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A comprehensive UHPLC–MS/MS screening method for the analysis of 98 New Psychoactive Substances and related compounds in human hair

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A comprehensive UHPLC–MS/MS screening method for the analysis of 98 New Psychoactive Substances and related compounds in human hair

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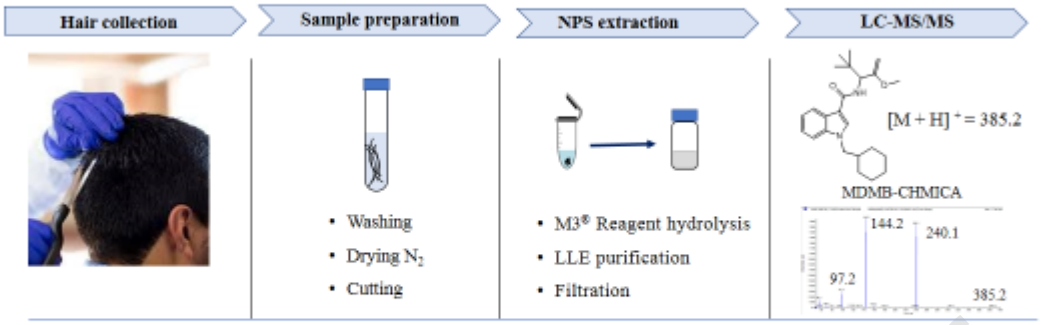
Graphical abstract



Graphical abstract

Highlights

- A fast LC-MS/MS method for the analysis of 98 NPS from hair is described.
- Various NPS targeted including tryptamines, cathinones, opioids, and cannabinoids.
- NPS extraction with M3® reagent followed by acidic and basic purification.
- The method validation showed good performance parameters and reduced matrix effect.
- It can be applied to routine toxicological studies in analytical laboratories.

Abstract

In this study, a rapid liquid chromatography-tandem mass spectrometry (LC-MS/MS) method has been developed and validated for the targeted analysis of 98 New Psychoactive Substances (NPS) from the hair matrix. The monitored compounds included various chemical classes (7 phenethylamines, 10 tryptamines, 18 cathinones, 24 synthetic opioids, and 38 synthetic cannabinoids) with emphasis given to newly emerged NPS. The method employed a direct

extraction process through the incubation of hair samples (25 mg) and internal standards with M3® reagent at 100 °C for 60 min, followed by extract purification through acid and basic liquid-liquid micro-extraction (LLME). Extracted compounds were analyzed through LC-MS/MS system operating in multiple reaction monitoring mode. NPS were separated in 9.5 min with a Poroshell 120 EC-C18 column (2.7 µm, 4.6 x 50 mm) using a gradient eluting mobile phase composed of water and acetonitrile/water (95:5) both containing 0.1% of formic acid. The developed and validated method shows a good precision (≤ 15%), linearity (R^2 between 0.993 and 0.999), selectivity, and sensitivity (LOD: 0.6 – 10.3 pg mg^{-1} and LOQ: 2.1 – 34.4 pg mg^{-1}). The method showed also reduced matrix effect and acceptable recovery for most of the targeted compounds. Our results showed that this method is suitable for quantifying NPS in hair matrix and could be employed in the context of routine analyses in analytical laboratories.

Keywords

Novel psychoactive substances (NPS); Abuse drugs; Hair analysis; UHPLC–MS/MS; opioids; cannabinoids.

1. Introduction

The constant emergence of new, unregulated drugs of abuse to bypass legislative controls represents a serious global concern [1]. Each year an important number of these substances appear on the drug market mimicking the effects of controlled drugs with unknown information on their toxicological profile and side effects [2, 3]. These substances grouped under the term New Psychoactive substances (NPS) are defined by the United Nations Office on Drugs and Crimes (UNODC) as "substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat" [4].

Up to January 2021, more than 1000 individual NPS have been reported including synthetic cannabinoids, synthetic cathinones, tryptamines, phenethylamines, piperazines, synthetic opioids, and other classes such as aminoindanes, phencyclidines-type substances, piperidines, and pyrrolidines [5, 6].

The response to the global phenomenon of NPS passes through the success of the screening methods to identify these substances in biological samples. However, the number of NPS is continuously increasing year after year, modifying the structure of previous illegal substances to circumvent drug-detection systems. For example, just in 2018, 55 NPS were reported for the first time in the European Union by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) [7]. Therefore, it is necessary to develop updated screening methods that can effectively identify and quantify known and newly introduced NPS.

Screening methods based on gas and liquid chromatography-mass spectrometry techniques (GC-MS and HPLC-MS) are the most used for the analysis of NPS within biological samples such as blood and urine [8-14]. These methods provide higher specificity and sensitivity compared to classical immunoassays or colorimetric screening tests [15]. More recently, the analysis of NPS from alternative biological matrices, allowing a non-invasive sampling such as oral fluid and hair has been reported in few studies [16-33].

Although less commonly tested, these alternative matrices especially hair, present some advantages over conventional matrices. Indeed, due to the incorporation of drugs into the keratin matrix before metabolism (polar metabolites being less incorporated into keratin), the analysis of hair samples allows targeting more efficiently parent drugs than their unknown metabolites [22]. Moreover, hair samples represent a less adulterated matrix due to the long stability of NPS into keratine. This stability allows a wider detection window with the possibility to evidence the chronic use of NPS [23]. However, hair sampling presents some limits such as the inability to detect recent drug consumption, the low concentrations, and the possible environmental contamination of hair, which can provide false-positive results [24, 25]

Among the limited papers reporting the analysis of NPS from hair samples, most studies focused on a restricted number of substances or specific groups of NPS such as cannabinoids [26], tryptamines [21], opioids [27], or cathinones [28]. Moreover, to our knowledge, few analytical methods have been reported to detect and quantify in keratin matrix newly emerged NPS such as novel synthetic cannabinoids (MMB-2201, APP-FUBINACA, 5-F-Cumyl-Pinaca, MDMB-CHMICA, 5-Cl THJ 018, and 2-naphthyl 5F-NNEI), novel synthetic opioids (carfentanyl), or novel phenylethylamines (ethylphenidate) (**Figure 1**).

Therefore, this study aims to develop a qualitative and quantitative UHPLC- ESI-MS/MS method for the screening in hair samples of 98 NPS from different classes through a single extraction procedure and a single chromatographic run. The novelty of this method will be the emphasis given to newly emerged NPS. This study will allow the implementation of a rapid, reliable, sensitive, and updated method, which can be routinizable in Toxicology laboratories for real sample analysis.

2. Materials and methods

2.1. Reagents and standards

Analytical standards of synthetic cannabinoids, synthetic opioids, cathinones, tryptamines, phenylethylamines, and internal standards (Methylone d_3, Ketamine d_4, Fentanyl d_5, and JWH-250-

4-Hydroxypentyl metabolite-d_5) were provided from Cerilliant, LGC Standards, Comedical, and Lipomed. All the standards were kept at -25 °C before analysis.

LC-MS grade methanol, isopropanol, acetonitrile, and dichloromethane stabilized with amylene (20 mg kg^{-1}), ammonia 30% for analysis, glacial acetic acid for ACS analysis, and diethyl ether stabilized with ethanol for pesticide analysis were supplied by Panreac Quimica (Barcellona, Spain). LC-MS grade formic acid was purchased from Honeywell-Fluka (Milan, Italy). The M3 reagent ® to extract the keratin matrix was purchased from Comedical® (Trento, Italy) and was stored at 2-8 °C. Deionized water was obtained from a Milli-Q Reagent Water System (Bedford, MA, USA) and other chemicals of high analytical grade were supplied by Sigma Chemical (Milan, Italy).

2.2. Preparation of calibration standards

The solutions used were prepared in methanol through suitable dilutions of the reference standards at a concentration of 25 and 250 pg μL^{-1}. The solutions were stored at - 20°C. These solutions were used for calibration, instrumental tuning, and the development of the analytical method. Working standard mixture solutions were prepared using the prepared solutions to enrich the drug- free human hair at 7 levels (5 - 500 pg mg^{-1}), while the solution of I.S (100 pg μL^{-1}) was added to obtain a final concentration in hair of 100 pg mg^{-1}.

2.3. Sample preparation

The correctly aliquoted keratin matrix (100-200 mg per sample) inserted inside 10 mL inert glass tubes has been subjected to the following decontamination cycle necessary to eliminate the main environmental contaminants (cosmetics, detergents, sebum, passive deposition of drugs and substances of abuse). Briefly, human hair samples were washed in 3 steps of vortexing followed by drying with 2 mL of water, then 2 mL of methanol twice, and 2 mL of butanol. After washing samples were dried under N_2. The washed sample was then transferred to a polypropylene jar and finely sliced into segments of about ½ mm through the use of scissors.

For each sample, 25 mg were weighed in a 10 mL hydrolysis tube and 25 µL of IS solution was added to the samples. The extraction of substances of abuse was performed using 500 µL of M3 reagent at 100°C for 60 min [27]. After the digestion process completion, samples were cooled to room temperature. At the end of the hydrolytic process, extracts were purified with two liquid-liquid micro-extraction (LLME) steps using a mixture of CH_2Cl_2 with 10% of isopropyl alcohol. The first LLME was performed in basic conditions adding 300 µL NH_4OH 3% and 500 µL of the CH_2Cl_2 mixture. The sample was vortexed for 90 seconds and the organic fraction was separated and collected through 5 min of centrifugation at 3400 rpm. The remained aqueous phase was purified through an acidic LLME adding 35 µL of CH_3COOH and 500 µL of the CH_2Cl_2 mixture, following the same conditions of vortex and centrifugation. The organic fractions derived from the basic and acid LLE were then combined, and the solvent evaporated under N_2 (**Figure 2**). The residue formed was reconstituted with 250 µL of mobile phase solution (65% of phase A and 35% of phase B) for HPLC-MS/MS analysis.

2.4. HPLC-MS/MS method

The LC-MS/MS system consisted of an HPLC Nexera X2 (SHIMAZDU USA Manufacturing inc. Canby, OR, USA) composed of two binary pumps LC-30AD, a degasser DGU-20Asr, the column oven CTO-30A, and a SIL-30A autosampler. The whole system was coupled with a 4000 Q TRAP triple quadrupole hybrid mass detector (ABSCIEX, Foster City, CA, USA) equipped with an ESI Turbo VTM Ion Source. The separation was performed on an HPLC column Poroshell 120 EC-C18 (2.7 µm, 4.6 x 50 mm, Agilent, Santa Clara, CA, USA) using a mobile phase consisting of phase A made of water and phase B made of acetonitrile/water (95:5) both phases containing 0.1% of formic acid. The elution was performed in gradient mode at a flow rate of 0.8 mL min^{-1}. The gradient started with 5% phase B for 0.5 min, then increased to 80% phase B within 7.5 min, increased again to 100% phase B in 0.1 min, where it held for 2.9 min, and then returned to the initial conditions within 0.1 min, and stayed on 5% phase B for 3 min. The total analytical time was 14.1 min and the column was thermostated at 45 °C. The autosampler was set to inject 4 µL and the samples were

thermostated at 10 °C. Positive electrospray ionization (ESI) was used for the ionization of the analytes: ion spray voltage, 2000 V; ion source nebulizer gas (gas 1), 40 psi; turbo heater gas (gas 2), 55 psi; curtain gas, 30 psi; ion source temperature, 550 °C. The masses acquisition was performed in Multiple Reaction Monitoring (MRM) with a scanning speed of 0.4 s using the scheduled algorithm of the Analyst® 1.6.2 software (ABSCIEX), with a detection window of 25 s. MRM transitions for the quantification and qualification of analytes are shown in **Table 1**.

2.5. Method validation

The validation of the method was performed assessing the linearity, selectivity, sensitivity, and reproducibility of the HPLC-MS/MS method. Moreover, the recovery of the extraction process and the matrix effect were also assessed. Analyses were performed at least in triplicates ($n \geq 3$). The method linearity was determined on calibration curves of 7 points (10, 25, 50, 75, 100, 250, and 500 pg mg^{-1}) considering coefficients of determination $R^2 \geq 0.995$. The sensitivity was assessed by determining the limits of detection and quantification (LOD and LOQ) of each analyte. Signal-to-noise ratios (S/N) of 3:1 and 10:1 were used to estimate the LOD and the LOQ respectively. The coefficient of variation (%CV) was used to evaluate the method precision. Accuracy (%) and precision (CV%) were both obtained from 5 replicated analyses of drug- free human hair samples spiked at 3 concentrations (10 pg mg^{-1}, 50 pg mg^{-1}, and 250 pg mg^{-1}).

The matrix effect (ME), recovery (RE), and process efficiency (PE) were determined by comparing the areas of the analytes prepared in standard solution (A) and the matrix with the addition of standards performed before (B) and after (C) the extraction according to the following equations:

$$ME\ (\%) = 100 - \left(100 \times \frac{C}{A}\right) \quad (1).$$

$$RE\ (\%) = 100 \times \frac{B}{C} \quad (2).$$

$$PE\ (\%) = 100 \times \frac{B}{A} \quad (3).$$

2.6. Application of the method to forensic analyses of human hair samples

The developed and validated method was applied to real human hair samples and on samples belonging to intra-laboratory circuits from Arvecon (Walldorf, Germania) and ISS (Istituto Superiore di Sanità, Roma, Italy). Each analytical session was built with a positive sample (with a mixture of standards) ahead for the verification and control of sensitivity, recovery, retention time, and the ratio of ions; and a negative sample for verification of the absence of potential external contamination. Analyses were performed in triplicate.

3. Results

3.1. HPLC-MS/MS

The MS characterization of the substances of abuse was performed by direct injection of analytes under consideration, in a solution consisting of phases A and B (50:50) at concentrations of 0.01 and 0.2 μg mL^{-1}. The molecular ions $[M + H]^+$ and any adducts were determined in Q1 scan mode and then, Product Ion Scan and MRM mode were operated by applying different collision energies, to characterize and identify the most abundant and characteristic ions. To improve the signal response, various parameters have been optimized such as the declustering potential (DP), entrance potential (EP), collision energy (CE), and collision exit potential (CXP). **Figure 3** reports the analytical characterization of some examined compounds from the main classes of the substances of abuse. The MS/MS parameters for 95 analytes and their MRM transitions are reported in **Table 1**. To optimize analytes separation in a fast and single run, chromatographic conditions were improved testing various reversed-phase columns, mobile phases, and gradient elution modes. The HPLC column Poroshell (2.7 μm, 4.6 x 50 mm) with its superficially porous particle (1.7 μm solid silica core + 0.5 μm porous outer layer) showed a fast and efficient separation with a high resolution and good peak shapes of analytes. **Figure 4** showed a chromatogram of the analyzed compounds. This method allowed a good chromatographic separation of isomers such as Trans-3-Methyl-Norfentanyl (3.46 min) and Cis-3-methyl-Norfentanyl (3.51 min); JWH007 (9.21 min) and JWH019 (9.34 min).

3.2. Sample preparation

The extraction of the substance of abuse from hair samples was performed with the M3 reagent®, which allowed a simple and fast extraction procedure. Moreover, the M3 reagent allowed the simultaneous extraction of a wide range of compound classes with acceptable recoveries (**Table 2**). Indeed, M3 reagent® is a solution patented by Comedical ® capable of extracting acid, neutral and basic compounds through a single hydrolytic step. Its use for the determination of some classes of new psychoactive substances (NPS) from the keratin matrix has been recently documented in the literature [29-31]. However, a remarkable matrix effect produced by the M3 reagent was observed through the direct injection of the diluted extract. Therefore, to reduce this matrix effect a purification phase was added after M3 extraction through two micro-extraction steps.

3.3. Method validation

Following US FDA guidelines for bioanalytical method validation, the developed analytical method was validated [32]. The determination of the validation parameters was made to guarantee the validation for at least one compound per family of determined substances, for a total of 52 analytes. Other compounds were not considered because the standards were available in insufficient quantities for complete validation (Methcathinone, Dimethylcathinone, Metilone, 4-Fluoromethcathinone, Ethcathinone, 4-Hydroxy DET, Ethylone, Methedrone, Naphyrone, Buphedrone, 4-FA, Buthylone, 4-Methylmethcathinone, 6-APB, Pentedrone, 6-MAPB, Penthylone, ±-cis-3-methyl Norfentanyl, 3,4 Dimethylmethcathinone, Tapentandol, α-PVP, MDPV, Pravadoline, 1-Naphyrone, AM-2233, JWH-200, AM-694, 5-F-NNEI 2-naphthyl isomer, AM-2201, JWH-302, RCS 4, JWH-250, JWH-016, JWH-251, JWH-203, JWH-081, JWH-007, JWH-098, JWH-307, JWH-122, RCS-8, JWH-019, JWH-210, and JWH-398). **Table 2** reports the validation parameters of the analytical method. Regarding the method linearity, the calibration curves of the analytes were linear in the range of analysis (10, 25, 50, 75, 100, 250, and 500 pg/mg) showing R^2 ranging from 0.990 to 0.999 for 94% of the targeted compounds, except for 5-MeO-AMT, 5-Meo-MiPT, and α-Ethyltryptamin, with R^2 between 0.981 and 0.987.

The LODs and LOQs were defined as the levels of analytes corresponding to signal-to-noise ratios (S/N) of 3:1 and 10:1 respectively. LODs and LOQs for studied compounds are within a range of 0.6 – 10.3 pg mg^{-1} and 2.1 – 34.4 pg mg^{-1}, respectively (**Table 2**). These levels are lower compared to LOD and LOQ reported in similar studies [33, 35] proving thus, the high sensitivity of the method although the small size of the hair samples used (25 mg).

For the accuracy (%) and method precision (%CV), 3 concentrations were considered in the range of the expected levels (10 pg mg^{-1}, 50 pg mg^{-1}, and 250 pg mg^{-1}). Precision was measured performing 5 replicated determinations per concentration and was obtained using the following formula: [(standard deviation/mean) x 100]. Except for 5-MeO-AMT and 5F-APINACA at 10 pg mg^{-1}, the method precision was lower than 15% for all the considered compounds at the 3 concentrations (**Table 2**). Accuracy, which was evaluated as the percentage deviation (%) between average value and expected value ranged between 80 and 120% for all analytes except for 5-MeO-AMT, 5-APB, 5-Meo-MiPT, and 5-MAPB at 10 pg/mg. Moreover, standard solutions and the reconstituted extracts were stable with degradation of less than 20% for at least 7 days of storage away from light at -20 °C.

The selectivity of the method was assessed by analyzing free-drug hair samples (blank samples) from 6 subjects to assess the presence of potentially interfering endogenous peaks. Subsequently, these samples were spiked with 40 analytes (opiates, stimulants, benzodiazepines, cannabinoids, etc ...) to evaluate the ability of the method to not produce false positives in the presence of potentially interfering xenobiotics. Analyses did not show interfering peaks in correspondence with the analytes studied, not even following the addition of potentially interfering exogenous substances. Moreover, high specificity was obtained through the selection of specific precursor/product ions transitions for each analyte, and their monitoring in MRM [35].

The carry-over effect of the method was also assessed through the analyses of blank samples immediately after standard samples at the highest concentration (500 pg mg^{-1}). Carry-over ranged from 0% to 0.1% for all the targeted compounds (**Table 2**).

3.4. Matrix effect, recovery, and process efficiency

The ME refers to the combined effects of interferences in samples on the quantitative analysis of targeted compounds [36]. The developed method showed a reduced matrix effect for most NPS analytes (**Table 2**). Indeed, for 92% of the tested compounds, the ME was found to be <15%. Ion suppression phenomenon was markedly observed on Mephedrone (-42.3%) and 5-Cl THJ 018 (-63.8%). During the development phase, other extraction methods were tested using solvents such as $CHCl_3$, CH_2Cl_2, $CH_3COOC_2H_5$, and Solid-Phase Extraction cleanup (SPE Oasis® PRiME HLB). The tested solvents and the SPE provided less satisfactory results. The double basic and acid LLME realized with a mixture of CH_2Cl_2 and 10% of isopropyl alcohol, allowed to effectively extract the analytes while reducing the matrix effect in most cases. Moreover, the extraction method offered acceptable levels of recoveries for the majority of the targeted compounds from samples spiked at 50 pg mg^{-1} concentration [37]. The PE, which represents the real recovery taking into account ME [36] ranged from 31.8% to 108.1% (**Table 2**). Almost 65% of the substances showed a PE between 70% and 100%.

3.5. Application of the method to forensic analyses of human hair samples

The use of the method for the analysis of fortified samples belonging to inter-laboratory circuits (Proficiency Testing, PT) provided interesting insights into the validity and application field of the method. PT consists of sending an unknown sample, in which known quantities of analytes have been added through a particular fortified absorption process, to several laboratories for qualitative-quantitative analyses, and outcomes are compared to the reference results released by the organizing body [39]. 3 classes of NPS were monitored, including fentanyl derivatives, cathinones, and synthetic cannabinoids. The developed method correctly identified all the NPS present in the analyzed samples without providing any “false positives” and “false negatives”. It also correctly quantified most of the substances for which the reference standard was available, with values within the reference ranges. Moreover, a good agreement of quantitative results was observed with 80% of the monitored compounds meeting the quantitative requirements of the circuit (data not shown).

The method was also used for the analysis of two real keratin samples for which the determination of fentanyl and cathinones was required. The analysis did not reveal the presence of NPS, which could be attributed to the lack of intake of the substances.

4. Discussion

The main challenge of NPS analysis is characterized by the emergence of novel NPS, requiring thus the updating of analytical methods able to detect and quantify a broad amount of compounds from different NPS classes. Considering these issues, many studies have been performed for the development of screening methods to analyze a large number of NPS from keratine matrix using HPLC-MS methods [20-34].

Being able to analyze NPS from keratin samples can be useful to study the epidemiologic distribution of these substances in the population. In this study, we intended to develop an analytical method that could determine in a single extraction process and chromatographic run numerous NPS characterized by different chemical-physical properties.

Therefore, a fast HPLC-MS/MS method has been developed and validated for the analysis of NPS from different chemical classes in 9.5 min: 7 phenethylamines, 10 tryptamines, 18 cathinones, 24 synthetic opioids, and 38 synthetic cannabinoids. In addition to the wide number of targeted NPS, the developed method allowed the identification of recently emerged NPS, which were poorly considered in previous screening methods. Indeed, taking into account the arrival of novel and irregulated NPS to bypass the drug-detection systems, it is a necessity to constantly update the analytical methods. 8 recently emerged NPS were targeted in this analytical method including Carfentanyl (novel synthetic opioid), Ethylphenidate (phenylethylamines), and MMB-2201, APP-FUBINACA, 5-F-Cumyl-Pinaca, MDMB-CHMICA, 5-Cl THJ 018, and 2-naphthyl 5F-NNEI as novel synthetic cannabinoids.

The method developed showed an excellent capacity in the qualitative determination of the substances sought in a complex matrix such as keratin, with sensitivity in line with other methods

described in the literature [40]. For all the compounds, the LOD was ≤ 10 pg/mg, and thus, suitable for the intended use [41]. Unlike similar studies, the direct extraction method ensured a good process efficiency in a reduced time of sample preparation [42].

Moreover, the HPLC method showed a high separation capacity of isomeric and isobaric substances, which is particularly difficult in the case of molecules with low molecular weights such as cathinones and phenylethylamines. Only two pairs of isomers 5/6-MAPB, and 5/6-APB, which differ in the position of the aminoalkyl chain on benzofuran were not well separated. For the unequivocal identification of these isomers, it is necessary to use additional technologies such as "ion mobility" mass spectrometry or complementary techniques such as NMR or GC-MS [43].

For reasons of availability of the standards, it was not possible to fully develop the quantitative aspect of the method. The data obtained from the restricted repeatability tests and the realization of the calibration batches showed however a good ability of the method to quantify most of the analytes. Moreover, this method showed a better sensitivity for compounds such as AB-FUCINACA, ADB-FUBINACA, and Cumyl-Pegaclone, for which sensitivity issues are reported in the literature [34].

Conclusions

In this study, a new method has been developed allowing the fast determination of 98 NPS from different chemical classes including phenethylamines, tryptamines, cathinones, synthetic opioids, and cannabinoids, with an emphasis given to newly emerged NPS. This analytical approach could find application in toxicological screenings applied to hair matrix in routine context through the direct extraction process and the rapid UHPLC-MS/MS method. The parameters of method validation (linearity, sensitivity, selectivity, precision, RE, ME, and PE) followed acceptable criteria for the majority of the targeted compounds. Finally, the method is foreseen to be used on a greater number of selected real samples and not only on fortified samples, to evaluate the real capacity of extraction from the matrix, and the possible distribution of NPS in populations.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Credit Author Statement:

Franks Kamgang NZEKOUE: Conceptualization, Methodology, Writing-Original draft preparation; **Marco AGOSTINI:** Methodology, Validation, Formal analysis; **Michele VERBONI:** Formal analysis, Investigation; **Caterina RENZONI:** Investigation; Methodology, Validation; **Lucia ALFIERI:** Data curation, Conceptualization, Methodology; **Simone BAROCCI:** Conceptualization; Data curation; **Massimo RICCIUTELLI:** Conceptualization, Data Curation; **Giovanni CAPRIOLI:** Data curation, Supervision, Writing- Reviewing and Editing; **Simone LUCARINI:** Data curation, Supervision, Writing- Reviewing and Editing;

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Figure 1. Structure of newly emerged synthetic cannabinoids (APP-FUBINACA, 5-F-Cumyl-Pinaca, MDMB-CHMICA, 5-Cl THJ 018, 2-naphthyl 5F-NNEI), tryptamines (5-MeO-DMT), synthetic opioids (Carfentanyl), and phenethylamines (ethylphenidate)

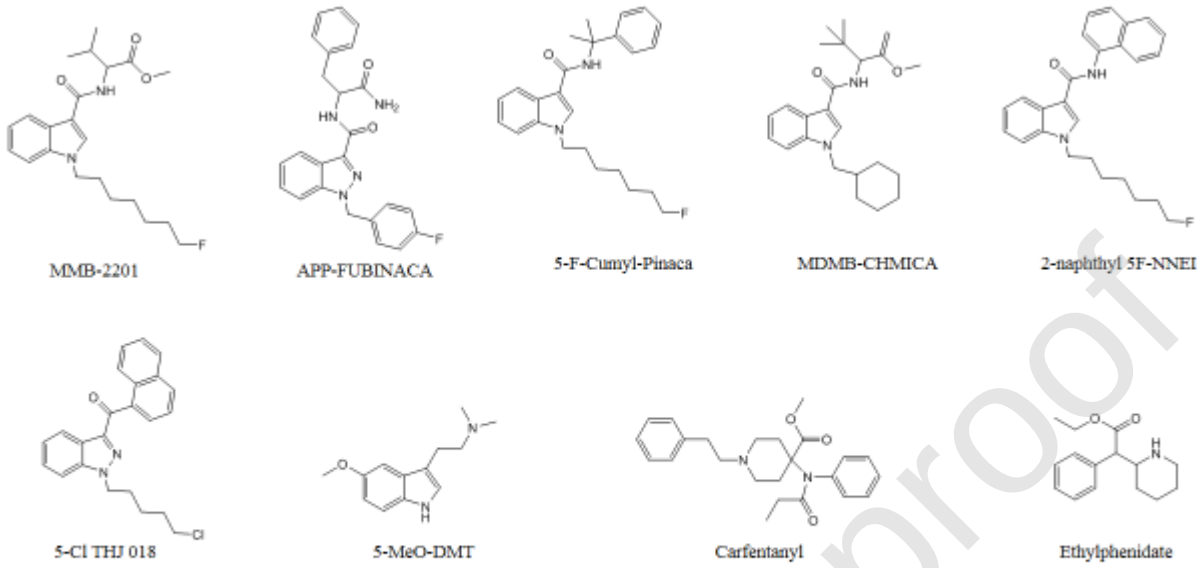

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Figure 2. Extraction process of NPS from hair samples

The phase A is made of water and phase B made of acetonitrile/water (95:5) both phases containing 0.1% of formic acid

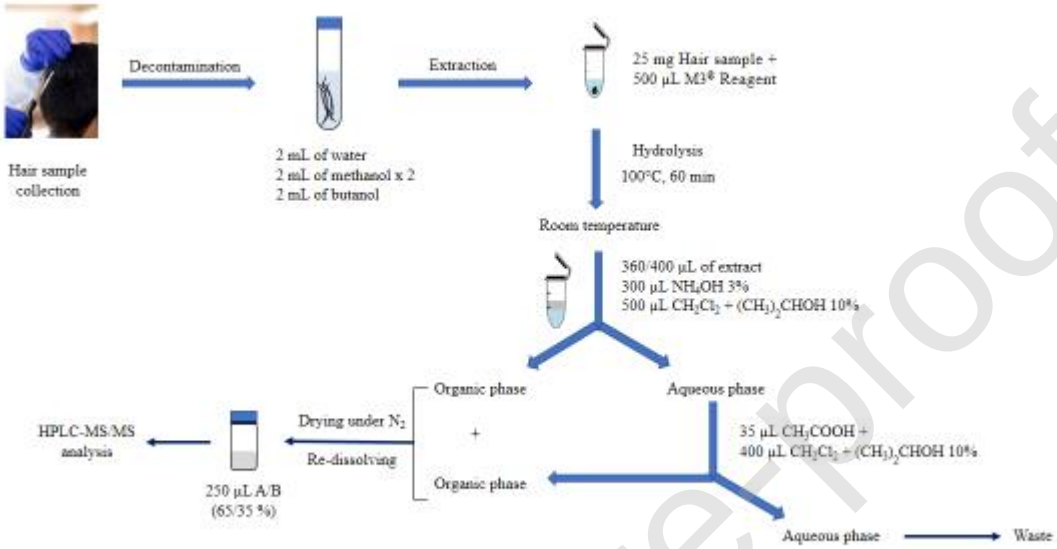

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Figure 3. Product ion scan of some examined compounds from the main classes of the substances of abuse: MDMB-CHMICA, 5-F-Cumyl-Pinaca, 5-MeO-DMT, and Carfentanyl

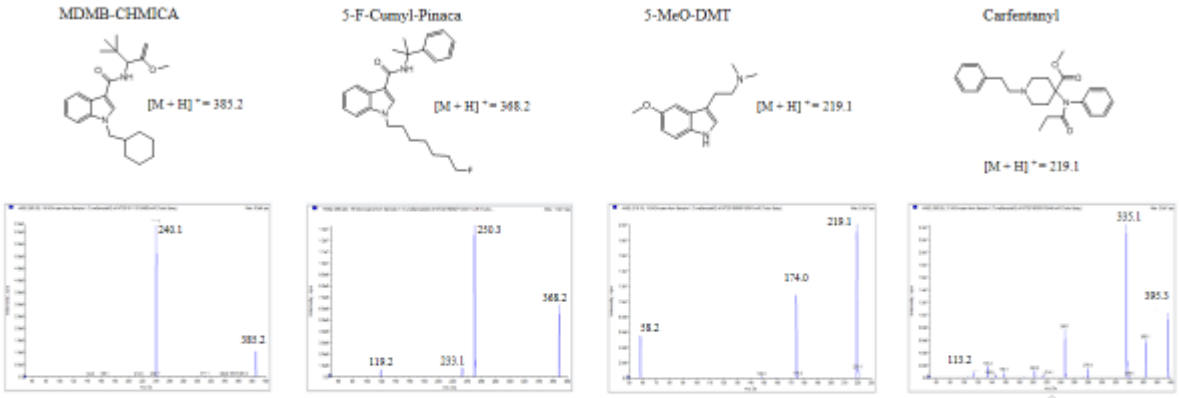

Figure 3. Product ion scan of some examined compounds from the main classes of the substances of abuse: MDMB-CHMICA, 5-F-Cumyl-Pinaca, 5-MeO-DMT, and Carfentanyl

Figure 4. Extracted Ion Chromatogram of targeted novel psychoactive substances in standard solution. +MRM (92 pairs)

1, Dimethylcathinone (2.44 min); 2, Metilone (2.50 min); 3, Methoxyacetyl NorFentanyl (2.54 min); 4, Ethcathinone (2.59 min); 5, Acetyl Norfentanyl (2.64 min); 6, 4-Fluoromethcathinone (2.69 min); 7, 4-Hydroxy DET (2.61 min); 8, Ethylone (2.76 min); 9, Methedrone (2.79 min); 10, N,N-DMT (2.82 min); 11, Buphedrone (2.86 min); 12, 4-FA (2.89 min); 13, 5-MeO-DMT (2.90 min); 14, Buthylone (2.95 min); 15, 5-MeO-AMT (3.00 min); 16, Mefedrone (3.04 min); 17, 6-APB (3.11 min); 18, Norfentanyl (3.25 min); 19, 4-Methylethcathinone (3.25 min); 20, Furanyl Norfentanyl (3.30 min); 21, β-Pentredone (3.32 min); 22, 5-Meo-MiPT (3.39 min); 23, 5-MAPB (3.39 min); 24, α-Ethyltriptamine (3.40 min); 25, Penthylone (3.42 min); 26, Trans 3-methyl Norfentanyl (3.45 min); 27, ±-cis-3-methyl Norfentanyl (3.45 min); 28, 3,4-Dimethylmethcathinone-1 (3.50 min); 29, Tapentadol (3.58 min); 30, 5-EAPB (3.57 min); 31, Butyryl Norfentanyl (3.60 min); 32, MDPV (3.67 min); 33, 5-MeO-DALT (3.68 min); 34, 4-AcO DiPT (3.74 min); 35, Butyryl Fentanyl

COOH (3.84 min); 36, Ethylphenidate (3.84 min); 37, β-OH Thiofentanyl (3.87 min); 38, Valeryl Fentanyl COOH (3.90 min); 39, Acetyl Fentanyl (3.90 min); 40, β-OH Fentanyl (3.93 min); 41, 5-MeO-DPT (3.96 min); 42, Alfentanyl (4.04 min); 43, 4-ANPP (4.07 min); 44, Fentanyl (4.28 min); 45, Despropionyl para-F-Fentanyl (4.31 min); 46, Cyclopropyl Fentanyl (4.33 min); 47, Pravadoline (4.44 min); 48, 1-Naphyrone (4.49 min); 49, Carfentanyl (4.50 min); 50, Butyryl Fentanyl (4.54 min); 51, Naphyrone (4.63 min); 52, AM-2233 (4.68 min); 53, Phenyl Fentanyl (4.69 min); 54, JWH-200 (4.75 min); 55, Phenylacetyl Fentanyl (4.78 min); 56, β-Phenyl Fentanyl (4.98 min); 57, AB-FUBINACA (5.13 min); 58, 5-F-APP-PICA (5.39 min); 59, 5-F-APP-PINACA (6.24 min); 60, 5-Cl AB-PINACA (6.24 min); 61, JWH-073 4-Butanoic Acid (6.43 min); 62, APP-FUBINACA (6.48 min); 63, ADB-FUBINACA (6.62 min); 64, JWH-018 5-Pentanoic Acid (6.66 min); 65, AM2201 4OH-Pentyl (6.70 min); 66, MMB-2201 (6.84 min); 67, AB-CHMINACA (6.99 min); 68, AM-694 (7.25 min); 69, 5-Fluoro ADB (7.32 min); 70, 5-F-NNEI 2-naphthyl isomer (8.04 min); 71, 5F-Cumyl-Pinaca (8.23 min); 72, AM 2201 (8.28 min); 73, RCS-4 (8.42 min); 74, JWH-302 (8.44 min); 75, JWH-250 (8.64 min); 76, Cumyl-PeGACLONE (8.64 min); 77, MDMB-CHMICA (8.81 min); 78, JWH-251 (8.91 min); 79, JWH-016 (8.95 min); 80, JWH-203 (9.00 min); 81, 5-Cl THJ 018 (9.01 min); 82, JWH-018 (9.03 min); 83, JWH-081 (9.08 min); 84, JWH-007 (9.13 min); 85, JWH-098 (9.20 min); 86, JWH-307 (9.23 min); 87, 5F-APINACA (9.28 min); 88, JWH-122 (9.28 min); 89, JWH-019 (9.29 min); 90, RCS-8 (9.31 min); 91, JWH-210 (9.34 min); 92, JWH-398 (9.34 min).

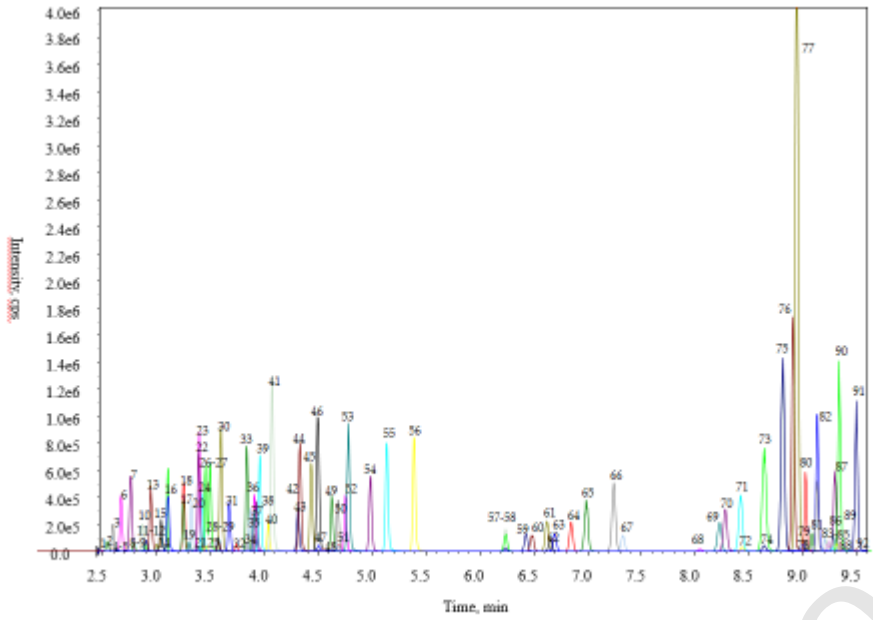

Table 1. High-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) acquisition parameters, including retention time (RT), molecular weight (MW), declustering potential (DP), precursor ion (Q1), product ion (Q3), collision energy (CE), and collision exit potential (CXP), for each transition

Analytes	Chemical classes	RT (min)	MW (Da)	DP (V)	Q1 (amu)	Q3 (amu)	CE (V)	CXP (V)
Methcathinone	Cathinones	2.34	163.2	40	164.2	105.0/146.2	30	8
Dimethylcathinone	Cathinones	2.45	177.1	55	178.1	105.1/133.1	30/20	8
Metilone	Cathinones	2.54	207.2	60	208.1	160.1/132.1	24/37	10
4-Fluoromethcathinone	Cathinones	2.56	181.2	50	182.2	149.2/103.2	28/38	8
Methoxyacetyl Norfentanyl	Synthetic opioids	2.60	248.3	58	249.1	84.2/106.0	24/33	13/4
Ethcathinone	Cathinones	2.61	177.2	30	178.2	132.1/117.1	22/35	8
Acetyl Norfentanyl	Synthetic opioids	2.68	218.1	62	219.1	84.1/55.2	25/53	14/8
4-Hydroxy DET	Tryptamines	2.76	232.1	70	233.2	86.0/160.0	21/31	14/8

Ethylone	Cathinones	2.77	221.1	50	222.1	174.2/204.1	25/20	10/12
Methedrone	Cathinones	2.77	193.2	35	194.2	146.0/161.2	37/25	8
Buphedrone	Cathinones	2.81	177.2	40	178.2	160.2/131.2	16/30	8/6
4-FA	Phenylethylamines	2.84	153.2	20	154.2	109.1/137.1	25/12.	8
N,N-DMT	Tryptamines	2.88	188.1	74	189.1	144.0/58.1	26/29	7/9
Buthylone	Cathinones	2.93	221.1	55	222.1	174.1/204.1	24/17	8/12
5-MeO-DMT	Tryptamines	2.96	218.1	51	219.1	58.2/174.0	24/31	9
5-MeO-AMT	Tryptamines	3.05	204.1	40	205.1	147.0/173.0	31	7/9
Mefedrone	Cathinones	3.11	177.1	46	178.1	145.4/119.2	29/31	10
4-Methylmethcathinone	Cathinones	3.23	191.1	60	192.2	174.2/145.1	16/25	8
6-APB	Phenylethylamines	3.26	175.1	50	176.1	91.1/131.1	25/40	9/15
Norfentanyl	Synthetic opioids	3.26	232.1	74	233.1	84.1/55.1	27/55	13/8
5-APB	Phenylethylamines	3.26	175.0	45	176.0	91.1/131.2	25/43	6/15
Furanyl Norfentanyl	Synthetic opioids	3.33	270.0	60	271.0	84.1/56.1	24/42	13/8
Pentedrone	Cathinones	3.36	191.2	33	192.2	174.2/132.2	17/26	8

6-MAPB	Phenylethylamines	3.39	189.0	50	190.0	159.0/131.2	19/28	8/9
5-MAPB	Phenylethylamines	3.39	189.1	74	190.1	159.0/131.2	19/30	8/7
5-MeO-MiPT	Tryptamines	3.40	246.0	67	247.0	86.2/174.0	24/28	5/9
Penthylone	Cathinones	3.41	235.1	60	236.1	188.1/175.2	25/30	8
α-Ethyltriptamina	Tryptamines	3.44	188.1	48	189.1	130.1/58.1	25/34	9
Trans-3-methyl Norfentanyl	Synthetic opioids	3.46	246.0	72	247.0	98.1/150.1	25	15/8
±-cis-3-methyl Norfentanyl	Synthetic opioids	3.51	246.1	62	247.1	98.2/69.1	25/45	16/10
3,4-Dimethylmethcathinone	Cathinones	3.55	191.2	30	192.2	159.2/144.2	28/41	8
Tapentandol	Synthetic opioids	3.59	221.1	70	222.1	107.1/121.0	35/29	4/5
α-PVP	Cathinones	3.60	231.1	80	232.1	91.0/126.1	30/32	10/12
5-EAPB	Phenylethylamines	3.61	203.1	61	204.1	131.2/159.0	31/19	6/8
Butyryl Norfentanyl	Synthetic opioids	3.69	246.1	60	247.1	84.1/177.1	28/23	13/9
MDPV	Cathinones	3.72	275.3	60	276.3	126.2/175.1	37/30	8
5-MeO-DALT	Tryptamines	3.84	270.1	82	271.1	110.2/174.1	20/27	8
4-AcO DiPT	Tryptamines	3.85	302.0	76	303.0	160.2/114.2	39/26	8/18

Butyryl Fentanyl COOH	Synthetic opioids	3.87	380.2	100	381.2	188.2/105.1	35/60	11/4
Ethylphenidate	Phenylethylamines	3.91	247.0	109	248.0	84.1/56.1	29/68	13/8
β-OH Thiofentanyl	Synthetic opioids	3.91	358.1	75	359.1	192.0/146.2	32	15/7
Valeryl Fentanyl COOH	Synthetic opioids	3.94	394.1	98	395.1	188.1/105.2	36/60	11/6
Acetyl Fentanyl	Synthetic opioids	3.96	322.1	110	323.1	188.1/105.1	33/53	9/4
β-OH Fentanyl	Synthetic opioids	4.04	352.2	82	353.2	204.0/186.2	29/34	16/15
5-MeO-DPT	Tryptamines	4.07	274.0	66	275.0	114.2/174.1	22/30	18/9
Alfentanyl	Synthetic opioids	4.28	416.2	100	417.2	268.3/197.1	27/38	6/10
4-ANPP	Synthetic opioids	4.31	280.2	102	281.2	105.2/188.1	46/26	4/10
Fentanyl	Synthetic opioids	4.33	336.1	70	337.1	188.2/105.1	35/50	4
Cyclopropyl Fentanyl	Synthetic opioids	4.50	348.1	82	349.1	188.1/105.1	33/55	9/17
Pravadoline	Synthetic opioids	4.53	378.3	50	379.3	135.1/114.0	29/46	8
Despropionyl para-F-Fentanyl	Synthetic opioids	4.55	298.3	74	299.4	188.1/105.1	26/47	10/4
1-Naphyrone	Cathinones	4.55	281.2	60	282.2	141.2/211.2	38/26	8
Carfentanyl	Synthetic opioids	4.63	394.2	130	395.2	335.1/113.1	27/42	8/18

Butyryl Fentanyl	Synthetic opioids	4.69	350.2	128	351.2	188.1/105.2	33/57	10/4
Naphyrone	Cathinones	4.70	281.2	60	282.2	141.2/211.2	38/26	8
Phenyl Fentanyl	Synthetic opioids	4.78	384.1	78	385.1	188.1/105.1	33/55	9/15
AM-2233	Synthetic cannabinoids	4.79	458.3	55	459.3	98.0/112.2	50/34	8
JWH-200	Synthetic cannabinoids	5.00	384.2	55	385.2	155.1/114.2	30/40	8
Phenylacetyl Fentanyl	Synthetic opioids	5.14	398.1	98	399.1	188.1/105.1	35/63	9/15
β-Phenyl Fentanyl	Synthetic opioids	5.40	412.0	82	413.0	188.1/105.1	36/63	14/17
AB-FUBINACA	Synthetic cannabinoids	6.20	368.2	58	369.2	253.2/324.2	23/33	8/5
5-F-APP-PICA	Synthetic cannabinoids	6.21	368.2	62	369.2	232.2/144.2	28/59	7
5-F-APP-PINACA	Synthetic cannabinoids	6.40	396.0	50	397.0	233.1/145.1	34/64	5/6
5-Cl AB-Pinaca	Synthetic cannabinoids	6.45	364.3	58	365.3	320.1/249.2	22/35	8/5
JWH-073 4-Butanoic Acid	Synthetic cannabinoids	6.59	357.3	75	358.3	155.2/127.1	34/74	7/5
APP-FUBINACA	Synthetic cannabinoids	6.62	416.2	66	417.2	253.1/109.1	35/60	15/4
ADB-FUBINACA	Synthetic cannabinoids	6.65	382.1	58	383.1	338.3/253.2	22/34	9/14
JWH-018 5-Pentanoic Acid	Synthetic cannabinoids	6.81	371.2	45	372.2	155.2/127.1	38/71	10/21

AM2201 4OH-Pentyl	Synthetic cannabinoids	6.94	375.2	50	376.2	155.1/127.2	35/72	11/5
MMB-2201	Synthetic cannabinoids	7.19	362.2	69	363.2	232.1/144.1	19/54	5/6
AB-CHMINACA	Synthetic cannabinoids	7.27	356.4	70	357.4	241.1/312.4	37/24	5/8
AM-694	Synthetic cannabinoids	8.03	435.2	65	436.2	231.0/203.0	38/64	8
5-Fluoro ADB	Synthetic cannabinoids	8.16	377.0	83	378.0	233.0/318.2	36/23	12/7
5-F-NNEI 2-naphthyl isomer	Synthetic cannabinoids	8.18	374.1	97	375.1	232.1/144.2	32/55	5/6
5-F-Cumyl-Pinaca	Synthetic cannabinoids	8.35	367.2	66	368.2	250.3/233.1	16/29	6/5
AM-2201	Synthetic cannabinoids	8,42	359.2	80	360.2	155.2/127.2	33/55	8/14
JWH-302	Synthetic cannabinoids	8.62	335.2	65	336.2	121.2/214.2	30/35	8
RCS 4	Synthetic cannabinoids	8.62	321.2	63	322.2	135.2/92.0	34/82	8
JWH-250	Synthetic cannabinoids	8.82	335.2	50	336.2	121.2/144.2	28/48	8
Cumyl-PeGACLONE	Synthetic cannabinoids	8.87	372.3	56	373.3	255.1/119.1	19/39	6/5
MDMB-CHMICA	Synthetic cannabinoids	8.90	384.2	102	385.2	240.1/144.1	25/53	5/7
JWH-016	Synthetic cannabinoids	8.97	341.3	64	342.3	155.2/127.2	35/68	8
JWH-251	Synthetic cannabinoids	8.98	319.2	63	320.2	214.2/144.2	35/50	8

JWH-203	Synthetic cannabinoids	9.01	339.2	69	340.2	125.0/214.1	40/36	8
5-Cl THJ 018	Synthetic cannabinoids	9.06	376.1	74	377.1	249.1/213.2	24/36	13/11
JWH-018	Synthetic cannabinoids	9.11	341.4	110	342.4	127.2/145.1	70/59	6/11
JWH-081	Synthetic cannabinoids	9.18	371.3	90	372.3	185.2/157.1	35/54	8
JWH-007	Synthetic cannabinoids	9.21	355.2	55	356.2	155.1/127.0	35/70	8
5F-APINACA	Synthetic cannabinoids	9.27	383.2	88	384.2	135.2/93.1	30/76	6/15
JWH-098	Synthetic cannabinoids	9.27	385.2	90	386.2	185.2/157.2	37/58	8
JWH-307	Synthetic cannabinoids	9.27	385.2	69	386.2	155.2/127.2	31/74	8
JWH-122	Synthetic cannabinoids	9.30	355.2	73	356.2	169.2/141.2	35/58	8
RCS-8	Synthetic cannabinoids	9.33	375.3	65	376.3	121.1/91.0	32/73	8
JWH-019	Synthetic cannabinoids	9.34	355.2	90	356,2	155.1/127.2	35/69	8
JWH-210	Synthetic cannabinoids	9.47	369.2	78	370.2	183.2/155.2	35/53	8
JWH-398	Synthetic cannabinoids	9.47	375.2	80	376.2	189.2/161.2	36/64	8

Table 2. Method validation. Concentration range, regression equation, linearity (R^2), coefficient of variation (CV%), carryover%, limits of detection (LODs), limits of quantification (LOQs), matrix effect (ME), recovery (RE), and process efficiency (PE) of targeted 52 NPS by HPLC–MS/MS.

Analytes	Chemical classes	LOD pg/mg	LOQ pg/mg	Carryover %	R^2	Accuracy % / CV %			ME (%)	RE (%)	PE (%)	IS 0.1 pg/mL
					10-500 pg/mg	10 pg/mg	50 pg/mg	250 pg/mg				
Methoxyacetyl NorFentanyl	Synt oppiods	2.5	8.2	/	0.995	99.4/7.2	92.3/3.3	105.4/3.6	-25.3	61.9	75.9	D5 Fentanyl
Acetyl Norfentanyl	Synt oppiods	1.7	5.6	/	0.996	90.9/6.0	99.0/3.1	106.4/3.6	3.9	66.6	64.2	D5 Fentanyl
N,N-DMT	Tryptamines	7.6	25.3	/	0.998	98.0/6.0	99.9/2.2	97.3/2.2	5.4	78.3	69.5	D3 Methylone
5-MeO-DMT	Tryptamines	2.6	8.5	/	0.995	80.4/5.6	117.7/4.9	103.3/2.6	22.1	86.3	58.9	D3 Methylone
Mephedrone	Cathinones	7.5	25.0	/	0.994	94.4/7.9	108.4/4.0	103.2/4.4	-42.3	44.4	62.5	D4 Ketamine
5-MeO-AMT	Tryptamines	4.3	14.2	/	0.986	65.2/21.3	104.9/8.2	110.6/3.4	-11.5	36.2	37.1	D5 Fentanyl

Norfentanyl	Synt oppiods	8.0	26.6	/	0.992	82.8/6.1	99.4/4.1	108.8/1.7	-8.3	75.8	77.8	D5 Fentanyl
5-APB	Phenylethylamines	3.1	10.3	/	0.991	63/6.4	125.2/5.3	101.3/4.1	-20.6	76.1	86.9	D4 Ketamine
Furanyl Norfentanyl	Synt oppiods	3.1	10.4	/	0.990	86.9/13.6	109.8/3.4	108.9/4.6	-10.9	84.8	87.8	D5 Fentanyl
5-Meo-MiPT	Tryptamines	2.7	9.1	/	0.987	65.7/10.2	118.8/2.3	109.3/2.8	9.3	72.1	65.0	D5 Fentanyl
5-MAPB	Phenylethylamines	5.3	17.7	/	0.990	64.9/13.5	128.2/5.0	101.3/3.4	-13.3	74.1	84.8	D4 Ketamine
α-Ethyltryptamin	Tryptamines	10.3	34.4	/	0.981	90.2/13.5	89.3/2.9	106.1/5.0	-24.4	53.2	65.2	D5 Fentanyl
Trans 3-methyl Norfentanyl	Synt oppiods	2.1	7.1	/	0.998	98.9/10.4	91.8/5.9	101.9/1.7	-18.5	76.3	90.1	D5 Fentanyl
5-EAPB	Phenylethylamines	2.5	8.5	/	0.997	94.7/4.8	99.7/2.5	105.8/2.8	-42.2	76.4	108.1	D5 Fentanyl
Butyryl Norfentanyl	Synt oppiods	3.5	11.7	/	0.994	98.2/5.5	92.9/1.6	101.8/3.4	-12.5	83.8	90.7	D5 Fentanyl
5-MeO-DALT	Tryptamines	1.3	4.3	/	0.993	90.5/4.8	102.9/1.7	107.6/3.5	3.7	65.4	63.3	D5 Fentanyl
4-AcO DiPT	Tryptamines	4.3	14.3	/	0.998	94.8/3.7	105.5/3.6	100.1/1.9	6.6	34.6	31.8	D5 Fentanyl
Butyryl Fentanyl COOH	Synt oppiods	0.6	2.1	/	0.995	121.6/8.1	99.9/5.3	95.2/4.2	-2.3	51.9	53.0	D5 Fentanyl
Ethylphenidate	Phenylethylamines	3.1	10.3	/	0.998	107.3/3.6	92.1/1.9	104.0/2.9	-24.8	77.0	96.1	D5 Fentanyl
ß-OH Thiofentanyl	Synt oppiods	3.4	11.2	/	0.998	96.5/6.6	103.4/3.9	102.2/3.3	10.1	77.8	70.1	D5 Fentanyl
Valeryl Fentanyl COOH	Synt oppiods	0.8	2.8	/	0.996	112.8/10.3	99.0/6.0	95.0/7.2	7.0	57.2	52.8	D5 Fentanyl

Acetyl Fentanyl	Synt oppiods	2.3	7.6	/	0.995	79.9/3.1	111.3/2.5	102.0/2.2	10.9	80.2	70.5	D5 Fentanyl
ß-OH Fentanyl	Synt oppiods	4.6	15.5	/	0.998	92.3/7.4	102.1/1.9	100.9/2.8	8.5	79.5	72.3	D5 Fentanyl
5-MeO-DPT	Tryptamines	1.5	5.1	/	0.999	99.5/1.7	98.9/2.0	99.5/1.6	1.3	71.3	70.7	D5 Fentanyl
Alfentanyl	Synt oppiods	2.3	7.6	/	0.996	118.7/10.6	108.5/12.3	99.1/4.3	5.3	79.7	75.5	D5 Fentanyl
4-ANPP	Synt oppiods	6.0	20.1	/	0.996	89.8/6.9	103.2/3.3	103.4/5.0	8.9	81.7	74.5	D5 Fentanyl
Fentanyl	Synt oppiods	1.0	3.3	0.03	0.999	100.2/4.8	100.4/0.8	99.1/2.2	5.4	82.0	76.4	D5 Fentanyl
Despropionyl para-F-Fentanyl	Synt oppiods	2.2	7.3	0.06	0.977	86.9/4.8	105.6/2.1	102.8/2.8	1.3	80.1	76.6	D5 Fentanyl
Cyclopropyl Fentanyl	Synt oppiods	1.0	3.3	0.03	0.999	99.0/2.7	102.0/1.7	99.2/2.8	5.8	80.6	75.1	D5 Fentanyl
Carfentanyl	Synt oppiods	1.7	5.6	0.03	0.999	101.1/2.8	98.7/1.3	100.6/3.7	9.1	81.1	73.3	D5 Fentanyl
Butyryl Fentanyl	Synt oppiods	3.5	11.7	0.03	0.998	89.6/6.1	104.3/3.1	100.9/1.5	8.1	80.0	73.5	D5 JWH-250 4-OH Pentyl
Phenyl Fentanyl	Synt oppiods	1.5	4.9	0.08	0.999	100.2/0.8	101.1/2.5	99.4/2.8	8.8	80.0	72.3	D5 Fentanyl
Phenylacetyl Fentanyl	Synt oppiods	0.9	2.8	0.08	0.996	86.2/1.4	108.1/0.8	103.2/1.8	13.4	80.7	68.1	D5 Fentanyl
ß-Phenyl Fentanyl	Synt oppiods	0.9	2.9	0.11	0.997	82.6/1.3	109.4/2.5	100.8/1.1	11.4	84.5	73.5	D5 JWH-250 4-OH Pentyl
AB-FUBINACA	Synt cannabinoids	1.2	4.2	/	0.999	96.8/1.8	101.4/3.1	100.1/1.9	-7.2	84.6	74.9	D5 JWH-250 4-OH Pentyl
5-F-APP-PICA	Synt cannabinoids	2.1	7.0	0.06	0.999	97.0/2.3	100.5/3.1	100.5/1.4	-1.4	73.3	73.0	D5 JWH-250 4-OH Pentyl

5-F-APP-PINACA	Synt cannabinoids	1.1	3.7	/	0.999	97.6/4.8	103.7/4.4	97.0/1.1	2.3	73.1	70.1	D5 JWH-250 4-OH Pentyl
5-Cl AB-Pinaca	Synt cannabinoids	5.1	17.1	/	0.999	95.1/6.3	103.2/2.0	99.2/2.4	-4.1	68.7	70.0	D5 JWH-250 4-OH Pentyl
JWH-073 4-Butanoic Acid	Synt cannabinoids	4.6	15.3	0.08	0.996	106.2/5.7	93.4/5.6	100.6/2.7	-0.1	50.2	49.1	D5 JWH-250 4-OH Pentyl
APP-FUBINACA	Synt cannabinoids	3.0	10.1	0.08	0.999	95.3/5.4	102.7/4.7	98.6/1.6	-3.5	67.2	69.6	D5 JWH-250 4-OH Pentyl
ADB-FUBINACA	Synt cannabinoids	1.8	6.0	/	0.999	95.3/6.0	102.1/1.8	99.9/1.7	-1.9	74.5	75.0	D5 JWH-250 4-OH Pentyl
JWH-018 5-Pentanoic Acid	Synt cannabinoids	2.9	9.8	0.11	0.997	103.6/2.6	94.3/2.9	99.8/1.1	0.9	53.4	51.8	D5 JWH-250 4-OH Pentyl
AM2201 4OH-Pentyl	Synt cannabinoids	1.5	5.2	0.09	0.997	89.1/6.1	106.8/1.3	100.8/1.1	6.5	68.2	64.4	D5 JWH-250 4-OH Pentyl
MMB-2201	Synt cannabinoids	1.1	3.8	/	0.998	90.9/2.1	105.1/1.2	101.2/2.0	-4.7	71.3	73.2	D5 JWH-250 4-OH Pentyl
AB-CHMINACA	Synt cannabinoids	1.6	5.5	/	0.999	95.8/6.2	104.6/2.9	97.9/1.5	1.7	79.1	76.9	D5 JWH-250 4-OH Pentyl
5-Fluoro ADB	Synt cannabinoids	2.3	7.6	0.07	0.999	93.7/4.9	103.8/4.1	98.4/1.2	6.5	68.9	64.4	D5 JWH-250 4-OH Pentyl
5F-Cumyl-Pinaca	Synt cannabinoids	0.8	2.6	/	0.999	93.4/5.0	105.1/3.3	100.0/1.7	-2.1	62.7	61.2	D5 JWH-250 4-OH Pentyl
Cumyl-PeGACLONE	Synt cannabinoids	1.0	3.4	0.09	0.999	94.2/6.7	106.4/4.7	97.6/2.1	15.9	65.8	55.0	D5 JWH-250 4-OH Pentyl
MDMB-CHMICA	Synt cannabinoids	1.5	5.0	0.10	0.999	95.9/6.5	103.2/1.5	98.4/1.4	10.9	81.9	71.3	D5 JWH-250 4-OH Pentyl
5-Cl THJ 018	Synt cannabinoids	5.7	19.1	0.04	0.993	108.8/9.1	92.7/3.7	103.0/9.9	-63.8	37.3	59.3	D5 JWH-250 4-OH Pentyl
JWH-018	Synt cannabinoids	3.7	12.2	/	0.994	118.7/11.0	86.0/5.4	105.4/5.0	-4.7	37.4	36.9	D5 JWH-250 4-OH Pentyl

5F-APINACA	Synt cannabinoids	6.0	20.0	/	0.998	86.1/25.0	104.4/12.1	98.8/12.4	-7.5	92.1	90.9	D5 JWH-250 4-OH Pentyl