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ANALYSIS OF TRICYCLIC ANTIDEPRESSANT DRUGS BY GAS CHROMATOGRAPHY USING NITROGEN-SELECTIVE DETECTION WITH PACKED AND CAPILLARY COLUMNS

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SUMMARY

A gas chromatographic method using either a conventional packed column (3% SP-2250) or a capillary column (SE-30) for the measurement of therapeutic plasma concentrations of tricyclic antidepressant drugs and their demethylated metabolites is described. The technique is based on a simple hexane extraction of drug from alkalinized plasma followed by derivatization with heptafluorobutyric anhydride for the measurement of demethylated compounds. Subsequently, parent drugs and derivatives are chromatographed and detected using a nitrogen-selective detector. A comparison of the results using both types of chromatographic systems is discussed.

INTRODUCTION

Tricyclic antidepressants continue to be one of the most widely used classes of psychotropic drugs employed in clinical practice on a long-term basis. Most of the available evidence [1—4] indicates that monitoring of plasma levels in individual patients during chronic administration is useful for optimizing the therapeutic response and avoiding toxic side-effects; however, there is no consensus on this matter at the present time [5, 6].

Among various analytical techniques used to measure tricyclic antidepressant plasma levels in recent years, gas chromatography (GC) employing a nitrogen-sensitive detector appears to be the most suitable for routine laboratory needs [7—10]. While this method is being successfully applied to monitoring steady-state levels of tricyclic antidepressants and their secondary amino derivatives, it does not appear to be sufficiently sensitive to measure reliably small concentrations of the latter compounds in single-dose pharmacokinetic studies or

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when low steady-state plasma levels are present. Derivatization of the secondary amine formed in vivo during treatment with the parent compound has occasionally been used to measure each amine separately in the same plasma sample [3, 11–13].

We now describe our results using a simple derivatization technique for the routine measurement of small quantities of secondary amine tricyclic antidepressants (desipramine, nortriptyline, demethylclomipramine, and maprotiline) along with their respective parent compounds (tertiary amines). Derivatization with heptafluorobutyric anhydride considerably increases the sensitivity of detection and allows the accurate measurement of plasma tricyclic antidepressant concentrations down to 5 ng/ml.

MATERIAL AND METHODS

Maprotiline (MAPRO), imipramine (IMI), desipramine (DMI), amitriptyline (AMI), nortriptyline (NT) and cyclobenzaprine (CBP) were checked by GC–MS analysis and kindly supplied by Dr. G. Belvedere (Istituto Mario Negri, Milan, Italy).

All the reagents [*n*-hexane–isoamyl alcohol (98.5:1.5, v/v), ethyl acetate and toluene] were of analytical grade (Merck, Darmstadt, G.F.R.). Heptafluorobutyric anhydride (HFBA), a 25% (v/v) solution in ethyl acetate, to derivatize the secondary amines was obtained from Pierce (Rockford, IL, U.S.A.). A solution of NaOH, 0.5 *N* (Merck) was also used.

Ethanolic solutions of the antidepressant drugs in the form of hydrochloride salts, containing 1 and 10 ng μl^{-1}, and CBP, containing 10 ng μl^{-1}, all calculated as free bases, were used. These solutions were stored at 4°C and they were found to be stable for up to three months.

All the glassware used was silanized using a solution (10%, v/v) of dimethylchlorosilane (DMCS) in toluene (Pierce); 2 ml of the DMCS solution were added to each tube. After agitation for 20–30 min the reagent was discarded and the remaining DMCS was evaporated with a nitrogen stream; the pipettes were rinsed with the DMCS solution and subsequently dried. This was done to avoid the absorption of tricyclic antidepressants, especially of the secondary amines, on to the glass surface of the tubes during extraction.

Extraction procedure

The internal standard marker (IS) used to measure MAPRO, CLOMI and DECLOMI was CBP (20 μl of a 10 ng μl^{-1} solution) for both packed and capillary columns. The IS for IMI and DMI, as well as for AMI and NT, was CLOMI (20 μl and 10 μl of a 10 ng μl^{-1} solution, respectively) for the packed column. With the capillary column, for IMI and DMI, the IS was NT (20 μl of a 10 ng μl^{-1} solution); while the IS for AMI and NT was CLOMI (10 μl of a 10 ng μl^{-1} solution). An ethanolic solution of IS was added to a conical tapered tube containing 1 ml of NaOH and 2 ml of plasma sample (to give pH ≈12). The samples were extracted with *n*-hexane–isoamyl alcohol (7 ml) for 20 min on a rock 'n roll shaker, centrifuged (5 min at 1000 *g* and 4°C), and then 6.5 ml of the organic phase were transferred to a clean tube. The hexane phase was evaporated to dryness in a water bath at 60°C under a gentle stream of nitrogen

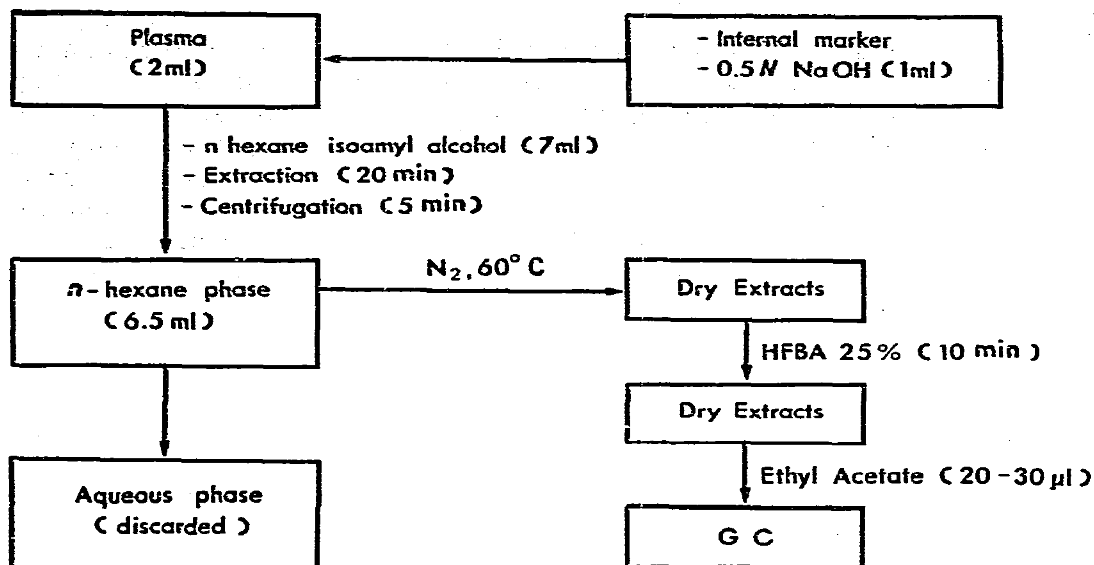

Fig. 1. Extraction scheme used for the analysis of tricyclic antidepressants and their metabolites.

and reacted with a solution of HFBA (200 µl) for 10 min at 60°C. The excess reagent was evaporated off (as described for hexane) and the dry residue was dissolved in 20 µl of ethyl acetate and 3—4 µl were injected into the gas chromatograph. The extraction scheme is shown in Fig. 1.

Gas chromatographic conditions

Two Perkin Elmer 910 gas chromatographs, dual column, equipped with nitrogen-selective detectors were used.

In the first chromatograph, two columns, 2 m × 4 mm I.D., were packed with 3% SP-2250 coated on Supelcoport 80—100 mesh (Supelco, Bellefonte, PA, U.S.A.). The columns were conditioned as follows: a temperature program (5°C/min) from 80 to 300°C with a helium flow-rate of 50 ml/min, then 4 h at 300°C without a gas flow, and finally 48 h at 300°C with a helium flow-rate of 50 ml/min. The oven temperature was 260°C, the injector port and detector temperatures were 300°C; the carrier gas (helium) flow-rate was 50 ml/min. The detector was heated at about 400—450°C (helipot at 650) with a hydrogen flow-rate of 2 ml/min and an air flow-rate of 100 ml/min.

An LKB capillary column, type 2101-502, 25 m × 0.37 mm I.D., was used in the second chromatograph. The open-tubular column was coated with SE-30. The GC conditions were the same as used for the packed columns for MAPRO, IMI, DMI, CLOMI and DECLOMI (see above). For AMI and NT the column temperature was 250°C. The carrier gas was helium at a flow-rate of 1.5 ml/min; helium was also used as make-up gas with a flow-rate of 40 ml/min. A solid injector of the type described by Ros [14] and supplied by Spiral (Dijon, France) was used for the application of samples.

Mass spectrometry

A VG 7070 mass spectrometer, equipped with a Hewlett-Packard 5710A gas chromatograph was used to determine the identity of the peaks obtained by the GC analysis. The GC column was packed with a 3% SP-2250 coated on Supelcoport 80—100 mesh (Supelco), the helium (carrier gas) flow-rate was ca. 15 ml/min, the oven temperature was 260°C, the molecular separator temperature was 250°C. The mass spectra were obtained using the electron-impact mode. The parameters were: electron-beam energy 70 eV, trap current 200 μA, acceleration energy 4 kV, ion-source temperature 250°C, the resolution was set at ≈1000.

RESULTS

Typical chromatograms with packed and capillary columns, obtained from extracts of spiked plasma samples of MAPRO, IMI, DMI, CLOMI, DECLOMI, AMI and NT are presented in Figs. 2 and 3; the retention times and other GC parameters obtained with both techniques are listed in Table I. Comparison with the chromatograms obtained from a blank plasma sample shows that

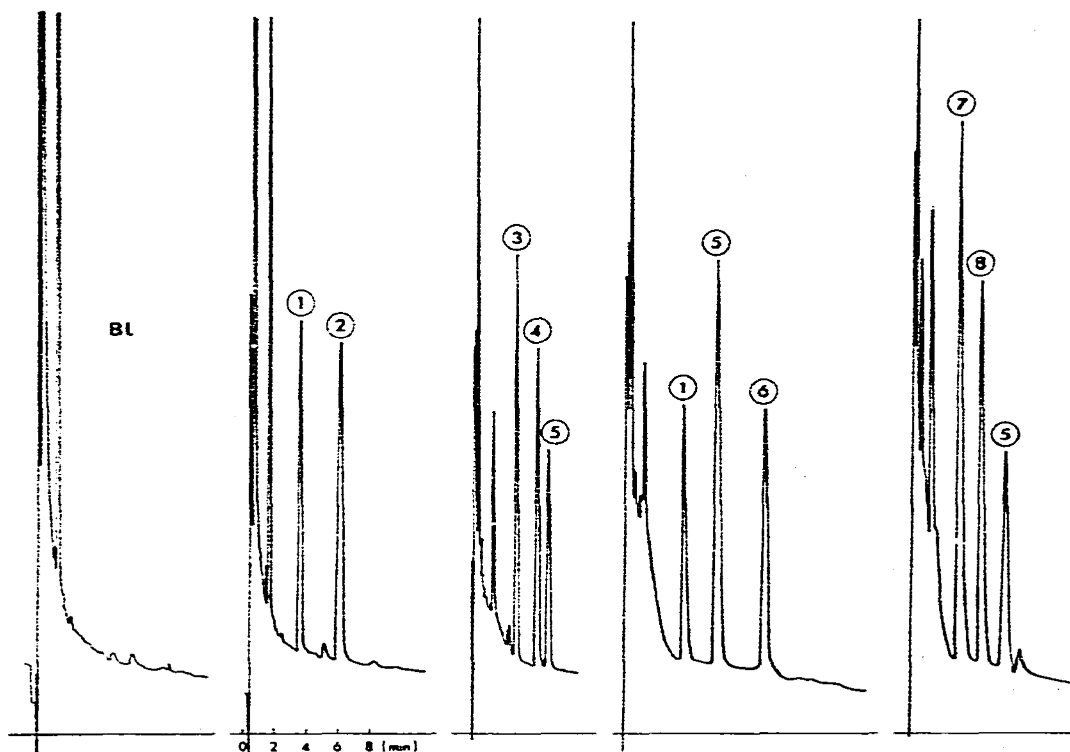

Fig. 2. Gas chromatograms of plasma sample extracts obtained with a packed column from a blank (Bl) and from spiked samples containing: 100 ng ml^{-1} of maprotiline (2), its internal standard cyclobenzaprine (1); 100 ng ml^{-1} of imipramine (3) and desipramine (4) with the internal standard clomipramine (5); 100 ng ml^{-1} of clomipramine and desmethylclomipramine (6), the internal standard being cyclobenzaprine; 200 ng ml^{-1} of amitriptyline (7) and nortriptyline (8), with the internal standard clomipramine (5).

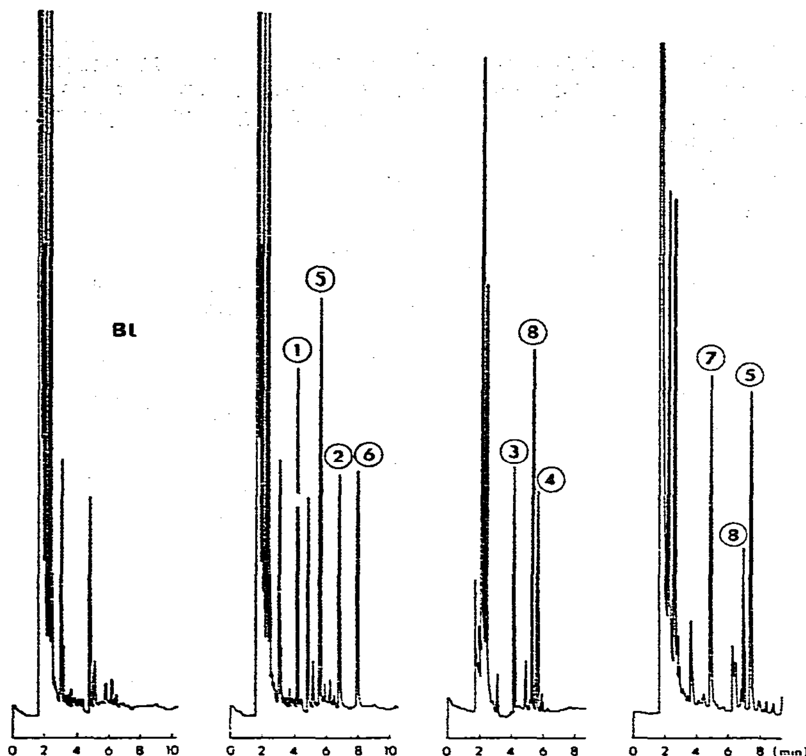

Fig. 3. Gas chromatograms of plasma sample extracts obtained with a capillary column from a blank (Bl) and from spiked samples containing: 100 ng ml^{-1} of maprotiline (2), 100 ng ml^{-1} of clomipramine (5) and desmethylclomipramine (6), the internal standard being cyclobenzaprine (1); 50 ng ml^{-1} of imipramine (3) and desipramine (4), with the internal standard nortriptyline (8); 100 ng ml^{-1} of amitriptyline (7) and nortriptyline (8), with the internal standard clomipramine (5).

endogenous compounds extracted from plasma did not interfere with drug measurement (Figs. 2 and 3).

The calibration curves prepared with tricyclic antidepressant plasma concentrations between 10 and 200 ng ml^{-1} were linear and the coefficient of variation (C.V.) of the day-to-day results at low concentrations did not exceed 15% (Fig. 4). Table II illustrates the accuracy of the analysis at low and high plasma concentrations with both packed and capillary columns.

All the secondary amines such as MAPRO and the demethylated metabolites DMI, DECLOMI and NT were derivatized on the secondary amino group with HFBA to the corresponding heptafluorobutyric amides. The GC—mass spectrometric (MS) analysis confirmed the formation of the amide derivatives of MAPRO (molecular ion at *m/e* 473), DECLOMI (*m/e* 496), DMI (*m/e* 462) and NT (*m/e* 459). The ethyl acetate solutions of the derivatives were stable even at room temperature for at least four days. The tertiary amines (IMI, CLOMI, AMI and CBP) did not appear to react with HFBA; this was evidenced by the same GC retention time and confirmed by GC—MS: IMI (molecular ion at *m/e* 280), CLOMI (*m/e* 314), AMI (*m/e* 277) and CBP (*m/e* 275).

TABLE I

RETENTION TIMES (t_R), NUMBER OF THEORETICAL PLATES (n) AND SYMMETRY FACTORS (Sym) OF TRICYCLIC ANTIDEPRESSANTS OBTAINED WITH PACKED AND CAPILLARY COLUMNS, BEFORE AND AFTER DERIVATIZATION OF THE SECONDARY AMINES WITH HFBA

	3% SP-2250 packed column*			SE-30 capillary column*		
	t_R (min)	n	Sym	t_R (min)	n	Sym
MAPRO	5.5	2300	0.75	7.2	27200	0.58
MAPRO-HFBA	7.5	3000	0.90	10.4	47300	0.86
IMI	3.5	2500	0.86	5.7	41900	0.69
DMI	4.3	2300	0.76	5.9	26300	0.58
DMI-HFBA	5.3	2800	0.96	8.6	50300	0.93
AMI	3.1	2500	0.88	5.4	45100	0.81
NT	3.9	2400	0.77	5.5	25700	0.58
NT-HFBA	4.6	2700	0.87	7.9	48200	0.88
CLOMI	6.1	2800	0.90	8.5	39900	0.73
DECLOMI	7.5	2500	0.79	9.0	33200	0.59
DECLOMI-HFBA	9.9	3100	1.00	11.8	48200	0.89
CBP	4.3	2600	0.91	5.8	36400	0.72

*Oven temperature = 250°C.

TABLE II

MEAN VALUES AND ACCURACY OF THE TRICYCLIC ANTIDEPRESSANT ANALYSES AT LOW- AND HIGH-SPIKED PLASMA CONCENTRATIONS WITH PACKED (3% SP-2250) AND CAPILLARY (SE-30) COLUMNS

	MAPRO	IMI	DMI	CLOMI	DE-CLOMI	AMI	NT
3% SP-2250 column							
Low conc. (20 ng ml^{-1})	20.2	18.9	20.4	18	21.4	20.2	20.8
± C.V. (n = 8)	14	8	14	3	4	4	6
High conc. (200 ng ml^{-1})	200.0	199.4	200.4	201.8	201.7	199.4	200.8
± C.V. (n = 8)	15	14	12	5	7	4	7
SE-30 capillary column							
Low conc. (10 ng ml^{-1})	9.9	9.5	9.9	10.3	9.6	9.8	10.3
± C.V. (n = 8)	11	8	8	9	4	11	11
High conc. (200 ng ml^{-1})	200.6	198.7	199.3	201.8	200.1	199.	199.5
± C.V. (n = 8)	10	8	8	5	5	11	12

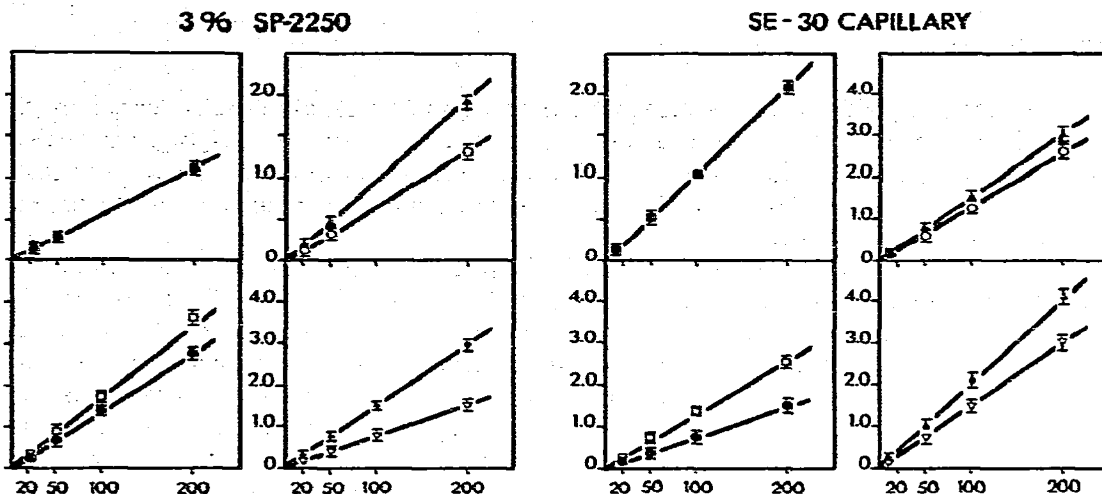

Fig. 4. Calibration curves of TCA plasma sample extracts obtained by the peak height ratio method (peak height tricyclic antidepressant/peak height internal standard) with a packed column (3% SP-2250) and a capillary column (SE-30). ■ = MAPRO, ▲ = IMI, ○ = DMI-HFBA, □ = CLOMI, ● = DECLOMI-HFBA, • = AMI, ▽ = NT-HFBA.

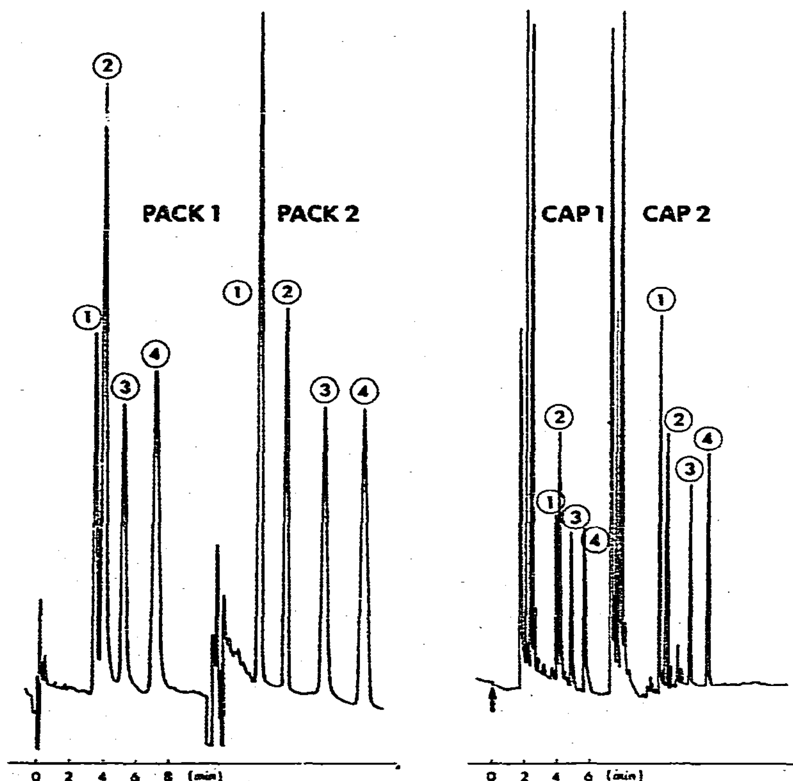

Fig. 5. Gas chromatograms of 30 ng of: nortriptyline (1), desipramine (2), maprotiline (3) and desmethylclomipramine (4) before (PACK 1, CAP 1) and after (PACK 2, CAP 2) derivatization with HFBA either on packed (PACK) or capillary (CAP) columns.

DISCUSSION

The simplicity of the procedure, which consists of a single *n*-hexane—isoamyl alcohol extraction, makes it particularly useful for routine analysis; the most common tricyclic antidepressants and their desmethyl congeners can be measured under the same GC conditions, using the respective internal standards, as indicated in the methodology.

All of the secondary amines are relatively polar molecules and when analysed by GC with semi-polar stationary phases such as SP-2250 (OV-17), tend to give tailing peaks. Derivatization with HFBA reduced the polarity of the compound and improved peak symmetry, hence, the sensitivity of the analysis (Fig. 5). As shown in Table I the chromatographic parameters such as the number of theoretical plates and peak symmetry factors improved considerably after derivatization of the secondary amines. Furthermore, formation of the derivatives minimized the possibility of column adsorption, especially at low concentrations. The reaction with HFBA was fast and complete within 5—10 min. The GC—MS analysis confirmed the structures of the compounds analysed and the formation of the heptafluorobutyric amide derivatives of the secondary amines. A prolonged reaction time should be avoided because it was found that IMI, DMI, CLOMI and DECLOMI can decompose and give rise to the formation of other peaks if the reaction time is more than 50—60 min. This was probably due to a reaction of HFBA on the side-chain of the drugs, as described [15] for DMI.

The results obtained with the packed and capillary columns show that both are reliable systems for the measurement of plasma concentrations of tricyclic antidepressants and their desmethyl metabolites at low therapeutic concentrations.

The drugs which are currently given in associated therapy with tricyclic antidepressants, such as benzodiazepines (diazepam, desmethyldiazepam, oxazepam, chlordiazepoxide) and phenothiazines (chlorpromazine, levomepromazine and thioridazine), were analysed using both systems (packed and capillary columns) under the analytical conditions used for tricyclic antidepressants. We found that with the SP-2250 packed column, chlorpromazine and levomepromazine could interfere with the analysis of DECLOMI-HFBA, and that with the SE-30 capillary column levomepromazine interfered with MAPRO-HFBA. These interferences, however, could be minimized by introducing a temperature program in the GC analysis.

The higher resolution of the GC peaks obtained with the capillary column makes this a superior technique for the determination of tricyclic antidepressants at very low plasma concentrations, or where there are interferences from concomitantly prescribed drugs or endogenous compounds. The capillary column appears to be particularly suitable for pharmacokinetic studies.

Analysis with the conventional packed column, however, is more simple and less expensive; also if one considers that a dual gas chromatograph enables one to work with two columns (which is not possible with a capillary column using a solid injector system), one can reduce the time of analysis by half. Thus, the packed column is more suitable for routine purposes such as monitoring plasma levels during chronic administration of tricyclic antidepressants.

In conclusion, we believe that the analytical methods described in the present report are suitable for the measurement of tricyclic antidepressants in plasma, both in pharmacokinetic studies and in routine therapeutic monitoring.

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