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Simultaneous Quantification of Opiates, Cocaine, and Metabolites in Hair by LC-APCI-MS/MS

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A quantitative LC-APCI-MS/MS method for simultaneous measurement of opiates, cocaine, and metabolites in hair was developed and validated. Cocaine and opiates were extracted from pulverized hair via sonication in methanol at 37 °C for 3 h. Samples were cleaned up using solid-phase extraction. LC separation was achieved in 20 min with identification and quantification by selected reaction monitoring. Calibration by linear regression analysis utilized deuterated internal standards and a weighting factor of $1/x$ ($R^2 > 0.998$). Limits of quantification (LOQ) ranged from 17 to 50 pg/mg for cocaine and metabolites and were 83 pg/mg for opiates. Standard curves were linear from the LOQ to 5000 pg/mg for cocaine and metabolites, except benzoylecgonine (2500 pg/mg). Opiate standard curves were linear from the LOQ to 12500 pg/mg. Accuracy ranged from 84 to 115% for all quantitative analytes. Precision, % relative standard deviation, was less than 11.0% for all analytes. Methanolic sonication produced less than 5% hydrolysis of cocaine and 6-acetylmorphine. The method should be useful for studying cocaine and opiate distribution into hair.

Analysis of hair samples for monitoring drug exposure is of considerable interest since drugs and metabolites incorporated into hair persist longer than in urine. A study evaluating urinary opiate excretion rates following single 120-mg intramuscular injections of codeine ($n = 4$) determined that total urinary codeine and morphine concentrations were less than the limit of detection (40 ng/mL via gas chromatography-mass spectrometry (GC/MS)) 5 days after administration.[1] In contrast, Rollins et al. conducted a clinical study in which 12 volunteers were administered a single 120-mg oral dose of codeine followed by measurement of codeine and morphine in hair. Hair collected 8 weeks after codeine administration revealed a mean codeine concentration of 30 pg/mg.[2] Thus, hair has been considered a useful alternative sample matrix for monitoring drug use in forensic and workplace testing programs. Additional useful applications for drug testing using hair are monitoring compliance during follow-up studies in drug treatment programs, as an alternative to urine testing and/or self-report for objectively documenting past drug use in research studies and when investigating cases of suspected drug-facilitated sexual assault.

There are a number of additional desirable characteristics, in addition to its wider window of drug detection, that make hair an attractive matrix for monitoring drug use. Hair collection is noninvasive and easily observed, minimizing the risk of sample switching or adulteration. Segmental analysis of hair may help determine the time of drug exposure.

There also are limitations for determining drug exposure with hair analysis. Since a number of drugs are commonly smoked (i.e., marijuana, cocaine, heroin, and methamphetamine), passive contamination of an individual's hair may occur. Typically, hair samples undergo a number of washing cycles in various solvents prior to analysis, in an attempt to prevent false positive results from external contamination.[3−6]

Another difficulty in interpreting hair testing results is that drug incorporation into hair can be influenced by the pigmentation, or more specifically the melanin content of hair. Wilkins, Rollins, and colleagues conducted a series of studies investigating the role of pigmentation on incorporation of drugs into hair. They administered cocaine, codeine, and phencyclidine intraperitoneally to male Long Evans rats, taking advantage of the fact that Long Evans rats have pigmented hair on the hood and nonpigmented hair on their flanks. They found that drug concentrations were greater in pigmented hair than nonpigmented hair.[7−9] Additionally, in controlled oral administration studies of codeine, significant positive relationships were found between the concentrations of codeine and melanin in hair.[10,11] The tendency for weakly basic drugs to be present in larger quantities in pigmented than nonpigmented hair contrasts findings for more neutral or acidic drugs, that is, phenobarbital and *n*-acetyl-amphetamine. Concentrations of these drugs in hair lack any relationship to hair pigment

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content.[12,13] The mechanisms of drug−melanin interaction remain to be clearly delineated, with studies demonstrating both ionic and covalent interactions.[14−17] A recent in vitro study suggests that drug−melanin interactions are due to ionic binding between drug molecules, which are positively charged under physiologic conditions, and negatively charged melanin polymers.[17] However, the use of matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry has identified covalent adducts of nicotine and amphetamine with the melanin intermediate L-3,4-dihydroxyphenylalanine (L-DOPA).[14,15]

Interpretation of hair analysis results is confounded by the limited understanding of mechanism(s) responsible for distribution of drugs into hair. This is in large part due to the limited number of controlled drug administration studies that include collection and analysis of plasma, sweat, and hair.

There are numerous methods, mostly via GC/MS, for simultaneously measuring cocaine and opiates in hair.[18−23] Many assays extract drug from the hair matrix via dilute hydrochloric acid.[18−20] However, the acetylated opiate, 6-acetylmorphine (6-AM), undergoes considerable hydrolysis to morphine under these conditions. Studies by Romolo et al. determined that 46.4% of 6-AM is hydrolyzed to morphine when 6-AM fortified pulverized hair is incubated in 0.1 M hydrochloric acid at 45 °C for 18 h.[24] 6-AM is a marker for differentiating heroin from other opiate use (i.e., codeine and morphine). Therefore, preservation of 6-AM during extraction from hair enables objective determination of heroin use. Recent studies attempted to identify extraction methods which minimized 6-AM hydrolysis. The best method employed sonicating pulverized hair samples in aqueous solutions.[24,25] Similarly, we sonicated pulverized hair in methanol to extract cocaine and opiates, while minimizing the conversion of 6-AM to morphine. However, methanol extraction yields considerable interferences, which required the use of tandem mass spectrometry to achieve adequate sensitivity. Methods for measuring cocaine and opiates in hair via LC-MS have been published; however, none simultaneously measure both drug classes and their metabolites.[5,26,27]

In this paper, we present, for the first time, a validated method for simultaneously measuring opiates, cocaine, and metabolites in hair using LC-MS/MS. Ultimately, this assay will be applied to hair samples collected during controlled administrations of cocaine and codeine to volunteers residing on a secure clinical research ward for 10 weeks. Analysis of hair samples in tandem with other matrixes, plasma and sweat, should improve our understanding of cocaine and codeine distribution into human hair.

EXPERIMENTAL SECTION

Clinical Samples. A volunteer with a history of cocaine and opiate use consented to reside on the secure clinical research unit at the Intramural Research Program, National Institutes on Drug Abuse, National Institutes of Health, to participate in a 10-week controlled cocaine and opiate administration study. This Intramural Research Board approved study was designed to investigate excretion of opiates, cocaine, and metabolites into hair following multiple subcutaneous (sc) cocaine hydrochloride and oral (po) codeine sulfate doses. The first 3 weeks of the study comprised the drug washout phase. During the low-dose drug administration week (week 4) 75 mg/70 kg cocaine, sc dose, and 60 mg/70 kg codeine, po dose, were administered on alternating days for a total of three doses for each drug. The high-dose drug administration week began on week 8 during which the subject received three doses of 150 mg/70 kg cocaine, sc dose, and 120 mg/70 kg codeine, po dose, on alternating days. An electric razor was used to collect hair from the frontal head region at the end of each week during the study.

Instrumentation. All experiments were carried out using a MDS Sciex API 3000 triple quadrupole mass spectrometer with an APCI source (Applied Biosystems, Foster City, CA). The mass spectrometer was interfaced with a Shimadzu HPLC system consisting of LC-10ADvp pumps and an SIL-HTc autosampler (Columbia, MD). All data were acquired and analyzed using Analyst software version 1.2. Pulverization of hair was performed using a Mini-bead beater 8 (BioSpec Products, Bartlesville, OK). A Beckman model J-6B centrifuge was used during sample preparation (Fullerton, CA). Evaporation under nitrogen was conducted in a TurboVap LV evaporator from Zymark (Hopkinton, MA).

Reagents. Standards and deuterated internal standards (listed in Table 1) were purchased from Cerilliant (Austin, TX). Ammonium acetate and formic acid were purchased from Sigma Chemical (St. Louis, MO). All solvents were HPLC grade. Fritted filters, part RFV2F15P, and Cleanscreen solid-phase extraction columns, part ZSDAU020, were used in preparing samples for analysis (United Chemical Technologies, Bristol, PA). Drug-free hair was obtained from healthy volunteers.

Preparation of Standard Solutions. One mg/mL solutions of cocaine, norcocaine, benzoylecgonine (BE), ecgonine methyl ester (EME), cocaethylene, codeine, norcodeine, morphine, and 6-acetylmorphine were diluted using acetonitrile to prepare calibration solutions. A stock solution containing 2 and 10 μg/mL of cocaine and opiate metabolite standards, respectively, was prepared in acetonitrile and stored at −20 °C. Serial dilutions of the stock solution created calibrators of 17−5000 (cocaine and metabolites) and 83−25000 pg/mg (opiates) when spiking 50 μL of standard solution into 20 mg of pulverized hair.

Quality control solutions were prepared in acetonitrile using different ampules of standards than were used for preparing

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Table 1. LC-APCI-MS/MS Method Parameters for Opiates, Cocaine, and Metabolites in Hair

compound	precursor ion (m/z)	collision energy (V)	product ion (m/z)	RT (min)
ecgonine methyl ester-d_3	203.0	36	85.1	3.1
ecgonine methyl ester	200.1	35	82.1	3.2
norcodeine	286.2	52	165.2	6.3
benzoylecgonine-d_3	293.3	28	171.3	6.7
benzoylecgonine	290.2	28	168.3	6.7
morphine-d_3	289.3	60	165.1	6.7
morphine	286.2	52	165.2	6.8
codeine-d_3	303.4	56	165.1	8.5
codeine	300.3	54	165.2	8.6
6-acetylmorphine-d_3	331.2	54	165.1	8.5
6-acetylmorphine	328.2	52	165.1	8.6
cocaine-d_3	307.3	28	185.1	10.9
cocaine	304.3	28	182.2	11.0
norcocaine-d_3	293.3	24	171.2	11.4
norcocaine	290.2	24	168.3	11.4
cocaethylene-d_3	321.3	29	199.3	11.9
cocaethylene	318.2	28	196.1	12.0

calibration standards. Serial dilutions produced low-, medium-, and high-quality controls spread across the linear dynamic range of each analyte. Quality control solutions were stored at −20 °C. A 50-μL spiking volume was used for each quality control.

Stock internal standard solution was prepared by dilutions of 100 μg/mL solutions of cocaine-d_3, norcocaine-d_3, benzoylecgonine-d_3, ecgonine methyl ester-d_3, cocaethylene-d_3, codeine-d_3, morphine-d_3, and 6-acetylmorphine-d_3 using acetonitrile. Deuterated norcodeine is not commercially available. Internal standard stock solution contained 1 and 5 μg/mL of d_3-cocaine metabolites and d_3-opiates, respectively, in acetonitrile, and was stored at −20 °C. A 10% solution (v/v) of the internal standard stock was prepared in acetonitrile and 50 μL of the diluted solution was added to each 20-mg hair sample, yielding a final deuterated internal standard concentration of 250 and 1250 pg/mg for deuterated cocaine and opiate metabolites, respectively.

Procedures. *Sample Preparation.* Twenty ± 0.2 milligrams blank hair was weighed into a 2-mL polypropylene tube half-filled with 3.2-mm chrome steel beads. Hair samples were pulverized in a minibead beater for 5 min with 50 μL of standard or quality control solution and 50 μL of internal standard solutions added to pulverized hair. Additionally, 50 μL of internal standard solution was added to 20 mg of pulverized hair samples collected during the clinical study. Following 20−30 min of incubation at room temperature, 1250 μL of methanol was added and tube contents were transferred into a 3.5-mL polypropylene tube. The 2-mL tube was rinsed again with 1250 μL of methanol and decanted into the same 3.5-mL tube. Extraction of drugs from hair was achieved by sonicating the samples at 37 °C for 3 h. Hair protein and beads were separated from the methanol solution by filtration through fritted filters. Methanol collected in clean 10-mL glass centrifuge tubes was centrifuged at 4 °C, 1850g for 7 min to pellet remaining protein. Supernatant was transferred to clean 10-mL glass centrifuge tubes and dried down under nitrogen at 40 °C in a Zymark evaporator.

Solid-Phase Extraction. Cleanscreen extraction columns were conditioned with 1 mL of elution solvent (dichloromethane:2-propanol:ammonium hydroxide, 78:20:2), 3 mL of methanol, 3 mL of distilled water, and 2 mL of 0.1 M phosphate buffer, pH 6.0. Following evaporation of methanol, sample residues were dissolved in 3 mL of 0.1 M phosphate buffer, pH 6.0, vortex-mixed for 30 s, and decanted onto conditioned columns. Columns were washed with 2 mL of distilled water, 2 mL of 0.1 M hydrochloric acid, and 3 mL of methanol. Columns were dried via vacuum at 5 psi for 5 min prior to eluting analytes with 5 mL of elution solvent into clean 10-mL glass centrifuge tubes. Eluates were dried under nitrogen at 40 °C in a Zymark evaporator. Samples were reconstituted with 50 μL of mobile phase A, vortexed for 30 s, centrifuged at 20 °C, 1500*g* for 2 min, and transferred to autosampler vials.

Liquid Chromatography. Chromatographic separation was performed using a Synergi Hydro RP column (150 × 2.0 mm, 4 μm) fitted with an end-capped C18 guard column (4 × 2.0 mm) (Phenomenex, Torrance, CA). The autosampler temperature was set to 4 °C. The column oven was maintained at 30 °C and 20 μL of sample was injected. Gradient elution was performed using (A) 10 mM ammonium acetate in water with 0.001% formic acid (pH 4.5) and (B) acetonitrile at a flow rate of 200 μL/min. The initial gradient conditions were 10% B with a final composition of 90% B at 20 min, which was maintained for 2 min. Initial conditions were re-established in 2 min and the column was re-equilibrated at initial gradient conditions for 6 min. LC eluant was diverted to waste for the first 0.5 min and after 20 min of the gradient.

Mass Spectrometry. All mass spectrometry data were acquired using positive APCI. Compound-specific optimization of MS/MS settings was performed via direct infusion at 10 μL/min of 100 ng/mL reference solutions in 50% aqueous methanol solution. The following APCI-MS settings were used: 50 psi nebulizer gas (high-purity nitrogen), 30 psi curtain gas, 3.0 μA corona discharge current, and 375 °C source temperature. Table 1 details the MS/MS parameter settings for each analyte. A dwell time of 200 ms was used for monitoring each transition. Quadrupole one was set to transmit the target mass ± 0.7 amu into the collision cell for monitoring cocaine and metabolites. Opiate monitoring was performed with quadrupole one set to transmit the target mass ± 1.0 amu into the collision cell. Quadrupole three was operated at unit resolution for all experiments.

Data Analysis. Calibration using internal standardization was performed using linear regression employing $1/x$ weighting. Peak area ratios of target analytes and their respective internal standards were calculated for each concentration. At least six analyte concentrations were used in each standard curve.

Validation. Sensitivity, linearity, intra- and interbatch precision, accuracy, recovery, and matrix effect were investigated to evaluate method integrity. Specificity was based on relative retention time, precursor mass, and fragment ion.

Sensitivity was evaluated by determination of the limit of detection (LOD) and limit of quantification (LOQ) (see Table 2). A series of decreasing concentrations of drug-fortified pulverized hair was analyzed to empirically determine LOD and LOQ. LOD was determined as the concentration with a signal-to-noise ratio of at least 3, while LOQ was the lowest concentration with a signal-to-noise ratio of at least 10 and acceptable accuracy and precision (target within at least 20% and relative standard deviation within at least 20%, $n = 5$). Any analyte for which accuracy did not fall within 80−120% of the target concentration was considered semiquantitative.

The linearity of the method was investigated by calculation of the regression line by the method of least squares and expressed

Table 2. Opiates, Cocaine, and Metabolites in Hair by LC-APCI-MS/MS: Limits of Detection (LOD), Limits of Quantification (LOQ), and Calibration Results

compound	internal standard	LOD (pg/mg)	LOQ (pg/mg)	equation	R^2	linear range (pg/mg)
cocaine	cocaine-d_3	8.5	17	$y = 1.03x + 0.016$	0.9995	17−5000
norcocaine	norcocaine-d_3	8.5	17	$y = 1.21x + 0.011$	0.9994	17−5000
BE[a]	BE-d_3	8.5	17	$y = 0.99x + 0.013$	0.9986	17−2500
EME[b]	EME-d_3	8.5	50	$y = 1.07x - 0.024$	0.9993	50−5000
CE[c]	CE-d_3	8.5	17	$y = 1.02x - 0.021$	0.9993	17−5000
codeine	codeine-d_3	41.5	83	$y = 1.26x - 0.015$	0.9995	83−12500
norcodeine	morphine-d_3	41.5	240	$y = 1.14x - 0.071$	0.9984	240−12500
morphine	morphine-*d3*	41.5	83	$y = 1.42x - 0.018$	0.9992	83−12500
6-AM[d]	6-AM-*d3*	41.5	83	$y = 1.15x - 0.026$	0.9995	83−12500

[a] Benzoylecgonine. [b] Ecgonine methyl ester. [c] Cocaethylene. [d] 6-Acetylmorphine.

by the squared correlation coefficient (R^2). A $1/x$ weighting factor was applied to compensate for heteroscedasticity. Linearity of each analyte was determined using at least six concentration levels, not including the blank matrix.

Precision and accuracy were evaluated over the linear range at three different concentrations, that is, low, medium, and high. Cocaine, norcocaine, benzoylecgonine, and cocaethylene low-, medium-, and high-quality control target concentrations were 40, 200, and 2000 pg/mg of hair. Ecgonine methyl ester low-, medium-, and high-quality control target concentrations were 80, 400, and 4000 pg/mg of hair. Opiate low-, medium-, and high-quality control target concentrations were 240, 1200, and 12000 pg/mg of hair. Intrabatch precision was evaluated by six determinations per concentration in 1 day. Interbatch precision was evaluated for six replicates per concentration on 4 days ($n_{total} = 24$). Interbatch precision was expressed as percent relative standard deviation calculated using all 24 individual values, equally weighted over four batches. Accuracy was determined comparing the mean measured concentration of six analyses to the target value. Accuracy was expressed as the percent of target concentration.

Recovery (%) for each analyte was also determined at the low, medium, and high control concentrations (see Table 4). For determination of recovery, quality control standard solution was added prior to or following solid-phase extraction (SPE). Recovery, %, was expressed as the mean analyte area of samples with control solution added before SPE ($n = 6$) divided by the mean analyte area of samples with control solution added after SPE ($n = 6$).

Matrix effect was investigated by comparing analyte peak areas of extracted blank samples that were spiked at the low quality control concentrations after SPE versus analyte peak areas of neat samples prepared in mobile phase A at equivalent concentrations. Matrix effect was computed by dividing the analyte areas of blank samples fortified after SPE by areas of neat samples, expressed as percent (Table 4).[28]

Hydrolysis of cocaine and 6-AM during sample processing was evaluated using blank hair fortified to 20000 pg/mg cocaine and 6-AM. Quantifying the amount of BE and morphine formed in these hydrolysis controls allowed the calculation of percent of cocaine and 6-AM hydrolysis.

Stability of extracted hair samples while in the 4 °C autosampler was evaluated over 60 h. Extracted low, medium, and high QC samples were analyzed immediately after extraction along with calibration standards ($n = 4$ for each QC level). Another set of four of each low, medium, and high QC samples were analyzed 60 h after extraction and subsequent storage in autosampler vials at 4 °C. All samples were quantitated using the initial calibration curve.

Safety Considerations. The method required no specific safety precautions aside from universal safety precautions for handling chemicals and biological samples.

RESULTS AND DISCUSSION

Calibration and Validation. Calibration using internal standardization with deuterated analogues was performed. In the absence of commercially available stable isotopic norcodeine, a deuterated version of morphine was used as the internal standard, which is structurally related and has a retention time close to norcodeine (see Tables 1 and 2). Norcodeine and morphine, which have identical masses and equivalent fragmentation, were resolved by at least 90% of peak height (Figure 1).

The method was validated according to the criteria described in the Experimental Section. Table 2 details the LODs, LOQs, and calibration results for all analytes. LODs were 41.5 and 8.5 pg/mg for opiates and cocaine metabolites, respectively. LOQs for cocaine, norcocaine, BE, and CE were 17 pg/mg and for EME 50 pg/mg. The higher LOQ for EME is caused by difficulties integrating the EME peak due to the presence of a shoulder (Figure 1). Opiate LOQs were 83 pg/mg for codeine, morphine, and 6-AM.

Linearity was achieved with a minimal squared correlation coefficient (R^2, $1/x$ weighting factor) of 0.998. Linearity ranged from the LOQ to 5000 pg/mg for cocaine and metabolites, except BE had an upper limit of 2500 pg/mg. Opiate linearity was from the LOQ to 12 500 pg/mg (Table 2).

Precision and accuracy were evaluated at three concentrations across the linear dynamic range of each analyte. Table 3 details the concentrations tested and data for accuracy and precision. Intrabatch precision (RSD) was less than 11.0% at low, medium, and high QC concentrations for all analytes ($n = 6$). Interbatch precision (RSD) was less than 8.1% for all analytes at low, medium, and high QC concentrations ($n = 24$). Accuracy calculated as the percent of target concentration of each analyte at low, medium, and high QC concentrations ranged from 84.0 to 115.0% of the target concentration ($n = 6$). Norcodeine failed to meet quantitative criteria for the low-quality control accuracy, as the intrabatch accuracy was 77.8% of target concentration. Therefore, we regard any data for norcodeine as semiquantitative. Accuracy issues for

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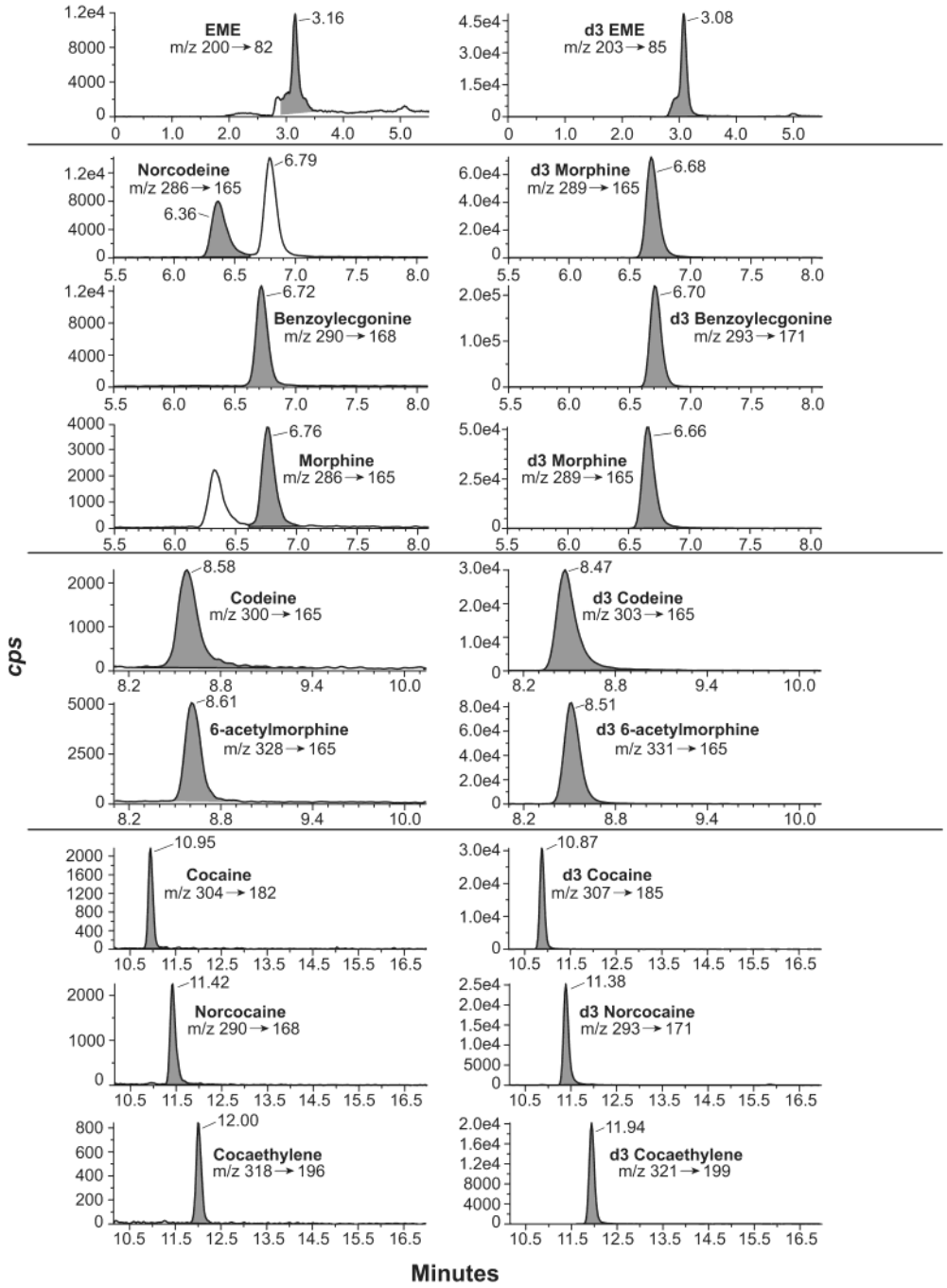

Figure 1. Selected reaction monitoring chromatograms of extracted hair samples illustrating opiates, cocaine, and metabolites fortified into blank hair samples at each analyte's respective limit of quantitation. LOQs for cocaine, norcocaine, BE, and CE were 17 pg/mg and for EME 50 pg/mg. Opiate LOQs were 83 pg/mg for codeine, morphine, and 6-AM. The concentration of norcodeine is 240 pg/mg. Internal standard concentrations are 250 and 1250 pg/mg for deuterated cocaine and opiates, respectively.

norcodeine might result from the unavailability of deuterated norcodeine for use as an internal standard. Morphine-d_3 was used as an internal standard for norcodeine as it elutes in a region of the chromatographic gradient nearby norcodeine and has a close, but different, structure.

Recovery ranged from 70.9 to 96.5% (Table 4). Matrix effect was investigated by comparison of extracted blank hair samples to which low QC solution was added following SPE to neat samples prepared equivalent to the low QC concentration in mobile phase A ($n = 6$ for each condition). Matrix enhancement was observed for BE, morphine, and 6-AM. Cocaethylene demonstrated matrix suppression (Table 4). We were able to achieve adequate quantitative assay characteristics since we used deuterated analogues as internal standards for each analyte, except norcodeine. It is important to note that neat standards quantitated within 20% of expected values, when they were quantitated using calibration curves generated from standards prepared in hair. Thus, the internal standards and analytes are equally affected by the matrix.

Quantification of BE and morphine formed in cocaine and 6-AM hydrolysis control samples was conducted over 15 runs. The average percentages of cocaine and 6-AM hydrolysis were 0.19 $\pm$ 0.06 and 4.5 $\pm$ 1.2%, respectively ($n = 15$).

Table 3. Opiates, Cocaine, and Metabolites in Hair by LC-APCI-MS/MS: Accuracy and Precision Data

	intrabatch precision (% RSD, $n = 6$)			interbatch precision (% RSD, $n = 24$)			accuracy (% of target, $n = 6$)		
compound	low	medium	high	low	medium	high	low	medium	high
cocaine[a]	11.0	3.0	2.2	8.1	4.5	5.0	115.0	100.2	92.5
norcocaine	2.5	1.8	2.6	4.4	3.7	3.5	104.3	99.7	96.0
benzoylecgonine	5.1	1.5	1.7	4.0	2.2	2.3	95.1	95.0	92.1
ecgonine methyl ester[b]	2.9	1.5	2.7	4.0	6.1	5.0	97.3	90.0	87.9
cocaethylene	2.7	1.7	2.7	3.8	6.0	7.3	98.8	91.0	84.0
codeine[c]	3.1	3.4	0.8	3.6	3.0	2.6	91.7	96.0	92.2
norcodeine[d]	3.4	2.4	1.7	7.7	3.8	3.6	77.8	85.1	92.1
morphine	2.4	1.9	2.5	3.6	2.6	2.2	85.1	99.6	92.4
6-acetylmorphine	1.8	3.7	0.9	3.8	3.3	2.3	93.5	97.9	95.6

[a] Cocaine, norcocaine, benzoylecgonine, and cocaethylene low-, medium-, and high-quality control target concentrations were 40, 200, and 2000 pg/mg of hair. [b] Ecgonine methyl ester low-, medium-, and high-quality control target concentrations were 80, 400, and 4000 pg/mg of hair. [c] Opiate low-, medium-, and high-quality control target concentrations were 240, 1200, and 12000 pg/mg of hair. [d] Since accuracy fell outside 80−120% target concentration for norcodeine low-quality control, norcodeine is considered semiquantitative.

Table 4. Recovery and Matrix Effect of Opiates, Cocaine, and Metabolites Extracted from Hair

	recovery (%, $n = 6$)			matrix effect (%, $n = 6$)
	low	mid	high	low
ecgonine methyl ester	80.3	83.4	82.4	111.0
benzoylecgonine	82.2	82.8	85.6	267.1
norcodeine	81.5	77.9	76.1	105.6
morphine	96.5	89.8	86.0	142.0
codeine	83.6	80.3	80.4	92.1
6-AM	82.4	80.2	79.8	152.3
cocaine	85.9	73.5	73.6	89.1
norcocaine	83.3	74.6	72.8	85.0
cocaethylene	85.5	72.6	70.9	68.2

Table 5. Codeine, Cocaine, and Metabolites Measured in Hair Samples Collected from a Volunteer during a Controlled Codeine and Cocaine Administration Study

		maximum concentrations (C_{max})	
(pg/mg)	baseline[a]	low dose[b]	high dose[b]
cocaine	113	2330	4570
benzoylecgonine	44.6	253	375
ecgonine methyl ester	<LOQ	217	459
norcocaine	<LOQ	45.7	210
cocaethylene	<LOQ	40.1[c]	91.8[c]
codeine	<LOQ	675	1750

[a] Hair shavings collected immediately preceding the start of drug dosings, representing previously self-administered cocaine. [b] Maximum concentrations observed 2−3 weeks after start of drug dosing. [c] Cocaethylene concentration is approximately 2.0% of cocaine C_{max}. Cocaine hydrolysis controls showed 0.91 ± 0.05% cocaethylene (n = 15) formed during sample processing.

Stability at 4 °C on the autosampler for 60 h was investigated. All analytes were stable under these conditions, differing from samples injected immediately by less than 17%.

Proof of Method. Since there is evidence that drugs interact with melanin in hair of drug users, we were concerned that methanolic sonication may not effectively disrupt these interactions.[14−17] Thus, we analyzed weekly head shavings from a single volunteer residing on the secure clinical research ward for 10 weeks who was administered three low doses of cocaine (75 mg/70 kg sc) and codeine (60 mg/70 kg p.o) during the fourth week of residence on the ward. Three high doses of cocaine (150 mg/70 kg sc) and codeine (120 mg/70 kg po) were administered during the eighth week of the study. The results in Table 5 show analyte concentrations just prior to the first drug administration along with maximum analyte concentrations (C_{max}) found after the low and high dosings. The C_{max}'s occurred in the second or third week following drug dosing. Norcodeine, morphine, and 6-acetylmorphine did not exceed the LOQ in any of this subject's samples. These data suggest a dose relationship for codeine, cocaine, and metabolites. This indicates that our method is capable of efficiently and reliably extracting drugs from hair and should be useful for studies investigating drug distribution into hair.

It is striking that we observed cocaethylene, a metabolite formed when cocaine and alcohol are co-administered, while the subject was residing on the clinical ward. We did observe approximately 1% of cocaethylene formed in our cocaine hydrolysis controls and the amounts of cocaethylene found in samples containing cocaine C_{max}'s were approximately 2% of the cocaine concentration.

It should be noted that we did not wash hair samples prior to analysis since this study did not involve the smoked route of administration and the subject was residing on the secure clinical research ward 24 h a day for the duration of this study. In the forensic or workplace setting, it is necessary to wash hair samples prior to analysis, to aid in distinguishing passive exposure (external contamination) and drug deposited into the hair follicle during self-administration.

CONCLUSION

Methanolic sonication of pulverized hair provides adequate recovery of opiates, cocaine, and metabolites from hair with minimal hydrolysis of cocaine or 6-AM. APCI was an efficient ionization technique which produced acceptable assay characteristics for cocaine, norcocaine, BE, EME, CE, codeine, morphine, and 6-AM. This method will enable us to analyze hair samples collected from participants administered cocaine and codeine in a study designed to investigate drug distribution into human hair.

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