Hair is a stable specimen and has a longer detection window (from weeks to months) than blood and urine. Through the analysis of hair, the long-term information of the drug use of the identified person could be explored. Our work is to establish an ultra-performance liquid chromatography–tandem mass spectroscopy (UPLC–MS/MS) method for simultaneous determination of methamphetamine, amphetamine, morphine, monoacetylmorphine, ketamine, norketamine, 3,4-methylenedioxyamphetamine (MDMA), and 3,4-methylenedioxyamphetamine (MDA) in hair. Methoxyphenazamine was used as an internal standard. The chromatographic separation was performed on a UPLC ethylene bridged hybrid (BEH) C18 (2.1 mm × 50 mm, 1.7 μm) column using a mobile phase of acetonitrile–water with 10 mmol/L ammonium acetate solution which containing 0.05% ammonium hydroxide. The multiple reaction monitoring in positive electrospray ionization was used for quantitative determination. The intra-day and inter-day precisions (relative standard deviation [RSD]) were below 15%. The accuracy ranged between 85.5% and 110.4%, the average recovery rate was above 72.9%, and the matrix effect ranged between 92.7% and 109.2%. Standard curves were in the range of 0.05–5.0 ng/mg, and the correlation coefficients were greater than 0.995. The established UPLC–MS/MS method was applied to analyze the hair samples successfully.

**Keywords:** UPLC–MS/MS, methamphetamine, amphetamine, morphine, monoacetylmorphine, ketamine, norketamine, MDMA, MDA, hair

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**Introduction**

Hair is used as a biological material and has an irreplaceable advantage over blood and urine [1–3]. Hair is a stable specimen and has a longer detection window (from weeks to months) than blood and urine [4–6]. Through the analysis of hair, could explore the long-term information of the drug use of the identified person. In particular, segmental hair analysis can provide useful information about drug abuse status and abuse history [6, 7]. Therefore, hair analysis has immeasurable value for forensic applications.

Because of the complex matrix of hair samples, the analysis is susceptible to interference from endogenous substances, so measuring drug abuse in hair is a challenge for analytical laboratories [8–10]. Gas chromatography–mass spectrometry (GC–MS) has been reported to detect drugs in hair samples. However, GC–MS technology has the following disadvantages: sensitivity is usually insufficient, sample pretreatment takes a long time, and derivatization steps such as morphine are required for compounds with poor gas chromatographic behavior. Compared with GC–MS, liquid chromatography with MS (LC–MS) has great advantages in biological sample analysis [11, 12]. Its use in forensic science is first directed to specific low-concentration polar analytes. In LC–MS analysis, the sensitivity is high, the amount of sample required is small, the sample pretreatment is simple, and no derivatization is required. Further, LC with tandem mass spectroscopy (LC–MS/MS) overcomes matrix background interference and improves signal-to-noise ratio, so it is more sensitive to complex biological samples and more suitable for analysis of low-level components.

The purpose of this study was to develop and validate an analytical method for the determination of methamphetamine, amphetamine, morphine, monoacetylmorphine, ketamine, norketamine, 3,4-methylenedioxyamphetamine (MDMA), and 3,4-methylenedioxyamphetamine (MDA) in human hair based on simple hair extraction, followed by ultra-performance liquid chromatography–tandem mass spectroscopy (UPLC–MS/MS) analysis. The main metabolite of methamphetamine was amphetamine; the main metabolites of heroin were morphine and monoacetylmorphine; the main metabolite of ketamine was norketamine; the main metabolite of MDMA was MDA. The selected toxicants represent the most common drugs such as methamphetamine, heroin, ketamine, and MDMA.

**Materials and Methods**

**Chemical Reagents.** Methamphetamine, amphetamine, morphine, monoacetylmorphine, ketamine, norketamine, MDMA, MDA, and methoxyphenamine (internal standard) (all purity >98%) were purchased from Sigma-Aldrich (Lewis, USA). Chromatographic grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Ultra-pure water (resistance >18 MΩ) was prepared by Milli-Q purification system (Bedford, USA).
Instruments and Conditions. ACQUITY UPLC and XevoXevo TQ-S Micro triple quadrupole mass spectrometer (Waters Corporation, USA) were use for determination of drugs in hair. An UPLC BEH C18 (2.1 mm × 50 mm, 1.7 μm) column with a column temperature of 40 °C was used, and the mobile phase was acetonitrile–10 mmol/L ammonium acetate solution (containing 0.05% ammonium hydroxide) at a flow rate of 0.4 mL/min with an injection volume of 1 μL. Gradient elution is with 10% initial acetonitrile for 0.2 min, increased into 80% in 1.3 min, keep at 80% for 0.5 min, then drop to 10% in 0.5 min, and hold for 1.5 min, with a total run time of 4.0 min. Nitrogen was used as desolvation gas (800 L/h) and cone gas (50 L/h). A capillary voltage of 2.5 kV, a source temperature of 150 °C, and a desolvation temperature of 400 °C were used. Multiple reaction monitoring (MRM) was used for quantitative analysis (Table 1).

Standard Solution Preparation. Methamphetamine, amphetamine, morphine, monoacetylmorphine, ketamine, norketamine, MDMA, and MDA were formulated as a 100 μg/mL methanol stock solution. The stock solution was diluted with methanol to the working solution, and all solutions are stored in a 4 °C freezer.

Preparation for Standard Curve. Healthy human hairs were given appropriate amounts of methamphetamine, amphetamine, morphine, monoacetylmorphine, ketamine, norketamine, MDMA, and MDA working solutions, constructed in the range of 0.02–5.0 ng/mg hair standard solutions (0.02, 0.05, 0.2, 1.0, 2.0, and 5.0 ng/mg). Quality control samples at concentrations of 0.05, 0.2, 0.8 and 4.0 ng/mg were prepared using the same method.

Sample Processing. A 10-mg hair was weighed, and washed with ultrapure water and acetone, then dried, cut, and ground. 0.3 mL methanol added, subjected to ultrasonic water bath for 1 h, with 13,000 rpm at 4 °C, and centrifuge for 10 min; 100 μL of the supernatant was transferred into a liner tube of a vial, and 1 μL was used for UPLC–MS/MS analysis.

Method Validation. The bioanalysis method validation was established according to the guidance of the US Food and Drug Administration. Validation projects include selectivity, matrix effects, linearity, precision, accuracy, recovery, and stability [13–22].

Applications. In 2018, the laboratory received 1193 hair samples and established test methods for common drug screening analysis.

Results and Discussion

Method Optimization. The hair pretreatment in this study used a simple pretreatment with water and acetone. Designed to meet the needs of fast hair treatment, it also cleans the drugs attached to the hair surface, as well as dust, inorganic salts, oils, and other impurities.

Various methods for extracting related drugs from hair have been reported, including enzymatic hydrolysis, acid hydrolysis, and alkaline hydrolysis. For example, the extraction of amphetamines and ketamine drugs is usually carried out by sodium hydroxide hydrolysis. Morphine is extracted by acid-hydrolysis, ultrasound-assisted, and liquid–liquid extraction methods, which has a long-term problem and cannot meet the needs of rapid experimental testing. In the direct methanol ultrasonic method, the measured drug is directly extracted with methanol by a simple dissolution extraction method. This reduces both the pre-processing time and the detection requirements.

As far as possible, the internal interfering substances are separated from the retention time by high-performance liquid chromatography (HPLC), and the mobile phase and chromatographic column determine the chromatographic behavior [23, 24]. We tried different chromatographic columns such as BEH C18 (2.1 mm × 50 mm 1.7 μm), BEH C18...
(2.1 mm × 100 mm, 1.7 µm), and HSS T3 (2.1 mm × 100 mm, 1.8 µm), and the results showed that BEH C18 (2.1 mm × 50 mm, 1.7 µm) had the best peak time and peak effect. We tried acetonitrile–0.1% formic acid, acetonitrile–10 mM ammonium acetate solution (containing 0.1% formic acid), methanol–0.1% formic acid, methanol–10 mM ammonium acetate solution (containing 0.1% formic acid), and acetonitrile–10 mM ammonium acetate solution (containing 0.05% ammonium hydroxide) with gradient elution. The results showed that acetonitrile–10 mM ammonium acetate solution (containing 0.05% ammonium hydroxide) results in the most satisfactory chromatographic peak shape and retention time. Thus, BEH C18 (2.1 mm × 50 mm, 1.7 µm) column and acetonitrile–10 mM ammonium acetate solution (containing 0.05% ammonium hydroxide) as the mobile phase were used in this work.

**Method Validation.** No impurities or endogenous substances that would interfere with the test could be identified, indicating that this method had good selectivity (Figure 1).

The equation for the standard curve of drugs was shown in Table 2. The intra-day precision RSD was below 15%, and the inter-day precision RSD was below 15%. The accuracy ranged between 85.5% and 110.4%, the average recovery rate was above 72.9%, and the matrix effect ranged between 92.7% and 109.2% (Table 3). The above results met the requirements of determination of drugs in biological tissues (acceptance criteria, intra-day, and inter-day accuracy: ±15% of the nominal concentrations; except ±20% at lower limit of quantification (LLOQ); intra-day and inter-day precision: ± 15% RSD, except ±20% RSD at LLOQ) [25].

UPLC–MS/MS was faster than traditional HPLC analysis and with enhanced signals [26–33]. Just 4 min to complete the analysis of plasma samples can save a lot of time. In addition, LLOQ (0.05 ng/mg) was relatively low, which can be used to determine low concentrations in hair.

**Applications.** In the laboratory, 1193 cases were detected. Positive concentration was set at 0.2 ng/mg. In fact, 381 cases were methamphetamine, followed by 6-acetylmorphine, ketamine, and MDMA (Table 4). When methamphetamine was positive, the methamphetamine and amphetamine were simultaneously detected at the same time. When heroin was positive, 6-acetylmorphine and morphine were simultaneously detected. When ketamine was positive, ketamine and norketamine were simultaneously detected. When MDMA was positive, MDMA and MDA were simultaneously detected.
## Conclusion

In this study, a simple, rapid, and selective simultaneous determination of amphetamine, morphine, monoacetylmorphine, ketamine, norketamine, MDMA, and MDA in hair by UPLC–MS/MS was developed and successfully applied to analysis of hair samples.

## Acknowledgements

This work was supported by grants from the start-up funding from Wenzhou Medical University (QJ17018).

## Table 4. Application to determine the hair sample in Wenzhou

| Area of inspection | Number of inspection | Positive number | Positive rate % | Methamphetamine Amphetamine Heroin 6-Acetylmorphine Morphine Ketamine Norketamine MDMA MDA |
|--------------------|----------------------|-----------------|-----------------|---------------------------------|-----------------|----------------|-----------------|-----------------|
| Yueqing            | 287                  | 75              | 26.13           | 63                             | 63              | 12             | 12              | 3               |
| Ouhai              | 247                  | 82              | 33.20           | 65                             | 65              | 18             | 18              | 3               |
| Lucheng            | 113                  | 48              | 42.48           | 42                             | 42              | 4              | 4               | 3               |
| Cangnan            | 113                  | 36              | 31.86           | 32                             | 32              | 1              | 1               | 3               |
| Kaiyuan            | 103                  | 17              | 16.50           | 16                             | 16              | 1              | 1               | 3               |
| Yongjia            | 98                   | 25              | 25.51           | 19                             | 19              | 7              | 7               | 3               |
| Ruian              | 79                   | 38              | 48.10           | 27                             | 27              | 1              | 1               | 1               |
| Taishun            | 56                   | 26              | 46.43           | 26                             | 26              | 1              | 1               | 3               |
| Longwan            | 40                   | 14              | 35.00           | 6                              | 6               | 1              | 1               | 3               |
| Dongtou            | 27                   | 6               | 22.22           | 6                              | 6               | 1              | 1               | 3               |
| Wencheng           | 24                   | 9               | 37.50           | 5                              | 5               | 2              | 2               | 3               |
| Pingyang           | 6                    | 5               | 83.33           | 4                              | 4               | 2              | 2               | 3               |
| Total              | 1193                 | 381             | 31.94           | 311                            | 311             | 45             | 33              | 15              |

Positive concentration of methamphetamine, 6-acetylmorphine, morphine, ketamine, and MDMA was set at 0.2 ng/mg, and the limit of detection of amphetamine, norketamine, and MDA was 0.02 ng/mg.