LC–MS/MS Determination of 88 Psychotropic Drugs in 1,865 Hair Samples from Addicts in Drug Abstinence

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Abstract
The emergence of novel drugs and the continuous expansion of the scope of the types of drugs under control have greatly increased requests for screening of a range of drugs in hair. Here, a multi-analyte method for the detection and quantification of 88 psychotropic drugs in the hair of addicts in drug abstinence was developed and fully validated using liquid chromatography–tandem mass spectrometry (LC–MS/MS). Hair samples (25 mg) were washed, cut into pieces, cryogenically ground and extracted in methanol. The extracted analytes were separated on an Allure PFP Propyl column (100 x 2.1 mm, 5 mm inside diameter, Restek, USA) and analyzed by LC–MS/MS in multiple reaction monitoring modes. The limits of detection and the limits of quantification ranged from 0.1 to 20 pg/mg and 0.2 to 50 pg/mg, respectively. The intra- and inter-assay precisions (relative standard deviation (RSD)) of all analyses ranged from 0.9% to 14.9% and 1.9% to 15.9%, respectively. Accuracy values were 100 ± 20%. The extraction recovery of quality control samples ranged from 50.9% to 99.6% for all analytes. The matrix effects for all analytes ranged from 46.8% to 99.7%. The method was successfully used to analyze 1,865 hair samples from addicts in drug rehabilitation at their own communities. Among the samples, 129 cases were positive; the majority of positive cases were from males (78.29%), 92.25% of whom were >35 years old. Traditional drugs, like methamphetamine and opioids, accounted for most positive cases, and 27 of the abstinence cases with a use history of methamphetamine were still positive. In addition to abused drugs, like methamphetamine, morphine and cocaine, the sedative-hypnotic and psychotherapeutic drugs, including clonazepam, alprazolam, estazolam, zolpidem and quetiapine, were detected in 26% of the hair samples, suggesting that these addicts may have insomnia and mental problems such as depression and psychosis, probably due to the long-term effects of drugs and withdrawal reactions. Three synthetic cannabinoids were also detected in four (2.7%) cases. A total of 37 cases were positive for methadone, tramadol and dextromethorphan, reflecting a new trend of alternative drug use when traditional drugs were not easy to obtain during the coronavirus disease 2019 outbreak.

Introduction

Hair analysis for drugs of abuse, therapeutic drugs and other chemicals has been conducted in forensic toxicology for >30 years. Compared with traditional biological samples, such as blood and urine, hair provides a long detection window while also being the only specimen that can provide chronological information on individual drug use (1). Moreover, segmental hair analysis can provide a retrospective calendar of an individual’s drug use or period of abstinence (2). For these reasons, drugs of abuse are routinely detected in hair by forensic toxicology laboratories for many purposes, such as criminal investigation, driver’s license renewal, workplace drug testing, child custody cases and abstinence monitoring (3–5).

The emergence of psychoactive drugs and the continuous expansion of the scope of new types of drugs under control have substantially increased requests for monitoring a range of drugs in the hair of addicts in abstinence (6–8). Hair analysis can also detect drug use in people who stop taking their medication a few days before a urine test. This increased demand for hair analysis has now led to a need for hair screening methods for a wider range of drugs. Generally, only methods for the detection of a single substance (9–11) or a single class of drugs (12–14) have been developed and reported for hair because analytical approaches aimed at identifying and quantifying different classes of drugs require time-consuming sample preparation (8, 15, 16).

Many methods are available for the extraction of drugs from hair. These include soaking the hair samples in an extraction solvent, sonication in methanol, dissolving under alkaline or acidic conditions or using enzymes (17–20), but these methods are very time-consuming. A freeze-milling technique can quickly crush hair into micron-sized particles by mechanical impact force, physical friction force and low-temperature freezing force, thereby increasing the specific surface area of hair and making rapid hair analysis a reality—on the premise that the analytes are not degraded. Liquid chromatography–tandem mass spectrometry (LC–MS-MS) methods provide high sensitivity while avoiding tedious derivatization steps. In addition, the high analytical sensitivity of LC–MS/MS assays has proven useful not only for the potential detection of a single-dose drug exposure but also for chronic use monitoring, especially in drug users who are frequently polydrug consumers (8).

The aim of the present study was to develop a wide-range LC–MS/MS analysis method for the screening and quantification of drugs in hair samples and to apply the developed...
method to determine the concentration of drugs in 1,865 hair samples from drug addicts who in drug rehabilitation at communities. Concomitantly, new trends in drug use for individuals in abstinence programs and drug abuse were assessed to provide guidance for drug control work.

Materials and Methods

Chemicals and reagents

The reference standards of antipyrine, carbamazepine, dexmedetomidine, dezocine, difenidol, diphenoxylate, haloperidol, lamotrigine, oxcarbazepine, paliperidone, promethazine, sertraline, sulpiride, trazodone and internal standard (IS) methoxyphenamine were purchased from the National Institutes for Food and Drug Control (Beijing, China). The other reference standards and IS were purchased from Cerilliant (Round Rock, TX, USA), and the details are shown in Table S1. High Performance Liquid Chromatography (HPLC)-grade methanol (99.9%) and acetoni- trile (99.9%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Formic acid (98%) and ammonium acetate (98%) for mass spectrometry were obtained from Fluka (Buchs, Switzerland). Water was purified by filtering deionized water through a Milli-Q system (Millipore, Burlington, MA, USA).

Standard solutions

A combined working standard solution at 10 µg/mL containing 88 analytes was prepared in methanol. From this mixed solution, working solutions with concentrations of 1,000, 100, 10, 5, and 1 ng/mL in methanol were prepared. The IS at 25 ng/mL was prepared in methanol, and all working standard solutions were stored at −20°C until use.

Instrumentation

LC–MS/MS analyses were performed with a UPLC Acquity I Class (Waters, Milford, MA, USA) interfaced to an AB Sciex 6500 plus Qtrap TM triple quadrupole mass spectrometer (AB Sciex, Foster City, CA, USA) with an electrospray ionization (ESI) ion source. Data were processed using the MultiQuan 3.0.2 software.

The 16-min chromatographic run used a gradient mobile phase composed of 20 mmol/L ammonium acetate with 0.1% formic acid in water (Mobile Phase A) and acetonitrile (Mobile Phase B). The samples were separated on an Allure PFPP column (100 × 2.1 mm, 5 mm i.d., Restek, USA). The LC mobile phase gradient is shown in Table S2. The autosampler was maintained at 4°C, and the sample injection volume was 10 µL per injection.

Mass spectrometry analysis was performed in the positive ion multiple reaction monitoring (ESI+ MRM) mode. The optimum conditions were an ion spray voltage of 5,500 V, a source temperature of 450°C, curtain gas at 30 psi, and nebulizing gas and heater gas both at 35 psi. Two transitions were observed per analyte, one for quantification and one for confirmation. All precursor ions, product ions, optimum fragmentor voltages, collision energy values and retention times are shown in Table S3.

Sample preparation

Hair samples were washed three times by manual shaking with acetone, dried at room temperature and cut into small snippets (<1 mm) with clean scissors for homogeneity. A 25 mg hair sample was accurately weighed and placed in a 2-mL reinforced tube (Omni International, Inc., Kennesaw, GA, USA). Ceramic beads were added, along with 10 µL IS mixture (25 ng/mL) and 490 µL methanol. The samples were pulverized using a freezing lapping apparatus (Jingxin, Shanghai, China) with the following settings: speed, 6 m/s; time, 20 s; and dwell, 40 s; each repeated 15 times. The samples were centrifuged at 12,500 rpm for 3 min. A water phase filter membrane (Φ 13 mm×0.22 µm, SCRC) is used for filtration. After filtration, the solution was transferred to an autosampler vial, and 10 µL was injected into the LC–MS/MS system.

Method validation

The method was fully developed and validated in hair in accordance with updated established international criteria (21, 22), including selectivity, limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy, precision, recovery and matrix effects.

For the examination of selectivity, 10 drug-free hair samples from nondrug users were used separately. The presence of endogenous interferences around the retention times of each analyte and the IS were investigated to demonstrate the selectivity of the method. The LOD and LOQ were measured by evaluating the signal-to-noise (S/N) ratio of three replicates for each compound at appropriate concentrations. The LOD was fixed at a concentration that gave S/N>3, while concentrations of analytes with S/N>10 were chosen for the LOQ. The linearity ranged from the LOQ of each analyte to 20,000 pg/mg. A five-point calibration curve was prepared by spiking with all substances at LOQ, 200, 1,000, 10,000, and 20,000 pg/mg hair, and the regression line was calculated using a weighted (1/x) linear regression model.

Intra-day precision and accuracy were calculated by analyzing three concentration levels (100, 4,000 and 15,000 pg/mg) in six replicates, while inter-day precision and accuracy were measured by analyzing the six sets of each sample on four different days. Precision was calculated as the coefficient of variation (CV) of the replicate analysis meeting the criterion set where precision was acceptable (i.e., <20% CV at low concentrations and <15% CV at medium and high concentrations for all analytes). Values of accuracy were also within the acceptance limits (≤±20% bias at LOQ and ≤±15% bias at medium and high concentrations).

The extraction recovery and matrix effects for three concentrations (100, 4,000 and 15,000 pg/mg) were estimated by a post-extraction addition approach. Matrix effects were investigated with hair samples from six different people, and it expressed as ion suppression or enhancement. Three sets of samples (low, medium and high concentrations) were prepared in six replicates to evaluate the extraction recovery and matrix effect. Matrix effects were reported as the percentage ratios of Set 2/Set 1, and recovery was obtained by the percentage ratios of Set 3/Set 2. Set 1 represented the peak area of the standard solution, Set 2 indicated the peak area of the fortified sample post-extraction (standard solution is added after hair grounding) and Set 3 was the peak area of the fortified sample pre-extraction (standard solution is added before hair grounding).
Collection and analysis of authentic hair samples

In total, we received 1,865 hair samples from addicts who in drug rehabilitation at community, and they were between 6 months to 5 years after the last test positive. Hair samples were cut by the police with scissors as close to the scalp as possible and then sealed in an envelope. Hair samples (~100 hair strands in each sample) were collected from the vertex posterior region of the scalp by cutting as close to the skin as possible. Approximately 25 mg of hair was washed and cut into small pieces, and the drugs were extracted and analyzed as described above.

Results and Discussion

Method development

The mass spectrometry settings were optimized for each drug by infusing the analytes directly into the mass spectrometer and identifying the optimum settings for the detection of the precursor and product ions. The two most intense and unique product ions were chosen for the construction of MRM transitions with protonated molecules for each analyte, and the transition with the greater intensity was selected as the quantitative transition.

In this paper, we present an LC–MS/MS for the simultaneous analysis of 88 drugs. We ensured a rapid analysis by evaluating a number of columns that were amenable to high-speed analysis without sacrificing chromatographic performance. These included the Allure PFPP column (100 × 2.1 mm, 5 µm) and the HSS T3 column (100 × 2.1 mm, 1.8 µm). The optimal combination of peak shape, tailing factor and retention time was achieved by the Allure PFPP column with a mobile phase of acetonitrile, aqueous ammonium acetate (20 mmol/L) and 0.1% formic acid. Gradient elution was used for its flexibility in injection time and flow rate, which facilitated the optimization of the peak shape and allowed separation over a short time. Analytes with different structures have longer retention times when separated at a steady flow rate; therefore, different flow rates were used to adjust the retention time and shorten the run time.

Method validation

No interfering compounds or significant signal losses or increases (ion suppression or enhancement) were observed. The chromatograms of fortified samples at 200 pg/mg are shown in Figure 1. The assay was selective for all tested compounds and for the IS. The LOD values ranged from 0.1 to 20 pg/mg, while the LOQ values ranged from 0.2 to 50 pg/mg. For all analytes, a linear weighted (1/x) model was deemed the best and was used for the calculation of the calibration curves. The correlation coefficients (r) were >0.993. The LOD, LOQ and correlation coefficient of each analyte are presented in Table S4.

The precision and accuracy were satisfactory for all analytes. The intra-assay precisions of all analyses at low, medium and high concentrations ranged similarly from 1.3% to 14.9%, 2.3% to 13.7% and 0.9% to 9.2%, respectively, while the inter-assay precisions ranged from 2.7% to 15.9%, 3.1% to 14.8% and 1.9% to 11.4%, respectively. The accuracy values were in the range of ±20%, which was within the accepted limits for this parameter.

The extraction recovery of the QC samples ranged from 50.9% to 99.6% for all analytes. Most analytes (74 of 88) were extracted more efficiently at high concentrations than at low and medium concentrations. The matrix effects for all analytes ranged from 46.8% to 99.7%. The presence of residual matrix components in the final extracts that could markedly affect the ionization process of the analytes was compensated for in the quantitative analyses by the use of an adequate IS. All results for the extraction, recovery and matrix effects for the low-, medium- and high-concentration samples are detailed in Table S5.

Application to authentic hair samples

General information of the 1,865 drug addicts

The information provided by the police for the 1,865 drug addicts indicated that 1,464 (78.5%) of the subjects were men. In all cases, 12.17% were young adults (ages <34 years); 166 (8.90%) were male and 61 (3.27%) were female, with 8 males and 1 female under the age of 25. Past data have also

![Figure 1. LC–MS/MS chromatogram of fortified hair samples at 200 pg/mg.](image-url)
shown that women tend to report their first drug use at a later age than men and that they are frequently initiated into drug use by their male partners (23). More than 50% of the addicts were middle-aged (ages 35–54 years), including 801 (42.95%) males and 236 (12.65%) females. Approximately 32.23% of the subjects were >55 years of age. The distributions of gender and age are shown in Figure 2.

Statistical analysis was carried out on the drug history of the 1,865 drug addicts. The abused drugs, like methamphetamine, opioids and cannabis, were the most commonly used drugs. Some cases of abuse of new psychotropic substances, such as flunitrazepam and 5-methoxy-N,N-dissopropyltriptamine (5-MeO-DiPT), were also reported. Some drug abuse, such as abuse of 5-MeO-DiPT, has a gender characteristic. In the present case, the main use of this drug was between two men who were sexual partners and got sexual pleasure by injecting 5-MeO-DiPT. Previous studies that investigated 106 suspected 5-MeO-DiPT users reported only two female cases (9). Polydrug use also appears to vary between men and women, with men more likely to combine marijuana and alcohol with the use of “harder” drugs, such as heroin, and with women more often using a combination of nonnarcotic drugs (24). The drug use histories of the 1,865 abstinent drug users are shown in Table I.

### Cases positive for psychotropic drugs

A total of 1,865 hair samples were analyzed according to the cut-off value (0.2 ng/mg for nonpsychotropic drugs and 0.05 ng/mg for psychotropic drugs) recommended by Society of Hair Testing and used in our laboratory (25). Of the 1,865 samples, 129 were positive for one or more substances. The distribution of age and gender in the positive drug abuse cases is depicted in Table II. The subjects ranged in age from 23 to 70 years old, and 92.25% of them were >35 years old. Only one female was under the age of 25. The latter finding is related to the anti-drug measures taken by China. In 2021, China’s Ministry of Public Security released data showing that the number of drug users under the age of 35 had declined by 30% as of 2020. The positive proportion was higher with increasing age, and the frequency of drug use in both female and male subjects peaked at an age >55 years. The majority of the positive cases were male (78.29%). The proportion of males was higher than females in all age groups, except for the group under the age of 25. This may reflect the greater social behaviors and wider social circles of males, so they are more likely to be exposed to drugs. However, the emergence of new psychoactive substances and the expansion of the national list of controlled drugs have resulted in an increase in the number of women with drug abuse problems (26).

### Drug trends

#### Methamphetamine

Among the drug records provided by the police, the largest group (45.2%) of addicts had used methamphetamine, and that drug accounted for a high proportion of the 129 positive cases (37.7%). This is related to the increase in the online market for amphetamines and fits with the trend of the present drug epidemic. The emergence of synthetic drugs, represented by amphetamine-type stimulants, including amphetamines, methamphetamine and 3,4-methylenedioxy-N-methylamphetamine, has resulted in their rapid spread in China since the 1990s and has posed a great challenge for the country (27). The 2012 National Drug Abuse Monitoring Annual Report of the Chinese State Food and Drug Administration documented that, in the past

### Table I. The History of Drug Use of 1,865 Abstinent Drug Users

| Drug                          | Male | Female |
|-------------------------------|------|--------|
| Methamphetamine              | 695  | 880    |
| Morphinheroin                 | 154  | 204    |
| Methylenedioxymethamphetamine| 7    | 7      |
| Cannabis                      | 25   | 27     |
| Cocaine                       | 3    | 3      |
| Flunitrazepam                 | 1    | 0      |
| Ketamine                      | 2    | 4      |
| 5-MeO-DiPT                    | 3    | 4      |
| Diazepam                      | 0    | 1      |
| Unknown                       | 633  | 817    |
| Total                         | 1,013| 1,948  |

*For some individuals, more than one drug was used, so the apparent total number is greater than the total number of cases (i.e., \( n = 1,865 \)).

### Table II. The Distribution of Age and Gender in the Positive Drug Abuse Cases

| Gender | Male  | Female | Total  |
|--------|-------|--------|--------|
| Age    | (n = 101, 78.33%) | (n = 28, 21.67%) | (n = 129, 100%) |
| <25    | 0     | 1      | 1      |
| 25–34  | 6     | 3      | 9      |
| 35–44  | 25    | 3      | 28     |
| 45–54  | 27    | 8      | 35     |
| >55    | 43    | 13     | 56     |

*For some individuals, more than one drug was used, so the apparent total number is greater than the total number of cases (i.e., \( n = 1,865 \)).

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Figure 2. Distribution of age and gender group of 1,865 cases.
5 years, the proportion of people abusing methamphetamines and other synthetic drugs has increased from 28.8% to 75.1% (28).

Comparison of the drug use before and after withdrawal, according to the information provided by the police about the drug ingestion history of the subjects in the current study, revealed that 27 cases of the people with a history of methamphetamine use were still positive for drugs 6 months to 5 years after the last positive test. The results indicated that relapses continue to occur among those who claimed abstinence of drug use. The police do not provide information on the history of drug use for all addicts who have undergone abstinence; therefore, only partial comparisons have been made here. In general, drug addicts who have undergone abstinence have a high relapse rate because of neuroadaptive processes within the central nervous system and the influence of individual psychology and environment. These addicts can shed their addiction physically in the short term, but getting rid of the addiction mentally is difficult (29, 30). In addition, compared with other drugs, methamphetamine has a higher relapse rate, which may indicate a higher prevalence of methamphetamine in public activities. Therefore, drug supervision needs to be strengthened.

**Synthetic cannabinoids**

Three synthetic cannabinoids (SCs; 4F-MDMB-PICA, 4F-MDMB-BUTINACA and MDMB-4en-PINACA) were detected in four (2.7%) of the positive cases: 4F-MDMB-PICA was detected in all four cases, while 4F-MDMB-BUTINACA and MDMB-4en-PINACA were detected in two of the four cases. SCs are a novel class of designer drugs that emerged as drugs of abuse in the late 2000s (31) and were sold as a “legal” replacement for cannabis to avoid the force of the law. Recently, the vaping of cannabis extracts and SCs in electronic cigarette devices has become increasingly popular and has provided a new method for the popularity of SCs.

**Other drugs**

Methadone (8.9%), tramadol (2.1%) and dextromethorphan (14.4%) were detected in 37 cases; among them, 30 had a drug history of morphine and methamphetamine abuse. Methadone belongs to the class of opioid analgesics, with similar potency to morphine and codeine. Tramadol and dextromethorphan were nonopioid analgesics. The current situation of the coronavirus disease 2019 pandemic has created difficulties in drug distribution and access; therefore, some of these drugs may represent substitutes for traditional drugs of abuse. At the same time, these results reflect a new trend in the current transition from traditional drugs to new-type drugs. Drug screening of hair from drug-abstinent persons can show drug intake by drug addicts during withdrawal and can reflect the new trends and characteristics of drug epidemics in a given region.

Some sedative-hypnotic and psychotherapeutic drugs were also detected in 26% of positive cases, with estazolam, alprazolam, zolpidem, clonazepam and quetiapine accounting for 8.2%, 2.1%, 8.2%, 4.1% and 3.4%, respectively, suggesting that the addicts may have insomnia and mental problems. Some studies have shown that the proportion of people with sleep disorders who abuse sedative-hypnotic drugs is significantly higher among drug users than among nondrug users, as are problems such as depression and anxiety (32, 33). The proportion of these drugs detected in positive cases is shown in Figure 3.

**Drug concentrations in the hair of positive cases**

The range and mean concentration of drugs in the positive cases are shown in Table III. Comparison of the concentrations of the parent drug and their metabolites revealed a lower concentration of clonazepam (20–90 pg/mg) than of its metabolite 7-aminoclonazepam (40–500 pg/mg); but a higher concentration of methadone (70–14,900 pg/mg) than 2-ethylene-1,5-dimethyl-3,3 diphenylpyrrolidine (EDDP).
### Table III. The Range and Mean of Concentration of Drugs of Positive Cases

| Drug                       | n  | Concentration ranges (pg/mg) | Median (pg/mg) |
|----------------------------|----|------------------------------|----------------|
| 4F-MDMB-BICA              | 4  | 5–260                        | 100            |
| 4F-MDMB-BUTINACA          | 2  | 20–49                        | 35             |
| MDMB-4EN-PINACA           | 2  | 150–500                      | 325            |
| Clonazepam                | 4  | 20–90                        | 45             |
| 7-aminoclonazepam         | 6  | 40–500                       | 230            |
| Alprazolam                | 3  | 50–120                       | 90             |
| Estrazol                  | 14 | 50–190                       | 135            |
| Zolpidem                  | 11 | 40–1,400                     | 130            |
| Dextromethorphan          | 21 | 47–800                       | 110            |
| Methadone                 | 13 | 70–14,900                    | 75             |
| EDDP                      | 13 | 50–5,900                     | 4,300          |
| Morphine                  | 3  | 200–500                      | 320            |
| 6-monoacetylmorphine      | 3  | 60–300                       | 180            |
| Methamphetamine          | 55 | 50–1,700                     | 170            |
| Amphetamine               | 14 | 30–120                       | 60             |
| Codeine                   | 4  | 140–1,200                    | 790            |
| Quetiapine                | 5  | 70–2,800                     | 1,000          |
| Cocaine                   | 4  | 60–230                       | 120            |
| Benzoylcegonine           | 2  | 50–60                        | 55             |
| Tramadol                  | 3  | 220–22,000                   | 1,000          |
| Sibutramine               | 1  | 70                           | 7              |
| Demethylsibutramine       | 1  | 70                           | 7              |
| Dimethylsibutramine       | 1  | 20                           | 20             |

(50–5,900 pg/mg); a higher concentration of cocaine (60–230 pg/mg) than benzoylecgonine (50–60 pg/mg) and a higher concentration of methamphetamine (50–1,700 pg/mg) than amphetamine (30–120 pg/mg). These relationships are generally consistent with published results (34–37). The amounts of a parent drug and its metabolites in hair are related to their physicochemical properties and parent drug intake.

### Conclusion

A sensitive LC–MS/MS method was established for the identification and quantification of 88 drugs, including drug withdrawal medicines and new-type drugs, in hair. The method was successfully tested on 1,865 hair samples provided by the police from people in drug withdrawal. Of the 1,865 hair samples, 129 were positive for drugs. The number of males was significantly higher than that of females, and 92.25% of them were >35 years old. Traditional illicit drugs, like methamphetamine, remain the most commonly used drugs, but some sedative-hypnotic and psychotherapeutic drugs, like tramadol and dextromethorphan, were also detected. These findings showed that addicts may have insomnia and mental problems, and they also reflect new trends and characteristics of the drug epidemic in the study region. The screening results also indicated the occurrence of drug relapse in some drug addicts, indicating the importance of improving the detection and control rates for community drug rehabilitation workers and especially police officers.

### Supplementary data

Supplementary data is available at Journal of Analytical Toxicology online.

### Funding

This work was supported by the National Natural Science Foundation of China (81772022), the Science and Technology Commission of Shanghai Municipality (15DZ1207500), the Shanghai Key Laboratory of Forensic Medicine (17DZ2273200) and the Shanghai Forensic Service Platform (19DZ2292700).

### References

1. Noriaki, S., Keiko, S., Tohru, K., Akihiro, M., Munehiro, K. (2019) Hair testing for drugs in the field of forensics. Yakugaku Zasshi: Journal of the Pharmaceutical Society of Japan, 139, 705–713.
2. Pragt, F., Balikova, M.A. (2006) State of the art in hair analysis for detection of drug and alcohol abuse. *Clinica Chimica Acta*, 370, 17–49.
3. Pragt, F., Broeckers, S., Hastelt, M., Herre, S., Andresen-Streicher, H., Sachs, H., et al. (2013) Methadone and illegal drugs in hair from children with parents in maintenance treatment or suspected for drug abuse in a German community. *Therapeutik Drug Monitoring*, 35, 737–752.
4. Vignali, C., Stramesi, C., Morini, L., PoZZi, F., Collo, G., Groppi, A. (2013) Workplace drug testing in Italy - critical considerations. *Drug Testing and Analysis*, 5, 208–212.
5. Johansen, S.S., Nielsen, M. (2012) A Suspended Facilitated Crime (DFC) Investigated by Hair Analysis: SOFT. University of Copenhagen: Boston, MA.
6. Fisichella, M., Morini, L., Sempio, C., Groppi, A. (2014) Validation of a multi-analyte LC-MS/MS method for screening and quantification of 87 psychoactive drugs and their metabolites in hair. *Analytical and Bioanalytical Chemistry*, 406, 3497–3506.
7. Thieme, D., Baumer, C., Sachs, H., Teske, J. (2013) Screening and long-term retrospection for psychoactive drugs in presumptive drug-facilitated crimes using segmented single hairs. *Drug Testing and Analysis*, 5, 736–740.
8. Fisichella, M., Morini, L., Sempio, C., Groppi, A. (2014) Validation of a multi-analyte LC-MS/MS method for screening and quantification of 87 psychoactive drugs and their metabolites in hair. *Analytical and Bioanalytical Chemistry*, 406, 3497–3506.
9. Wang, R., Xiang, P., Yu, Z., Shi, Y. (2019) Application of hair analysis to document illegal 5-methoxy-N,N-dissopropyltrptamine (5-MeO-DiPT) use. *Forensic Science International*, 304, 1–9.
10. Alves, M., Zanchetti, G., Piccinotti, A., Tameni, S., Martinis, B., Polettini, A. (2013) Determination of cocaine and metabolites in hair by column-switching LC-MS-MS analysis. *Analytical and Bioanalytical Chemistry*, 405, 6299–6306.
11. Kaur, S., Ghosh, P., Varshney, K.M., Shukla, S.K., Satyanarayana, S. (2014) Determination of clonazepam in hair and nail using liquid chromatography tandem mass spectrometry (LC-MS/MS). *Journal of Liquid Chromatography & Related Technologies*, 37, 1917–1928.
12. Hutter, M., Kneisel, S., Auwarter, V., Neukamm, M.A. (2012) Determination of 22 synthetic cannabinoids in human hair by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences*, 903, 95–101.
13. Lee, S., Dajeong, J.I., Kang, S. (2014) A sensitive LC-MS/MS method for the simultaneous determination of 18 opioids and metabolites in human hair. *Yakugaku Zasshi: Journal of the Pharmaceutical Society of Japan*, 134, 737–752.
14. Rust, K.Y., Baumgartner, M.R., Meggiorino, N., Kraemer, T. (2012) Detection and validated quantification of 21 benzodiazepines and 3 "z-drugs" in human hair by LC–MS/MS. *Forensic Science International*, 215, 64–72.
15. Nielsen, M.K.K., Johansen, S.S., Dalsgaard, P.W., Linnet, K. (2010) Simultaneous screening and quantification of 52 common pharmaceuticals and drugs of abuse in hair using UPLC-TOF-MS. Forensic Science International, 196, 85–92.

16. Boumba, V.A., Di Rago, M., Peka, M., Drummer, O.H., Gerostamoulos, D. (2017) The analysis of 132 novel psychoactive substances in human hair using a single step extraction by tandem LC/MS. Forensic Science International, 279, 192–202.

17. Musshoff, F., Lachenmeier, K., Lichtermann, D., Madea, B. (2009) Cocaine and opiate concentrations in hair from subjects in a heroin maintenance program in comparison to a methadone substituted group. International Journal of Legal Medicine, 123, 363–369.

18. Corcia, D.D., Durso, E., Gerace, E., Salomone, A., Vincenti, M. (2012) Simultaneous determination in hair of multiclass drugs of abuse (including THC) by ultra-high performance liquid chromatography-tandem mass spectrometry. Journal of Chromatography B, 899, 154–159.

19. Harun, N., Anderson, R.A., Cormack, P.A. (2010) Analysis of ketamine and norketamine in hair samples using molecularly imprinted solid-phase extraction (MISPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Analytical and Bioanalytical Chemistry, 396, 2449–2459.

20. Qian, Z.H., Yang, H.X., Liu, C.M. (2018) Rapid detection of designer benzodiazepine flubromazolam by GC-MS and UPLC-Q-TOF MS. Chinese Journal of Analysis Laboratory, 37, 1133–1136.

21. Peters, F.T., Wissenbach, D.K., Busardo, F., Marchei, E., Pichini, S. (2017) Method development in forensic toxicology. Current Pharmaceutical Design, 23, 5455–5467.

22. Wille, S.M.R., Coucke, W., Baere, T.D., Peters, F.T. (2017) Update of standard practices for new method validation in forensic toxicology. Current Pharmaceutical Design, 23, 5442–5454.

23. Amaro, H., Hardy-Fanta, C. (1995) Gender relations in addiction and recovery. Journal of Psychoactive Drugs, 27, 325–337.

24. Grella, C.E., Joshi, V. (1999) Gender differences in drug treatment careers among clients in the National High Performance Drug Abuse Treatment Outcome study. American Journal of Drug & Alcohol Abuse, 25, 385–406.

25. The Society of Hair Testing. (2003) Recommendations for hair testing in forensic cases. http://soht.org/images/pdf/Consensus_on_Hair_Analysis.pdf (Accessed Oct 7, 2003).

26. Pleym, H., Spigset, O., Kharasch, E.D., Dale, O. (2010) Gender differences in drug effects: implications for anesthesiologists. Acta Anaesthesiologica Scandinavica, 47, 241–259.

27. Fang, Y.X., Wang, Y.B., Shi, J., Liu, Z.M., Lin, L.U. (2006) Recent trends in drug abuse in China. Acta Pharmacologica Sinica, 207, 140–144.

28. (2012) National Surveillance Center on Drug Abuse 2012 Annual report on drug abuse in China. Beijing, China: National Surveillance Center on Drug Abuse, China Food and Drug Administration. http://www.sda.gov.cn/WS01/CL0051/93340.html (Accessed Oct 17, 2013).

29. Weiss, F., Cicciocioppo, R., Parsons, L.H., Katner, S., Richter, R.R. (2010) Compulsive drug-seeking behavior and relapse. Neuroadaptation, stress, and conditioning factors. Annals of the New York Academy, 937, 1–26.

30. Cao, X.M., Jiang, H.P. (2005) Sleep disturbances in drug abusers. Neuropharmacology, 47, 279–287.

31. Liao, Y., Tang, J., Liu, T., Chen, X., Hao, W. (2011) Sleep and mood problems among Chinese heroin-dependent individuals. The American Journal of Drug and Alcohol Abuse, 37, 179–183.

32. Huang, L., Guozhu, X., Jiawang, Z. (2000) Changes in protein expression in rat brain after 6-hydroxydopamine treatment. Neuropharmacology, 39, 614–620.

33. Frost, M., Khler, H., Blaschke, G. (2010) Enantioselective determination of methadone and its main metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) in serum, urine and hair by capillary electrophoresis. Electrophoresis, 31, 1026–1034.

34. Nakahara, Y., Kikura, R. (1994) Hair analysis for drugs of abuse. VII. The incorporation rates of cocaine, benzoylecgonine and ecoinine methyl ester into rat hair and hydrolysis of cocaine in rat hair. Archives of Toxicology, 68, 54–59.

35. Negusz, A., Moore, C.M., Kern, J.L., Janicak, P.G., Strong, M.J., Levy, N.A. (2000) Quantitation of clonazepam and its major metabolite 7-aminoclonazepam in hair. Journal of Analytical Toxicology, 24, 614–620.

36. Kikura, R., Nakahara, Y., Kojima, S. (2000) Simultaneous determination of dimethylamphetamine and its metabolites in rat hair by gas chromatography–mass spectrometry. Journal of Chromatography B, Biomedical Sciences and Applications, 741, 163–173.