Journal of Chromatography, 231 (1982) 25—39
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1289

SIMPLE AND FAST SOLVENT EXTRACTION SYSTEM FOR SELECTIVE AND QUANTITATIVE ISOLATION OF ADRENALINE, NORADRENALINE AND DOPAMINE FROM PLASMA AND URINE

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(First received December 15th, 1981; revised manuscript received March 2nd, 1982)

SUMMARY

A very simple solvent extraction system for the selective and quantitative isolation of adrenaline, noradrenaline and dopamine from plasma and urine is described. The extraction system makes use of the complex formation, in alkaline medium, between diphenylborate and the diol group in the catecholamines in combination with ion-pair formation. The influence of various parameters on the distribution coefficient was investigated by analysis of the liquid phases by high-performance liquid chromatography with electrochemical detection. From these results the optimal extraction conditions can be selected. With hexane + 1% *n*-octanol containing 0.25% (w/v) of tetraoctylammonium bromide as extraction solvent, the catecholamines can be quantitatively isolated from plasma and urine at pH 8.6 in the presence of 0.1% (w/v) of diphenylborate. For urine the recovery was 101.5 ± 1.9% for adrenaline, 100.6 ± 2.0% for noradrenaline and 99.9 ± 1.5% for dopamine. For plasma the recoveries were, respectively, 101.8 ± 3.3%, 100.5 ± 2.6% and 92.9 ± 3.5%. The recovery of dihydroxybenzylamine, included in the study as internal standard, was determined to be 96.3 ± 1.6% for urine and 89.9 ± 2.7% for plasma. The applicability of the developed extraction system as clean-up and concentration step for the analysis of catecholamines in plasma and urine by high-performance liquid chromatography with electrochemical detection is demonstrated.

INTRODUCTION

Various analytical methods, such as fluorimetry [1, 2], gas chromatography [3, 4] and in particular liquid chromatography [5—8], are used for the analysis of catecholamines in body fluids and tissues. With all these methods an extensive clean-up procedure, sometimes followed by a concentration step, is inevitable in order to obtain accurate and precise data. Various separation methods have been used as clean-up and concentration step: solvent extrac-

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tion [9, 10], adsorption on alumina [11, 12] and hydrophobized materials [13, 14], ion exchange [8, 15, 16] and isolation on boric acid gels [8, 17, 18]. The adsorption on alumina is by far the most frequently used method because of its unique selectivity towards catechol groups in alkaline medium by which rather clean extracts can be obtained. In this respect the recently developed boric acid gel, which shows a similar selective behaviour towards catechol groups as alumina, becomes increasingly popular, in particular because sometimes problems arise with the reproducibility of the activity of Al_2O_3, which can differ from batch to batch and requires extensive pretreatment before use. The chromatographic methods, although very useful, are rather time consuming and can not be easily automated, which is a great disadvantage when many samples have to be analyzed.

From the point of view of simplicity, reproducibility and automation, isolation by means of solvent extraction offers many possibilities [19]. However, until now the results of the isolation of catecholamines by means of solvent extraction have been mainly restricted to brain tissues [9, 20]. For that purpose *n*-butanol was found to be a suitable solvent for the extraction of catecholamines from acidified solution [20]. However, for plasma and urine samples far too many endogenous substances, interfering seriously with the analysis of catecholamines, are co-extracted with *n*-butanol. An improvement was obtained when the catecholamines were extracted as ion-pairs with diethylhexylphosphoric acid as pairing anion [21, 22]. However, also under these conditions the co-extraction of endogenous substances is still significant.

It is common knowledge that the co-extraction of endogenous (and exogenous) substances always present in body fluids will be suppressed when less polar extraction solvents can be used. In the present paper the results are reported of an investigation to isolate catecholamines from plasma and urine samples by solvent extraction with high recovery and very low co-extraction of endogenous substances, using the complex formation in alkaline medium between diphenylborate and catechol groups. The selectivity and the recovery of the isolation of catecholamines from aqueous, plasma and urine samples was determined using high-performance liquid chromatography (HPLC) with electrochemical detection.

EXPERIMENTAL

Apparatus

The HPLC system used to analyze the extracts consisted of a reciprocating pump (Orlita, Giessen, G.F.R.) equipped with a home-made flow-through bourdon-type manometer and pulse dampener (custom), a coulometric detector (Kipp Analytica, Emmen, The Netherlands) with a potential setting of +0.6 V vs. Ag/AgCl, and a high-pressure sampling valve (type 7010; Rheodyne, Berkeley, CA, U.S.A.) equipped with a 500-μl sample loop. The columns used were of 316 stainless steel and of dimensions of 150 × 4.6 mm (Fast LC-8, Technicon, Tarrytown, NY, U.S.A.).

Chemicals

All chemicals were of analytical grade and used without any further pre-

treatment. The catecholamines were obtained from Sigma (St. Louis, MO, U.S.A.), diphenylborate–ethanolamine was from Aldrich (Milwaukee, WI, U.S.A.), the quaternary amines from BDH (Poole, Great Britain) and octanol from Riedel de Haen (Seelze-Hannover, G.F.R.).

PROCEDURES

Chromatography

After equilibration of the phases, the concentrations of the catecholamines in the aqueous and organic phase (after back-extraction) were determined by means of HPLC with electrochemical detection. For this purpose use was made of a dynamic cation-exchange system as described previously [23]. The column packing was a C_8 bonded silica (Fast LC-8) and the mobile phase was composed of a water–methanol mixture (75:25, v/v) containing 0.05 *M* sodium acetate, 0.15 *M* acetic acid, 0.01% (w/v) of sodium dodecyl sulfate (SDS), 0.01% (w/v) sodium chloride and 0.01% (w/v) EDTA. Depending on the solute concentration, the injection volume ranged between 20 and 450 μl. In order to decrease the background current in detection, the pump, manometer, capillaries and injection port were pumped through, once before use, with 200 ml of 15% nitric acid.

Extraction

The distribution coefficient (D_i) of the catecholamines, defined as the ratio of the total catecholamine concentrations in the organic and aqueous phase, was determined by mixing 5 ml of the organic phase with 5 ml of the aqueous phase. The pairing cations were dissolved in the organic phase, except for the inorganic cations, which were dissolved in the aqueous phase. Diphenylborate–ethanolamine complex (DPBEA) was usually dissolved in the aqueous phase.

The pH of the aqueous phase was adjusted with an NH_4Cl–NH_4OH buffer, except in the case where the influence of pH on D_i was investigated, when an $(NH_4)_2HPO_4$–NH_4OH buffer was used. Known concentrations of the catecholamines (ca. 10 nmol/ml) in the aqueous phase were prepared from a stock solution of the solutes in 0.05 *M* phosphoric acid, stored at 4°C.

The two phases were shaken by hand for 2 min and if necessary centrifuged (1200 *g*) to improve phase separation. The concentrations of the solutes in the organic phase were determined by back-extraction into an aqueous phase; 1 ml of the organic phase was mixed with 1 ml of 0.05 *M* trichloroacetic acid (TCA) and 20–450 μl of the aqueous phase were injected into the HPLC system.

For relatively small D_i values the concentrations in the aqueous phase could be measured directly by HPLC, after adjustment of the pH to 4 with sulphuric acid, by injecting a maximum 20 μl of the aqueous phase. Larger injection volumes were not possible because of serious disturbance of the chromatographic system due to the high salt concentration. In such cases the solutes in the aqueous phase had to be extracted into an organic phase and back-extracted into a small volume (concentration step) of 0.05 *M* TCA before injection into the HPLC system.

Urine and plasma samples

Urine. To 0.5 ml of urine (preserved with 0.025 *M* HCl) 100 µl of a solution of the internal standard (equivalent to 35 ng) dihydroxybenzylamine (DBHA) + 1 ml of 2.0 *M* NH_4Cl—NH_4OH (pH 8.5) buffer containing 0.2% (w/v) of DPBEA and 0.5% (w/v) of EDTA are added. After the addition of 4 ml of *n*-heptane containing 1% (v/v) *n*-octanol and 0.25% (w/v) of tetraoctylammonium bromide (TOABr) the mixture is shaken by hand for 2 min and then centrifuged at 1200 *g*. To 3 ml of the organic phase 2 ml of *n*-octanol and 0.4 ml of 0.08 *M* acetic acid are added and the mixture is shaken by hand for 2 min, followed by centrifugation (5 min); 100 µl of the aqueous phase are injected into the HPLC system.

Plasma. To 2 ml of plasma 100 µl of a solution of DHBA (equivalent to 700 pg) + 1 ml of 2 *M* NH_4OH—NH_4Cl buffer (pH 8.5) containing 0.2% (w/v) DPBEA and 0.5% (w/v) of EDTA are added. After the addition of 5 ml of *n*-heptane + 1% *n*-octanol containing 0.25% (w/v) TOABr, the sample is shaken by hand for 2 min and centrifuged at 1200 *g* for 5 min. Then 4 ml of the organic phase are transferred to a conical tube and mixed with 2 ml of *n*-octanol and 250 µl of 0.08 *M* acetic acid, shaken by hand for 2 min, then centrifuged; 200 µl of the aqueous phase are injected into the HPLC system.

RESULTS AND DISCUSSION

For the isolation of catecholamines by solvent extraction use was made of the complex formation between borate and catechol (diol) groups in alkaline medium [11] and of ion-pair formation. The applicability of this complex formation for the selective isolation of catecholamines from body fluids has been demonstrated in the last years with the so-called boric acid gels [17, 18]. Although the exact reaction scheme has not been elucidated, our opinion is that the extraction proceeds via the scheme given in Fig. 1.

In the present study diphenylborate was used because of its hydrophobic substituents which favour the extraction of borate—diol complexes into organic solvents. Diphenylborate is commercially available as the diphenyl-

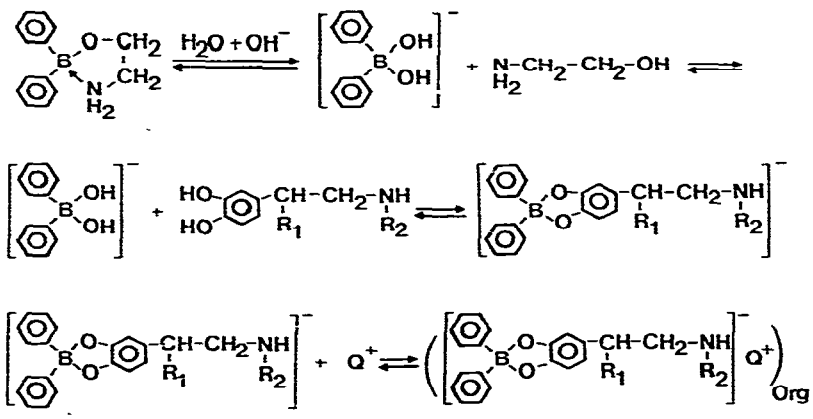

Fig. 1. Tentative equilibrium reactions involved in the extraction of catecholamines using diphenylborate as complexing agent and ion-pair formation.

borate—ethanolamine complex. This complex dissociates in aqueous alkaline medium into a negatively charged diphenylborate and ethanolamine. The diphenylborate forms a negatively charged stable complex with the catecholamines via the diol group, which cannot be easily extracted into an organic solvent. In order to realize this an ion-pair has to be formed with a cation Q^+ (pairing ion) added to the aqueous or organic phase [24]. According to this reaction scheme the distribution coefficient (D_i) will be influenced by the pH of the aqueous phase, the diphenylborate and pairing ion (Q^+) concentration, on the type of pairing ion and on the composition of the organic solvent.

In order to verify the reaction scheme and to determine the optimal conditions for the extraction of catecholamines from plasma and urine samples, the D_i values of these solutes were measured under a variety of conditions. As organic solvents chloroform, *n*-octanol and mixtures of these solvents with *n*-heptane or hexane were chosen because these are found to be useful in ion-pair extraction [24].

Besides the catecholamines dihydroxybenzylamine (DHBA) was also included in the study because of its possible use as an internal standard.

Effect of pH of the aqueous phase

The influence of pH on the distribution coefficient, using NH_4^+ as the Q^+ and *n*-octanol as extraction solvent, is given in Fig. 2. It shows that the D_i increases significantly with increasing pH of the aqueous phase. This can be ex-

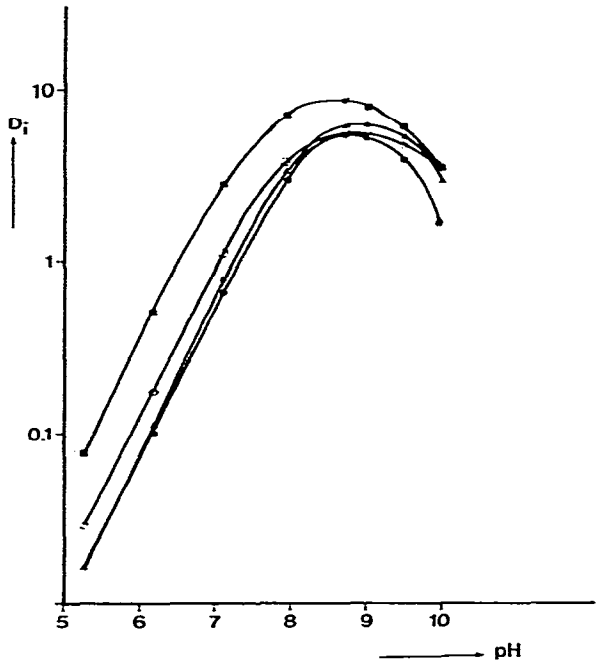

Fig. 2. Effect of the pH of the aqueous phase on the distribution coefficient D_i with NH_4^+ as pairing ion (Q^+) and *n*-octanol as extractant. (▲) Adrenaline; (●) noradrenaline; (■) dihydroxybenzylamine; (○) dopamine.

pected as at low pH the diphenylborate—ethanolamine complex is quite stable [25]. Therefore, the catecholamines can only be transferred to the organic phase as ion pairs with the inorganic anions (HPO_4^{2-}) present in the aqueous phase, which was shown before to be rather ineffective [24]. With increasing pH the diphenylborate—ethanolamine complex starts to dissociate and the negatively charged diphenylborate will be formed and reacts with the diol group of the catecholamines, thus promoting the extractability of the catecholamines (i.e. by masking the hydroxy groups) as a complex ion pair (with Q^+ as pairing ion) into the organic phase. The D_i values reach a maximum at pH 8—9 and then decrease again. This latter effect can be attributed to dissociation of the hydroxy groups of the catecholamines at high pH. Also losses due to oxidation are observed in this region. It should be noted that usually decomposition, due to oxidation, of the catecholamines already starts to occur at about pH 7. However, we found that the decomposition is almost negligible up to pH 9 when diphenylborate is present.

From Fig. 2 it can be concluded that the optimal pH of the aqueous phase must be in the range 8—9, which is in agreement with earlier reports [26]. A similar behaviour of D_i as function of the pH is observed when octanol is replaced by other solvents such as chloroform.

Effect of the type of pairing ion (Q^+)

According to the assumed reaction scheme the diphenylborate—catecholamine complex will be extracted into the organic phase as an ion pair with a

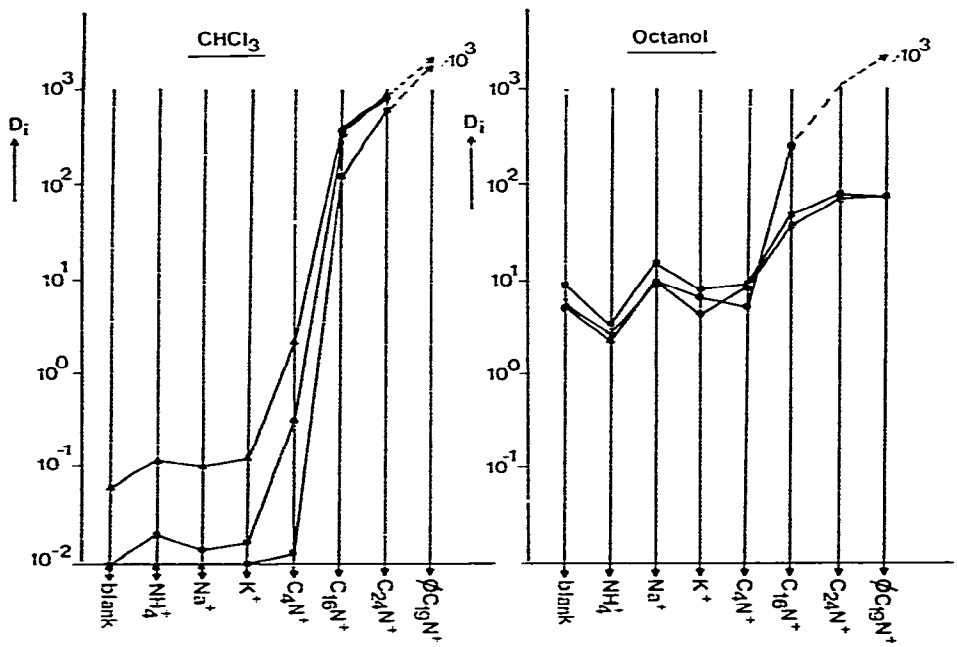

Fig. 3. Effect of the type of pairing ion (Q^+) on the distribution coefficient D_i of catecholamines with chloroform and *n*-octanol as extraction solvents. C_4N^+ = tetramethylammonium chloride; $C_{16}N^+$ = tetrabutylammonium hydrogen sulfate; $C_{24}N^+$ = tetrahexylammonium bromide; $\phi C_{19}N^+$ = benzyldimethyl *n*-hexadecylammonium chloride.

cation Q^+. It is known from ion-pair extraction studies that the extractability of ion pairs is also dependent on the type of pairing ion [24]. Usually a pairing ion with a large hydrophobic moiety enhances the extractability.

In order to investigate this for the present ion-pair extraction system, the distribution coefficient was measured with various types of inorganic and organic cations with chloroform and *n*-octanol as extraction solvents. The results of these measurements are shown in Fig. 3. It shows that the type of cation significantly influences the value of D_i. Further, some significant differences between chloroform and *n*-octanol can be noticed. With the inorganic cations and with tetramethylammonium, very small D_i values (0.01—1) were found with chloroform and significantly larger values (2—10) with *n*-octanol. This can be attributed to the better solvation ability of octanol compared to chloroform [24]. In agreement with previous findings the extraction (D_i value) inproves significantly for all solutes when using more hydrophobic quaternary amines. With chloroform the D_i value of all solutes increases with increasing hydrophobic part of the quaternary amine, reaching D_i values $> 10^3$ with benzyldimethyl *n*-hexadecylammonium chloride. With octanol, however, the D_i values of adrenaline and noradrenaline also reach values $> 10^3$ with benzyldimethyl *n*-hexadecylammonium, but for dopamine and dihydroxybenzylamine significantly smaller D_i values (ca. 70) were found. This might be caused by side-reactions in one or both phases [24].

The results as given in Fig. 3 show that, with hydrophobic quaternary amines as pairing ion, the catecholamines can be almost quantitatively isolated from an aqueous phase with *n*-octanol or even better with chloroform as the extractant.

It must be noted that perchlorate ions, often used for deproteinization of plasma samples, must be absent in the aqueous phase because these anions form extremely stable ion pairs with hydrophobic quaternary amines and thus block the ion-pair formation of the diphenylborate—catecholamine complex with these quaternary amines.

Effect of the amount of chloroform and octanol added to hexane

It is common knowledge that the co-extraction of interfering substances from, for instance, plasma and urine samples is the lowest with very non-polar extraction solvents. Although chloroform and octanol are promising extraction liquids, it would be an advantage if these solvents could be replaced by a less polar solvent or could be used mixed with a non-polar solvent. In order to investigate this, the distribution coefficient was measured with pure hexane and mixtures of hexane—chloroform and hexane—*n*-octanol. The results of these measurements are given in Fig. 4. Pure hexane could not be used as an extraction solvent because a precipitate of unknown identity was formed. By analysis of both phases it was found that the catecholamines adsorb strongly on this precipitate. This precipitate does not occur when about 3% (v/v) of chloroform and 0.5% of octanol is added to hexane. It should be noted that much lower concentrations of chloride as used here lead to an increase of this precipitate problem.

Fig. 4 shows the effect of the amount of chloroform and octanol present in hexane on the distribution coefficient. With chloroform the log D_i values of

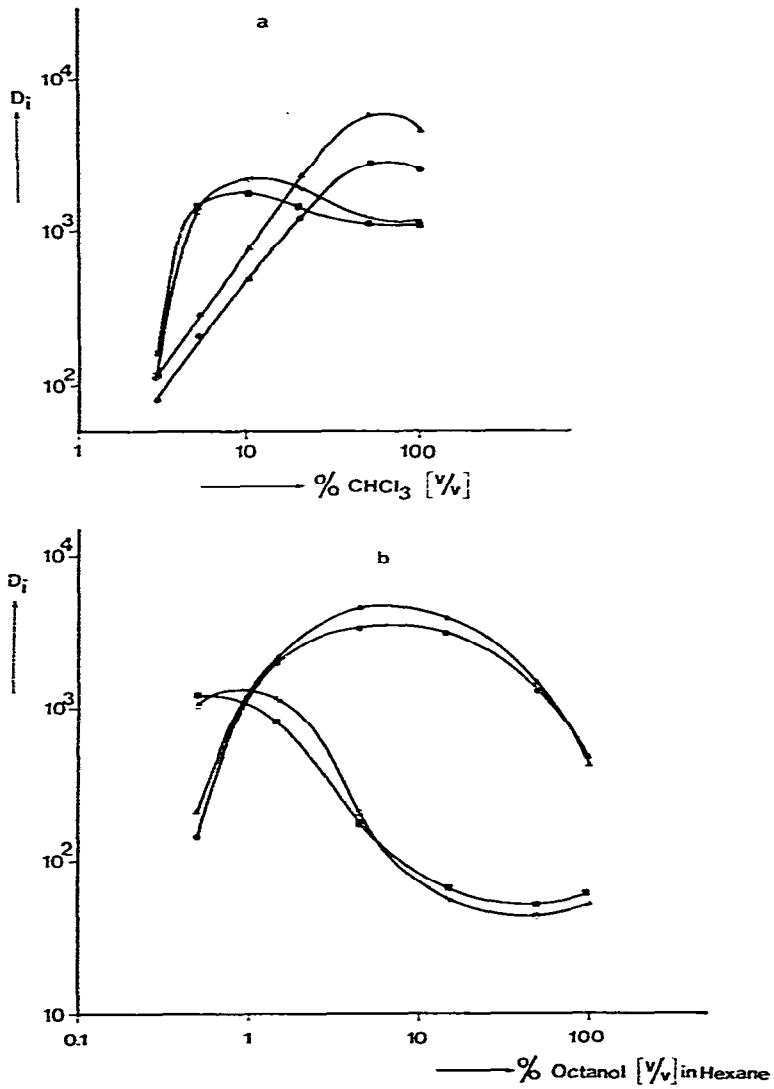

Fig. 4. Effect of the amount of chloroform (a) and octanol (b) in hexane on the distribution coefficient of catecholamines with tetraoctylammonium as pairing ion. (▲) Adrenaline; (●) nonadrenaline; (■) dihydroxybenzylamine; (○) dopamine.

adrenaline and noradrenaline increase almost linearly with increasing chloroform content up to 50% of chloroform and then decrease again towards 100% of chloroform. Dopamine and dihydroxybenzylamine show a significantly different dependence. The D_i values increase with increasing chloroform content and pass through a maximum at about 10% (v/v) of chloroform. With octanol the distribution coefficients of adrenaline and noradrenaline increase with increasing octanol content, pass through a maximum value at about 4% of octanol and then sharply drop again. For dopamine and dihydroxybenzylamine a maximum D_i value is found at about 1% of octanol and also for these solutes the D_i value decreases sharply with increasing octanol content.

From Fig. 4 it can be seen that with about 20% of chloroform or 1% of octanol for all solvents a D_i value $> 10^3$, large enough for a quantitative isolation, can be obtained. With respect to co-extraction of interfering substances, 1% of octanol is preferable.

Effect of the diphenylborate concentration

The influence of the diphenylborate concentration on the distribution coef-

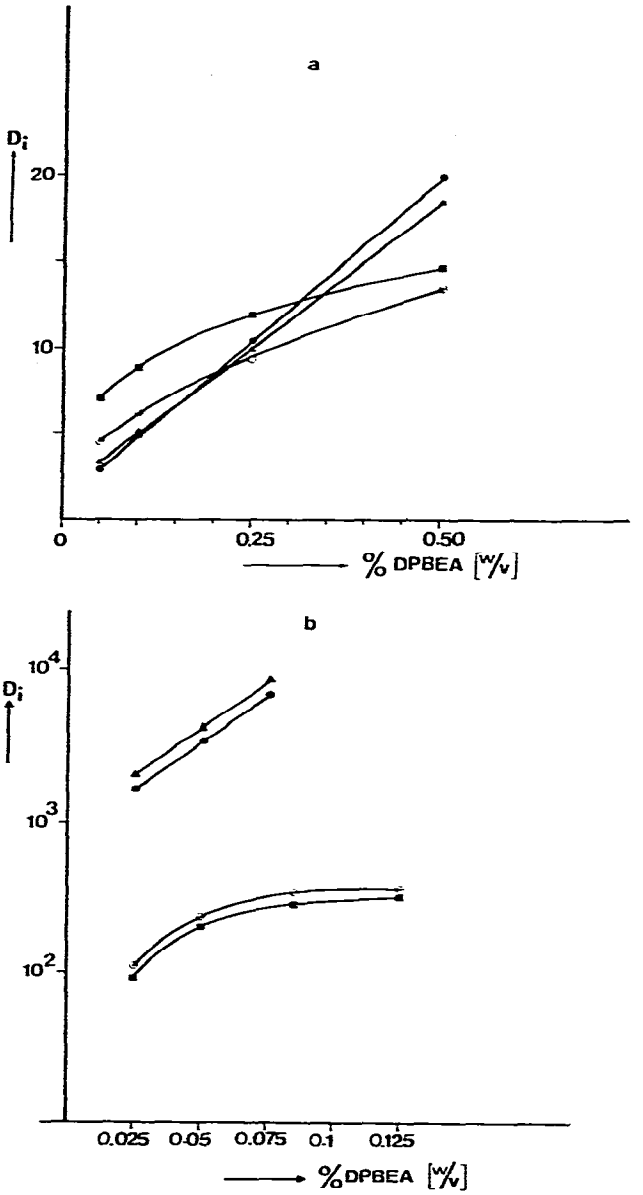

Fig. 5. Effect of the diphenylborate concentration on the distribution coefficient of catecholamines with NH_4^+ (a) and tetraoctylammonium (b) as pairing ion. (▲) Adrenaline; (●) noradrenaline; (■) dihydroxybenzylamine; (○) dopamine.

ficient was investigated with NH_4^+ as the pairing ion and octanol as the extractant, and with tetraoctylammonium as Q^+ and hexane + 2.5% (v/v) octanol as the extraction solvent. The results of these measurements are given in Fig. 5. In both extraction systems the D_i values of adrenaline and noradrenaline increase steeply with increasing diphenylborate concentration as expected on the basis of the assumed reaction scheme given in Fig. 1. With tetraoctylammonium as the Q^+ and diphenylborate concentrations > 0.075%, the D_i values of adrenaline and noradrenaline could no longer be accurately determined because of the extremely low catecholamine concentration in the aqueous phase after phase equilibration (i.e. the D_i values of these two solutes became extremely large). The D_i values of dopamine and dihydroxybenzylamine also increase with increasing diphenylborate concentration (although less steeply than those of adrenaline and noradrenaline) with NH_4^+ as pairing ion and octanol as extractant. However, with tetraoctylammonium the D_i value first increases and then levels off at higher diphenylborate concentrations. This deviating behaviour must be attributed to side-reactions occurring in one or both liquid phases and indicates the imperfectness of the assumed reaction scheme.

From Fig. 5 it can be seen that the diphenylborate concentration should be about 0.1%, which is less than half of the solubility of diphenylborate in water.

Effect of the pairing-ion concentration

The influence of the Q^+ concentration on the distribution coefficient was investigated with tetraoctylammonium as pairing ion and octanol as extractant. The results are shown in Fig. 6. The D_i value of all solutes increases steeply with increasing tetraoctylammonium concentration in agreement with findings in ion-pair extraction systems and in accordance with Fig. 1. A Q^+ concentration as large as possible seems to be of advantage. However, when using hexane

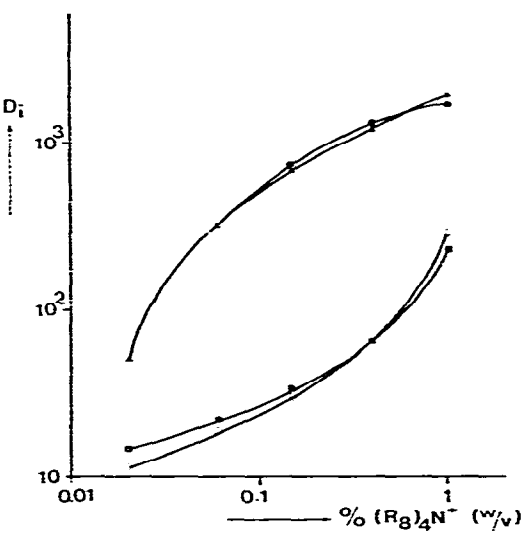

Fig. 6. Effect of the pairing ion concentration [$(R_8)_4N^+$ = tetraoctylammonium] on the distribution coefficient of catecholamines. Extraction solvent: *n*-octanol. (▲) Adrenaline; (●) noradrenaline; (■) dihydroxybenzylamine; (○) dopamine.

+ 1% octanol as extractant, the D_i values are much larger than with pure octanol and become more or less constant (D_i values 10^3 to $5 \cdot 10^3$) at 0.3—0.4% (w/v) of tetraoctylammonium. From the point of view of co-extraction of anions via ion-pair formation with tetraoctylammonium, this concentration seems to be a good choice.

Selection of the extraction conditions

The results as reflected in Figs. 2—6 can be used to determine optimal extraction conditions for the catecholamines. From the point of view of extraction efficiency and selectivity of extraction, a mixture of hexane + 1% of octanol as extractant and 0.1% (w/v) of diphenylborate and 0.2—0.3% of tetraoctylammonium and a pH of the aqueous phase of 8.6 appears to be a good compromise. Under these conditions plasma and urine samples were extracted as described under Procedures and the extracts were analyzed by HPLC using electrochemical detection. It can be noticed that hexane + 20% of chloroform is also favourable as an extractant. However, unfortunately chloroform generates a baseline disturbance at or near the position of dopamine in the chromatogram, at least with the chromatographic and detection system used in this study.

Fig. 7 shows chromatograms of extracts of a standard mixture, a plasma sample and a urine sample. The chromatogram of the plasma sample was recorded at the most sensitive setting of the electrochemical detector (25 nA full-scale). As can be seen the background is almost free of interfering substances, allowing the determination of adrenaline, noradrenaline and dopamine. Adrenaline and noradrenaline can be determined in plasma of normal persons and abnormal levels can easily be detected. However, the concentration of dopamine in plasma of normal persons is usually too low to be detected with the present system. If one wants to determine dopamine, the mobile phase composition has to be changed, by variation of the methanol content, in such a way that the capacity factor k' of dopamine is as small as possible accounting for the disturbance of the chromatogram near the unretained position. Under these conditions adrenaline and noradrenaline can not be determined as they disappear in the front peak.

The chromatogram of an extract of urine is shown in Fig. 7b. Also here the background is excellent and allows a precise determination of the three major catecholamines.

From Figs. 2—6 it can also be seen that dopamine and dihydroxybenzylamine behave significantly differently compared to adrenaline and noradrenaline. Because of this, dihydroxybenzylamine is not the ideal internal standard for adrenaline and noradrenaline. In this respect N-ethylnoradrenaline would probably be a better internal standard for these two solutes.

Quantitative aspects of the extraction

The recovery and reproducibility of the developed extraction method was tested with plasma and urine samples of healthy volunteers. The recovery was determined by spiking the plasma and urine samples with known amounts of the catecholamines (usually ten times the endogenous concentration) and

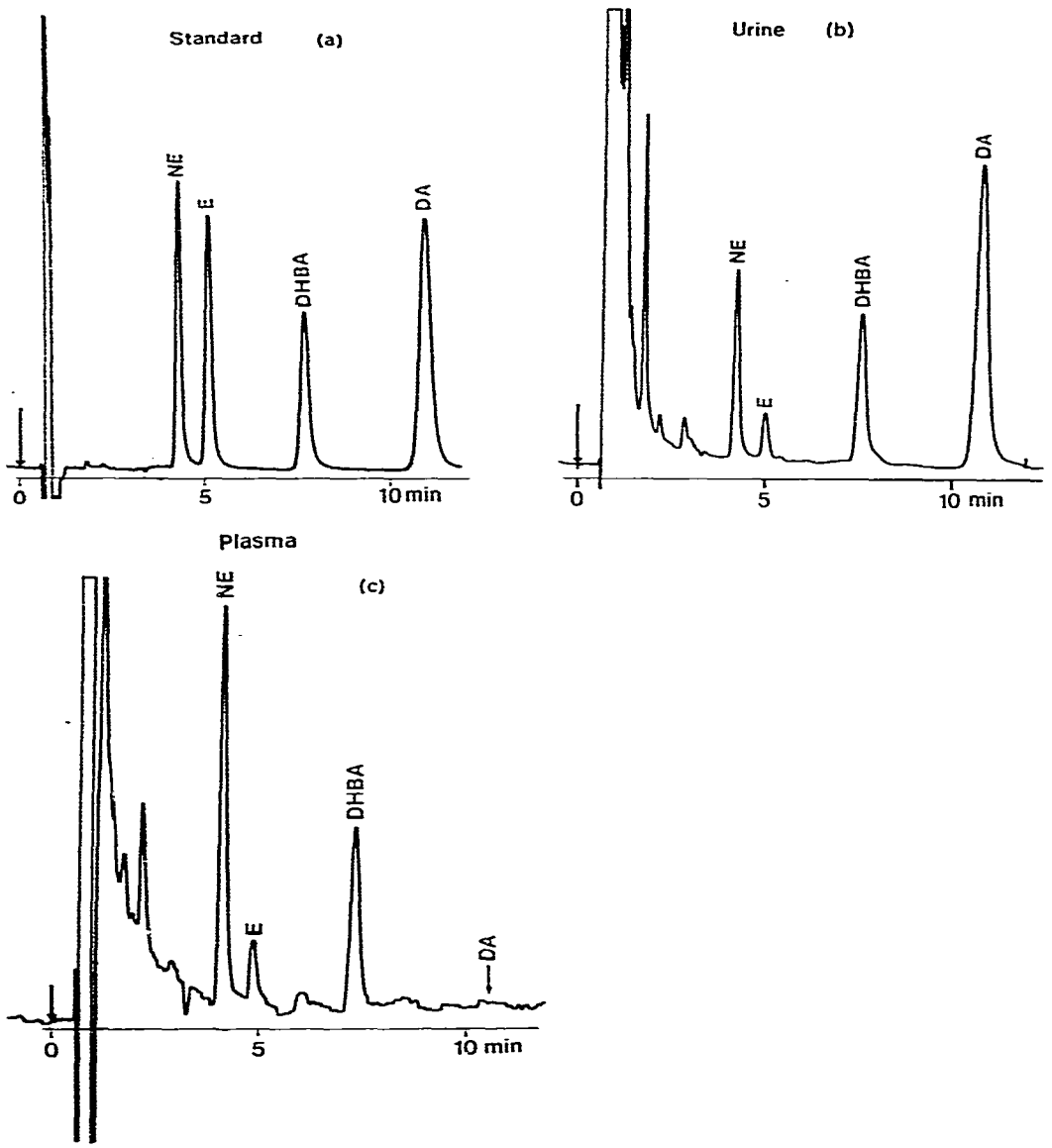

Fig. 7. Chromatograms of extracts of a standard solution (a), urine (b) and of a plasma sample (c) obtained with solvent extraction and HPLC with electrochemical detection. (a) containing 70 ng of noradrenaline (NE), 76 ng of adrenaline (E), 60 ng of dihydroxybenzylamine (DHBA), and 127 ng of dopamine (DA) per ml; (b) containing 56 ng of Ne, 16 ng of E, 60 ng of DHBA, 190 ng of DA per ml; (c) containing 515 pg of NE, 125 pg of E, 600 pg of DHBA per ml.

extracting these samples as described under Procedures, followed by analysis of the extracts by HPLC. The concentrations found were corrected for endogenous concentrations determined in parallel experiments with the non-spiked samples. The results of these measurements are given in Table I. As can be seen from the table, the three catecholamines are completely recovered from urine. However, the recovery of dihydroxybenzylamine is somewhat

TABLE I

RECOVERY OF CATECHOLAMINES* FROM PLASMA AND URINE BY SOLVENT EXTRACTION

	Amount added (ng)	Recovery (%)
Urine (0.5 ml) (n = 7)	NE = 210	100.6 ± 2.0
	E = 230	101.5 ± 1.9
	DHBA = 170	96.3 ± 1.6
	DA = 380	99.9 ± 1.5
Plasma (2 ml) (n = 7)	NE = 7.0	100.5 ± 2.6
	E = 7.0	101.8 ± 3.3
	DHBA = 6.0	89.9 ± 2.7
	DA = 12.0	92.9 ± 3.5

*NE = noradrenaline; E = adrenaline; DHBA = dihydroxybenzylamine; DA = dopamine.

TABLE II

REPRODUCIBILITY OF THE SOLVENT EXTRACTION HPLC METHOD FOR THE DETERMINATION OF CATECHOLAMINES* IN PLASMA AND URINE OF A HEALTHY VOLUNTEER (UNSPIKED SAMPLES)

	Urine (ng/ml) (n = 5)	Plasma (pg/ml) (n = 5)
NE	56.6 ± 1.5	515 ± 17
E	15.7 ± 0.55	125 ± 6
DA	191.4 ± 6.9	—

*Abbreviations as in Table I.

lower (96 ± 1.6%), which raises doubts concerning the use of this solute as an internal standard.

From plasma adrenaline and noradrenaline are completely recovered. The recoveries of dopamine and again dihydroxybenzylamine were found to be significantly lower: 93% and 90%, respectively. An explanation for this has not yet been found. Deproteinization before extraction may improve the recovery, but this was not investigated.

The reproducibility of the method was tested by extraction of a series of non-spiked plasma and urine samples of a healthy volunteer. The catecholamine levels, including the standard deviation, in plasma and urine of this volunteer are given in Table II. As can be seen from this table, the reproducibility of the method is excellent, as is reflected in the standard deviation which ranges between 3 and 4%. The catecholamine levels determined agree with those reported in the literature, except for adrenaline in plasma, which is somewhat higher. However, this might be attributed to the way in which the blood sample was taken, as this might significantly influence the actual concentration level of adrenaline in the plasma.

CONCLUSIONS

The developed solvent extraction system, based on the complex formation in alkaline medium between diphenylborate and catechol groups and ion-pair formation, was found to be extremely suitable for the selective and quantitative isolation of adrenaline, noradrenaline and dopamine from plasma and urine. From the point of view of simplicity, recovery and reproducibility, this solvent extraction system has definite advantages compared to the chromatographic isolation techniques. Further, the simple solvent extraction system lends itself to automation. Preliminary experiments in this direction show that urine samples can be automatically handled with a Technicon Fast LC system. A report on this subject is in preparation.

When combining the solvent extraction with HPLC and electrochemical detection, it is possible to determine the catecholamines in plasma and urine of healthy persons and to detect increased catecholamine levels in patients. However, the described isolation method might be also of great value as a clean-up step for other analytical methods such as fluorimetry, radioimmunoassay, or radioenzymatic methods.

Future work in our laboratory will be devoted to the application of the present extraction principle for the isolation of other compounds containing diol groups and to apply diphenylborate, dynamically coated on various packings, for on-column isolation and/or as a chromatographic HPLC system.

ACKNOWLEDGEMENTS

This work was carried out partly under a Technicon Grant. Thanks are due to Mr. Eric Endert for valuable advice and technical assistance.

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