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Short Communication

Simultaneous determination of trimipramine and its major metabolites by high-performance liquid chromatography

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ABSTRACT

Trimipramine is a tricyclic antidepressant drug often assayed by gas chromatographic or gas chromatographic–mass spectrometry techniques. A high-performance liquid chromatographic method with electrochemical detection is described for the assay of trimipramine and its major metabolites, monodesmethyltrimipramine and 2-hydroxytrimipramine, in plasma. The method is sensitive, accurate and robust and thus suitable for routinely assaying samples following single doses of trimipramine to man. The assay was applied to plasma samples obtained following a single 50-mg dose of trimipramine to healthy volunteers.

INTRODUCTION

Trimipramine, 5-(3-dimethylamino-2-methylpropyl)-10,11-dihydro-[5*H*]-dibenz[b,f]azepine (Surmontil™, Fig. 1), is a tricyclic antidepressant drug. Tricyclic antidepressants are used extensively in psychiatry in the treatment of a wide range of depressive states occurring with anxiety and neurosis. The efficacy and tolerance of trimipramine is well documented by comparative clinical trials [1–5] and its safety has been established during more than twenty years of use in clinical practice [6].

Only a few reports describing trimipramine pharmacokinetics in man are available [7,8]. Previous studies [9,10] showed that monodesmethyltrimipramine and 2-hydroxytrimipramine are the major metabolites of trimipramine (Fig. 1).

Methods to assay trimipramine in plasma previously reported include gas chromatography [7,8], liquid chromatography [9,11] and recently gas chromatography–mass spectrometry [10]. Reported gas and liquid chromatographic methods lack the specifity or sensitivity required to monitor the low plasma levels of

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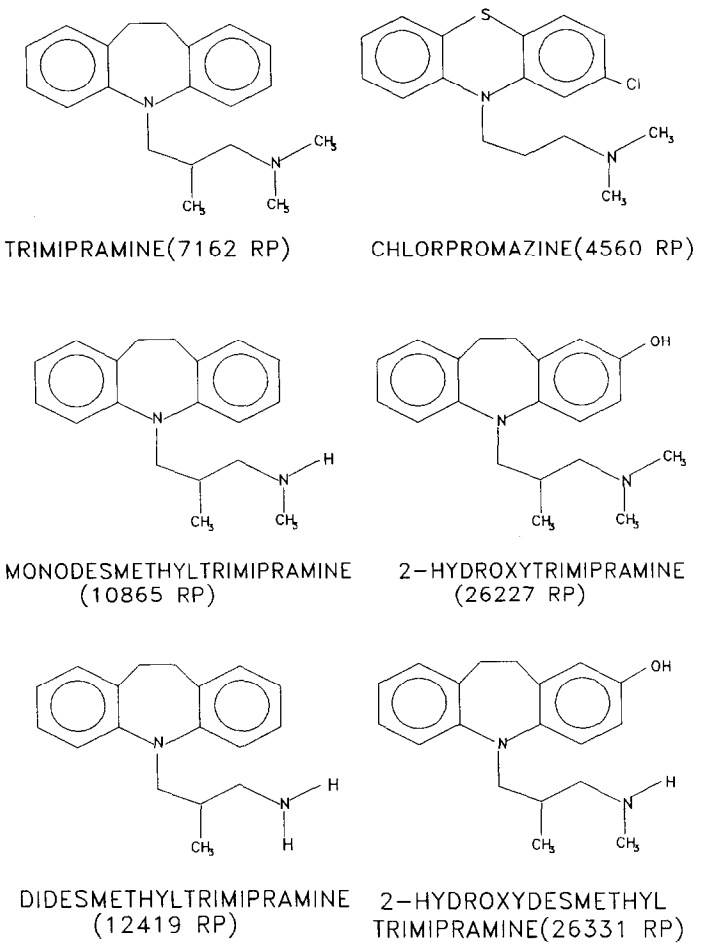

Fig. 1. Structures of trimipramine, its metabolites and internal standard.

trimipramine following single therapeutic doses. The gas chromatographic–mass spectrometric method involves a derivatization procedure and requires more specialised equipment.

This communication describes a simple high-performance liquid chromatographic (HPLC) method using electrochemical detection (ED), suitable for the assay of trimipramine in plasma following doses used in bioequivalence studies [12]. Concentrations of the major metabolites may also be assayed.

EXPERIMENTAL

Chemicals and reagents

Trimipramine, its major metabolites (monodesmethyltrimipramine and 2-hydroxytrimipramine), its minor metabolites (didesmethyltrimipramine and 2-hy-

droxymonodesmethyltrimipramine) and the internal standard (I.S., chloropromazine) were supplied by Rhône-Poulenc. Diethyl ether, chloroform, orthophosphoric acid, sodium hydroxide, methanol, acetonitrile, hexane and isoamylalcohol were from May & Baker (Dagenham, U.K.). Convol pH 11 and pH 7 buffers were from BDH (Poole, U.K.). All solvents were HPLC grade.

Chromatographic conditions

A stainless-steel HPLC column (25 cm × 4.6 mm I.D., HPLC Technology, Macclesfield, U.K.) packed with Spherisorb nitrile bonded phase (5 μm) was used. Analyses were performed using a mobile phase of dipotassium hydrogen orthophosphate (adjusted to pH 6.5 with orthophosphoric acid)–methanol–acetonitrile (1:1:1, v/v) at a flow-rate of approximately 0.8 ml/min, giving a pressure of 9000 MPa (HPLC pump, Model 351, supplied by Applied Chromatography Systems, Macclesfield, U.K.) and at a column temperature of 40°C. An ESA Coulochem electrochemical detector (Model 5100A supplied by Seven Analytical, Shefford, U.K.) and a high-sensitivity analytical cell with electrode 1 set at +0.3 V and electrode 2 set at +0.85 V was used to detect trimipramine. Data capture and processing were carried out using Perkin Elmer Nelson Model 2600 chromatography software.

Samples were injected by an automatic sample injector (WISP 710B, Waters Assoc., Watford, U.K.). Peak-height ratios were used for the calibration graph and for determining the concentration of trimipramine. Detector response for chlorpromazine (I.S.) was 60% of that of trimipramine.

Extraction and assay method

The internal standard (chlorpromazine, 20 ng, Fig. 1) was added to plasma (2 ml) and the pH adjusted to 11 (1 ml, Convol buffer). The extracting solvent, hexane–isoamyl alcohol (10 ml, 98:2, v/v), was added and the sample vortex-mixed. The sample was shaken for 15 min and then centrifuged for a further 15 min at 840 *g* at a temperature of 0–2°C. The upper organic layer was removed and evaporated to dryness at 50°C under oxygen-free nitrogen. The residue was redissolved in 100 μl of the mobile phase and aliquots were injected onto the HPLC column.

RESULTS AND DISCUSSION

The assay method was able to separate the monodesmethyl metabolite and the 2-hydroxy metabolite of trimipramine (Fig. 2). The method did not separate the didesmethyl metabolite from trimipramine but the former has been shown to be absent from human plasma [9].

Calibration graphs were obtained by analyses of plasma samples to which trimipramine was added (1–40 ng/ml range). Linear correlation between concentration and peak-height ratio of trimipramine to the internal standard (chlorpro-

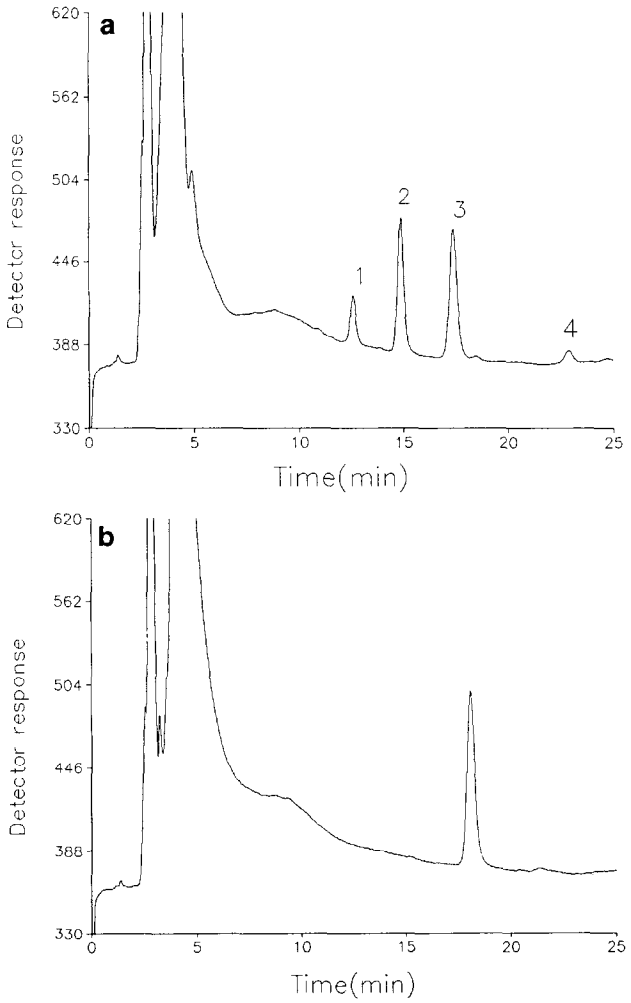

Fig. 2. Chromatogram of an extracted plasma sample: (a) 10 h post-dose after a 50-mg oral dose of trimipramine; (b) blank plasma containing internal standard. Peaks: 1 = 2-hydroxytrimipramine (metabolite); 2 = trimipramine (5.8 ng/ml measured concentration in plasma); 3 = chlorpromazine (internal standard); 4 = desmethyltrimipramine (metabolite).

mazine) yielded a correlation coefficient (r) of at least 0.999. The coefficient of variation (C.V.) above 1 ng/ml was $< 10\%$. (See Table I for intra-assay reproducibility.) The limit of accurate quantitation was therefore set at 1 ng/ml.

The stability of trimipramine was evaluated by analysing spiked standards stored at $-20°C$. There was no loss after several months of storage. The coefficient of variation was 6.9% over a period of 166 days. This is in agreement with an earlier storage stability study by Bougerolle *et al.* [10]. The recovery of trimipramine in this assay was 56% with a coefficient of variation of 6.4% at 10

TABLE I

INTRA-ASSAY REPRODUCIBILITY FOR DETERMINATION OF TRIMIPRAMINE

Nominal concentration (ng/ml)	Measured concentration (ng/ml)							C.V. (%)
	Individual values						Mean ± S.D.	
1.00	1.15	1.00	1.13	0.95	1.06	0.91	1.03 ± 0.10	9.7
2.00	1.58	1.88	1.73	1.71	1.84	1.83	1.76 ± 0.11	6.3
5.00	5.01	5.07	5.03	5.13	5.10		5.07 ± 0.05	1.0
25.00	24.99	25.60	24.80	25.30	25.76		25.29 ± 0.40	1.59

ng/ml. Mean recovery of desmethyltrimipramine was 86% and of chlorpromazine was 54%.

Plasma trimipramine concentrations were determined in a healthy male volunteer following a single oral dose of trimipramine (Surmontil; 50 mg). The maximum plasma concentration–time profile depicted in Fig. 3 shows the observed maximum plasma concentration (C_{max}) to be 16 ng/ml at the time of peak concentration (T_{max}) of around 3–4 h.

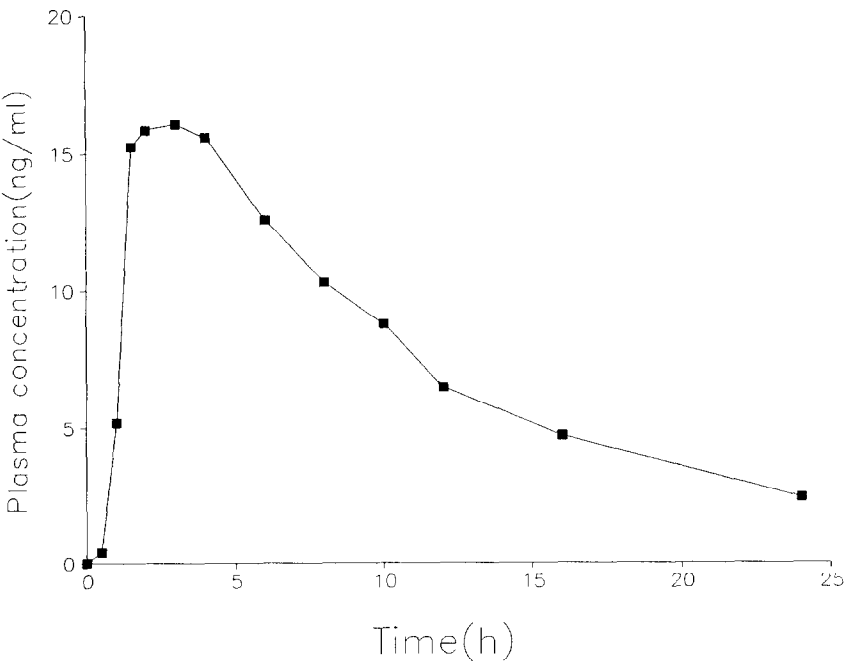

Fig. 3. Plasma level *versus* time profile in a healthy volunteer given a single oral dose (50 mg) of trimipramine.

The plasma concentration then falls to about 2.0 ng/ml at 24 h after dosing. The plasma concentrations of the monodesmethyl and hydroxy metabolite were less than 3 ng/ml.

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