Journal of Chromatography, 273 (1983) 151—160
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1534

SOLVENT EXTRACTION OF TRICYCLIC AMINES FROM BLOOD PLASMA AND LIQUID CHROMATOGRAPHIC DETERMINATION

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SUMMARY

The extraction of seven tricyclic antidepressant amines from human plasma at different pH values was investigated for dichloromethane, diethyl ether and hexane—1-pentanol (95:5). The amines were extracted as bases and back-extracted to sulphuric acid, 0.10 mol/l, prior to the separation by bonded-phase liquid chromatography. Ether and hexane—1-pentanol (95:5) were most suitable, tertiary amines being best extracted at pH 8, and secondary amines at pH 10. Using ether, both tertiary and secondary amines required 30 min extraction time for a quantitative yield while 15 min was sufficient for hexane—1-pentanol (95:5). UV detection allowed concentrations down to 10 ng in 1 ml of plasma to be determined.

Three ammonium ions — octylammonium, dimethylammonium, and trimethylammonium — were added as modifiers to the mobile phase containing acetonitrile in phosphoric acid, 0.10 mol/l. In the concentration interval 0.010—0.030 mol/l all of the amine modifiers gave on Polygosil C_8 peak asymmetry factors of sufficiently low magnitude, while on LiChrosorb RP-18 this was so only for di- and trimethylammonium in a concentration of 0.030 mol/l.

INTRODUCTION

Tricyclic amines used as drugs are in many instances subject to plasma level monitoring as a support to rational drug therapy. In the last few years a great number of chromatographic methods for tricyclic antidepressants have been published in particular by liquid chromatography (e.g. refs. 1—7).

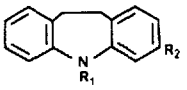
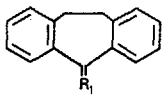
For liquid chromatography of tricyclic and other hydrophobic amines on chemically bonded stationary phases the use of an amine modifier in the aqueous mobile phase has been adapted to improve the chromatographic performance [8]. In addition these substances are of analytical interest since published methods often exhibit much lower recoveries in the work-up procedure, down to 65%, than would be expected from their distribution coefficients [9—11]. This effect may to some extent be due to adsorption losses not being prevented but the major reason must be inconsistency in the ex-

0378-4347/83/0000—0000/$03.00

traction procedure used. In the present study three common organic solvents were used in the extraction from blood plasma of a number of tricyclic amines, and secondary and tertiary aliphatic amines (Table I). The extraction as a function of time was determined for each solvent at three different pH values.

TABLE I

LIST OF TRICYCLIC ANTIDEPRESSANT AMINES

Formula	Name	R_1	R_2
	Imipramine	$-(CH_2)_3N(CH_3)_2$	-H
	Desipramine	$-(CH_2)_3NHCH_3$	-H
	Trimipramine	$-CH_2CH(CH_3)CH_2N(CH_3)_2$	-H
	Clomipramine	$-(CH_2)_3N(CH_3)_2$	-Cl
	Desmethylclomipramine	$-(CH_2)_3NHCH_3$	-Cl
	Amitriptyline	$=CH(CH_2)_2N(CH_3)_2$	
	Nortriptyline	$=CH(CH_2)_2NHCH_3$	

The liquid chromatographic studies comprised two bonded phases — LiChrosorb RP-18 and Polygosil C_8 — and amine modifiers of different structures to elucidate conditions for a satisfactory chromatographic behaviour of the tricyclic amines.

EXPERIMENTAL

Apparatus

The liquid chromatograph was composed of an Altex solvent metering pump Model 110 A (Berkeley, CA, U.S.A.) and a Waters 440 (Waters Assoc., Milford, MA, U.S.A.) UV detector operated at 254 nm. The injector was from Rheodyne (Berkeley, CA, U.S.A.) with 20-, 100-, and 150-μl loops. The separation column of stainless steel (length 150 mm, O.D. 6.35 mm, I.D. 4.5 mm) had end fittings of modified Swagelok connections. Room temperature was used.

The spectrophotometer used in the determination of molar absorptivities was a Beckman DU®-8 UV—Vis spectrophotometer (Fullerton, CA, U.S.A.).

The pH meter used in the extraction experiments was a PHM 64 research pH meter (Radiometer, Copenhagen, Denmark). The mixer used was a table universal shaker TU-1 (B. Braun Melsungen AG, Melsungen, G.F.R.) which operated in an orbital mode at a frequency of 200 rpm with the tubes being placed horizontally.

Chemicals and packing material

Octylamine (puriss, Fluka, Buchs, Switzerland), N,N-dimethyloctylamine (DMOA) (ICN Pharmaceuticals, Plainview, NY, U.S.A.) and N,N,N-trimethyloctylammonium (TMOA) bromide (Department of Organic Chemistry, Hässle, Mölndal, Sweden) were used.

The drug compounds were supplied as hydrochlorides except for trimipramine (maleate). They fulfilled the quality requirements of the Pharmacopoeia Nordica.

All other chemicals used were of analytical grade. The column packing material was Polygosil C_8, average diameter 5 μm (Macherey-Nagel & Co., Düren, G.F.R.) and LiChrosorb RP-18, 5 μm (E. Merck, Darmstadt, G.F.R.).

Column packing procedure

Microporous silica particles (1.8 g) were suspended in 12 ml of isopropanol—dichloromethane (1:1). The slurry was filled in a reservoir connected to the separation column which was packed downwards with methanol—water (60:40) at 400 bar.

Chromatographic systems

(A) Stationary phase: LiChrosorb RP-18, 5 μm. Mobile phases: 30% of acetonitrile in phosphoric acid, 0.10 mol/l, containing octylamine (OA), dimethyloctylamine (DMOA) and trimethyloctylammonium (TMOA) bromide in concentrations of 0.005, 0.010 and 0.030 mol/l. Flow-rate: 1 ml/min.

(B) Stationary phase: Polygosil C-8, 5 μm. Mobile phases: 25—30% of acetonitrile in phosphoric acid, 0.10 mol/l, containing OA, DMOA and TMOABr in concentrations of 0.005, 0.010 and 0.030 mol/l. Flow-rate: 1 ml/min.

Determination of distribution constants

The distribution constant (K_D) for each amine between diethyl ether or hexane—1-pentanol (95:5) and water was determined with equal volumes of organic and aqueous phases in centrifuge tubes at room temperature. As the aqueous phase phosphate buffer solutions (ionic strength = 0.10) of three different pH values around log D = 0 ($D = C_{org.}/C_{aq.}$) were used. The total concentration of each amine in the two phases was $2.5 \cdot 10^{-4}$ mol/l. The tubes were mechanically shaken for 30 min. After centrifugation the organic phase was transferred to another tube and the pH was measured in the aqueous phase. The concentrations of the amines in the aqueous phase were determined chromatographically after washing the phase with hexane and centrifugation.

The concentrations of amines in the organic phase were determined by back-extraction to sulphuric acid, 0.10 mol/l, for 10 min. After centrifugation, washing with hexane and centrifugation again, the sample was injected in the chromatograph; 20-μl samples of the aqueous phases were injected.

The molar absorptivities of the amines at 254 nm were determined to be around $8 \cdot 10^3$.

Determination of the extraction yield of amines from human blood plasma

Volumes of 2.00 ml of blank plasma and of buffer solution (Table II) were mixed with 200 μl of 0.001 *M* hydrochloric acid containing 100 nmol of each amine in centrifuge tubes. Then 4.00 ml of the organic solvent, dichloromethane, diethyl ether or hexane—1-pentanol (95:5), were added for each pH and amine solution. The plasma phases for each pH and organic solvent were then extracted for 2, 5, 10, 15, 30, 45 and 60 min.

TABLE II

BUFFER SOLUTIONS USED IN EXTRACTION AND BLANK PLASMA STUDIES

pH	Buffer	Volume (μl)
8	Tris 1 mol/l, pH 8.0	50
10	Na_2CO_3 0.5 mol/l	140—180*
11**	NaOH 1 mol/l	110
12**	NaOH 1 mol/l	180—200*

*The volume was determined by the buffering capacity of the plasma used.
**pH 11 was used for CH_2Cl_2 and pH 12 for $(C_2H_5)_2O$ and C_6H_{14}—n-$C_5H_{11}OH$ (95:5).

After centrifugation for 10 min, 3.00 ml of organic phase were transferred to a centrifuge tube containing 3.00 ml of sulphuric acid, 0.10 mol/l, and the amines were back-extracted to the aqueous phase by shaking for 10 min.

After centrifugation, the organic phase was removed by aspiration and 1 volume of hexane was added to remove dissolved organic solvent. After shaking for approx. 1 min and centrifugation for 3 min the hexane was removed by aspiration.

A 100-μl aliquot of the aqueous phase was injected onto the chromatographic column. The absorbance of the eluate was measured at 254 nm.

Quantitative evaluation

The extraction yield of the amines was estimated by peak height or area measurement and comparison with direct injection of a reference sample in sulphuric acid, 0.10 mol/l, which prior to injection was shaken with the relevant organic solvent followed by hexane as described above.

Determination of the sensitivity of the analytical method

The determination of the sensitivity was performed according to the analytical method above, except for some minor modifications. Half the volume of blank plasma and buffer solution was used; 100 μl of the 0.001 mol/l hydrochloric acid containing 0.1 nmol of each of desipramine, trimipramine and clomipramine were added to blank plasma. The extraction was performed with diethyl ether and hexane—1-pentanol (95:5), respectively, for 45 min at pH 8 and 10. The organic phase was transferred to a conical centrifuge tube containing 300 μl of sulphuric acid, 0.10 mol/l, which after aspiration of the organic phase was purified by shaking twice with hexane. Finally, 150 μl of the aqueous phase were injected.

RESULTS AND DISCUSSION

Extraction studies

In methods to determine tricyclic antidepressant amines in biological material, samples buffered to pH 12 and 10 are mostly used. The extraction times vary between 1 and 60 min and the volume ratio organic/aqueous phase

between 1 and 8. At pH 12 imipramine and desipramine in human plasma have been reported to be extracted in 90% yield after 10 min extraction time with hexane—1-pentanol (99:1) [1], in 68.5% and 65.5% yield, respectively, by 15 min with hexane—1-butanol (80:20) [2], > 80% yield by 60 min with hexane—isopentanol (99:1) [3], and 88.2% and 103.2% yield by 5 min with butyl chloride [4]. At pH 10 imipramine and desipramine have been extracted in 65.5% and 67.5% yield, respectively, in 10 min with hexane [5], 86.4 ± 4.3% and 100 ± 3.2% yield in 15 min with methanol—ethyl acetate—hexane (0.4:20:79.6) [6], and 89% and 84% yield by 15 min shaking with ether [7].

The extraction yield depends on the pH of the plasma phase and on the extraction time as is illustrated in Figs. 1—3 for ether, hexane—1-pentanol (95:5) and dichloromethane.

The distribution ratio, D, at pH 8, 10 and 12 (11 for dichloromethane) and the theoretical extraction yield from pure aqueous solutions can be estimated from the distribution constant, log K_D (Table III). More than 99% of the amines should be extracted, with the exception of the secondary amines at pH 8 where ether and hexane—1-pentanol (95:5) give between 90 and 99% yield.

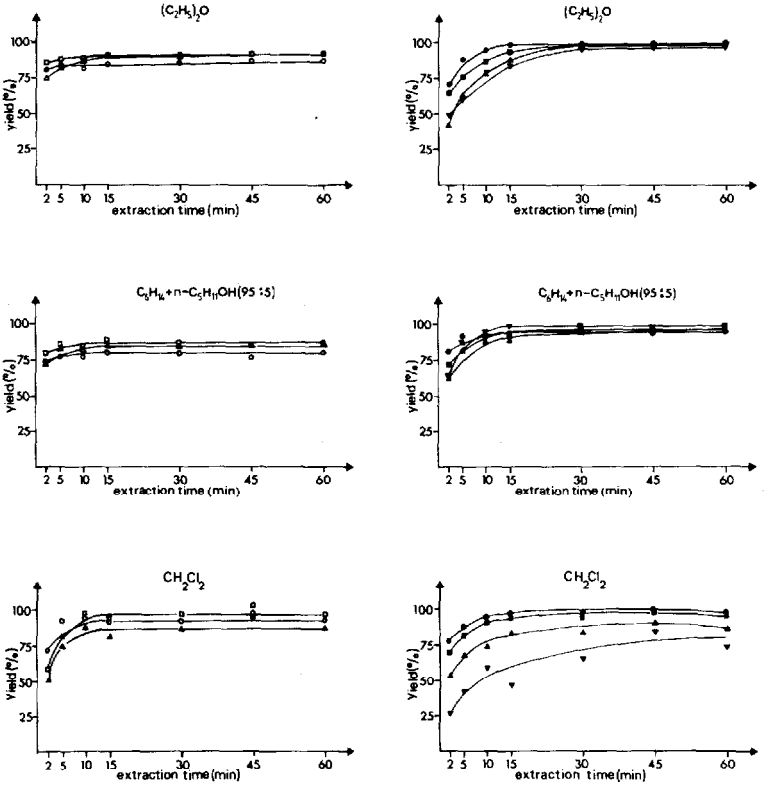


Fig. 1. Extraction yield of seven tricyclic antidepressant amines from plasma at pH 8 for three organic solvents. (○) Desipramine, (□) nortriptyline, (△) desmethylclomipramine, (●) imipramine, (■) amitriptyline, (▲) clomipramine, (▼) trimipramine.

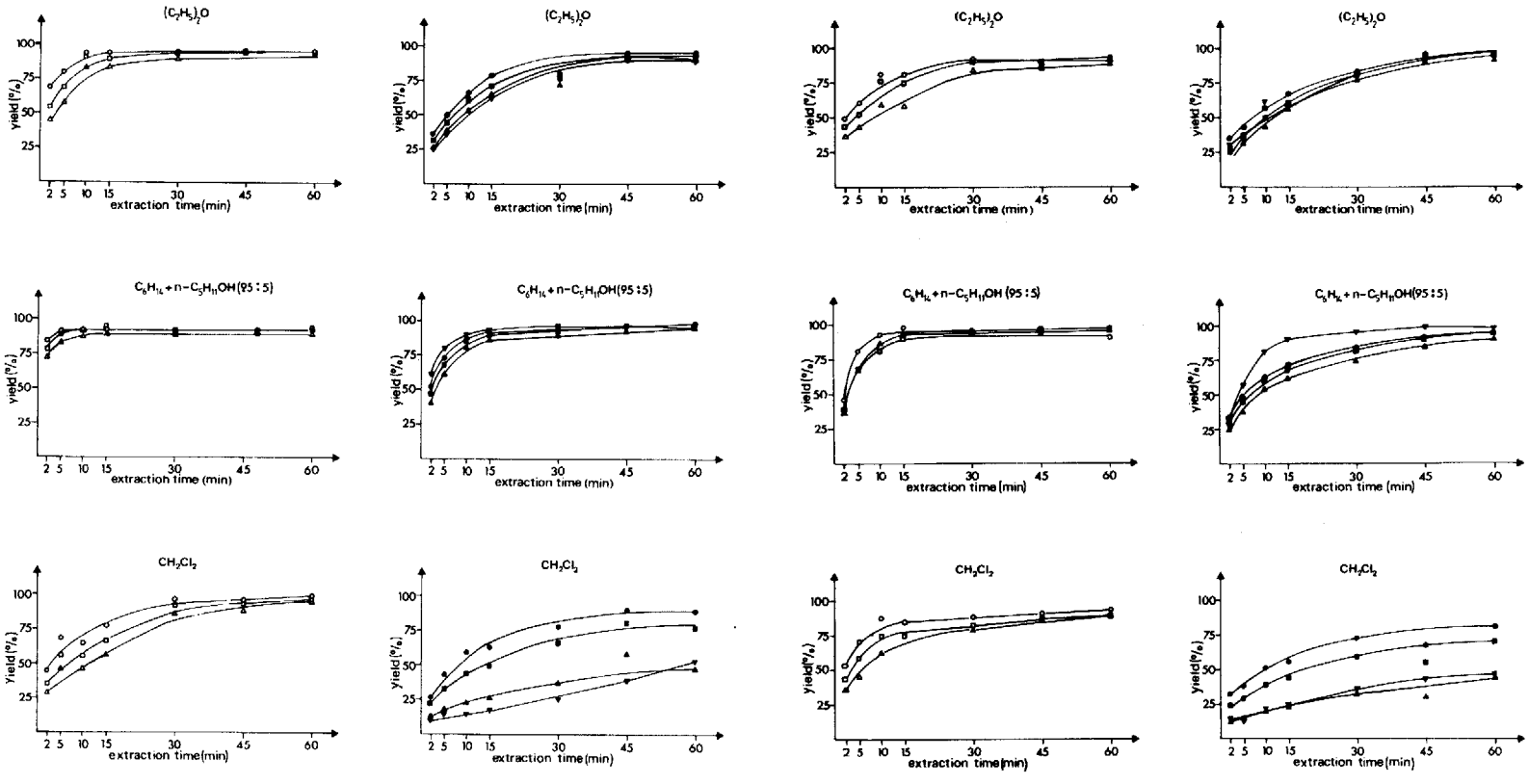


Fig. 2. Extraction yield of seven tricyclic antidepressant amines from plasma at pH 10 for three organic solvents. Symbols as in Fig. 1.

Fig. 3. Extraction yield of seven tricyclic antidepressant amines from plasma at pH 12 (pH 11 for CH_2Cl_2). Symbols as in Fig. 1.

TABLE III

DISTRIBUTION COEFFICIENTS

Ionic strength = 0.10.

Amine	log $K_{D(A)}$*			pK'_{HA}***
	CH_2Cl_2**	$(C_2H_5)_2O$	C_6H_{14}—n-$C_5H_{11}OH$ (95:5)	
Imipramine	5.8	4.27	4.33	9.4
Desipramine	5.1	3.38	3.17	10.2
Amitriptyline	6.2	4.72	4.82	9.4
Nortriptyline	5.6	3.87	3.78	10.2
Clomipramine	6.6	5.14	5.15	9.4
Desmethylclomipramine	5.8	4.26	4.11	10.2
Trimipramine	6.6	5.18	5.17	9.4

*Calculated from log ($K_{D(A)} \times K'_{HA}$).
**Values of imipramine and desipramine were taken from ref. 9. All other values in CH_2Cl_2 estimated from data for $CHCl_3$ from refs. 9 and 10.
***Taken from ref. 11.

The extraction yields were much lower than predicted for some of the extraction systems (Figs. 1—3). With dichloromethane as organic solvent, a very low extraction from plasma was achieved for the tertiary amines at pH 10 and 11, especially for clomipramine and trimipramine which also at pH 8 gave a much too low yield. Dichloromethane gave an extensive protein denaturation in the plasma which increased with increasing pH. This precipitation may cause inocclusion of the strongly protein-bound amines and insufficient access of the organic solvent. Hexane—1-pentanol (95:5) gave only a moderate protein denaturation, which also increased with increasing pH, while ether gave only a slight protein denaturation. In spite of the low protein denaturation of hexane—pentanol and ether, maximum extraction was achieved more rapidly at pH 8 than at pH 10 and 12. This may indicate that the binding of tricyclic antidepressant amines to plasma components are pH dependent and increase on going from pH 8 to pH 10 and 12.

As shown in Figs. 1—3, the secondary amines required shorter extraction times than the tertiary amines to give maximal extraction yield, which may reflect a weaker protein binding. However, dichloromethane should be avoided as it generally gave a lower yield and large deviation between the duplicate samples.

The secondary amines have to be extracted at pH 10 (Fig. 2) if a quantitative recovery is to be obtained when ether or hexane—1-pentanol (95:5) constitutes the organic phase. For ether, 30 min extraction time gave a maximum yield for nortriptyline and desipramine of 94% and 95%, respectively, but only 89% of N-desmethylclomipramine. Using hexane—1-pentanol (95:5) a 15 min extraction time was sufficient to obtain maximum yield, 89% for N-desmethylclomipramine and 92% for the other two amines.

The highest yields for the tertiary amines were obtained at pH 8 (Fig. 1)

using ether or hexane—1-pentanol (95:5) as organic solvent. Maximum extraction in ether was achieved after 30 min while 15 min extraction time was sufficient with hexane—1-pentanol (95:5) to obtain yields of about 95%.

Chromatographic studies

The tricyclic amines were separated as ion-pairs with $H_2PO_4^-$ at pH 3.5 on two types of reversed-phase columns — LiChrosorb RP-18, 5 μm, and Polygosil C_8, 5 μm. The asymmetry factor, Asf, and the capacity factor, k', were investigated without any alkylamine modifier present in the mobile phase and when OA, DMOA and TMOA were added to the mobile phase in the concentrations 0.005, 0.010 and 0.030 mol/l. The tricyclic amines were injected in sulphuric acid, 0.10 mol/l, as in the extraction studies. The results for trimipramine are presented in Fig. 4 and are representative of the other amines studied. In Table IV, Asf and k' values for the tricyclic amines on Polygosil C_8 and LiChrosorb RP-18 are presented using the same mobile phase containing OA as modifier.

The mobile phase content of acetonitrile had to be diminished when OA, DMOA and TMOA were added, because the alkylammonium ions reduce the capacity factors by competing with the tricyclic ammonium ions for the adsorption sites [8, 12].

Acceptable peak symmetry (Asf < 2) was obtained on Polygosil C_8 for all tricyclic ammonium ions at all three concentrations of alkylammonium ions

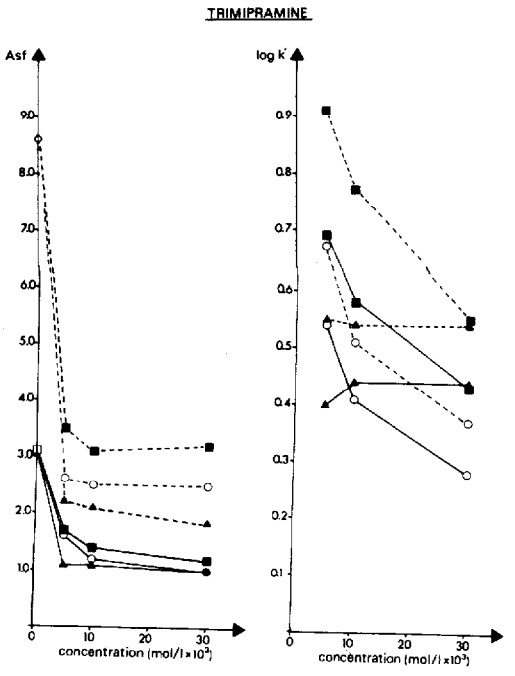


Fig. 4. Peak asymmetry factor (Asf) and capacity factor for trimipramine in the presence of different amine modifiers in the eluent: (■) OA, (○) DMOA, (▲) TMOA. Stationary phases: (— — —) LiChrosorb RP-18, (———) Polygosil C_8.

TABLE IV

COMPARISON OF ASYMMETRY FACTORS (Asf) AND CAPACITY FACTORS (k') OF TRICYCLIC AMINES SEPARATED ON TWO STATIONARY PHASES

Mobile phase: 30% of acetonitrile and OA 0.005 mol/l in H_3PO_4 0.10 mol/l.

Amine	LiChrosorb RP-18, 5 μm		Polygosil C_8, 5 μm	
	Asf	k'	Asf	k'
Desipramine	3.5	4.5	2.3	1.8
Trimipramine	3.5	8.1	2.3	2.8
Desmethylclomipramine	3.0	10.2	2.4	3.3
Clomipramine	3.5	12.3	2.2	3.8

(Fig. 4). At these concentrations TMOA gave approximately constant Asf and k' values.

On LiChrosorb RP-18, TMOA gave acceptable peak symmetry (Asf < 2) at higher concentrations of the modifier, while OA and DMOA improved the chromatographic performance less efficiently.

Sensitivity

The sensitivity of the determination for the tricyclic antidepressant amines in plasma was evaluated by extraction with ether and hexane—1-pentanol (95:5) at pH 8 and 10. A 1-ml volume of plasma was used and 150 μl of aque-

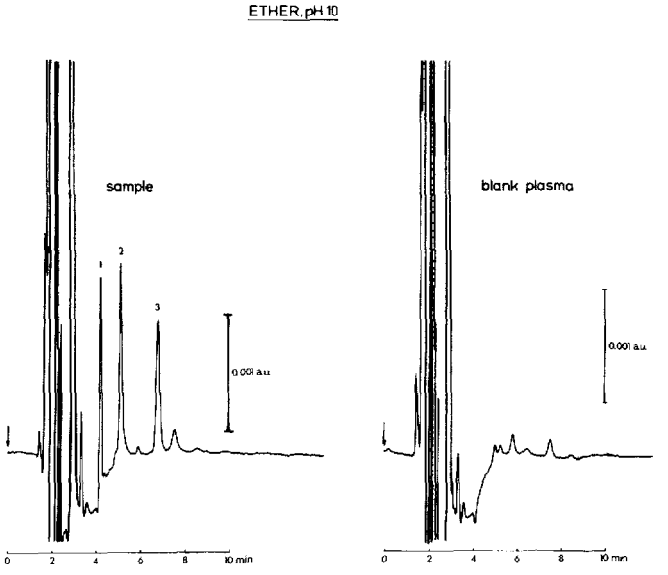


Fig. 5. Chromatograms of extract from 1 ml plasma spiked with 28.2 ng of desipramine (1), 40.1 ng of trimipramine (2), and 41.3 ng of clomipramine (3). Stationary phase: Polygosil C_8, 5 μm. Mobile phase: 25% acetonitrile and 0.015 mol/l DMOA in 0.1 mol/l H_3PO_4.

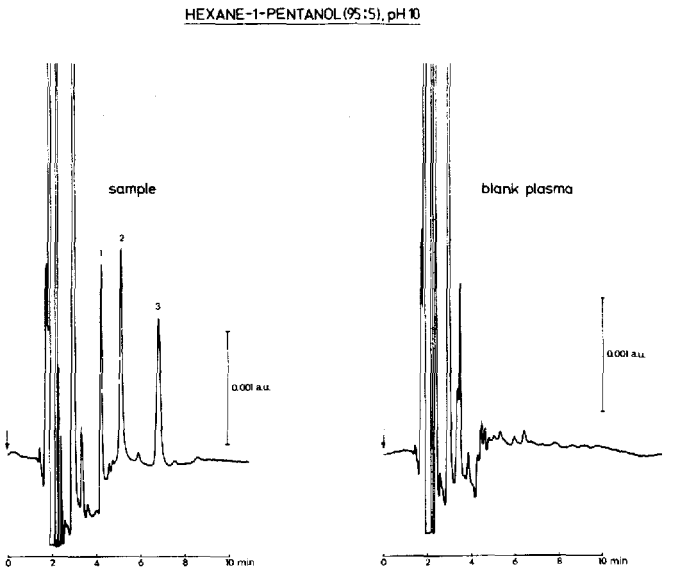


Fig. 6. Same conditions as in Fig. 5.

ous phase were injected onto the column, as these volumes were adequate. This analytical method allows determinations down to 10 ng/ml of plasma sample. The sensitivity limit is set by the background in the blank plasma at the place of the tricyclic amines (Figs. 5 and 6).

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