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Note

Gas chromatographic determination of maprotiline and its N-desmethyl metabolite in human blood using nitrogen detection

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Maprotiline (Ludiomil®) is an antidepressive agent that has been in use for several years. It is found in erythrocytes in concentrations about 2.5 times the plasma concentration [1]. Desmethyl-maprotiline is one of its metabolites.

A double radioisotope derivative (DRID) assay of maprotiline in blood has been described [2] and its clinical applications reported [3]. Electron-capture gas—liquid chromatography (GLC—ECD) using the heptafluorobutyryl derivative of maprotiline was applied to the determination of maprotiline in plasma, blood and urine [4, 5]. Gas chromatography—mass spectrometry (GC—MS) was also used to assay maprotiline from post mortem material [6]. Tricyclic antidepressants and their metabolites were determined in serum by high-performance liquid chromatgraphy (HPLC) [7].

Antipsychotic drugs containing either a secondary or a tertiary amine were determined by gas chromatography using thermionic nitrogen-selective detectors (GLC N-FID) [8—14]. Maprotiline and desmethyl-maprotiline were estimated in serum by GLC N-FID [15]. A comparison between GLC N-FID and DRID for the determination of maprotiline in human plasma was reported [9, 16].

In order to allow the quantitative assay of maprotiline in whole blood by laboratories not equipped with the instrumentation necessary for the DRID method, we describe here a GLC N-FID method for the determination of maprotiline and desmethyl-maprotiline in blood.

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EXPERIMENTAL

Chemicals and reagents

Maprotiline hydrochloride, desmethyl-maprotiline hydrochloride and desipramine hydrochloride were supplied by Ciba-Geigy (Basle, Switzerland). All reagents and solvents are of analytical grade.

The content of four vials of pH 10 Titrisol (Merck 9890; E. Merck, Darmstadt, G.F.R.) is diluted to 500 ml with distilled water to yield the pH 10 buffer used as the extraction buffer.

The solutions of maprotiline hydrochloride, desmethyl-maprotiline hydrochloride and desipramine hydrochloride are prepared in 0.01 *N* sulphuric acid. The aqueous solution of desipramine hydrochloride (internal standard) contains 1 ng/μl.

Equipment

A Hewlett-Packard Model 5710A gas chromatograph equipped with a nitrogen detector (Model 18789A) is used. The column is operated at 245°C, the injector and the detector at 300°C, with a nitrogen flow-rate of 40 ml/min. The flow-rates of hydrogen and air are, respectively, 3 and 30 ml/min. The heating pulse of the cell is 14 V adjusted as recommended by Hewlett-Packard.

The column packing is 3% HI-EFF-8BP on Chromosorb W HP 100–120 mesh (Applied Science Labs., State College, PA, U.S.A.). The packed column (0.30 m × 3 mm I.D.) is flushed with the carrier gas at a flow-rate of 20 ml/min and heated to 250°C at a rate of 20°C per 15 min. The column temperature is held overnight at 250°C.

Extraction and derivatization

Two hundred microlitres of the internal standard solution are measured into a stoppered glass tube. Then, 1 ml of the sample, 2 ml of pH 10 buffer and 5 ml of heptane containing 3% isoamyl alcohol are added. The tube is shaken mechanically (Infors shaker) for 15 min at 300 rpm and centrifuged at 2000 *g*.

An aliquot volume of the organic phase is transferred to another tube and 2 ml of 0.1 *N* sulphuric acid is added. The tube is shaken mechanically for 15 min at 300 rpm and centrifuged at 2400 *g*. The interface between the two layers must be well defined.

The organic phase is discarded, 1 ml of 0.2 *N* sodium hydroxide, 2 ml of pH 10 buffer and 4 ml of hexane containing 5% ethyl acetate are added. The tube is shaken mechanically for 15 min at 300 rpm and centrifuged at 2000 *g*. A maximum volume of the organic phase is transferred to another tube (care being taken not to withdraw any of the aqueous phase), 50 μl of anhydrous pyridine, 20 μl of acetic anhydride are added and the stoppered tube is heated at 60°C for 1 h. After cooling to room temperature, 1 ml ethyl acetate and 2 ml of 1 *N* sodium hydroxide are added. The tube is shaken mechanically for 15 min at 300 rpm and centrifuged at 2000 *g*.

A maximum volume of the organic phase is transferred to a conical tube and taken to dryness under a nitrogen stream at 40°C. If the GC run cannot be made until the next day, the organic phase should be stored at −20°C without taking to dryness.

Gas chromatography

Just before injection onto the column, 50 µl of ethyl acetate are added. The tube is shaken on a Vortex mixer and a 2-µl portion of the ethyl acetate solution is injected into the gas chromatograph.

The maprotiline (or desmethyl-maprotiline) content is calculated from the peak-area ratio by reference to a calibration curve. This curve is obtained by extraction of blood spiked with increasing amounts of maprotiline (20—400 ng/ml) or increasing amounts of desmethyl-maprotiline (50—400 ng/ml) and a constant amount of internal standard (200 ng/ml).

The calibration curve is stable for 24 h and valid for one week. It must be checked every week to assess its reproducibility.

The authenticity of the chromatographic peaks obtained with the monoacetylated derivatives of maprotiline, desmethyl-maprotiline and desipramine was verified by injecting solutions of the pure synthetic monoacetylated derivatives.

RESULTS AND DISCUSSION

Precision and recovery

Tables I and II give the results obtained when the described procedure is applied to blood samples spiked with maprotiline hydrochloride and desmethyl-maprotiline hydrochloride, respectively. Gupta et al. [15] obtained the same results for the estimation of the precision of their method.

TABLE I

PRECISION AND RECOVERY FOR THE DETERMINATION OF MAPROTILINE APPLIED TO SPIKED HUMAN BLOOD SAMPLES

Amount added (ng/ml)	Mean amount found (ng/ml; $n = 6$)	Precision Reproducibility (± S.D.)	Recovery Accuracy (mean ± C.V.%)
20	23.3	± 1.2	117.0 ± 5.2
50	47.7	± 9.4	95.3 ± 4.7
100	90.7	± 4.0	90.7 ± 4.4
200	205.0	± 8.6	102.7 ± 4.4
400	392.7	± 30.4	98.0 ± 7.4

TABLE II

PRECISION AND RECOVERY FOR THE DETERMINATION OF DESMETHYL-MAPROTILINE APPLIED TO SPIKED HUMAN BLOOD SAMPLES

Amount added (ng/ml)	Mean amount found (ng/ml; $n = 6$)	Precision Reproducibility (± S.D.)	Recovery Accuracy (mean ± C.V.%)
50	51.0	± 1.7	103.0 ± 3.1
100	104.0	± 9.6	104.0 ± 9.6
200	198.5	± 6.0	99.0 ± 3.0
400	363.4	± 33.0	91.0 ± 8.2

Sensitivity

Concentrations down to 50 ng of maprotiline and desmethyl-maprotiline per ml of blood can be determined accurately. Attempts to use 2 ml of blood instead of 1 ml to improve sensitivity were unsuccessful, because they resulted in a high chemical background.

Plasma interference and selectivity

Fig. 1 shows that there is no interference from the normal constituents of the blood. Known metabolites of maprotiline, other than desmethyl-maprotiline, are hydroxylated or conjugated compounds [1] and they cannot be extracted or detected by our method. A re-extraction step is involved in the extraction procedure. This step is used to purify the extract, thus avoiding some interfering peaks obtained after derivatization.

GLC N-FID and DRID methods are suitable for maprotiline estimation in patient plasma and their precisions are comparable [9, 16], but DRID is a technique not readily accessible to standard laboratories. GLC—ECD using the heptafluorobutyryl derivative of maprotiline [4, 5] was very sensitive when applied to the pure compound. Its application to biological extracts yields a high chemical background.

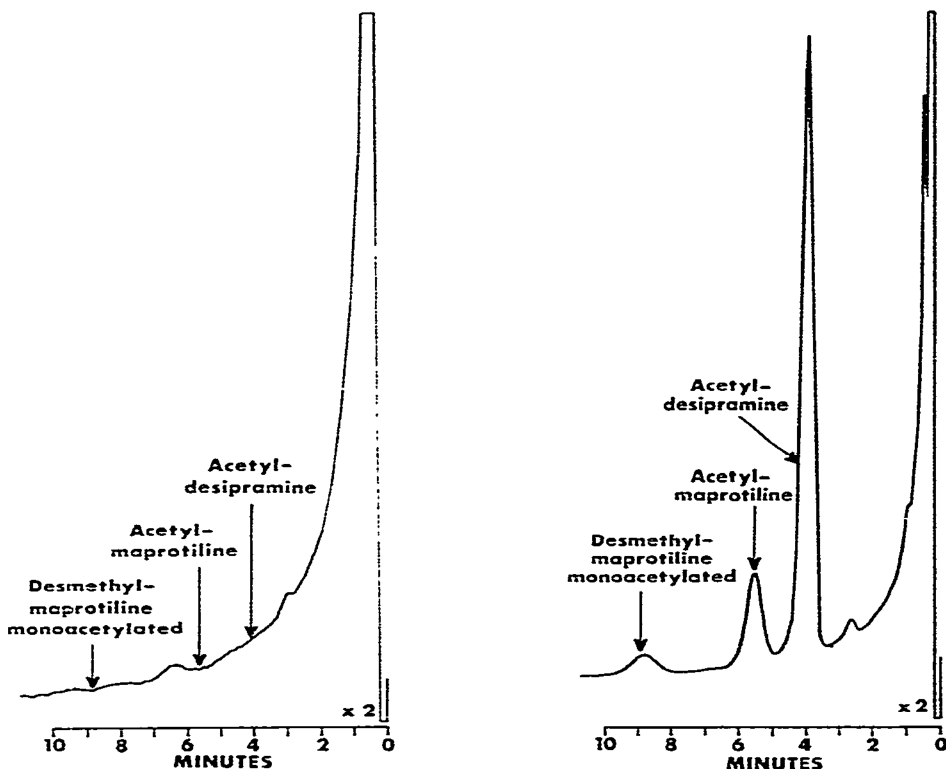

Fig. 1. Example of a chromatogram of a 1-ml human blood blank (the arrows indicate the location of the derivatized amines).

Fig. 2. Example of a chromatogram of an actual blood sample containing (see text) 200 ng of desipramine, 70 ng of maprotiline, and 51 ng of desmethyl-maprotiline.

Our results are comparable to those of Gupta et al. [15], but our method offers the advantage of determining maprotiline and desmethyl- maprotiline in whole blood.

Fig. 2 shows the chromatogram of a blood sample withdrawn on the 19th day from a subject who received one 25-mg Ludiomil® tablet twice a day (50 mg of maprotiline hydrochloride per day for a period of 19 days).

Chromatographic conditions

Silicone stationary phases such as OV-17 (50—50 Me—Ph silicone), SP 2250 DB (50—50 Me—Ph silicone modified for use with basic compounds), OV-25 (25—75 Me—Ph silicone) or SP 2401 DB (trifluoropropyl silicone modified for use with basic compounds) are not suitable for the GLC of acetyl-maprotiline. Acetylated maprotiline and desmethyl-maprotiline either do not separate from each other or are not separated from interference peaks.

More polar stationary phases such as DEGS (diethylene glycol succinate, polyester) or SP 1000 (Carbowax + substituted terephthalic acid) are not suitable for temperatures higher than 220°C.

High-temperature stationary phases such as Poly-S-179 (polyphenyl ether sulfone) and Poly-MPE (polymetaphenoxylene) give peaks with considerable tailing.

The cyclohexanedimethanol succinate (HI-EFF-8BP) has proved very satisfactory for the GLC of acetylated maprotiline and its metabolite; peaks due to extractable impurities elute early, while the desired peaks appear well separated and symmetrical.

CONCLUSION

The described GLC N-FID method makes it possible to determine maprotiline and desmethyl-maprotiline quantitatively in whole blood. In laboratories that are not equipped with the instrumentation necessary for the DRID method [2, 3], this method can be used to monitor maprotiline levels during chronic treatment.

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