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COLUMN LIQUID CHROMATOGRAPHY OF TRICYCLIC ANTIDEPRESSANTS

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SUMMARY

A column liquid chromatographic system for the analysis of tricyclic antidepressants in serum is described. A high separation efficiency can be obtained with a mixture of ethyl acetate, *n*-hexane and methylamine as eluent on a silica gel column. The retention is easily regulated by varying the concentration of *n*-hexane, the modifier methylamine and the water content of the ethyl acetate.

Examples are given of separations of test mixtures of tricyclic antidepressants and of some of these drugs in serum. UV detection permits determinations down to the 10-ng level in serum.

INTRODUCTION

Tricyclic antidepressants (TCA) are psychotherapeutic agents, the use of which for the treatment of depression has increased considerably in recent years. A rapid, selective and sensitive method for the qualitative determination of TCA is essential in cases of overdose, in the chronic treatment of endogene depression and in the quality control of pharmaceutical products. Intoxication resulting from synergism or antagonism of several medicines, accumulation of drugs in the human body and suicide attempts requires a rapid qualitative analysis in order to be able to take adequate counter measures.

The application of column liquid chromatography for the analysis of TCA has many advantages over gas-liquid chromatography[1–4], thin-layer chromatography[5,6] and spectrophotometric techniques[7,8]. Knox and Jurand[9] examined the application of amine–perchlorate ion pairs on silica gel with dichloromethane and a higher aliphatic alcohol (*n*-butanol or isoamyl alcohol) as eluent, while Persson and Lagerström[10] preferred methanesulphonic acid as the stationary phase and dichloromethane, *n*-hexane and n-butanol as the mobile phase in ion-pair partition chromatography. Ion-pair chromatography in an adsorption mode was performed on a chemically modified silica gel by Knox and Pryde[11]. Adsorption chromatography of TCA on alumina[9] and

silica gel[12–15] was reported by several workers. The potential of column liquid chromatography has been demonstrated in the development of moderately successful procedures for TCA[9–15]. However, investigations with serum have been largely neglected.

The aim of this study was to develop a complete separation of all TCA by liquid–solid chromatography on silica gel using a versatile mobile phase system. The procedure should also permit the routine determination of TCA in serum samples.

EXPERIMENTAL

Apparatus, chemicals and materials

The liquid chromatograph was constructed from custom-made and commercially available parts[16].

In all experiments, organic solvents of analytical grade (Merck, Darmstadt, G.F.R.) were used, including the 35% aqueous solution of methylamine. Solvents were de-gassed by ultrasonication immediately before use. LiChrosorb SI-60 (Merck) with a mean particle diameter of 5 μm was used as the packing material.

Chromatography

The columns were packed by using the balanced density slurry technique. Mixtures of ethyl acetate (or dichloromethane), *n*-hexane and methylamine were used as eluents. Capacity ratios (k) were calculated from the retention times of the components and an unretarded compound (monochlorobenzene). The samples were dissolved in the eluent and injected by means of a sampling valve device. The volume of the loop was varied during the experiments.

Extraction

The procedure for the extraction of the TCA from serum is outlined in Table I.

TABLE I

SCHEME FOR EXTRACTION OF TCA FROM SERUM

Stage	*Operation*
2 ml of serum	(1) Add internal standard, add 40 μl of 1 *N* NaOH solution.
	(2) Add 10 ml of diethyl ether.
	(3) Homogenize for 5 min.
	(4) Decant organic phase.
	(5) Repeat steps (2), (3) and (4) twice with aqueous phase.
	(6) Pour aqueous phase into glass tube, centrifuge for 1 min at 1000 *g*.
Diethyl ether phase	(1) Collect diethyl ether phases from steps (4) and (6).
	(2) Dry over anhydrous Na_2SO_4.
	(3) Evaporate solvent with a warm stream of nitrogen.
Residue	(1) Dissolve in 200 μl of eluent.
	(2) Ultrasonicate for 1 min.
	(3) Analyse aliquot by liquid chromatography (volume injected: 48 μl)

RESULTS AND DISCUSSION

Choice of the phase system

The composition of the mobile phase was varied in order to find the optimal separation conditions. The influence of the percentage of *n*-hexane and of the modifier

methylamine on the capacity ratio was measured. The presence of methylamine is essential: when no methylamine is present in the eluent the basic TCA are irreversibly adsorbed on the column. Caude *et al.*[17] obtained similar results for the separation of phenothiazines on silica gel with a mobile phase consisting of ethyl acetate, methanol and ethylamine.

In order to elucidate the influence of the modifier methylamine, the capacity ratios (k) of some compounds were plotted against the percentage of methylamine at a constant water content of the eluent (Fig. 1). It can be seen that the retention decreases rapidly at higher methylamine contents. Ammonia or other weak bases can be used instead of methylamine. Increasing the percentage of *n*-hexane produces a small increase in the capacity ratios, as can be seen in Fig. 2.

The order of elution of the components depends primarily on the acid–base properties of the substituents; primary amines are less basic than secondary, while

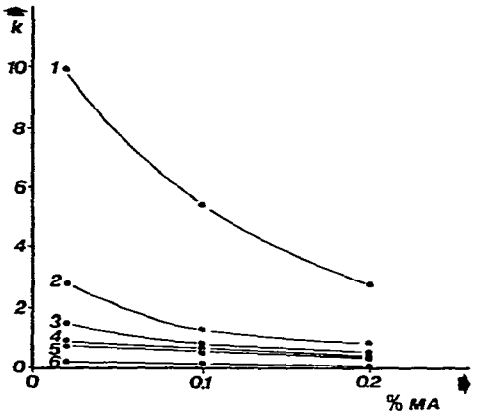

Fig. 1. Dependence of k on volume percentage of methylamine (MA) in eluent consisting of ethyl acetate (20% saturated with water) and a 35% aqueous solution of methylamine. 1 = Desipramine; 2 = promazine; 3 = imipramine; 4 = amitriptyline; 5 = clomipramine; 6 = trimipramine.

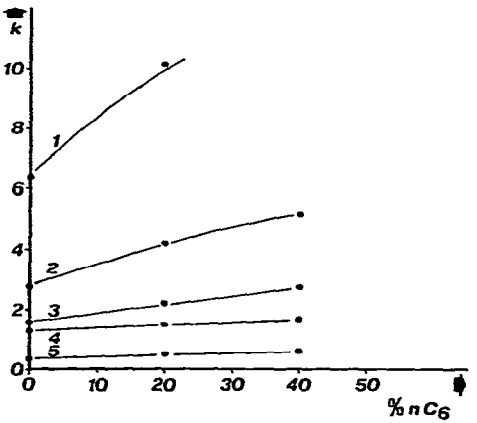

Fig. 2. Effect of volume percentage of *n*-hexane (nC_6) on the capacity ratio. Mobile phase: ethyl acetate, (dry), *n*-hexane and 0.1% (v/v) methylamine. 1 = Nortriptyline; 2 = promazine; 3 = imipramine; 4 = amitriptyline; 5 = trimipramine.

secondary are more basic than tertiary amines, because of steric hindrance. Hence imipramine wil be eluted before desipramine and amitriptyline before nortriptyline.

From Table II, it can be concluded that mobile phase compositions based on ethyl acetate give slightly less selective but faster separations than those based on dichloromethane.

Some preliminary experiments had already shown a smaller selectivity on alumina with mobile phases consisting of dichloromethane, *n*-hexane and acetic acid. The presence of acetic acid in the eluent was also necessary for symmetrical elution of the components. Hence separations on silica gel were to be preferred, and this preference is clearly demonstrated in Fig. 3.

TABLE II

CAPACITY RATIOS OF TCA ON LICHROSORB SI-60 WITH DIFFERENT ELUENT COMPOSITIONS

Eluent 1: ethyl acetate (20% saturated with water) + 0.2% (v/v) of methylamine. Eluent 2: dichloromethane (20% saturated with water) + 0.2% (v/v) of methylamine. Eluent 3: dichloromethane (20% saturated with water) + 20% (v/v) of *n*-hexane + 0.2% (v/v) of methylamine.

Sample	*Capacity ratio (k)*		
	Eluent 1	*Eluent 2*	*Eluent 3*
Trimipramine	0.08	0.17	0.30
Clomipramine	0.38	0.44	0.55
Amitriptyline	0.40	0.51	0.62
Imipramine	0.56	0.67	0.90
Nortriptyline	—	2.61	2.44
Desipramine	2.74	4.11	4.33
Opipramol	3.5	10.3	10.1

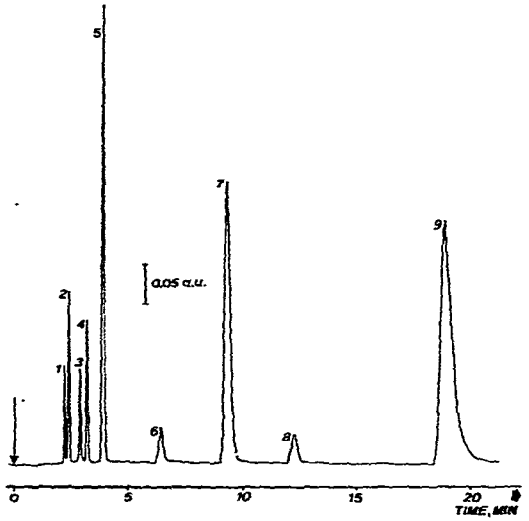

Fig. 3. Chromatogram of TCA test mixture. Column: 300 × 4.6 mm I.D. LiChrosorb SI-60, mean particle diameter 5 μm (HETP 20–30 μm for all components). Eluent: ethyl acetate (20% saturated with water) + 0.2% (v/v) of methylamine. Flow-rate: 1.7 ml/min. Detection: UV (254 nm). Components: 1 = monochlorobenzene; 2 = trimipramine; 3 = clomipramine; 4 = amitriptyline; 5 = imipramine; 6 = promazine; 7 = nortriptyline; 8 = desipramine; 9 = protriptyline. Volume injected: 48 μl.

Serum samples

In order to determine TCA concentrations in serum samples, it is important to combine high sensitivity with high selectivity. The selectivity can be regulated by varying the content of *n*-hexane and methylamine and by the degree of water saturation of ethyl acetate. When working with a UV detector, the sensitivity is dependent mainly on the molar absorbance at the optimal wavelength and noise of the detector. UV detection in this instance was limited by the choice of ethyl acetate (cut-off at 260 nm). Other workers[18,19] have considered the determination of sensitivity (S),

TABLE III

CHARACTERISTIC PARAMETERS FOR DETECTION

Column: 300 × 4.6 mm LiChrosorb SI-60, mean particle diameter 5 μm. Eluent: ethyl acetate (dry) + 0.05% (v/v) of methylamine. Flow-rate: 1.3 ml/min. Detection: UV (254 nm); noise (peak to peak), $2 \cdot 10^{-4}$ a.u.; time constant, 0.7 sec.

Parameter	*Amitriptyline*	*Imipramine*
Sensitivity, S (a.u.·ml·g^{-1})	$1.9 \cdot 10^{13}$	$2.5 \cdot 10^{13}$
Minimal detectable amount, M (g)	$13.5 \cdot 10^{-9}$	$13 \cdot 10^{-9}$
Capacity ratio, k	2.5	4.0
Molar absorbtivity at 254 nm, ε (a.u.·l·mole^{-1}·cm^{-1})	4900	6300

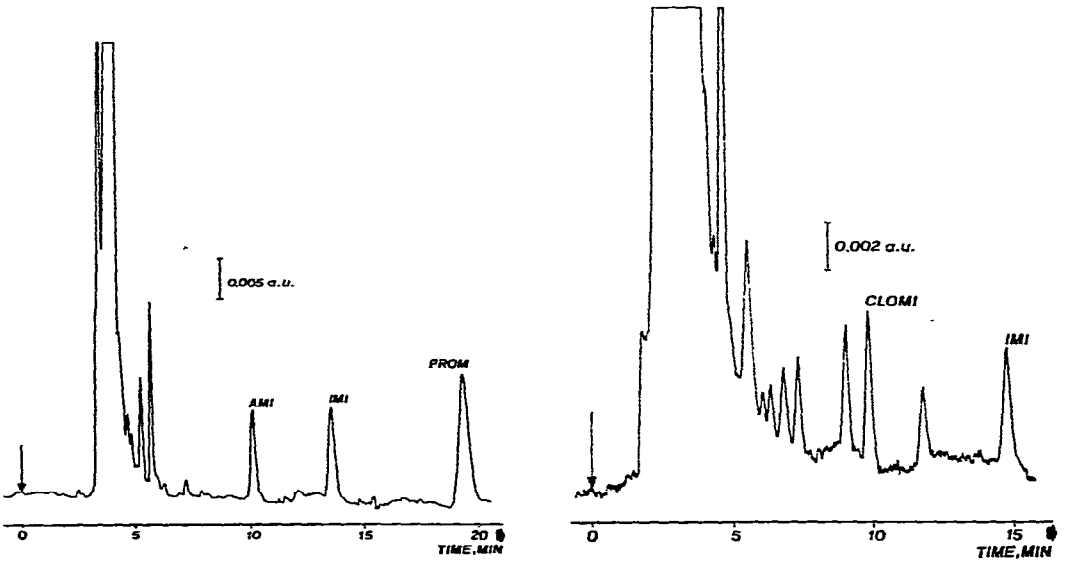

Fig. 4. Chromatogram of an extract of serum. Column: 300 × 4.6 mm I.D. LiChrosorb SI-60, mean particle diameter 5 μm. Eluent: ethyl acetate (dry) + 0.05% (v/v) of methylamine. Flow-rate: 1.0 ml/min. Pressure drop: 95 atm. Detection: UV (254 nm). Components: AMI = amitriptyline (126 ng) (HETP = 25 μm); IMI = imipramine (132 ng) (HETP = 26 μm); PROM = promazine (186 ng) (HETP = 30 μm). Volume injected: 48 μl.

Fig. 5. Chromatogram of an extract from serum. Column: 300 × 4.6 mm I.D. LiChrosorb SI-60, mean particle diameter 5 μm. Eluent: ethyl acetate (dry) + 0.04% (v/v) of methylamine. Flow-rate: 1.1 ml/min. Pressure drop: 110 atm. Detection: UV (254 nm). Components: CLOMI = clomipramine (67 ng) (HETP = 24 μm); IMI = imipramine (70 ng) (HETP = 20 μm). Volume injected: 48 μl.

minimal detectable amount (M) and maximal allowable injection volume (V_o), and these properties are summarized in Table III.

The response of the UV detector was linear with amounts of sample injected of up to at least 300 ng, and injections of amounts up to 100 μl could be allowed without causing more than a 5% loss in resolution.

The extraction procedure resulted in recoveries of 91 $\pm$ 4% for amitriptyline and 95 $\pm$ 4% for imipramine. Figs. 4 and 5 demonstrate the analysis of amitriptyline, imipramine and clomipramine in human serum (with promazine and imipramine, respectively, as internal standards).

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NOTE ADDED IN PROOF

During review, we became aware of a similar effort, using dichloromethane–propan-2-ol–ammonia as eluent on silica gel. Readers interested in the estimation of TCA in plasma by column liquid chromatography may wish to consult ref. 20.

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