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ARTICLE *in* JOURNAL OF CHROMATOGRAPHY A · DECEMBER 1972

Impact Factor: 4.17 · DOI: 10.1016/S0021-9673(01)86150-1 · Source: PubMed

Reprinted from:

Journal of Chromatography
Elsevier Publishing Company, Amsterdam - Printed in The Netherlands

CHROM. 6314

A METHOD FOR THE DETERMINATION OF IMIPRAMINE IN HUMAN PLASMA BY GAS–LIQUID CHROMATOGRAPHY–MASS FRAGMENTOGRAPHY

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(Received July 21st, 1972)

SUMMARY

A sensitive and specific method for the quantitative determination of imipramine in human biological fluids is described.

After extraction with *n*-hexane, imipramine is detected by gas–liquid chromatography–mass fragmentography. The procedure permits routine analysis on relatively small amounts of plasma (1–2 ml) containing as little as 10 ng/ml of imipramine without any interference from endogenous substrates.

INTRODUCTION

In the field of tricyclic antidepressant drugs, the pharmacokinetics of secondary amines, *i.e.*, monomethylated compounds such as desipramine and nortriptyline, have been the object of extensive studies[1–6]. However, very little information is available on the plasma concentrations of tertiary amines, such as imipramine or amitriptyline, in depressed patients undergoing chronic treatment with these drugs[7–9]. Several methods have been described for the determination of imipramine in biological fluids[10–14], but they either lack specificity and sensitivity, require large amounts of blood, or necessitate very long procedures. Hence they do not meet the requirements of routine clinical assays.

Our interest in the pharmacokinetics of tricyclic antidepressant drugs has prompted us to use the gas chromatography–mass fragmentography technique introduced by HAMMAR *et al.*[15]. In this technique, the mass spectrometer is used as a very sensitive gas chromatographic detector that continuously monitors one, two or three mass numbers of compounds eluted from the gas chromatographic column. Hence the quantitative determinations of imipramine in small volumes of biological fluids of patients undergoing chronic treatment are possible.

In this paper we describe a new procedure based on this technique of gas–liquid chromatography–mass fragmentography that satisfies the requirements for the routine determination of imipramine in small amounts of plasma.

EXPERIMENTAL

Standards and reagents

Imipramine was provided by Ciba-Geigy, Milan, and promazine (used as internal marker) by Pierrel, Milan.

The following reagents were used: *n*-hexane R.P. (C. Erba), NaOH (Merck), absolute ethanol R.P.E. (C. Erba).

Apparatus for gas–liquid chromatography–mass fragmentography (GLC–MF)

An LKB Model 9000 mass spectrometer equipped with a gas chromatograph, an accelerating voltage alternator (A.V.A.) and a peak matcher were used.

The gas chromatographic conditions were as follows. The chromatographic column was a glass tube, 2 m long and 2 mm I.D., packed with 3% OV-17 on Gas-Chrom Q, 100–120 mesh (Applied Science Laboratories); the column temperature was 240° and the injection port temperature was 260°; and the carrier gas (helium) flow-rate was 30 ml/min.

The mass spectrometer was set to the following conditions: separator temperature 280°; ion source temperature 290°; trap current 60 μA; electron energy 30 eV; multiplier potential 3.5 kV; and filters 20 c.p.s.

The A.V.A. conditions were: accelerating potentials 3.5 and 3.456 kV; switching frequencies 2 times/sec; and filters ± 3 Hz.

In order to increase the sensitivity of the equipment and to obtain a better evaluation of the results, the mass fragmentograms were recorded directly on the pen–ink recorder normally used for GLC. The apparatus arrangement is illustrated in Fig. 1.

The output of the mass spectrometer amplifier was connected to the input of the peak matcher amplifier (LKB 9020), set for amplification $\times 10$. The signal was then

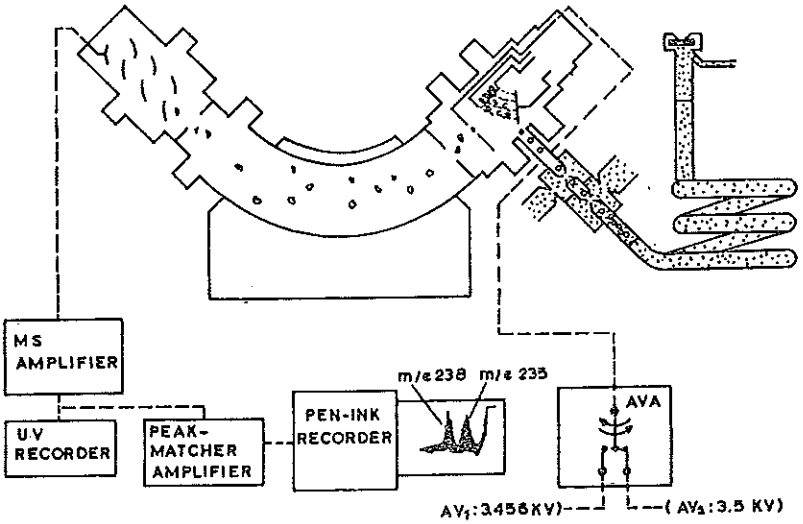

Fig. 1. Schematic diagram of the combined GLC–MS apparatus (LKB 9000) for mass fragmentography of imipramine and promazine.

introduced into the paper recorder pre-set for a 10 mV full-scale sensitivity (chart speed 5 mm/min). With these settings, it was possible to obtain a signal ten times more intense than the normal signal obtained using the mass spectrometer amplifier. The peak shape so obtained was very similar to that obtained in GLC (Fig. 2).

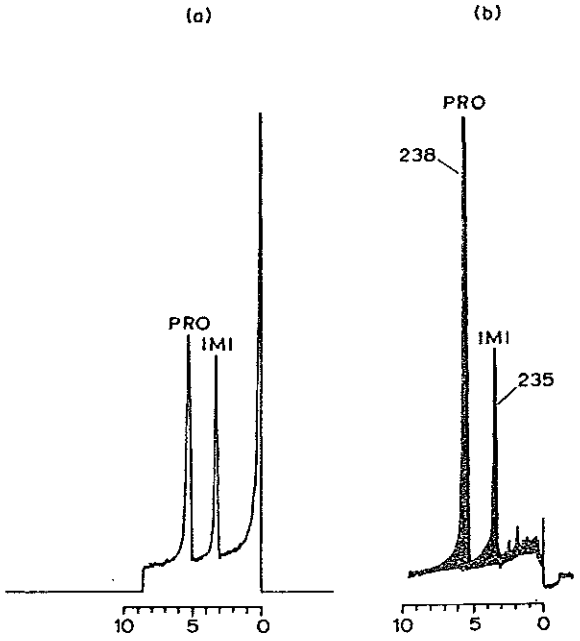

Fig. 2. (a) Gas chromatogram and (b) mass fragmentogram of imipramine (IMI) and its internal marker, promazine (PRO).

Determination of the calibration curves and quantitative analysis of imipramine

Imipramine was dissolved in absolute ethanol in concentrations varying from 4 to 400 ng/ml. Three 1-ml aliquots of each of the solutions were evaporated to dryness under a gentle stream of nitrogen in a water-bath at 60°. A 100-μl volume of absolute ethanol containing 1 ng/μl of promazine was then added to the dry residue.

A 1-μl volume of this solution was injected on to the gas chromatographic column. A calibration curve was constructed relating the imipramine peak areas to the internal marker (promazine) areas and was found to be linear for a total amount of drug added to the sample from 5 to 200 ng.

The minimum detectable amount of imipramine by injection on to the gas chromatographic column was 50 pg with constant linearity up to 2 ng. The same procedure was followed with plasma samples containing imipramine in known amounts (Fig. 3).

Extraction procedure

A 2-ml volume of plasma was made alkaline with 0.5 ml of 2.5 *M* NaOH and then extracted with 3 ml of *n*-hexane. Then 2 ml of the organic phase were evaporated to dryness under a gentle stream of nitrogen at 60° in a water-bath. The dry residue

was redissolved in 100 μl of absolute ethanol containing promazine (1 ng/μl) and 1 μl of this solution was injected on to the gas chromatographic column. The recovery from plasma was found to be 81 $\pm$ 2%.

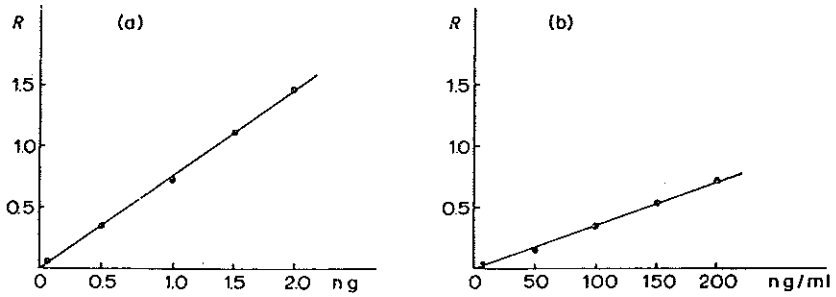

Fig. 3. (a) External calibration curve of imipramine. (b) Calibration curve obtained adding known amounts of imipramine to 2 ml of plasma and then processing as indicated in the extraction procedure. R = ratio of the peak areas of imipramine and its internal standard, promazine.

RESULTS AND DISCUSSION

A typical mass fragmentogram of a patient's plasma samples before starting imipramine treatment and after 21 days of treatment (imipramine, 125 mg/day) is shown in Fig. 4. It can be seen that desipramine (DMI), the demethylated derivative of

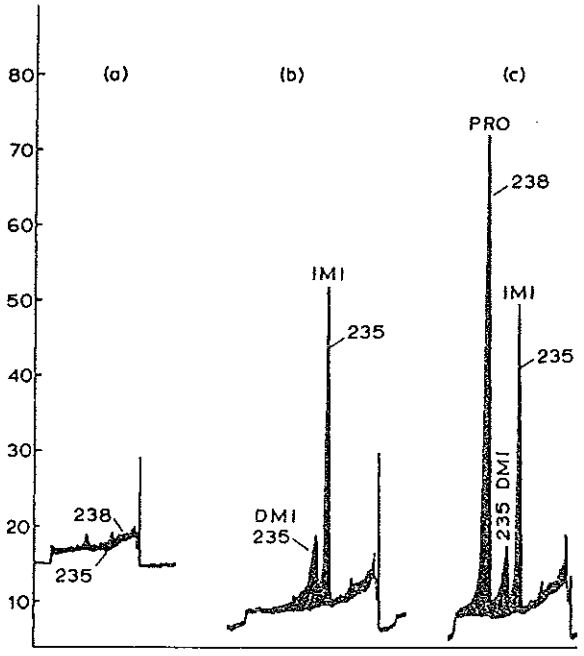

Fig. 4. Mass fragmentograms obtained from plasma of a patient undergoing chronic treatment with imipramine (125 mg/day): (a) before treatment; (b) after treatment with imipramine (IMI) without internal marker; (c) as for (b) after addition of the internal marker, promazine (PRO).

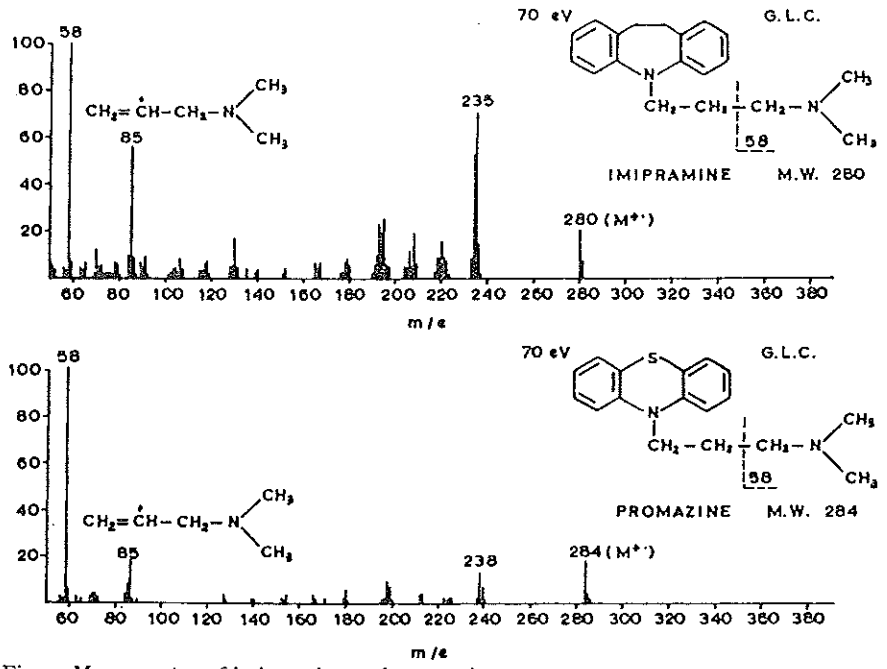

Fig. 5. Mass spectra of imipramine and promazine.

imipramine, does not interfere with the analysis. The use of promazine as an internal marker for quantitation by the relative peak area technique is therefore proved to be satisfactory. Promazine was chosen as the internal marker because of its suitable retention time and the fact that the two compounds (imipramine and promazine) give

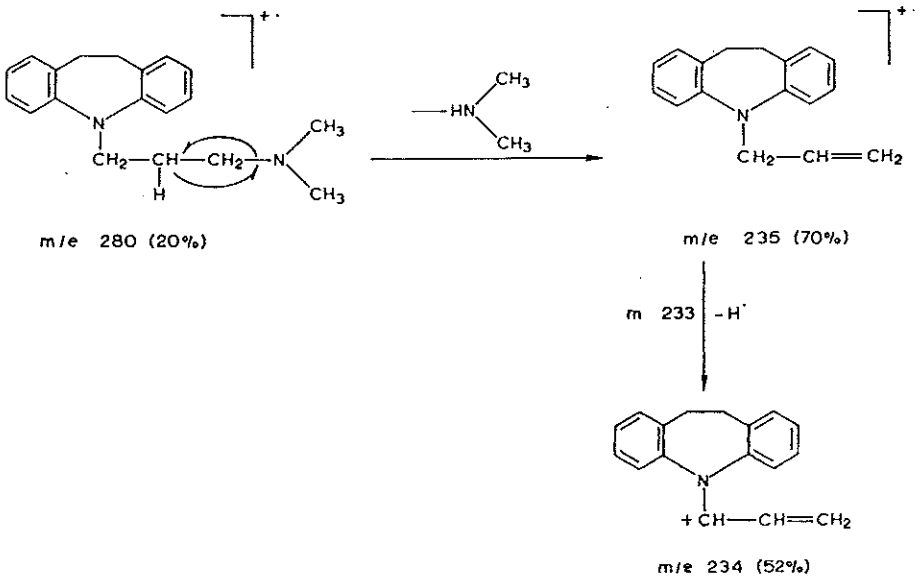

Fig. 6. Fragmentation pathway of imipramine.

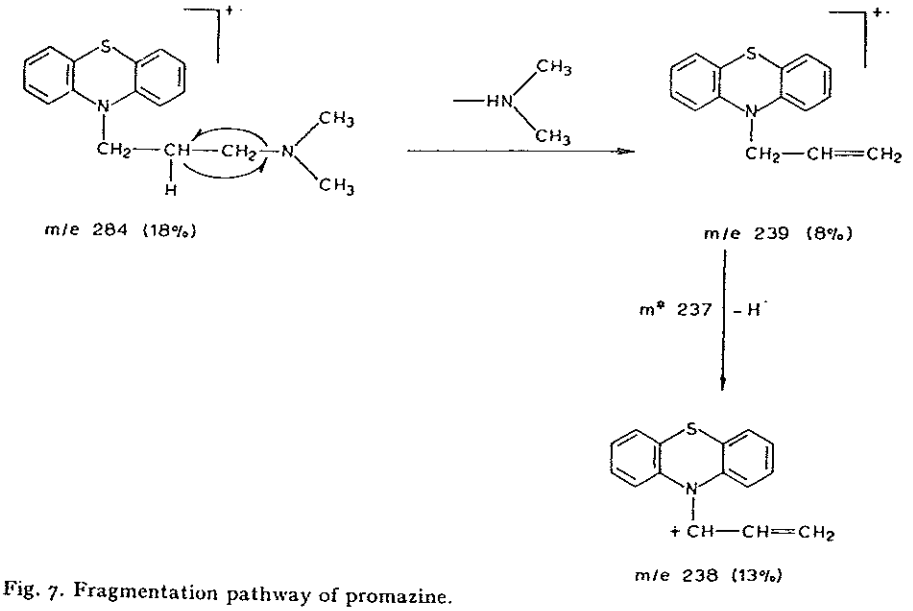

Fig. 7. Fragmentation pathway of promazine.

rise, after electron impact, to two fragment ions at *m/e* 235 and *m/e* 238, respectively (Fig. 5), which are characteristic and arise from similar fragmentation pathways (Figs. 6 and 7). Most important, however, is the fact that the two fragment ions are present within a mass range of 10% in accordance with the technical requirements of the instrument. The mass spectrum of imipramine (Fig. 5) shows the base peak at *m/e* 58, corresponding to a β-bond fission with respect to the nitrogen atom of the side-chain, giving rise to N,N-dimethylformimine with retention of the positive charge. Other intense peaks are present at *m/e* 85 and 235, that at *m/e* 85 being due to a cleavage of the bond between the ring nitrogen and the first carbon atom of the side-chain, with a rearrangement of a hydrogen atom on the ring nitrogen.

Promazine shows analogous behaviour and the peaks at *m/e* 58 and *m/e* 85 can be explained as before.

The origin of the two peaks at *m/e* 235 and *m/e* 238 is explained in Figs. 6 and 7. Imipramine undergoes a 1,4-rearrangement and by losing N,N-dimethylamine gives rise to a fragment ion at *m/e* 235 (relative intensity 70%). Promazine, after a similar 1,4-rearrangement, loses N,N-dimethylamine, giving rise to the ion at *m/e* 239 with a subsequent loss of a hydrogen radical to form the *m/e* 238 fragment (relative intensity 13%).

Routine spectra of imipramine and promazine were obtained at 70 eV, but for the mass fragmentographic analysis an electron energy of 30 eV was used because the relative abundance of the ions at *m/e* 235 and 238 was much greater.

No interference from endogenous substrates, nor from other drugs such as barbiturates or benzodiazepines administered concurrently, were noted in a series of samples obtained from different patients. Plasma values obtained in three patients monitored for 28 days during chronic treatment with imipramine are shown in Table I.

The relationship between imipramine plasma levels and the plasma levels of its

TABLE I

PLASMA LEVELS (ng/ml) OF IMIPRAMINE (IMI) AND DESIPRAMINE (DMI) IN DEPRESSED PATIENTS DURING CHRONIC TREATMENT WITH IMIPRAMINE

The dosages were between 75 and 125 mg/day; blood sampling was performed 6 h after the morning administration. Desipramine plasma concentrations were determined according to HAMMER AND BRODIE[16]. (—) = not performed.

Case	*Body weight (kg)*	*Sex*	*Days of treatment*							
			7		*14*		*21*		*28*	
			IMI	*DMI*	*IMI*	*DMI*	*IMI*	*DMI*	*IMI*	*DMI*
D.M.R.	57	F	30	98	50	160	120	260	70	340
V.C.	62	M	25	6	125	15	115	22	(—)	(—)
B.S.	52	M	15	3	15	12	(—)	(—)	(—)	(—)

main metabolite were the subject of a preliminary communication[17]. From the results presented, we think that the GLC–MF technique described here satisfies the requirement for its applicability to monitoring imipramine plasma levels in human biological fluids. It is specific and sensitive, covering the range of the drug plasma concentrations normally present in depressed patients undergoing chronic treatment. The clinical relevance of determining imipramine steady-state plasma levels with particular reference to the therapeutic outcome and the incidence of side-effects is now under evaluation.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. M. RIZZO for her cooperation and help in following up the cases reported in this study.

This work was supported by N.I.H. Grant No. 1PO1-GM1 8376 01 PTR.

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