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REVERSED-PHASE LIQUID CHROMATOGRAPHY FOR THE SEPARATION OF CHLORPROMAZINE, IMIPRAMINE AND SOME OF THEIR METABOLITES

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SUMMARY

Reversed-phase liquid chromatography on a C_{18} bonded silica with water–alcohol mixtures containing decylamine as the mobile phase was investigated for its applicability to the separation of chlorpromazine, imipramine and their metabolites. The addition of decylamine to the mobile phase was found to be necessary in order to obtain symmetrical peaks. The influence of the decylamine and ethanol concentrations and of the pH of the mobile phase was investigated and these parameters were found to be useful for the adjustment of the retention. The phase system developed was found to be suitable for the required separation. The phase system was applied to the analysis of imipramine and its metabolites, desmethylimipramine and didesmethylimipramine, and desmethylchlorpromazine in human plasma.

INTRODUCTION

The tricyclic antidepressants are by far the most commonly prescribed drugs for the treatment of psychiatric patients suffering depressions. The monitoring of the level of the antidepressant drugs and their metabolites in plasma is important for various reasons: (i) it provides more insight into the relationship between the level of the drug and its clinical effect; (ii) it is useful for discriminating poor biological availability from fast metabolic degradation or unreliable drug administration to patients; and (iii) it provides valuable information for pharmocokinetic studies.

Of the variety of analytical techniques applied for this purpose, high-performance liquid chromatography (HPLC) has been shown to be pre-eminently suitable[1–4] and has been applied succesfully to the analysis of imipramine and desmethylimipramine and also its two hydroxy metabolites[5]. However, the didesmethyl metabolite of imipramine was not included in these studies. Chlorpromazine has only been determined by HPLC in drug formulations[6]. A main drawback of the liquid chromatography of basic substances such as tricyclic antidepressent, is frequently the poor peak shape, probably caused by interactions of these bases with the support.

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In this paper we report the results of an investigation to apply reversed-phase (RP) HPLC with alkyl-modified silica as the stationary phase and water–alcohol mixtures containing a long-chain alkylamine as the mobile phase for the separation of imipramine, chlorpromazine and their metabolites. The phase system was tested for its suitability for the analysis of plasma extracts from patients receiving these tricyclic drugs.

EXPERIMENTAL

Apparatus

The liquid chromatograph was constructed from commercially available parts and consisted of a Varian 8500 syringe pump, a Rheodyne 7105 high-pressure sampling valve, a Pye Unicam variable-wavelength UV detector and a linear potentiometric recorder (Servogor, Goerz, Austria). In all experiments stainless-steel columns of I.D. 4.6 mm and length 125 mm were used.

Materials

In all experiments doubly distilled water was used. All solvents and chemicals were of analytical-reagent grade and used without any further pre-treatment. The alkyl-modified silica was Hypersil ODS, mean particle size 5 μm (Shandon, London, Great Britain). The drugs and their metabolites were kindly donated by Dr. R. G. Muusze, St. Joris Gasthuis, Delft, The Netherlands. Their structures and abbreviations are given in Table I.

Procedures

The columns were packed by a balanced slurry technique and were washed successively with 100 ml of methanol, 50 ml of the appropriate water–organic solvent mixture and finally with the eluent until constant retention of the solutes was achieved. Blank pooled plasma and plasma samples containing chlorpromazine, imipramine or desmethylimipramine were stored at −20°C. After thawing, the plasma samples were treated according to the following extraction scheme[6]: a mixture of 2 ml of plasma, 2 ml of 1 *M* sodium hydroxide solution and 5 ml of *n*-heptane–isoamyl alcohol (99:1) was shaken for 10 min and then centrifuged for 3 min at 300 *g*. To 4 ml of the organic layer was added 0.2 ml 0.05% orthophosphoric acid and the mixture was shaken for 2 min and then centrifuged for 3 min at 300 *g*. A 100-μl volume of the aqueous layer was injected into the column. The amount of adsorbed alkylamine was determined by means of the breakthrough method[7].

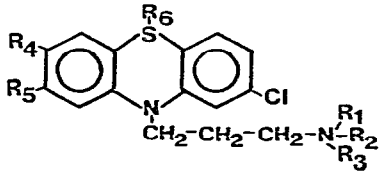
RESULTS AND DISCUSSION

As the metabolites of the antidepressants under investigation are more polar and usually present in plasma at considerable lower concentrations than the parent drugs, RP-HPLC was chosen as the separation method. In this type of liquid chromatography the metabolites will usually be eluted with a smaller capacity ratio than the parent drugs, which is advantageous with respect to the detection limit of these metabolites[3].

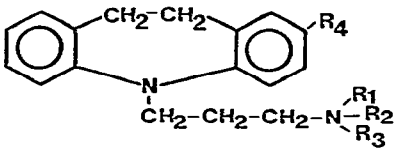
A general problem in the liquid chromatography of basic substances is severe

TABLE I

STRUCTURES OF THE ANTIDEPRESSANTS AND THEIR METABOLITES



Compound	*Code*	R_1	R_2	R_3	R_4	R_5	R_6	R_7
Nor II CPZS → O	1	H	H	—	—	—	O	—
CPZS → O	2	CH_3	CH_3	—	—	—	O	—
Nor I CPZS → O	3	CH_3	—	—	—	—	O	
7-OH-CPZ	4	CH_3	CH_3	—	OH	—	—	—
6-OH-CPZ	5	CH_3	CH_3	—	—	OH	—	—
Nor II-CPZ	6	H	H	—	—	—	—	—
Nor I-CPZ	7	CH_3	H	—	—	—	—	—
Chlorpromazine (CPZ)	8	CH_3	CH_3	—	—	—	—	—
Dichlorpromazine	9	CH_3	CH_3	—	—	—	—	Cl



Compound	*Code letters*	R_1	R_2	R_3	R_4
Imipramine	a	CH_3	CH_3	—	—
Desmethylinipromino	b	CH_3	H	—	—
Didesdemethylimipiramine	c	H	H	—	—
2-Hydroxyimipramine	d	CH_3	CH_3	—	OH
Imipramine-N-oxide	e	CH_3	CH_3	O	—

peak tailing, probably due to the specific interactions of the bases with the support. These interactions can be supressed in three ways: (i) by post-silanization of the RP support[8]; (ii) by applying basic eluents[3,8,9]; or (iii) by using ion-pair chromatography[7,10,11].

All of these possibilities were initially investigated using a C_{18} modified silica (Hypersil ODS) as the column support. Post-silanization of the Hypersil ODS material by refluxing with a 10% (v/v) solution of dichloromethylsilane in toluene for 24 h did not result in any significant improvement in the peak shapes of the solutes. On applying ion-pair chromatography[7,11], by adding 0.1% (w/v) of sodium lauryl sulphate to the mobile phase, it was found that, apart from a considerable difference in retention behaviour, the peak shape improved significantly for a number of solutes. However, for some metabolites (in particular the hydroxylated ones) a poor peak shape was still observed.

By far the best results were obtained when basic eluents were applied. For this purpose 0.2% (v/v) of decylamine was added to the mobile phase. Under these con-

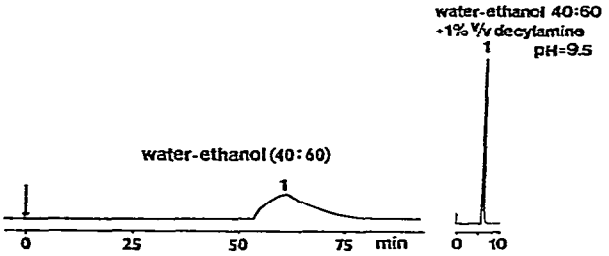


Fig. 1. Effect of the presence of decylamine in the mobile phase on the peak shape and retention of chlorpromazine (1).

ditions the peak shape of all solutes improved greatly (see Fig. 1). Therefore, in all further experiments basic eluents containing decylamine were used.

In order to determine the optimal conditions for the separation of the solutes, the capacity ratio (k_i') was measured as a function of the organic modifier content, the amount of decylamine and of the pH of the mobile phase. Fig. 2 shows the effect of the methanol content of the mobile phase on the k_i' of chlorpromazine and its metabolites. It can be seen that k_i' decreases with increasing methanol content. For some solutes a linear dependence of log k_i' on the percentage of methanol was found, whereas, particularly for the hydroxylated metabolites, a deviation from linearity

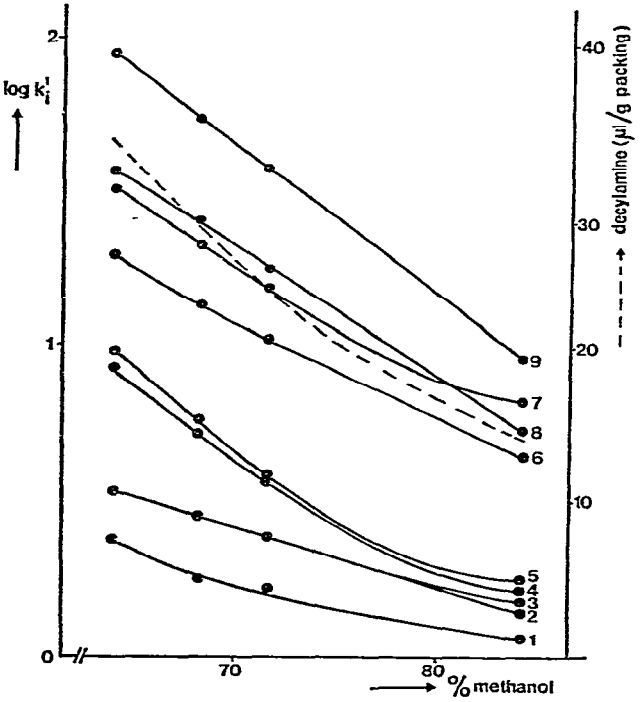


Fig. 2. Influence of the methanol content on the capacity ratio (k_i') of chlorpromazine and its metabolites. Mobile phase: water–methanol + 0.28% (v/v) of decylamine. For identification of compounds, see Table I.

occurred at higher methanol contents. As the amount of adsorbed decylamine is influenced by the methanol content (see the broken line in Fig. 2), this deviation from linearity might be partly attributed to a change in the specific nature of the dynamically modified stationary phase.

The influence of the amount of decylamine in the mobile phase on the k_i' of chlorpromazine and its metabolites is shown in Fig. 3. It can be seen that, the k_i' values decrease sharply from 0.02% (v/v) to about 0.1% (v/v) of decylamine and then decrease gradually with further increase in the amount of decylamine. The change in k_i' coincides well with the shape of the adsorption isotherm of decylamine, as indicated by the broken line in Fig. 3. For imipramine and its metabolites a similar dependence of k_i' on the methanol and decylamine concentrations was found.

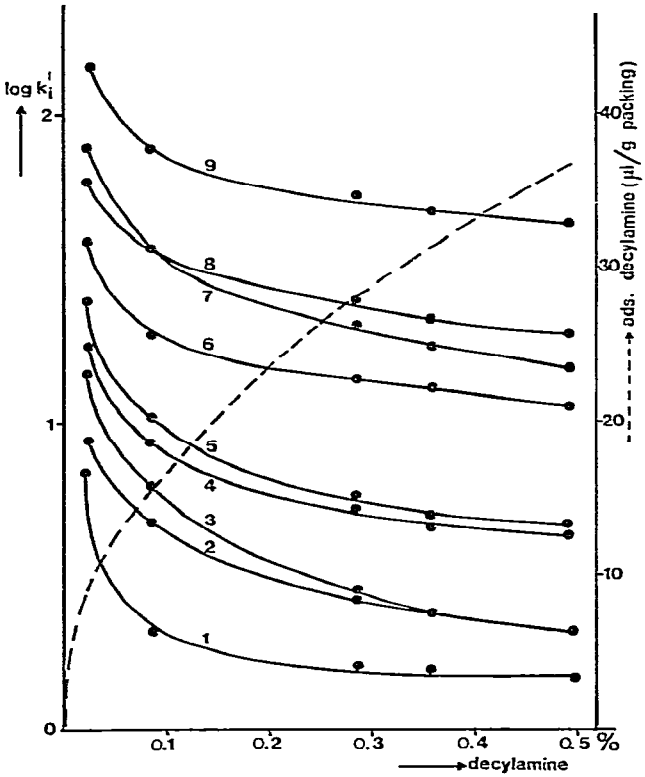


Fig. 3. Influence of the amount of decylamine on the k_i' of chlorpromazine and its metabolites. Mobile phase: water–methanol (3:7) + decylamine. For identification of compounds, see Table I.

To obtain symmetrical peaks it was necessary to add at least 0.1% (v/v) of decylamine with chlorpromazine and its metabolites and about 0.05% (v/v) with imipramine and its metabolites.

When the decylamine content of the mobile phase is changed the pH will also be changed. In order to investigate the effect of the pH of the mobile phase on the retention, k_i' values were measured at pH 9.5 and 11.0 (adjusted with perchloric acid) and the results are shown in Fig. 4. It can be seen that the k_i' values of chlorpromazine

and its metabolites are much larger at pH 9.5 than at pH 11.0. Moreover, the elution order changes for some pairs of solutes. For imipramine and its metabolites the changes are smaller.

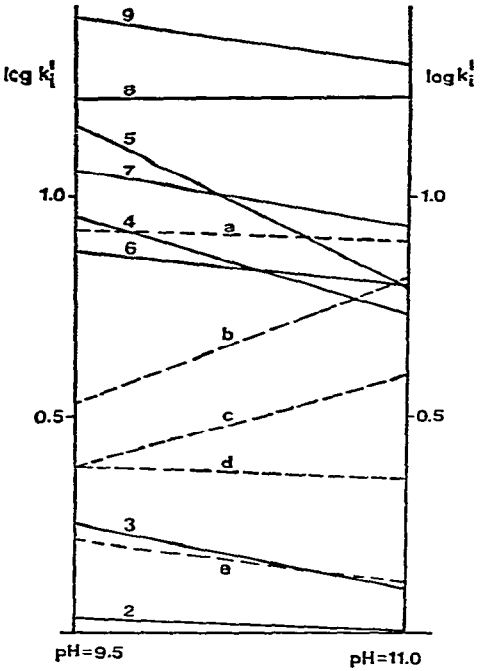


Fig. 4. Influence of the pH of the mobile phase on the k'_i of chlorpromazine (solid line) and imipramine (broken line) and their metabolites. Mobile phase: for chlorpromazine and its metabolites, water–ethanol (1:1) + 2% of decylamine; for imipramine and its metabolites, water–ethanol (4:6) + 0.2% of decylamine. For identification of compounds, see Table I.

With respect to the amount of adsorbed decylamine, it was found that at pH 9.5 about 80 μl of decylamine per gram of silica were adsorbed and about 200 μl/g at pH 11.0. This difference certainly has an influence on the nature of the stationary phase and hence on the retention. With respect to the type of organic modifier, ethanol was also tested. Although ethanol acts as a strong modifier, no significant differences were found in comparison with methanol. However, it was found that decylamine dissolves better in water–ethanol than in water–methanol mixtures, and therefore the final separations were performed with ethanol instead of methanol as organic modifier.

The results in Figs. 2–4 show that the organic modifier and decylamine concentrations and the pH are useful parameters for adjusting the retention of the antidepressants and their metabolites.

Fig. 5 shows chromatograms of the separation of test mixtures of (a) chlorpromazine and (b) imipramine and their metabolites. Dichlorpromazine, a less common antidepressant, was included in the study as a possible internal standard.

Quantitative aspects

The precision and linearity of the HPLC method were determined by injecting

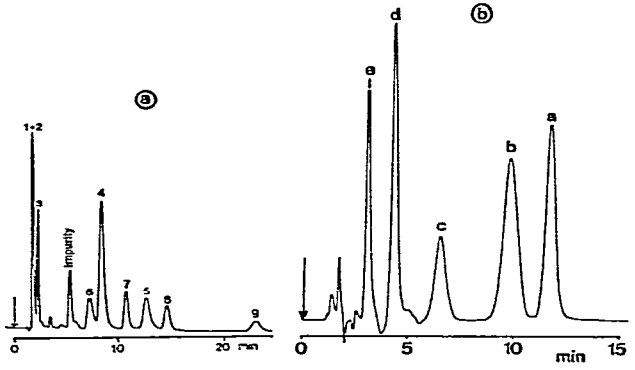


Fig. 5. Separation of test mixtures of (a) chlorpromazine and (b) imipramine and their metabolites. Mobile phase: (a) water–ethanol (1:1) + 2% of decylamine, pH = 9.5 adjusted with $HClO_4$; (b) water–ethanol (4:6) + 0.2% of decylamine, pH = 11.0. For identification of compounds, see Table I.

100 μl of solutions of the various solutes at different concentrations and measurement of the peak heights. The calibration graphs were linear up to 1 μg/ml for the parent drugs and main metabolites with correlation coefficients ranging from 0.998 to 0.999. The precision of the method was determined from repeated injections ($n = 5$) of 0.1 μg/ml solutions of imipramine and chlorpromazine and was found to be $\pm$ 4% for both solutes. The peak-to-peak value of the baseline noise was determined to be 10^{-5} absorbance unit. This leads to a calculated limit of detection, for a signal-to-noise ratio of 3, for imipramine and its metabolites of about 1 ng for the most UV-active compound (2-hydroxyimipramine) and about 3 ng for the least UV-active compound (didesmethylimipramine). For chlorpromazine and its desmethylated metabolites the limit of detection was calculated to be 2 ng.

The recovery and reproducibility of the extraction were determined by spiking blank plasma samples with known amounts of the parent drugs and their main metabolites (40 ng/ml) and extracting as described under *Procedures*. The recoveries were 85 $\pm$ 3% for imipramine, 81 $\pm$ 2% for desmethylimipramine and 74 $\pm$ 4% for didesmethylimipramine ($n = 4$).

Chlorpromazine was not extracted at all under the chosen extraction conditions. This is in contradiction with reports in the literature[12,13] in which excellent recoveries of this drug were obtained with extraction procedures similar to that used in this study. Also with more polar extraction solvents such as diethyl ether or dichloromethane only a small percentage of chlorpromazine was extracted. Adsorption on to the surface of the glassware used for the extraction or decomposition of chlorpromazine might be the reason for the very low recovery. Both possibilities were investigated in more detail.

Stability tests with solutions of chlorpromazine showed that this compound decomposes rapidly in basic solutions. However, with respect to the extraction time this cannot fully explain the very low recovery of this drug. The main losses of chlorpromazine were due to adsorption onto the surface of the glassware and on to the synthetic material in the screw-stopper of the extraction tubes. Replacement of these tubes with tubes fitted with glass stoppers decreased the adsorption losses, but the recoveries were still poor (5–20%) and varied significantly from tube to tube.

So far we have not been able to overcome the losses due to adsorption. An alternative might be to add an excess of a compound that is preferentially adsorbed on the glass surface, as proposed by Fenimore *et al.*[12]. However, this has not yet been investigated.

Desmethylchlorpromazine could be extracted with a recovery of about 60%. However, also for this compound large variations in recovery were found (± 20%), which seriously hampers its quantitative measurement.

Analysis of plasma extracts of patients

The developed RP system was applied to the analysis of the plasma from patients receiving daily doses of imipramine or desmethylimipramine. Although chlorpromazine could not be extracted for the reasons outlined above, plasma from a patient receiving chlorpromazine daily was also investigated for the presence of desmethylchlorpromazine. Fig. 6a and b show the chromatograms of extracts of blank plasma and of plasma spiked with 100 n*M* of imipramine and some of its metabolites.

Fig. 6c shows the chromatogram of the plasma from a patient receiving 100 mg of imipramine daily. It can be seen that this patient shows an unusually high metabolic conversion into desmethyl- and didesmethylimipramine. The plasma levels in this patient were determined to be 29 nmol/l of imipramine, 86 nmol/l of desmethylimipramine and 10 nmol/l didesmethylimipramine. This example shows the need to determine the main metabolites of drugs in cases of rapid metabolization.

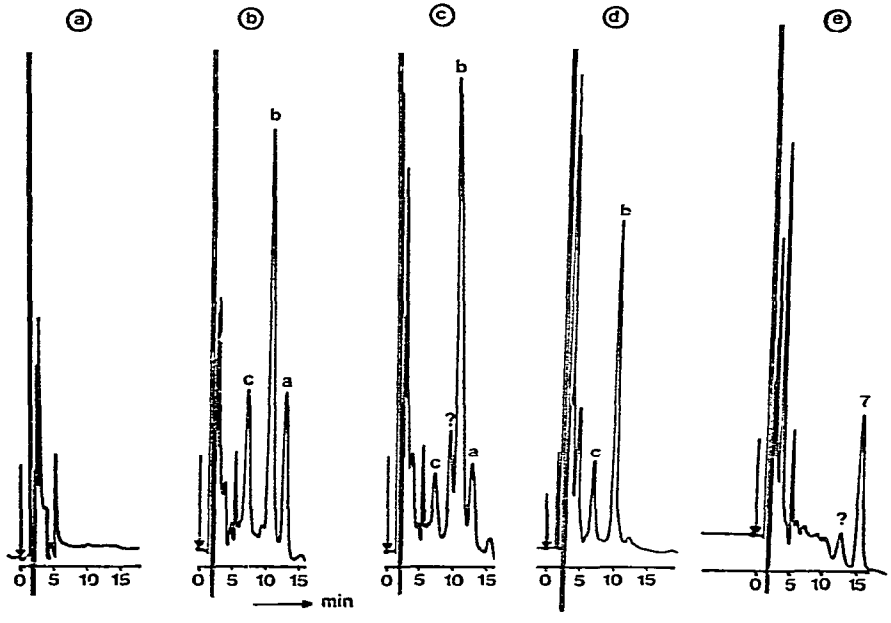


Fig. 6. Chromatograms of extracts of (a) blank and (b) spiked plasma and of plasma of patients receiving daily (c) 100 mg of imipramine, (d) 100 mg of desmethylimipramine and (e) 75 mg of chlorpromazine. Conditions as in Fig. 5. For identification of compounds, see Table I.

Fig. 6d shows the chromatogram of the plasma from a patient receiving 100 mg of desmethylimipramine daily. It can be seen that the parent drug and the didesmethyl metabolite can easily be determined by the proposed method. The plasma levels in this patient were determined to be 75 nmol/l of desmethylimipramine and about 40 nmol/l of didesmethylimipramine.

Fig. 6e shows the chromatogram of the plasma from a patient receiving 75 mg of chlorpromazine daily. No chlorpromazine could be detected in this sample because of the failure of the extraction procedure. However, the main metabolite of this drug, desmethylchlorpromazine, was found in the plasma at a concentration of about 40 nmol/l (estimated value).

CONCLUSION

RP-HPLC using mobile phases containing decylamine was found to be useful for the separation of chlorpromazine, imipramine and their metabolites. The presence of decylamine in the mobile phase improves considerably the peak shapes of these basic substances.

With the column material used (Hypersil ODS), no damage of the column packing due to the basic eluent was observed during a period of 6 months. The RP system is applicable to the analysis of imipramine and its two desmethyl metabolites in human plasma extracts. The same would apply to chlorpromazine and its desmethyl metabolite, provided that the extraction of these substances, which in this study was seriously affected by adsorption on to the surface of the glassware, can be improved.

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