Journal of Chromatographic Science, Vol. 36, March 1998

Simultaneous Quantitation of Cocaine, Opiates, and Their Metabolites in Human Hair by Positive Ion Chemical Ionization Gas Chromatography–Mass Spectrometry

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Abstract

A sensitive method is developed for the combined extraction of cocaine (COC), cocaethylene (CE), benzoylecgonine (BE), ecgonine methyl ester (EME), norcocaine (NORCOC), 6-acetylmorphine (6-MAM), codeine (COD), norcodeine (NORCOD), morphine (MOR), and normorphine (NORMOR) from human head hair using an enzyme-based digestion technique (Protease VIII/DTT/Tris-buffer pH 6.5 at 22°C). After pH adjustment to 5.5, the digests are extracted with a solid-phase extraction procedure using Bond-Elut Certify columns. The extract residues are evaporated at 40°C, reconstituted in 20 µL of ethyl acetate, and derivatized with the reagents *N*-methyl-*N*-trimethylsilylheptafluorobutyramide (MSHFBA), *N*-methyl-bis-heptafluorobutyramide (MBHFBA), and *N*-trimethylsilylimidazole (TMSIM). Analyses are performed by positive ion chemical ionization gas chromatography–mass spectrometry using a DB-1 capillary column. Two injections are performed on each extract to optimize sensitivity for all analytes. The assay is capable of reliably quantitating 500 pg/mg of all compounds and is linear to 50 ng/mg, except for BE, which is linear to 25.0 ng/mg. The method was used to analyze human hair samples obtained from cocaine and heroin users. COC, BE, and EME are detectable in all samples, whereas NORCOC, CE, COD, 6-MAM, and MOR are detected in only some samples. Norcodeine and normorphine are not detected. The assay is currently being used to analyze hair samples from a study investigating the mechanisms of drug disposition in hair.

Introduction

Selective and sensitive analytical methods for the quantitative determination of drugs and their metabolites in biological samples are important for the interpretation of data. For instance, the selection of a method to digest drugs from hair is critical for the quantitative outcome of drug concentrations in hair. Complete dissolution of the hair would be preferred to ensure that all of the bound analytes are released from the hair and measured. However, strong alkali solutions such as NaOH used for total digestion of the hair may degrade drugs, particularly cocaine (1).

Methods used to digest drugs from hair can be grouped into three classes: chemical hydrolysis, solvent extraction, and enzymatic digestion (2). Cocaine (COC) and its metabolites, cocaethylene (CE), benzoylecgonine (BE), ecgonine methyl ester (EME), and the opiates, including morphine (MOR), codeine (COD), and 6-acetyl morphine (6-MAM) have been extracted from hair samples after hydrolysis by acid (3,4), enzyme (5), or directly by methanol (6). Cirimele and coworkers concluded that acid or β-glucuronidase/arylsulfatase gave higher extraction recoveries than methanolic extraction for these drugs (7). Other enzymes commonly used for digestion are proteinase K and pronase (1). In the present study, the efficiency of different enzymatic methods to digest hair samples obtained from cocaine and opioid users was compared with an acid hydrolysis procedure. A sensitive gas chromatographic–mass spectrometric (GC–MS) method was developed for the combined digestion, extraction, and derivatization of cocaine and opiates and their metabolites, including norcocaine (NORCOC), norcodeine (NORCOD), and normorphine (NORMOR). Although other articles have reported the simultaneous digestion, extraction, and detection of cocaine and codeine and their metabolites from hair samples by GC–MS with electron-impact MS (Table I) (3,4,6), to our knowledge, no studies have reported the use of chemical-ionization MS for the analysis of both cocaine and codeine and their metabolites. Chemical-ionization MS was used in this study to enhance sensitivity. In addition, a new combination of reagents was used for the

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Table I. List of Analytical Conditions Used for Hair Analysis of Cocaine and Opiates

Reagent	Conditions*	Compounds	References
MTBSTFA	CI	Cocaine	1
BSTFA + 1% TMCS	EI	Cocaine and opiates	3,6,7
PFPA/HFIP	EI	Cocaine	12
PFPA/PFPOH	EI	Cocaine	5
HFPA/HFPOH	EI	Cocaine and opiates	4

* CI = chemical ionization; EI = electron impact.

derivatization of cocaine and opiates and their metabolites to form trimethylsilyl–heptafluorobutyryl (TMS–HFB) derivatives.

Experimental

Reagents

COC, BE, CE, NORCOC, EME, COD, MOR, 6-MAM, NORCOD, NORMOR, heroin, and the internal standards COC-d_3, BE-d_3, CE-d_3, EME-d_3, COD-d_3, MOR-d_3, and 6-MAM-d_3 were obtained from the Radian Corporation (Austin, TX). NORCOC-d_5 was obtained from the Research Triangle Institute (Rockville, MD). Capillary GC–MS-grade methylene chloride, ethyl acetate, 2-propranol, and methanol were obtained from Baxter (McGraw Park, IL). Tris (hydroxymethyl) aminomethane hydrochloride (Trizma HCl), Tris (hydroxymethyl) aminomethane (Trizma base), D,L-dithiothreitol (DTT), protease VIII (Subtilisin Carlsberg; 13.5 U/mg solid), and proteinase K (14 U/mg solid) were obtained from Sigma (St. Louis, MO). Potassium hydroxide, hydrochloric acid, and potassium phosphate monobasic were obtained from Mallinckrodt (St. Louis, MO). Sodium acetate was obtained from Fisher (Pittsburgh, PA). *N,O*-Bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA + 1% TMCS) was obtained from Pierce (Richfield, IL). *N*-Methyl-*N*-trimethylsilylheptafluorobutyramide (MSHFBA) and *N*-methyl-bis-heptafluorobutyramide (MBHFBA) were obtained from Macherey-Nagel (Düren, Germany). *N*-Trimethylsilylimidazole (TMSIM) was obtained from Alltech (Deerfield, IL). Bond-Elut Certify columns (10 mL, 130 mg) were purchased from Varian (Harbor City, CA).

GC–MS analysis

Analyses were performed on a Finnigan-MAT 4500 GC–MS equipped with INCOS software (Finnigan MAT, San Jose, CA). The chromatographic column was a DB-1 capillary column (30 m × 0.32-mm i.d., 1.0 µm) (Bellefonte, PA). The initial column temperature of 120°C was held for 0.1 min, then programmed to 270°C at the rate of 20°C/min, then programmed to 310°C at 10°C/min, and held for 1.4 min at the final temperature. The carrier gas was helium with a head pressure of approximately 8 psi. Temperatures of the injection port, interface, transfer line, and ionizer were 285, 285, 275, and 130°C, respectively. The reagent gas was methane–ammonia (1:2) adjusted to a source pressure of 0.60 Torr. The emission current, electron energy, conversion dynode, and multiplier were set at 0.16 mA, –70 eV, –3 kV, and –1500 V, respectively. The MS was operated in the positive chemical ionization (PICI) detection mode. Two injections were performed on each extract. First, 1 µL was injected in splitless mode for the compounds NORCOC, BE, COD, MOR, 6-MAM, NORCOD, and NORMOR. Second, 0.3 µL of the sample was reinjected for the compounds COC, CE, and EME. Two separate injections of different volumes (1 and 0.3 µL) were needed because of the significantly different signal responses of the various analytes. Performance of two separate injections prevented saturation of both the column and the detector for all analytes of interest. The MS was operated in the selected ion monitoring (SIM) mode and programmed for detection of *m/z* 272 (EME-d_0), *m/z* 275 (EME-d_3), *m/z* 304 (COC-d_0), *m/z* 307 (COC-d_3), *m/z* 318 (CE-d_0), *m/z* 321 (CE-d_3), *m/z* 362 (BE-d_0), *m/z* 365 (BE-d_3), *m/z* 503 (NORCOC-d_0), *m/z* 508 (NORCOC-d_5), *m/z* 372 (COD-d_0), *m/z* 375 (COD-d_3), *m/z* 430 (MOR-d_0), *m/z* 433 (MOR-d_3), *m/z* 400 (6-MAM-d_0), *m/z* 403 (6-MAM-d_3), *m/z* 571 (NORCOD-d_0), and *m/z* 629 (NORMOR-d_0). Peak height ratios were calculated based on the ratios of each analyte's peak height to that of the corresponding internal standard. For quantitation of NORMOR and NORCOD (compounds with no corresponding deuterated internal standard), 6-MAM-d_3 was used as the internal standard. The concentration of each analyte was determined from least-squares equations generated from peak height ratios of the calibrators.

Stock solutions and preparation of standard curves

Drug reference solutions were diluted in methanol to obtain mixed working solutions containing 0.1, 1.0, and 10.0 ng/mL of A (COC, BE, EME, CE, and NORCOC) and B (COD, MOR, 6-MAM, NORMOR, and NORCOD). Internal standard solutions were diluted in methanol to obtain mixed internal standard solutions containing 1.0 ng/mL of C (deuterated COC, BE, EME, CE, and NORCOC) and D (deuterated COD, MOR, and 6-MAM). Stock, mixed working solutions and mixed internal standard solutions were stored at –20°C until use. The diluted working solutions A and B were used to prepare daily standard curves by fortifying drug-free human hair with known concentrations of standards. To fortify hair, drug-free hair was carefully cut into small segments, 20 mg was placed into silanized glass vials, and the standard solution was added. The final fortified hair concentrations for standard curves were: 0.1, 0.2, 0.3, 0.5, 0.8, 1.0, 2.0, 3.0, 5.0, 10.0, 25.0, 50.0, and 100.0 ng/mg.

Preparation of quality control samples

Positive quality control samples (0.2, 0.5, 5.0 and 50.0 ng/mg of all compounds in fortified hair) were prepared daily. Stock solutions used to prepare quality control samples were prepared independently from those used to prepare standards and from separate sources or lot numbers when possible. Drug-free human hair was also digested, extracted, and analyzed as a negative control in each assay.

Subject hair samples

Hair samples (anterior and posterior) were collected from five healthy Africoid or Caucasian males who participated in re-

search protocols at the Addiction Research Center (NIDA, Baltimore, MD). All subjects provided written informed consent. All subjects had a history of cocaine and heroin use.

Digestion and extraction

Prior to digestion, 50 µL of the mixed internal standard solutions C and D were added to the hair standards, controls, or human samples (final concentration, 5 ng/mg). The hair was digested with 5.2 mL of 0.01M Tris buffer, along with 0.8 mL of fresh 0.4M DTT in 10mM sodium acetate buffer and 110 µL of 10 mg/mL Protease Type VIII in 1M Tris buffer at 37°C overnight.

Before extraction, the pH of the samples was adjusted to 5.5 with 0.1M HCl, and the tubes were mixed. Bond-Elut Certify solid-phase extraction (SPE) columns were conditioned by sequentially adding 3 mL methanol, 3 mL distilled water, and 1 mL phosphate buffer (pH 6.0). Care was taken to ensure that the columns did not dry between conditioning steps. Hair digest samples were added to the columns, and a vacuum was applied. Each column was then washed with 2 mL deionized water, 2 mL 0.1M HCl, and 3 mL methanol. All columns were dried under a vacuum for 10 min. Analytes were eluted with 3 mL of freshly prepared methylene chloride–2-propanol–ammonium hydroxide (78:20:2, v/v/v). The eluents were collected in silanized conical tubes, and the solvent was evaporated at 40°C under a stream of air. Ethylacetate (20 µL) was added to the tubes, and the tubes were mixed. MSHFBA–TMSIM (30 µL, 1000:40, v/v) was added; the tubes were mixed and then heated at 80°C for 2 min. After cooling to room temperature (RT), 5 µL MBHFBA was added. The solution was mixed and heated at 80°C for 25 min. The tubes were allowed to cool to RT, and the liquid was transferred to labelled autosampler vials for injection into the GC–MS. The three highest points of the standard curve (25.0, 50.0, and 100.0 ng/mg) were diluted with MSHFBA–TMSIM before injection into the GC–MS.

Recovery

Recovery studies were performed by preparing two sets of samples (A and B). Set A consisted of samples with three replicates at three different concentrations: 0.5, 5, and 50 ng/mL. This sample set was digested and extracted using the method as presented. Samples in set B (three replicates) were digested and extracted as described, except the internal standards were added to the organic solvent just prior to evaporation. The ratio of analyte height to corresponding deuterated internal standard height was obtained, and mean ratios were calculated for samples in sets A and B. The mean ratio at each of the three concentrations for set B was divided by the mean ratio for the corresponding concentration in set A to obtain the recovery ratio. The recovery ratio was multiplied by 100 to calculate the percent recovery.

Results and Discussion

Derivatization

A number of procedures for the combined derivatization of cocaine metabolites and opiates and metabolites were evaluated by GC–MS with PICI. Table I lists the reagents previously used for the derivatization of cocaine metabolites and/or opiates and their metabolites from hair samples. For chemical ionization, the derivatized analyte must be easily protonated and should have limited fragmentation. In this study, a new combination of reagents (MSHFBA–TMSIM–MBHFBA), which forms TMS derivatives with the OH groups and HFB derivatives with the NH groups, was used and compared with BSTFA + 1% TMCS, which forms only TMS derivatives. COC and EME do not derivatize.

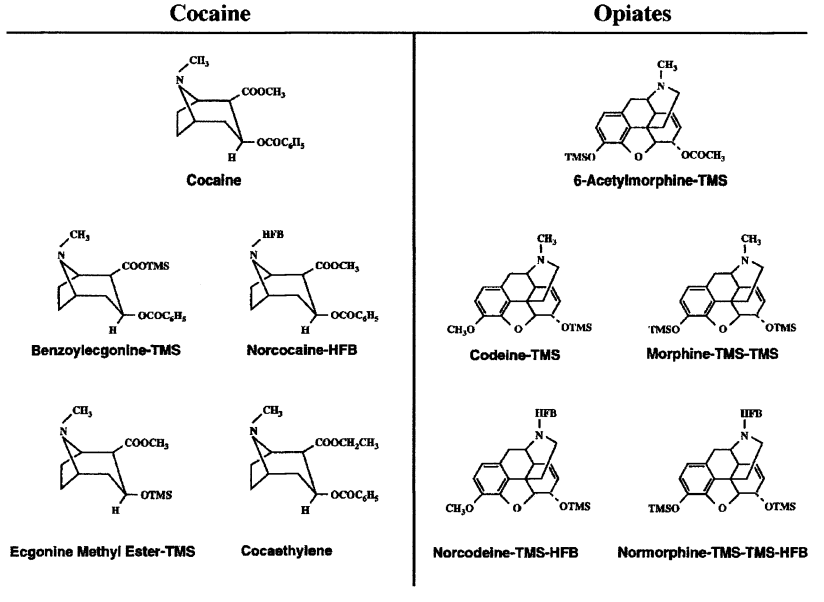

Figure 1. TMS–HFB derivatives of cocaine and opiates and their metabolites.

BSTFA + 1% TMCS derivatization resulted in protonated molecular ions for EME, BE, NORCOC, and 6-MAM. However, COD, MOR, NORCOD, and NORMOR showed additional fragment ions (loss of *m/z* 90) in addition to the protonated molecular ions. COD and MOR showed a loss of *m/z* 90 and an ammonia adduct (+*m/z* 17) in addition to the protonated molecular ion.

Figure 1 shows the formed TMS–HFB derivatives of cocaine metabolites, opiates, and opiate metabolites. The MSHFBA–TMSIM–MBHFBA derivatizion resulted in protonated molecular ions for all compounds except COD and MOR, which also showed –*m/z* 90 and +*m/z* 17 in addition to the protonated molecular ion. NORCOD and NORMOR were derivatized through formation of both TMS and HFB derivatives, which resulted in more stable compounds. Because the TMS–HFB derivatives demonstrated protonated molecular ions for more

compounds than the derivatives formed after using BSTFA + 1% TMCS, the MSHFBA–TMSIM–MBHFBA mixture was selected for assay development and validation.

Chromatography

Figures 2A and 2B show the chromatographic separation of COC and opiates, respectively, with their metabolites; the column and temperature conditions are described. Table II lists the $[M+H]^+$ ions monitored.

Digestion

Five procedures for digesting hair samples were studied: (*a*) protease VIII at RT, (*b*) protease VIII at 37°C overnight, (*c*) proteinase K at RT, (*d*) proteinase K at 37°C overnight, and (*e*) 0.1N HCl 45°C overnight. For the enzymatic digestion, the cocaine method of Harkey et al. was first evaluated (1). In this method, sodium dodecylsulfate (SDS) is used in the digestion procedure. However, for the analysis of opiates from hair samples, SDS was not suitable; after extraction of the samples, the recovery for the opiates was negligible. Without SDS in the digestion buffer, the recovery of cocaine was unaffected. Therefore, SDS was not added to the digestion buffer. None of the hair samples were completely dissolved after overnight digestion by the different procedures. Therefore, it is possible that some drugs and metabolites were not recovered from the hair. The chromatograms of the proteinase K digests showed no 6-MAM-d_0 or -d_3 peak for both digestion temperatures. Therefore, this enzyme was not suitable for our method. However, all the compounds were effectively extracted in the same amounts after acid or protease VIII hydrolysis. Protease VIII hydrolysis was chosen over acid hydrolysis for further method development because of our previous experience with the enzymatic digestion.

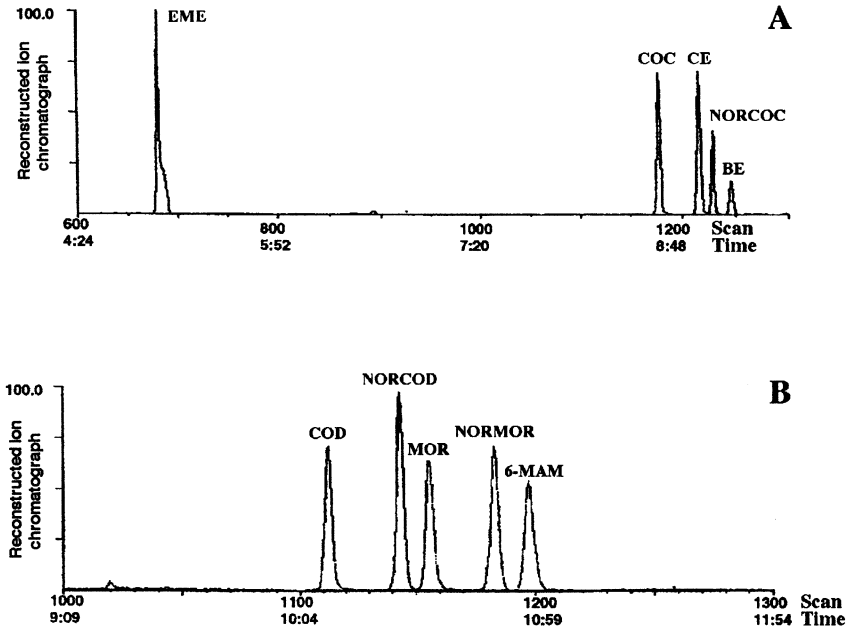

Figure 2. Selected ion chromatograms (1 AMU) of (A) COC, BE, EME, CE, and NORCOC and (B) COD, MOR, 6-MAM, NORCOD, and NORMOR after derivatization (20 ng on column).

Because the subjects were cocaine and heroin addicts, it was important to determine whether the digestion and extraction technique caused degradation of COC to BE, heroin to 6-MAM and MOR, and 6-MAM to MOR. For the COC conversion study, 25 ng/mg of COC-d_0 and the internal standard BE-d_3 was added to 20 mg of hair. The mean conversion of COC to BE was 5.2% ± 0.5 standard deviation (SD) (five replicates). For the heroin and 6-MAM conversion study, heroin-d_0 and 6-MAM-d_0 were added separately in triplicate to 20 mg of hair samples at 5 ng/mg together with MOR-d_3 as the internal standard. The amount of heroin conversion to 6-MAM and MOR during the digestion and extraction procedure was

Table II. Molecular Weight and *m/z* Data for Cocaine and Opiates and Their Metabolites

Analyte	Molecular weight of underivatized analyte	Molecular weight of derivatized analyte	$[M+H]^+$ (*m/z*) of analyte after derivatization
COC	303	underivatized	304
BE	289	361	362
NORCOC	289	485	503 (ammonia adduct)
EME	199	271	272
CE	317	underivatized	318
COD	299	371	372
MOR	285	429	430
6-MAM	327	399	400
NORCOD	285	570	571 (ammonia adduct)
NORMOR	271	628	629 (ammonia adduct)
Heroin	369	underivatized	370

Table III. Summary of Recovery Results* for COC, BE, NORCOC, EME, CE, COD, MOR, 6-MAM, NORCOD, and NORMOR (Three Replicates) Added to Drug-Free Hair Specimens

Concentration (ng/mg)	EME	COC	CE	BE	NORCOC
0.5	64.2	71.0	70.8	71.2	55.0
5.0	79.2	71.0	71.5	66.4	89.4
50	96.2	78.8	77.8	79.5	77.8
	COD	**MOR†**	**6-MAM†**	**NORCOD**	**NORMOR**
0.5	85.9	103.5	24.0	33.5	40.5
5.0	66.4	127.7	32.7	37.2	39.4
50	84.7	102.7	52.9	34.4	40.6

* Values are expressed as recovery percentage.
† The results of the morphine and 6-MAM recovery should be interpreted with caution because of the conversion of 6-MAM to morphine during digestion.

73.5%. During the extraction step only, 11.6% of heroin was converted to 6-MAM and MOR. The amount of 6-MAM conversion to MOR during the digestion and extraction procedure was 4.6%; 3.2% of the loss was due to the extraction step. Consequently, the digestion step affected heroin to a greater degree than the extraction step (61.9% of the conversion). Most of the converted heroin remained as 6-MAM; only a small amount was converted to MOR.

Some papers have reported methanol extraction of drugs from hair (6,8). This resulted in no degradation of COC or heroin. However, it is possible that some drug and metabolite incorporated in hair was not recovered from the intact hair.

Extraction

All compounds were extracted from the digested hair samples by a previously developed SPE procedure for cocaine and its metabolites (9). This method was also suitable for opiates. Because some procedures used 0.1M acetate buffer (pH 4.0) during the wash step instead of 0.1N HCl, these wash solvents were compared (10). The recovery for BE dropped with the acetate buffer wash. Therefore the 0.1N HCl wash step was used in this method.

Extraction efficiency

As shown in Table III, the recovery percentage for cocaine and its metabolites added to drug-free hair, digested and extracted, was between 55 and 96%. The recovery results of the opiates, especially MOR and 6-MAM, should be interpreted with caution because of the conversion of 6-MAM to MOR during digestion.

Linearity

Regression analysis of the calibration graphs demonstrated linearity from 0.1 to 50 ng/mg, except for BE, which was linear to 25 ng/mg. Correlation coefficients were typically greater than 0.990. Although the method was linear to 50 ng/mg, the calibration plot from 0.1 to 50 ng/mg was used only for the points higher than 5 ng/mg. If the concentration was less than 5 ng/mg, accuracy was much better when a calibration plot from 0.1 to 5.0 ng/mg was used.

Accuracy and precision

For intra-assay determination, each concentration studied was analyzed in replicates of five. Accuracy and precision were calculated from the mean and SD (Tables IV and V). At 0.2 ng/mg, accuracy and precision were not acceptable according to established validation criteria (11). At 0.5 and 5 ng/mg, accuracy was within 25% of the target for all compounds, except for NORMOR. The precision at these concentrations was also within 25%, except for NORMOR and NORCOD. These two compounds did not have their own internal standard. The 50-ng/mg samples, which were diluted, were within 20% of the target for cocaine and metabolites. The precision was also within 20%. However, diluting the samples for the opiates resulted in values not within acceptable limits for validation.

Application

The described method was used to analyze hair samples obtained from cocaine and heroin users. The concentrations of all compounds are summarized in Tables VI and VII. From each

Table IV. Intra-Assay Accuracy and Precision for EME, COC, CE, BE, and NORCOC

Concentration	EME	COC	CE	BE	NORCOC
0.2 ng/mg					
Mean	0.31	0.15	0.18	0.20	0.17
Accuracy (%)	156	73	94	102	83
CV* (%)	7.1	2.1	5.3	33	18.3
Replicates	5	5	5	5	5
0.5 ng/mg					
Mean	0.63	0.49	0.53	0.48	0.51
Accuracy (%)	125	98	106	97	102
CV* (%)	10	5.7	2.9	24	13.4
Replicates	5	5	5	5	5
5 ng/mg					
Mean	4.6	4.7	4.7	4.7	5.2
Accuracy (%)	93	94	94	94	104
CV* (%)	4.3	5.6	6	6.5	12.1
Replicates†	4	4	4	4	3
50 ng/mg					
Mean	52.7	40.3	52.1	‡	59.8
Accuracy (%)	105	81	104		120
CV* (%)	3.9	4.9	8.0		19.2
Replicates	5	5	5		4

* Coefficent of variation.
† One of five quality control samples had no internal standard and could not be calculated for all analytes.
‡ BE was not linear after 25 ng/mg.

Table V. Intra-Assay Accuracy and Precision for COD, MOR, 6-MAM, NORCOD, and NORMOR

Concentration	COD	MOR	6-MAM	NORCOD	NORMOR
0.2 ng/mg					
Mean	0.21	0.21	0.23	0.20	0.18
Accuracy (%)	105	105	115	100	90
CV* (%)	27.9	27.0	15.5	16.7	18.4
Replicates	5	5	5	5	5
0.5 ng/mg					
Mean	0.50	0.50	0.53	0.62	0.57
Accuracy (%)	100	100	106	124	114
CV* (%)	11.6	9.3	10.8	16.3	26.2
Replicates	5	5	5	5	5
5 ng/mg					
Mean	5.7	5.8	5.3	5.3	6.3
Accuracy (%)	114	116	107	106	126
CV* (%)	16.8	11.9	11.4	30.2	22.8
Replicates	4	4	3	4	4
50 ng/mg					
Mean	47.5	46.3	71.4	53.4	72.5
Accuracy (%)	95.1	92.6	148	106.8	145
CV* (%)	14.3	7.0	16.1	20.3	23.0
Replicates	5	5	5	5	5

* Coefficient of variation.

Table VI. Concentrations of COC, BE, EME, NORCOC, and CE in Head Hair Specimens Collected from Five Human Subjects Using Heroin and Cocaine

Subject*	COC (ng/mg)	BE (ng/mg)	EME (ng/mg)	NORCOC (ng/mg)	CE (ng/mg)
A (ant)	9.5	3.0	0.6	< LOQ†	< LOQ
A (post)	18.3	9.0	0.8	< LOQ	< LOQ
B (ant)	8.6	1.1	0.5	< LOQ	< LOQ
B (post)	7.1	1.6	< LOQ	< LOQ	< LOQ
C (ant)	27.1	11.0	1.5	< LOQ	< LOQ
C (post)	40.7	11.8	1.8	0.6	< LOQ
D (ant)	11.5	2.6	0.5	< LOQ	< LOQ
D (post)	10.9	2.3	< LOQ	< LOQ	< LOQ
E (ant)	20.5	3.6	0.5	0.5	0.8
E (post)	17.3	2.8	0.5	0.5	0.8

* Ant = anterior hair sample; post = posterior hair sample.
† The limit of quantitation (LOQ) was 0.5 ng/mg.

subject, an anterior and posterior part of the hair was analyzed. COC and BE were detectable in all samples, whereas EME, NORCOC, CE, 6-MAM, and MOR were detected in only some samples. COD, NORCOD, and NORMOR were not detected. Because the subjects were cocaine and heroin addicts and did not take codeine, no COD was detected in their hair samples. For some subjects, there was a substantial difference in the concentrations of the drugs in the anterior and posterior hair samples. The reasons for this variation are unknown, but one possible explanation could be due to different amounts of sweat exposure containing drugs in the two areas of collections.

Conclusion

This paper described a PICI MS procedure with methane–ammonia as a reagent gas for the combined digestion, extraction, and derivatization of cocaine and opiates and their metabolites in human hair. All compounds were effectively and accurately extracted after acid or protease VIII digestion. Heroin was converted during the digestion process to 6-MAM and MOR. Most of the converted heroin became 6-MAM; only a small amount of MOR was formed. TMS–HFB derivatives were utilized and were well-separated under the conditions described. The method was successfully used for the analysis of hair from cocaine and heroin users. The assay is currently being used to analyze samples from a study investigating the mechanisms of drug disposition in hair after administration of cocaine and codeine.

Acknowledgments

This study was supported by the Intramural Research Program of the National Institute on Drug Abuse, Baltimore, MD and NIDA Grant No. DA09096.

Table VII. Hair Concentrations of COD, MOR, 6-MAM, NORCOD, and NORMOR from Five Subjects

Subject*	COD (ng/mg)	MOR (ng/mg)	6-MAM (ng/mg)	NORCOD (ng/mg)	NORMOR (ng/mg)
A (ant)	< LOQ†	< LOQ	< LOQ	< LOQ	< LOQ
A (post)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
B (ant)	< LOQ	< LOQ	1.4	< LOQ	< LOQ
B (post)	< LOQ	< LOQ	0.8	< LOQ	< LOQ
C (ant)	< LOQ	0.9	1.6	< LOQ	< LOQ
C (post)	< LOQ	1.4	1.5	< LOQ	< LOQ
D (ant)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
D (post)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
E (ant)	< LOQ	< LOQ	0.5	< LOQ	< LOQ
E (post)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ

* Ant = anterior hair sample; post = posterior hair sample.
† The limit of quantitation (LOQ) was 0.5 ng/mg.

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Manuscript accepted January 9, 1998.