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Analysis of drugs of abuse and contaminants in individuals under drug surveillance programs: A study in hair samples

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ABSTRACT

In this study, over 900 hair samples from individuals using drugs of abuse in São Paulo and Rio Grande do Sul, Brazil were analysed for classical drugs and new psychoactive substances (NPS) in hair samples. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods were developed for the detection of some drugs (25B-NBOMe, 25C-NBOMe, 25E-NBOH, 2C-C, 2C-I, 3,4-MDPHP, 3-MeO-PCP, 5F-MDMB-PINACA, 5-MeO-DMT, cannabidiol, ethylone, JWH 073, ketamine, mephedrone and UR-144) and drug adulterants (levamisole, phenacetin, strychnine); other substances including cocaine, amphetamines, benzodiazepines, opioids, cannabinoids were also analysed. The methods demonstrated good selectivity, linearity with lower limits of quantification ranging from 0.02 to 0.26 ng/mg, as well as precision and accuracy meeting ISO/IEC 17025 criteria. A total of 29.8 % samples were positive for at least one drug. Cocaine and its metabolites were the most frequently detected, with benzoylecgonine (111 cases), cocaine (88 cases), and cocaethylene (37 cases), highlighting prevalent cocaine use. Cannabinoids were also common, with THC (57 cases) and CBN (48 cases) detected. The presence of adulterants such as phenacetin (76 cases), levamisole (14 cases), and strychnine (3 cases) were also identified. Additionally, 5F-MDMB-PINACA was also detected (5 cases), along with ketamine (9 cases). Findings also highlighted a prevalence of poly-drug consumption, where THC was frequently detected with cocaine (20 cases) and benzoylecgonine (26 cases). CBN also showed notable associations with cocaine (16 cases) and benzoylecgonine (22 cases). These findings provide important insights into the evolving drug landscape in Brazil and highlight the effectiveness of hair analysis as a tool for monitoring drug use.

1. Introduction

The abuse of drugs, both legal and illegal, is a global public health concern that continues to pose significant challenges. The UNODC *World Drug Report 2023* [1] highlights several key concerns about drug abuse at the global level. In 2021, more than 296 million people used drugs, a 23 % rise over the past decade. Furthermore, 39.5 million people suffer from drug use disorders, representing a 45 % increase during the same period. Cannabis remains the most widely used drug globally, with approximately 209 million users in 2021. Despite increasing legalization in some countries, there is concern about rising use among young people and the risks of high-THC cannabis to mental health. Opioids, particularly synthetic ones like fentanyl, are responsible for the highest number of drug-related deaths. Although Afghanistan's opium ban has reduced production, synthetic opioids are easier to produce and distribute, posing a significant ongoing threat. The opioid crisis in North America

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continues to be severe, with high overdose rates and fatalities. Concerning cocaine, there has been a rise in cocaine consumption and trafficking, especially in Europe and Africa. Cocaine production increased by 35 % in the past decade, contributing to higher availability in global markets. Finally, synthetic drugs, such as amphetamines and new psychoactive substances (NPS), are on the rise due to their low cost and ease of manufacture. In Brazil, the problem of drug abuse is particularly acute, with reports from the "*Sistema de Vigilância Toxicológico Brasileiro*" and the "*Subsistema de Alerta Rápido de Drogas*" from 2023 indicating widespread use of not only classical drugs, but also the emerging new psychoactive substances (NPS) [2–4]. These systems report a concerning rise in polydrug use among younger individuals, revealing an evolving drug landscape that includes both well-known substances and an increasing number of NPS. The latter represent a significant challenge to both law enforcement agencies and forensic laboratories worldwide [5]. These substances are synthesized to mimic the effects of controlled drugs while evading legislations, and their rapid proliferation, coupled with ever-changing chemical structures, makes them difficult to detect and ultimately quantify. This poses a major hurdle for toxicologists, as conventional screening methods may fail to identify these substances [5]. Furthermore, legal systems vary significantly between countries. For example, while Brazil enforces stricter penalties for drug use, its response to NPS remains inconsistent due to the rapid emergence of these substances in the drug market. In contrast, Portugal has focused on implementing robust harm reduction and rehabilitation measures, addressing drug-related issues through a public health approach rather than solely punitive actions, but the problem for NPS also persists. In order to address the challenge of detecting and quantifying both traditional drugs and NPS, hair analysis offers a valuable approach. In contrast to urine or blood, which only reveal recent drug use, hair provides a longer window of detection, allowing assessing up to several months of substance exposure. Hair analysis is particularly helpful in evaluating patterns of drug consumption over time and can be used to identify chronic drug use. Its non-invasive collection method and the stability of drug compounds also make it ideal for studying long-term exposure in populations at risk of substance abuse [6].

Considering these developments, 911 samples from individuals under drug surveillance from the city of São Paulo and Rio Grande do Sul (Brazil) presenting high incidence of drug trafficking and drug use in public, were collected and analysed to assess patterns of abuse. For that, a fast liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed to detect and quantify both classical drugs of abuse and NPS in hair samples; cocaine contaminants (levamisole, phenacetin and strychnine) were also included in the method. The study focused on a population already known to consume classical drugs, with the goal of assessing NPS use and determining which new substances had been introduced. This analysis offers important insights into emerging drug trends and the extent of NPS penetration within established drug-using populations.

2. Materials and methods

2.1. Materials and instrumentation

Analytical standards of 2-(4-chloro-2,5-dimethoxyphenyl)ethan-1-amine (2C-C), 2,5-dimethoxy-4-iodophenethylamine (2C-I), (1-butyl-1H-indol-3-yl)(naphthalen-1-yl)methanone (JWH 073), 4-bromo-2,5-dimethoxy-N-[(2-methoxyphenyl)methyl]-benzeneethanamine (25B-NBOMe, 2-(((4-ethyl-2,5-dimethoxyphenethyl)amino)methyl)phenol (25E-NBOH), benzodioxol-5-yl)-2-(1-pyrrolidinyl)-1hexanone, monohydrochloride (3,4-MDPHP), were purchased from Cayman Chemicals (Michigan, United States), 5-methoxy-*N*,*N*-methyltryptaline (5-MeO-DMT) and methyl 2-{[1-(5-fluoropentyl)-1H-indazole-3-carbonyl] amino}- 3,3-dimethylbutanoate (5F-MDMB-PINACA or 5-ADB) were acquired form Supelco (Darmstadt, Germany), 2-(4-chloro-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethan-1-amine (25C-NBOMe), 3-methoxyphencyclidine (3-MeO-PCP), mephedrone, ethylone, 3-[(2,2,3,3-tetramethylcyclopropyl)carbonyl]-1H-indole-1-pentanoic acid (UR-144), cannabidiol, ketamine, and phenacetin were purchased from LGC Promochem (Teddington, United Kingdom), strychnine and levamisole were purchased from Ehrenstorfer (Augsburg, Germany). Formic acid (Merck, Darmstadt, Germany), acetonitrile (JTBaker, New Jersey, United States), methanol (JTBaker, New Jersey, United States), were all HPLC grade. Deionized water was obtained from a Milli-Q system (Elga Pure Chorus 1, Veolia, MA, USA).

The working solutions of the analytical standards were prepared by dilution with methanol:acetonitrile (1:1) to the final concentrations of 10000 ng/mL for all analytes, while internal standards (IS) were diluted to the final working solution of 150 ng/mL. All stock and working solutions were kept protected from light and stored at 4 °C.

The LC-MS/MS instrumentation is a Waters Xevo TQ-S Micro with an Acquity UPLC System. The method was developed and validated at *Laboratórios* ChromaTox Ltda, São Paulo, Brazil, and testing for all analytes. The method for the classic drugs of abuse has been accredited to ISO/IEC 17025. Two different methods were developed: one for classic drugs (Method 1) and another for NPS and adulterants (Method 2). Method 1 is under corporate protection, and therefore further details cannot be disclosed. Regarding Method 2, all configurations and instrumental parameters are detailed in Tables 1 and 2. The chromatographic column used, ACQUITY UPLC® BEH C_{18} (2.1 × 50 mm; 1.7 µm), was maintained at 40 °C, and the analyses were performed under gradient elution with binary phase A (deionized water, 0.1 % formic acid) and B (acetonitrile, 0.1 % formic acid) with a flow rate of 0.5 mL/min according to the profile reported in Table 3. The analysis takes a total of 2.2 min.

2.2. Sample collection and preparation

Nine hundred and thirty-one hair samples were collected between 2021 and 2024 from individuals under drug surveillance, in the city of São Paulo and Rio Grande do Sul with a high incidence of drug use, particularly crack cocaine. The Government of São Paulo has established the Hub of Care for Crack and Other Drugs in this area, with the purpose of providing treatment for substance use disorders. The hub offers urgent and emergency care for individuals experiencing acute episodes of drug dependence. With an average flow of 250 patients per week, the

Table 1
Detailed MRM transitions monitored for analytes and internal standards from Method 2 in study.

Analyte	Parent ion> Quantifier, Qualifier (*m/z*)	Retention time (min)	Internal standard used
25B-NBOMe	381 > 121, 90	1.30	Metamphetamine-d5
25C-NBOMe	337 > 121, 91	1.29	Metamphetamine-d5
25E-NBOH	316 > 178, 193	1.29	Metamphetamine-d5
2C-C	216 > 184, 199	1.10	Amphetamine-d11
2C-I	308 > 291, 276	1.15	Mephedrone-d3
3,4-MDPHP	290 > 135, 149	1.18	Ephedrine-d3
3-MeO-PCP	274 > 189, 121	1.22	PCP-d5
5F-MDMB-PINACA	378 > 233, 145	1.81	Canabinol-d3
5-MeO-DMT	219 > 58, 174	1.03	PCP-d5
Cannabidiol	315 > 193, 123	1.80	Canabidiol-d3
Ethylone	222 > 174, 204	1.02	MDEA-d5
JWH 073	328 > 155, 127	1.92	Canabidiol-d3
Ketamine	238 > 125, 179	0.78	Ketamine-d4
Levamisole	205 > 178, 123	0.74	Benzoylecgonine-d3
Mephedrone	178 > 145, 119	1.05	Mephedrone-d3
Phenacetin	180 > 138, 110	1.27	Cocaethylene-d3
Strychnine	335 > 184, 156	1.02	Cocaine-d3
UR−144	312 > 214, 125	2.10	THC-d3

Table 2
Internal standards MRM transitions and retention time used in Method 2.

Internal standard	Parent ion> Quantifier (*m/z*)	Retention time (min)
Amphetamine-d11	147 > 130	0.7
Benzoylecgonine-d3	293 > 171	0.79
Canabidiol-d3	314 > 223	1.93
Cocaethylene-d3	321 > 199	0.94
Cocaine-d3	307 > 185	0.87
Ephedrine-d3	169 > 151	0.67
Ketamine-d4	242 > 211	0.77
MDEA-d5	213 > 105	0.77
Mephedrone-d3	181 > 163	0.77
Metamphetamine-d5	155 > 82	0.73
PCP-d5	249 > 96	0.95
THC-d3	318 > 196	2.00

Table 3
Gradient profile of method 2.

Time (min)	Flow rate (mL/min)	Mobile phase A (%)	Mobile phase B (%)
0.00	0.5	98	2
1.50		5	95
2.00		5	95
2.01		98	2
2.20		98	2

Mobile phase A: 0.1 % formic acid in water
Mobile phase B: 0.1 % formic acid in acetonitrile

service can be considered an epicentre for the most severe cases within the spectrum of addictive disorders, where crack addiction is intertwined with extreme conditions of social vulnerability. This study was conducted in accordance with the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the *Comitê de Ética em Pesquisa* from *Universidade Federal de São Paulo* (CAAE: 39437220.4.0000.5505). A lock of hair (of about the thickness of a pencil) was collected from persons attending said Hub, and the distal and proximal regions of the samples were identified. In individuals where scalp hair was not available, body hair was collected. Hair analysis was performed in the first 3 cm of sample (corresponding to an approximate time span of 90 days). In the case of body hair, the sample was analysed as a whole, 1 cm corresponding approximately to 120 days (due to the higher proportion of telogen hair) [7].

Both blank and authentic samples were stored in paper envelopes at room temperature, away from direct sunlight, in a dry atmosphere, according to Chromatox internal protocol.

For the classical drugs method information about sample treatment and extraction procedure cannot be disclosed due to proprietary methods of Chromatox Dasa. As for the NPS and cocaine contaminants method hair samples were washed with methanol to remove excess of dirt and some possible external contamination that could influence interpretation. After the decontamination step, 10 mg of hair was weighed in 2 mL tubes. Briefly, 750 µL of Chromatox extraction solution (proprietary of Chromatox Dasa, thus details about the composition cannot be disclosed), 25 µL of internal standard, and two metal beads were added to the tubes, which were securely sealed. The hair samples were pulverized for 4 min in Biotage® Lysera. Subsequently, the samples were transferred to 96-well plates and processed using Biotage® Pressure+ 96, facilitating both hair filtration and enhancing recovery rates, while reducing variability during solid-phase extraction. After this process, samples were ready for analysis by LC-MS/MS equipment. The following substances were screened for: amphetamines (amphetamine, ephedrine, diethylpropion, mazindol, and fenproporex), methamphetamines (methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA), 3,4-methylenedioxy-*N*-methyl-α-ethylphenylethylamine (MBDB)), benzodiazepines (alprazolam, clonazepam, diazepam, lorazepam, midazolam, nitrazepam, nordiazepam, oxazepam, temazepam, flurazepam, chlordiazepoxide), hypnotics and sedatives (zopiclone, zolpidem, flunitrazepam), cannabinoids (tetrahydrocannabinol (THC), cannabinol (CBN), cannabidiol), cocaine (anhydroecgonine methylester; AEME (crack), benzoylecgonine, cocaethylene, cocaine, norcocaine), opiates (morphine, 6-acetylmorphine, 6-acetylcodeine, codeine, dihydrocodeine, diacetylmorphine), opioids (oxycodone, fentanyl, pethidine, propoxyphene, norpropoxyphene, tramadol, desmethyltramadol, methadone and 2-ethylene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)), other substances (LSD, phencyclidine (PCP), cathinone, methcathinone), and 14 NPS (3-MeO-PCP, mephedrone, ethylone, 2,4-MDPHP, ketamine, UR-144, JWH-073, 5F-MDMB PINACA, 25C-NBOMe, 25B-NBOMe, 25E-NBIOH, 2C-C, 2C-I, 5-MeO-DMT), norketamine (ketamine's metabolite) and cocaine adulterants (phenacetin, levamisole and strychnine).

2.3. Method validation

The validation of the analytical method was conducted according to the guidelines /recommendations established in the Brazilian Norm '*Associação Brasileira de Normas Técnicas Norma Brasileira ISO/IEC 17025*' (NIT-DICLA-069 REV Nº07) [8], and those from the Society of Hair Testing (SoHT) [9,10]. Selectivity, linearity and limits of detection and quantification, accuracy, repeatability, robustness, stability, and carry over were assessed.

2.3.1. Selectivity

Selectivity, also referred to as matrix effect in NIT-DICLA-069 rev. 07 guidelines, was evaluated by analysing spiked samples both with, and without matrix (hair (methanol:acetonitrile (1:1)), at 0.2 ng/mg (n = 10). The ability of the method to distinguish the analyte from other components in the matrix was confirmed, ensuring that the method is selective for the target analytes. The criteria employed for confirmatory identification were as follows. (1) the retention time of the peak detected in the sample should not differ from that of the positive control by more than 0.1 minutes or 1 %, whichever is greater and, (2) for the comparison of ions/transitions observed via mass spectrometry, the following criteria were applied: a. All transitions associated with the compound under investigation must be assessed for relative intensity, using the positive control as a reference standard; b. Comparisons of retention time and mass spectra must be conducted using positive controls analysed within the same analytical batch; c. A minimum of two transitions must be detected and compared to confirm the identity of the substance; d. All ions/transitions employed in compound identification must exhibit a signal-to-noise ratio greater than 3:1 and, e. The relative intensity values observed in the sample must fall within the tolerance ranges specified according to the guidelines of the European Workplace Drug Testing Society in Urine – Version 2.0.

2.3.2. Linearity and limits

The linearity was assessed by analysing different concentrations (six calibrators excluding the origin) of the analyte across a specified range 0.02 (depending on the compounds) – 1 ng/mg (Supplementary table 1). Calibration curves were constructed by plotting the peak area ratio between each analyte and the respective IS peak areas against concentration, and the determination coefficient (r^2) was calculated to confirm the linear relationship between the concentration and the response. The criteria to assess the fitness of this linear model included a weighted r^2 higher than 0.986. A weighted least square regression (1/x) was used to compensate for heteroscedasticity. Three quality control samples (low, medium and high) were analysed in quadruplicate along with each calibration curve.

The limit of detection (LOD) was determined as the lowest concentration of the analyte that could be reliably detected but not necessarily quantified. The lowest limit of quantification (LLOQ) was determined as the lowest concentration that could be quantified with acceptable precision and accuracy and is the first calibration point in the linear range.

Both were calculated based on the standard deviation of the response and the slope of the calibration curve.

2.3.3. Precision and accuracy

Repeatability was assessed by analysing the same sample multiple times under identical conditions within the same day. Three concentrations: low, medium, and high, with four replicates at each level were used. The coefficient of variation (CV) was calculated to determine the precision of the method under these conditions. CV of $\leq$ 20 % was required to ensure the method's precision under repeatability conditions.

According to the guideline NIT-DICLA-069 rev. 07, intermediate precision "should be evaluated by repeating the experimental design used for the assessment of repeatability, but varying one or more of the operational conditions, such as the day of analysis, analyst, or equipment. Moreover, the guideline states that "the evaluation of the results should statistically demonstrate (using hypothesis tests or ANOVA as applicable) the equivalence of the groups of samples analysed with the similar groups (low, medium, and high concentrations) analysed for repeatability." In this work, intermediate precision was evaluated considering changes in the day of analysis and in the analyst (n = 25).

Accuracy, referred to as "trueness" in NIT-DICLA-069, was evaluated by comparing the measured values with known reference values within the calibration curve (n = 6). The accuracy was expressed as the percentage recovery of the known amount of analyte added to the matrix.

2.3.4. Stability

The stability of the analyte was done using six different concentrations values within the calibration curve in duplicate, evaluated after seven days of storage, and the processed samples were kept at −20°C, with no exposure to light. The results were compared to those obtained from freshly prepared samples to determine any significant changes in concentration. Analytes were considered stable if the concentration remained within ± 20 % of the initial value throughout the testing period.

2.3.5. Carry-over

Carry-over was assessed by analysing a blank sample following the analysis of a high-concentration sample (10 ng/mg). The presence of the analyte in the blank sample was evaluated to ensure that there was no significant carry-over effect. Carry-over was considered negligible if the analyte signal in the blank sample was $\leq$ 20 % of the signal obtained at the lower QC sample (0.2 ng/mg).

3. Results and discussion

3.1. Method validation

3.1.1. Selectivity

For all analytes, the method proved to be selective with no interference from the matrix.

3.1.2. Linearity and limits

Linearity data and both LOD and LLOQ are summarized in Supplementary table 1. The LLOQ for Method 2 ranged from 0.07 ng/mg (strychnine, phenacetin) to 0.13 ng/mg (2C-I, 3,4-MDPHP and 25C-NBOMe). Supplementary figure 1 shows a chromatogram at the LLOQ.

Concerning method 1, the LLOQs ranged from 0.02 ng/mg (nordiazepam, flunitrazepam, lorazepam, midazolam, nitrazepam, oxazepam, CBN) to 0.26 ng/mg (cocaine).

In addition, in method 2 the LODs were in the range of 0.04 ng/mg (strychnine, phenacetin, and 5F-MDMB-PINACA) to 0.06 ng/mg (2C-C, 2C-I, 3,4-MDPHP, 25C-NBOMe, 25E-NBOH, 25B-NBOMe, and cannabidiol). Concerning method 1, the LODs ranged from 0.01 to 0.13 ng/mg.

3.1.3. Precision and accuracy

Repeatability proved to be acceptable. Supplementary table 2 shows the results. Supplementary table 3 summarizes all data from intermediate precision. For all analytes tested, the F values obtained in the ANOVA analysis did not exceed the critical F value (4.0427), and the corresponding p values were greater than the 0.05 significance level adopted for this study. This indicates that there were no statistically significant differences between the different conditions (e.g., days, analysts). Thus, the variability observed across the runs is within acceptable limits of random variation, and no systematic bias was introduced by varying these conditions, which demonstrates that the method is highly precise under intermediate precision conditions. Accuracy ranged from 80 % to 118 %, for all analytes. All data (method 1 and 2) is presented in Supplementary table 2.

3.1.4. Stability

Stability values ranged from 82.61 % to 119.47 % (mean values), which respects the values established by the guideline used in this study, showing that all analytes present a good stability across the tested conditions. All data is presented in Supplementary table 4.

3.1.5. Carry-over

According to the criteria for carry-over assessment, the analytes signal in the blank were below 20 % of that obtained from the lower QC sample (0.2 ng/mg), indicating that no significant carry-over was observed.

3.2. Application to real samples

The method was successfully applied to authentic samples collected between 2021 and 2024. A total of 931 samples were collected, but classical drugs of abuse were analysed in only 885 due to limited sample availability. The results are presented in Supplementary Table 5 and summarised in Table 4. Out of the 931 samples, 277 (29.8 %) yielded positive results. NPS were detected in 14 samples, 10 in combination with other drugs of abuse, and 4 alone (ketamine).

In the amphetamine group, 37 samples tested positive, some of them for more than one compound. Amphetamine and methamphetamine were detected once (1.04 ng/mg; concentration below LLOQ, respectively). Conversely, MDA was detected 17 times (ranging from 0.09 to 4.18 ng/mg), MDMA 13 times (ranging from 0.11 to 1.53 ng/mg) and ephedrine 16 times (ranging from 0.10 to 0.95 ng/mg). In the cocaine group, 117 samples tested positive. Cocaine concentrations ranged from 0.26 to 977.26 ng/mg, while benzoylecgonine ranged from 0.03 to 507.85 ng/mg. Cocaethylene ranged from 0.05 to 25 ng/mg, norcocaine from 0.03 to 14.33 ng/mg and AEME (marker of crack consumption) from 0.03 to 36.57 ng/mg. Phenacetin was the contaminant more often found (76 samples), followed by levamisole (14 samples). Their concentrations ranged from 0.07 to 218.89 and from 0.38 to 9.04 ng/mg respectively. Strychnine was detected in 3 samples, and the concentrations ranged from 0.25 to 0.85 ng/mg. It is important to refer that strychnine is a toxic alkaloid from the seeds of the *Strychnos nux-vomica* tree, native to Asia. It is highly poisonous and can cause severe muscle spasms and convulsions, sometimes fatal. Strychnine was used as an adulterant in drugs like heroin and cocaine primarily to enhance the perceived effects by stimulating the nervous system, giving a false impression of potency or quality. In Brazil, its commercialisation has been prohibited since 1998 [Ordinance No. 344 of 12 May 1998]. However, at present, its use as an adulterant is uncommon due to its extreme toxicity and the high risk of severe or fatal adverse effects. Although rare, serious cases of poisoning have been reported by the Brazilian media in recent years. It was therefore surprising to detect it in some of the analysed samples.

Cannabinoids were the second most frequently detected group. THC was found in 57 samples, and cannabidiol detected in one sample (0.06 ng/mg, lower than LLOQ). CBN was detected in 48 samples. The

Table 4
Summarized information on real samples' application positive cases (drugs and metabolites, frequencies, concentration).

Analyte	Positive cases (Frequency, n)	Lowest concentration (ng/mg)	Highest concentration (ng/mg)	Average (ng/mg)	Median (ng/mg)	Standard deviation (ng/mg)
Amphetamine	1	1.04	1.04	n.a	n.a.	n.a
Methamphetamine	1	<LLOQ	<LLOQ	n.a	n.a.	n.a
MDA	17	0.09	4.18	0.84	0.45	1.01
MDMA	13	0.11	1.53	0.43	0.38	0.39
Ephedrine	16	0.10	0.95	0.35	0.27	0.24
Clonazepan	5	0.03	0.10	0.07	0.07	0.03
Diazepam	5	<LLOQ	0.97	0.57	0.55	0.40
Nordiazepam	9	0.04	1.12	0.37	0.29	0.38
Flunitrazepam	6	0.02	0.02	0.02	0.02	0.004
Lorazepam	4	0.06	0.07	0.07	0.07	0.004
CBN	48	0.02	0.60	0.10	0.07	0.11
Cannabidiol	1	<LLOQ	<LLOQ	n.a	n.a.	n.a
THC	57	0.03	0.74	0.14	0.07	0.15
AEME	44	0.03	36.57	5.14	0.35	9.86
BZE	111	0.03	507.85	21.06	0.23	72.78
Cocaethylene	37	0.05	25.00	2.45	0.79	5.26
Cocaine	88	0.26	977.26	59.40	4.84	167.54
Norcocaine	35	0.03	14.33	2.50	0.30	4.31
Codeine	5	0.09	0.34	0.18	0.13	0.10
Tramadol	12	0.12	1.22	0.33	0.22	0.30
Desmethyltramadol	1	<LLOQ	<LLOQ	n.a	n.a.	n.a
Zolpidem	2	0.16	0.34	0.249	0.25	0.13
Levamisole	14	0.38	9.04	2.62	1.44	2.76
Phenacetin	76	0.07	218.89	11.22	0.51	39.01
Strychnine	3	0.25	0.85	0.45	0.48	0.40
Ketamine	9	0.09	0.22	0.10	0.09	0.09
5FMDMB-PINACA	5	0.93	3.71	1.62	1.48	1.25
Fentanyl	1	16.48	16.48	n.a	n.a.	n.a

concentration range detected was 0.02–0.60 ng/mg for CBN and 0.030–0.74 ng/mg for THC, respectively. THC-COOH was not analysed in this study.

Regarding benzodiazepines, their prevalence was low, with diazepam and nordiazepam being the most detected substances (9 samples). It is important to note the presence of flunitrazepam, which was found in 3 samples at concentration levels ranging between 0.01 and 0.02 ng/mg. Flunitrazepam is prohibited in several countries due to its potential use in drug facilitated crimes.

The consumption of opioids follows the global trend, showing a significant decrease, and as observed in this study, the number of positive cases for opioids was also low. Tramadol was the most detected substance (12 samples), with concentration levels ranging from 0.02 to 1.22 ng/mg. It is important to note that none of the analysed samples showed the presence of 6-monoacetylmorphine (a marker for heroin use) or morphine. Codeine was found in in 5 samples.

NPS were detected in 14 samples, among which ketamine was found in 9 samples, with concentration levels ranging from 0.09 to 0.22 ng/mg. 5F-MDMB-PINACA was found in 5 samples, with concentrations between 0.93 and 3.71 ng/mg. There was only one case of fentanyl, with a concentration of 16.48 ng/mg. In this sample 5F-MDMB-PINACA was also detected (2.09 ng/mg). The current global trend in the consumption of NPS shows an increase in the variety of available compounds, although some reports indicate that overall consumption may be stabilising or even declining in certain areas. As for the most consumed or detected NPS, ketamine and synthetic cannabinoids, such as 5F-MDMB-PINACA, are among the compounds that have shown notable prevalence in several studies. These compounds are popular among consumers, and their detection in samples is becoming increasingly common. However, trends can vary by region and cultural context, so it is essential to consult specific data for a clearer understanding [11,12].

However, it is important to mention that, since 2020, the use of esketamine has been authorised in Brazil for the treatment of refractory severe depression, and fentanyl is used as an analgesic and anesthetic. Nevertheless, its use is restricted to hospital settings.

Considering the studied population and the difficulty for this group to obtain esketamine and fentanyl, we believe that the presence of ketamine and fentanyl is due to their recreational rather than therapeutic use.

It is also important to highlight the initiatives implemented by the Brazilian government to address substance use disorders. In particular, the Hub of Care for Crack and Other Drugs, launched in April 2023, is a public service offering addiction treatment and social support in São Paulo's largest open drug scene, known as "Cracolândia" (*Crackland*). This centre focuses on the most severe cases, particularly crack addiction, often associated with extreme social vulnerability. As example, during its first 12 months of operation, the facility has treated 3759 patients reporting synthetic cannabinoid use, underscoring the urgent need for intervention within this population [13].

Given these factors, along with the potential involvement of individuals in detoxification treatments, the relatively low percentage of positive results in the samples may be explained.

We would like to state that cut-off criteria from the SoHT were not used in interpreting these results; had they been, the number of positive cases would likely have been lower.

3.2.1. Concomitant substance use patterns

For substances with a high number of positive cases (cocaine and its metabolites, phenacetin, levamisole, THC, and CBN – cannabinoid derivatives), an evaluation was conducted to determine whether concomitant drug consumption was associated with various analytes. Table 5 illustrates the simultaneous quantification.

These results highlight patterns of poly-substance use among

Table 5
Number of concomitant substances use patters.

Analyte group	Number of samples with positive for both analytes
Cocaine and metabolites & Phenacetin	26
Cocaine and metabolites & Levamisole	43
Cocaine and metabolites & THC	76
Cocaine and metabolites and & cannabis derivatives	63
Total number of positives	208

individuals consuming cocaine and cannabis, as well as exposure to harmful adulterants such as levamisole and phenacetin. However, it is important to note that the number of adulterant-positive samples for cocaine and its metabolites was relatively small compared to the total number of samples (~280), suggesting that cocaine contamination with adulterants may not be uniformly distributed across the entire cohort. Despite this, the observed correlations—both statistically and clinically meaningful—indicate that some individuals are simultaneously exposed to multiple psychoactive substances. For instance, cocaine use was frequently correlated with cannabinoid exposure (THC and, to a lesser extent, CBN), suggesting a pattern of poly-substance use among individuals who consume stimulants and cannabis concurrently. The results demonstrate a pervasive pattern of co-occurrence between cocaine, its key metabolites, and known adulterants such as levamisole and phenacetin. These findings highlight that heavy cocaine users are not solely exposed to cocaine and its metabolites in isolation but rather to a complex matrix of adulterants and additional psychoactive substances. This poly-substance landscape carries significant implications for clinical practice, addiction treatment, and public health interventions, underscoring the need for integrated approaches that address the interplay between cocaine, its contaminants, and co-consumed cannabinoids.

4. Conclusions

A rapid LC-MS/MS method for detecting a range of traditional drugs and NPS in hair samples from individuals in São Paulo, Brazil was developed and validated within the scope of this study. This method, which complies with ISO/IEC 17025 and the Society of Hair Testing standards, showed high precision, accuracy, and robustness, confirming hair analysis as a reliable tool for chronic drug use monitoring.

Key findings revealed the presence of various classical drugs, primarily cocaine and its metabolites, along with significant levels of cannabinoids, benzodiazepines, and opioids. Notably, contaminants like levamisole and strychnine were identified in cocaine samples, underlining health risks due to adulterants. Additionally, the detection of emerging NPS such as ketamine and synthetic cannabinoids (5F-MDMB-PINACA) could suggest a shifting drug landscape in Brazil, with users increasingly combining classical drugs with NPS. Notwithstanding, care should be taken in results interpretation, as the number and kinds of NPS available to consumers is always changing. Therefore, the possibility of missing the detection of potentially used compounds is big, because the number and kind of substances screened for in analytical methods cannot adequately accompany the production of new compounds.

This research highlights hair analysis as a valuable method for long-term drug exposure assessment, herein demonstrated by analysis of a large number of samples, offering insights into evolving drug use patterns. Also, the simplicity of the sample preparation process and the rapid analysis time further enhance its utility for forensic and toxicological applications.

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Declaration of Competing Interest

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.forsciint.2025.112493.

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