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Determination of opiates in human fingernail—Comparison to hair

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ABSTRACT

6-Monoacetylmorphine in keratinized matrices can be used to discriminate between heroin users and individuals exposed to other sources of morphine alkaloids. Frozen pulverization is effective in preventing 6-monoacetylmorphine hydrolysis. The main aim of this study was to develop an LC-MS/MS method for the determination of five opiates in human fingernails using a frozen pulverization preparation method and to investigate the correlation between the concentration of opiates in nail and hair samples from subjects whose urine specimens were positive for morphine. Borate buffer (500 μL; pH 9.2) was added to 20 mg of pulverized fingernail, followed by ultrasonication and liquid–liquid extraction. Analytes were analyzed on an Allure PFP propyl column by gradient elution. The mass spectrometer was operated in the positive electrospray ionization mode and multiple reactions monitoring mode. A total of 12 of 18 fingernail samples contained detectable 6-monoacetylmorphine (mean = 0.43 ng/mg, range = 0.10–1.37 ng/mg), morphine (mean = 1.74 ng/mg, range = 0.58–3.16 ng/mg) and codeine (range from <limit of quantification to 0.27 ng/mg). Similarly, 12 of 18 hair samples obtained from the same subjects were positive at the revised Society of Hair Testing cutoff level of 0.2 ng/mg. The concentrations of 6-monoacetylmorphine, acetylcodeine and codeine in hair were significantly higher than those in nails. However, the concentration of MOR in nails was significantly higher than that in hair, except for one sample. All of the ratios of 6-MAM/MOR were below 0.57. It is proposed that nails may be an alternative to hair for documenting heroin abuse.

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1. Introduction

Keratinized matrices (such as fingernails and hair) are important supplementary samples to body fluid for the determination of past drug exposure. It is generally accepted that keratinized matrices have the ability to accumulate and retain drugs taken in the past [1,2], enabling keratinized matrices to provide a historical profile of drug intake. Over the past several years, hair analysis has routinely been used in forensic toxicological analysis, occupational medicine and clinical toxicology [3]. As is the case for hair, drugs may remain in nails for an extended period of time, which can be useful in determining an individual's past drug exposure [4]. Because hair samples cannot always be obtained because of alopecia, personal habits of tonsure, religious customs, etc., nail samples could be potentially useful in these cases. However, the mechanism of drug entry and incorporation into nails is not known, and relatively few publications are available in the drug-abuse field based on nail samples [5].

Based on reports from the United Nations Office on Drugs and Crime in 2013 [6], the use of opioids (heroin, opium and prescription opioids) has increased in Asia since 2009. The injection of drugs remains a serious public health concern in a number of countries in East and Southeast Asia. The abuse of heroin is a serious social problem in China [7]. 6-Monoacetylmorphine (6-MAM), a unique metabolite of heroin [8], hydrolyzes to morphine (MOR) rapidly [9] and is difficult to detect in urine hours after heroin intake. However, MOR present in urine samples may also originate from the ingestion of therapeutic drugs [10]. Therefore, the interpretation of positive MOR findings in urine samples is a problem in toxicology. In China, cough syrup and some traditional Chinese medicines that contain opiates are widely used. The so-called poppy seed problem could be solved by testing hair for 6-MAM because 6-MAM is not detected in hair after the consumption of MOR, codeine (COD), seeds or other substances [8,11,12].

As a specific marker for heroin abuse, 6-MAM is an important target compound in keratinized matrices [8]. Compared to other fluid matrices, keratin materials are a much more complex substrate to address in the development of analytical methods [13–15]. Because keratin materials are solid matrices, they must be digested/hydrolyzed to extract the target analytes. 6-MAM is likely

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to convert into MOR during typical hair incubation procedures, such as those performed under acidic or alkaline conditions. As a consequence: (i) the concentration of opiates measured in hair depends on the extraction method used and (ii) the ratios between different analytes (e.g., 6-acetylmorphine vs. morphine) may reflect the rate of hydrolysis during sample preparation rather than different types of exposure to opiates [13,16].

This issue can also be addressed using fingernail samples. To date, few papers have investigated the presence of opiates in nail samples. Lemos et al. [17] analyzed morphine in fingernail clippings after alkaline hydrolyzation by HPLC. Cingolani et al. [18] determined morphine, 6-acetylmorphine and cocaine in toe nails simultaneously after hot acid hydrolysis (1 mL of HCl 37%). The correct and reliable interpretation of opiate detection results for an authentic nail sample requires the optimization of incubation procedures. Frozen pulverization is a new sample preparation method commonly used in chemistry, biology and other fields through which samples are pulverized completely at cold temperatures generated by liquid nitrogen. In our previous work, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the simultaneous analysis of 11 opiates in hair samples was developed using frozen pulverization preparation, which is suitable for chemically unstable compounds, such as 6-MAM and acetylcodeine (AC) [19].

In this paper, an LC-MS/MS method was developed for the determination of 6-MAM, MOR, COD, AC and heroin in human fingernails prepared by frozen pulverization. The purpose of this study was to investigate the correlation between the concentration of opiates in nail and hair samples from subjects whose urine specimens were positive to MOR and to evaluate the usefulness of fingernails as an analytical specimen in the discrimination between heroin users and individuals exposed to other sources of morphine alkaloids.

2. Experimental

2.1. Reagents

6-MAM, MOR, COD, AC, heroin, 6-MAM-d6 and MOR-d3 were all purchased from Cerilliant (Round Rock, TX, USA). Acetonitrile, methanol and 2-propanol, both of HPLC gradient grade, were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-solvent-grade chloroform was purchased from Baker (Baker, USA). HPLC-grade ammonium acetate and formic acid (p.a., 50%) were purchased from Fluka Chemical Co. (Buchs, Switzerland). The acetone used for decontamination was of analytical grade. Ultra-purified water was prepared by a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Sample and preparation

Drug-free nail samples were donated by six laboratory personnel with a known non-opioid history.

Nail samples were obtained from all 10 fingers using commercial cosmetic nail clippers and were stored independently in a clean paper bag at room temperature in a dry and lightproof location prior to analysis. The nail samples were rinsed for 2 min using deionized water twice and then washed with acetone twice. The final acetone wash was collected and analyzed to exclude contamination. After washing, the nail samples were air-dried. The dried nails were pulverized in an SPEX 6700 Freeze Mill (Freezer/Mill, SPEX CertiPrep) with liquid nitrogen. The nail powder (approximately 20 mg) was transferred to a clean Eppendorf tube. Then, 500 μL of borate buffer (pH 9.2) (containing 50 ng of 6-MAM-d6 and 50 ng of MOR-d3 as internal standards) was added into the tube. The sample tubes were sonicated in an ultrasonic bath for 30 min at room temperature. Samples were extracted with 500 μL of chloroform:2-propanol (9:1, v/v). After centrifugation (12 000 rpm), the organic solvent was evaporated at 45 °C, and the residue was reconstituted with 100 μL of acetonitrile–20 mM ammonium acetate buffer (20 mM ammonium acetate buffer with 0.1% formic acid, pH 4.0) (8:2, v/v); a 5-μL sample was used for analysis.

The hair sample was prepared according to the procedure described in the previous work [19]. Briefly, a 3-cm hair follicle (as measured from the root of the hair) was cut and washed twice with water and acetone. The last acetone wash was collected and analyzed to exclude contamination. After drying, the hair sample was pulverized with an SPEX 6700 Freeze Mill. A 20-mg sample of powered hair was sonicated in an ultrasonic bath in 500 μL of borate buffer (pH 9.2) for 30 min at room temperature in the presence of 50 ng of 6-MAM-d6 and 50 ng of MOR-d3 as internal standards. The sample was then mixed with 500 μL of chloroform:2-propanol (9:1, v/v), vortexed and centrifuged (12 000 rpm). After solvent evaporation at 45 °C, the residue was reconstituted with 100 μL of acetonitrile–20 mM ammonium acetate buffer (8:2, v/v), and 5 μL of the sample was injected for analysis.

2.3. Apparatus and conditions

LC–MS/MS analysis was performed on a Waters Acquity UPLC system (Waters Corp., Milford, MA, USA) coupled with an API 4000 QTRAP mass spectrometer (Applied Biosystems/MSD Sciex, Foster City, CA). The software Analyst 1.5 (ABSciex, Darmstadt, Germany) was used for LC–MS/MS operation and data analysis.

2.3.1. Chromatographic conditions

The LC consisted of an online degasser, a quaternary pump and an autosampler. The analytical column was an Allure PFP Propyl column (2.1 × 100 mm, 5 μm i.d. Restek, Bellefonte, USA) fitted with a Zorbax Extend-C18 narrow-bore guard column (2.1 × 12.5 mm 5 μm i.d., Agilent Technologies, Espoo, Finland). The mobile phase consisted of acetonitrile (phase A) and an ammonium acetate buffer (phase B). Chromatographic separation was achieved by gradient elution at a flow rate of 0.3 mL/min. The gradient was as follows: initial condition (18% A), 0–2 min (18% A), 2–3 min (18–27% A), 3–8 min (27% A) and 8–10 min (27–18% A). The autosampler temperature was maintained at 4 °C, and the column temperature was 45 °C.

2.3.2. Mass spectrometric conditions

The mass spectrometer was operated in the positive electrospray ionization (ESI+) mode and multiple reactions monitoring (MRM) mode. The optimized mass parameters are presented in Table 1. The other mass spectrometric settings were optimized as

Table 1
Multiple reaction monitoring (MRM) parameters for five opiates and internal standards.

Compound	Precursor ion (m/z)	Product ion (m/z)	DP (V)	CE (V)
6-MAM	328.3	165.3	100	57
		211.3		35
MOR	286.1	201.2	80	70
		165.2		56
AC	342.4	229.3	80	37
		282.3		60
COD	300.2	165.3	84	62
		199.2		37
Heroin	370.4	310.1	100	61
		268.2		48
6-MAM-d6	334.3	165.3	100	40
		211.5		37
MOR-d3	289.2	201.1	80	59
		165.3		56

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