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Development of a standardized analysis strategy for basic drugs using ion-pair extraction and high-performance liquid chromatography — VII. Determination of drugs in plasma

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Abstract: A standardized analysis strategy for basic drugs which had been applied previously to pharmaceutical dosage forms, cosmetics and saliva has been applied to plasma. Low extraction recoveries that were obtained at first were shown by protein binding studies to be due to interactions between plasma proteins, the drug and the organic ion-pairing reagent. These interactions could be avoided by removal of the plasma proteins from the sample prior to addition of the counterion solution. The standardized analysis strategy was shown to be applicable to the determination of both polar and non-polar drugs in plasma.

Keywords: *Ion-pair extraction applied to plasma; octylsulphate; di(2-ethylhexyl) phosphoric acid; interaction with plasma proteins; standardized analysis strategy; HPLC.*

Introduction

Previous papers in this series have reported the concept, philosophy and advantages of a standardized analysis strategy for basic drugs [1, 2] and its application to pharmaceutical dosage forms [3, 4], cosmetics [5] and saliva [6]. The present paper is intended to demonstrate the possible applications of this strategy for the determination of various basic drugs in plasma. A second aim is to cast some light on drug interactions with plasma proteins, which may occur when applying an ion-pair extraction technique to plasma using an organic ion-pairing reagent.

Because of its efficiency, the ion-pair extraction technique has found a number of applications in drug analysis [7–10]. Most of these applications, however, are concerned with the extraction of drugs from aqueous solution or urine. By comparison, fewer applications to plasma are known and it is striking that inorganic counterions, such as Cl^-, I^- and ClO_4^- are mainly used for extraction. Organic ion-pairing reagents, which should guarantee higher extraction recoveries owing to the more hydrophobic nature of the resulting ion-pair, are rarely applied to plasma; in re-extractions, however, these organic reagents are more frequently used.

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The analyte is first extracted from the matrix as the non-ionized species [11] or as the ion-pair with an inorganic counterion [12, 13], after which it is back-extracted into an aqueous phase. Re-extraction into an organic phase can then be carried out with an organic counterion. However, direct extraction of the analyte from plasma with an organic counterion would be much faster. Apart from the present work as described in this paper, only a few workers [11, 14] have so far reported a similar approach.

Experimental

Chemicals and reagents

All drugs were of pharmacopoeial or equivalent purity. Sodium-*n*-octylsulphate (analytical grade) was purchased from E. Merck (Darmstadt, FRG). Di(2-ethylhexyl) phosphoric acid (HDEHP) was obtained from Koch-Light Laboratories Ltd. (Colnbrook, Bucks, UK) and purified as described previously [1].

All organic solvents were HPLC-grade and purchased either from E. Merck (Darmstadt, FRG) or from Fluka AG (Buchs, Switzerland), except for chloroform (E. Merck) and propylamine (Fluka AG) which were of analytical grade. Bovine albumin containing 96–99% of albumin, the remainder being mostly globulins, was purchased from Sigma Chemical Company. ^{14}C-imipramine (10 mCi/mmol) was purchased from The Radiochemical Centre Ltd. (Amersham, UK). The liquid scintillation cocktail employed was Lumagel (Lumac Systems AG, Basel, Switzerland). All other reagents were of analytical grade. Water was de-mineralized, double-distilled and further purified using a Water-I system (Gelman Sciences, Ann Arbor, Michigan, U.S.A.).

Apparatus

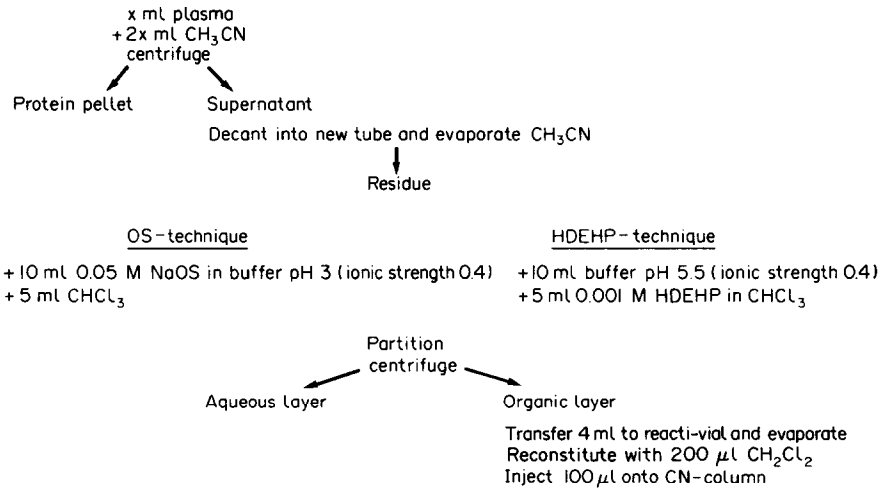
Chromatography was performed using Varian 5060 liquid chromatographs equipped with a Valco or a Rheodyne loop injector (sample loop volume, 100 μl) and a standard fixed wavelength (254 nm) detector or a model UV-100 variable wavelength detector. A Varian 9176 or a Kipp and Zonen model BD8 recorder was used. The chromatographs were interfaced to a Vista CDS 401 chromatographic data system. Liquid scintillation counting was performed with a Packard model 3255 Tri-Carb liquid scintillation spectrometer. For the protein binding studies an Amicon 52 ultrafiltration cell was used, equipped with Amicon YM 10 Diaflo ultrafiltration membranes.

Chromatographic columns

Normal phase chromatography was performed on a 300 × 4 mm i.d. column packed with 10-μm MicroPak CN-10 (Varian). Reversed-phase chromatography was carried out on a 250 × 4 mm i.d. column packed with 10-μm LiChrosorb CN (E. Merck), protected with a 30 × 4 mm i.d. pre-column dry-packed with the same packing material. The system was back-flushed overnight using a slow acetonitrile–dichloromethane–acetonitrile gradient at a flow rate of 0.1 ml/min.

Procedures

Final preferred extraction technique (Scheme 1). The 4-ml plasma sample was transferred by pipette into a glass centrifuge tube equipped with a PTFE-covered screw-cap. Acetonitrile (8 ml) was added dropwise from a burette during continuous vortex mixing. After centrifugation for about 10 min at 2500–3000 rpm, the supernatant was transferred to a clean centrifuge tube and the acetonitrile was removed by evaporation at



Scheme 1
Final preferred extraction scheme.

about 60°C under a gentle stream of nitrogen. Either phosphate buffer (10 ml) pH 5.5 (ionic strength = 0.4) and 5 ml of 0.001 M HDEHP in chloroform (HDEHP-technique) or 10 ml of 0.05 M sodium-*n*-octylsulphate in phosphate buffer pH 3.0 (ionic strength = 0.4) and 5 ml chloroform (NaOS-technique) were added. Partitioning was performed by gently shaking the tubes longitudinally in a shaking bath for 30 min. Mixing with a vortex mixer for a few minutes might be equally effective but was not investigated.

After centrifugation for about 10 min at 2500–3000 rpm, the aqueous layer was discarded and 4 ml of the organic phase was transferred by pipette into a conically tapered vial (Reacti-Vial, Pierce Chemical Company). Prior to chromatography, the extracts were evaporated to dryness under a gentle stream of nitrogen at *ca* 45°C and reconstituted with 200 µl of dichloromethane. One-half of the reconstituted extract was injected into the chromatograph.

Determination of extraction recoveries. Standard solutions in dichloromethane (normal phase mode) or in water (reversed-phase mode) were used to construct calibration graphs of peak area against concentration. All calibration graphs were rectilinear in the concentration range studied. The amount of drug in the extract was determined by interpolation in the usual way.

Liquid scintillation counting. A 100-µl sample (organic phase or aqueous phase) together with 10 ml of scintillation cocktail were transferred by pipette into liquid scintillation counting vials. The number of radioactive desintegrations was counted over 10 min, related to an external standard (^{226}Ra) and corrected for quenching using a previously constructed quenching curve; chloroform was used as the quenching substance.

Protein binding studies. In the ultrafiltration cell 12 ml of 4% m/v bovine albumin solution in phosphate buffer pH 7.4 (ionic strength = 0.1) containing either papaverine or mepyramine in a concentration of 200 ng/ml, was equilibrated with 30 ml of phosphate buffer pH 3.0 (ionic strength = 0.4), with or without octylsulphate; this procedure ensured, as in the extraction procedure, that the volume of reagent solution was in

excess. After 10 min equilibration time, the free drug fraction was separated from the protein and the drug–protein complex, through a membrane with a cut-off at 10,000 M.W., by applying nitrogen under pressure. The first 3-ml fraction of the ultrafiltrate was used to test the absence of protein in the ultrafiltrate by application of the biuret reaction. The drug content of the next 10-ml ultrafiltrate fraction was assayed. The percentage of drug bound to albumin was calculated by subtracting the amount in the ultrafiltrate from the initial amount.

Results and Discussion

The standardized analysis strategy for basic drugs comprises an ion-pair extraction step using chloroform as the solvent and either sodium-*n*-octylsulphate (NaOS) at pH 3.0 or di(2-ethylhexyl)phosphoric acid (HDEHP) at pH 5.5 as the ion-pairing reagent. The extracts are then injected without back- or re-extraction onto a CN-bonded phase using either acetonitrile–water–propylamine (90:10:0.01 v/v/v) or *n*-hexane–dichloromethane–acetonitrile–propylamine (50:50:25:0.1 v/v/v/v) as the preferred eluents. The volume ratio of the mobile phase components should be 'fine tuned' for each particular analytical application.

The first attempts to apply the strategy to plasma were made using NaOS as the ion-pairing reagent; papaverine, mepyramine, methapyrilene and thonzylamine were examined as the test drugs and the normal phase mode was used (for chromatographic conditions see Table 1). In order to evaluate the purity of the extracts, relatively large sample volumes (4 ml) were used. The extraction procedure employed is presented in Scheme 2.

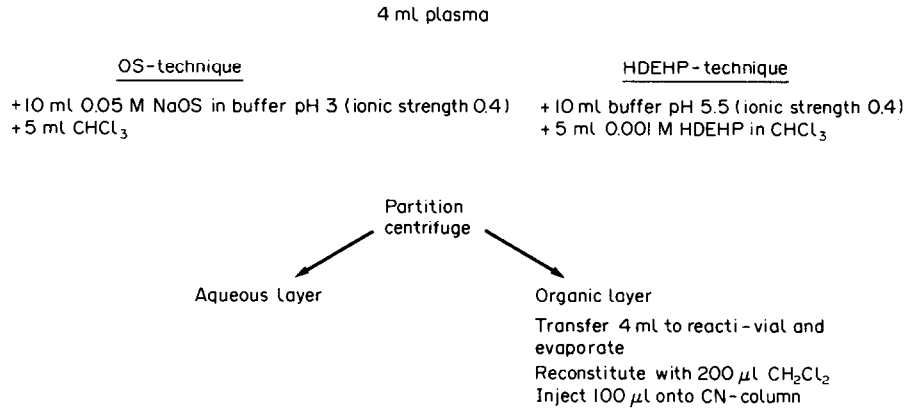
The results were encouraging in that pure extracts were obtained, the chromatographic peaks of the test drugs had a good shape, and the drugs were well resolved from endogenous plasma constituents. However, the extraction yields were only 60–85%. At first it was thought that this low yield could be due to consumption of the ion-pairing reagent by other cations present in the matrix. Thus the counterion concentration was increased in an attempt to overcome the problem; however, this modification failed to do so.

Table 1
Chromatographic conditions

Mode	Analyte	t_r (min)	Detection wavelength (nm)
Normal phase*	Papaverine	4.2	254
	Mepyramine	5.8	254
	Thonzylamine	4.8	254
	Methapyrilene	4.5	254
Reversed-phase†	Melperone	6.5	243
	Metoclopramide	7.1	254
	Thioridazine	4.0	262
	Mesoridazine	8.1	262
	Acebutolol	8.0	235
	Diacetolol	9.8	235

*Column, Micropack-CN. Mobile phase: *n*-hexane–dichloromethane–acetonitrile–propylamine (50:25:25:0:1, v/v/v/v). Flow rate, 2 ml/min.

†Column, Lichrosorb-CN with a pre-column. Mobile phase: acetonitrile–water–propylamine (80:20:0:1, v/v/v). Flow rate, 2 ml/min.



Scheme 2

The extraction step was then investigated using a radioactive tracer which allows quantitation of the analyte in both the organic and the aqueous layer after partitioning. Imipramine was used as the test drug and ^{14}C-imipramine as the tracer. The results of the experiments with the HDEHP-technique are presented in Table 2 which tabulates the percentage of the tracer, corrected for quenching losses, in both the organic and aqueous phase after partitioning. As could be expected from earlier findings [15] the extraction from aqueous solution was almost quantitative. The extraction recoveries from plasma were only *ca* 80% and the non-extracted analyte was not present in the aqueous phase.

Table 2
Percentage of radiolabelled imipramine in the organic and aqueous phases after partitioning*

Matrix	Imipramine concentration (µg/ml)	Organic phase (%)	Aqueous phase (%)	Total (%)
Aqueous solution	15	99.6	0.2	99.8
Plasma	15	82.1	0.4	82.5
Plasma	15×10^{-3}	78.2	0.4	78.6
Deproteinized plasma	15	100.6	0.6	101.2

* Ion-pairing reagent was HDEHP.

However the dense protein layer which was formed between the organic and aqueous phases after partitioning and centrifugation was found to be highly radioactive; this observation suggests that incomplete recovery may have been due to interactions with plasma proteins. This was confirmed by the quantitative recovery from plasma after deproteinization before spiking with the imipramine solution. Further confirmation was gained by repeating the first HPLC-experiments with papaverine, mepyramine and the other drugs, using plasma which had been deproteinized by ultrafiltration before spiking with the drugs, as reported in Table 3. Quantitative recoveries and excellent precision were achieved.

The interaction between drug, counterion and plasma proteins was further investigated by measuring the percentage of drug bound to albumin both in the presence and in

Table 3
Extraction* yields (%) from 4 ml of plasma†

Drug	Drug concentration (ng/ml)	Recovery (%)	RSD‡ ($n = 6$)
Papaverine	200	100.8	1.7
Mepyramine	200	99.4	2.3
Thonzylamine	150	100.1	2.0
Methapyrilene	100	100.0	1.0

* By Extraction Scheme I. NaOS was the ion-pairing reagent.
† Ultrafiltered before spiking with the drug solution.
‡ Relative standard deviation (%).

the absence of the counterion. The experiments were carried out using papaverine and mepyramine as the test drugs and octylsulphate as the counterion. The concentrations and volumes of the albumin, drug and counterion solutions were chosen in order to simulate the conditions which exist when 10 ml of counterion solution is added to 4 ml of plasma (extraction scheme 1). The percentage of each drug bound to albumin, corrected for losses due to adsorption on the ultrafiltration membrane, was calculated, as shown in Table 4.

It can be observed that binding of the drugs to albumin is enhanced upon addition of the counterion and with increasing counterion concentration. These findings demonstrate an interaction between drugs, plasma proteins and organic ion-pairing reagents. As stated above, only a few workers have reported on the application of an ion-pair extraction technique to plasma using an organic ion-pairing reagent. Eriksson *et al.* [11] attempted to use 3,5-di-tert-butyl-2-hydroxybenzene sulphonate as the counterion for the extraction of apomorphine from plasma. However, they obtained low recoveries which they ascribed to interactions between the counterion and the plasma proteins. The ion-pair technique was therefore abandoned in favour of a conventional approach for extracting apomorphine from plasma. After back-extraction into an aqueous phase, re-extraction into a small volume of dichloromethane was then carried out, using the ion-pair technique with 3,5-di-tert-butyl-2-hydroxybenzene sulphonate as the counterion.

Jarvie and Stewart [14] used dodecylsulphate as the counterion for the extraction of paraquat from plasma and obtained a recovery of 75%. These authors also suggested that the reduction of the extraction efficiency from plasma, compared to that from aqueous solution, is due to ion-pairing of the counterion with charged side-chains on proteins. Furthermore, they suggested that this effect could be overcome by further increasing the concentration of dodecyl sulphate. However, it appears (Table 4) that enhancing the counterion concentration would only enhance protein binding of the drug.

Table 4
Percentage of drug bound to bovine albumin at pH 3.0

Concentration of added octylsulphate	Papaverine (% bound)	Mepyramine (% bound)
0	22	21
0.05 M	61	79
0.5 M	94	87

Consequently, in order to avoid interactions between plasma proteins, the drugs and the counterion, proteins should be removed from the sample before addition of the counterion solution. The ultrafiltration technique is useful in this respect only if the unbound (pharmacologically active) fraction of the drug is to be determined.

If the determination of the total plasma concentration of the drug is required, a technique which precipitates the proteins and liberates the drug bound to them should be used. This can be achieved by addition of solutions of strong acids such as perchloric acid and trichloroacetic acid, solutions of heavy metals or organic solvents such as acetonitrile, methanol and ethanol.

The majority of these deproteinization techniques have been evaluated. The use of perchloric acid and trichloroacetic acid resulted in impure extracts and resulted in competition between, for example, the perchlorate ions and the octylsulphate ions for ion-pair formation with the drugs. This problem can be overcome by precipitating the perchlorate ions as their potassium salt but it introduces an additional step in the extraction scheme. In addition, precipitation techniques with heavy metals were unsuccessful. Excellent results were obtained, however, using acetonitrile as the protein-precipitating agent. When the addition of acetonitrile is carried out with sufficient care (dropwise addition during continuous mixing with a vortex mixer), co-precipitation of the drug is minimal.

The NaOS-extraction of 4-ml plasma samples spiked with papaverine (or one of the other drugs) was repeated using Scheme 1, the final preferred extraction technique. The results (Table 5) show that high recoveries and good precision were obtained. Typical chromatograms are shown in Fig. 1; it can be seen that the shape of the chromatographic peaks was good and that the drugs were well resolved from endogenous compounds. Other drugs that have been extracted from plasma by the NaOS-technique and determined by chromatography using the normal phase mode include aprindine and its active desethylmetabolite; carbamazepine and its 10,11-epoxide metabolite; and amidopyrine. These analyses are reported separately [6, 16].

Some of the substances mentioned above, such as papaverine, mepyramine and aprindine, were also extracted by the HDEHP-technique, when the results obtained were similar to those obtained with the NaOS-technique. However, these drugs are rather non-polar and the use of a very non-polar ion-pairing reagent such as HDEHP is not necessary. In contrast, for very hydrophilic drugs, the HDEHP-technique is to be preferred to the OS-technique. Additional problems arise when the extract is then chromatographed in the reversed-phase mode. These problems are illustrated with acebutolol and its active metabolite diacetolol, melperone and metoclopramide as the

Table 5
Extraction* yields (%) from 4 ml of plasma†

Drug	Drug concentration (ng/ml)	Recovery (%)	RSD† ($n = 6$)
Papaverine	200	96.2	3.1
Mepyramine	200	94.2	3.8
Thonzylamine	150	98.8	3.9
Methapyrilene	100	100.8	3.5

* By Extraction Scheme II. NaOS was the ion-pairing reagent.
† Relative standard deviation (%).

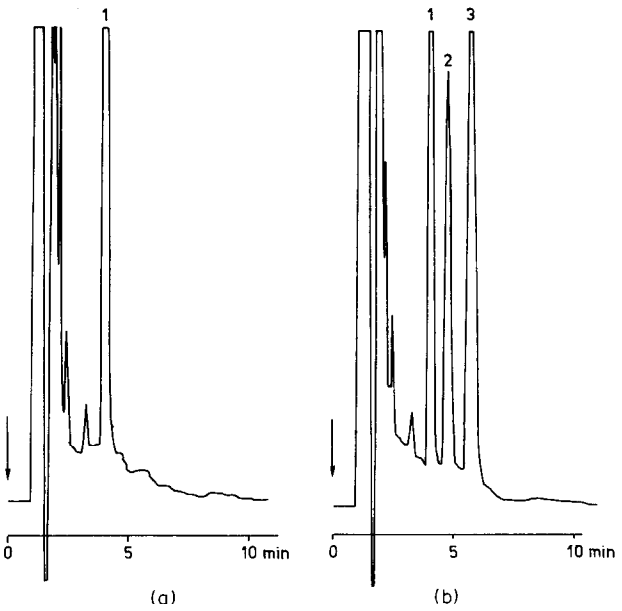


Figure 1
Chromatograms of an OS-extract of: (a) 4 ml of blank plasma; and (b) 4 ml of plasma spiked with thonzylamine (150 ng/ml) and mepyramine (200 ng/ml). Peak identification: 1, caffeine; 2, thonzylamine; 3, mepyramine. Column: 10-μm MicroPak-CN, 300 × 4 mm i.d. Mobile phase: *n*-hexane–dichloromethane–acetonitrile–propylamine (50:25:25:0.1 v/v/v/v). Flow rate, 2 ml/min. Detection: 254 nm, 0.02 AUFS.

test drugs. Although some of these compounds can be chromatographed in the normal phase mode, only the reversed-phase mode was used in order to briefly examine the initial difficulties experienced when injecting the ion-pair extracts into the reversed-phase eluent without back- and re-extraction.

The first problem concerns the nature of the solvent used for reconstituting the evaporated plasma extracts. At first attempts were made to use the mobile phase (acetonitrile or acetonitrile with various proportions of propylamine, methanol and other solvents) for this purpose. However, particularly with the HDEHP-extracts, it was impossible to obtain a clear solution unless large volumes were used. It was then decided to proceed as previously when using the normal phase mode and to use dichloromethane in order to dissolve the highly non-polar ion-pairs. A volume of dichloromethane as small as 20 μl was sufficient to obtain a clear solution.

However, irreproducible and unsatisfactory chromatograms were obtained upon injection of these extracts, probably due both to perturbation of the chromatographic equilibria and to the partial and temporary precipitation of the counterion (particularly HDEHP) at the top of the column. These problems could be overcome, however, by using a 3-cm pre-column dry-packed with the same packing material as that of the analytical column. The use of a pre-column also allows injection of larger volumes of dichloromethane to minimize injection errors. Moreover, it was found that the life of the analytical column could be extended two- or even three-fold when the pre-column was used.

The life, stability and efficiency were also extended if the pre-column and the analytical column were back-flushed with dichloromethane overnight, as described in the

Experimental section. After about 20–25 working days the pre-column produced excessive back-pressure and tailing peaks. The pre-column was then cleaned and refilled.

In summary, the problems encountered at first when combining the ion-pair extraction technique, particularly the HDEHP-technique, with the reversed phase eluent were overcome by: reconstituting the evaporated extracts with 200 μl of dichloromethane, half of which is injected into the chromatographic system; using a pre-column; and regularly back-flushing the system with dichloromethane.

The possible value of the standardized analysis strategy using the reversed-phase eluent for assaying drugs in plasma was evaluated with metoclopramide, melperone, acebutolol, diacetolol, thioridazine and mesoridazine as the test drugs. The extraction recoveries using both ion-pairing reagents can be compared from Table 6. As could be anticipated by reference to earlier findings [1, 3], HDEHP is a more appropriate pairing agent than NaOS for hydrophilic drugs, since it gives rise to superior extraction recoveries. Typical chromatograms are shown in Figs 2–5. It can be seen that each extract was pure, that the shape of the chromatographic peaks was good, and that the solutes were well resolved from endogenous plasma constituents.

Table 6
Extraction recoveries from plasma following extraction scheme 1. Chromatography was performed in the reversed-phase mode

Drug	Drug concentration (ng/ml)	OS-technique Recovery (%)	RSD* ($n = 6$)	HDEHP-technique Recovery (%)	RSD ($n = 6$)
Thioridazine	100	94.0	2.6	98.9	1.4
Mesoridazine	100	94.9	8.1	95.2	4.3
Acebutolol	50	25.6	7.6	99.7	3.2
Diacetolol	50	10.7	8.5	89.7	4.1
Metoclopramide	50	74.5	9.0	93.6	5.6
Melperone	100	89.9	5.8	91.6	2.8

* RSD = relative standard deviation.

Entire analytical methods have been constructed using either the NaOS- or the HDEHP-technique combined with the reversed-phase mode for the assay of mebeverine and an alcohol derivative in plasma and urine [17], for the assay of aprindine and its desethylmetabolite in plasma [16] and for the simultaneous determination of imipramine, 2-hydroxy-imipramine, desipramine and 2-hydroxy-desipramine in plasma [18]. The development and characteristics of these assay methods and their application to samples from patients are reported elsewhere.

Note added in proof

During editorial review of the present paper, the authors became aware of some papers [19–22] using related ion-pairing approaches. Garrett *et al.* [19] determined biguanides in urine by UV-spectrophotometry following extraction of the analytes as ion-pairs with bromothymol blue into dichloromethane and back-extraction into an aqueous phase after addition of excess tetrabutyl-ammonium hydroxide. This method was not directly applicable to plasma [20]. The authors stated that no ion-pair of bromothymol blue-biguanide could be extracted from plasma and they attributed this result to

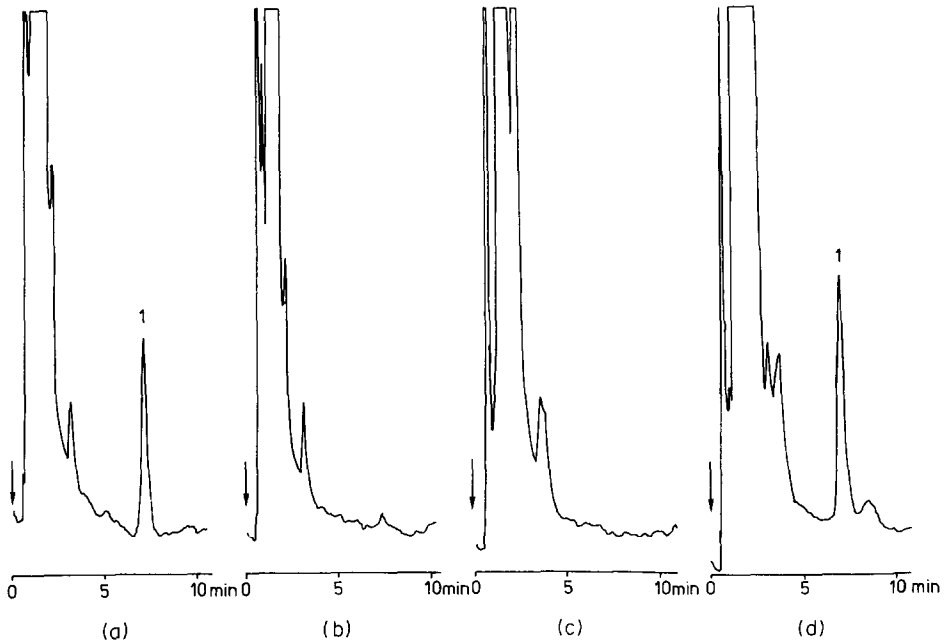


Figure 2
Chromatograms of: (a) an OS-extract of 4 ml of blank plasma spiked with metoclopramide (50 ng/ml); (b) an OS-extract of 4 ml of blank plasma; (c) an HDEHP-extract of 4 ml of blank plasma; and (d) an HDEHP-extract of 4 ml blank plasma spiked with metoclopramide (50 ng/ml). Peak identification: 1, metoclopramide. Column: 10-μm LiChrosorb CN, 250 × 4 mm i.d., protected by a 30 × 4 mm i.d. precolumn dry-packed with 10-μm LiChrosorb CN. Mobile phase: acetonitrile–water–propylamine (80:20:0.01 v/v/v). Flow rate, 2 ml/min. Detection: 254 nm, 0.005 AUFS.

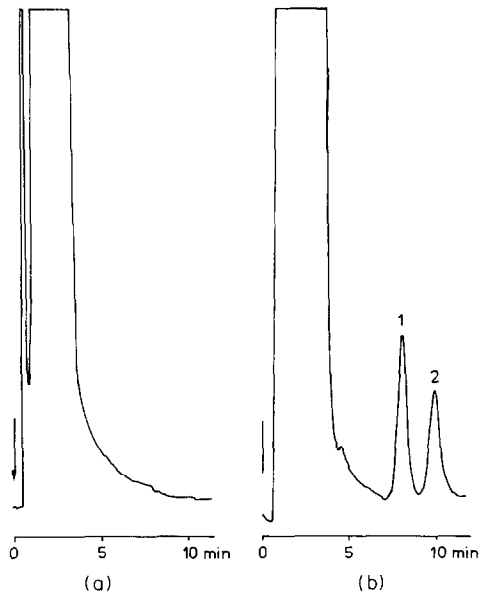


Figure 3
Chromatograms of an HDEHP-extract of: (a) 4 ml of blank plasma; and (b) 4 ml of blank plasma spiked with acebutolol (50 ng/ml) and diacetolol (50 ng/ml). Peak identification: 1, acebutolol; 2, diacetolol. Chromatographic conditions as in Fig. 2, except for detection: 235 nm, 0.01 AUFS.

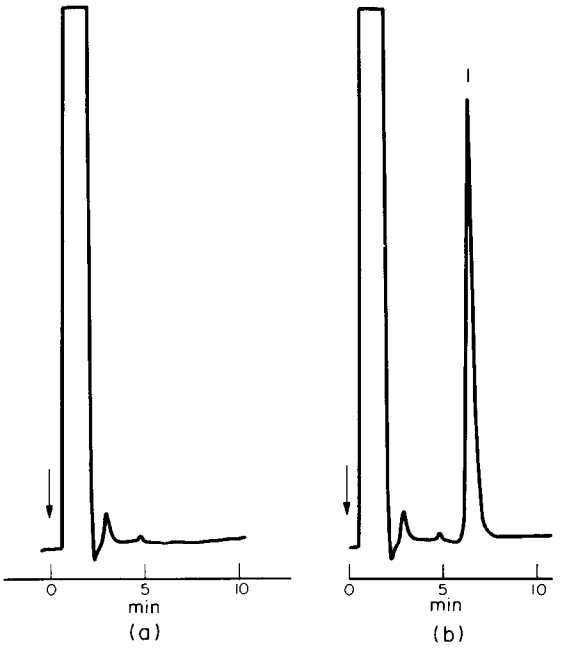


Figure 4
Chromatograms of an HDEHP-extract of (a) 4 ml of blank plasma; and (b) 4 ml of blank plasma spiked with melperone (100 ng/ml). Peak identification: 1, melperone. Chromatographic conditions as in Fig. 2, except for detection: 243 nm, 0.01 AUFS.

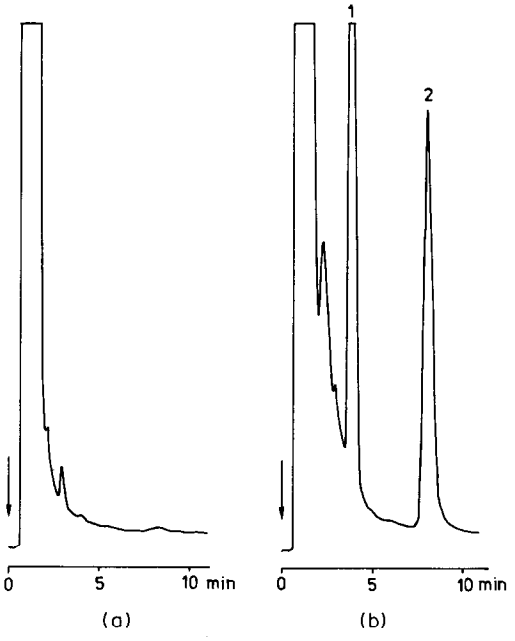


Figure 5
Chromatograms of an HDEHP-extract of: (a) 4 ml of blank plasma; and (b) 4 ml of blank plasma spiked with thioridazine (100 ng/ml) and mesoridazine (100 ng/ml). Peak identification: 1, thioridazine; 2, mesoridazine. Chromatographic conditions as in Fig. 2, except for detection: 262 nm, 0.01 AUFS.

coprecipitation of the biguanides with plasma proteins, which are denatured by organic solvents such as dichloromethane [20].

The results of the protein binding studies in the present work contradict this hypothesis and suggest rather that hydrophobic binding may occur between the pairing agent and the proteins. In fact Garrett *et al.* [20] earlier proposed a solution to the problem similar to that developed here; that is, the removal of plasma proteins prior to extraction. The recoveries of the biguanides could be increased to 40–70% by either trichloroacetic acid precipitation or ultrafiltration.

Eksborg *et al.* [22] used dipicrylamine as the pairing agent for the extraction of methylguanidine from plasma into dichloromethane. The efficiency of extraction was low owing to binding of methylguanide and dipicrylamine to plasma proteins. An absolute recovery of 95 ± 5% was obtained by mixing the sample and the reagents with Celite 545 and performing the extraction in an extraction column.

Eksborg and Persson [21] used the same pairing agent (dipicrylamine) to extract choline from rat brain samples into dichloromethane. The tissue samples were homogenized in 0.1 M perchloric acid. Since perchloric acid simultaneously precipitates proteins and since the extraction was carried out on the supernatant, a high recovery (95 ± 5%) was obtained.

Conclusions

The following conclusions can be drawn:

(1) Low extraction recoveries from plasma obtained with an ion-pair extraction technique using organic ion-pairing reagents are due to interactions between plasma proteins, the drug and the pairing agent.

(2) These interactions can be avoided by removing plasma proteins from the sample prior to addition of the ion-pairing agent. When the preferred extraction procedure is used, pure extracts and high extraction recoveries are obtained for both non-polar and polar compounds as well as for drugs such as thioridazine and imipramine, which are highly protein bound in physiological conditions.

(3) Both the NaOS- and the HDEHP-extracts can be directly chromatographed in both the normal- and the reversed-phase mode on the CN-column, without back- and re-extraction. In all instances the evaporated extracts should be reconstituted with dichloromethane. When the reversed-phase mode is employed, use of a pre-column and back-flushing of the system with dichloromethane are essential.

(4) The standardized analysis strategy is applicable to a wide variety of basic drugs and matrices (syrups, emulsions, ointments, saliva, urine and plasma). It is a very helpful tool for laboratories in which widely divergent pharmaceutical analyses are performed.

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