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ISOLATION OF BASIC DRUGS FROM PLASMA USING SOLID-PHASE EXTRACTION WITH A CYANOPROPYL-BONDED PHASE

GREET MUSCH and DESIRE L. MASSART*

Vrije Universiteit Brussel, Farmaceutisch Instituut, Laarbeeklaan 103, 1090 Brussels (Belgium)

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SUMMARY

The use of a CN sorbent for the isolation of basic compounds from plasma is described. Adsorption from water and plasma was investigated for a test set of 30 basic drugs. It was found that compounds with a carbon chain length $\geqslant 11$ are totally retained and that the competitive effect of the matrix on the adsorption is minimal. Methanol–phosphate buffer (pH 3, $\mu = 0.05$) (50:50) yielded good recoveries for more polar compounds; apolar basic drugs can be efficiently eluted using methanol containing 0.1% propylamine. Water up to 3 ml can be used for the washing step. This approach was applied to the determination of eight drugs in plasma at therapeutic levels. The absolute recoveries ($n = 6$) obtained were $98.8 \pm 7.3\%$ for papaverine, $82.3 \pm 3.9\%$ for practolol, $83.4 \pm 2.6\%$ for metoclopramide, $87.3 \pm 5.8\%$ for imipramine, $82.8 \pm 3.3\%$ for procaine, $82.7 \pm 4.6\%$ for morphine, $87.5 \pm 7.2\%$ for propranolol and $90.4 \pm 6.2\%$ for yohimbine.

INTRODUCTION

Drug assays in biological materials usually consist of two parts: sample pretreatment followed by appropriate analysis of the compounds of interest in the resulting extract. For the development of a high-performance liquid chromatographic (HPLC) analysis, this means that the sample pretreatment determines the minimal concentration that must be detected and the required selectivity, and so has implications for the selection of an appropriate detector, which in turn has implications for the selection of appropriate separation parameters [1].

This explains the interest in a general approach to sample pretreatment. Such a general strategy was successfully developed in our laboratory for the extraction of basic substances from plasma using ion-pair extraction [2]. However, this technique is very time-consuming, labour-intensive and difficult to automate, and therefore solid-phase extraction (SPE) has been investigated.

The off-line approach was preferred to the on-line trace enrichment technique because of the greater flexibility of the former and also because in the column-

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switching technique various additional critical issues such as the length of the precolumn, straight or backflushing and precolumn coupling time must be optimized [3–6], all of which make the off-line approach more attractive for a systematic study of the SPE of basic drugs.

The object of this study was not to propose new methods or to give a deeper insight into SPE, but to show that a strategic, generalized approach to SPE is possible, thereby making method development much easier. It means that instead of finding ad hoc solutions for all new problems, one can try to follow a strategy which in many instances will lead rapidly to a solution. In this first attempt the sorbent selected was a CN-bonded silica. It was shown previously [1,7] that CN-bonded phases permit a strategy to be developed that allows nearly all drugs to be chromatographed. The experience obtained in chromatography can be applied here, although the aim is different. In chromatography one tries to obtain partition coefficients resulting in capacity factors in the region $k' = 1$–10. In solid-phase extraction the equilibrium constant for the isolate retention should be high, for instance greater than 1000, and conversely, when elution is desired, the equilibrium constant of the compound of interest should be very low, for instance less than 0.001.

A second reason for this selection is the intermediate polarity of the CN cartridge. One can hope that this type of sorbent can be used for the isolation of compounds from both an aqueous matrix and an apolar medium. Compared with C_{18} and other apolar sorbents, the elution should be easier in most instances as the less apolar the sorbent, the easier is the elution in the reversed-phase mode. For these reasons the same kind of solid phase is used in both the precolumn and the separation column, although the selectivity is better in dual-column systems with different solid phases.

A test set of 30 basic substances with different basicities and polarity properties was selected. The adsorption on a CN cartridge from water was investigated for each drug and the effects of several possible interferences were studied.

A second crucial point is the selection of an appropriate washing and eluting agent. The ideal wash solvent would remove all the possible plasma interferences without loss of the active compound. The optimal eluent elutes totally the drug of interest and must be compatible with the selected chromatographic conditions. For those bases which are completely adsorbed we tried to develop such optimal eluents. From these results, starting conditions for the isolation of basic compounds from plasma were derived and applied.

The conditions derived were then used for the determination of eight basic substances in plasma in order to illustrate their compatibility with the chromatographic parameters described in earlier work, namely a CN stationary phase in combination with a mobile phase composed of acetonitrile–phosphate buffer (pH = 3, $\mu = 0.05$) and three modes of detection: UV, amperometric and fluorescence.

EXPERIMENTAL

Apparatus

Chromatography was carried out using a Varian (Walnut Creek, CA, U.S.A.) 5000 liquid chromatograph equipped with a Rheodyne injector (100 μl) and a

1040-A diode array detector (optical pathlength 0.6 cm, cell volume 4.5 μl) from Hewlett-Packard (Avondale, PA, U.S.A.), coupled in series with a Perkin-Elmer (Überlingen/See, F.R.G.) LS 4 fluorescence detector. The chromatograms were recorded with a BD 9 two-pen recorder (Kipp and Zonen, Delft, The Netherlands) and a Vista CDS 401 instrument (Varian, Palo Alto, CA, U.S.A.) was used as an integrator.

As an amperometric detector an LKB (Bromma, Sweden) Model 2143 instrument was used (glassy carbon electrode, palladium reference electrode, cell volume 5.5 μl) coupled to a Varian 8500 pump equipped with a 100-μl Valco injector. The chromatograms were recorded with a Shimadzu (Kyoto, Japan) IC-R3A data processor.

Chromatography

The stationary phase was CN-bonded phase LiChrosorb (Merck, Darmstadt, F.R.G.) with a particle size of 5 μm in a 25 cm $\times$ 0.4 cm I.D. column. The mobile phase was acetonitrile–phosphate buffer (pH 3, $\mu = 0.05$) containing 0.001 *M* sodium chloride. The concentration of organic modifier ranged from 3 to 40%.

Chromatography was carried out at a flow-rate of 1 ml/min at ambient temperature. A pre-column tube (3 cm $\times$ 0.4 cm I.D.) packed with CN-bonded LiChrosorb (10 μm) was used in order to protect the analytical column.

Drugs and reagents

All drugs were of pharmacopeial purity. Stock solutions (100 ppm) were prepared and diluted with water. This solution was used to load the sample (water or plasma). Standard solutions were obtained by diluting the stock solution with water (for the study of the adsorption and wash step), with methanol–phosphate buffer (pH 3, $\mu = 0.05$) or with mobile phase (for the study of the elution). The stock solutions were stored at 4 °C. The standard solutions were prepared fresh daily.

Acetonitrile and methanol were purchased from Merck. For the chromatography the acetonitrile was of liquid chromatographic grade; for other purposes analytical-reagent grade solvents were used. Phosphoric acid, sodium dihydrogenphosphate and sodium chloride were also purchased from Merck. Propylamine was obtained from Fluka (Buchs, Switzerland). All glass tubes were silanized using Surfasil (Pierce, Oud Beyerland, The Netherlands) diluted 1:10 with acetone.

Sample pretreatment

A Baker (Deventer, The Netherlands) 10 SPE vacuum manifold was used. The CN cartridges (1-ml capacity) were also purchased from Baker. During this study several batches were used. However, the different stages (adsorption, wash and elution, extraction from plasma) were carried out with cartridges belonging to the same batch.

The sorbent was conditioned using 2 $\times$ 1 ml of methanol and 2 $\times$ 1 ml of water. The sorbent was not allowed to dry out at the end of the conditioning step.

Frozen plasma was allowed to thaw and warm to room temperature. Then, 1

ml of plasma was transferred into a silanized glass tube loaded with 100 μl of an aqueous drug standard and deproteinized by adding 2 ml of acetonitrile dropwise under continuous vortexing. The supernatant was transferred into a clean silanized glass tube and applied as such or after dilution with water (1:5) on the cartridge. The plasma interferences were removed by washing the cartridge with 3×1 ml of water. Finally, the drug was recovered using 1 ml of eluent [methanol–phosphate buffer (pH 3, μ=0.05) (50:50) or methanol+0.1% propylamine]. The former eluent was injected directly into the chromatographic system; the latter was evaporated and reconstituted in 1 ml of mobile phase.

Experimental conditions for the assay of eight basic drugs

Papaverine. UV detection was performed at 252 nm. An amount of 1 ml of plasma was spiked and deproteinized with 2 ml of acetonitrile. For this drug it was necessary to dilute the supernatant sufficiently with water in order to provide 100% adsorption. The eluent used was methanol+0.1% propylamine and after evaporation this was reconstituted in 1 ml of mobile phase.

Practolol. This drug was monitored using UV detection at 247 nm and the concentration of organic modifier for HPLC was 5%. The eluent was methanol–phosphate buffer.

Metoclopramide. Amperometric detection was used at a potential of 1.1 V. The recommended procedure for solid-phase extraction was used as such; the eluent was methanol–phosphate buffer.

Imipramine and desipramine. Amperometric detection was used at 1.1 V. The eluent used was methanol+0.1% propylamine.

Procaine. The same chromatographic and extraction conditions were used as for metoclopramide.

Propranolol. Propranolol possesses native fluorescence detection and was monitored at an excitation wavelength of 287 nm and an emission wavelength of 338 nm. The eluent used for the SPE procedure was methanol containing 0.1% propylamine.

Yohimbine. Yohimbine was monitored with fluorescence detection at an excitation wavelength of 272 nm and an emission wavelength of 350 nm. The recommended procedure was carried out using methanol–phosphate buffer as eluent.

RESULTS AND DISCUSSION

Adsorption

The retention power of the cyanopropyl phase for basic drugs in water was studied for a test set of 30 compounds. The sorbent was the same as the CN-bonded phase used in HPLC for the reasons mentioned before. As it is well known [7] that the primary interactions of the CN sorbent in polar solvent environments are due to hydrophobicity, drugs possessing a different number of carbon atoms were incorporated into the set. The adsorptive properties of bonded silica are also due to the polar character of the silica and the residual silanols remaining on the surface; in aqueous solutions this means that there is a possible ionic in-

teraction with protonated bases. For this reason drugs with various basic properties were studied.

The results are presented in Table I. In general, the drugs with a carbon chain length $\geqslant 11$ can be considered as a group giving 99–100% adsorption. This phenomenon is observed for both relatively weak bases such as nicotinamide and benzocaine and for relatively strong bases such as epinephrine, ephedrine and chlorphentermine.

The retention of basic drugs on a CN sorbent in aqueous conditions yields good results only when the substance is sufficiently apolar. The secondary interactions are not strong enough to result in maximal adsorption even when the concentration of the drug is decreased or when cartridges with a higher content of sorbent are used. This is confirmed by the fact that neutral compounds with a carbon chain length < 11 are also only partially retained on a CN sorbent under the same

TABLE I

ADSORPTION OF 30 BASIC DRUGS FROM WATER ON A CN SORBENT

Compound	Number of C atoms	Adsorption (%) ($n=2$)
Nicotinamide	6	36
Amiloride	6	100
Benzocaine	9	81
Epinephrine	9	75
Ephedrine	10	84
Chlorphentermine	10	71
Mexiletine	11	100
Metoclopramide	12	100
Triamterene	12	100
Terbutaline	12	100
Salbutamol	13	100
Procaine	13	100
Amylocaine	14	99
Practolol	14	100
Trimethoprim	14	100
Tiapride	15	100
Propranolol	16	100
Morphine	17	100
Chlorpromazine	17	100
Fenoterol	17	100
Desipramine	18	100
Imipramine	19	100
Prochlorperazine	20	100
Papaverine	20	100
Yohimbine	21	100
Strychnine	21	100
Vincamine	21	100
Verapamil	27	100
Narcotine	22	99
Reserpine	33	100

conditions. This is the case for phenacetin (86%), sulphanilamide (43%) and *p*-aminophenol (52%). An exception to this is the basic drug amiloride. Although this drug contains only six carbon atoms it is totally adsorbed on the CN cartridge. It was thought at first that this could perhaps be explained by the presence of three primary amine functions, favourably localized so that secondary interactions might become more important. Amiloride was also totally retained on a C_2 cartridge. In order to study the influence of residual silanols on the adsorption of amiloride, the CN cartridge was conditioned twice with 1 ml of methanol followed by two cartridge volumes of water containing 0.1% propylamine so that the silanols were masked. The pH of this solution was adjusted to neutral. Amiloride was still totally adsorbed on the CN cartridge so that the exception cannot be explained by secondary interactions. As the pK_a of amiloride is relatively high (8.7), meaning that the greater part of the drug is protonated, this effect also cannot be explained by possible hydrogen bonding.

The drug–sorbent interaction can be influenced by the matrix. Some plasma components can occupy the active sites of the sorbent, resulting in a decreased adsorption of the drug of interest. Therefore, adsorption from plasma was studied and compared with adsorption from water. The results are reported in Table II. The competitive effect of the matrix is low. This was investigated for relatively high drug concentrations (1 μg/ml). It is not possible to study the adsorption step separately (it is not part of a complete adsorption–wash–elution cycle) at lower levels owing to the plasma impurities resulting in a large blank peak. However, as will be shown later with eight practical examples, the results of a complete extraction procedure from plasma (i.e., at therapeutic and therefore low levels) are similar to those from water. It can be concluded that, if there is an effect on the adsorption, it must be minimal.

TABLE II

COMPARATIVE STUDY OF THE ADSORPTION OF DRUGS FROM WATER AND FROM PLASMA

Compound	Adsorption from water (%) ($n=2$)	Adsorption from plasma (%) ($n=2$)
Imipramine	100	97
Morphine	100	100
Practolol	100	97
Narcotine	99	98
Prochlorperazine	100	98
Chlorpromazine	100	96
Metoclopramide	100	93
Tiapride	100	92
Procaine	100	100
Chlorphentermine	71	66
Amiloride	100	92
Ephedrine	84	75
Amylocaine	99	93

A further important aspect in this context is the drug–matrix interaction, which with the plasma matrix can be described as protein binding. As in general proteins are large molecules, the protein–drug complex is excluded from the sorbent pores (60 μm). This implies that the protein–isolate binding must be broken, freeing the drug for total retention. This may not be necessary in all instances, but it certainly is in some. For instance, we have shown (unpublished work) that when pentoxiphylline is determined at therapeutic levels in plasma, omission of the deproteinization step leads to losses of 50%. Therefore, it was necessary to implement a deproteinization step before the adsorption step. The proteins were precipitated with a double amount of acetonitrile. It was shown to be unnecessary to dilute the supernatant as most drugs were still retained on the CN cartridge even in this more apolar medium. There were some exceptions, however. Among the 25 substances tested we found two, papaverine and narcotine, which were retained only 46 and 63%, respectively.

Elution

Different eluents were tried for all the basic drugs that are efficiently adsorbed on the CN cartridge. First the pure solvents used in general in reversed-phase chromatography such as water, methanol and acetonitrile were investigated. The recoveries obtained using 1 ml of solvent are given in Table III. Water up to 3 ml can be used as the washing solvent so that polar plasma interferences are removed from the cartridge without loss of the pharmaceutical compound of interest.

In general the recoveries with pure solvents were not good, except for narcotine. Methanol yielded significantly better results than acetonitrile and for procaine,

TABLE III

ELUTION RECOVERIES FOR SOLVENTS USED IN THE REVERSED-PHASE MODE

Compound	Recovery (%) ($n=2$)		
	Water	Methanol	Acetonitrile
Mexiletine	0	39	23
Salbutamol	0	0	0
Practolol	0	60	60
Desipramine	0	55	45
Chlorpromazine	0	62	24
Propranolol	0	0	0
Procaine	0	86	64
Amiloride	0	92	64
Metoclopramide	0	67	50
Prochlorperazine	0	89	32
Morphine	0	83	58
Imipramine	0	41	25
Papaverine	0	82	2
Narcotine	0	100	103
Yohimbine	0	0	0
Tiapride	0	61	59

amiloride, morphine and papaverine more than 80% was eluted from the cartridge. This can be explained by the fact that methanol can act as both a hydrogen donor and acceptor whereas acetonitrile has only hydrogen-accepting properties.

In order to find more suitable eluents the experience obtained with chromatography was applied. As in earlier work with amperometric detection it was found that a CN column in combination with the mobile phase acetonitrile–phosphate buffer (pH 3, μ=0.05) (40:60) provided relatively small k' values for a large amount of drugs, the use of this mobile phase as eluent was investigated. The elution yields were considerably increased (see Table IV). Nevertheless, this eluent was not suitable owing to co-elution of impurities from the cartridge. These interfering peaks were especially observed with amperometric detection but even with UV detection this eluent was not compatible (see Fig. 1).

For this reason an analogous eluent for which the interferences were not observed, namely methanol–phosphate buffer (pH 3, μ=0.05) (50:50), was investigated in order to study the extraction yields. The recoveries obtained were similar except for the phenothiazines, chlorpromazine and prochlorperazine.

For some drugs, namely propranolol and imipramine (relatively strongly basic drugs), papaverine and triamterene (relatively weakly basic compounds) the influence of the percentage of methanol, the pH of the buffer and the ionic strength on the elution yield were investigated. A representative graph is shown for imipramine in Fig. 2. It can be concluded that an acidic environment (pH 3) is necessary in order to obtain satisfactory recoveries. The use of buffer systems offers the advantge that the amount of organic modifier can be lowered when the ionic strength is high enough (Fig. 2). This explains the use of 50% methanol in combination with 50% phosphate buffer (pH 3, μ=0.05) as eluent.

As the extraction yields were not good for the phenothiazines, we investigated if the recoveries could be enhanced by using a higher amount of eluent. An ex-

TABLE IV

COMPARATIVE STUDY OF ELUTION RECOVERIES WITH ACETONITRILE–PHOSPHATE BUFFER VERSUS METHANOL–PHOSPHATE BUFFER

Compound	Recovery (%) (n=2)	
	Acetonitrile–phosphate buffer (pH 3, μ=0.05) (40:60)	Methanol–phosphate buffer (pH 3, μ=0.05) (50:50)
Practolol	84	104
Desipramine	90	95
Imipramine	85	85
Chlorpromazine	77	60
Procaine	102	91
Imipramine	100	85
Amiloride	83	88
Metoclopramide	95	93
Prochlorperazine	85	20
Morphine	85	97

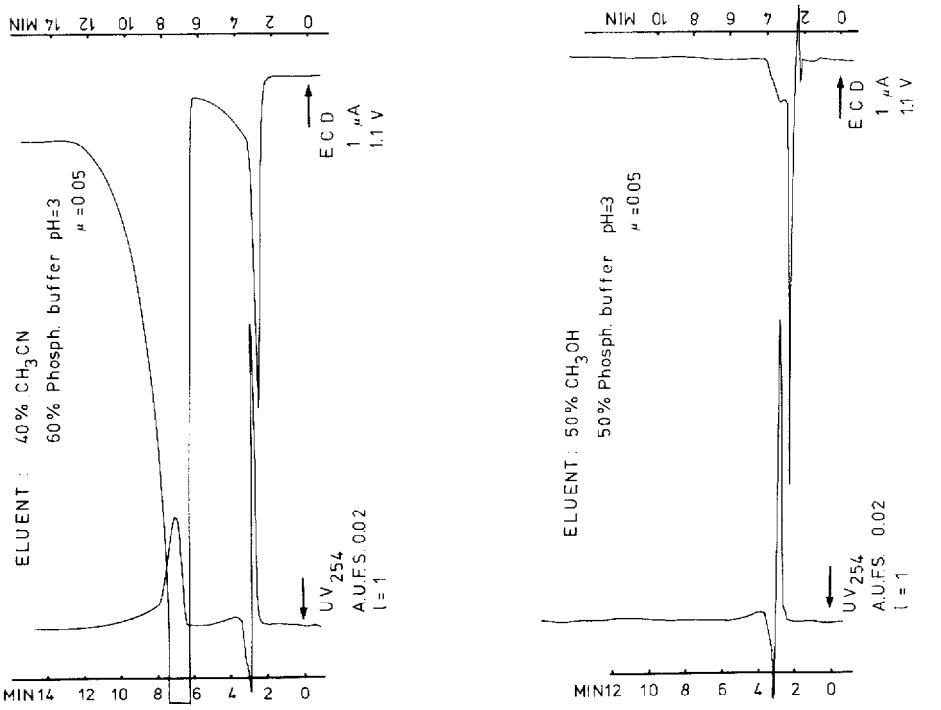

Fig. 1. Left: chromatogram of the impurities obtained after elution of the cartridge with acetonitrile–phosphate buffer (pH 3, $\mu=0.05$) (40:60). Right: clean chromatogram obtained after elution of the cartridge with methanol–phosphate buffer (pH 3, $\mu=0.05$) (50:50).

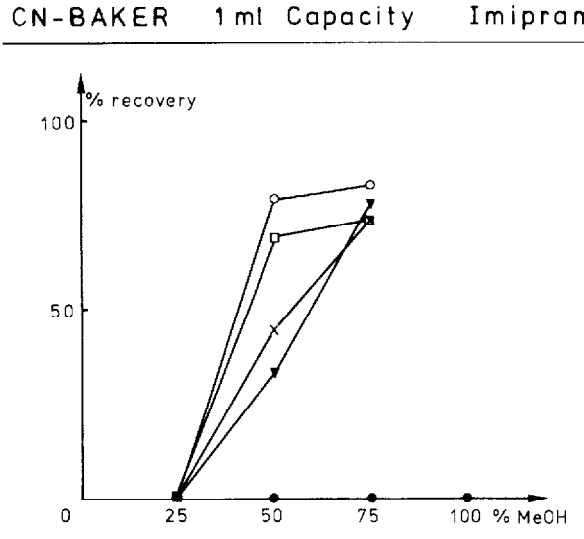

Fig. 2. Recovery of imipramine as functions of pH, ionic strength and percentage of methanol in the eluent. ●, Water, pH 7; ▼, water, pH 3; ×, $\mu=0.025$; ○, $\mu=0.05$; □, $\mu=0.1$.

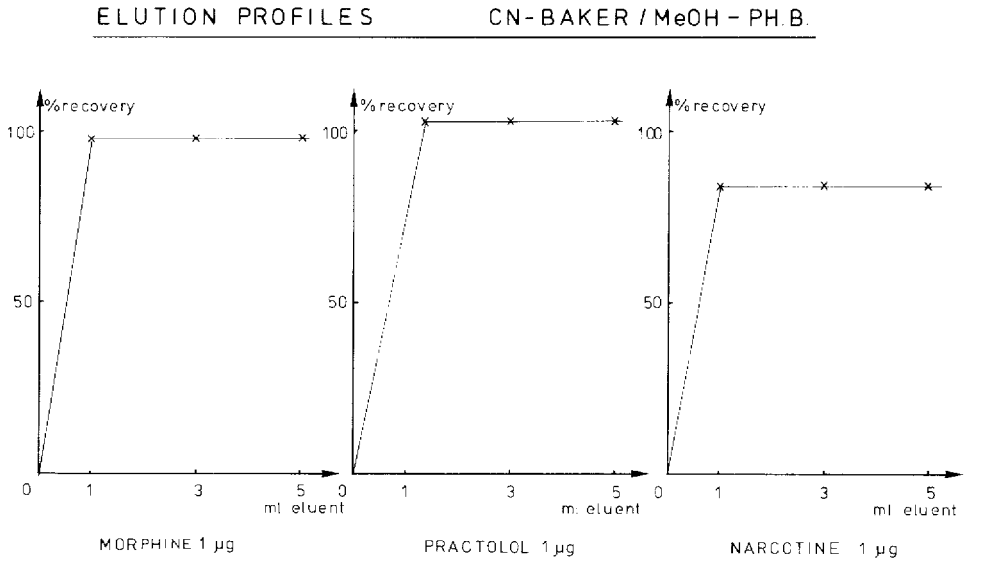

Fig. 3. Recovery of morphine, practolol and narcotine as a function of the amount of eluent.

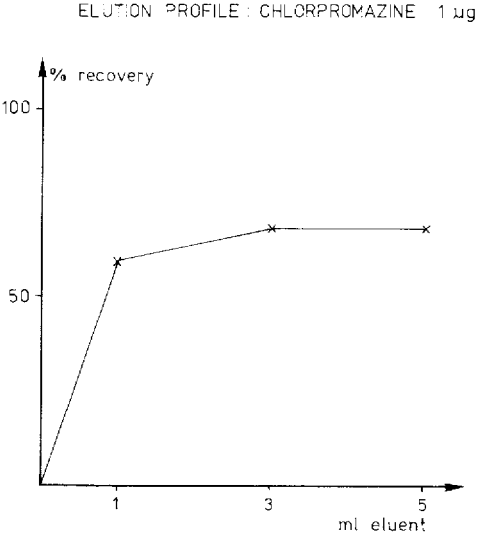

Fig. 4. Recovery of chlorpromazine as a function of the amount of eluent.

ample of such an elution profile is given for a few drugs in Fig. 3. We conclude that the extraction yields are not enhanced by using more eluent; this means that some active sites are still not reached and/or that the properties of the sorbent are changed by the eluent so that an increased amount of eluent does not offer better recoveries. This implies that one needs a more efficient eluent for more apolar drugs (e.g., for chlorpromazine in Fig. 4).

As it is known that basic drugs interact with residual silanol functions, the use of methanol containing 0.1% propylamine was investigated. The results are pre-

TABLE V

COMPARATIVE STUDY OF ELUTION RECOVERIES WITH METHANOL–PHOSPHATE BUFFER VERSUS METHANOL–PROPYLAMINE

Compound	Recovery (%) ($n=2$)	
	Methanol–phosphate buffer (pH 3, $\mu=0.05$) (50:50)	Methanol + 0.1% propylamine
Mexiletine	95	40
Terbutaline	105	83
Triamterene	95	102
Salbutamol	102	83
Procaine	91	65
Practolol	104	102
Trimethoprim	94	92
Tiapride	88	92
Propranolol	86	92
Chlorpromazine	60	75
Morphine	97	75
Fenoterol	100	35
Desipramine	95	80
Imipramine	85	86
Prochlorperazine	20	75
Papaverine	72	86
Yohimbine	91	74
Vincamine	93	94
Strychnine	92	89
Narcotine	84	96
Verapamil	61	89
Reserpine	25	87

sented in Table V, from which it is clear that this eluent provides better elution yields for more apolar compounds such as for prochlorperazine, papaverine, narcotine, verapamil and reserpine. However, for more polar compounds such as mexiletine and terbutaline better recoveries were obtained using methanol–phosphate buffer as eluent. Even when higher amounts of propylamine were added to methanol (e.g., 1%) the elution recoveries did not increase.

This tendency was observed for both strongly and weakly basic drugs. The methanol–phosphate buffer eluent offers the advantage that it can be injected directly in the mobile phase used here, i.e., an acetonitrile–buffer system. It has the advantage that it is compatible with the three modes of detection. The other eluent, methanol containing 0.1% propylamine, must first be evaporated and reconstituted in the mobile phase. This drawback is compensated for by the possibility of trace enrichment on the one hand and because this approach is compatible with all mobile phases even under normal-phase conditions, which is more difficult for the first eluent, on the other hand.

Applications

The use of the recommended approach is illustrated for eight basic drugs at a level typical of therapeutic levels of basic drugs (100 ng/ml in plasma) using UV,

amperometric and fluorescence detection. In general the recommended concentration of acetonitrile in the HPLC mobile phase was 40%. The detailed experimental conditions are given under Experimental for each of the eight drugs separately.

Papaverine. The recovery obtained was $98.8 \pm 7.3\%$ ($n=6$). Chromatograms are shown in Fig. 5.

Practolol. For this drug the SPE yield obtained was $82.3 \pm 3.9\%$ ($n=6$).

Metoclopramide. The extraction yield was $83.4 \pm 2.6\%$ ($n=6$).

Imipramine and desipramine. This is a second example where amperometric detection was used. The extraction yield obtained was $87.3 \pm 5.8\%$ ($n=6$) for imipramine and $81.4 \pm 3.7\%$ ($n=6$) for desipramine. This is illustrated in Fig. 6.

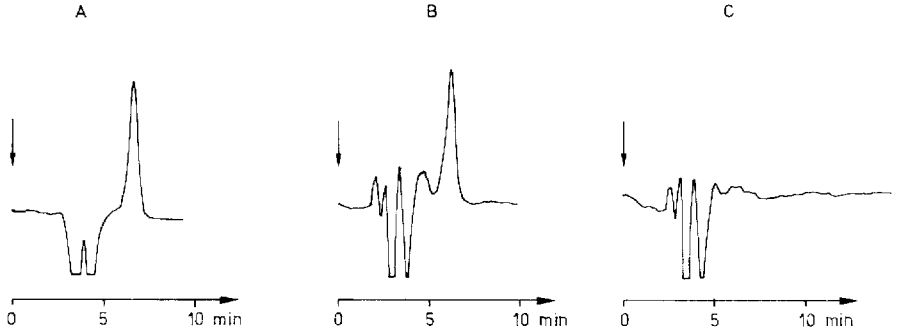

Fig. 5. Determination of papaverine in plasma: (A) aqueous standard; (B) plasma standard; (C) blank plasma.

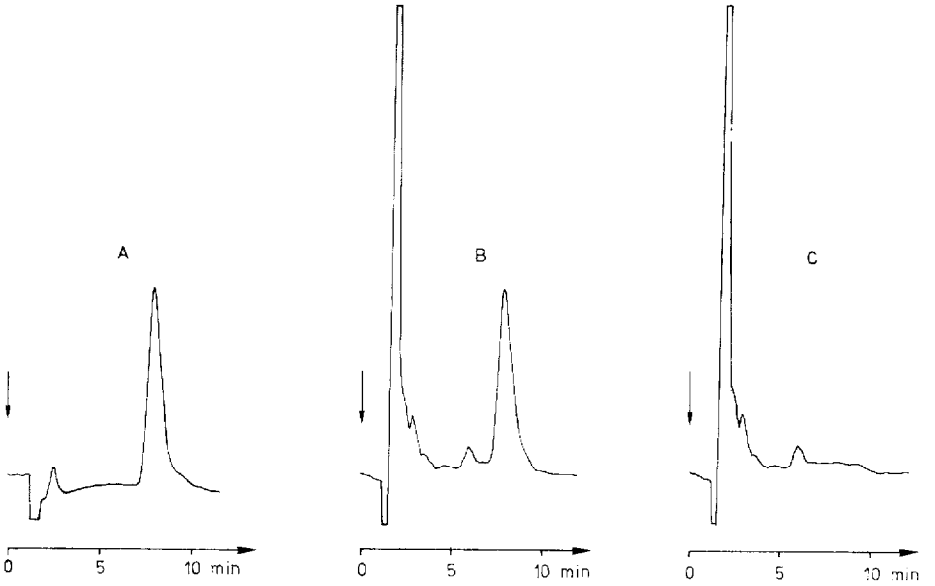

Fig. 6. Determination of imipramine in plasma: (A) aqueous standard; (B) plasma standard; (C) blank plasma.

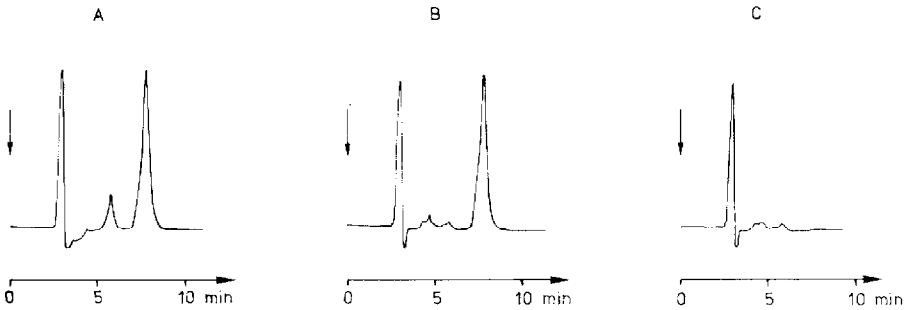

Fig. 7. Determination of yohimbine in plasma: (A) aqueous standard; (B) plasma standard; (C) blank plasma.

Procaine. An extraction yield of $82.8 \pm 3.3\%$ ($n=6$) was obtained for this local anaesthetic.

Morphine. The SPE yield for this drug was $82.7 \pm 4.6\%$ ($n=6$).

Propranolol. The recommended procedure of the β-blocker resulted in a recovery of $87.5 \pm 7.2\%$ ($n=6$).

Yohimbine. The recovery of this basic drug was $90.4 \pm 6.2\%$ ($n=6$). Chromatograms are presented in Fig. 7.

CONCLUSIONS

This work represents a first approach to the development of a general method for the isolation of basic drugs from an aqueous medium and plasma using SPE with a CN sorbent. From the results obtained, it appears that acceptable results can be obtained for 24 of the 30 drugs, counting only the drugs with more than 11 carbon atoms. The adsorption step causes problems only for more polar drugs with a carbon chain shorter than 11 and no competitive effect of the matrix was observed during the adsorption step.

For all the drugs investigated water can be used to remove selectively the plasma interferents from the cartridge so that the blanks obtained are acceptable for UV, amperometric and fluorescence detection. In general, the pure solvents used in reversed-phase chromatography do not yield good recoveries. However, good results were obtained using 1 ml of methanol–phosphate buffer (pH 3, $\mu=0.05$) (50:50) and for less polar basic substances methanol containing 0.1% propylamine. The eluents are compatible with the candidate chromatographic systems that can be selected by an expert system [1].

The use of a CN sorbent for the isolation of acidic and neutral compounds is under investigation.

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