Journal of Chromatography, 277 (1983) 347—351
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1813

Note

Gas chromatographic routine analysis of five tricyclic antidepressants in plasma

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(First received March 7th, 1983; revised manuscript received June 3rd, 1983)

Since the beginning of the clinical trials with tricyclic antidepressants, clinicians have been looking for a simple and fast procedure for monitoring the plasma levels of these drugs. Several analytical methods have been proposed for the determination of drugs and their metabolites, as reported in a recent review [1]; routinely, they are most frequently assayed by gas—liquid chromatography (GLC) [2—5] or, more recently, by high-performance liquid chromatography (HPLC) [6—10].

Gas chromatographic procedures, however, often yield variable results because of some problems in concentration of organic extracts or in derivatisation of demethylated metabolites. We therefore report a simple and reliable gas chromatographic method with nitrogen detection which allows the determination of plasma therapeutic concentrations of amitriptyline, nortriptyline, imipramine, desipramine or clomipramine and requires no secondary amine derivatisation or organic solvent evaporation.

EXPERIMENTAL

Chemicals and reagents

Amitriptyline and nortriptyline were supplied by Merck, Sharpe and Dohme Research Lab. (Rahway, NJ, U.S.A.), imipramine, desipramine, maprotiline and clomipramine by Ciba-Geigy (Basle, Switzerland).

The reagents used in the extraction procedure were of analytical grade. *n*-Heptane was purified as reported in a previous paper [2].

Stock standard solutions were prepared by dissolving in 10 ml of water, 10 mg, calculated as free base, of amitriptyline, nortriptyline, imipramine, desipramine, maprotiline or clomipramine. These solutions are stable at 4°C for at least six months.

0378-4347/83/$03.00

Maprotiline or desipramine were used as internal standards: working solution was prepared by diluting the stock solution in water (100 ng in 10 μl).

Gas chromatography

The analysis was carried out on a Fractovap Series 2200 chromatograph (Carlo Erba) equipped with a nitrogen—phosphorus detector and Minigrator digital integrator.

The column was a 120 cm × 3 mm I.D. silanized glass column rinsed with methanolic potassium hydroxide 0.1% (v/w) and packed with 10% OV-17 on Gas-Chrom Q (100—120 mesh) washed with methanolic potassium hydroxide 0.1% (v/w).

The instrument was set at the following conditions: column temperature, 235°C, detector temperature 500°C, injection port temperature 290°C, hydrogen flow 3 ml/min, carrier gas (helium) flow 40 ml/min, air flow 200 ml/min.

Extraction procedure

All glassware used was rinsed with methanol containing 0.1% of potassium hydroxide. To 1 ml of serum or plasma 10 μl of internal standard, 1 ml of 0.5 *M* sodium hydroxide, 6 ml of *n*-heptane and 0.3 ml of isoamyl alcohol were

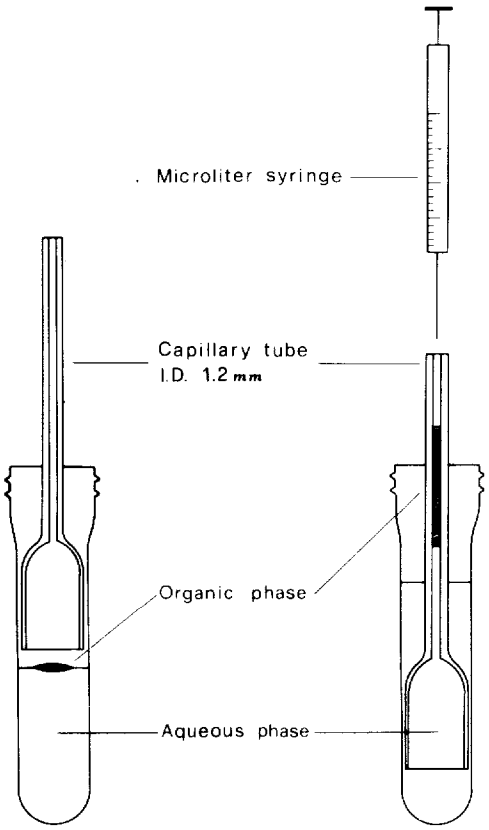

Fig. 1. Apparatus for the recovery of a small volume of organic phase.

added. After extraction for 2 min in a vortex mixer and centrifugation at 2500 *g* for 10 min, the test tube was placed in glycol antifreeze solution at −25°C. The organic phase, separated from the frozen aqueous phase, was reextracted with 2.5 ml of 0.2 *M* hydrochloric acid for 1 min and centrifuged. The acid extract was transferred to a 15-ml specially designed test tube and after addition of 2.5 ml of 0.5 *M* sodium hydroxide and 25 μl of *n*-heptane, was extracted for 1 min. After centrifugation, a bubble cap with a capillary collector (Fig. 1) was inserted and 10 μl of *n*-heptane were injected into the chromatograph.

The concentrations of drugs in unknown samples were determined by calculating the ratios of each drug peak area with that of the internal standard. These ratios were then compared with equivalent ratios obtained from a chromatogram of known quantities of a standard mixture, extracted and separated using the described procedure.

RESULTS AND DISCUSSION

A typical chromatogram of a plasma sample obtained from an amitriptyline-treated depressed in-patient, is shown in Fig. 2.

The detection limit for all the antidepressants studied was between 10 and 20 ng: the retention times of the compounds studied are reported in Table I.

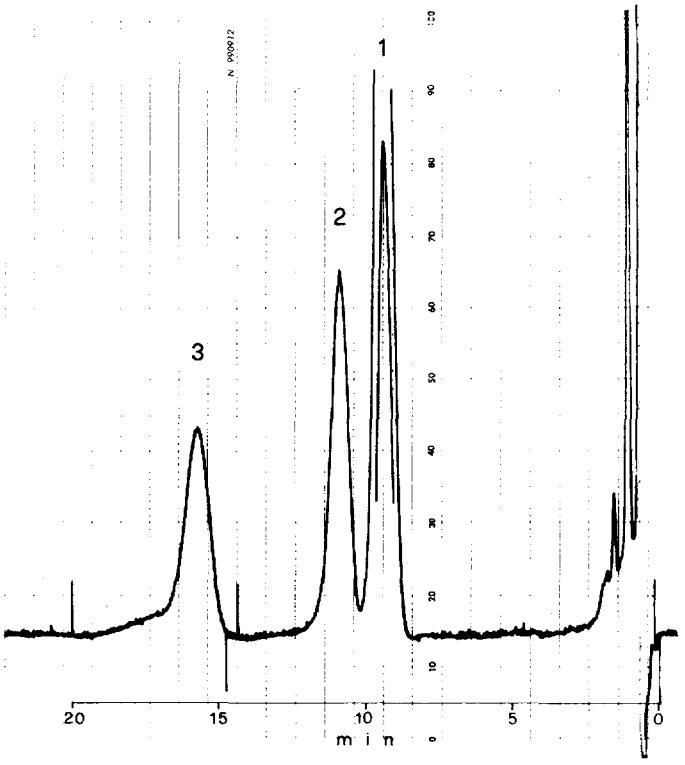

Fig. 2. Chromatogram of a plasma sample from a depressed in-patient treated with amitriptyline (50 mg, intramuscularly, twice a day): 1 = amitriptyline, 308 ng; 2 = nortriptyline, 168 ng; and 3 = internal standard (maprotiline, 100 ng). Chromatographic conditions: see text.

Drug-free plasma samples of healthy subjects and of untreated in-patients were extracted and analyzed for possible interference by endogenous constituents: no background interference was observed. Furthermore, we also noted that the commonly used benzodiazepines (nitrazepam, flurazepam) did not interfere with the analysis.

Table II gives the results obtained when the described procedure was applied to drug-free blood samples spiked with increasing amounts of amitriptyline, nortriptyline or imipramine, desipramine with maprotiline as internal standard or with clomipramine using desipramine as internal standard.

The assay precision calculated from six samples had a coefficient of variation ranging from 3.1% to 10.5%.

TABLE I

RETENTION TIMES OF STANDARDS

Compound	Time (sec)
Amitriptyline	573
Imipramine	621
Nortriptyline	666
Desipramine	731
Maprotiline	972
Clomipramine	1090

TABLE II

PRECISION OF THE ASSAY IN THE DETERMINATION OF AMITRIPTYLINE, NORTRIPTYLINE, IMIPRAMINE, DESIPRAMINE AND CLOMIPRAMINE ADDED TO HUMAN PLASMA SAMPLES

n=6.

Compound	Amount added (ng/ml)	Amount found (ng/ml, mean ± S.D.)	C.V. (%)
Amitriptyline*	50	50.3 ± 4.97	9.87
	100	91.9 ± 5.86	6.37
	200	201.0 ± 14.12	7.24
Nortriptyline*	50	49.8 ± 4.78	9.59
	100	98.2 ± 3.41	3.46
	200	198.8 ± 11.09	5.57
Imipramine*	50	50.8 ± 3.95	7.77
	100	100.0 ± 5.50	5.50
	200	198.2 ± 15.27	7.70
Desipramine*	50	52.0 ± 1.60	3.07
	100	99.2 ± 4.80	4.83
	200	197.6 ± 10.00	5.06
Clomipramine**	50	49.1 ± 2.21	4.49
	100	101.9 ± 10.70	10.49
	200	202.0 ± 13.11	6.48

*Internal standard: maprotiline.
**Internal standard: desipramine.

TABLE III

PLASMA CONCENTRATION OF AMITRIPTYLINE AND NORTRIPTYLINE IN CHRONICALLY TREATED* DEPRESSED WOMEN

Day of treatment	Amitriptyline (ng/ml)						Nortriptyline (ng/ml)					
Patient No:	1	2	3	4	5	6	1	2	3	4	5	6
7	70	90	95	120	145	280	20	30	20	85	30	40
14	80	140	140	130	190	410	20	60	25	150	50	110
21	70	130	140	120	185	435	20	50	30	155	50	160
28	55	120	120	100	190	445	20	55	30	125	45	135

*For treatment conditions see text.

The described method was applied to the determination of plasma concentrations of amitriptyline and nortriptyline of several depressed in-patients chronically treated with amitriptyline 50 mg intramuscularly, twice a day. Blood samples were drawn in the morning, 10 h after the last administration. The data are reported in Table III.

In conclusion, although other analytical methods for tricyclics appear to be adequate, the modification presented in this report resulted in an improved system. First, the organic solvent does not need to be evaporated since the concentration of drug in the small final volume is sufficient for detection. Second, the evaluation of the demethylated metabolites nortriptyline and desipramine is performed without derivatisation. Finally, drugs are evaluated in a small blood sample and in a total assay time of approximately 2 h.

ACKNOWLEDGEMENT

This work was supported in part by CNR Grant No. 82.01068.83: research project "Monitoraggio degli aspetti biologici degli psicofarmaci".

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