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Development of a screening method for cocaine and cocaine metabolites in urine using solvent microextraction in conjunction with gas chromatography

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Abstract

A simple, quick and inexpensive screening method for cocaine and cocaine metabolites has been developed. Drug extraction was achieved using the relatively new technique of solvent microextraction (SME). Complete analysis is achieved in 13 min, using, a 6-min extraction with a 2-μl drop followed by separation on a gas chromatograph. The developed procedure was tested as a screening method for cocaine and cocaine metabolites in spiked urine samples. Using SME, concentrations as low as 0.125 μg ml^{-1} of cocaine, ecgonine methyl ester, cocaethylene and anhydroecgonine methyl ester were measurable with relative standard deviation values averaging 9.0%.

Keywords: Solvent microextraction; Cocaine; Cocaethylene; Ecgonine methyl ester; Anhydroecgonine methyl ester

1. Introduction

Screening for drugs of abuse is becoming increasingly common in the workplace. Federally regulated drug testing programs have protocols which involve an initial screen using immunoassay followed by a confirmatory gas chromatography–mass spectrometry (GC–MS) analysis on immunoassay-positive samples [1]. Despite widespread application, immunoassay has several drawbacks. Standard immunoassay kit responses are susceptible to interferences caused by the presence of adulterants in the urine. Results do not differentiate between metabolites and give only minimal quantification of the analyte. In addition to federally regulated workplace drug testing programs, many non-regulated companies also employ drug testing [2]. Because these companies are not bound by federal regulation they may use alternative methods of analysis as well as other specimens such as saliva or hair. Companies may test for a variety of reasons including pre-employment, post-accident, post-incident, for-cause, random, safety sensitive and contractual requirements. As drug screening of employees becomes more widespread, a large burden is placed on laboratories making development of a new fast and inexpensive screening method appealing.

According the National Institute on Drug Abuse at the National Institutes of Health, a 1996 study showed that ''crack'' cocaine continues to dominate the nation's illicit drug problem [3]. In 1996, 1.75 million people were using cocaine, in both the acid

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and base form, in the United States. Cocaine undergoes reaction in the body forming a number of major metabolites such as benzoylecgonine and ecgonine methyl ester (EME) [4,5] that are then excreted in the urine, sweat, saliva and faeces. When cocaine is ingested with alcohol, another metabolite, cocaethylene, is produced in the liver causing intensified euphoric effects [6]. Anyhydroecgonine methyl ester (AEME), the thermal degradation product of cocaine, is also excreted in the urine and is indicative of ''crack'' cocaine use [7,8].

The Substance Abuse and Metal Health Services Administration (SAMHSA) mandatory guidelines for federal workplace drug testing programs sets the initial test cut-off level for cocaine metabolites at 0.300 μg ml^{-1} [9]. Using these cut-off levels as a guide, any potential screening method developed must be able to detect cocaine and cocaine metabolites at this concentration. Besides this requirement, any potential screen must also be quick, easy to use, and inexpensive.

Solvent microextraction (SME) is a fairly new method of sample preparation which has recently been described in several papers [10–14]. Jennot and Cantwell described a relatively simple technique in which a microdrop of toluene was suspended on the tip of either a PTFE rod or microsyringe which was immersed in the stirred aqueous sample solution [11,12]. SME provides analyte extraction using small volumes of organic solvent which is quick, inexpensive and uses simple equipment found in most analytical laboratories.

In the present study, SME is employed by suspending a 2-μl solvent drop from the tip of a microsyringe immersed in a stirred aqueous solution (Fig. 1). The extraction drop remains on the tip of the syringe for a set extraction time after which the drop is withdrawn from the solution into the syringe. The contents of the syringe are then injected into a GC system for preliminary identification and quantification of the extracted solute. An extraction protocol for cocaine and three metabolites: EME, cocaethylene and AHME from distilled water and synthetic urine was developed by varying extraction solvent, pH and oven temperature programming. Studies on extracting these compounds from human urine samples showed that an additional filtration step was required to remove particulate matter formed on addition of base.

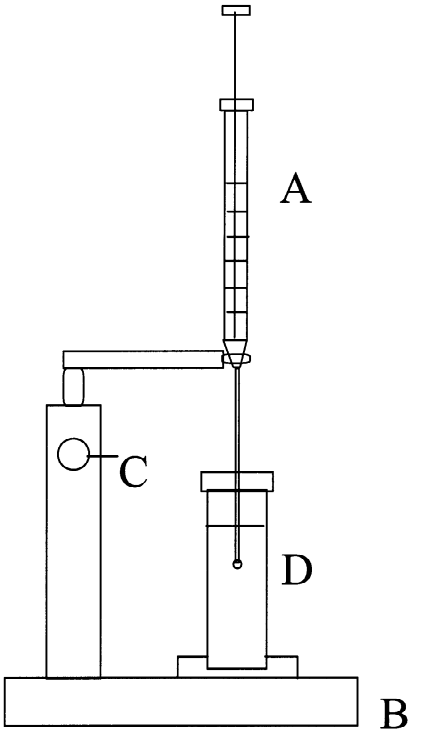

Fig. 1. Schematic of syringe stand. A, Syringe; B, stand; C, screw used to adjust the height of the syringe and D, extraction vial.

2. Experimental

2.1. Reagents

The cocaine, cocaethylene, EME and AEME (1.0 mg ml^{-1} in acetonitrile) were obtained from Radian International (Austin, TX, USA). All solvents used were HPLC grade unless otherwise stated. Solvents used were methanol, octane (Aldrich, Milwaukee, WI, USA), toluene (Spectrum, New Brunswick, NJ, USA), dichloromethane, chloroform, and butyl acetate (Fisher Chemicals, Fairlawn, NJ, USA). Synthetic urine tablets were obtained from Alltech Associates (Deerfield, IL, USA) and were dissolved in ultrapure, distilled, deionised (18.2 MΩ) water obtained from a Milli-Q water purification system (Millipore, Milford, MA, USA). Trisodium phosphate buffer was made by dissolving 23.8 g trisodium phosphate (Mallinckrodt, Paris, KY, USA) in 250 ml of ultrapure water. All glassware was deactivated using dimethyldichlorosilane (Supelco, Bellefonte, PA, USA) as described by the manufacturer

and extraction vials were bought pre-silanized (Alltech Associates, State College, PA, USA). All gases were supplied by Pallini Industries (Athens, OH, USA).

2.2. Instrumentation

A Varian 3400 GC system modified with a Varian 1077 split/splitless injection port (Varian Associates, Walnut Creek, CA, USA) was used in all experiments. Ultrapure helium (99.999%) passed through hydrocarbon traps, oxygen traps and moisture traps (Alltech) was used as the carrier gas at a constant flow of 2.4 ml min^{-1}. The injection port was held at 280°C and used in the split mode with split flow of 24 ml min^{-1}. Separation was carried out on a RTX-5 30 m×250 μm, 0.25 μm 95% dimethyl–5% diphenyl polysiloxane copolymer column (Restek, Bellefonte, PA, USA). Oven temperature programming was used to facilitate separation with an initial oven temperature of 140°C ramping at a rate of 25°C min^{-1} to a final temperature of 280°C. A Valco pulsed-discharge helium ionization detection (PDHID) system (Valco Instruments, Houston, TX, USA) was used as the detector. The detector temperature was held at 280°C and a helium flow of 30 ml min^{-1} was used to induce formation of the plasma. A Gateway 2000 4DX2-66V desktop computer (Gateway 200, North Sioux City, SD, USA) with EZChrom software (6.7; Scientific Software, CA, USA) was used to collect and analyze the data.

Standard solutions of cocaine, cocaethylene, ecgonine methyl ester and anhydroecgonine methyl ester were made in methanol. These solutions were analyzed with GC using the parameters outlined above. From the resulting chromatograms, retention times were determined for each of the compounds of interest. Quantification throughout the experimental work was accomplished using peak areas measured by EZChrom.

2.3. Extraction procedure

The extraction procedure was carried out using a Hamilton 701SN 10-μl microsyringe fitted with a Chaney adapter (Hamilton, Reno, NV, USA), an 8 mm×1.5 mm magnetic stir bar (VWR Scientific Products, West Chester, PA, USA), a magnetic stir plate, a syringe stand (constructed in the laboratory) and a silanized 7-ml extraction vial with a PTFE–silicon septum (Supelco). Using the Chaney adapter the maximum syringe volume and the delivery volume were set to 2.0 μl.

For an extraction, solvent was drawn into the syringe to the maximum volume (2.0 μl). The syringe was then inserted into the syringe stand above the extraction vial in such a way that the needle passed through the septa of the extraction vial and the needle tip protruded to a depth of about 8 mm below the surface of the stirred analyte solution. To form the extraction drop, the plunger was depressed, causing the 2 μl of organic solvent to be suspended from the needle tip. The drop was exposed to the analyte solution for a set extraction time after which the drop was drawn back into the syringe with the needle still immersed in the analyte solution. The syringe was then removed from the syringe holder and the extraction solvent with the extracted analytes was injected into the gas chromatograph for analysis.

3. Results

3.1. Method development

Method development was examined from a univariate optimization approach. Standard solutions with concentrations of each of the drugs ranging from 10 to 0.25 μg ml^{-1} were made in methanol. Injections of 2 μl were made of these solutions using the oven temperature program outlined above and calibration curves with good linearity were obtained (R^2=0.9477–0.9989). All quantification in this study was determined using peak area. Any relative standard deviation (RSD) values stated were calculated from three replicate measurements unless otherwise stated.

In order to determine which organic solvent would be optimal for this extraction, several solvents and solvent mixtures were examined. Each extraction solvent was tested using aqueous solutions containing 1 ml of 8 μg ml^{-1} of cocaine, cocaethylene and ecgonine methyl ester and 0.75 ml of trisodium phosphate buffer with pH 10.5. Toluene, octane and dichloromethane–toluene (1:9) were tested and although these gave good extraction of cocaine and cocaethylene, no peak was observed for EME.

Chloroform and chloroform–toluene (1:1) yielded peaks for all three compounds with chloroform extractions giving larger EME peaks than the chloroform–toluene mix. Butyl acetate and chloroform–propanol (1:1) were also tested but the drop dislodged before extraction was complete. In light of these results, chloroform was chosen as the extraction solvent for the remainder of the study.

The pH of the solution is known to play and essential role in extraction of drugs from urine. The pH of aqueous solutions containing the drugs at a concentration of 4 μg ml^{-1} was adjusted to 8.5, 9.8, 10.5, and 11.4 and the optimal extraction was found to occur at a pH of 10.5.

In previous studies [10], an extra 0.9 μl was drawn up into the syringe following extraction to ensure that the entire extraction drop was drawn up into the syringe for injection into the GC system. In this case, the chloroform drop appeared to be completely drawn up by retracting the plunger to the 2.0 μl mark. The volume drawn up following the extraction was varied from 1.8 to 2.2 μl and it was found that no advantage resulted from injecting the extra volume.

A 6-min extraction time was used throughout the experimental work to correspond to the GC run time. Previous studies [10] showed that a stir rate of below 240 rpm provided good extraction with a low rate of drop loss. We chose to work with a 2-ml sample as this volume would be compatible with other body fluids in future studies.

Extractions from aqueous solutions containing cocaine, cocaethylene and EME were then carried out using the optimized extraction conditions. The pH was adjusted by addition of 100 μl of trisodium phosphate buffer with pH of 10.5, to a 2-ml sample of the drug mixture. Replicate extractions on aqueous solutions containing the three compounds at concentrations of 4.0 to 0.125 μg ml^{-1} were made and calibration lines plotted. Good linearity (R^2= 0.9920–0.9956) and reproducibility were obtained with RSD values ranging from 4 to 32% and averaging 16%.

Solutions of cocaine, cocaethylene and EME were then prepared in synthetic urine for extraction. Initial extractions yielded poor results and it was discovered that because the synthetic urine has a acidic pH more base had to be added to adjust the pH to 10.6. In order to compensate, a new trisodium phosphate buffer was made at a pH of 13.1. It was found that addition of 100 μl of this buffer to 2 ml of the synthetic urine resulted in a pH of 10.6. After making this adjustment to the procedure, five-point calibration lines from solutions containing 4.0 to 0.250 μg ml^{-1} of each compound were constructed. Good linearity was obtained (R^2=0.9801–0.9958) and RSD values varied from 4.7 to 26.1% with an average of 14.3%.

Urine was then obtained from several subjects. Male 1 was a 27-year-old male subject taking cough medicine containing guaifenesin and pseudoephedrine hydrochloride. The second subject, female 1, was a 26-year-old female who was taking a variety of prescription and over-the-counter medication including: clemastine fumarate, phenylpropanolamine hydrochloride, loratadine, pseudoephedrine hydrochloride, zithromaz, doxycycline, norethindrone, ethinyl estradiol, carbamazepine, naproxen sodium, acetaminophen, guaifenesin. Female 2 was a 23-year-old female taking oral contraceptives and female 3 was a 24-year-old female on no medication.

Initial attempts at extracting these urine samples found that drop dislodgement occurred 100% of the time. Closer examination revealed that addition of base to the urine samples caused a fine precipitate to form, which upon stirring would collide with the extraction drop causing it to fall off the tip of the needle. In order to overcome this, the extraction procedure was amended in the following way. A 3-ml volume of urine sample and 150 μl of trisodium phosphate buffer were pipetted into a silanized 10-ml beaker and stirred for 1 min. A 2-ml volume of this solution was then drawn up into a 10-ml plastic disposable syringe and passed through a 13-mm nylon syringe filter (Alltech) with 0.2 μm pores into the extraction vial where the extraction procedure continued as previously described.

Aliquots of the urine samples, spiked with cocaine, cocaethylene, EME and AEME were adjusted to pH 10.5, filtered and analyzed. Extractions on the urine samples prior to addition of drugs were also carried out with no interfering peaks occurring at the retention times of interest. A four-point calibration curve was prepared from triplicate extractions of female 2s' urine spiked to concentrations between 1.0 and 0.25 μg ml^{-1}. These curves showed

Table 1
Equations and R^2 values of calibration lines produced in this study[a]

	Cocaine	Cocaethylene	EME	AEME
Standards	$1171.3x+710.9$ $R^2=0.9987$	$1337.5x+1292.7$ $R^2=0.9997$	$858.95x-38.8$ $R^2=0.9992$	$982.6x-81.1$ $R^2=0.9951$
Aqueous samples	$15\,259x+690.7$ $R^2=0.9969$	$18\,736x+415.1$ $R^2=0.9993$	$6473.1x+250$ $R^2=0.9999$	$15\,988x+222.6$ $R^2=0.9952$
Synthetic urine	$19\,342x+683.0$ $R^2=0.9925$	$21\,291x+744.3$ $R^2=0.9926$	$6266x+152.8$ $R^2=0.9993$	$15\,771x+602.2$ $R^2=0.9960$
Human	$18\,024x+915.3$ $R^2=0.9989$	$20\,607x+1716.3$ $R^2=0.9979$	$6134.7x+266.6$ $R^2=0.9993$	$15\,520x+133.1$ $R^2=0.9947$

[a] Slopes are given in V min ml g^{-1} and intercepts in μV min^{-1}.

good linearity ($R^2=0.9910$–0.9986) and reproducibility with RSD values ranging from 1.2 to 23.9% and averaging 9.0%. Several extraneous peaks were observed in the extractions from the subjects' urine with female 1s' urine producing the largest and most predominant peaks.

In addition to these preliminary calibration studies, multiday calibration studies were undertaken of cocaine, cocaethylene, EME and AEME. This entailed daily preparation of four point calibration lines for five consecutive days with triplicate extractions at each concentration. Standard calibrants ranged in concentration from 10 to 2 μg ml^{-1} and extraction calibration solutions for aqueous, synthetic urine and human urine (female 1) ranged in concentration from 1 to 0.2 μg ml^{-1}. These lines had good linearity and reproducibility with RSD values for human urine extractions ranging from 1.6 to 28% and averaging 10%. Equations and correlation coefficients from the pooled data are given in Table 1, and the daily slopes from AEME calibration lines are given in Table 2.

In order to test the feasibility of using this method as a screening technique, four samples of urine from female 3 were spiked with cocaine, cocaethylene and ecgonine methyl ester by a colleague. Extractions were then carried out and the concentrations were then calculated from extraction calibration curves. Good correlation between actual concentration and observed concentration was obtained. The results of this study are summarized in Table 3.

4. Discussion

The majority of current urinalysis techniques, including federal drug testing programs, use the

Table 2
Slope values from AEME multiday calibration study[a]

Day	Sample medium			Mean slope of three mediums	RSD (%)
	Aqueous	Synthetic	Human		
1	16 309	14 694	13 485	14 829	9.6
2	19 629	16 659	15 131	17 140	13.3
3	16 839	15 143	14 329	15 437	8.3
4	15 266	17 739	15 281	16 095	8.8
5	12 122	14 619	17 055	14 599	16.9
Mean slope of 5 days	16 033	15 771	15 056		
RSD (%)	16.9	8.7	8.8		

[a] Slopes given are from four-point calibration lines over 1.0 to 0.2 μg ml^{-1} prepared daily from triplicate extractions at each concentration.

Table 3
Results of blind study on spiked urine samples of female 3[a]

Sample		Cocaine	Cocaethylene	EME
A	**Experimental**	**0.0**	**0.0**	**0.0**
	Actual	0.0	0.0	0.0
B	**Experimental**	**0.2**	**0.0**	**0.5**
	Actual	0.2	0.0	0.5
C	**Experimental**	**0.5**	**0.7**	**0.0**
	Actual	0.5	0.7	0.0
D	**Experimental**	**0.1**	**0.5**	**0.3**
	Actual	0.1	0.6	0.2

[a] All values reported in μg ml^{-1}. Bold values indicate the experimental results obtained by SME extraction and normal font values indicate the actual concentrations.

metabolite benzoylecgonine as indicative of cocaine use. Although this metabolite accounts for 35 to 54% of the dose excreted and has a relatively long half-life of 4.5 h [2], chromatography of this compound can be difficult and derivatization is often required [5]. In order to avoid time-consuming derivatization reactions, detection of other metabolites of cocaine were investigated. EME is accountable for 32 to 49% of the administered dose and has a half-life of 3.1 h. Although the half-life of EME is lower than that of benzoylecgoinine, the difference is acceptable in exchange for a reduced sample preparation time. Approximately 1–9% of the cocaine dose is excreted unchanged in the urine. Cocaine has a very short half life of only 0.8 h [2] and thus its presence would indicate recent cocaine use. Similarly, the presence of cocaethylene would indicate alcohol ingestion and AEME would reveal the route of administration of the drug.

The solvent microextraction study was initiated using a dichoromethane–toluene (1:9) mix as our extraction solvent at a pH of 10.5, as these conditions had been reported for the extraction of cocaine and cocaine metabolites [15]. Although the cocaine and cocaethylene were successfully extracted using this solvent mix, no extraction of EME had occurred. As the suitability of other solvents for extraction of the compounds of interest was tested, it became evident that more polar organic solvents yielded better extraction of EME while not reducing the amount of cocaine and cocaethylene extracted. It was found that chloroform (polarity index=4.1) gave superior extraction of EME when compared to toluene (2.4), octane (0.0) and dichloromethane (3.1) as well as mixes of any of these solvents. Butyl acetate and chloroform–propanol drops could not be maintained on the syringe tip for the extraction time. This may be due to the increased solubility of butyl acetate and propanol in aqueous solutions in comparison to chloroform as well as their decreased interfacial tensions [16]. Although exposure to high levels of chloroform has been linked to liver and kidney damage [17], the volume of organic solvent used in SME is unlikely to cause harmful toxicological effects.

Although previous work in our laboratory using hexane as the extraction solvent [10] required an extra 0.9 μl to be drawn into the syringe to ensure the entire drop was withdrawn, this was not required in this study. The partial solubility of chloroform (0.097 g/100 ml water) may be an explanation to this. Because chloroform is partially soluble in aqueous solutions, it is feasible that sufficient solvent dissolves into the water to negate the need for the extra volume. This would not be the case for hexane because of its reduced solubility in water (0.0138 g in 100 ml).

Since our cut-off level for cocaine and cocaine metabolites was set to 0.300 μg ml^{-1}, equal to the SAMHSA guidelines, this value was used as our goal for limits of detection. In the aqueous extraction and synthetic urine extractions it was possible to quantify each compound at concentrations below 0.125 μg ml^{-1}, well below the screening cut-off level. Extractions from our subjects' urine spiked with drugs also yielded quantification at the 0.125 μg ml^{-1} level. It may be possible to reduce these values further by extending the extraction time but this is not necessary for the objectives of a screening method. Also by keeping the extraction time synchronized with the separation time, the amount of time required for a complete analysis of a sample was minimized while maximizing the sample throughput. Using the extraction protocol for urine, a complete analysis can be performed in 13 min. This allows seven samples to be analyzed per hour if the extraction and separation are run in tandem.

Multiday calibration studies of standard solutions and extractions yielded interesting results. Compari-

son of extractions from the same medium between days showed no statistically significant differences in the slope values ($\alpha = 0.05$) with RSD values for the 5 days averaging 10.5%. The results of this study are shown for AHEME in Table 2. Additionally, comparison of slopes from extraction calibration lines of aqueous, synthetic and human solutions showed no statistical differences with the daily RSD values between mediums averaging 11.4%. Results for the other compounds of interest were similar with no clear trends in the slopes evident.

The amount extracted was calculated for all the urine extractions using peak area measurements and calibration curves of standards. The preconcentration factors and extraction efficiency were calculated for the extraction of the four compounds of interest from human urine samples. The preconcentration factor is the ratio between the final concentration of the analyte in the extracted drop and the concentration of the analyte in the original solution. Preconcentration factor values were calculated from average extractions of female 1s' urine obtained in the multiday calibration study using standard calibration lines. The results range from 7.4 to 17.5 (Table 4). Examination of the data revealed a slight trend of increasing preconcentration factor with decreasing concentration of analyte in the original solution that was experienced in previous work [10]. Extraction efficiency was calculated by determining the percent of the total analyte present in the original solution ($n_{s,initial}$) that was extracted into the organic drop ($n_{a,final}$) as shown in Eq. (1):

$$\%E = (n_{a,final}/n_{s,initial}) \cdot 100 \quad (1)$$

The extraction efficiency values are very small ranging from 0.74 to 1.75%. From the solubility data [18], the K_D value for the extraction of cocaine was determined to be 857. Using this value, if extraction were to continue to completion approximately 46% of the cocaine would be extracted into the organic drop. Since the focus of this study is the development of a fast, high throughput screening method, allowing the extraction to continue to equilibrium would be impractical and unnecessary as the amount extracted is sufficient for detection and quantification at the required level.

RSD values of the triplicate extractions from female 2s' urine were acceptable with an average of 9%. These values are considerably lower than those obtained in other solvent microextraction studies performed in this laboratory [10]. A possible explanation for this improved reproducibility is that the extraneous aqueous volume previously injected into the GC system is no longer required. The variability in extraction experienced may be attributed to the irreproducible formation of the drop on the tip of the syringe.

Examining urine samples from several subjects allowed us to determine if any interferences occurred due to natural variation in the biomatrix or due to other factors such as drug ingestion. Although some extraneous peaks occurred in urine samples of subjects not taking any medication, the degree of sample clean up that resulted was remarkable. Using

Table 4
Preconcentration factor and extraction efficiency (in italics) values for SME of human urine sample spiked to concentrations ranging from 1.0 to 0.2 μg ml^{-1} [a]

Concentration of solution (μg ml^{-1})	Cocaine	Cocaethylene	EME	AEME
1.00	15.7 *1.57%*	16.0 *1.60%*	7.5 *0.75%*	16.1 *1.61%*
0.8	15.3 *1.53%*	15.4 *1.54%*	7.6 *0.76%*	15.5 *1.55%*
0.5	15.8 *1.58%*	15.9 *1.59%*	8.0 *0.80%*	17.2 *1.72%*
0.2	16.5 *1.65%*	17.5 *1.75%*	8.6 *0.86%*	15.8 *1.58%*

[a] Values calculated from average peak areas and calibration lines from multiday calibration study.

solvent microextraction in conjunction with GC our screening method provides selectivity by two means. Initial sample clean up occurs due to the discriminating nature of a liquid–liquid extraction. Only those compounds that have affinity for the organic phase will partition into the organic drop, hence excluding many compounds present in the urine sample. The compounds of interest are further separated from interfering compounds on the GC column allowing separation and identification by retention time.

Extractions on the urine sample of female 1 produced many extraneous peaks possibly due to the medications that were being ingested (Fig. 2). Although many of the peaks are large, no interferences to this study were observed, since the peaks did not occur at the retention times of interest. Through this initial study we have excluded 12 over-the-counter and prescription drugs as interferents including antibiotics, antihistamines, decongestants, expectorants, anti-inflammatory, anti-seizure and oral contraceptive drugs. The retention times of the illicit drugs amphetamine, methamphetamine, phencyclidine, methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA) and methylenedioxyethylamphetamine (MDEA) differ from those of the compounds of interest and hence their presence in a sample would not hamper this screening method. Similarly other drugs of abuse should be tested to determine if they will be extracted from the urine at the given conditions and if so if they have retention times which would interfere with the compounds of interest. Although the results of this study are promising, the effect of possible interferents and adulterants must be tested more comprehensively and on a larger number of urine samples before this technique could be employed routinely as a screening method.

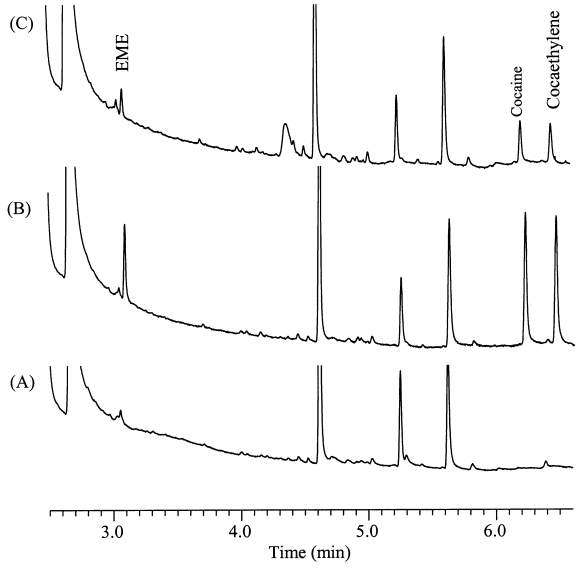

Fig. 2. Chromatogram of extraction from urine sample of female 1. (A) An extraction on the urine prior to spiking with drug mixture, (B) extraction on the urine spiked to a concentration of 1 μg ml^{-1} cocaine, cocaethylene, and EME and (C) spiked to a concentration of 0.250 μg ml^{-1}. Analyte volume used was 2 ml with addition of 100 μl phosphate buffer to yield a pH of 10.5. SME drop was 2 μl of chloroform. Extraction time was 6 min and stir rate was <240 rpm.

Fig. 2 also illustrates the ability of this technique to detect cocaine, cocaethylene and EME at concentrations below the cut-off level mandated for a screening test. The extraction shown in Fig. 2c shows distinct peaks for these compounds when present at a concentration of 0.250 μg ml^{-1}. Extractions of AEME from the spiked urine sample of female 2 gave similar results with AEME eluting at 2.76 min. These chromatograms show clearly the ability of this screening technique to detect at concentrations below 0.300 μg ml^{-1}.

To test the feasibility of this technique as a screening method it was important to ascertain whether differentiation between samples containing the compounds of interest and those that did not was possible. The results obtained from these studies (Table 3) show good correlation between measured and actual concentrations in the urine. If the concentration of drug constituting a positive screen was set at 0.300 μg ml^{-1}, no false positives and one false negative would occur.

5. Conclusion

In the present work, we have described a novel extraction method for the screening of cocaine and cocaine metabolites in urine using inexpensive equipment found in a typical analytical laboratory. SME was capable of extensive sample clean up and provided sufficient preconcentration of the drugs of interest required for a screening method. Although

this method does not meet guidelines required for federally regulated drug screening programs, it offers an alternate method of testing for those not subject to government guidelines. This initial screening method could easily be extended to encompass a large number of theraputic and illicit drugs. This coupled to the fact that SME is fast, simple, requires little solvent and produces little waste makes SME an attractive alternative to conventional screening methods.

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