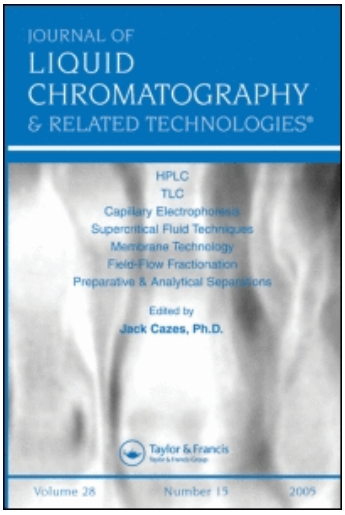

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A Comparative Study of Different Solid Phase Extraction Procedures for the Analysis of Alkaloids of Forensic Interest in Biological Fluids by RP-HPLC/Diode Array

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A COMPARATIVE STUDY OF DIFFERENT SOLID PHASE EXTRACTION PROCEDURES FOR THE ANALYSIS OF ALKALOIDS OF FORENSIC INTEREST IN BIOLOGICAL FLUIDS BY RP-HPLC/DIODE ARRAY

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ABSTRACT

Different Solid Phase Extraction (SPE) procedures are studied for the pretreatment of urine, blood plasma and serum samples, for the analysis of alkaloids of general clinical-toxicological interest. Various SPE cartridges, extraction conditions and solvents were used in the search of the best recovery and clean-up. In the whole study the internal standard was used as a chromatographic standard e.g. it was added in the sample after the end of the extraction. Analysis of the extracts was performed by RP-HPLC with photodiode array detection. Quantitation was performed in four selected wavelengths and comparative results are provided. The extraction reproducibility was also studied employing cartridge-to-cartridge variations. The method was also applied in the analysis of urine samples of heroin and cocaine users.

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INTRODUCTION

Solid phase extraction is established over the last years as a very effective method for sample pretreatment and clean-up. The majority of the recent works related with toxicological analysis report the use of SPE, using a variety of microcolumns and extraction conditions. SPE offers various advantages compared to liquid-liquid extraction such as higher efficiency (therefore higher selectivity and recovery), usage of smaller quantities and solvent volumes, ease and convenience in handling, absence of emulsions, smaller time consumption, and automation options, thus it is the method of choice especially in the clean-up of complex mixtures like biological fluids, feedstuff, foods etc [1].

SPE is widely used in the pretreatment of forensic and clinical samples prior to a chromatographic determination of alkaloids. Both free and conjugated morphine have been extracted from blood on Extrelut silica columns and analysed by GC [2, 3]. Morphine, 6-monoacetylmorphine and codeine were extracted from urine on Clean-Screen Dau columns (silica based cation exchange copolymer) prior to the GC analysis [4]. This extraction column gave better recovery and purification than a C_{18} column or a liquid-liquid extraction procedure for the extraction of 6-monoacetylmorphine from urine [5]. Morphinone and other urinary metabolites of morphine have been extracted from guinea pig urine and bile on C_{18} columns [6]. Morphine and codeine have been extracted from whole blood on a C_{18} extraction column and analysed by HPLC/UV [7]. The analysis of morphine, normorphine, codeine, norcodeine and their glucuronides has been reported by HPLC electrochemical detection following SPE on C_8 cartridge [8]. Morphine and its glucuronides have been extracted from human plasma [9, 10] and neonatal guinea pig plasma [11] with the use of C_{18} cartridges or from blood plasma with the use of Clean Screen bonded silica columns [12]. Morphine and hydromorphine have been analysed in plasma by HPLC combined with coulometric detection after SPE on C_{18} columns

[13]. Similar methods employing silica based C_{18} extraction columns, have been applied for the extraction of codeine and its metabolites from blood plasma [14, 15, 16] and urine [15, 16]. SPE of cocaine and its metabolites has been reported on some types of columns. Extrelut [17], Amberlite XAD-2 [18], and Chem-Elut [19] columns have been used for the pretreatment of urine [17, 18, 19] or whole blood and plasma [17]. Extraction of such basic drugs takes place in alkaline environment (pH 9.0-10.0), so that the alkaloids obtain their non-protonated form and are strongly retained on the reversed phase microcolumn by non-polar interactions. Graphitised carbon columns have also been used for the SPE of basic drugs [20].

A comparison of different extraction (liquid-liquid or SPE) techniques for the analysis of drugs in biological fluids was the purpose of some studies. Octadecylsilane materials proved to give the best overall results for the extraction of ten representative compounds of acidic, basic, amphoteric hydrophobic and hydrophilic classes, but depending on the class of the compound other materials gave very satisfactory results. Basic drugs were best extracted on polystyrene divinyl benzene or cyanopropyl columns; amphoteric compounds could also be extracted on polymers [21]. Morphine was best extracted from urine by a combination of liquid-liquid and SPE on C_{18} columns [22]. Variation in the type of sorbent, results in variations in solute-sorbent interactions and secondary to matrix-sorbent interactions which finally result in variations in the recovery and the clearness of the samples [23]. The same authors reported manufacturer-to-manufacturer and batch-to-batch variations of the examined C_{18} cartridges. Differences also occurred after SPE from aqueous solutions and serum, indicating inclusion of endogens in the extraction process. Batch-to-batch variations can not be avoided, and are attributed to the variation on content and availability of polar groups on silica [24, 25]. The reproducibility of a SPE extraction procedure is critical, since there are numerous factors affecting the extraction mechanism namely: type of extracted compound, type of sample matrix and extraction sorbent, preconditioning procedure, composition, volume and pH value of the eluting

solvent, mass of the sorbent [1]. The presence of all these factors result to a much more versatile and finally more selective system comparing to a liquid-liquid system, but they also result to low reproducibility. The latter is attributed mainly to the sorbent condition which varies not only between the different types but also between different manufacturers, or even lots of the same manufacturer.

In an earlier report, we compared different SPE cartridges for the RP-HPLC of morphine and codeine in biological fluids [26]. This subject is now expanded in the study of the whole extraction procedure for the analysis of a variety of drugs by HPLC diode array detection. The scope of the work was the study of SPE of selected basic drugs for systematic toxicological analysis. The selected compounds of general clinical-forensic interest were analysed and extracted in a variety of SPE cartridges with different procedures and analysed by HPLC-diode array as previously described [27]. The study included the optimisation of the SPE in order to achieve the best recovery and removal of the interfering compounds.

EXPERIMENTAL

Apparatus

The experiments were carried out in a Shimadzu quaternary low pressure gradient system. The solvent lines were mixed in a FCV-9AL mixer and an LC-9A pump was used to deliver the mobile phase to the column which was thermostated in a CTO-6A oven. Introduction of the samples on the column was achieved by a SIL-9A auto sampler and detection was performed on a SPDM 6A photodiode array detector. Chromatograms were stored on the hard disk of a Laptop 286 PC and printed on a Seikosha SP-1900 printer. Data was analysed both on the Laptop 286 PC and on a Vip 386 PC. Degassing of the solvents was achieved by ultrasonication

under vacuum and continuous helium sparging in the solvent flasks through a DGU-2A degassing unit. All the mentioned apparatus were from Shimadzu (Kyoto, Japan). Separation of the alkaloids was made on an Adsorbospher HS C_{18} 5 μm 250 x 4.6 mm I.D. columns obtained from Alltech Associates (Deerfield, IL).

Materials

Morphine, codeine, 6-monoacetylmorphine, diamorphine, nalorphine, cocaine and benzoylecgonine were obtained from Alltech as methanolic solutions at concentrations of 1000 ppm. Papaverine was obtained from the Forensic Medicine and Toxicology Laboratory of the Aristotle University of Thessaloniki. Flufenamic acid was from ELPEN (Athens, Greece). All the stock solutions were prepared by dissolving the appropriate amount in HPLC grade methanol and kept refrigerated.

Ammonium acetate solutions were prepared by dissolving the appropriate quantity of the analytical grade compound, which was obtained from Merck (Darmstadt, Germany), in double-deionised water. Glacial acetic acid was purchased from Merck. The aqueous buffers after their preparation and pH adjustment, were filtered in a glass vacuum solvent filtration apparatus through a 0.2 μm Anodisk 47 mm glass filter obtained from Alltech. All the organic solvents used in this study, were of HPLC grade and obtained from Merck.

The borate buffer pH 9.2 was prepared by mixing 250 ml of 0.025 M sodium borate ($Na_2B_4O_7 \cdot 10H_2O$) and 18 ml of 0.1M sodium hydroxide. Both compounds were analytical reagent grade and obtained from Merck.

The SPE cartridges used were Bond Elut C_{18}, Bond Elut C_8 (Analytichem *International*, Varian, Harbor City, CA), Alltech C_{18}, Alltech C_8, Alltech Toxiclean (Alltech Associates, Deerfield, IL), Altech C_{18} Rigas (Rigas Labs, Thessaloniki Greece), Bakerbond C_{18} (J.T. Baker, Gross Gerau, Germany), Separcol C_{18} (Anapron spol Sr.O, Bratislava, Slovakia).

Chromatographic conditions

The chromatographic conditions used were reported previously [27]. The mobile phase was a mixture of MeOH-ACN-1.2% CH_3COONH_4, 40:15:45 (v:v:v) as the mobile phase, at a flow rate of 0.8 ml/min. Quantitation was performed in four selected wavelengths 225, 239, 254 and 289 nm. Especially for the quantitation of cocaine and benzoylecgonine an additional wavelength (232 nm) was chosen due to the low absorbance signal at 289 nm.

Extraction of standard solutions

The first step of the extraction optimisation was the extraction of standard methanolic solutions of the analysed compounds. The microcolumns were preconditioned by elution with 3 ml of methanol, 2 x 3 ml of double deionised water and 2 ml of borate buffer (pH 9.2). Then 100 µl of the standard solutions were applied on column. The columns were washed with 2 x 3 ml of water, dried with application of vacuum (15-20 psi) for 10 min and the alkaloids were eluted from the sorbent with 2 ml of a suitable organic solvent. The resulting solutions were evaporated to dryness in a water bath at 45 ^{o}C, under a gentle stream of nitrogen. The residues were reconstituted with 100 µl of a solution of the internal standard. Aliquots of 20 ml of the resulting solutions were analysed on the HPLC.

All the fractions collected after buffer and sample application or washing during the SPE optimisation in all the samples, were tested for the presence of the analysed alkaloids in order to confirm that no loss occurred in these steps.

The use of the internal standard as a chromatographic standard was chosen in order to have the standard in constant concentration. In most of the reported studies the standard is added before the extraction. In such a case the quantitative results are

also affected by the standards extraction recovery and may be misleading. For example if the absolute recovery of both the standard and the analyte is about 50%, the final results will show a recovery of about 100%. The recovery calculated when the addition of the standard is made just prior to the HPLC analysis, is the absolute recovery of the extraction.

Extraction of blood plasma-serum samples.

The extraction cartridges that proved to be most suitable for the SPE of the alkaloids were tested for the pretreatment of blood plasma and serum samples. Samples of 100 µl of plasma or serum were combined in an Eppendorf tube with 100 µl of a standard solution of opium alkaloids. 200 µl of acetonitrile and 100 µl of the borate buffer were added in the mixtures and the tubes were shaken in the vortex for 1 min and centrifuged for 15 min at 4000 rpm. The supernatants were applied on the SPE columns which had previously been conditioned as already reported. The extraction procedure reported for the extraction of standard solutions, was also followed and the final solutions were evaporated to dryness as previously described. The residues were reconstituted with 100 µl of a 2.5 ng/µl solution of flufenamic acid.

The same procedure was applied for the extraction of cocaine and benzoylecgonine from plasma samples. The final solutions obtained from the SPE were evaporated to dryness and the residues were reconstituted with 100 µl of a 5 ng/µl solution of nalorphine (internal standard)

Extraction of urine samples.

Urine samples could be analysed without a protein precipitation step, however for general toxicological analysis it is advisable to apply such a step together with a

dilution of an unknown sample for better protection of the apparatus. Samples of 100 μl of urine were mixed in Eppendorf tubes with 200 μl of acetonitrile, 500 μl of borate buffer and 100 μl of opiate (or cocaine) standard solution. The mixtures were vortexed for 1 min and centrifuged at 2000 rpm for 5 min. The liquid of each tube was applied to the SPE cartridge which had been previously conditioned as already reported. The extraction procedure reported for the extraction of standard solutions, was also followed and the final solutions were evaporated to dryness as previously described. The residues were reconstituted with 100 μl of the internal standard solution. Especially for the Alltech Toxiclean column an additional preconditioning procedure (the one that is suggested by the manufacturer) was also followed. The column was eluted with 2 x 1 ml MeOH, 2 x 1 ml H_2O and 2 x 1 ml aq. 1 mole/lt CH_3COOH. The urine sample was applied on column and the column was eluted with 2 x 1 ml H_2O and let dry with application of vacuum. The compounds were eluted from the column with 2 x 1 ml of MeOH (procedure B) or with 2 x 1 ml MeOH containing 2% NH_4OH (procedure C).

Quantitative study

Quantitative studies were again divided in two sections: study of the major opium alkaloids and study of cocaine and its metabolites. Calibration curves for the determination of opium alkaloids in plasma samples were constructed using the standard addition method. The samples were spiked at eight different concentrations (0.1-15 ng/μl for morphine, 6-MAM, codeine and 0.02-3 ng/μl for papaverine) and after the selected pretreatment they were repeatedly (five times) analysed on the HPLC. In the urine samples the added concentrations were 0.3-15 ng/μl for morphine, 6-MAM, codeine and diamorphine and 0.06-5 ng/μl for papaverine. The concentration of flufenamic acid (internal standard) was fixed at 2.5 ng/μl.

Cocaine and benzoylecgonine were added on plasma in eight different concentrations in the range 0.1-15 ng/μl and on urine in eight different concentrations in the range 0.083-12.499 ng/μl. Nalorphine (internal standard) was present in all samples at 5 ng/μl.

In both analyses the internal standard was used as chromatographic standard e.g. it was added on the sample just prior to the analysis, in order to have a constant concentration. The final reconstitution of the residues of the extraction was always made with the internal standard solution.

RESULTS AND DISCUSSION

Extraction optimisation

The solvents tested for the elution of the alkaloids from the extraction columns were methanol and a (1:1) mixture of dichloromethane-acetone. The recoveries obtained after the extraction in a variety of columns are given in Table I. Other elution solvents tested, were ethanol and acetonitrile which resulted to lower recoveries. Preconditioning of the columns with a higher pH buffer (pH 10) resulted in losses (especially for cocaine) which were attributed to the alkaline hydrolysis of the analysed compounds. As it is clearly seen in Table I methanol resulted to the best overall recovery for the alkaloids. Elution with the mixture of dichloromethane-acetone gave very good results for the non-polar alkaloids like cocaine, papaverine and diamorphine, but poor recovery for the polar compounds like morphine, codeine and benzoylecgonine.

The recoveries obtained for the extraction from spiked plasma samples are presented in Table II. Diamorphine was rapidly hydrolysed in plasma under the tested conditions, therefore no quantitative analysis could be performed. As it is seen in

TABLE 1.

Absolute Recoveries of Various Cartridges for the SPE of Standard Solutions with Concentrations 5 ng/μl for the Opium Alkaloids and 4 ng/μl of Cocaine Alkaloids. Eluting Solvent is Methanol (A), and a (1:1) Mixture of Dichloromethane-Acetone (B). Details in the Experimental Section.

SPE microcolumn	Alkaloid recovery (%)													
	Morphine		6 MAM		Codeine		Diacetylmor-phine		Papaverine		Benzoylec-gonine		Cocaine	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Bond Elut C_{18}	50.2	33.9	72.5	39.7	55.6	26.3	--	--	79.2	68.17	39.93	31.23	30.26	74.21
Bond Elut C_8	45.2	49.5	51.1	55.7	43.9	59.9	--	--	55.3	109.2	80.22	26.25	89.12	77.16
Altech C_{18}	48.8	30.1	66.9	46.3	37.9	32.5	29.3	48.3	56.8	83.4	47.59	19.56	48.71	68.59
Altech C_8	88.3	37.9	87.5	61.7	105.2	45.5	75.9	32.6	104.9	103.1	32.95	18.65	39.75	96.12
Altech Extrelut	90.9	56.1	95.75	54.2	76.9	19.5	72.1	--	106.2	83.6	--	--	--	--
Altech Toxiclean	85.5	45.2	93.4	46.7	71.2	39.3	70.9	--	100.3	80.9	79.56	29.32	68.82	75.25
Altech C_{18} Rigas	42.1	NT	40.6	NT	39.5	NT	39.1	NT	63.1	NT	NT	NT	NT	NT
Bakerbornd C_{18}	42.6	NT	50.2	NT	42.9	NT	--	NT	59.1	NT	41.21	25.62	35.12	67.95
Separcol C_{18}	48.3	NT	42.7	NT	39.4	NT	22.5	NT	76.2	NT	NT	NT	NT	NT

NT= Not tested

TABLE 2.

Absolute Recoveries of Various Cartridges for the SPE of Spiked Plasma Samples with 5 ng/μl of Opium Alkaloids (1 ng/μl for Papaverine) and 4 ng/μl of Cocaine Alkaloids. Elution Solvent is Methanol, Except (*) Where the Elution Solvent is a (1:1) Mixture of Dichloromethane-Acetone. Details in the Experimental Section.

Analyte	Recovery (%)						
	Morphine	6-MAM	Codeine	Diamorphine	Papaverine	Benzoylecgonine	Cocaine
Altech Toxiclean	88.9	79.6	67.5	19.6	79.9	69.36	71.6
(*)	<10	<10	<10	<10	60.4	NT	NT
Bond Elut C_8	90.1	43.5	29.4	12.5	43.2	55.1	78.25
(*)	<10	<10	<10	<10	59.1	NT	NT
Bakerbond C_{18}	<10	<10	<10	55.2	103.2	34.4	<10
(*)	25.4	63.5	48.9	<10	100.1	NT	NT
Altech C_{18}	NT	NT	NT	NT	NT	31.1	<10
Altech C_8	NT	NT	NT	NT	NT	42.8	46.8
Bond Elut C_{18}	NT	NT	NT	NT	NT	36.0	32.1

NT=Not tested

Table II the best results were obtained with the use of the Altech Toxiclean column. The same column achieved a very satisfactory clean-up, so it was chosen for the SPE of the plasma samples for the rest of the study. Elution of the Bakerbond C_{18} column with methanol, gave very low recoveries for the polar opium alkaloids but high for diamorphine and papaverine. On the contrary, relatively higher recoveries were obtained, with the use of the apolar mixture of dichloromethane-acetone as the eluting solvent. This was in contrast with the results obtained in the other cartridges, and it can be attributed to the high coverage of the silica material on the Bakerbond column and the domination of the apolar interactions in retention of the alkaloids on column, in contrast with the other columns (Bond Elut C_8, Altech Toxiclean) where the sorbent material is assumed to be more polar. Precipitation of the proteins of the plasma or

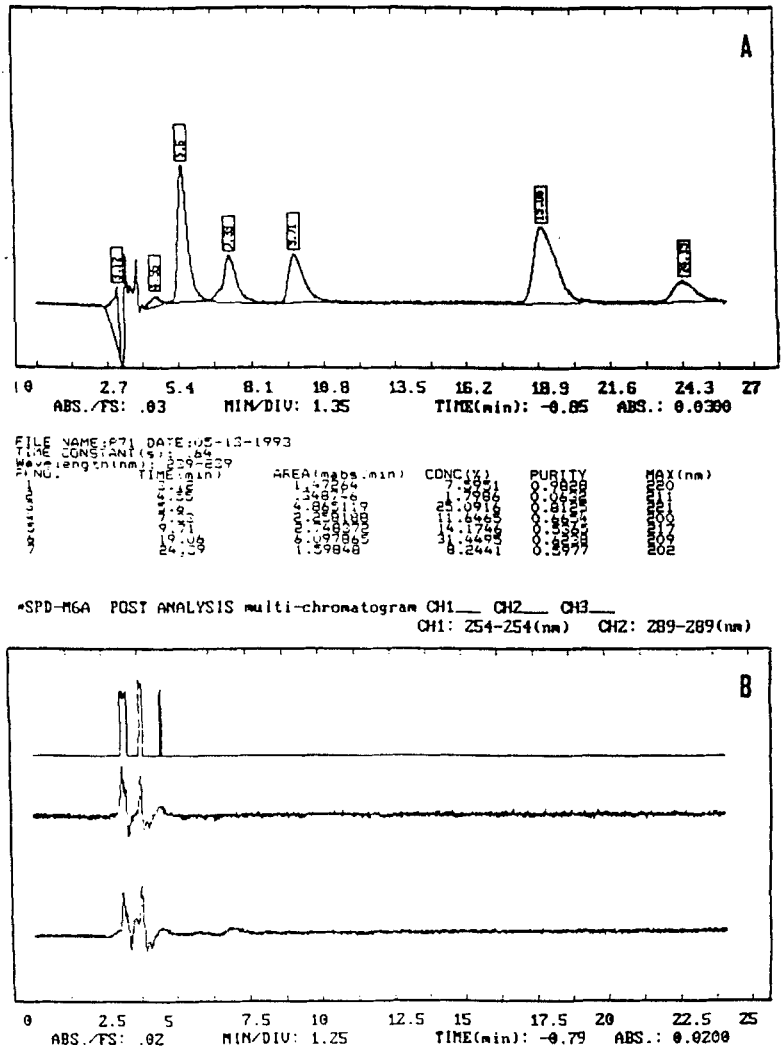

Fig. 1. A: Chromatographic analysis of plasma spiked with morphine (5 ng/μl)=5.6 min, 6-MAM (5 ng/μl)=7.33 min, codeine (5 ng/μl)=9.17 min, papaverine (1 ng/μl)=19.06 min and the internal standard flufenamic acid (2.5 ng/μl)=24.39 min. Quantitation at 239 nm.
B: Chromatographic analysis of the same plasma sample prior to the addition of the alkaloids. Quantitation at 254 and 289 nm.

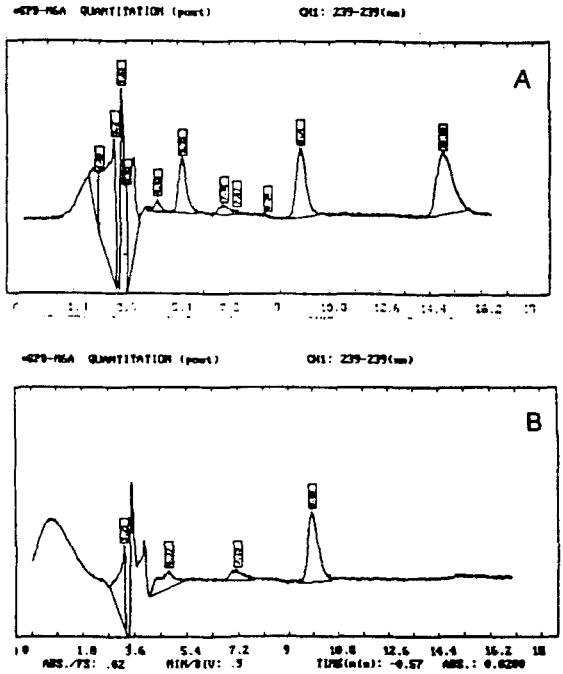

Fig. 2. **(A)** : Chromatographic analysis of plasma spiked with cocaine=14.55 min, benzoylecgonine=5.55 min and the internal standard nalorphine=9.72 min (at concentration 5 ng/μl for all the three compounds).
(B) : Chromatographic analysis of blank plasma with the addition of the internal standard.
Quantitation at 239 nm on both chromatograms.

serum samples was also tried with addition of trichloroacetic acid and it actually resulted in quite "cleaner" samples. Unfortunately the following pH adjustment of the sample, for the SPE was not successful and resulted in low recoveries. A typical chromatogram of a plasma sample spiked with opium alkaloids is given in Fig. 1 A whille Fig. 1 B shows the chromatographic analyis of the same sample prior to the addition of the alkaloids. Fig. 2 shows the chromatographic analysis of a spiked with coca alkaloids (Fig. 2 A) and a blank (Fig. 2 B) plasma sample.

TABLE 3.

Absolute Recoveries of Various Cartridges for the SPE of Urine Spiked with 5 ng/μl of the Opium Alkaloids and 4 ng/μl of Cocaine Alkaloids. Elution Solvnet is Methanol, except Procedure (C) where the Elution Solvent is Methanol with 2% NH_4OH. Details in the Experimental Section.

SPE Cartridge	Alkaloid Recovery (%)						
	Morphine	6 MAM	Codeine	Diacetyl morphine	Papaverine	Benzoylecgonine	Cocaine
Altech Toxiclean (A)	136.9	59.6	70.5	<10	101.2	85.4	84.6
(B)	25.2	<10	<10	<10	35.9	NT	NT
(C)	39.6	42.2	46.5	26.8	129.5	NT	NT
Altech C_{18} Rigas	136.2	39.2	86.5	<10	123	71.4	56.2
Altech C_8	NT	NT	NT	NT	NT	32.6	80.2
Bond Elut C_{18}	139.4	36.9	79.5	36.5	119.3	91.3	78.6
Bond Elut C_8	108.8	72.6	89.5	60.64	112.2	90.1	89.3
Bakerbond C_{18}	51.7	49.6	40.9	21.9	100.0	75.7	79.3

NT=Not tested

The recoveries obtained after the SPE from spiked urine samples are presented in Table III. Bond Elut C_8 proved to be the best fitted column for the pretreatment of urine samples and it was used for the rest of the study. Preconditioning of the Altech Toxiclean column in acidic environment (procedures B and C) did not improve the experimental results. Typical chromatograms of spiked urine and blank samples and urine samples of drug users are given in Fig. 3 for the analysis of opium alkaloids and in Fig. 4 for the analysis of coca alkaloids.

As a general result from the literature [24] but also from the above study and our experience, there should be no judgement of a "bad" or a "good" cartridge. Like in

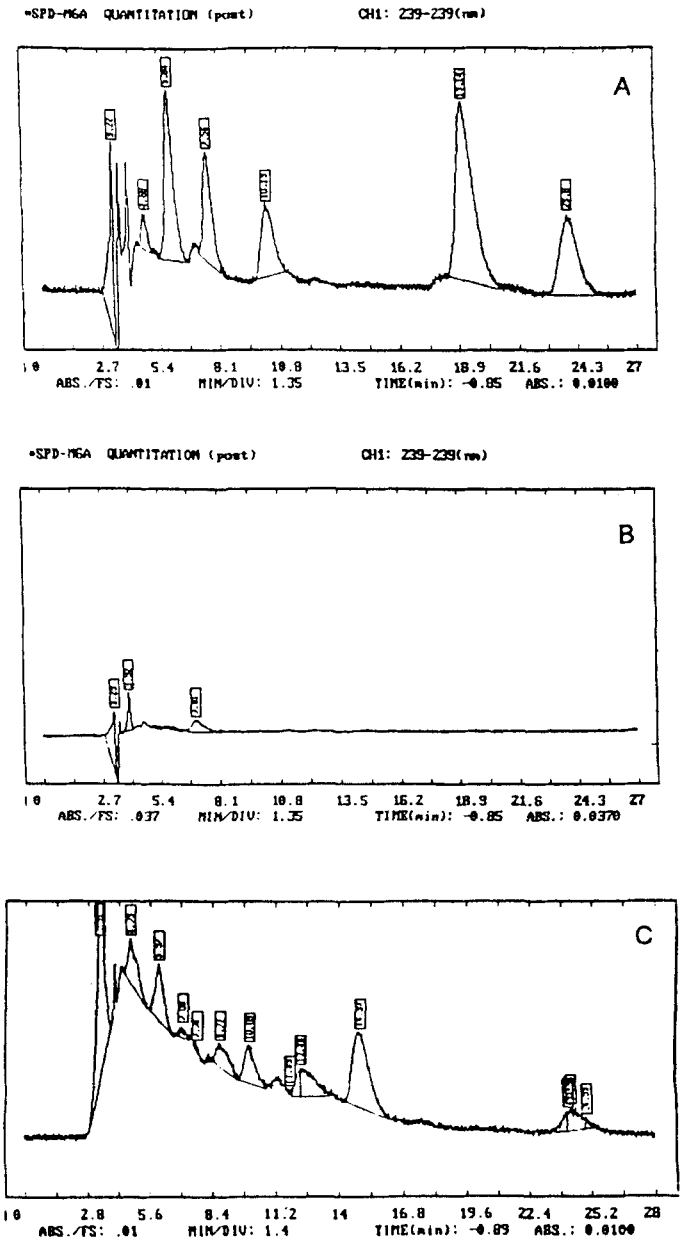

Fig. 3. A: Chromatographic analysis of urine spiked with morphine (5 ng/µl)=5.84 min, 6-MAM (5 ng/µl)=7.56 min, codeine (5 ng/µl)=10.19 min, papaverine (1 ng/µl)=19.14 min and the internal standard flufenamic acid (2.5 mg/ml)=25.8 min.
B: Analysis of a blank urine sample.
C: Analysis of urine of a heroine user.
Quantitation at 239 nm.

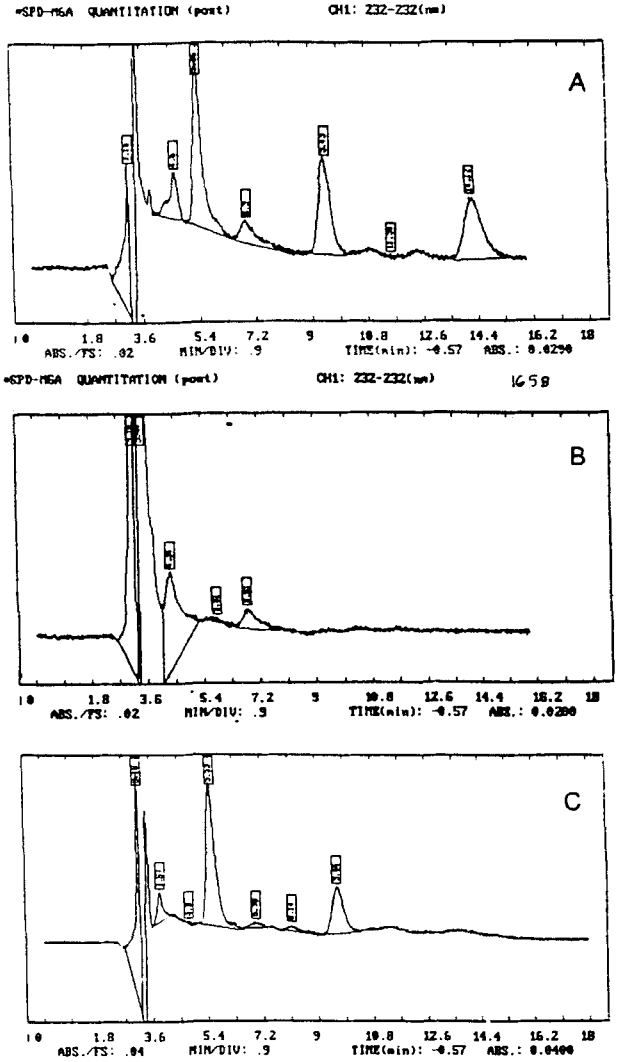

Fig. 4. (A) : Chromatographic analysis of urine spiked with cocaine=14.10 min, benzoylecgonine=5.37 min and the internal standard nalorphine=9.41 min(5 ng/μl).
(B) : Chromatographic analysis of blank urine and
(C) : Chromatographic analysis of urine of a cocaine user
Quantitation at 232 nm.

HPLC, some materials are most suitable for a specific application and give very good results for a type of compounds (e.g. polyaromatic compounds) but do not fit for the analysis of other types (alkanes etc.). Multiple interactions take place during the extraction procedure and the presence of the secondary interactions (like ionic interactions of the analyte with the free silanol groups) may prove very useful in the SPE [1, 28].

Quantitation

The simultaneous determination of morphine, 6-MAM and codeine in urine is of great interest for the toxicological analysis since it offers an undoubtful indication of the use of heroine. In the same way the determination of cocaine and its major metabolite benzoylecgonine in urine is the proof of cocaine usage. These two analyses are of the most common tasks of a toxicology laboratory. The linear regression equations obtained after the analysis of spiked plasma samples are presented in Table IV. The best results were obtained with 225 or 239 nm as the detection wavelength. The higher correlation coefficient observed at 254 nm is due to the smaller number of points of the calibration curve at this wavelength. The linear regression equations for the analysis of spiked urine samples are given in Table V.

Reproducibility of the SPE

The reproducibility of the SPE procedure can be evaluated by run-to-run, day-to-day, cartridge-to-cartridge and lot-to lot reproducibilities [1, 26]. The main reason for the different performance of cartridges of the same type is the irreproducibility of the sorbent preparation and packing procedure which according result in variation in the

TABLE 4.

Calibration Curves for the Analysis of Spiked Plasma Samples.

Alkaloid	Wavelength (nm)	Calibration Curve Equation *	Correlation Coefficient (R) *
Morphine	225	Y= - 0.186505 + 0.367174 X	0.99628
	239	Y= 0.11279 + 0.190643 X	0.99964
	254	Y= 0.09163 + 0.362636 X	0.99965
	289	Y= 0.005393 + 0.059263 X	0.99671
Codeine	225	Y= -0.01391 + 0.19836 X	0.99721
	239	Y= -0.00937 + 0.22943 X	0.99847
	254	Y= 0.032505 + 0.244442 X	0.99931
	289	Y= 0.03241 + 0.029747 X	0.99820
6-MAM	225	Y= -0.01391 + 0.1983 X	0.99624
	239	Y= 0.22623 + 0.190643 X	0.99817
	254	Y= 0.04478 + 0.134858 X	0.99935
	289	Y= -0.00236 + 0.02396 X	0.99332
Papaverine	225	Y= 0.040989 + 0.596276 X	0.99375
	239	Y= 0.262112 + 1.52974 X	0.99351
	254	Y= -0.07495 + 1.70751 X	0.99925
	289	Y= 0.004088 + 1.70751 X	0.99991
Benzoylecgonine	225	Y = 0.0010451 + 0.040302 X	0.99861
	232	Y = 0.025065 + 0.051979 X	0.99895
	239	Y = 0.020731 + 0.036586 X	0.99639
	254	Y = 0.035059 + 0.024408 X	0.98224
Cocaine	225	Y = 0.04674 + 0.040147 X	0.99828
	232	Y = 0.004525 + 0.12652 X	0.99884
	239	Y = 0.154942 + 0.069656 X	0.99912
	254	Y = 0.060245 + 0.024408 X	0.98666

TABLE 5.

Calibration Curves for the Analysis of Spiked Urine Samples.

Analyte	Wave-length (nm)	Calibaration Curve Equation	Correlation Coefficient (R)
Morphine	225	Y= 0.216705 + 0.189115 X	0.99854
	239	Y= 0.160075 + 0.176587 X	0.99654
	254	Y= 0.12709 + 0.200581 X	0.99355
	289	Y= 0.03319 + 0.0300719 X	0.99915
Codeine	225	Y=0.149443 + 0.16999 X	0.99594
	239	Y= 0.102895 + 0.12965 X	0.99357
	254	Y= 0.07361 + 0.157462 X	0.99941
	289	Y= 0.020538 + 0.027523 X	0.99630
6-MAM	225	Y= -0.029689 + 0.149111 X	0.99459
	239	Y= 0.11234 + 0.1354113 X	0.99976
	254	Y= 0.108323 + 0.115248 X	0.99959
	289	Y= 0.026214 + 0.020304 X	0.99832
Diamorphine	225	Y= 0.070489 + 0.042835 X	0.99635
	239	Y= 0.07845 + 0.022182 X	0.99712
	254	Y= 0.03645 + 0.023023 X	0.99642
	289	Y= 0.020892 + 0.014042 X	0.99696
Papaverine	225	Y= -0.09392 + 0.420322 X	0.99393
	239	Y= 0.13548 + 1.614382 X	0.99659
	254	Y= 0.133587 + 1.32986 X	0.99695
	289	Y= 0.016933 + 0.088413 X	0.99564
Benzoylecgonine	225	Y = 0.91954 + 0.092464 X	0.99908
	232	Y = 0.21600 + 0.222987 X	0.99932
	239	Y = 0.45642 + 0.24384 X	0.99910
	254	Y = 0.18958 + 0.11288 X	0.99958
	289	Y = 0.07951 + 0.07895 X	0.99509
Cocaine	225	Y = 0.0031656 + 0.07577 X	0.99807
	232	Y = 0.107571 + 0.1876004 X	0.99911
	239	Y = 249856 + 0.223251 X	0.99852
	254	Y = 0.052504 + 0.094247 X	0.99873
	289	Y = -0.02478 + 0.0655867 X	0.99860

TABLE 6.

Reproducibility of SPE of Standard Solutions, Spiked Plasma and Spiked Urine Samples. Sample Concentration 5 ng/μl of morphine, 6-MAM, Codeine and 1 ng/μl for Papaverine in the Plasma Sample. The Urine Sample was also Spiked with 5 ng/μl of Diamoprhine.

Analyte	Altech Toxiclean (plasma)		Bond Elut C_8 (urine)	
	Mean Recovery (%)	RSD (%)	Mean Recovery (%)	RSD (%)
Morphine	88.9	4.64	108.8	6.12
6-MAM	79.6	6.42	72.6	2.98
Codeine	67.5	5.90	89.5	3.87
Diamorphine	--	--	60.64	8.97
Papaverine	79.9	3.78	112.2	4.71

coverage of the hydroxyl groups of the silica base material. Therefore there are differences on the content and the type and activity of polar silanol groups in the sorbent. The reproducibility of the selected SPE procedures was checked as cartridge-to-cartridge reproducibility. Five Altech Toxiclean cartridges of the same batch were used for the repeated SPE of a plasma sample spiked with opium alkaloids. Five Bond Elut C_8 cartridges were used for the analysis of spiked urine samples. The results of this study are given in Table VI. Diamorphine showed the highest variations, phenomenon which was attributed both to the irreproducibility of the quantitation of the chromatographic peak [27] and to the instability of the compound and its possible hydrolysis.

Reusability of the SPE columns

The reusability of the SPE columns was tested by repeating five times the extraction of a spiked with plasma sample on an Altech Toxiclean column and the

TABLE 7.

Recoveries of the Alkaloids from Spiked Plasma and Urine Samples after Extraction on Reused Columns. Plasma Spiked with 5 ng/μl of Morphine, 6-MAM, Codeine and 1 ng/μl of Papaverine. Urine Sample was also Spiked with 5 ng/μl of Diamoprhine.

Analyte	Recovery (%)				
	1st use	2nd use	3rd use	4th use	5th use
Morphine	93.5	94.4	87.1	80.2	72.1
6-MAM	85.3	82.1	77.6	72.3	70.1
Codeine	66.4	68.3	62.1	60.5	58.8
Papaverine	83.4	78.4	73.2	68.9	63.2

extraction of a spiked urine sample on a Bond Elut C_8 column. In both samples an opiate standard solution (5 ng/μl for morphine, 6-MAM and codeine, 1 ng/μl for papaverine) was added and the selected extraction and preconditioning procedure was followed. The regeneration of the columns was made with elution with 3x 1 ml hexane, 5 x 1 ml MeOH and 2 x 1 ml deionised water. Hexane was applied in order to remove the apolar endogenous components that have been retained mostly by hydrophobic interactions and methanol and water in order to remove the polar endogenes that have been retained by ionic interactions and did not elute with the alkaloids. Directly after the regeneration, the borate buffer and the samples were applied on the columns. The results are given in Table VII. As can be seen the recoveries are quite satisfactory for both columns, with a significant loss of performance after each use. The cleanliness of the samples was also satisfactory showing no extra peaks in the late chromatograms. However as it's also recommended in the literature [1], it's not advisable to reuse the SPE columns, especially after the use of a high pH buffer, which may deteriorate the silica based material.

CONCLUSIONS

Different SPE columns and procedures were tested for the pretreatment of blood plasma-serum and urine samples. The type of the sorbent, the preconditioning procedure and the nature of the eluting solvent are of the most important factors in the extraction optimisation. The selected extraction procedure was found reproducible and precise. The method was also applied in the analysis of urine samples of heroin and cocaine users. Detection of the alkaloids should preferably be performed at the low UV region. Reuse of the SPE columns results in decreased extraction performance.

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REFERENCES

1 X. H. Chen, *Thesis*, Groningen University 1993.

2 R. Wasels, F. Belleville, P. Paysant, P. Nabet and I.Krakowski, J. Chromatogr., 489: 411-418 (1989).

3 C. Lora-Tamayo, T.Tena and G. Tena, J. Chromatogr., 422: 267-273 (1987).

4 T. V. Duc and A. Vernay, Biomed. Chromatogr., 4: 65-69 (1990).

5 T. V. Duc and A. Vernay, J. High Resol. Chromatogr., 13: 162-166 (1990).

6 Y. Kumagai, T. Ishida and S. Toki, J. Chromatogr., 421: 155-160 (1987).

7 I. R. Tebbett, Chromatographia, 23: 377-378 (1987).

8 C. P. W. C. M. V. van Wissen, P. M. Koopman-Kimenai and T. B. Vree, J. Chromatogr., 570: 309-320 (1991).

9 J. L. Mason, S. P. Ashmore and A. R. Aitkenhead, J. Chromatogr., 570: 191-197 (1991).

10 S. P. Joel, R. J. Osborne and M. L. Slevin, J. Chromatogr., 430: 394-399 (1988).

11 L. J. Murphey and G. D. Olsen, J. Liq. Chromatogr., 16: 2545-2561 (1993).

12 D. Wielbo, R. Bhat, C. Ghari, D. Vidyasagar, I. R. Tebbett and A. Gulati, J. Chromatogr., 615: 164-168 (1993).

13 A. I. Bouquillon, D. Freeman and D. E. Moulin, J. Chromatogr., 577: 354-357 (1992).

14 K. Persson, B. Lindstrom, D. Spalding, A. Wahlstrom and A. Rane, J. Chromatogr., 491: 473-480 (1989).

15 Z. E. Chen, G. Reynolds, F. Bochner and A. Somogyi, J. Chromatogr., 493: 313-324 (1989).

16 R. J. Stubbs, R. Chiu and W. F. Bayne,J. Chromatogr., 377: 447-453 (1986).

17 P. P.Pop, F. Grimaldi, M. Bresson, M. Fornaris and A. Viala, J. Liq. Chromatogr., 16: 2797-2811 (1993).

18 R. W. Taylor, F. C. Jain and M. P. George, J. Anal. Toxicol., 11: (1987) 233-234.

19 D. S. Isenchmid, B. S. Levine and Y. H. Caplan, J. Anal. Toxicol., 12: 242-245 (1998).

20 S. H. Cosbey and I. Craig, Proceedings of the 31st International Meeting of The International Association of Forensic Toxicologists, Leipzig 1993, R. K. Mueller, edr., Molin Apress 1994, pp. 153-156.

21 J. T. Stewart, T. S. Reeves and I. L. Honiberg, Anal. Letters, 17: 1811-1826 (1984).

22 M.E. Soares, V. Seabra and M. de L. A. Bastos, J. Liq. Chromatogr., 15: 1533-1541 (1992).

23 V. Marko and K. Radova, J. Liq. Chromatogr., 14: 1671-1682 (1991).

24 V. Marko and K. Radova, J. Liq. Chromatogr., 14: 1645-1658 (1991).

25 V. Marko K. Radova and I. Novak, J. Liq. Chromatogr., 14: 1659-1670 (1991).

26 I. Papadoyannis, A. Zotou, V. Samanidou, G.Theodoridis and F.Zougrou, J. Liq. Chromatogr., 16: 3017-3040 (1993).

27 G. Theodoridis, I. Papadoyannis, H. Tsoukali, J. Chromatogr., Subm. for publication.

28 K. C. Van Horne (Editor), Sorbent Extraction Technology, Analytichem International, Harbor City, CA 1985.

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