Technical Note

Simultaneous Detection and Quantitation of Morphine, 6-Acetylmorphine, and Cocaine in Toenails: Comparison with Hair Analysis

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Abstract

This study describes the results of the simultaneous detection and quantitation of morphine, 6-acetylmorphine, and cocaine in toenail and hair samples obtained from 18 forensic autopsies of drug abusers who had died in various manners. After external decontamination, each specimen was submitted to hot acid hydrolysis (1 mL of HCl 37%) in the presence of internal standards, followed by liquid–liquid and solid-phase extraction techniques. The extracts were then derivatized with propionic anhydride and analyzed by gas chromatography–mass spectrometry, operating in the selected ion monitoring mode. The limit of quantitation for all analytes was 0.5 ng on column. Results showed that both cocaine and morphine are more concentrated in toenails than in hair. Mean concentrations were 0.99 ng/mg (toenails) versus 0.48 ng/mg (hair) for cocaine and 1.27 ng/mg (toenails) versus 0.79 ng/mg (hair) for morphine. Distribution of 6-acetylmorphine showed no significant variations between the two (mean concentrations 0.46 ng/mg vs. 0.50 ng/mg in hair).

Introduction

Using samples of nails is an alternative or a complement to hair specimens in the detection and quantitation of therapeutic substances and drugs of abuse in humans and is an interesting field of forensic toxicology in the laboratory (1–8).

The mechanism of drug entry and incorporation into the nail matrix is not well known (2). However, it is assumed that the dividing cells responsible for nail formation also incorporate drugs. During formation, drugs may be incorporated continuously or as a single event. It has been reported that drugs gain rapid access to the distal nail plate during nail production by incorporation into cornified cells of the nail bed. Incorporation of drugs by diffusion from the nail bed to the ventral portion of the nail plate is thought to be minimal (2,9,10). Studies have also shown that nail production and drug incorporation occur in the lunular germinal matrix, as the nail plate grows distally from the base of the nail (2,11,12). Another potential mechanism of drug entry is through exposure to environmental contamination and biological fluids, including sweat, sebum, saliva, and urine. In addition, processes previously identified in hair may also influence incorporation (2,13). These include the chemical nature of the drug analyte (e.g., lipophilicity and state of ionization), the metabolic profile of the drug, and the composition of the matrix.

Drugs of abuse and their metabolites, including cocaine, amphetamines, and opiates, have been successfully detected in nails. The first studies go back to 1984, when Suzuki and others identified amphetamines and methamphetamines in nails obtained from abusers by sensitive gas chromatography–chemical ionization mass spectrometry (GC–MS) (1).

Since then, attention has focused much more on the detection of a wide variety of drugs of abuse in nails, with not always concordant data.

In 1994, Miller and others reported comparisons between hair and nails and showed that the cocaine/benzoylecgonine ratio was approximately 5:1 in hair and close to 1:1 in nails. These authors also reported that the total concentrations of target drugs in hair were substantially higher than those found in nails (14).

In 1995, Cirimele and others showed that amphetamine (AP), 3,4-methylenedioxyamphetamine (MDA), and 3,4-methylenedioxymethamphetamine (MDMA) can be detected in both hair and fingernails, concentrations of AP, MDA, and MDMA detected in fingernails being slightly higher than those in hair (7). They found 10.2 and 12.0 ng/mg of AP, 8.0 and 9.7 ng/mg of MDA, and 53.4 and 60.2 ng/mg of MDMA in head hair and fingernails, respectively. These data are not in accordance with the results of Miller's study.

In 2000, Miller and others, investigating the disposition patterns of cocaine and opiates in hair and fingernail specimens, demonstrated that higher concentrations of drug were found in hair than in fingernails (3).

More recently, the suitability of nails for forensic purposes has been studied by others (8,15–17).

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The aims of our study were to detect and quantitate morphine, 6-acetylmorphine, and cocaine in toenails obtained from forensic autopsies of drug abusers and to compare results with those from head hair analysis to ascertain the suitability of nail analysis as a complement to the study and interpretation of drug use.

Materials

Analyses were performed on toenail samples from 18 habitual heroine/cocaine abusers who had died in various manners. Toxicological findings in body fluids and causes of death in the 18 cases are listed in Table I. Entire nails were removed, postmortem, from the big toe, including the matrix portion; hair was cut from the vertex of the scalp as close as possible to the skin. Samples for analysis were selected from the proximal portions of both toenails and hair.

Analytical-grade chemicals and reagents were ammonium hydroxide (Sigma Aldrich Srl, Milano, Italy), Bond Elute Certify extraction columns (Varian, Torino, Italy), chloroform (Carlo Erba, Milano, Italy), cocaine (Alltech, State College, PA), ethyl acetate (Sigma Aldrich Srl), hydrochloric acid (Carlo Erba), isopropyl alcohol (Sigma Aldrich Srl), methanol (Carlo Erba), methylene chloride (Sigma Aldrich Srl), morphine (Alltech), 6-MAM (Alltech), nalorphine (Sigma Aldrich Srl), propionic anhydride (Sigma Aldrich Srl), and sodium hydroxide (Merck, Darmstadt, Germany). Borate buffer (pH 9.0) was prepared from a mixture of boric acid (6.20 g), deionized water (500 mL), and sodium hydroxide (41.5 mL), making the volume up to 1000 mL with deionized water.

Equipment included a homogenizer (Ultra Turrax, Ica Labor Technik, Milan, Italy), sonicator (model 1210, Bramson, Damburg, CO), centrifuge (model 4218, ALC, Milan, Italy), vortex mixer (Falc, Bergamo, Italy), Analytichem Vac Elut (Varian SPS 24, Palto Alto, CA), temperature bath (Carlo Erba thermostatic bath), and GC–MS (Varian Saturn 4200).

Table I. Quantitation of Morphine and Cocaine in Body Fluids

	Cocaine (mg/L)			Morphine (mg/L)		
Case	Blood	Urine	Bile	Blood	Urine	Bile
1 SD*	–	–	–	–	–	–
2 CRF	–	–	–	0.300	–	–
3 VD	–	–	–	–	–	–
4 CRF	–	ns	–	0.058	ns	82.18
5 CRF	–	ns	ns	0.900	ns	ns
6 CRF	–	ns	ns	1.490	ns	ns
7 CRF	–	ns	–	1.680	ns	0.067
8 CRF	1.500	0.190	–	11.480	1.750	5.840
9 VD	–	–	–	–	–	–
10 CRF	0.068	0.020	4.035	0.480	0.028	0.480
11 CRF	0.013	traces	–	0.526	0.627	0.807
12 CRF	0.002	0.006	ns	0.180	2.550	ns
13 CRF	–	–	–	7.990	–	–
14 CRF	0.440	0.360	11.63	0.620	0.420	0.450
15 CRF	–	–	–	3.850	–	–
16 CRF	–	–	–	0.930	–	–
17 CRF	0.350	–	–	2.330	2.710	–
18 CRF	–	–	–	0.450	–	–

* Abbreviations: SD, sudden death, –, not detected, CRF, cardio-respiratory acute failure due to acute overdose; VD, violent death; and ns = no specimen available for analysis.

Methods

Preparation of samples

Toenails. Fifty milligrams of toenails was cut into small pieces and placed in a test tube. The samples were washed twice with methylene chloride (2 mL) by stirring for 10 min.

Hair. Fifty-milligram samples were drawn and placed in a test tube. The samples were washed twice with methylene chloride (2 mL) by stirring for 10 min. All decontamination washes were eliminated. The following procedure was adapted from a study previously published by Cingolani et al. (18).

Acid hydrolysis

One milliliter of nalorphine solution (1 mcg/mL in methanol) as internal standard and 1 mL of HCl (37%, v/v in water) were added to each sample (both toenails and hair). The mixture was incubated at 100°C for 30 min in a 10-mL glass tube sealed with a Teflon screw cap in a thermostat bath and then kept for 12 h at room temperature. At the end of incubation, the solution was alkalinized to pH 8.9–9.0 using sodium hydroxide (25%, w/v in water).

Table II. Quantitative Results in Hair and Toenails

	Hair (ng/mg)			Toenails (ng/mg)		
Case	Cocaine	Morphine	6-MAM	Cocaine	Morphine	6-MAM
1	0.41	0.21	–*	–	2.98	–
2	–	–	–	–	1.03	–
3	0.24	–	–	–	1.19	–
4	–	0.17	–	–	2.03	–
5	0.22	3.05	0.61	–	-	0.36
6	0.47	0.67	0.65	–	0.78	–
7	–	0.65	0.23	–	0.52	–
8	0.15	1.29	–	–	3.05	–
9	0.37	–	–	0.10	0.60	–
10	–	0.11	–	0.10	–	–
11	–	0.14	–	0.12	–	–
12	–	0.12	–	–	–	–
13	–	–	0.85	–	0.41	–
14	–	–	–	–	–	–
15	0.6	0.5	–	0.47	0.54	0.33
16	1.07	-	–	4.6	0.77	0.54
17	0.92	1.8	–	1.10	2.2	0.83
18	0.38	–	0.17	0.47	0.47	0.25

* –, not detected.

Liquid–liquid extraction

The procedure was carried out with 10 mL of chloroform/isopropylic alcohol solution (3:1, v/v). The separated organic layer was then transferred to another silanized tube and evaporated to dryness under a stream of nitrogen at 90°C. The residue was reconstituted in 2 mL of borate buffer (pH 9.0).

Solid-phase extraction

Bond Elute Certify extraction columns were used.

Preparation of columns. Columns were conditioned sequentially with 2 mL of methanol and 2 mL of phosphate buffer (0.1M) at pH 6.0–7.0.

Specimen application. Samples were transferred to the cartridge and allowed to flow slowly through the columns, under low vacuum.

Column rinsing. Columns were rinsed sequentially with 2 mL of distilled water, 3 mL of 0.1M hydrochloric acid, and 5 mL of methanol and then dried under a vacuum of 15 mmHg for 10 min.

Analyte elution. Extracts were eluted twice, respectively, with 1 mL of methylene chloride/isopropylic alcohol solution (8:2, v/v) containing 2% ammonium hydroxide, freshly prepared. Eluates were transferred to 7-mL silanized gradual tubes and evaporated to dryness under a gentle stream of nitrogen at 30°C.

Derivatization

Dried elutes were derivatized with 50 µL of propionic anhydride for 1 h at 90°C (tubes closed and vortex mixed) (19). The solution was evaporated to dryness at 30°C, and the residue was dissolved in 50 µL of ethyl acetate; 1 µL of this was subjected to GC–MS analysis, as described.

GC–MS analysis

The following conditions were applied: a DB-5 MS capillary column (fused silica, 30 m × 0.25 mm) was used; carrier gas was helium, at a flow rate of 1.8 mL/min. The temperature program was started at 140°C for 1 min and increased to 300°C at a rate of 15°C/min. The injection volume was 1 µL in splitless mode. The mass detector operated in the selective ion monitoring mode on ions obtained for electronic impact at 70 EV. Mass spectra were recorded in a mass range *m/z* 40–650.

Quantitative results in hair and toenails are listed in Table II. Morphine, 6-MAM, and cocaine were identified by comparisons with a retention time of the internal standard and the mass spectra of morphine, 6-MAM, and cocaine standards (morphine/dipropionyl = *m/z* 268, 341, and 397; 6-MAM/propionyl = *m/z* 268, 327, and 383; and cocaine = *m/z* 82, 182, and 303). Quantitation was carried out by monitoring the abundance of *m/z* 341 ions (morphine/dipropionyl), *m/z* 383 (6-MAM/propionyl), *m/z* 182 (cocaine), and *m/z* 367 (nalorphine/dipropionyl) (13). To verify the linearity of the detector and to calibrate the method, five calibrators were tested, 0.1, 0.5, 1.0, 2.0, and 4.0 ng/mg (morphine, 6-MAM, and cocaine in drug-free tested toenails). Linear regression analysis of calibration data showed a correlation coefficient approaching 1.0. The limit of morphine, 6-MAM, and cocaine quantitation was set at 0.1 ng/mg.

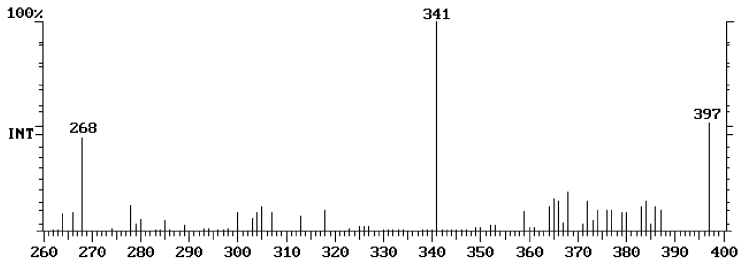

Figure 1. Chromatographic results of toenail sample (case 17 of Tables I and II). Mass spectrum of peak at retention time 11.66 min (*m/z* 268, 341, and 397 morpine/dipropionyl).

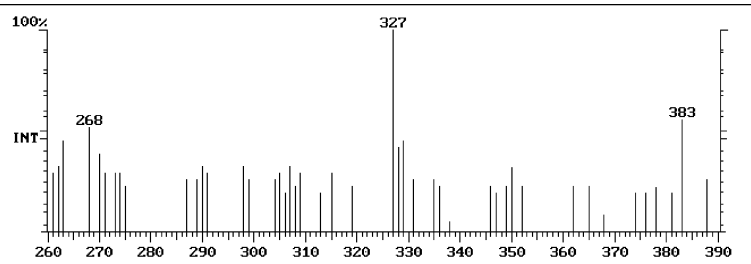

Figure 2. Chromatographic results of toenail sample (case 17 of Tables I and II). Mass spectrum of peak at retention time 11.73 min (*m/z* 268, 327, and 383 6-MAM/propionyl).

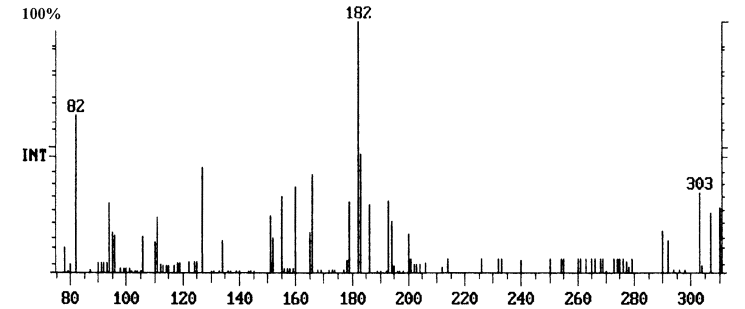

Figure 3. Chromatographic results of toenail sample (case 17 of Tables I and II). Mass spectrum of peak at retention time 10.42 min (*x* 82, 182, and 303 cocaine).

Results and Discussion

Chromatograms of positive morphine, 6-MAM, and cocaine toenail samples are shown in Figures 1–3, obtained from subject 17. The quantitative values of each drug in toenails and hair proved their different distribution in the keratinized matrices.

Both cocaine and morphine were more concentrated in toenails than in hair. Mean concentrations were 0.99 ng/mg (toenails) versus 0.48 ng/mg (hair) for cocaine and 1.27 ng/mg (toenails) versus 0.79 ng/mg (hair) for morphine (Figure 4). Instead, 6-MAM showed no significant variations between the two (mean

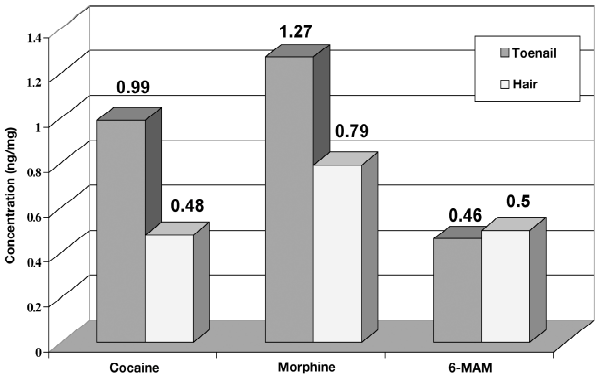

Figure 4. Means of morphine, 6-MAM, and cocaine concentrations (ng/mg) in hair and toenail samples.

concentrations 0.46 ng/mg in toenails vs. 0.50 ng/mg in hair). These results are in contrast with those published by Miller and others, who reported that total concentrations in hair were substantially higher than in nails (3). The different distributions may probably be explained in view of the different speeds of growth of these keratinized matrices and their different mechanism of incorporation. Drugs are incorporated into nails by a double mechanism of deposition, into the root via blood flow in the nail matrix and the nail bed (20). This mechanism explains the observed difference, and thus allows us to assume the two matrices are able to cover different time windows.

In order to compare the analyses of biological fluids (blood, urine, and bile) and keratinized matrices, positive results were observed in both hair and nails (cases 1, 3, and 9), whereas corresponding biological fluids were negative in the tested substances. The presence of these substances in keratinized matrices only contributed additional information regarding subjects' drug exposure, revealing their past abuse. These data are clear when we consider the causes of death of subjects 1, 3, and 9, which were not due to acute overdose, but to sudden death (case 1) and violent death (cases 3 and 9); all the other subjects died of acute overdose. Case 14 was slightly different, in that positive results were only obtained in biological fluids, negative ones being found in nails and hair. This was probably due to the fact that drugs had been used immediately prior to death (i.e., the time interval in question was too short to enable us to detect drugs already distributed in keratine samples).

Conclusions

Results indicate that morphine, 6-MAM, and cocaine show good distributions in toenails, improving our knowledge about the use of these matrices and the different mechanisms of drug incorporation in nails and hair. The stability of drugs in nails makes their analysis a valuable tool for postmortem investigation. In most cases, there are sufficient positivity matches between hair and toenail analysis. Therefore, toenails may be used as a complement or alternative to hair analysis in all cases in which it is impossible to obtain specimens of hair. Quantitative data obtained from toenails, for both morphine and cocaine, are higher than those obtained from hair; this difference may be attributed to the differing processes of their distribution and speeds of growth.

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