Pergamon

Journal of Pharmaceutical & Biomedical Analysis, Vol. 12, No. 4, pp. 463–481, 1994

0731–7085/94 $7.00 + 0.00

Solid-phase extraction of small drugs on apolar and ion-exchanging silica bonded phases: towards the development of a general strategy

M. MOORS,* B. STEENSSENS, I. TIELEMANS and D.L. MASSART

Vrije Universiteit Brussel, Pharmaceutical Institute Laarbeeklaan 103, B-1090 Brussels, Belgium

Abstract: In connection with the development of a general strategy for solid-phase extraction (SPE) of drugs, the use of the apolar octadecyl silica bonded phase and ion-exchanging phases with a benzene sulphonic acid or quaternary amine bonded functionality is investigated for the SPE of small polar drugs. This investigation was performed on a set of 15 drugs, belonging to varying pharmacological groups and with varying structures. For each analyte, its adsorption on the C_{18} and the ion-exchanging phase was controlled for an aqueous solution and for a spiked plasma sample. For those analytes retained on the sorbent, different elution solvents were compared. Although SPE methods could successfully be developed for some drugs, no general solution can be proposed and no solution was found for a few drugs. The main problems are that for these few drugs no sufficient retention is obtained on any SPE phase investigated or the selectivity is too low.

Keywords: *Solid-phase extraction; octadecyl- and ion-exchanging silica bonded phases; small, relatively polar analytes.*

Introduction

Most solid-phase extraction (SPE) methods described in the literature involve the selective extraction of one drug and its metabolites from the biological matrix [1–7] or the extraction of a limited number of structurally related compounds (e.g. barbiturates [8], xanthines [9], sulphonamides [10]), whereby the selection of the solid phase and the solvents are specific for the application. Some SPE methods are developed for a limited number of drugs belonging to the same pharmacological group, but with varying structures and chemical properties (e.g. some anti-inflammatory drugs [11], antiepileptic drugs [12], anticonvulsant drugs [13], the toxicological screening for drugs with abuse potential [14]). In our laboratory a more general strategy was developed for the SPE of drugs from aqueous samples or plasma samples: this was achieved by investigating the SPE for a set of test compounds, i.e. drugs belonging to different pharmacological groups and with varying molecular structures and properties [15–17], and deducing rules and SPE procedures applicable in the majority of cases. In this general SPE method, the cyanopropyl bonded phase is used and a limited number of elution solvents were proposed, between which a selection should be made in function of the drug's polarity and acid–base character. Contrary to most methods described in the literature, this constitutes a general approach for SPE, not optimized towards a given substance or a given class. It should be seen as a procedure that will, for a majority of drugs, lead to acceptable results in terms of recovery and selectivity and may require some optimization for specific applications. The knowledge derived from all the experiments was organized in a decision tree, enabling its implementation in an expert system [18].

The developed SPE strategy was however only applicable for relatively apolar drugs: the polarity of the drugs was expressed as the number of carbon atoms in the molecule's structure and the cyanopropyl sorbent could not sufficiently retain drugs with less than 11 carbon atoms. The number of carbon atoms is a very rough criterion to classify the drugs as relatively polar or apolar and for a number of drugs, classified as 'small and polar (<11 C-atoms)', it will be possible to extract them according to the general SPE method on a CN-phase, just as polar functional groups can make a large molecule (>11 carbon atoms) too polar to be withheld on the CN-phase.

In the work presented in this article, the aim

* Author to whom correspondence should be addressed.

was to investigate two types of sorbents, namely the more apolar and most often used octadecyl phase (C_{18}) and the strong ion-exchanging phases benzene sulphonic acid (strong cation-exchanging phase SCX) and quaternary amine (strong anion-exchanging phase SAX), for the SPE of polar drugs from aqueous samples or plasma samples.

The number of drugs with less than 11 carbon atoms is rather limited when compared with the bulk of drugs on the market: the main classes containing drugs with a small carbon skeleton are the amphetamines, barbiturates, sulphonamides, nicotinic acid and its esters, *p*-hydroxybenzoic acid and its esters, xanthines and further some diuretics (e.g. acetazolamide, amiloride, hydrochlorthiazide), some anti-inflammatory drugs (e.g. salicylic acid, paracetamol) or other drugs, belonging to various pharmacological and structural groups (e.g. the anticancer drug fluorouracil, the antihypertensive captopril, the antimycotic drug ciclopirox, the muscle relaxant baclofen, the histamine H_2 receptor antagonist cimetidine). The 15 drugs selected for this work are listed in Table 1: they all contain not more than 12 carbon atoms, they show varying pK_a values and pharmacological properties and it is known from earlier experiments that they were insufficiently retained on the cyanopropyl bonded phase. For these 15 drugs, the different SPE steps (adsorption, washing and elution) were investigated on both the C_{18} and the ion-exchanging phases, if possible a SPE procedure was developed on an aqueous standard solution and its application on plasma samples was evaluated.

Experimental

Chemicals and reagents

The drugs for which the SPE was studied are listed in Table 1. Stock solutions were prepared in methanol or water in a concentration of 100 mg/100 ml and kept in the refrigerator at 4°C. Standard solutions were prepared daily by appropriate dilution in water or the solvent required for the SPE.

Milli-Q water (Millipore corporation, Bedford, MA) was used to prepare the standard solutions, the mobile phase and the eluents for SPE. The reagents propylamine (PA), triethylamine (TEA) and N,N-dimethyloctylamine (DMOA) were delivered by Fluka (Buchs, Switzerland). All other reagents were of analytical grade and delivered by Merck (Darmstadt, Germany).

The different buffer solutions were prepared by mixing the appropriate amounts of basic and acid salts for 1 l of buffer solution [20]. Before making up the volume to 1 l, the pH was checked and, if necessary, corrected with 1 M NaOH or 1 M HCl. Phosphate buffer pH 3 was prepared with 1 M ortho-phosphoric acid and sodium dihydrogenphosphate monohydrate, for pH 7 and 10 the sodium salts

Table 1
List of the drugs for which the SPE was investigated

Drug name	Number of C-atoms	pK_a [19]	Acid/base*	Pharmacological group [19]
Phenobarbital	12	7.4	WA	barbiturate
Barbital	8	8.0	WA	barbiturate
Salicylic acid	7	3.0	A	
Nicotinic acid —N=	6	2.0	A	vitamin B group
—COOH		4.8		
Paracetamol	8	9.5	WA	aniline derivative
Propyl hydroxybenzoate	10	8.4	WA	
Hydrochlorthiazide	7	7.0	WA	thiazide diuretic
		9.2		
Sulphathiazole	9	7.1	WA	sulphonamide
Theophylline	8	8.6	WA	xanthine derivative
Cimetidine	10	6.8	WB	histamine H_2-receptor
		7.1		antagonist
Benzocaine	9	2.5	WB	local anaesthetic of the *p*-amino-benzoic acid group
Ephedrine	10	9.6	B	sympathomimetic agent
Chlorphentermine	10	9.6	B	sympathomimetic agent
Nicotinamide	6	3.3	WB	vitamin B group
Amiloride	6	8.7	B	diuretic

*(W)A = (weak) acid/(W)B = (weak) base.

dihydrogenphosphate monohydrate and sodium monohydrogenphosphate dihydrate were used and the phosphate buffer pH 12 was prepared with 1 M NaOH and sodium monohydrogenphosphate dihydrate. For the citric buffer pH 3, 2 M citric acid and 2 M NaOH were mixed in a ratio 5:3. For the ammonia buffer of pH 10 ammonium chloride and a 25% NH_3 solution were used.

Equipment

The SPE was performed manually with a Baker-10 or Baker-21 vacuum manifold. The octadecylcartridges were from Baker (Phillipsburg, KS, USA); the ion-exchanging cartridges quaternary amine (SAX) and benzene sulphonic acid (SCX) were from Analytichem (Varian Sample Preparation Products, Harbor City, CA, USA). All cartridges contained 100 mg silica bonded phase and had a reservoir of 1 ml.

Extracts were chromatographed on a Lichrosorb® C_{18}-stationary phase, packed with 5 μm particles in a stainless steel column of 250 mm length and an internal diameter of 4 mm. The HPLC column was protected with a guard-column, 25 mm in length, 4 mm i.d. and packed with 10 μm Lichrosorb® C_{18}-bonded silica particles. A Perkin–Elmer Series 10 Liquid Chromatograph was used. Samples were injected by means of a Rheodyne injector, through a 100 μl loop. The detection system consisted of a Perkin–Elmer LC 90 UV detector, which has a cell volume of 8 μl and a path length of 1 cm. Chromatograms were recorded and integrated with an Intermat IC-R3A data processor.

Solid-phase extraction of aqueous standard solutions

The SPE cartridges were in a first step wetted by aspirating two reservoir volumes (1 ml) of methanol through the sorbent bed. The vacuum pressure is maintained between 30 and 40 kPa during the whole SPE procedure on the apolar C_{18} silica bonded phases; on the ion-exchanging sorbents the vacuum is reduced to 20 kPa. Care is taken that the sorbent does not become dry after the wetting, i.e. the flow of the solvent is stopped as soon as the meniscus reaches the upper frit. In a second step the sorbent is conditioned with 2 ml of an appropriate solvent (water or an acid solution). Next 1 ml of the aqueous solution of the drug is brought in contact with the sorbent. The retention of the drug is investigated from different solutions (water, 1% H_3PO_4, 1% acetic acid) depending on the acid–base character of the drug and the type of solid phase. In the wash step two column volumes of the same solvent as used in the adsorption step is aspirated through the cartridge. For the elution different solvents are investigated (methanol; methanol containing amines; buffer solutions). In each step the fraction flowing through the cartridge is collected in a tube and chromatographed. If the collected solvent had a solvent strength lower than the solvent strength of the mobile phase, direct chromatography is possible. Samples with a too high solvent strength are diluted with water prior to injection, or evaporated at 50°C under a stream of nitrogen, whereafter the residue was dissolved in 1 ml of mobile phase. Samples with a too high pH (above pH 8 and below pH 2) are diluted with the buffer used in the mobile phase, i.e. phosphate buffer pH 3, until the pH of the extract falls between 2 and 8 or until a dilution of 1/6. A dilution beyond 1/6 was undesirable for reasons of detectability.

Pretreatment and solid-phase extraction of spiked plasma samples

The frozen blank plasma was thawed at room temperature. To 1 ml plasma 100 μl of a 50 ppm solution of an analyte in water was added and vortex-mixed. The plasma was deproteinized by adding, dropwise and under continuous vortex-mixing, 2 ml of acetonitrile. The deproteinized plasma sample was centrifuged at 3000 rpm for 15 min. The upper liquid was poured off in a vial. The remaining protein pellet was rinsed with approximately 200 μl of acetonitrile, which was added to the vial. Next the acetonitrile was evaporated under a stream of N_2 and at a temperature of 50°C, until a volume of ±750 μl deproteinized plasma remained in the vial. The volume of the deproteinized plasma sample was enlarged by adding 1 ml of water or 1 ml of 1% acetic acid or phosphoric acid, depending on the kind of analyte and the sorbent type. In this way a sample with similar characteristics (pH, aqueous) as the standard solution used in the SPE of aqueous standard solutions is obtained. The aqueous sample was aspirated through a conditional cartridge. The SPE procedure was further carried out as described in the previous paragraph. In the wash and elution steps the

solvents selected during the SPE method development on aqueous solutions were used.

HPLC and UV detection

All drugs are chromatographed in reversed phase on a Lichrosorb® C_{18} column. The mobile phase consisted in each case of a mixture of phosphate buffer pH 3, $I = 0.05$ and methanol. The percentage of organic modifier in the mobile phase was chosen in such a way that the drug eluted with a retention time between 5 and 12 min. Detection was performed at the λ_{max} for each drug. Table 2 gives an overview of the chromatographic parameters (mobile phase, retention times, capacity factors) and the detection wavelength.

Results

Earlier work established that a 100 mg cyanopropyl cartridge was inappropriate for the extraction of small molecules [15–17]: this type of sorbent was too polar to retain the polar neutral, acidic and basic molecules with less than 11 carbon atoms in their structure. Starting from this knowledge, the first sorbent investigated for the SPE of small drugs was the most general used, apolar octadecyl cartridge.

1. Octadecyl silica bonded phase

Adsorption and wash step for aqueous solutions on a C_{18}-sorbent. The C_{18}-sorbent was wetted by aspirating 2 ml methanol through it. Then the C_{18}-sorbent was conditioned with water, the drugs were adsorbed from an aqueous solution and the cartridge was rinsed with 2 ml water. As can be seen in Table 3, all basic drugs, except for nicotinamide, were totally retained on the C_{18}-phase. The more polar character of nicotinamide when compared with the other bases explains the fact that this compound co-elutes in the washing: nicotinamide has a log P value <0, whereas cimetidine, benzocaine, ephedrine and chlorphentermine have log P values between 0.4 and 2. For the (weak) acid molecules, the retention from water is for five out of nine drugs too weak, so that breakthrough is observed immediately when the sample is put on the sorbent, or during the wash step with water. The two barbiturates are relatively well retained, showing only a small loss of not more than 6% in the washing with water: theophylline and propyl hydroxybenzoate are completely retained on the C_{18}-sorbent. Nicotinic acid shows the least retention because of its more polar character. The primary interactions supposed to hold a compound on the C_{18}-phase are apolar van der Waals forces, but secondary interactions at the residual silanol groups can also occur. As the pH of the solutions brought on the sorbent is approximately 5, basic drugs will mostly be positively charged (depending on the pK_a-value) and can interact with the silanol groups. The acids can, depending on their pK_a-value, be neutral or negatively charged. In the former case the silanol groups are of no importance. In the case they are negatively charged, it is not impossible that a repulsion between the silanols and the drug can prevent the retention. Therefore the adsorption for the acids was checked under acidic conditions, so that the acid and/or the silanols are unionized. To suppress the ionization of

Table 2
Chromatographic parameters and detection

Drug	Mobile phase*	t_R (min)	k'	λ_{max} (nm)
Phenobarbital	67–32.5	12.5	4.2	220
Barbital	70–30	6.4	1.7	220
Salicylic acid	65–35	9.0	2.8	234
Nicotinic acid	100–0	6.0	1.5	260
Paracetamol	75–25	6.5	1.7	247
Propyl hydroxybenzoate	30–70	5.5	1.3	256
Hydrochlorthiazide	75–25	6.1	1.5	270
Sulphathiazole	75–25	8.2	2.4	258
Theophylline	70–30	6.1	1.5	269
Cimetidine	90–10	11.1	3.6	220
Benzocaine	60–40	11.3	3.7	220
Ephedrine	75–25	5.9	1.5	220
Chlorphentermine	60–40	10.5	3.4	220
Nicotinamide	98–2	10.0	3.2	260
Amiloride	75–25	8.1	2.4	286

* % Phosphate buffer pH 3, $I = 0.05$–% methanol.

Table 3
Retention on 100 mg octadecylsorbent after conditioning with methanol, wetting the sorbent with water and dissolving the drugs in water

Drug	% Adsorption on C_{18} from water	% Co-elution with 2 ml wash solvent, i.e. water
Acids		
Phenobarbital	100	5.9
Barbital	100	3.3
Salicylic acid	100	87.6
Nicotinic acid	14.8	9.4
Paracetamol	100	17.1
Propyl hydroxybenzoate	99.8	0.1
Hydrochlorthiazide	100	26.1
Sulphathiazole	99.8	20.5
Theophylline	100	0
Bases		
Cimetidine	100.0	0
Benzocaine	98.5	0
Ephedrine	100.0	0
Chlorphentermine	99.6	0
Nicotinamide	98.9	68.6
Amiloride	99.3	0

Table 4
Retention of the (weak) acidic drugs on 100 mg octadecylsorbent after wetting with methanol, conditioning the sorbent with 1% acetic acid or 1% phosphoric acid in water and dissolving the drugs in 1% acetic acid or 1% phosphoric acid

Drug	% Adsorption on C_{18} from 1% HAc	% Elution with washsolvent, i.e. 1% HAc	% Adsorption on C_{18} from 1% H_3PO_4	% Elution with washsolvent, i.e. 1% H_3PO_4
Phenobarbital	100.0	0.0	100	2.5
Barbital	96.7	44.2	87.8	28.8
Salicylic acid	99.5	0.0	100	0.3
Nicotinic acid	62.4	26.8	18.1	8.4
Paracetamol	81.5	52.6	53.1	30.4
Propyl hydroxybenzoate	100.0	0.0	100	0.0
Hydrochlorthiazide	99.7	68.9	53.1	27.9
Sulphathiazole	88.1	41.1	53.9	17.4
Theophylline	99.4	35.3	88.7	13.1

the silanols, the pH must be lower than 4 [21, 22]. The C_{18}-phase was conditioned with 1% acetic acid (pH ±3) or 1% phosphoric acid in water (pH ±1). The same solvent as used in the conditioning step was also used to dissolve the drug and during the wash step. However, the results in Table 4 show that the use of acid solutions in the conditioning, adsorption and wash steps does not improve the adsorption of acid molecules to a large extent. Only three drugs are now totally retained on the C_{18}-sorbent when working at low pH, namely phenobarbital, salicylic acid and propyl hydroxybenzoate and only for salicylic acid a significant improvement is observed when comparing the adsorption from water and an acid solution (phenobarbital and nipasol were also well retained from water). The other six acid molecules are insufficiently retained and again co-elute when rinsing the cartridge with the acid solution. The log P values, reveal that the three retained drugs are less polar than the other acids: their log P values are 1.43, 2.26 and 3.04 for phenobarbital, salicylic acid and propyl-parahydroxybenzoate, respectively, whereas the log P values of the other acids are around zero or even negative (−0.2 for nicotinic acid, 0.0 for hydrochlorthiazide and 0.01 for sulphathiazole). In some cases contradictory observations were made: theophylline and barbital are, for example, well retained from water, while a loss of up to 44% is observed from the acetic acid solution. No changes occur when decreasing the pH by using a stronger acid, namely phosphoric acid instead of acetic acid.

Elution for aqueous solutions from a C_{18}-sorbent. The elution was studied for those compounds completely adsorbed on the C_{18}-

sorbent, i.e. the five basic compounds cimetidine, benzocaine, ephedrine, chlorphentermine and amiloride and the five acids phenobarbital, salicylic acid and propyl hydroxybenzoate, theophylline and barbital (the former three acids are adsorbed from an acid solution, the latter two drugs from water).

For the basic drugs, the elution was investigated with the following solvents: methanol, methanol containing 0.1% propylamine and a mixture of methanol and phosphate buffer pH 3, $I = 0.05$ (1:1). These eluents are the same as used in the SPE of relatively apolar, basic drugs (more than 11 C-atoms in their structure) on cyanopropyl cartridges [15] and the selection of these eluents is based on HPLC experience [22–24]. The buffer–methanol mixture is similar to the mobile phase used to chromatograph the drugs: the pH 3 of this eluent is supposed to suppress the ionisation of the residual silanol groups; using the less polar methanol (compared with the water used in the adsorption and wash step) the van der Waals forces can be disrupted and the percentage of methanol (organic modifier) chosen is rather high (50%) to elute the drugs from the solid phase in an as small as possible volume. Amine modifiers, such as propylamine, are used to block the residual silanol groups and compete with the basic drugs for the binding at these sites. Table 5 presents the elution recoveries obtained for the five basic drugs. If an acceptable recovery of at least 80% was obtained with one of the eluents, the repeatibility of the SPE was checked for a 5 ppm standard solution of the drug and in that case the mean of six extractions and the standard deviation are shown in Table 5. Acceptable recoveries and standard deviations were obtained for all five bases with CH_3OH–phosphate buffer pH 3, $I = 0.05$. For cimetidine and amiloride 0.1% propylamine in CH_3OH also gave good results. For ephedrine and chlorphentermine, no elution at all is observed with methanol, so methanol could possibly be used as a wash solvent for these drugs.

For the acid drugs methanol could be used as an eluting solvent. As can be seen in Table 5, mean recoveries of at least 85% are obtained and the standard deviation is not greater than 5%.

SPE on the C_{18}-sorbent for spiked plasma samples. In a following stage the SPE method developed on a C_{18} solid phase for an aqueous solution of a small acid (phenobarbital, theophylline, barbital) or base (cimetidine, benzocaine, amiloride, ephedrine and chlorphentermine) was applied to a plasma sample, spiked with 5 μg ml^{-1} of the analyte (for analytical convenience the same concentration was taken for all analytes). The recovery as well as the cleanness of the extract, or in other words the selectivity of the SPE on C_{18}, were evaluated.

For three of the five basic drugs, a recovery was obtained comparable with the result for the SPE of the aqueous solutions: 91.2% and 91.5% for cimetidine when eluting with 0.1% propylamine in methanol and CH_3OH–phosphate buffer pH 3, $I = 0.05$, respectively; 84.9% for chlorphentermine when eluting with CH_3OH–phosphate buffer pH 3, $I = 0.05$; and 90.7% and 84.8% for amiloride when eluting with 0.1% propylamine in methanol and CH_3OH–phosphate buffer pH 3, $I = 0.05$,

Table 5
Elution of the drugs adsorbed on the octadecyl bonded silica phase

Drug	CH_3OH (%)	Elution with 1 ml of 0.1% propylamine in CH_3OH (%)	CH_3OH–phosphate buffer pH 3, $I = 0.05$ (1:1) (%)
Basic			
Cimetidine	88.7	89.8 ± 7.7	90.6 ± 5.3
Benzocaine	3.2	30.1	87.3 ± 2.5
Ephedrine	0	27.3	95.9 ± 4.3
Chlorphentermine	0	23.6	92.1 ± 1.8
Amiloride	25.2	98.3 ± 1.6	98.2 ± 4.7
Acid			
Phenobarbital	98.3 ± 1.8		
Propyl hydroxybenzoate	88.0 ± 5.0		
Salicylic acid	85.3 ± 5.0		
Theophylline	97.5 ± 2.4		
Barbital	97.6 ± 1.0		

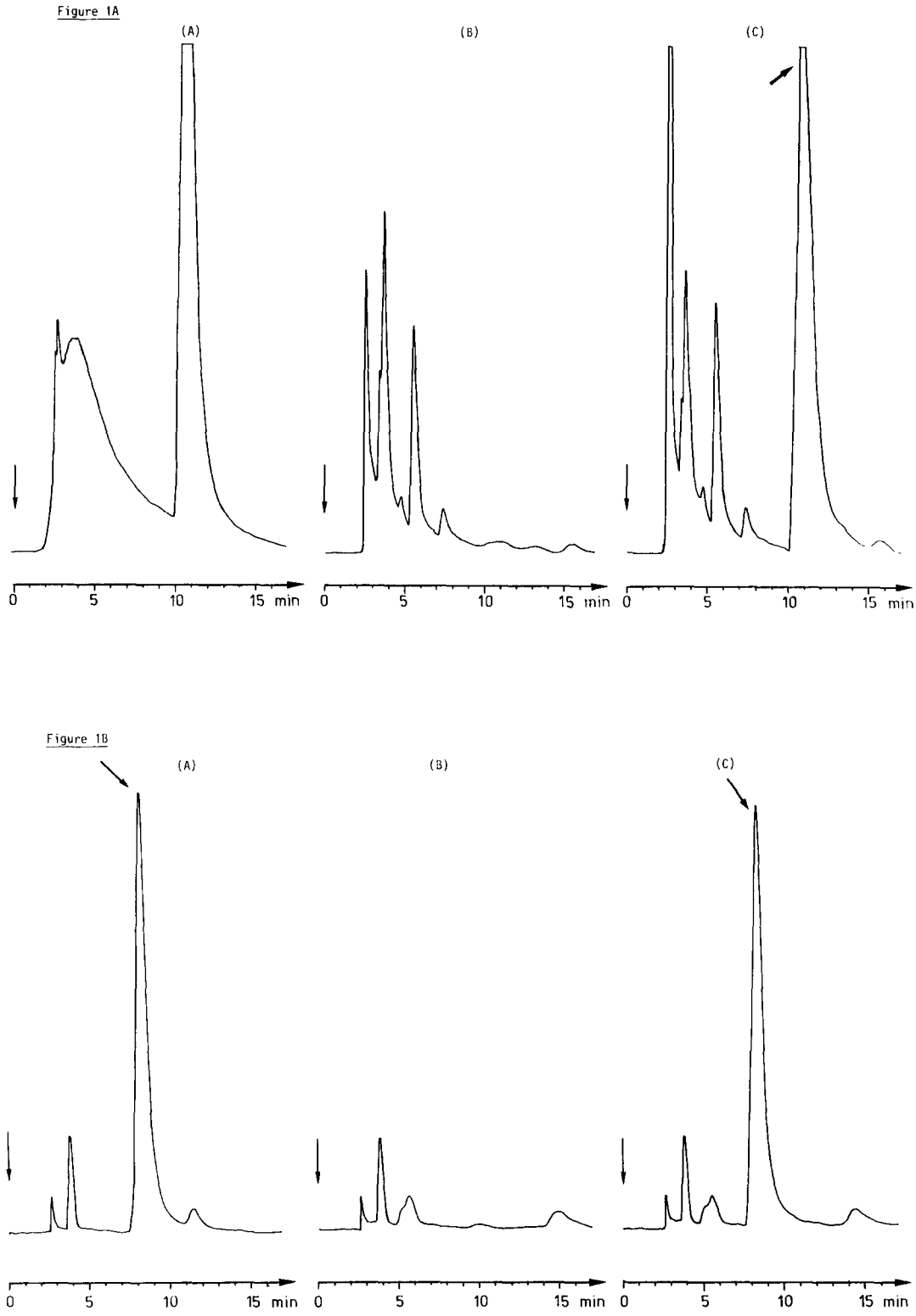

Figure 1
(Top) Chromatograms of (A) a 5 ppm standard solution of chlorphentermine, (B) a blank plasma extract and (C) the extract of plasma spiked with 5 μg ml^{-1} chlorphentermine. SPE on C_{18}; elution with CH_3OH–phosphate buffer pH 3, I = 0.05. For HPLC and detection conditions: see Table 2 (0.008 AUFS). (Bottom) Chromatograms of (A) a 5 ppm standard solution of amiloride, (B) a blank plasma extract and (C) the extract of plasma spiked with 5 μg ml^{-1} amiloride. SPE on C_{18}; elution with 0.1% propylamine in methanol. HPLC and detection conditions: see Table 2 (0.008 AUFS).

respectively. The chromatograms of the blank plasma extracts and the extracts of chlorphentermine and amiloride are shown in Fig. 1. For benzocaine the recovery was lower (61.4%). Further investigation revealed that a second millilitre of the eluent was necessary to increase the recovery to 90.2%.

The blank extract of the plasma was sufficiently clean, except for the extract of ephedrine. This chromatogram showed a large peak with approximately the same retention time as ephedrine and made a quantitation of ephedrine impossible.

For the acid phenobarbital, the SPE method of the C_{18}-sorbent also yielded insufficiently clean extracts: the chromatogram of the methanol eluent still showed numerous matrix peaks, interfering with the phenobarbital peak (see Fig. 2). For the weak acids theophylline and barbital, the problem arises that the drugs are not completely retained on the C_{18}-phase from the deproteinized plasma. Losses of up to 76% are observed. Possible explanations are that both analytes are better dissolved in plasma, which has a higher polarity than water, and show therefore less affinity for the C_{18}-phase, or that endogenous plasma components compete with these weak acids for the adsorption sites.

2. Ion-exchanging silica bonded phase

The ion-exchanging phases investigated for the SPE of the small drugs were the benzene sulphonic acid cation (SCX) exchanging phase for the bases and the quaternary amine anion (SAX) exchanging phase for the acids. Adsorption and elution of ionic compounds on ion-exchanging phases are mainly determined by the pH, the ionic strength and the type of counterions in the solutions. In the adsorption step both the silica bonded ion exchanging moiety and the analyte must have opposite charges. Therefore the pH of the solution needs to be 2 pH units below the pK_a value of the basic analyte or anion exchange function and 2 pH units above the pK_a value of the acid analyte or cation exchange function. The ionic strength must be low and the presence of counterions with a high affinity for the ion exchange phase must be avoided. In the elution, the conditions need to be opposite: the pH must be adjusted so that the analyte or the ion exchanging group is uncharged, the ionic strength must be high and counterions with a high affinity for the ion exchanging moiety favour elution. Changing the eluent's polarity by adding an organic solvent can improve the elution.

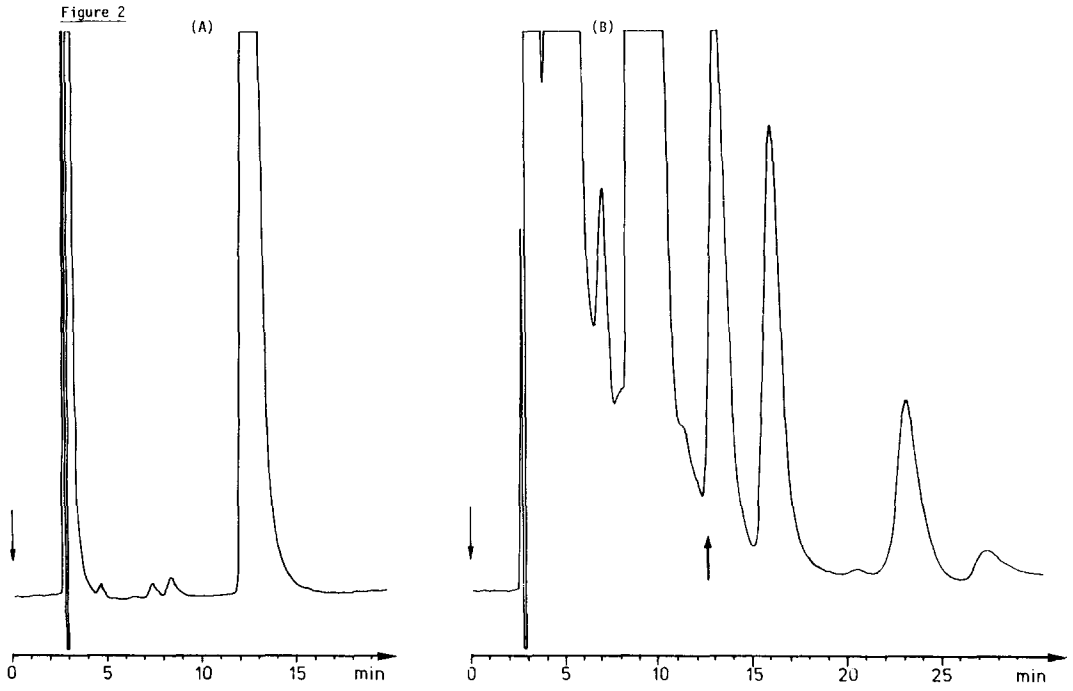

Figure 2
Chromatograms of (A) a 5 ppm standard solution of phenobarbital and (B) a blank plasma extract. SPE on C_{18}; elution with methanol. For HPLC and detection conditions: see Table 2 (0.008 AUFS).

Adsorption of aqueous solutions of basic analytes on the benzene sulphonic acid phase (SCX). Aqueous solutions containing 5 μg ml^{-1} of the basic analyte were prepared and adsorbed on the benzene sulphonic acid phase, prior wetted with methanol and conditioned with water. All six drugs were completely adsorbed on the sorbent and remained retained when washing with up to 3 ml water and 1 ml methanol.

The bases having a pK_a value >7 (Table 1) are positively charged in an aqueous solution (pH ± 5). The pK_a value of the sulphonic acid functional group is very low and at pH 5 it is negatively charged, so that adsorption of the basic analytes through electrostatic forces is possible. Besides electrostatic interactions, apolar van de Waals forces can occur at the benzene ring of the solid phase or dipole–dipole interactions between the sulphonic acid group and the bases' functional groups containing a nitrogen atom. The latter interactions explain the retention of nicotinamide and benzocaine, that are not ionized at the pH studied.

Adsorption of aqueous solutions of acid analytes on the quaternary amine phase (SAX). For the small acid drugs, the adsorption on the quaternary amine ion-exchanging phase was investigated in a similar way to the basic compounds on the benzene sulphonic acid phase. Only the two relatively strong acids salicylic acid and nicotinic acid, which both have an ionizable carboxylic functional group, were retained. Both drugs have low pK_a values, so that, in water, at pH ± 5, an electrostatic interaction can take place with the positively charged ammonium functions of the sorbent. All other drugs are weak acids or even rather neutral molecules, with high dissociation constants ($pK_a > 7$) and no distinct ionizable functions (e.g. xanthine, barbiturates), for which ion exchange is not the appropriate system for extraction.

Elution of basic drugs from the benzene sulphonic acid phase (SCX). Different elution solvents were evaluated systematically for two of the six bases, namely for the weak base benzocaine and for chlorphentermine, whereby the effect of the pH, the ionic strength, the kind of buffer, the amount of organic solvent and the use of an amine modifier were investigated. For the other basic drugs, only those eluents leading to an acceptable elution recovery for benzocaine and chlorphentermine were tested.

The recoveries observed for benzocaine and chlorphentermine, using 3 ml of each eluent are presented in Table 6. Water and methanol were not able to elute the analytes from SCX. The effect of methanol containing an organic amine was investigated: the selected amines were the primary amine propylamine (PA), already used as an eluent on the apolar C_{18}-phase, and the tertiary amines triethylamine (TEA) and dimethyloctylamine (DMOA). It was supposed that these amines would compete with the analyte for the adsorption on the SCX and in this way bring about the analyte's elution. At the concentration of 0.1% (= 12.1 mM PA, 7.17 mM TEA, 4.86 mM DMOA) no significant elution is observed with any of the amines. At the concentration of 121 mM, different results were observed for benzocaine and chlorphentermine (Fig. 3). For benzocaine high recoveries are obtained, namely 97.1% with PA, 90.7% with TEA and 74.3% with DMOA. The lower recovery with DMOA might indicate that the size of the competitive amine is important rather than its primary or tertiary character. With the smaller molecules propylamine and triethylamine, a higher recovery is obtained than with the larger dimethyloctylamine. For chlorphentermine the results are somewhat surprising because of the large differences observed with PA and TEA or DMOA. With the highest concentration of 121 mM PA in methanol, 86.4% of the analyte elutes in a volume of 3 ml; with the two other amines on the contrary, chlorphentermine does not elute at all (or only an insignificant amount of less than 10%).

Besides displacing the analyte from SCX with competitive molecules like the amines, the elution caused by a pH change was investigated. A 0.1 N NaOH solution, which has a pH of ±12, yielded 46.4% recovery for benzocaine and only 9.0% for chlorphentermine.

Using buffers of varying pHs and varying ionic strength, the effect of both a pH increase and a competition between the buffer ions and the adsorbed basic compound can be controlled in the elution step. For benzocaine, the lowest pH tested was 7. As its pK_a value is only 2.5, at pH 7 its aromatic amine function is undissociated. The two other pH values were 10 and 12. As the pK_a value of chlorphentermine is 9.6, only the buffer solutions with pH

Table 6
Elution of benzocaine and chlorphentermine adsorbed on a benzene sulphonic acid phase

Eluting solvent	Elution recoveries in percentage of the retained amount							
	Benzocaine				Chlorphentermine			
	1st ml	2nd ml	3rd ml	Total	1st ml	2nd ml	3rd ml	Total
Water	0	0	0	0	0	0	0	0
Methanol	0	0	0	0	0	0.8	2.3	3.1
0.1% PA or 12.1 mM PA in CH_3OH	0.6	0.4	2.9	3.9	0	0	0	0
1% PA or 121 mM PA in CH_3OH	68.6	28.5	0	97.1	53.4	31.9	1.1	86.4
0.1% TEA or 7.17 mM TEA in CH_3OH	0.4	0	2.0	2.4	0.9	0.9	0	1.8
1% TEA or 71.7 mM TEA in CH_3OH	1.1	84.4	1.5	87.0	0	0	0	0
1.7% TEA or 121 mM TEA in CH_3OH	54.3	33.1	3.3	90.7	0	0	3.0	3.0
0.1% DMOA or 4.86 mM DMOA in CH_3OH	0	0	0	0	0	0	0	0
1% DMOA or 48.6 mM DMOA in CH_3OH	0	16.3	26.4	42.7	0	8.1	0	8.1
2.5% DMOA or 121 mM DMOA in CH_3OH	29.4	37.0	7.9	74.3	0	0	0	0
0.1 N NaOH (pH ± 12)	1.8	1.2	43.4	46.4	0.3	1.1	7.6	9.0
Phosphate buffer								
pH 7, I = 0.05	0.3	0.6	15.0	15.9	/	/	/	/
pH 10, I = 0.05	0.2	0	5.2	5.4	0.4	0	0	0.4
pH 12, I = 0.05	8.1	2.9	13.1	24.1	0	0.4	0.7	1.1
pH 7, I = 0.1	1.4	16.9	24.6	42.9	/	/	/	/
pH 10, I = 0.1	0.3	9.8	37.4	47.5	0	3.6	4.1	7.7
pH 12, I = 0.1	0	9.7	58.4	68.1	0	4.9	5.8	10.7
pH 7, I = 0.5	3.2	14.5	14.6	32.3	/	/	/ 1.9	/
pH 10, I = 0.5	3.4	14.5	13.9	31.8	0.8	1.1	6.5	3.8
pH 12, I = 0.5	11.8	18.3	9.0	39.1	0	0		6.5
Phosphate buffer pH 12, I = 0.1 with								
25% methanol	0.6	24.7	23.8	49.1	0.3	5.3	18.0	23.6
50% methanol	0.2	6.1	51.3	57.6	0.6	3.5	45.0	49.1
NH_3/NH_4^+ buffer								
pH 10, I = 0.05	15.4	35.7	22.4	73.5	0	0	0	0
pH 10, I = 0.1	18.3	16.3	23.4	58.0	0	0	0	0
pH 10, I = 0.5	48.3	35.9	11.0	95.2	0	0	2.0	2.0
2.5 N NH_3 in CH_3OH	76.4	1.2	1.0	78.6	50.1	0.8	0	50.9

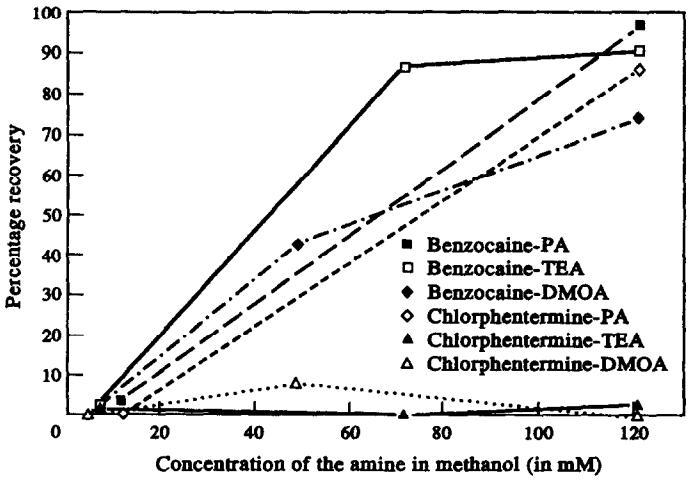

Figure 3
Elution of benzocaine and chlorphentermine from a SCX solid phase with different concentrations of amines in methanol.

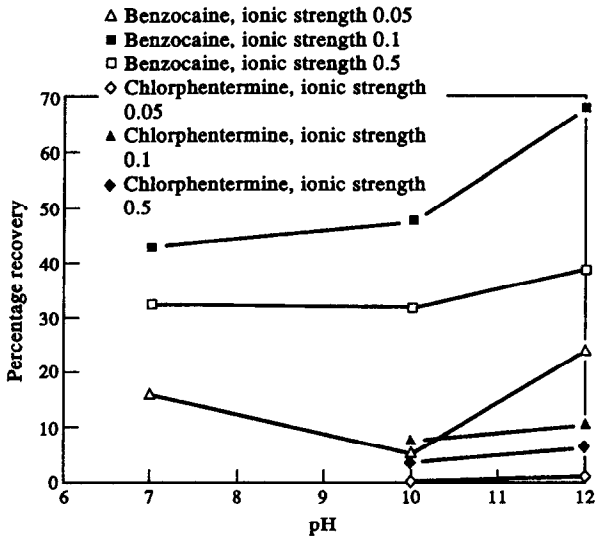

Figure 4
Elution of benzocaine and chlorphentermine from a SCX solid phase with 3 ml of phosphate buffer, with varying pH and ionic strength.

10 and 12 were tested. The selected levels of the ionic strength were 0.05, 0.1 and 0.5.

From Figure 4 and Table 6 it can be seen that the elution recoveries are extremely different for benzocaine and chlorphentermine. For chlorphentermine, with the highest pK_a value, not more than 10% elution can be effected, whereas the maximum elution recovery for benzocaine is 68.1%. The effectiveness of a buffer as eluent largely depends on the pK_a of analyte.

The effect of the pH can only be described for benzocaine. Increasing the pH from 7 to 10 does not improve the elution: the total recovery remains the same or even decreases. Only when the pH was raised to 12 were distinct higher recoveries obtained (Fig. 4). The reason why the pH must be so high in order to attain an acceptable recovery is not clear.

For the ionic strength the optimal value lies around 0.1, as at this ionic strength a maximum elution is observed.

The addition of CH_3OH to the phosphate buffer in the eluent again gave different results for benzocaine and chlorphentermine: for benzocaine the result is slightly lower than the recovery obtained without the addition of methanol, whereas for chlorphentermine the elution increases with the amount of methanol added.

Generally the results obtained with phosphate buffer are not sufficiently good: the recoveries are too low (maximum 49.1% for chlorphentermine and 68.1% for benzocaine) and the elution is too slow, so that large volumes of phosphate buffer are needed to complete the elution.

Besides the phosphate buffer, an ammonia buffer was investigated. The ammonium gives the possibility to prepare a high pH buffer (pH 10) and the NH_4^+ ion has a different selectivity for the SCX than the Na^+ counterions in the phosphate buffer. However, when aspirating the 3 ml of NH_3/NH_4^+ buffer pH 10, $I = 0.05$ through the cartridge on the vacuum manifold, a blue, precipitating compound was observed in the extract. Further research revealed that the blue compound came from the taps, placed on the cover of the vacuum manifold. To overcome this problem, all steps of the SPE were carried out by means of the vacuum manifold, except for the elution step, which was completed by means of centrifugation. The cartridge was filled with the ammonia buffer, placed in a screw-capped tube and centrifuged for 5 min at 1000 rpm.

Chlorphentermine did not elute with the ammonia buffer, whereas for benzocaine the ammonia buffer is clearly more effective as eluent than the phosphate buffer: the recoveries obtained with the ammonia buffer pH 10 are significantly higher than with a phosphate buffer of the same pH and ionic strength. The effect of ionic strength is different for both types of buffer: for the ammonia

buffer the highest ionic strength value yields the highest elution, contrary to the observations made with a phosphate buffer. The disadvantage of the use of the ammonia buffer is, as with the phosphate buffer and probably any other type of buffer, that at least 3 ml of eluent are necessary to elute the analyte completely from the sorbent. This dilution of the sample leads to a loss in sensitivity.

SPE methods on a SCX whereby the elution of the drugs was brought about with NH_3 and methanol, have been described in the literature [10, 25]. The solvent used by Süss *et al.* was investigated for benzocaine and chlorphentermine, namely 2.5 N NH_3 in methanol. For both analytes, a significant elution was obtained with the first millilitre fraction, in contrast with the buffers or amine modifiers. For benzocaine, a recovery of 76.4% is obtained; for chlorphentermine the recovery of 50.1% is still low, but the result is markedly better than with most of the other eluents. Although not all the drug eluted with the first millilitre of 2.5 N NH_3, no significant elution is observed with the two following fractions. It seems that a fraction of the drug remains irreversibly bound on the solid phase.

As the drugs did not elute with the first millilitre of phosphate buffer pH 12, $I = 0.1$, but only in the second and third millilitre, 1 ml of this alkaline buffer was used in the wash step and eluted subsequently with 2.5 N NH_3 in methanol, which gave acceptable results for both small basic analytes benzocaine and chlorphentermine. By applying this SPE method, a nearly complete elution was obtained, for both benzocaine and chlorphentermine: the recoveries were 99.2 and 95.8%, respectively. With the combination of the aqueous alkaline buffer and 2.5 N NH_3 in methanol, it was again necessary to centrifuge the solvent through the solid phase.

Because of the good results obtained for both benzocaine and chlorphentermine when eluting with 1% PA in methanol or when combining 1 ml phosphate buffer pH 12, $I =$ 0.1 and 2.5 N NH_3 in methanol, these solvents were also investigated for the four small basic drugs ephedrine, cimetidine, nicotinamide and amiloride. The results are summarized in Table 7. With 1% PA in methanol recoveries between 62.6 and 97.1% were observed. Better results were obtained for the six basic drugs with 2.5 N NH_3 in methanol, after washing the solid phase with water and 1 ml of the alkaline phosphate buffer. The recoveries are all greater than 87% and a 1 ml volume was in most cases sufficient to bring about the elution. The mean, standard deviation and RSD for the repeated SPE on the SCX sorbent for 5 ppm aqueous solutions, is shown in Table 8. The relative standard deviations are not higher than 3%. This SPE method has the disadvantage of using a strong alkaline eluent with a high solvent strength, which cannot immediately be injected into a chromatograph. Dilution of the extract can reduce the solvent strength, but even a 1/6 dilution with a buffer cannot decrease the pH below 8, the upper limit for not damaging the silica bonded HPLC column. The extract thus needs to be evaporated and the residue dissolved in the mobile phase, prior to injection. This additional step can possibly cause a loss of the analyte due to thermal decomposition or the incomplete dissolution of the analyte in the mobile phase and is the time-limiting step in the whole procedure.

SPE on SCX for plasma spiked with basic analytes. When applying the SPE on SCX for spiked plasma samples, three different problems arose, namely insufficient adsorption, co-elution in the wash step with the alkaline phosphate buffer and the presence of interfering peaks in the chromatograms of the extracts. The insufficient adsorption, which was observed for benzocaine, and co-elution in the wash step, observed for benzocaine, cimetidine and ephedrine, can be explained by the competition between the analytes and ionic, endogenous plasma substances. For benzocaine the volume of extracted plasma needed to be reduced from 1 ml to 500 or 250 μl, so that the adsorption of benzocaine increased from 90.7%, to 98.8 and 100%, and the amount co-eluting in the washing with 2 ml water diminished from 47.1% to 0%. For cimetidine and ephedrine the volume of 1 ml plasma could be maintained, but the washing with phosphate buffer needed to be omitted. Thus, when extracting an analyte from an aqueous solution free from possible competing ions, the use of phosphate buffer was needed to favour the subsequent elution with ammonia in methanol; however, when plasma samples are extracted, it might be necessary to omit washing with the alkaline buffer because of the ionic strength of the matrix. The extent of the influence of the ionic strength depends on the analyte, chlorphentermine, adsorbed on a SCX

Table 7
Elution of the small basic drugs from a SCX solid phase with water, methanol, phosphate buffer pH 12, $I = 0.1$ and 2.5 N NH_3 in methanol, and with 1% propylamine in methanol

Analyte	Solvent	% Recovery in the 1st ml	2nd ml	3rd ml	Total recovery
Benzocaine	1% PA in methanol	68.6	28.5	0	97.1
	phosph. buffer pH 12	0	9.7	58.4	68.1
	2.5 N NH_3 in methanol after wash with water	76.4	1.2	1.0	78.6
	2.5 N NH_3 in methanol after wash with phosph. buffer pH 12	97.3	1.9	0	99.2
Chlorphentermine	1% PA in methanol	53.4	31.9	1.1	86.4
	phosph. buffer pH 12	0	4.9	5.8	10.7
	2.5 N NH_3 in methanol after wash with water	50.1	0.8	0	50.8
	2.5 N NH_3 in methanol after wash with phosph. buffer pH 12	95.8	0	0	95.8
Amiloride	1% PA in methanol	6.2	53.0	15.6	74.8
	phosph. buffer pH 12	0	8.3	21.8	30.1
	2.5 N NH_3 in methanol after wash with water	89.8	7.8	0	97.6
	2.5 N NH_3 in methanol after wash with phosph. buffer pH 12	93.8	3.9	0	97.7
Cimetidine	1% PA in methanol	24.0	43.2	2.1	69.3
	phosph. buffer pH 12	0	17.7	22.6	40.3
	2.5 N NH_3 in methanol after wash with water	96.0	0	0	96.0
	2.5 N NH_3 in methanol after wash with phosph. buffer pH 12	89.6	0	0	89.6
Ephedrine	1% PA in methanol	19.8	74.1	0	93.9
	phosph. buffer pH 12	0	0	0	0
	2.5 N NH_3 in methanol after wash with water	0	94.6	0	94.6
	2.5 N NH_3 in methanol after wash with phosph. buffer pH 12	93.6	0	0	93.6
Nicotinamide	1% PA in methanol	13.7	48.1	0.8	62.6
	phosph. buffer pH 12	1.6	71.7	0	73.1
	2.5 N NH_3 in methanol after wash with water	99.4	4.1	1.7	105.2
	2.5 N NH_3 in methanol after wash with phosph. buffer pH 12	87.4	0	0	87.4

Table 8
Repeatability of the SPE on SCX for 5 ppm aqueous solutions of the small basic drugs: elution with 1 ml 2.5 N NH_3 in methanol, after washing with an alkaline buffer ($n = 6$)

Analyte	Mean recovery ±SD (%)	RSD (%)
Benzocaine	98.1 ± 1.4	1.4
Chlorphentermine	96.0 ± 2.3	2.4
Amiloride	89.1 ± 1.1	1.2
Cimetidine	93.3 ± 1.3	1.4
Ephedrine	102.5 ± 2.7	2.6
Nicotinamide	98.7 ± 0.8	0.8

from an aqueous solution, could not be eluted with buffer solutions and for a plasma sample it is seen that the drug is completely retained and no co-elution occurs with the alkaline buffer, notwithstanding the higher ionic strength of the plasma. For benzocaine, on the contrary, buffer solutions were able to desorb the analyte from SCX and the ionic strength of the plasma sample is sufficiently high to prevent benzocaine's adsorption. For other drugs, such as cimetidine, the influence of the ionic strength lies somewhere in between, because the drug is well retained on a SCX from plasma, but washing with phosphate buffer introduces an excess of ions and co-elution occurs. The fact that competitive ionic substances from the plasma interfere in the adsorption and elution already indicate that the cation-exchanger does not show enough selectivity. This also becomes obvious in the chromatography of the extracts. The chromatograms of nicotinamide and ephedrine show peaks interfering near their retention time; for cimetidine and amiloride late eluting peaks, originating from the matrix, drastically increase the chromatographic run (±40 min for cimetidine or ephedrine; ±15 min for amiloride). An example is given in Fig. 5. An additional wash step with methanol did not

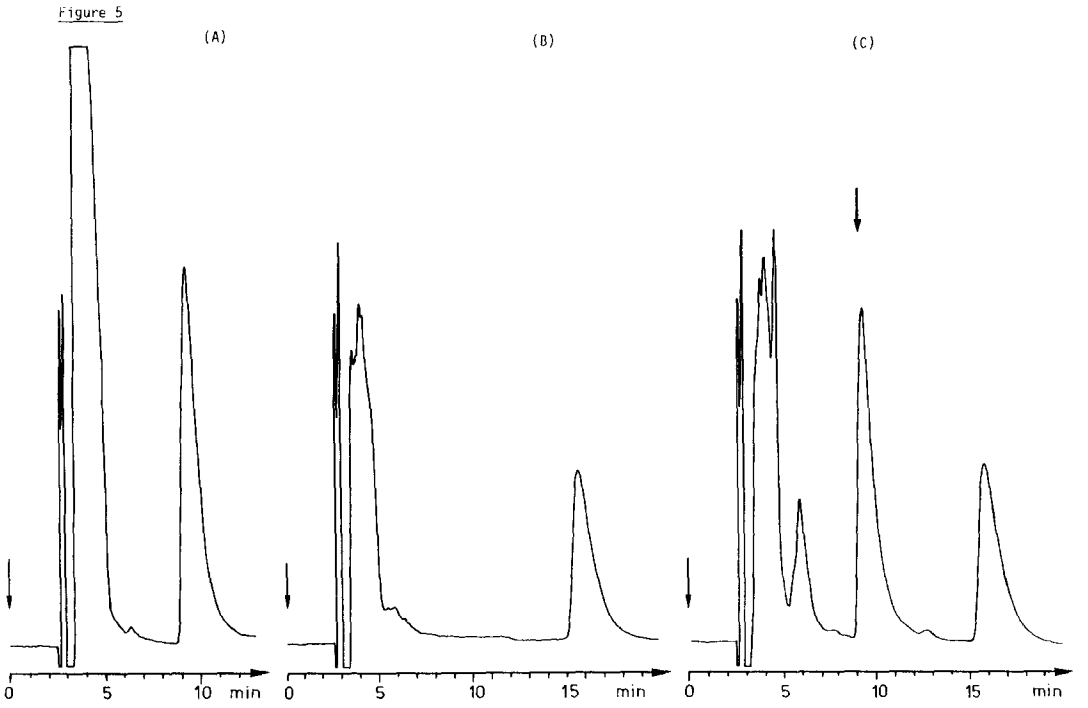

Figure 5
Chromatograms of (A) a 5 ppm standard solution of amiloride, (B) a blank plasma extract and (C) the extract of plasma spiked with 5 μg ml^{-1} amiloride. SPE on SCX; elution with 2.5 N NH_3 in methanol. For HPLC and detection conditions: see Table 2 (0.008 AUFS).

offer a solution: the interfering peaks in the chromatogram of ephedrine, nicotinamide and amiloride were still present, and cimetidine co-eluted with methanol. Only for chlorphentermine, which is chromatographed with the highest solvent strength, the interfering plasma peaks elute in front of the chromatogram and do not disturb the determination of the analyte. But for this latter compound significant losses are observed when evaporating the extract and dissolving the residue in the mobile phase, so that the only solution is to dilute the extract prior to chromatography, which consequently goes with a loss in sensitivity.

The recoveries obtained when extracting plasma samples spiked with 5 μg ml^{-1} were: 89.4% for cimetidine and 84.4% for amiloride when extracting 1 ml of plasma and using only water as a wash solvent; 90.7% for chlorphentermine when extracting 1 ml of plasma and using water and methanol as wash solvents; and 101.4% for benzocaine when extracting 250 μl of plasma and using water and methanol as wash solvents.

Elution of acid drugs from the quaternary ammonium phase (SAX). The eluents investigated for the elution of nicotinic acid and salicylic acid from SAX all had a pH <3, so that the ionization of the carboxylic group could be suppressed. Besides phosphate buffer pH 3, a citrate buffer was used. The citrate anions have a higher affinity for the ammonium functional group of the solid phase than the phosphate ions [26]. As for the basic compounds on the SCX, three different ionic strengths and the addition of methanol were investigated. Also the use of strong sulphuric acid in water or water–methanol were tried out. The results are summarized in Table 9. Different results are observed for both acids. Nicotinic acid can be easily eluted with phosphate buffer: if the ionic strength is 0.05, 2 ml are necessary to elute the total amount of analyte, but when the ionic strength is increased or 10% of methanol is added, more than 85% elutes in the first millilitre. Salicylic acid is more strongly retained on the SAX phase: the recovery also increases with the increasing ionic strength and the percentage of methanol, but a complete elution in the first millilitre is only achieved with 50% methanol added to the phosphate buffer, $I = 0.1$. Using the citrate buffer the problem arises that the

Table 9
Elution of salicylic acid and nicotinic acid adsorbed on a quaternary ammonium phase

Eluting solvent	Elution recoveries in percentage of the retained amount					
	Salicylic acid			Nicotinic acid		
	1st ml	2nd ml	Total	1st ml	2nd ml	Total
Water	0	0	0	0	0	0
Methanol	0	0	0	0	0	0
Phosphate buffer pH 3						
$I = 0.05$	0	2.2	2.2	61.4	36.1	97.5
$I = 0.1$	0	6.4	6.4	85.0	1.0	86.0
$I = 0.5$	4.1	50.5	54.6	103.1	0.8	103.9
Phosphate buffer pH 3, $I = 0.1$ with						
10% methanol	1.7	45.5	47.2	98.9	1.8	100.7
25% methanol	44.5	58.5	103.0	92.8	0	92.8
50% methanol	102.6	9.1	111.7	†	†	†
Citrate buffer pH 3						
$I = 0.06$	0	7.3	7.3	*	*	*
$I = 0.12$	0.3	8.4	8.7	*	*	*
$I = 0.6$	38.7	49.8	88.5	*	*	*
1% H_2SO_4 in H_2O	93.0	6.2	99.2	98.7	4.8	103.5
1% H_2SO_4 in H_2O–methanol (1:1)	107.1	0.6	107.7	†	†	†

* Determination of nicotinic acid not possible by means of the applied HPLC system: the solvent peak of the citrate buffer is not separated from the peak of nicotinic acid.

† Determination of nicotinic acid not possible by means of the applied HPLC system: the solvent strength of the extract was too high, so that the peak of nicotinic acid was deformed and not separated from the solvent peak.

citrate itself shows a broad peak in front of the chromatogram and this citrate peak can interfere with the analyte. This problem is seen for nicotinic acid, which is chromatographed with 100% phosphate buffer pH 3, $I = 0.05$, without the addition of an organic modifier, and has a retention time of only ±6.0 min. The retention of salicylic acid can more easily be shifted to higher values by adapting the percentage of organic modifier, so that a separation between the citrate peak and the salicylic acid peak can be achieved. When using citrate buffer to elute salicylic acid from the SAX sorbent the maximum attainable recovery was 88.5%. Better results were obtained with a 1% H_2SO_4 solution: a recovery higher than 93% was reached with 2 ml of the eluent and when adding 50% of methanol only 1 ml is needed. For nicotinic acid an almost complete recovery was obtained with 1 ml 1% H_2SO_4.

The repeatability of the SPE on SAX for a 5 ppm aqueous solution of both acids was checked. (1) The mean recovery and standard deviation of six replicate analyses was 100% ± 2.7% for nicotinic acid when eluting with 2 ml phosphate buffer pH 3, $I = 0.1$–CH_3OH (90:10) and 96.5% ± 1.5% when eluting with 1 ml of a 1% H_2SO_4 solution. (2) For salicylic acid, a mean recovery of 87.4% ± 7.7% was obtained when eluting with 2 ml phosphate buffer pH 3, $I = 0.1$–CH_3OH (75:25) and 98.5% ± 1.5% when eluting with 1% H_2SO_4–methanol (1:1).

SPE on SAX for plasma spiked with acid analytes. When applying the SPE procedure for nicotinic acid or salicylic acid on plasma samples, the same problem occurs as noticed for the SPE of basic compounds from plasma on SCX, namely the incomplete adsorption of both acids on SAX and co-elution in the washing with water. Again a solution is found by decreasing the volume of plasma sample from 1 ml to 500 or 250 µl. When starting from only 250 µl plasma, spiked with nicotinic acid, a clean extract was obtained, no matrix peaks interfered with the nicotinic acid peak and the recovery was 93.6% when eluting with 2 ml phosphate buffer pH 3, $I = 0.1$–methanol (90:10). The chromatograms of this SPE are shown in Fig. 6. For salicylic acid, complete retention on SAX was observed when using 500 µl of plasma or less. The chromatogram here however, showed interfering peaks, independent of the eluent used.

Discussion and Conclusions

The SPE of polar drugs, here roughly defined as drugs with less than 11 carbon atoms in their structure, is more difficult to develop than the SPE of larger, relatively apolår drugs, because the small drugs are difficult to retain

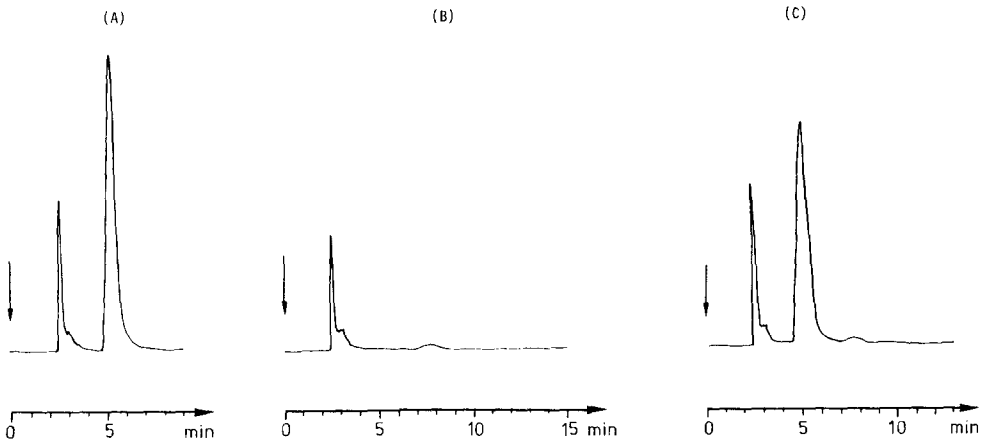

Figure 6
Chromatograms of (A) a 2.5 ppm standard solution of nicotinic acid, (B) a blank plasma extract and (C) the extract of plasma spiked with 5 μg ml^{-1} nicotinic acid. SPE on SAX; elution with 2 ml CH_3OH–phosphate buffer, pH 3 $I = 0.05$ (1:9). For HPLC and detection conditions: see Table 2 (0.016 AUFS).

by apolar van der Waals forces. The experiments above show that, for the SPE of aqueous solutions, the octadecyl sorbent could be used for five of the six small, basic molecules and for five out of nine small, (weak) acids. These observations lead to the conclusion that the use of the C_{18}-sorbent is more appropriate for small basic drugs than for (weak) acids. Not only the van der Waals forces assure their adsorption, but also the electrostatic binding on the residual silanol groups.

C_{18} is known to be a generally applicable, but less selective sorbent type and this is also evident from the experiments. For spiked plasma samples, the methods developed on aqueous solutions gave no acceptable recovery and purity for the basic drug ephedrine or for the weak acids.

Ion-exchanging silica bonded phases are, on the contrary, known as more selective phases. Their application is limited to ionic analytes, so that its use was excluded for a number of drugs under investigation, more precisely all neutral or weak acid analytes. For all six basic drugs and for the acids salicylic acid and nicotinic acid, the ion-exchanging phase could be used. The use of these anion- or cation-exchanging phases shows a number of disadvantages: firstly, elution can only be brought about with a relatively large volume of high ionic buffers, or with a strong acid or alkaline solution. Both kinds of eluent give rise to compatibility problems with the subsequent HPLC analysis. Secondly, when applying a SPE procedure on an ion-exchanging sorbent for the extraction of a plasma sample, endogenous, ionic compounds will prevent the adsorption of the analyte.

In some cases, it might be worthwhile to consider a combination of an apolar and an ion-exchanging sorbent. This is, for example, the case for the basic drug chlorphentermine. When extracting this drug on the SCX phase with the methanolic ammonia solution as

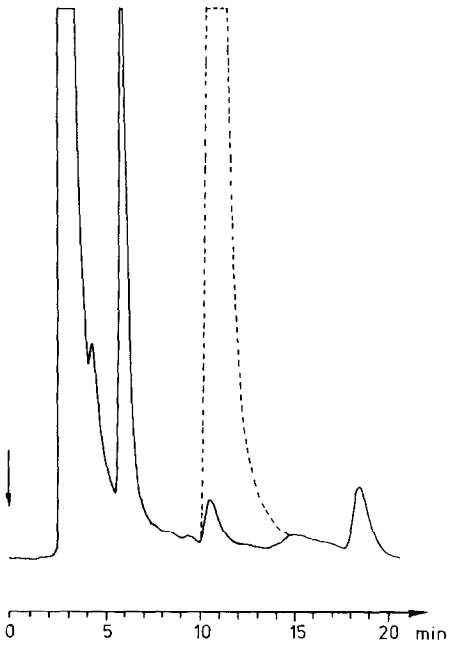

Figure 7
Chromatogram of a blank plasma extract after SPE on SCX + C_{18}. SPE and HPLC and detection conditions for chlorphentermine (0.008 AUFS). Peak of a standard solution of chlorphentermine is indicated by a dashed line.

eluent, significant losses (up to 44%) are observed when evaporating the eluent and reconstituting the residue in the mobile phase. When combining the SPE on SCX and C_{18}, the evaporation step can be omitted.

A combined use of an apolar and ion-exchanging phase offers however no solution to the problem of selectivity. For chlorphentermine, for example, the extraction on SCX, followed by an SPE on C_{18}, is not more selective than the SPE on C_{18} alone: as can be seen in Fig. 7, the chromatogram presents a small interfering peak.

Towards a strategy for SPE

On the basis of the observations made in

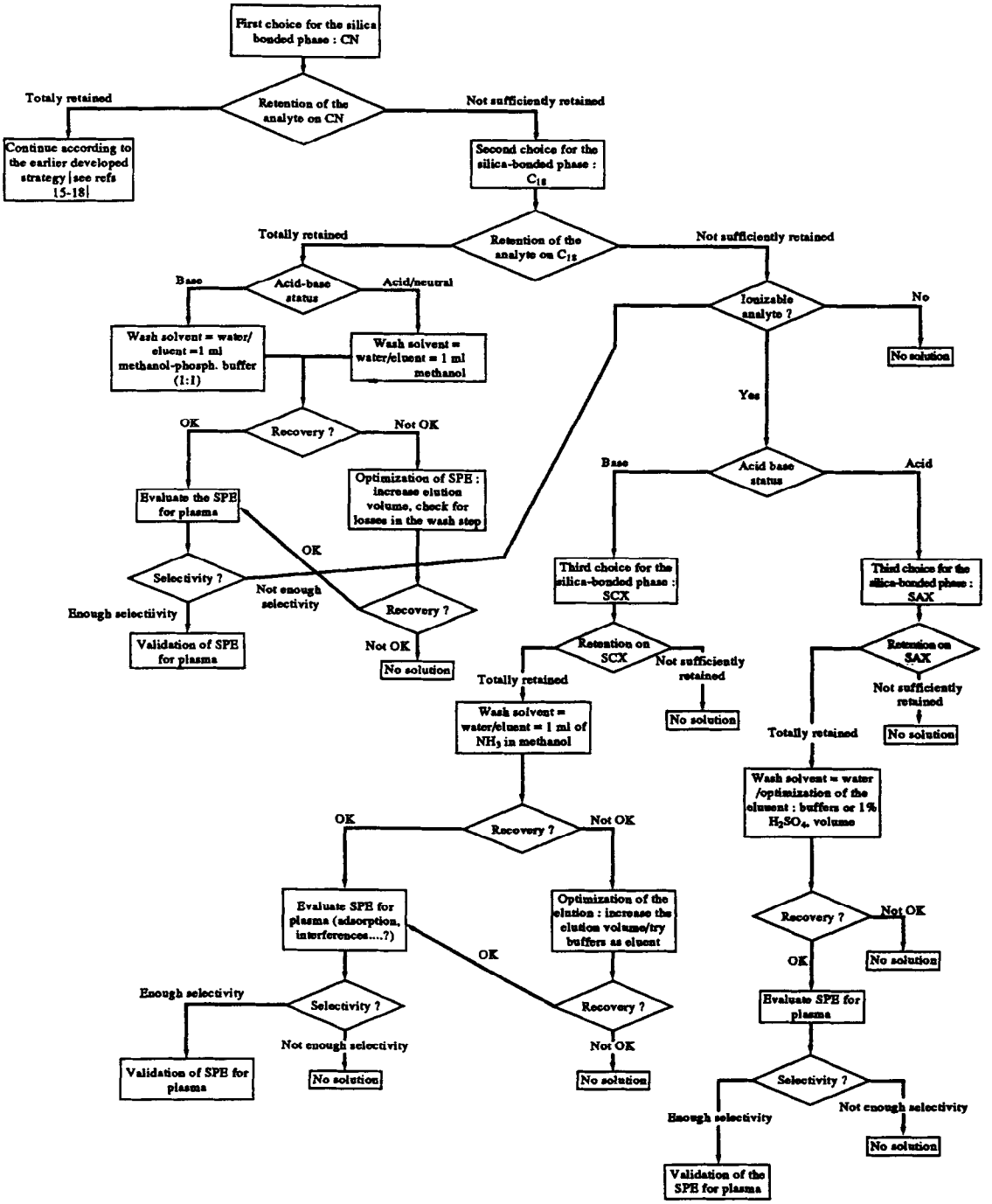

Figure 8
Decision tree for the SPE of small, relatively polar analytes.

these experiments, an attempt can be made to extend the earlier developed general approach for SPE [15–18]. A decision tree for the SPE of relatively polar analytes is outlined in Fig. 8. The cyanopropyl silica bonded phase remains the preferred and first choice sorbent type. For small polar drugs, which are insufficiently retained on a cyanopropyl cartridge, the octadecyl sorbent will be the first alternative to explore. If the drug is retained on the C_{18}-phase, then a SPE procedure using water as wash solvent and, according to the drug being an acid or a base, methanol or methanol–phosphate buffer pH 3, $I = 0.05$ as eluent can be evaluated. If this procedure yields an acceptable and reproducible recovery, its selectivity for a plasma sample must be evaluated. Whether the octadecyl sorbent will show enough selectivity largely depends on the concentration levels to determine and the HPLC system used (mobile phase composition, detection system).

If the drug does not show enough retention either on the cyanopropyl or on the octadecyl sorbent, this clearly indicates that an extraction procedure based on other interactions than the van der Waals forces must be applied. For drugs with ionizable functions, the use of an ion-exchanging solid phase can be considered. For basic drugs, the SPE procedure on the SCX, using ammonia in methanol as eluent is advised. Whether an alkaline phosphate buffer or methanol should be used in the wash steps or how large the volume of the plasma sample can be, must be investigated. To ensure the compatibility with the subsequent HPLC analysis, the eluent needs to be evaporated to dryness and the residue is reconstituted in the mobile phase. For drugs with negatively charged groups, the SPE procedure on the SAX phase, using a mixture of phosphate buffer pH 3, $I = 0.1$–methanol or sulphuric acid–methanol can be investigated. The ratio of the phosphate buffer or sulphuric acid and methanol, as well as the elution volume and the volume of plasma that can be extracted needs to be optimized. It is important to check in an early stage whether the ion-exchanging phase results in a sufficiently clean extract: the chromatograms of the plasma extracts obtained after SPE on an ion-exchanging phase can show interfering peaks, partly caused by endogenous plasma compounds, and partly because of the extreme elution conditions (extreme pH, high ionic and solvent strength) incompatible with the HPLC mobile phase.

For polar drugs not retained on the octadecyl sorbent and having no ionizable functional groups, no alternative can be presented for the moment.

The criteria used in the decision tree (Fig. 8) to follow a certain branch may appear vague. How should one decide whether an analyte is sufficiently retained or not, what is a good recovery and what does enough selectivity mean? These decisions have to do with the evaluation of a result by a user and depend on his context. On the basis of our own experience with SPE, we think an analyte must be adsorbed for at least 95% and should not elute when the solid phase is washed with the same volume and type of solvent as the analyte was dissolved in. Recoveries should be larger than 80%. The evaluation of the selectivity includes several points, namely whether the adsorption is influenced by the matrix or not, and whether matrix compounds interfere in the subsequent analysis.

Acknowledgement — The authors thank Mrs K. Decq for skilful technical assistance and the FGWO for financial support.

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[*Received for review 10 August 1993*]