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Simultaneous assay of cocaine, heroin and metabolites in hair, plasma, saliva and urine by gas chromatography–mass spectrometry

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Abstract

As part of an ongoing research program on the development of drug detection methodology, we developed an assay for the simultaneous measurement of cocaine, heroin and metabolites in plasma, saliva, urine and hair by solid-phase extraction (SPE) and gas chromatography–mass spectrometry (GC–MS). The analytes that could be measured by this assay were the following: anhydroecgonine methyl ester; ecgonine methyl ester; ecgonine ethyl ester; cocaine; cocaethylene; benzoylecgonine; cocaethylene; norcocaethylene; benzoylnorecgonine; codeine; morphine; norcodeine; 6-acetylmorphine; normorphine; and heroin. Liquid specimens were diluted, filtered and then extracted by SPE. Additional handling steps were necessary for the analysis of hair samples. An initial wash procedure was utilized to remove surface contaminants. Washed hair samples were extracted with methanol overnight at 40°C. Both wash and extract fractions were collected, evaporated and purified by SPE. All extracts were evaporated, derivatized with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) and analyzed by GC–MS. The limit of detection (LOD) for cocaine, heroin and metabolites in biological specimens was approximately 1 ng/ml with the exception of norcodeine, normorphine and benzoylnorecgonine (LOD = 5 ng/ml). The LOD for cocaine, heroin and metabolites in hair was approximately 0.1 ng/mg of hair with the exception of norcodeine (LOD = 0.3 ng/mg) and normorphine and benzoylnorecgonine (LOD = 0.5 ng/mg). Coefficients of variation ranged from 3 to 26.5% in the hair assay. This assay has been successfully utilized in research on the disposition of cocaine, heroin and metabolites in hair, plasma, saliva and urine and in treatment studies.

1. Introduction

The detection and measurement of drugs and metabolites in biological specimens provides treatment specialists, health officials, and law enforcement officers with an objective means for the diagnosis of drug abuse by human subjects. A variety of techniques have been utilized for specimen analysis, but gas chromatography–mass spectrometry (GC–MS) is the required methodology for drug testing in the United States as specified by the Mandatory Guidelines for Federal Workplace Drug Testing [1]. Presently, only urine is routinely collected and analyzed in workplace drug testing programs. Recently, there has been growing interest in the

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utilization of other types of biological specimens for drug testing such as hair, saliva and blood. Different specimens provide unique pharmacologic information regarding drug exposure by the individual who is undergoing testing. Generally, urine drug testing provides evidence of recent usage for 2–4 days following acute drug administration. Hair testing can provide information on drug exposure dating back months to years, depending on the length of an individual's hair. Blood and saliva provide a short-term measure of drug exposure (< 1 day), but can be correlated more closely with drug-induced effects than either urine or hair testing results.

Cocaine and heroin are two of the most commonly encountered illicit drugs in drug testing programs in the United States. Often, these drugs are used in combination by the intravenous route. Data from the Drug Abuse Warning Network (DAWN) for the year 1990 indicate that cocaine in combination with heroin is the most frequently used drug combination, except alcohol, reported by both emergency rooms and medical examiners [2,3]. Although there have been numerous assays reported for the separate detection of cocaine and opiates, simultaneous assays for cocaine and heroin by GC–MS are limited [4]. Further, each drug is extensively metabolized to a number of major and minor metabolites (Figs. 1 and 2) adding to the complexity of detection. Recently, we reported a simultaneous assay for cocaine and heroin in hair samples [5]. The assay utilized solid-phase extraction (SPE) and analysis by GC–MS. This report describes the optimization of this assay for the analysis of cocaine, heroin and fourteen related analytes in hair, urine, saliva and plasma specimens.

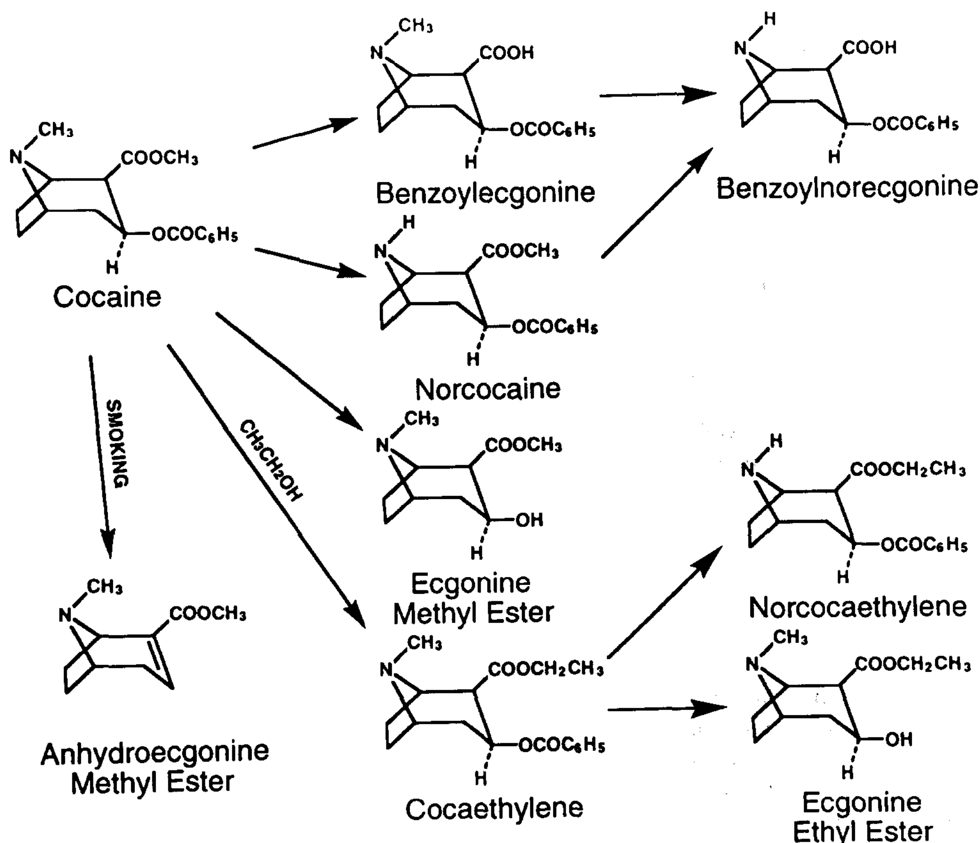

Fig. 1. Metabolic and degradation pathways of smoked cocaine ("crack").

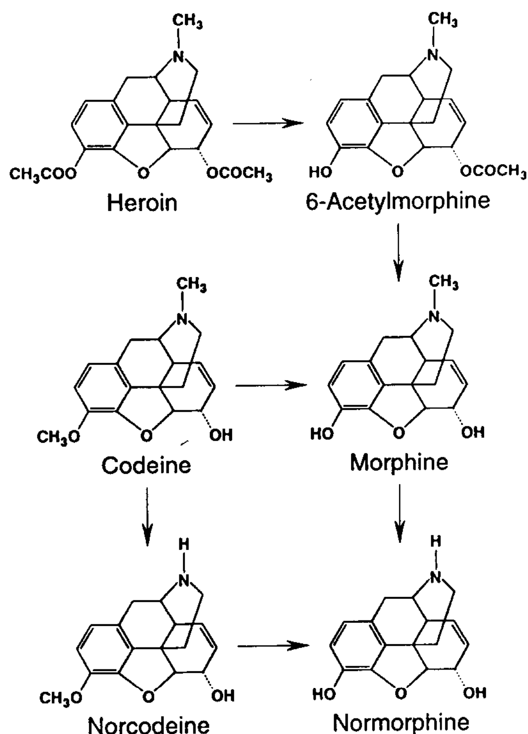

Fig. 2. Metabolic pathways of heroin, morphine and codeine.

2. Experimental

2.1. Chemicals and materials

Cocaine hydrochloride was obtained from Mallinckrodt (St. Louis, MO, USA). Benzoylecgonine tetrahydrate, ecgonine methyl ester hydrochloride, benzoylnorecgonine and norcocaine hydrochloride were obtained from the Research Technology Branch, National Institute on Drug Abuse (Rockville, MD, USA). Cocaethylene and norcocaethylene were generous gifts from Dr. Ivy Carroll, Research Triangle Institute (Research Triangle, NC, USA). Anhydroecgonine methyl ester and ecgonine ethyl ester were generous gifts from Dr. Andrew Allen, Addiction Research Center, National Institute on Drug Abuse (Baltimore, MD, USA). Morphine sulfate pentahydrate was obtained from Sigma (St. Louis, MO, USA). Codeine phosphate, norcodeine hydrochloride and normorphine hydrochloride were purchased from Merck (Rahway, NJ, USA). Heroin hydrochloride was obtained from Alltech-Applied Science (State College, PA, USA). 6-Acetylmorphine, $[^2H_3]$-6-acetylmorphine and $[^2H_3]$-cocaethylene were purchased from Radian Corporation (Austin, TX, USA). $[^2H_3]$Cocaine, $[^2H_3]$benzoylecgonine tetrahydrate, $[^2H_3]$ecgonine methyl ester HCl, $[^2H_3]$codeine and $[^2H_3]$morphine were purchased from Sigma. $[^2H_9]$Heroin was synthesized at the Addiction Research Center, National Institute on Drug Abuse by a published procedure [6]. Methanol, methylene chloride, 2-propanol and acetonitrile (J.T. Baker, Phillipsburg, NJ, USA) were HPLC-grade solvents. N,O-Bis-(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) was purchased from Pierce (Rockford, IL, USA). Solid-phase extraction (SPE) columns (Clean Screen DAU, 200 mg, 10 ml), filtration columns (10 ml), and the 24-position vacuum manifold system were purchased from United Chemical Technologies (Horsham, PA, USA). GC–MS autosampler microvials were purchased from Sun Brokers (Wilmington, NC, USA). Acetate buffers (pH 4.0 and pH 6.0) were prepared as varying mixtures of 0.5 *M* sodium acetate and 0.5 *M* acetic acid. Sodium fluoride was added to the pH 6.0 buffer to give a final concentration of 0.5% (w/v).

2.2. Collection of plasma, saliva, urine and hair samples

Clinical specimens were collected from healthy volunteers with a history of drug abuse who participated in research protocols at the Addiction Research Center (NIDA, Baltimore, MD, USA). All protocols were approved by the Institutional Review Board for Human Subjects Research at the Francis Scott Key Medical Center (Baltimore, MD, USA). The study was conducted under the guidelines for the protection of human subjects, and each volunteer gave informed consent. Blood, saliva, urine and head hair samples were collected, processed and frozen immediately at −30°C. Samples remained frozen until analysis.

2.3. Preparation of drug solutions for hair analysis

Standard drug solutions were prepared in methanol and stored at −30°C until used. Separate solutions were employed for the preparation of standard curves and control samples. Standard curve and control samples were prepared by the addition of stock drug solutions (0.5 mg/ml) to washed drug-free hair samples at the beginning of the extraction procedure. The concentrations of cocaine, heroin and metabolites ranged from 0.1–100 ng/mg of hair. Tri-deuterated analogs of cocaine, cocaethylene, ecgonine methyl ester, benzoylecgonine, 6-acetylmorphine, morphine and codeine were available as internal standards and were added in concentrations of 5 ng/mg of hair for cocaine and metabolites and 2.5 ng/mg of hair for opiates. [$^2H_9$]Heroin was used as the internal standard for heroin. Standard solutions of heroin and [$^2H_9$]heroin were prepared immediately before use to minimize chemical hydrolysis.

2.4. Preparation of drug solutions for urine, plasma and saliva analysis

Standard drug solutions were prepared in deionized water and were stored at −30°C until used. Standard curve and control samples were prepared by the addition of stock drug solutions to the biological matrix in a ratio of 9:1 (v/v). The concentrations of cocaine and cocaine metabolites ranged from 12.5 to 1000 ng/ml. Concentrations of heroin and heroin metabolites ranged from 2.5 to 100 ng/ml. Internal standards were identical to those used for hair analysis, but concentrations were adjusted to 100 ng/ml for cocaine and metabolites and to 50 ng/ml for opiates. Due to the instability of heroin in aqueous solution, heroin and [$^2H_9$]heroin were prepared as acetonitrile solutions immediately before use on a daily basis.

2.5. Instrumentation

Quantitative analyses were performed on a Hewlett-Packard 5890A gas chromatograph equipped with an autosampler (HP7673A) and interfaced with a Hewlett-Packard 5970B mass-selective detector (MSD). A split–splitless capillary inlet system and a HP-1 fused-silica capillary column (12 m × 0.2 mm I.D., 0.33 μm film thickness) were utilized for the analyses.

2.6. Chromatographic conditions

The splitless injection mode with a purge-off time of 0.5 min was used for all analyses. Ultrapure grade helium was utilized as the carrier gas at a flow-rate of 1 ml/min. The initial oven temperature was 70°C, held for 1 min, programmed to 220°C at 35°C/min, held at 220°C for 0.25 min, programmed to 250°C at 10°C/min and held for 3 min. The injection port and transfer line temperatures were maintained at 250°C and 280°C, respectively.

The mass-selective detector was operated in the selected-ion monitoring mode. Three ions for analytes and two ions for internal standards were monitored. The ions for each compound were monitored in the following elution order (quantitative ion indicated in parenthesis): anhydroecgonine methyl ester, m/z (152), 166, 181; [$^2H_3$]ecgonine methyl ester, m/z (99), 85; ecgonine methyl ester, m/z (96), 82, 271; ecgonine ethyl ester, m/z (96), 83, 285; [$^2H_3$]cocaine, m/z (185), 85; cocaine, m/z (182), 82, 303; [$^2H_3$]cocaethylene, m/z (199), 85; cocaethylene, m/z (196), 82, 317; [$^2H_3$]benzoylecgonine, m/z (243), 85; benzoylecgonine, m/z (240), 82, 361; norcocaine, m/z (140), 240, 346; norcocaethylene, m/z (254), 140, 360; benzoylnorecgonine, m/z (404), 140, 298; [$^2H_3$]codeine, m/z (374), 237; codeine, m/z (371), 234, 178; [$^2H_3$]morphine, m/z (432), 239; morphine, m/z (429), 236, 414; norcodeine, m/z (429), 250, 292; [$^2H_6$]-6-acetylmorphine, m/z (405); [$^2H_3$]-6-acetylmorphine, m/z (402), 343; 6-acetylmorphine, m/z (399), 340, 287; normorphine, m/z (487), 254, 308; [$^2H_9$]heroin, m/z (334), 378; heroin, m/z (327), 310, 369. Mass axis calibration for all quantitative ions was determined daily with an unextracted standard. Resolution was maintained at 0.1 amu. The GC–MS was autotuned daily according to manufacturer's instruction. The electron multiplier was

operated at +200 eV relative to the tune value. Daily maintenance of the GC–MS included clipping of the GC column and replacement of the injector septum, liner and seal.

Quantitation of ecgonine methyl ester, cocaine, cocaethylene, benzoylecgonine, codeine, morphine, 6-acetylmorphine and heroin was based upon ratios of peak area to the corresponding deuterated internal standard of each analyte. Quantitation of compounds without deuterated internal standards was based upon ratios of peak area to the nearest corresponding deuterated internal standard. Analytes were identified based upon comparison of retention time and relative abundance of the confirming ions to the corresponding values of authentic standards assayed in the same run.

2.7. Processing, washing and extraction of hair samples

Hair samples (10 mg) were placed in a filtration column and weighed. The bottom of the column was sealed and 1 ml of methanol was added as wash solvent. The hair was cut into fine particles with surgical scissors. The total time of cutting and washing lasted approximately 1 min. The columns were placed in a vacuum manifold and the methanol wash fraction was collected in 13 × 100 mm borosilicate culture tubes. The remaining hair was rinsed with methanol (2 × 1 ml) with vacuum assisted elution. The wash fractions were collected, combined and internal standards (100 μl containing 25 ng of opiate analytes and 50 ng of cocaine analytes) were added. The solution was evaporated to dryness at room temperature in a water bath. The residue was treated with 3 ml of 0.5 *M* acetate buffer (pH 6) containing 0.5% (w/v) of sodium fluoride and extracted by the SPE procedure.

The remaining hair sample was extracted by resealing the bottom of the column and addition of 1 ml of methanol. Internal standards (100 μl containing 25 ng of opiate analytes and 50 ng of cocaine analytes) were added and a mini magnetic stirring bar (1.5 × 8 mm) was placed in the column. The top of the column was sealed and the column was placed in a 1-dram glass vial. The vial was incubated with stirring in a water bath at 40°C for 18 h. After cooling, the extract was collected and the remaining hair sample was rinsed with 0.5 ml of methanol. The extract and rinse fractions were combined and evaporated to dryness. The residue was treated with 3 ml of 0.5 *M* acetate buffer (pH 6) containing 0.5% (w/v) of sodium fluoride and extracted by SPE.

2.8. SPE of hair and biological specimens

Prior to extraction, plasma and saliva samples (1 ml) were treated with internal standards, diluted with acetate buffer (pH 6) and filtered through a filtration column. SPE columns were conditioned with methanol (2 × 2 ml), water (2 × 2 ml) and acetate buffer (1 ml, pH 6.0). Vacuum was removed prior to addition of the acetate buffer to prevent column drying. Hair washes, hair extracts, plasma, urine and saliva filtrates were added to the wet columns. Samples were eluted through the SPE columns and the columns were washed with water (2 × 1 ml) and acetate buffer (1 ml, pH 4.0). Columns were aspirated for 5 min, then washed with acetonitrile (2 × 1 ml). The columns were dried for 5 min and eluted with 3 × 2 ml of freshly prepared elution solvent (methylene chloride–2-propanol–ammonium hydroxide, 80:20:2, v/v). Column flow-rate was controlled at 1–2 ml/min during processing. The eluate was collected, evaporated to dryness, and the residue was reconstituted with 20 μl of acetonitrile and transferred to an autosampler vial. BSTFA (20 μl containing 1% TMCS) was added and the mixture was heated at 60°C for 30 min. An aliquot (1 μl) of the derivatized extract was analyzed by GC–MS.

3. Results

3.1. Hair analysis

Linearity and limit of detection

The limits of detection (LODs) for extracted cocaine, heroin and metabolites were approximately 0.1 ng/mg of hair (10 mg hair sample) for all analytes (signal-to-noise ratio >3) with the exception of norcodeine (LOD = 0.3 ng/mg) and

normorphine and benzoylnorecgonine (LOD = 0.5 ng/mg). Responses for all compounds were linear in the range of 0.1–10.0 ng/mg of hair. Correlation coefficients were typically > 0.995 for all analytes.

Occasionally, hair samples were encountered that contained analytes at a concentration > 10 ng/mg. When this occurred, new standard curves were constructed with an extended concentration range (10–100 ng/mg of hair) and samples were reanalyzed.

Accuracy and precision of GC–MS assay for cocaine, heroin and metabolites in hair

Between-run precision and accuracy for cocaine, heroin and metabolites were determined at concentrations of 1 ng/mg and 5 ng/mg per analyte added to control hair. The results are shown in Table 1. Generally coefficients of variation (C.V.) were ≤ 10% for analytes at a concentration of 5 ng/mg. C.V.s ranged from 2.9 to 26.5% for analytes at 1 ng/mg concentration. Relative accuracy was ≥ 90% for all analytes with the exceptions of norcodeine (80% at 1 ng/mg) and normorphine (70% at 1 ng/mg).

Extraction and recovery of cocaine, heroin and metabolites from hair

Hair samples were analyzed for cocaine, heroin and metabolites according to the analytical scheme shown in Fig. 3. During the development and optimization of this process, the efficiency of methanol as a solvent for the extraction of drug analytes was evaluated. A relative comparison was made between methanol and acetonitrile for removal of cocaine, heroin and metabolites from 10 drug users' hair samples. Acetonitrile was less efficient in the removal of cocaine with relative recoveries ranging from 18–58%; even lower recoveries were obtained for other analytes.

The efficiency of methanol for the removal of cocaine, heroin and metabolites from hair was evaluated by re-extraction of drug users' hair samples previously extracted with methanol. The second methanolic extract resulted in the recovery of 0–7% of drug analytes, indicating that > 90% of extractable drug had been removed by methanol in the first extraction step.

GC–MS analysis of drug users' hair

Head hair samples were collected from 19 subjects who abused both heroin and cocaine. These samples together with standards and drug-free control samples were analyzed according to the procedure shown in Fig. 3. All 16 analytes were monitored simultaneously by GC–MS. A typical selected-ion monitoring chromatogram of a standard drug extract, control sample and a drug user's hair sample is shown in Fig. 4. The result of the analysis is shown in Table 2. The predominant analyte in all samples was cocaine, ranging in concentration from 0.4 ng/ml to 172.7 ng/ml. Benzoylecgonine was present in all samples, but in substantially lower concentration than cocaine. Anhydroecgonine methyl ester, ecgonine methyl ester, cocaethylene, norcocaine, codeine, morphine, 6-acetylmorphine and heroin were present in some samples in concentrations ranging from 0.1–7.6 ng/mg. Ecgonine ethyl ester and norcocaethylene were present in trace amounts (Fig. 4). Benzoylnorecgonine, norcodeine and normorphine were not detected in any of the samples tested.

3.2. Urine, plasma and saliva analysis

Assay performance characteristics

The LODs for extracted cocaine, heroin and metabolites extracted from biological specimens was approximately 1 ng/ml (1 ml aliquot) for all analytes (signal-to-noise ratio > 3) with the exception of norcodeine, normorphine and benzoylnorecgonine (LOD = 5 ng/ml). Responses for all compounds were linear in the range of 1–100 ng/ml of biological fluid. Correlation coefficients were typically > 0.99 for all analytes. For concentrated samples (e.g. > 100 ng/ml of analyte), separate standard curves were utilized (100–1000 ng/ml). In some cases, dilution was employed to obtain concentrations on the standard curve.

Table 1
Between-run precision and relative accuracy of analysis for cocaine and opiates in hair samples

Analyte	Number of runs	Concentration (ng/mg)		S.D. (ng/mg)	C.V. (%)	Accuracy (%)
		Added	Measured			
Anhydroecgonine methyl ester	7	1.0	1.1	0.2	19.1	90
	7	5.0	4.9	0.3	5.1	98
Ecgonine methyl ester	8	1.0	1.1	0.1	6.4	90
	9	5.0	4.9	0.3	5.6	98
Ecgonine ethyl ester	6	1.0	1.1	0.1	10.2	90
	6	5.0	4.9	0.2	3.7	98
Cocaine	9	1.0	1.0	0	2.9	100
	9	5.0	5.1	0.4	7.2	98
	3	25.0	25.7	0.8	3.0	97
Cocaethylene	9	1.0	1.0	0.1	7.8	100
	9	5.0	4.9	0.1	2.9	98
Benzoylecgonine	9	1.0	1.0	0.1	10.5	100
	9	5.0	5.0	0.3	5.4	100
Norcocaine	9	1.0	1.0	0.3	26.5	100
	9	5.0	5.2	0.5	10.3	96
Norcocaethylene	8	1.0	1.0	0.1	13.6	100
	9	5.0	5.1	0.4	7.8	98
Benzoylnorecgonine	8	5.0	5.3	0.4	7.9	94
Codeine	9	1.0	1.1	0.1	5.7	90
	9	5.0	4.9	0.2	3.2	98
Morphine	9	1.0	1.1	0.1	7.0	90
	9	5.0	5.0	0.3	5.4	100
Norcodeine	6	1.0	1.2	0.1	7.3	80
	6	5.0	4.9	0.6	13.1	98
Normorphine	5	1.0	1.3	0.3	21.8	70
	6	5.0	4.9	1.0	19.1	98
6-Acetylmorphine	9	1.0	1.0	0.1	13.1	100
	9	5.0	5.1	0.2	4.4	98
Heroin	5	1.0	1.1	0.1	9.5	90
	5	5.0	5.0	0.4	7.4	100

GC–MS analysis of drug users' urine

Urine samples were collected from 5 polydrug abusers who abused both heroin and cocaine. The samples were analyzed by the procedure illustrated in Fig. 3. The results are shown in Table 3. Benzoylecgonine, ecgonine methyl ester, and benzoylnorecgonine were detected in all samples. Cocaine was present in low concentrations in four samples. Heroin and 6-acetylmorphine were detected in only one speci-

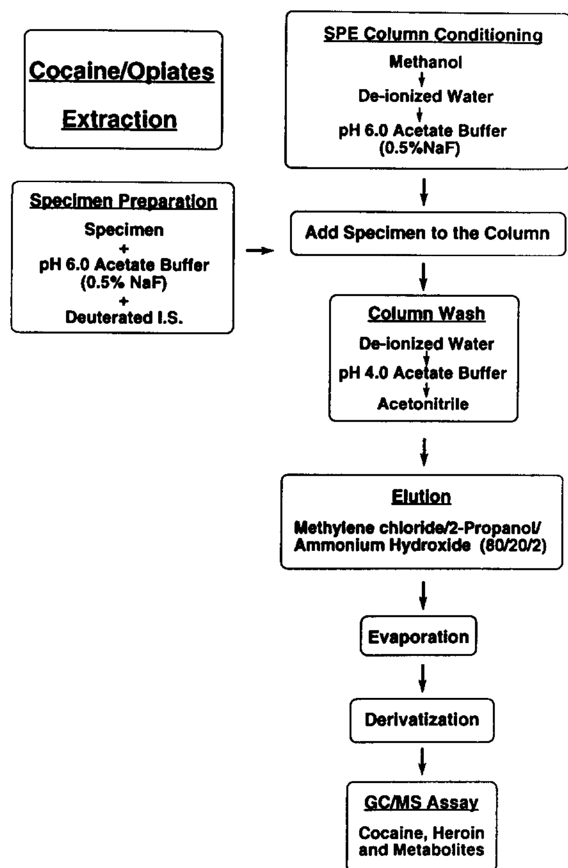

Fig. 3. SPE extraction scheme for biological specimens.

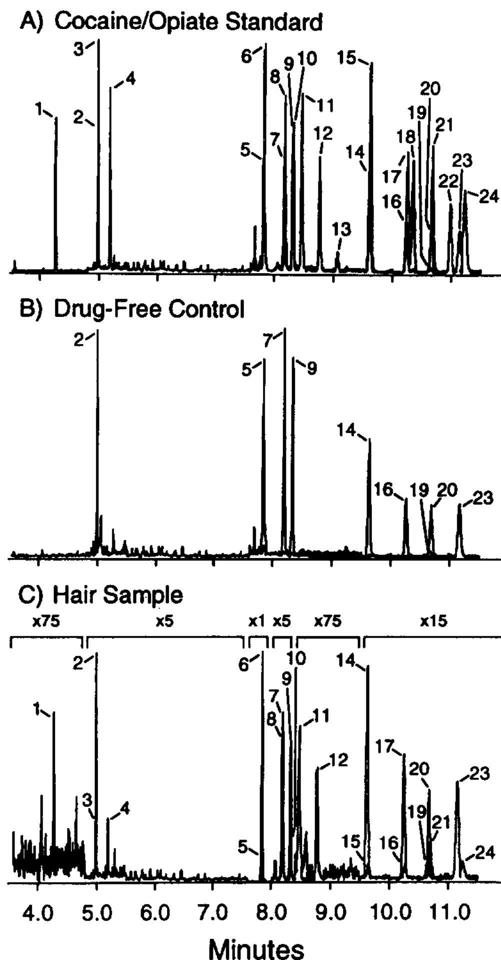

Fig. 4. SIM recordings of extracts from standard cocaine/opiate hair, drug-free control hair and a hair sample collected from a heroin user. Analytes are identified as follows: anhydroecgonine methyl ester (1), [$^2H_3$]ecgonine methyl ester (2); ecgonine methyl ester (3); ecgonine ethyl ester (4); [$^2H_3$]cocaine (5); cocaine (6); [$^2H_3$]cocaethylene (7); cocaethylene (8); [$^2H_3$]benzoylecgonine (9); benzoylecgonine (10); norcocaine (11); norcocaethylene (12); benzoylnorecgonine (13); [$^2H_3$]codeine (14); codeine (15); [$^2H_3$]morphine (16); morphine (17); norcodeine (18); [$^2H_6$]-6-acetylmorphine (19); [$^2H_3$]-6-acetylmorphine (20); 6-acetylmorphine (21); normorphine (22); [$^2H_9$]heroin (23); heroin (24).

men, whereas morphine, codeine, normorphine and norcodeine were present in all samples.

GC–MS analysis of heroin users' saliva

Saliva samples were analyzed from subjects who had received 12 mg of heroin hydrochloride by intranasal administration. Table 4 shows the results for a typical subject. Heroin and 6-acetylmorphine concentrations peaked at 10 min after drug administration, then decreased rapidly over a period of 1 h. Morphine concentrations increased slowly and peaked at 1 h after administration. Thereafter, the concentrations of the three analytes declined to levels approaching the LOD of the assay (1 ng/ml) by 3 h. Occasional resurgence of heroin and its two metabolites in saliva specimens was likely a result of the subject's coughing and clearing his nasal passage, thus, re-contaminating his oral cavity with heroin.

Table 2
Cocaine and opiate concentrations (ng/mg) in head hair of heroin/cocaine abusers

Subject	AEME	EME	COC	CE	BE	NCOC	COD	MOR	6AM	HER
1	0.0	0.0	5.8	0.0	1.1	0.0	0.0	0.2	0.3	0.0
2	0.0	0.7	29.1	7.6	3.1	0.0	0.2	0.0	0.1	0.0
3	0.0	0.0	15.5	0.3	1.1	0.0	0.6	1.3	0.8	0.3
4	0.0	0.0	5.1	0.0	1.3	0.0	0.0	0.0	0.1	0.0
5	0.0	0.3	20.4	0.0	2.4	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	7.3	0.8	0.9	0.0	0.0	0.0	0.2	0.0
7	0.0	0.2	25.8	0.2	1.8	0.0	0.0	0.0	0.0	0.0
8	0.0	0.0	13.4	0.9	1.6	0.0	0.3	0.3	1.1	0.4
9	0.0	0.0	2.6	0.9	0.2	0.0	0.0	0.0	0.1	0.0
10	0.0	0.6	21.2	3.6	1.2	0.0	0.0	0.3	0.3	0.0
11	4.6	2.4	172.7	0.2	24.4	3.3	0.3	0.7	1.6	0.2
12	0.0	0.0	0.4	0.3	0.1	0.0	0.0	0.0	0.0	0.0
13	0.3	0.0	21.4	0.8	4.8	0.0	0.0	0.2	0.1	0.0
14	0.0	0.0	12.4	0.0	1.3	0.0	0.0	0.0	0.4	0.0
15	0.0	0.0	1.3	0.0	0.3	0.0	0.0	0.0	0.1	0.0
16	0.0	1.2	48.2	0.0	4.1	1.2	0.0	0.3	0.0	0.0
17	0.3	0.0	12.2	0.4	6.2	0.0	0.3	0.0	0.4	0.0
18	0.0	0.0	5.5	0.4	1.0	0.0	0.0	0.3	0.5	0.0
19	0.0	0.0	4.8	0.0	0.2	0.0	0.0	0.0	0.0	0.0

Abbreviations: AEME = anhydroecgonine methyl ester; EME = ecgonine methyl ester; COC = cocaine; CE = cocaethylene; BE = benzoylecgonine; NCOC = norcocaine; COD = codeine; MOR = morphine; 6AM = 6-acetylmorphine; HER = heroin.

GC–MS analysis of cocaine users' plasma

Plasma samples from subjects who had been administered cocaine by the intravenous (50 mg) or subcutaneous (100 mg) were analyzed. Fig. 5 shows the concentration–time curves of cocaine and benzoylecgonine for a typical subject. Cocaine concentration peaked immediately following intravenous administration. In contrast, subcutaneous administration of cocaine resulted in delayed absorption with the peak concentration occurring at 1 h. Benzoylecgonine concentrations also increased slowly after subcutaneous administration, but thereafter remained elevated in a way similar to that observed following intravenous cocaine administration. Substantially smaller amounts of ecgonine methyl ester were present throughout the collection period (data not shown).

Table 3
Cocaine, opiate and metabolite concentrations in urine samples of drug abusers (ng/ml)

Subject	EME	COC	BE	NBE	COD	NCOD	MOR	NMOR	6AM	HER
1	297	29	4876	92	18	29	144	33	0	0
2	286	0	289	9	961	456	127	683	0	0
3	2434	363	9197	626	20	33	224	60	0	0
4	608	31	3773	38	261	63	685	35	25	2
5	1656	70	5758	73	115	30	416	29	0	0

Abbreviations: EME = ecgonine methyl ester; COC = cocaine; BE = benzoylecgonine; NBE = benzoylnorecgonine; COD = codeine; NCOD = norcodeine; MOR = morphine; NMOR = normorphine; 6AM = 6-acetylmorphine; HER = heroin.

Table 4
Heroin and metabolite concentrations (ng/ml) in saliva of a subject who received a 12-mg intranasal dose of heroin hydrochloride

Time (min)	Heroin (ng/ml)	6-Acetylmorphine (ng/ml)	Morphine (ng/ml)
−30	0	0	0
5	82.9	51.9	10.5
10	307.8	58.7	5.2
15	22.4	35.8	18.5
30	0.9	25.3	22.6
45	27.3	20.8	20.1
60	15.7	38.4	25.4
90	0	5.6	14.5
120	0.9	2.2	6.2
180	0	1.7	1.7
240	3.6	8.1	10.0
360	0	0.9	0
720	0	0	0
1440	0	0	0

4. Discussion

The development of a simultaneous GC–MS assay for the measurement of cocaine, heroin and 14 metabolites in hair, urine, plasma and saliva was an analytical challenge that required optimization of numerous chemical and chromatographic parameters. Initially, it was necessary to develop a purification step in which

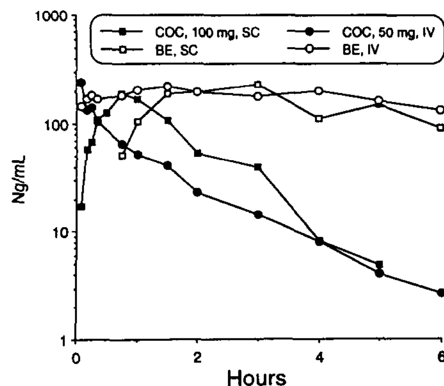

Fig. 5. Plasma concentrations of cocaine (COC) and benzoylecgonine (BE) following subcutaneous (SC) and intravenous (IV) drug administration to a human subject.

cocaine, heroin and metabolites could be extracted in an efficient, reproducible manner. Speed of extraction and pH conditions were important considerations since both cocaine and heroin are highly susceptible to hydrolysis, particularly under high pH conditions [6]. Liquid–liquid extraction was not considered as a viable option because of time and pH considerations. Consequently, SPE techniques were employed in which the pH was adjusted to minimize heroin and cocaine hydrolysis. With optimization of the SPE conditions, high recoveries were obtained for all analytes. Measurement of analytes was accomplished by GC–MS operated in the selected-ion monitoring mode. Three ions were monitored for each analyte. Quantitation was based on internal standardization with deuterated analogs of the major analytes. This provided sufficient sensitivity and specificity for the measurement of drugs in the different biological specimens. Both reproducibility (precision) and relative accuracy were within acceptable limits for most analytes.

The stabilization and recovery of heroin was an important goal of this assay. Intact heroin has been reported to be present in hair [7] and biological fluids after drug administration [6]; consequently, it was important to include this rather unstable analyte in the assay. It was known that heroin hydrolyzed readily to 6-acetylmorphine in blood (enzymatic) and aqueous solutions at elevated pH [6]. In the present assay, the hydrolysis of heroin was minimized ($<10\%$) by the addition of sodium fluoride to biological specimens during collection. For additional protection, sodium fluoride was also added to the buffer utilized in SPE. Initial testing of SPE without sodium fluoride produced erratic results for the recovery of heroin. Addition of sodium fluoride improved both precision and recovery. Another improvement was the use of $[^2H_9]$heroin as an internal standard. This allowed independent monitoring of heroin hydrolysis in each run by measurement of the m/z 405 ion of $[^2H_6]$-6-acetylmorphine, the hydrolysis product of deuterated heroin. Cor-

rection of quantitative results could be made with this technique when heroin hydrolysis exceeded 10%.

Several digestion procedures have been described for removal of drug from hair utilizing acid, base, enzymes and organic solvents [8–11]. Neither acid or base digestion was suitable in the present assay because of the instability of cocaine and heroin. Initially enzymatic hydrolysis was attempted, but poor recoveries of all analytes were obtained. Methanol was eventually selected as the wash and extraction solvent of choice for hair analysis because of the stability of heroin, 6-acetylmorphine, and cocaine in this solvent. Another organic solvent, acetonitrile, was also evaluated as a potential solvent for hair extraction. Although most drug analytes could be detected in acetonitrile extracts, recoveries of all analytes were substantially lower than with methanol extraction. The efficiency of recovery of the analytes from hair by extraction with methanol was examined by re-extraction of hair samples previously extracted with methanol. In the second extraction, less than 10% of the analytes measured in the first determination were present. This indicated that nearly all extractable compound was removed in the first extraction.

Analysis of 19 hair samples from known drug users revealed the presence of cocaine and related metabolites in all samples. Heroin, 6-acetylmorphine or morphine was present in 15 samples. The presence of heroin and 6-acetylmorphine was an important finding that clearly established heroin abuse and effectively ruled out other opiate sources such as poppy seed and codeine as the cause of the positive results. Codeine was present in 5 samples and 4 samples were negative for all opiates. In all cases, cocaine was present in substantially higher amounts than heroin-related analytes, consistent with our earlier findings [5]. Anhydroecgonine methyl ester was present in 3 hair samples indicating that cocaine had been used by the smoking route of administration. Cocaethylene was present in 12 samples, but in a lower concentration than cocaine. This metabolite is formed when cocaine and ethanol are ingested together [12] and its presence can be used as a marker of combined cocaine/alcohol abuse.

Urine, saliva and plasma samples also could be analyzed for cocaine and opiates with the GC–MS assay. Urine samples were often highly concentrated and required dilution. In addition, the standard curve had to be extended to accommodate a wider range of concentrations for some samples. Three ions monitored for each analyte provided the confidence needed for forensic drug identification. This was particularly important for the identification of minor metabolites. Minor metabolites were most often encountered in urine (Table 3), whereas saliva and plasma most often contained parent drug and major metabolites. Presently, this assay is being utilized in pharmacokinetic studies of cocaine and opiate compounds in human subjects and allows collection of a wide array of data on multiple metabolites in a variety of biological fluids and tissues.

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