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Preliminary studies of a fast screening method for cocaine and cocaine metabolites in urine using hollow fibre membrane solvent microextraction (HFMSME)

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A simple and efficient screening method for cocaine and cocaine metabolites has been developed using an inexpensive, disposable hollow fibre membrane. Drug extraction was achieved using hollow fibre membrane solvent microextraction (HFMSME). Extraction and separation, using a gas chromatograph, was achieved in less than 7.5 min. Using HFMSME, concentrations below 0.050 μg mL^{-1} were measurable. Good reproducibility was achieved when an internal standard was used, producing relative standard deviation values averaging 5.4%. The effects of various adulterants and interferents on the screening technique were studied and a direct comparison to drop solvent microextraction was made.

Introduction

In 1999, an estimated 6.7% of the nation's adult population were illicit drug users.[1] In an attempt to deter drug abuse, workplace drug testing programs are becoming widespread, both in the public and private sector. Because of the large number of samples requiring testing, a fast, inexpensive and reliable drug screen is desirable. This would reduce the number of samples requiring full evaluation, allowing a more cost-effective and timely means of testing a large number of samples. Currently, immunoassay is widely used as a preliminary screen for urinalysis, but these kits are often susceptible to interferences caused by the presence of adulterants in the urine.[2] Results also do not differentiate between metabolites and give only minimal quantification.

One of the most crucial steps in testing for drugs of abuse in biological samples is sample preparation. Because the concentrations of the drugs and metabolites are low, sample preparation of these complex samples involves analyte preconcentration as well as sample clean-up. Traditionally, drug testing methods employ liquid–liquid extraction or solid phase extraction for sample preparation prior to GC or HPLC analysis.[3] These methods provide sufficient analyte enrichment, but they require time consuming processes such as evaporation of solvent and reconstitution in order to provide sufficient preconcentration. These steps require large amounts of solvent, are labor intensive and have the potential to produce loss of analyte.

Although liquid–liquid and solid phase extractions have many advantages, it is desirable to develop an extraction technique in which the analytes are contained in a volume suitable for direct analysis. This has been partially addressed by the development of solid phase microextraction (SPME). Although this method offers many advantages, SPME is not widely used in drug testing due to the long equilibrium times required to achieve the required limits of detection. SPME also suffers from the disadvantage that the fibres are expensive and thus not disposable. Several studies have shown carryover of analytes on the SPME fibre between extractions.[4,5] This is particularly undesirable in a forensic setting and can only be eliminated by cleaning the fibre with a 5 min procedure, limiting sample throughput.

Solvent microextraction (SME) techniques, in which the acceptor solution volume is reduced to 1–30 μL, have recently been used as a way of simplifying sample preparation. Jeannot and Cantwell have described a method in which a single drop of organic solvent is suspended on the tip of a microsyringe into a stirred sample solution.[6] This technique has been applied to the extraction of pesticides in river water,[7,8] drugs from urine,[9,10] polyaromatic hydrocarbons from soil,[11] chlorobenzenes[12] and explosives from water samples.[13]

Although this research had several advantages over solid phase extraction, solid phase microextraction and traditional liquid–liquid extraction, drop SME has several drawbacks. As a result of drop instability at high stir rates, stir rate speeds are limited. Samples with particulate matter must also undergo some form of filtration in order to prevent the particles from colliding with the drop and dislodging it.[10,11]

Another approach to extraction on the microliter scale involves the use of a hollow fibre membrane filled with the acceptor solution. Rasmussen *et al*. described a microextraction system in which the acceptor solution is contained in a piece of polypropylene hollow fibre.[14] The fibre is filled from one end with 15–25 μL of the acceptor solution and placed into the sample where partitioning of the analytes occurs. The acceptor solution is then removed from the fibre on the other end and introduced into a GC, CE or HPLC for analysis. Because HFMSME fibres are inexpensive, the fibres are not reused, thus eliminating the potential for carryover. This type of HFMSME has been used for analysis of several drugs in biomatrices demonstrating good sample clean-up, high preconcentration factors and low detection limits.[14–16] These reports have all used protocols with relatively long extraction times (30–45 min) that limit sample throughput.

Cocaine undergoes reactions in the body to form a number of major metabolites such as benzoylecgonine and ecgonine methyl ester (EME),[17,18] which are then excreted in the urine, sweat, saliva and faeces. A pyrolysis product, anhydroecgonine methyl ester (AEME), formed when cocaine is smoked, is also excreted in the urine.[19,20] Another cocaine metabolite, cocaethylene, is excreted in the urine when cocaine is ingested with ethanol.[21] Federal workplace drug testing program regulations set screening cut-off levels for cocaine metabolites at 0.300 μg mL^{-1}.[22] Using this cut-off level as a guide, any potential screening method must be able to detect cocaine and cocaine metabolites at this concentration.

This paper examines whether a quick HFMSME method can be developed that, when combined with a short GC separation,

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would provide a fast screening technique for cocaine and cocaine metabolites in urine.

Experimental

Reagents

Cocaine, cocaethylene, EME, AEME, (1.0 mg mL^{-1} in acetonitrile), and phencyclidine (PCP) (1.0 mg mL^{-1} in methanol) were obtained from Radian International (Austin, TX, USA). Standard solutions of amphetamine, methamphetamine, methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), and methylenedioxyethylamphetamine (MDEA) were also obtained from Radian International. All solvents used were HPLC grade unless otherwise stated. Solvents used were methanol (Aldrich, Milwaukee, WI, USA) and chloroform (Fisher Chemicals, Fairlawn, NJ, USA). Synthetic urine tablets were obtained from Alltech Associates, Inc. (Deerfield, IL, USA) and were dissolved in ultrapure, distilled, deionised water (18.2 MΩ) 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. Ammonia and sodium chloride were purchased from Aldrich and sodium phosphate was purchased from Fischer for the adulterant studies. All glassware was deactivated using dimethyldichlorosilane (Supelco, Bellefonte, PA, USA), as described by the manufacturer, and extraction vials were bought pre-silanized (Alltech Associates Inc., State College, PA, USA). All gases were supplied by Air Products (Parkersburg, WV, USA).

Urine samples were obtained from volunteers taking several prescription and over-the-counter medications. These included loratadine, pseudoephedrine sulfate, norethindrone, ethinyl estradiol, carbamazepine, oxcarbazepine, naproxen sodium, and acetaminophen.

Instrumentation

A Varian 3400 GC 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. During the preliminary work, separation was carried out using an Rtx-5, 30 m × 0.25 mm × 0.25 μm 95% dimethyl–5% diphenyl polysiloxane copolymer column (Restek Corporation, 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 with a carrier gas flow of 2.4 mL min^{-1} and a split flow of 24 mL min^{-1}. The remainder of the research was carried out using an Rtx-5, 5 m × 0.1 mm × 0.1 μm column with a carrier gas flow of 1.4 mL min^{-1} and a split flow of 20 mL min^{-1}. Separation was achieved using an oven temperature program starting at 85 °C, ramping at a rate of 30 °C min^{-1} to a final temperature of 240 °C. A Valco pulsed-discharge helium ionisation detector (PDHID) (Valco Instruments, Houston, TX, USA) was used. The detector temperature was held at 290 °C and a helium flow of 30 mL min^{-1} was used to induce formation of the plasma. A Dell Dimension XPS R400 desktop computer (Dell Computer Corporation, Round Rock, TX, USA) with EZChrom software (version 6.7, Scientific Software, CA, USA) was used to collect and analyze the data.

Standard solutions of cocaine, cocaethylene, EME, AEME, and PCP were made in methanol. These solutions were analyzed using the parameters outlined above. From the resulting chromatograms, retention times were determined for each of the compounds of interest. pH adjustment of the sample prior to extraction was achieved using phosphate buffer. Previous studies have shown that optimal extraction occurs at pH 10.6.[10] Each extraction analysis was performed on an 8 mL sample in an 8 mL sample vial. Quantification throughout the experimental work was accomplished using peak areas measured by EZChrom. Any reported percent relative standard deviation (%RSD) values were calculated from three replicate measurements unless otherwise stated.

Extraction procedure

Two types of fibres were used during this study, Q 3/2 Accurel KM polypropylene hollow fibre tubing (Akzo Nobel, Wupertal, Germany) with inner diameter of 600 μm and Cellmax® Implant Membrane modified polyvinyldene difluoride tubing (Spectrum Labs, Rancho Dominguez, CA, USA) with inner diameter of 1000 μm. The fibre was cut into 6 cm pieces and sealed at one end by singeing with a flame. The fibre was then soaked in the extraction solvent for at least 10 min to ensure that the pores were filled with the extraction solvent. It was found that, without pre-treatment, the chloroform was being adsorbed into the pores of the fibre making solvent withdrawal impossible. Using this pre-treatment method it was possible to withdraw 4 μL of the chloroform from the Accurel fibres and 8 μL from the Cellmax® fibres after a 3 min extraction. In order to position the fibre in such a way that the open end was above the sample surface, it was threaded through a polypropylene septum with a slightly larger diameter than that of the sample vial. This allowed the fibre to be reproducibly positioned in such a way that the sealed end was approximately 1 cm from the base of the vial and the open end protruded approximately 1 cm above the surface of the sample liquid. A schematic diagram of the extraction set up can be seen in Fig. 1.

A Hamilton 701 SN 10 μL syringe was used to fill the fibre with 10 μL of extraction solvent. The solution was then stirred at 1600 rpm for 3 min. Following the extraction time, 4 μL of the extraction solvent was recovered from the fibre using a Hamilton 701 SN syringe fitted with a Chaney adaptor. This extract was then injected into the GC for analysis.

Results and discussion

Method development

In choosing the metabolites to be screened in this analysis, concentration of the analyte and chromatographic properties were considered. The presence of benzoylecgonine in a urine sample is currently used as an indication of cocaine use because this metabolite has a relatively long half-life (4.5 h) and accounts for 35–54% of the dose excreted.[23] Although these factors make benzoylecgonine appealing as an analyte, chromatography of this compound is difficult and derivatisation is often

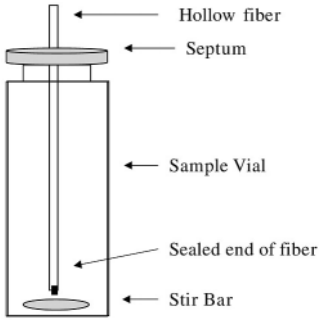

Fig. 1 Schematic diagram of the HFMSME apparatus.

required prior to GC separation.[18] Since little or no sample preparation is favored in a fast screening technique, other metabolites of cocaine were investigated. EME is accountable for 32–49% of an administered dose and has a half-life of 3.1 h. In addition, EME is advantageous as no derivatisation is required prior to chromatographic analysis. Although the half-life of EME is lower than that of benzoylecgonine, the difference is acceptable in exchange for a reduced sample preparation time. The presence of other metabolites in a urine sample can give additional information about when and how the cocaine was administered. The presence of cocaine in a urine sample would indicate recent use as it has a very short half-life (0.8 h).[23] AEME present in a urine sample would indicate the route of administration while the presence of cocaethylene is evidence of ethanol ingestion.

Two different types of fibres were tested in the initial development of this HFMSME method. The extraction solvent, chloroform, and initial GC conditions were optimized in previous studies.[10] Extractions were performed on several solutions of varying concentrations of cocaine, cocaethylene, EME, and AEME. It was found that when using the Accurel fibres with a 3 min extraction time cocaine, cocaethylene, and AEME were detectable at concentrations down to 50 ng mL^{-1} and EME was detectable to 75 ng mL^{-1}, well below the target screening cut-offs. Longer extraction times caused reduction in the amount of extraction solvent that could be reproducibly recovered from the Accurel fibres. As the aim of this study was the development of a fast screening method, 3 min was selected as our extraction time. In order to take full advantage of the fast extraction times, the GC conditions were altered to yield a shorter run time. This was done by using a 5 m column with a smaller inner diameter and thinner film thickness. Using this column, in conjunction with altered oven temperature programming, shortened the GC run time to 4 min.

Standard solutions with concentrations of each drug ranging from 10 to 0.5 μg mL^{-1} were made in methanol. Injections of 4 μL were made using the optimized oven temperature program previously outlined. Peak area calibration curves were obtained with good linearity (R^2 = 0.994–0.997). Initial investigations with both types of fibres found that replicate extractions yielded poor peak area reproducibility (%RSD 17–21%). The use of PCP as an internal standard was investigated. This was accomplished by addition of 80 μL of a 100 μg mL^{-1} solution to 25 mL samples resulting in a final PCP concentration of 0.320 μg mL^{-1}. It was found that measuring the peak area ratio between the analyte peak and the PCP peak significantly decreased variability (%RSD 1–13%). As a result of this, peak area ratio measurements were used for all quantification.

In order to determine which of the two fibres gave optimal extraction, the extractions were performed on synthetic urine solutions containing 0.40 μg mL^{-1} of cocaine, cocaethylene, EME, and AEME as well as internal standard. Triplicate extractions were carried out at this concentration for each fibre type, with a fresh piece of fibre and a fresh aliquot of sample being used for each analysis. Peak areas and peak area ratio measurements were compared and the results can be seen in Fig. 2. The Accurel fibres yielded significantly larger peak area measurements with better reproducibility compared to the Cellmax® fibres. The peak area ratios for the two types of fibres were similar, with the Cellmax® fibres having lower reproducibility compared to the Accurel fibres, as illustrated by the error bars in Fig. 2. Because the Accurel fibres yielded peak areas that were 2.3 to 7 times larger than those obtained with the Cellmax® fibres, these were chosen as optimal and were used in the remainder of the experiments. The cause of the differences in extraction between the fibres is not clear. A number of parameters such as pore size, wall thickness, and fibre composition could affect extraction efficiency. The Accurel fibres have a much larger pore size of 0.64 μm compared to the Cellmax® fibres (0.035 μm), which, in itself, may account for the increased extraction. This phenomenon should be studied more closely in the future by comparing extractions done with fibres of varying pore size, wall thickness, and composition to see which of these parameters affect the extraction.

The next parameter optimized was the stir rate. Previous studies done with drop SME were done at low stir rates in order to ensure that the drop was not dislodged from the tip of the syringe.[7,8,10] As drop dislodgement is no longer a concern, stir rates can be increased as a means of reducing the Nernst diffusion layer and increasing extraction rate. A urine solution containing the drugs of interest at a concentration of 0.4 μg mL^{-1} was made and triplicate measurements were performed at 4 stir rates: 2000, 1600, 600 and 0 rpm. The results of this study are shown in Fig. 3.

Peak areas generally increased with increasing stir rate except for AEME and EME where the peak area decreased at 2000 rpm compared to 1600 rpm. The peak area ratios did not show a distinct trend. Although 2000 rpm did give increased extraction for cocaine and cocaethylene, %RSD for the area ratios also increased (5.0–12.2%) compared to those obtained at 1600 rpm (1.6–8.7%). This, combined with the increased AEME and EME areas, led to choosing 1600 rpm as the optimal stir rate.

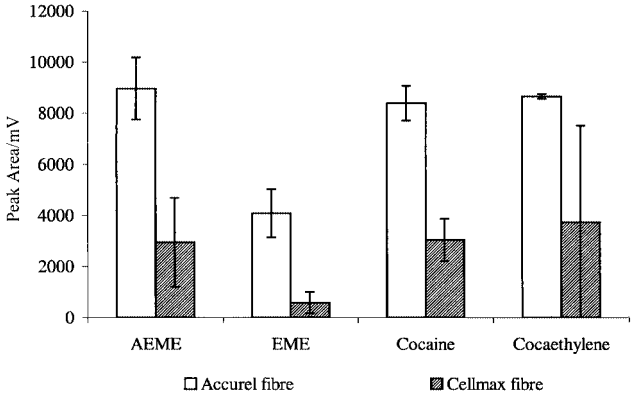

Fig. 2 Comparison of HFMSME extractions made with Accurel fibres and Spectrum fibres. Extractions were made from urine samples containing 0.4 μg mL^{-1} of AEME, EME, cocaine, cocaethylene, and 0.32 μg mL^{-1} PCP. Error bars represent 95% confidence intervals using Student's *t* test.

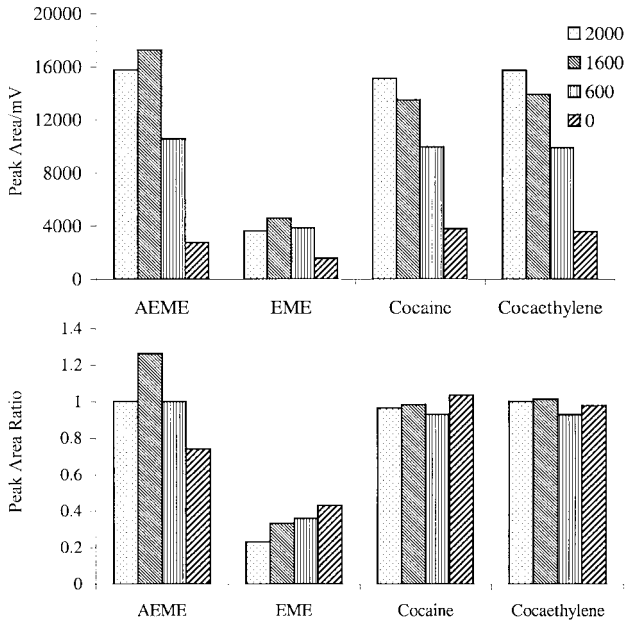

Fig. 3 Comparison of HFMSME extractions made using Accurel fibres at various stir rates. Extractions were made from urine samples containing 0.4 μg mL^{-1} of AEME, EME, cocaine, cocaethylene and 0.32 μg mL^{-1} PCP.

Calibration

Using these optimized conditions, extraction calibration lines were constructed. Urine solutions containing concentrations of 1.0, 0.8, 0.4, 0.2, and 0.1 μg mL^{-1} of cocaine, cocaethylene, AEME, EME and internal standard were made in 25 mL volumetric flasks. Extractions were performed and calibration lines were constructed with 3 replicate extractions performed at each concentration. In order to test the interday reproducibility of the extractions, this calibration procedure was repeated for 3 days. The results of these calibration studies can be seen in Table 1. Fig. 4 illustrates the ability of this technique to detect AEME, EME, cocaine, and cocaethylene at concentrations below the cut-off level mandated for a screening test. The chromatogram in Fig. 4(a) shows distinct peaks for all four compounds present at a concentration of 0.2 μg mL^{-1}. Detection limits (defined as a peak giving a response equal to a blank signal plus three times the noise) for this HFMSME technique were estimated using extraction calibration lines based on height: 27 ng mL^{-1} for AEME, 48 ng mL^{-1} for EME, 26 ng mL^{-1} for cocaine, and 11 ng mL^{-1} for cocaethylene. These detection limits are well below the screening cut-off levels established for this analysis.

The amount extracted was calculated for the urine extractions, using peak area measurements and calibration curves of standards. The preconcentration factor is defined as the ratio between the final concentration of the analyte in the acceptor solution and the concentration of the analyte in the original solution. Preconcentration factors were calculated for the extraction of the four compounds of interest, from the pooled extraction data obtained during the calibration procedure. The results range from 10.5 to 29.1 (Table 2).

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

Interferents and adulterants

Interferents. While performing the calibration studies, solutions were produced using urine samples obtained from donors taking various prescription and non-prescription drugs. These included anti-inflammatory, oral contraceptive, antihistamine, decongestant, and anti-convulsant medications. Although extractions performed using these samples afforded many extraneous peaks, as can be seen in Fig. 4, analysis of the drugs of interest was not impeded as they had different GC retention times.

In addition to these licit drugs, several illicit drugs were added to urine samples to determine if their presence would affect this analysis. Using this temperature program, the retention times of amphetamine, methamphetamine, methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), and methylenedioxyethylamphetamine (MDEA) differ from those of the compounds of interest and hence their presence in a sample would not interfere with this screen. Preliminary studies indicate that these drugs are also extracted into the acceptor solution and can be analyzed concurrently with the cocaine metabolites, generating a broader drug screen.

Adulterants. A number of common products have been added to urine samples in the hopes of producing a false negative screen. A number of these compounds have been reported in the literature to interfere with the detection of cocaine metabolites when screening urine samples with immunoassay.[3,24] The addition of 5% ammonia, 10% ammonia, 50 mg mL^{-1} sodium chloride, 75 mg mL^{-1} sodium chloride, 42 μL mL^{-1} bleach, 42 μL mL^{-1} Drano, 42 μL mL^{-1} detergent, and 5% phosphate to the urine samples spiked to a concentration of 0.4 μg mL^{-1} with AEME, EME, cocaine, cocaethylene and internal standard were tested. The results for cocaine are shown in Table 4. The addition of several of the adulterants caused significant changes to the pH and in these cases the amount of phosphate buffer added was modified. When detergent was added to the urine sample, a very dirty chromatogram was obtained and a large signal was obtained between 2 and 3 minutes, masking the PCP peak and making quantification impossible. The presence of this adulterant was evident as foaming was observed with stirring, distinguishing it from a non-adulterated sample.

Comparison of HFMSME to SME

Extractions from synthetic urine solutions with 1 μg mL^{-1} AEME, EME, cocaine, cocaethylene, and internal standard were performed using SME and HFMSME. In order to compare the two extraction methods directly, the HFMSME method had to be modified. Lower stir rates were used as rapid stirring causes drop dislodgment in SME and only 2 μL of $CHCl_3$ was withdrawn from the fibre for injection in HFMSME. When performing SME on human urine, samples must be filtered before extraction to remove particulate matter. This prevents particulate matter from colliding with the drop and causing it to dislodge from the tip of the syringe. In order to eliminate the need for filtration, synthetic urine was used for SME/HFMSME comparison. Although no large differences occurred in area ratios, large differences in peak areas were obtained (Fig. 5). Reproducibility was considerably better using HFMSME with %RSD values for the area ratios averaging 6.7% compared to 10.0% obtained with SME. In order to

Table 1 Results from multiday extraction calibration curves

Area ratio calibration data	AEME	EME	Cocaine	Cocaethylene
Day 1 R^2	0.9956	0.9987	0.9912	0.9916
Day 2 R^2	0.9977	0.9947	0.9974	0.9994
Day 3 R^2	0.9953	0.9947	0.995	0.9952
Pooled Data R^2	0.9916	0.9978	0.9924	0.9936
Day 1 Line Equation	$3.07x + 0.048$	$1.13x + 0.086$	$2.66x + 0.240$	$2.67x + 0.187$
Day 2 Line Equation	$3.20x - 0.047$	$1.24x + 0.011$	$2.83x + 0.147$	$2.88x + 0.085$
Day 3 Line Equation	$3.02x + 0.064$	$1.34x + 0.043$	$2.64x + 0.262$	$2.63x + 0.217$
Pooled Data Line Equation	$3.15x + 0.023$	$1.26x + 0.049$	$2.76x + 0.212$	$2.78x + 0.157$
Day 1 %RSD	5.1	5.5	4.1	6.5
Day 2 %RSD	6.8	8.1	4.9	5.9
Day 3 %RSD	6.0	5.6	3.3	3.8
Pooled Data %RSD	7.0	9.5	6.1	7.7

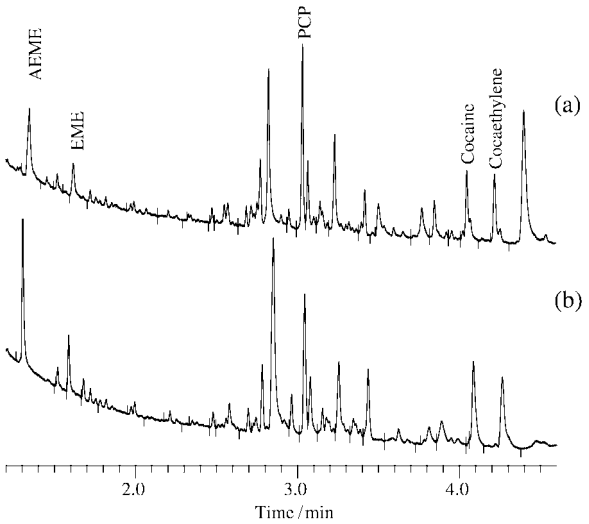

Fig. 4 Chromatogram of extractions from urine solutions containing (a) 0.20 μg mL^{-1} and (b) 0.4 μg mL^{-1} of AEME, EME, cocaine, cocaethylene, and 0.32 μg mL^{-1} of PCP as internal standard.

Table 2 Amount of drug in 4 μL extract (μg mL^{-1}) and preconcentration factors (in italics) for HFMSME of human urine samples spiked to concentrations ranging from 1.00 to 0.10 μg mL^{-1}

Concentration of drug in solution/ μg mL^{-1}	AEME	EME	Cocaine	Cocaethylene
1.00	0.091	0.044	0.083	0.088
	22.8	*11.0*	*20.8*	*21.0*
0.80	0.083	0.037	0.074	0.074
	25.8	*11.7*	*23.3*	*23.1*
0.40	0.032	0.017	0.033	0.032
	20.2	*10.5*	*20.5*	*20.0*
0.20	0.017	0.011	0.020	0.018
	21.2	*14.0*	*25.2*	*22.7*
0.10	0.009	0.006	0.012	0.011
	22.3	*15.7*	*29.1*	*26.8*

Table 3 Results of blind study on spiked urine samples

Sample		AEME	EME	Cocaine	Cocaethylene
A	Experimental	0.12	0.00	0.00	0.16
	Actual	0.12	0.00	0.00	0.10
B	Experimental	0.00	0.00	0.00	0.00
	Actual	0.00	0.0	0.00	0.00
C	Experimental	0.64	0.80	0.72	0.56
	Actual	0.73	0.80	0.61	0.43
D	Experimental	0.20	0.13	0.16	0.00
	Actual	0.25	0.05	0.15	0.00
E	Experimental	0.00	0.40	0.36	0.34
	Actual	0.00	0.45	0.31	0.31

determine the extraction efficiency, peak area calibration curves of 2 μL injections of standard solutions containing 1–20 μg mL^{-1} of AEME, EME, cocaine, and cocaethylene were made in methanol. Using this information, concentration of the analyte in the extract and the preconcentration factors were calculated (Table 5). Preconcentration factors using both extraction methods are small, but the fibre yielded extraction enhancement by a factor of 1.9 to 2.4 compared to the drop. Increasing the stir rate during the HFMSME 2μL extraction causes a reduction in the Nernst diffusion layer and a further increase in enhancement factor (2.7–4.3). These values correlate well with the preconcentration values calculated for the 4 μL HFMSME extraction. These preconcentration values suggest that extraction is dependent on both surface area and stir rate, which is expected.

In addition to increased peak area, preconcentration factor, and reproducibility, HFMSME has the added advantage that no filtration of the urine sample is required prior to extraction. When a urine sample is adjusted to pH 10.6, a fine precipitate forms. When an SME is performed, this precipitate may collide with the extraction drop, causing it to fall off the tip of the needle. Since the HFMSME extraction solvent is contained in a hollow fibre, the possibility of complete loss of acceptor solution is eliminated and no filtration is required.

To estimate the surface area of acceptor solution exposed to the urine solution, one must consider the porosity of the fibre as well as the wall thickness and inner diameter. Since the inner diameter of the fibre is 0.6 mm, for every centimeter of fibre the internal volume is 2.8 μL. The thickness of the fibre walls is 0.1 mm giving a total fibre volume of 7.8 μL cm^{-1}. This allows us to calculate the volume of the walls as being 5.0 μL cm^{-1}. Accurel reports the walls of the fibre to be 70% porous, making the volume of the pores 3.5 μL cm^{-1}. Hence, every centimeter of fibre saturated with acceptor solution contains a maximum of 6.3 μL of solvent. Thus, for every 10 μL of acceptor solution we load into the fibre we fill at least 1.59 cm. Using this value we can calculate the minimum exposed acceptor solution surface area (SA) where d is the outer diameter of the fibre and h is 1.59 cm [eqn. (1)].

$$SA = 0.7\,\pi\,d\,h \quad (1)$$

Table 4 Results of adulterant studies for the extraction of cocaine from a urine sample spiked to a concentration of 0.4 μg mL^{-1}. Extractions of AEME, EME and cocaethylene showed similar trends in area ratio and %RSD

Adulterant	Visual effect	pH	Cocaine Area Ratio	%RSD
None	Normal	5.5	0.92	3.8
5% Ammonia	Normal	10.5	0.89	2.8
10% Ammonia	Normal	11.5	1.00	5.8
50 mg mL^{-1} NaCl	Normal	5.5	0.92	4.0
75 mg mL^{-1} NaCl	Normal	5.5	1.18	8.4
42 μL mL^{-1} bleach	Yellow–brown color	5.5	2.23	19.0
42 μL mL^{-1} Drano®	Blue color	12	1.46	11.2
5% Phosphate	Normal	5.5	0.89	16.7
Detergent	Frothy	5.5	—	—

Table 5 Comparison study of SME and HFMSME

		AEME	EME	Cocaine	Cocaethylene
Area	SME	5576	3788	6709	6962
	HFMSME	12881	6880	12228	12703
	HFMSME Fast	23086	8996	21153	21930
Preconcentration factor	SME	5.9	3.8	5.4	5.1
	HFMSME	14.0	7.7	10.6	9.9
	HFMSME Fast	25.4	10.4	18.9	17.5
Enhancement due to fibre	HFMSME	2.39	2.05	1.94	1.93
	HFMSME Fast	4.32	2.77	3.47	3.41

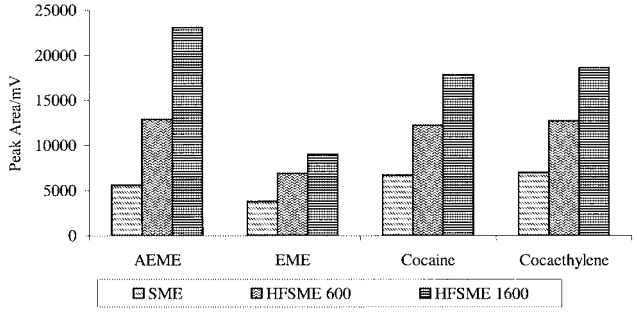

Fig. 5 Comparison of extractions using drop SME at 600 rpm, HFMSME at 600 rpm and HFMSME at 1600 rpm.

This calculation gives an exposed surface area of 34.9 mm^2, 4.5 times larger than the 7.7 mm^2 calculated for the 2 μL SME drop.[8] If preconcentration was dependent on surface area alone, then the extraction enhancement due to the fibre would be expected to be 4.5, but this is not the case. This suggests that more complex diffusion processes are occurring in the fibre walls. Another factor in this discrepancy are the physical properties of chloroform, which may allow evaporation and dissolution into the bulk solution. When this occurs, drug that has partitioned into the chloroform will be removed from the surface of the fibre back into the sample solution making the final concentration of drug in the extract lower than optimal. This could be prevented if a solvent with higher boiling point and lower solubility such as octane was used. It should be pointed out that these calculations do not account for fibre swelling due to plasticisation or solvent and/or analyte absorbing into the polymer surface, which may also account for the difference between actual and calculated enhancement. These factors should be more closely examined in future HFMSME studies.

The rate of the extraction seems to be limited to a degree by the diffusion of the analyte through the acceptor solution in the walls of the fibre. This is because the acceptor solution within the inner volume of the fibre is withdrawn rather than the solution contained in the wall pores. If a fibre with a thinner wall was used, a thinner diffusion layer would occur and a greater extraction would be expected.

Conclusion

This paper describes a novel extraction procedure for the screening of cocaine and cocaine metabolites in urine using an inexpensive hollow fibre membrane. HFMSME was capable of extensive sample clean-up and provided sufficient preconcentration of the drugs of interest to detect them well below the screening cut-off level. HFMSME provides superior preconcentration compared to SME, and the disposable nature of the fibre eliminates the possibility of carryover seen in SPME. This preliminary study could easily be extended to encompass a large number of therapeutic and illicit drugs. These characteristics make HFMSME a very attractive alternative sample preparation technique for screening methods.

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