinical and laboratory findings. *Addiction*. 2013;108(3):534-544. https://doi.org/10.1111/j.1360-0443.2012.04078.x
48. Giorgetti A, Mogler L, Halter S, et al. Four cases of death involving the novel synthetic cannabinoid 5F-Cumyl-PEGACLONE. *Forensic Toxicol*. 2020;38(2):314-326. https://doi.org/10.1007/s11419-019-00514-w
49. EPS NPS PROJECT. The demand of New Psychoactive Substances. Italy, 2016. https://www.rissc.it/wp-content/uploads/2020/05/NPS_Final_Demand_report_def.pdf. Accessed 12 September, 2021.
50. Freni F, Bianco S, Vignali C, et al. A multi-analyte LC-MS/MS method for screening and quantification of 16 synthetic cathinones in hair: application to postmortem cases. *Forensic Sci Int*. 2019;298:115-120. https://doi.org/10.1016/j.forsciint.2019.02.036

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