Journal of Chromatography, 309 (1984) 81—93
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2109

RELIABLE ROUTINE METHOD FOR THE DETERMINATION OF ANTIDEPRESSANT DRUGS IN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

TIM VISSER*,*, MARC C.J.M. OOSTELBOS and PETER J.M.M. TOLL

Laboratory of Clinical Pharmacology and Toxicology, Pharmacy of the Groot Ziekengasthuis, Nieuwstraat 34, 5211 NL 's-Hertogenbosch (The Netherlands)

(First received September 15th, 1983; revised manuscript received February 13th, 1984)

SUMMARY

We describe a rapid and reliable method using high-performance liquid chromatography for the simultaneous measurement of plasma concentrations of currently prescribed antidepressants and their main metabolites (amitriptyline, nortriptyline, *trans*-10-hydroxynortriptyline, clomipramine, desmethylclomipramine, imipramine, desipramine, zimeldine, norzimeldine, doxepin, desmethyldoxepin, trimipramine and mianserin). The method involves a single extraction of plasma at pH 10.1 with hexane—acetonitrile (98:2), solvent transfer to and evaporation in a disposable glass tube and subsequent chromatography of the residue on a CN bonded-phase column using acetonitrile—methanol—phosphate buffer (pH 7.0) as mobile phase. Protriptyline is used as the internal standard. Calibration curves remain linear up to at least 200 μg/l, detection limits are 5 μg/l, absolute recoveries are over 92%, and precision (coefficient of variation) is 6.9%. Norzimeldine and 10-hydroxynortriptyline show lower recoveries, protriptyline and 10-hydroxynortriptyline higher detection limits. Adsorption to glassware and chemical decomposition during analysis are shown to be negligible. Psychoactive and other drugs frequently prescribed in combination with antidepressants have been tested for their chromatographic properties under the same conditions.

INTRODUCTION

Antidepressant drugs are frequently used in psychiatric clinics for treatment of major depression. Large interindividual variations in plasma concentrations among patients receiving the same dosage of these drugs have been shown in several reports [1]. There is no consensus concerning the relationship between

*Present address: Laboratory of Clinical Pharmacology and Toxicology, Central Hospital Pharmacy, Boerhaavelaan 24, 2035 AC Haarlem, The Netherlands

plasma concentration and clinical response [2, 3]. However, measurement of serum concentrations of antidepressants and their metabolites is justified by the pharmacokinetic variability, and in cases of intoxication or patient non-compliance. Reliable and sensitive methods should be available. A review of methodology has recently been published [4].

For application in the clinical laboratory, a large number of gas chromatographic methods (GLC) have been reported, especially for amitriptyline and its metabolite nortriptyline. The earliest methods used flame-ionization detection [5, 6] or electron-capture detection [7]. In more recent studies the introduction of nitrogen-sensitive detectors, which leads to improved sensitivity, has been described [8, 9]. Some papers report analytical methods that have been worked out for several of the currently prescribed antidepressants [6, 8]. High-performance liquid chromatography (HPLC) has also been used successfully for quantifying antidepressant drugs in plasma [10—13]. Techniques like ion-pair partition, adsorption and reversed-phase liquid chromatography have all been applied with a number of different types of column packing and elution solvents. Methods described in the literature vary widely with regard to sample treatment. Recoveries and detection limits of the drugs may differ as well. Some investigators have advocated elaborate sample preparation techniques [11, 12].

Other techniques that have been used for the determination of antidepressants include GLC combined with mass spectrometry and immunoassays. Gas chromatography in combination with mass spectrometry is sensitive and specific but not easily adaptable to the clinical laboratory [14]. Immunoassays are prone to interference [15]. We report a procedure for determining amitriptyline, nortriptyline, *trans*-10-hydroxynortriptyline, clomipramine, desmethylclomipramine, imipramine, desipramine, zimeldine, norzimeldine, doxepin, desmethyldoxepin, trimipramine and mianserin in plasma samples by HPLC. All but two of these antidepressants can be measured simultaneously. Sample pretreatment is easy and fairly rapid, while recovery and detection limit of the method are excellent.

MATERIALS AND METHODS

Instrumentation

A high-pressure liquid chromatograph (Waters Assoc., Etten-Leur, The Netherlands), Model 6000 A, in conjunction with an ultraviolet detector (Waters Assoc.), Lambda Max Model 480, was used. Extinction was measured at 254 nm, the sensitivity being 0.02 a.u.f.s. The column (stainless steel, 30 cm × 3.9 mm I.D.) was packed with 10 μm diameter cyanopropylsilane-coated silica beads (μBondapak-CN®, reversed phase). The column was obtained commercially (Waters Assoc.).

The mobile phase was prepared by mixing 625 ml of acetonitrile, 155 ml of methanol and 220 ml of phosphate buffer pH 7.0. The mixture was filtered (0.45 μm) before use. Chromatography was performed at room temperature, the flow-rate being 1.5 ml/min.

Chemicals and reagents

Aqueous solutions were prepared using glass-distilled water. Acetonitrile, hexane and methanol were analytical reagent (p.a.) grade (Merck, Darmstadt, F.R.G.).

Sodium carbonate (Ph. Eur.) was used for preparation of the 1.0 *M* sodium carbonate solution. The phosphate buffer used for the elution solvent was made by dissolving $Na_2HPO_4 \cdot 2H_2O$ (Merck) in water (5 mmol/l) and adjusting the pH to 7.0 with 0.1 *M* H_3PO_4 (Ph. Eur.). Higher pH values result in decreased stability of the column packing materials.

The antidepressants were obtained as hydrochloride salts except for trimipramine (maleate). Amitriptyline, nortriptyline and *trans*-10-hydroxynortriptyline were obtained from Lundbeck (Copenhagen, Denmark). Desipramine, imipramine, clomipramine and desmethylclomipramine were from Ciba-Geigy (Basel, Switzerland). Doxepin and desmethyldoxepin were from Pfizer (New York, NY, U.S.A.). Zimeldine and norzimeldine were from Astra (Södertälje, Sweden). Trimipramine was from Rhône-Poulenc (Paris, France), mianserin from Organon (Oss, The Netherlands) and protriptyline from Merck Sharp and Dohme (Rahway, NJ, U.S.A.). Methadone hydrochloride was Ph. Eur. grade.

Materials

Tubes used for extraction were glass, 100 × 16 mm round-bottomed (Renes, Zeist, The Netherlands). The tubes were stoppered with polyethylene stoppers and used once only.

Serum and internal standard were pipetted using disposable tips. The standard solutions were dispensed by an analytical syringe.

Procedures

Preparation of standard solutions. The experiments were carried out with two working standard solutions. One working standard (I) was a mixture of amitriptyline, nortriptyline, *trans*-10-hydroxynortriptyline, imipramine, desipramine, doxepin, desmethyldoxepin, mianserin and trimipramine. The other working standard solution (II) was a mixture of zimeldine, norzimeldine, clomipramine and desmethylclomipramine. Both working standards contained 4.0 mg/l of each antidepressant in 0.005 *M* hydrochloric acid in methanol and were prepared by 50-fold dilution of stock standards in 0.005 *M* hydrochloric acid in methanol. The working internal standard was a 4.0 mg/l solution of protriptyline, prepared in the same way. An external standard solution, containing methadone 32 mg/l in 0.005 *M* hydrochloric acid in methanol was used for determination of the recoveries. All standard solutions were stored in the dark at 4°C. No decomposition could be detected after one month of storage.

Calibration curve measurements. Of the working standard solutions 12.5, 25, 50 and 100 µl were measured into test tubes and 2 ml of plasma were added. Equilibration took place overnight. These solutions, like patient plasmas, were treated as described under *Plasma sample handling.* The ratios between the peak areas of each drug and the internal standard were plotted against the

plasma concentration of the drug. Slopes and correlation coefficients were calculated using a least-squares procedure.

Plasma sample handling. Mix 2.0 ml of plasma, 200 μl of working internal standard solution (protriptyline) and 200 μl of 1.0 *M* sodium carbonate solution in a test tube. Add 8 ml of hexane—acetonitrile (98:2, v/v). Close the tube with a polyethylene stopper and extract for 10 min by mixing on a rotator rack. Centrifuge for about 2 min to separate the layers. Put the organic layer into a second test tube and evaporate *almost* to dryness under a stream of nitrogen while warming the tube in a water bath (40°C). Add 200 μl of mobile phase, mix on a Vortex mixer and keep the test tube in an ultrasonic bath for 10 sec. Inject about 100 μl into the liquid chromatograph.

For routine measurement, patient blood samples are centrifuged immediately after collection. The plasma is removed and placed in tubes used for the assay. If not analysed promptly plasmas are frozen at −20°C and not permitted to thaw until assay.

Procedure for determination of coefficient of variation. Of the working standard solutions 125 μl and 250 μl were mixed with 10.0 ml of plasma. In each of the four spiked plasmas drugs were measured three times, using each time 2.0 ml of plasma.

Recovery studies. Recoveries of the antidepressants at the 25 μg/l and 100 μg/l levels were determined by adding after extraction 50 μl of an external standard solution (methadone) to the extract while evaporating the organic layer. At both concentrations the peak areas relative to this external standard (Q_1) were calculated. These values were compared with the ratios (Q_2) obtained by injecting a comparative reference solution, prepared without extraction. The recovery is the ratio between Q_1 and Q_2.

The reference solutions were prepared by pipetting appropriate quantities of the methanolic working standards in extraction tubes, evaporating the methanol and adding mobile phase to the residue. For determining the recovery at 25 μg/l, the reference solution consisted of 50 ng of the drugs, 800 ng of internal standard (protriptyline) and 1600 ng of methadone as external standard in 200 μl of mobile phase. The reference solution for determining the recovery at 100 μg/l contained 200 ng of the drugs, 800 ng of protriptyline and 1600 ng of methadone.

Adsorption studies. In order to find out whether our reference solutions could be interpreted as real references, a comparison was made between the reference solutions and so-called control solutions. Reference and control solutions were prepared at both the 25 μg/l and 100 μg/l levels. The control solutions contained 40-fold quantities of antidepressant, protriptyline (internal standard) and methadone (external standard) compared to the reference solutions, in a volume of 8 ml of mobile phase (40-fold volume). On preparing the control solutions, factors critical for adsorption onto glass, like evaporation of the methanolic standards and exposure of a small volume of liquid to a large glass surface area (when dissolving the residue), were avoided. Preparation took place by pipetting into a test tube appropriate volumes of the methanolic working and stock standard solutions (to keep the quantity of methanol minimal) and diluting to volume without evaporating the methanol. The peak

areas relative to methadone after injecting about 100 μl of the control solutions and reference solutions were compared.

Extraction studies. Norzimeldine and *trans*-10-hydroxynortriptyline were investigated for incomplete extraction. At the 100 μg/l level the recovery of both metabolites was determined as described under *Recovery studies.* After extraction, the pH of the remaining plasma was measured for control. The plasma was extracted for a second time after addition of 200 μl of the protriptyline internal standard solution. Peak areas were determined relative to methadone. As a control amitriptyline was run through the same procedure.

RESULTS

Drug determination

The resolution of both standard mixtures of antidepressants after chromatography is shown in Fig. 1. If the antidepressants were injected as a single mixture, clomipramine would not be completely separated from amitriptyline and zimeldine would interfere with trimipramine. There is no interference with possible peaks from blank plasma.

Calibration curves of some of the drugs are shown in Fig. 2. For all antidepressants a good linear relationship between peak area ratios and plasma levels over a large concentration range is found.

Retention times, coefficients of correlation and variation, recoveries and analytical detection limits at 254 nm are summarized in Table I. The detection limit in plasma, defined as twice the noise level by 0.001 a.u.f.s., for most antidepressants is 2 μg/l and for their desmethyl metabolites 5 μg/l. Incomplete extraction causes the detection limit of 10-hydroxynortriptyline to be a little higher. For protriptyline, the internal standard, the detection limit has not been determined. However, because this compound has a minimum extinction at 250 nm, the detection limit will be high and can be lowered by measuring nearer to the extinction maximum at 292 nm [16]. As shown in Table I, recoveries of norzimeldine and *trans*-10-hydroxynortriptyline are rather low. In order to find out to what extent adsorption onto glass or incomplete extraction influenced our results, adsorption and extraction studies were carried out.

The results of the adsorption studies are shown in Table II. Between the 200-μl reference solutions and the 8-ml control solutions no significant differences were found when peak area ratios of antidepressant relative to methadone were calculated for either method.

Table III shows the results of the extraction studies. Equal fractions of norzimeldine and *trans*-10-hydroxynortriptyline are extracted at the first and second extraction step. Incomplete extraction appears to be the cause of the low recovery (Table I) of both forenamed antidepressant metabolites. The relating chromatograms are shown in Fig. 3.

Selectivity of the method

Several drugs were tested for potential interference with our procedure by comparing the retention times of these drugs with those of the antidepressants. We did not determine the extraction of these materials from plasma. Table IV shows the absolute and relative retention times of the investigated drugs.

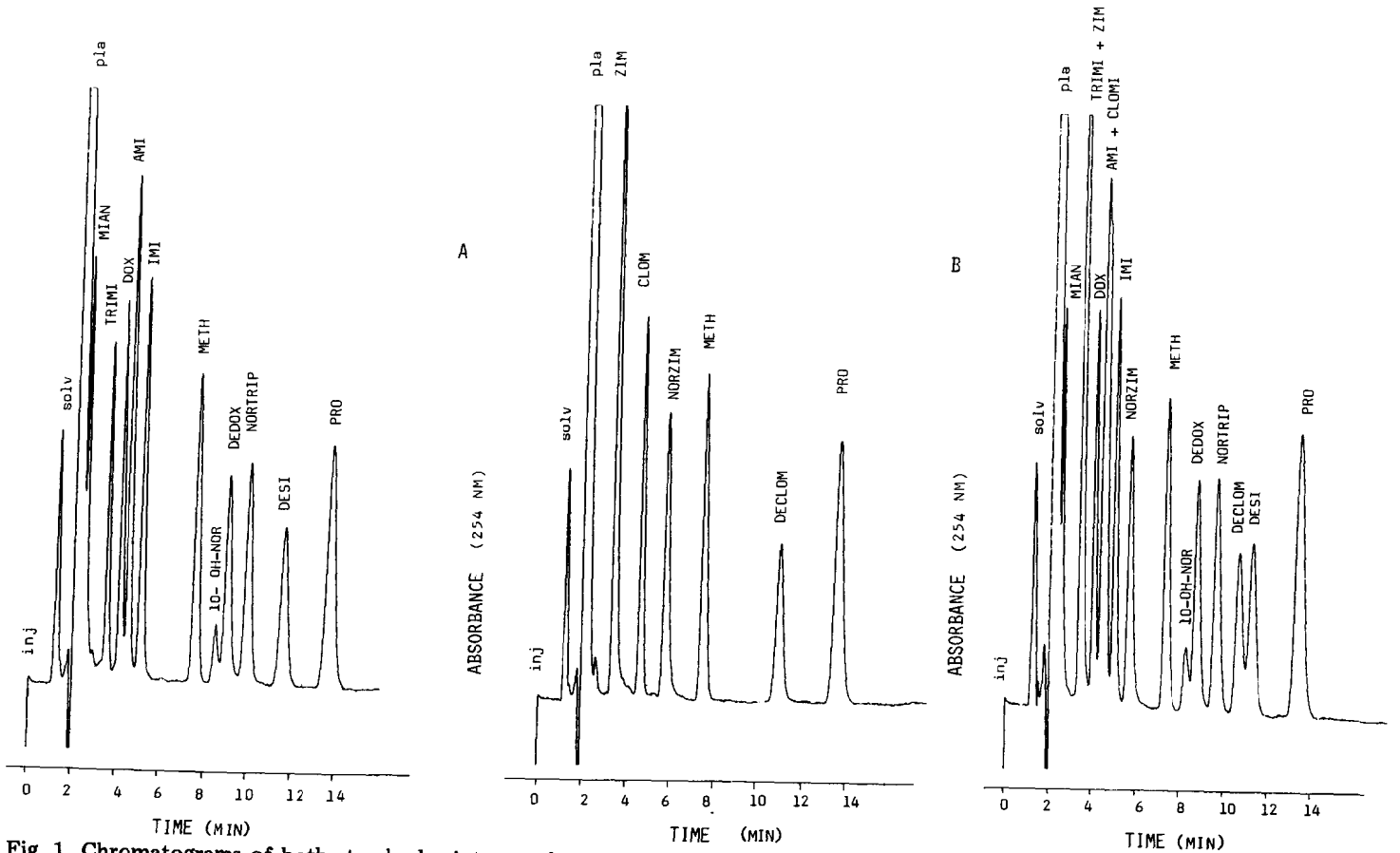

Fig. 1. Chromatograms of both standard mixtures of antidepressants, after spiking (100 μg/l) and extraction from plasma; protriptyline (400 μg/l) and methadone were added as internal and external standards. Method as described in the text. A, standard mixture I; B, standard mixture II; C, a combination of both standard mixtures. Abbreviations: inj = injection, solv = solvent peak, pla = plasma peak, MIAN = mianserin, TRIMI = trimipramine, DOX = doxepin, AMI = amitriptyline, IMI = imipramine, METH = methadone, 10-OH-NOR = *trans*-10-hydroxy-nortriptyline, DEDOX = desmethyldoxepin, NORTRIP = nortriptyline, DESI = desipramine, PRO = protriptyline, ZIM = zimeldine, CLOM = clomipramine, NORZIM = norzimeldine, DECLOM = desmethylclomipramine.

TABLE I

RESULTS FOR THE DETERMINATION OF ANTIDEPRESSANTS

Generic name	Brand name	Retention time		Correlation coefficient*	Coefficient of variation (%) (n = 3)		Recovery (n = 3)		Detection limit (254 nm) (μg/l plasma)
		Absolute (min)	Relative to protriptyline		50 μg/l	100 μg/l	25 μg/l	100 μg/l	
Mianserin	Tolvon	2.56	0.185	0.9995	5.7	6.5	92	92	3
Zimeldine	Zelmid	3.50	0.253	0.9998	2.3	3.6	96	92	2
Trimipramine	Surmontil	3.59	0.260	0.9990	4.2	4.8	106	96	2
Doxepin	Sinequan, Quitaxon	4.20	0.304	0.9992	1.7	5.7	107	100	2
Amitriptyline	Sarotex, Tryptizol	4.58	0.331	0.9998	3.7	4.7	107	98	2
Clomipramine	Anafranil	4.73	0.342	0.9996	1.3	3.0	98	94	3
Imipramine	Tofranil	5.19	0.376	0.9997	3.8	3.9	111	101	2
Norzimeldine		5.89	0.426	0.9999	4.6	4.4	51	50	5
trans-10-Hydroxynortriptyline		8.61	0.623	0.9998	6.9	5.9	40	43	12
Desmethyldoxepin		9.14	0.661	0.9999	4.6	2.2	99	93	5
Nortriptyline		10.07	0.729	0.9998	1.8	6.6	111	96	4
Desmethylclomipramine		11.10	0.803	0.9999	0.9	2.6	99	93	5
Desipramine	Pertofran	11.72	0.848	0.9999	1.5	3.6	102	95	4
Protriptyline	Concordin	13.82	1.000	—	—	—	97	92	—

*Calibration curve: 25, 50, 100, 200 μg/l plasma.

TABLE II

RESULTS OF THE ADSORPTION STUDIES

Data represent peak area ratios of antidepressant to external standard (methadone).

Generic name	Corresponding plasma level = 25 μg/l		Corresponding plasma level = 100 μg/l	
	Ratio for reference solution (n = 3)	Ratio for control solution (n = 3)	Ratio for reference solution (n = 3)	Ratio for control solution (n = 3)
Mianserin	0.130 ± 0.006	0.136 ± 0.005	0.658 ± 0.017	0.704 ± 0.031
Zimeldine	0.532 ± 0.024	0.576 ± 0.009	2.107 ± 0.036	2.236 ± 0.030
Trimipramine	0.218 ± 0.005	0.238 ± 0.006	0.866 ± 0.021	0.867 ± 0.010
Doxepin	0.278 ± 0.008	0.292 ± 0.007	1.075 ± 0.024	1.069 ± 0.009
Amitriptyline	0.379 ± 0.009	0.409 ± 0.011	1.480 ± 0.040	1.457 ± 0.009
Clomipramine	0.288 ± 0.004	0.305 ± 0.007	1.118 ± 0.009	1.188 ± 0.017
Imipramine	0.347 ± 0.008	0.377 ± 0.012	1.370 ± 0.039	1.357 ± 0.009
Norzimeldine	0.435 ± 0.019	0.445 ± 0.011	1.575 ± 0.045	1.672 ± 0.012
trans-10-Hydroxynortriptyline	0.181 ± 0.009	0.199 ± 0.004	0.731 ± 0.015	0.731 ± 0.008
Desmethyldoxepin	0.284 ± 0.013	0.328 ± 0.005	1.146 ± 0.014	1.109 ± 0.007
Nortriptyline	0.290 ± 0.010	0.326 ± 0.014	1.161 ± 0.017	1.135 ± 0.006
Desmethylclomipramine	0.246 ± 0.002	0.241 ± 0.013	0.886 ± 0.031	0.937 ± 0.014
Desipramine	0.256 ± 0.006	0.287 ± 0.005	1.003 ± 0.021	0.988 ± 0.018
Protriptyline	1.901 ± 0.043	1.842 ± 0.031	1.830 ± 0.075	1.707 ± 0.041

TABLE III

RESULTS OF THE EXTRACTION STUDIES

Spiked quantity = 200 ng of each antidepressant in 2 ml of plasma (n = 3).

Generic name	First extraction			Second extraction			Total recovery	
	Absolute recovery (ng)	Relative recovery (%)	C.V. (%)	Absolute recovery (ng)	Relative* recovery (%)	C.V. (%)	Absolute (ng)	Relative (%)
Amitriptyline	189.2	94.6	4.6	11.0	102	13.3	200.2	100.1
trans-10-Hydroxynortriptyline	79.2	39.6	6.0	47.8	39.6	16.2	127.0	63.5
Norzimeldine	98.2	49.1	4.1	48.6	47.7	15.2	146.8	73.4

*Relative to the quantity present after the first extraction.

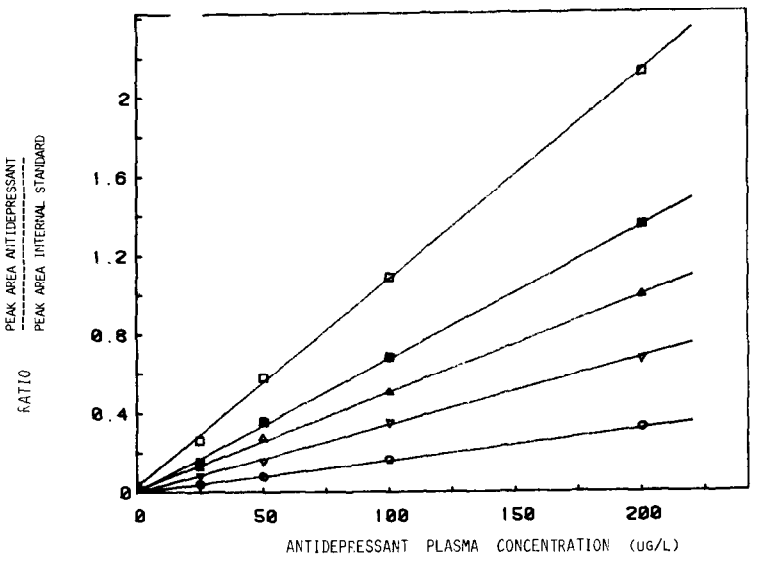

Fig. 2. Calibration curves of spiked plasma. □, ZIM; ■, AMI; △, CLOMI; ▽, MIAN; ○, 10-OH-NOR (abbreviations as in Fig. 1).

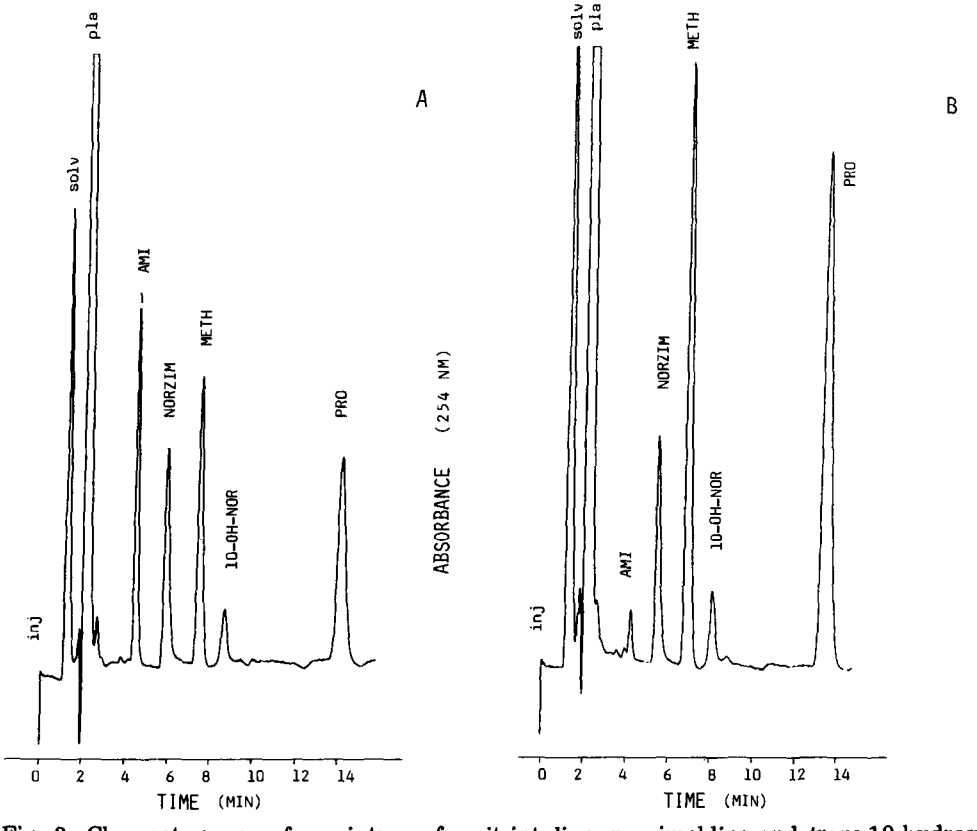

Fig. 3. Chromatograms of a mixture of amitriptyline, norzimeldine and *trans*-10-hydroxy-nortriptyline (each 100 μg/l plasma) after the first (A) and after the second (B) extraction from plasma; protriptyline and methadone were added as internal and external standards. Method as described in the text. Same abbreviations as used in Fig. 1. Each figure is presented on a different scale. Note the relative ratios of the peak heights.

TABLE IV

RETENTION TIMES FOR SOME COMMON DRUGS TESTED WITH THE HPLC PROCEDURE

Generic name	Retention time	
	Absolute (min)	Relative to protryptyline
Phenobarbitone	1.83	0.13
Theophylline	2.07	0.15
Acetaminophen	2.21	0.16
Flupentixol	2.21	0.16
Caffeine	2.25	0.16
Carbamazepine	2.30	0.17
Chlordiazepoxide	2.37	0.17
Nitrazepam	2.37	0.17
Diazepam	2.38	0.17
Oxazepam	2.38	0.17
n-Desalkylflurazepam	2.38	0.17
Fluphenazine	2.49	0.18
Clopentixol	2.54	0.18
Perphenazine	2.56	0.19
Opipramol	2.58	0.19
Penfluridol	2.89	0.21
Promethazine	3.07	0.22
Sulpiride	3.17	0.23
Levomepromazine	3.24	0.23
Desmethylmianserin	3.26	0.24
Prochlorperazine	3.47	0.25
Chlorpromazine	3.81	0.28
Sulforidazine	4.24	0.31
Orphenadrine	4.28	0.31
Promazine	4.59	0.33
Phenytoin	5.25	0.38
Thioridazine	5.25	0.38
Propanolol	5.26	0.38
Mesoridazine	5.46	0.40
2-Hydroxyimipramine	6.31	0.46
Methadone	7.66	0.55
2-Hydroxydesipramine	10.51	0.76
Disopyramide	11.35	0.82
Maprotiline	13.32	0.96
Protriptyline	13.82	1.00

DISCUSSION

With the described assay many commonly used antidepressants and their major therapeutically active metabolites can be measured in plasma. The method is highly selective within the group of the tricyclics. Antidepressant therapy in psychiatric patients can be monitored and therapy can be evaluated in the light of the acquired data.

Since protriptyline itself is only rarely administered as a drug, while having about the same solubility properties as the other antidepressants, we selected it as the internal standard for the assay. In clinical situations where knowledge of protriptyline or maprotiline concentration (Table IV) is required, an alternative internal standard should be used. Favourable results will be obtained with one of the other tricyclic antidepressants. In rare cases, when clomipramine and amitriptyline or trimipramine and zimeldine are prescribed together, modification of the eluent will be necessary to achieve peak separation.

Our assay is well suited for routine application in the clinical laboratory because the extraction procedure is simple (one-step extraction) and fairly rapid. Extraction procedure and chromatography take about 15 min each. The assay provides adequate sensitivity and precision for monitoring steady-

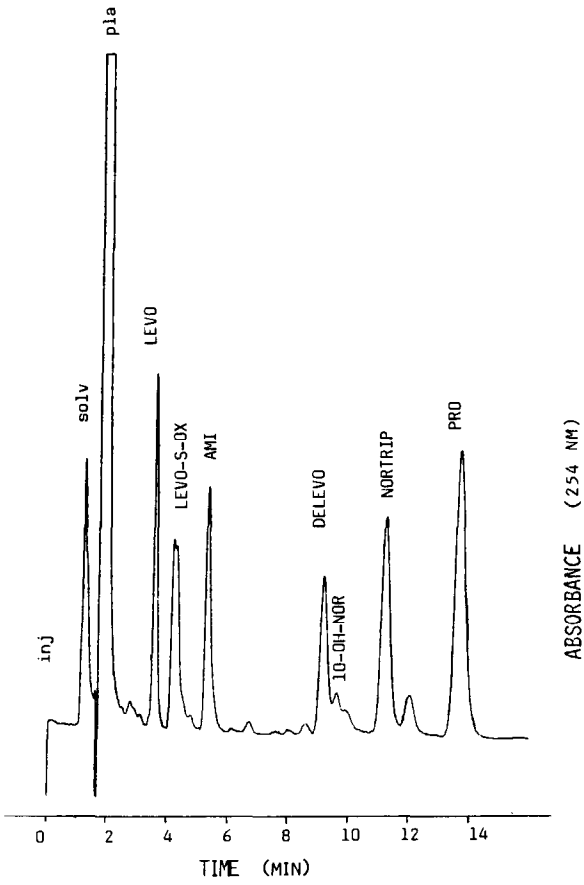

Fig. 4. Chromatogram of a patient's plasma after extraction. Patient was treated daily with 2 × 75 mg of Tryptizol® and 3 × 50 mg of Nozinan®. Method as described in text. Abbreviations: inj = injection, solv = solvent peak, pla = plasma peak, LEVO = levomepromazine, LEVO-S-OX = compound peak, one of which is levomepromazine sulfoxide, AMI = amitriptyline (85 μg/l plasma), DELEVO = desmethyllevomepromazine, 10-OH-NOR = *trans*-10-hydroxynortriptyline, NORTRIP = nortriptyline (117 μg/l plasma), PRO = protriptyline (internal standard).

state concentrations, as well as subtherapeutic and toxic concentrations of antidepressants. Recoveries of the antidepressants were determined at concentrations of 25 and 100 μg/l. We found high recoveries at both concentrations (Table I). Low recoveries were found only for norzimeldine and 10-hydroxynortriptyline. Irregular losses of norzimeldine when concentrated by gentle evaporation have been noted before [17]. However, in our case the low recovery is explained by incomplete extraction. The incomplete extraction of 10-hydroxynortriptyline compared to nortriptyline can be understood because of the introduction of a polar hydroxyl group. No reasonable explanation, however, has been found for the large difference in extraction properties between norzimeldine and zimeldine. Koteel et al. [13], though working with similar chromatographic conditions, report very low recoveries of antidepressant drugs. Our results suggest that their extraction procedure is not suitable.

Several authors report loss of antidepressant during determination, most likely through adsorption to the glassware [18, 19]. According to some, recovery decreases with decreasing amounts of drug [12, 20]. Evaporation conditions seem to be critical [18, 21, 22]. We investigated whether interaction with the glass surface influenced our results. As shown in Table II, neither glass surface area nor our way of evaporating significantly influences the results. So our reference solutions used for the recovery studies can be interpreted as real references. Lack of adsorption is also suggested by the high correlation coefficients of the calibration curves. This information underlines the value of the described assay method.

Since patients treated with antidepressants frequently receive other psychoactive drugs, among them sedatives and tranquillizers that are structurally related to the antidepressants, the clinical usefulness of any method for monitoring antidepressants will largely be determined by the degree of freedom from interferences by these drugs. Of the drugs tested some interfere with the described assay of antidepressants, the relevance of which is determined by the individual drug and by the individual antidepressant.

The described method has been successfully used in our laboratory for monitoring clinical and forensic cases. As an illustration, a chromatogram obtained by working up plasma from a patient undergoing antidepressant therapy is shown in Fig. 4. Also, participation in quality control schemes for antidepressant drugs has yielded good results.

REFERENCES

1 L.F. Gram, Clin. Pharmacokin., 2 (1977) 237.
2 S.C. Risch, L.Y. Huey and D.S. Janowsky, J. Clin. Psychiat., 40 (1979) 4.
3 S.C. Risch, L.Y. Huey and D.S. Janowsky, J. Clin. Psychiat., 40 (1979) 58.
4 B.A. Scoggins, K.P. Maguire, T.R. Norman and G.D. Burrows, Clin. Chem., 26 (1980) 5.
5 H.B. Hucker and S. Stauffer, J. Pharm. Sci., 63 (1974) 296.
6 G. Nyberg and E. Mårtensson, J. Chromatogr., 143 (1977) 491.
7 O. Borgå and M. Garle, J. Chromatogr., 68 (1972) 77.
8 S. Dawling and R.A. Braithwaite, J. Chromatogr., 146 (1978) 449.
9 D.N. Bailey and P.I. Jatlow, Clin. Chem., 22 (1976) 777.

10 D.R.A. Uges and P. Bouma, Pharm. Weekbl. Sci. Ed., 1 (1979) 45.
11 F.L. Vandermark, R.F. Adams and G.J. Schmidt, Clin. Chem., 24 (1978) 87.
12 H.F. Proelss, H.J. Lohmann and D.G. Miles, Clin. Chem., 24 (1978) 1948.
13 P. Koteel, R.E. Mullins and R.H. Gadsen, Clin. Chem., 28 (1982) 462.
14 R.G. Jenkins and R.O. Friedal, J. Pharm. Sci., 67 (1978) 17.
15 K.P. Maguire, G.D. Burrows, T.R. Norman and B.A. Scoggins, Clin. Chem., 24 (1978) 549.
16 R.B. Moyes and I.C.A. Moyes, Postgraduate Med. J., 53 (Suppl. 4) (1977) 117.
17 B. Emanuelsson and R.G. Moore, J. Chromatogr., 146 (1978) 113.
18 P.K. Sonsalla, T.A. Jennison and B.S. Finkle, Clin. Chem., 28 (1982) 1401.
19 P.M. Edelbroek, E.J.M. de Haas and F.A. de Wolff, Clin. Chem., 28 (1982) 2143.
20 A. Jørgensen, Acta Pharm. Toxicol., 36 (1975) 79.
21 E. Antal, S. Mercik and P.A. Kramer, J. Chromatogr., 183 (1980) 149.
22 J.J. Thoma, P.B. Bondo and C.M. Kozak, Ther. Drug Monit., 1 (1979) 335.