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Note

Gas—liquid chromatographic procedure with alkali flame ionization detection for the determination of maprotiline in plasma

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Maprotiline is a tetracyclic antidepressant drug with structural similarities to the tricyclic antidepressants. Steady-state plasma concentrations of 50—550 ng/ml were observed when 150 mg was given daily as reported by Riess et al. [1]. Several analytical procedures have been employed for the estimation of maprotiline in biological specimens such as the double radioisotope derivative technique by Riess [2], the gas—liquid chromatographic (GLC) procedures of Geiger et al. [3], who employed the electron-capture detector, and Gupta et al. [4] who used a nitrogen-specific detector, and recently the GLC—mass spectrometric (MS) assay of Alkalay et al. [5] who used selected ion monitoring in the chemical ionization mode with isotopically labelled internal standard.

A GLC procedure using an alkali flame ionization detector (AFID) was recently developed in our laboratories [6] for the determination of imipramine and desipramine. This method has been applied successfully to the determination of maprotiline from plasma and is described herein.

EXPERIMENTAL

Instrumentation

A gas chromatograph (Hewlett-Packard Model 5730A) equipped with an alkali flame ionization detector was used. The 1.83 m × 2 mm I.D. coiled-glass column was packed with 5% OV-17 on Gas-Chrom Q (100—120 mesh). The

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column was conditioned at 315°C for 48 h with a gentle flow of nitrogen and then the oven temperature was maintained at 255°C. Injection port and detector temperatures were 300°C while the detector bead current was adjusted for maximum sensitivity. Helium, was maintained at a flow-rate of 30 ml/min and the air and hydrogen flow-rates were adjusted for optimum sensitivity.

Extraction procedure

An analytical method used for tricyclic drugs [6] has been adapted for maprotiline. To a 0.5—2.0-ml plasma sample (control, spiked or from a dosed subject) were added 0.5 ml of an aqueous solution of the internal standard (desipramine) and 0.5 ml of a borate buffer solution (pH 9). The sample was extracted twice with 4-ml portions of cyclohexane. The combined organic layers were back extracted with a 2% methanolic solution of 1 *N* hydrochloric acid. After evaporation of the methanolic layer, the residue was redissolved in 50 μl amyl acetate and reacted for 2 min at 85°C with 10 μl acetic anhydride. The mono-N-acetyl derivatives of maprotiline and desipramine were analysed by GLC—AFID.

Standard solutions

Stock solutions of maprotiline and desipramine (100 μg base per ml) were prepared daily by dissolving their hydrochloride salts in distilled water. Appropriate dilutions in the concentration range required for the maprotiline calibration curve were prepared in control blank plasma using the stock solution. The internal standard working solution was prepared by diluting the stock solution of desipramine with distilled water to a concentration of 400 ng/ml.

Calibration curve

Peak height ratios were calculated by dividing the height of the peak due to mono-N-acetylmaprotiline by the height of the peak due to mono-N-acetyldesipramine, the internal standard. Calibration curves were assembled from results of spiked plasma samples by plotting the peak height ratios against the concentrations of the drug.

RESULTS AND DISCUSSION

When maprotiline and desipramine standards were derivatized with acetic anhydride and submitted to GLC—AFID analysis, sharp, symmetrical peaks were obtained, eluting at 11.5 and 9.8 min respectively. The authenticities of the derivatives thus formed were confirmed by GLC—MS to be the mono-N-acetyl derivatives of maprotiline and desipramine. Blank and spiked plasma samples were extracted, derivatized and submitted to GLC—AFID analysis. A typical chromatogram of blank plasma is shown in Fig. 1A and no peaks were seen beyond the 6-min retention time. The spiked plasma sample was analysed according to the procedure where the two acetyl derivatives eluted at the same retention times as the standards (Fig. 1B). When MS analysis was performed on these eluted peaks they were found to be identical to the authentic mono-N-acetyl derivatives, with no interferences from endogenous materials.

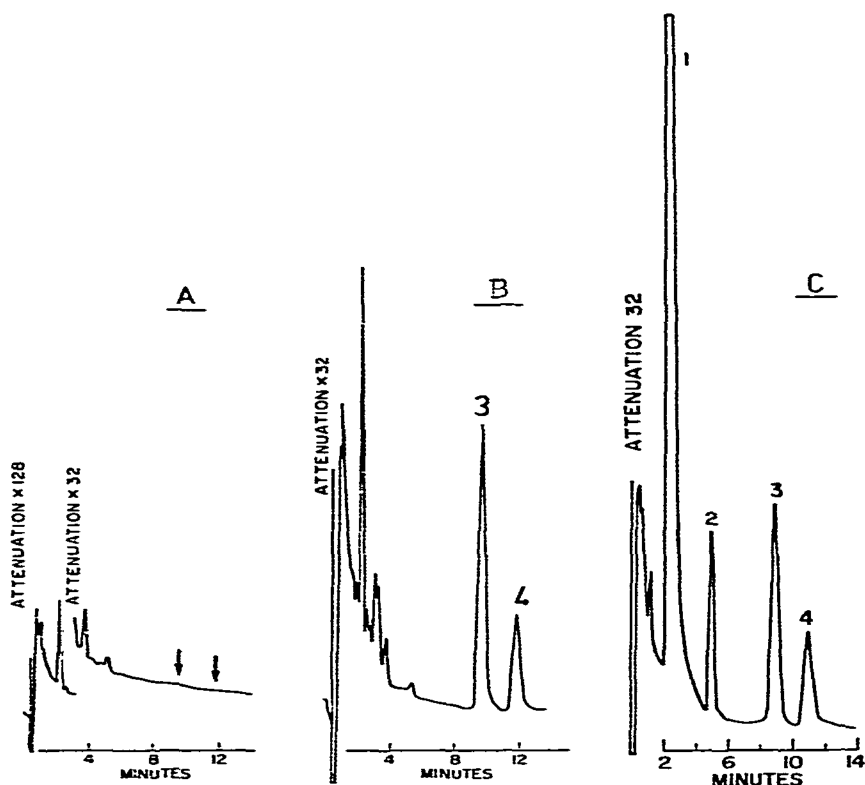

Fig. 1. Chromatograms of (A) blank plasma; (B) spiked plasma containing 200 ng of the internal standard desipramine, and 76.4 ng/ml of maprotiline (1 ml plasma) and (C) plasma from a patient overdosed on maprotiline (2 ml plasma were used and the concentration estimated was 50.3 ng/ml for maprotiline; 200 ng internal standard). Peaks: 1 = tris(2-butoxyethyl) phosphate (contaminant); 2 = unidentified; 3 = mono-N-acetyldesipramine; 4 = mono-N-acetylmaprotiline.

Typical calibration curves over the range 10—225 ng/ml gave a coefficient of determination, $r^2 = 0.997$ for the equation $Y = a + bX$ (where $a = 0.0193$, $b = 0.0041$ and $N = 32$). A calibration curve was run with every set of unknown samples.

The overall recoveries of maprotiline at 25, 75 and 150 ng/ml concentrations in plasma were 53.3, 51.4 and 50.7% with an overall relative standard deviation of 5.6%.

This method has been applied to plasma level determination for a patient suffering from a maprotiline overdose. The patient, a 67 year old male, was brought to the emergency unit in coma. During emergency treatment, including hemoperfusion, blood samples were taken and it was possible to monitor maprotiline plasma concentration for thirteen days following ingestion.

The authenticity of maprotiline was confirmed by GLC—MS. The highest maprotiline concentration attained was 449 ng/ml at the initiation of the hemoperfusion and this fell to ca. 300 ng/ml at the end of hemoperfusion; levels of ca. 28 ng/ml were found on days 12 and 13. The levels determined

were obtained from at least duplicate analysis of each sample (Table I).

A typical chromatogram of the patient's plasma sample is shown in Fig. 1C. Peak 1, eluting at 2.2 min, was due to tris(2-butoxyethyl) phosphate, a contaminant from the rubber stopper of Vacutainers. Peak 2, with a 5.0-min retention time, was not identified. Peaks 3 and 4 were due to mono-N-acetyldesipramine and mono-N-acetylmaprotiline, respectively. Other cases have also been monitored.

TABLE I

MAPROTILINE PLASMA LEVELS ESTIMATED

Time after suspected overdose		Concentration of maprotiline in plasma (ng/ml)
Hours	days	
40.0*	1.67	300.2
41.7	1.74	449.0
42.5	1.77	341.0
44.0	1.83	385.5
46.0	1.92	304.4
47.5	1.98	300.6
48.0**	2.00	
78.5	3.27	417.2
116.5	4.85	330.6
126.5	5.27	193.9
150.5	6.27	214.1
174.5	7.27	280.4
246.5	10.27	50.3
294.5	12.27	28.0
318.5	13.27	27.5

*Beginning of hemoperfusion.
**End of hemoperfusion.

The GLC—electron-capture detection procedure of Geiger et al. [3] is long (ca. 3 h) and tedious. Precautions have to be taken with lower plasma concentrations (less than 50 ng/ml) because of the electron-capture detection linearity response. The procedure of Gupta et al. [4], linear over the range 50—400 ng/ml, requires the use of silanized glassware, an initial washing step before extracting the drug, with additional drying being necessary to remove any pyridine traces. Use of the GLC—MS method of Alkalay et al. [5], although specific and sensitive (0.5 ng/ml), would be limited because the expensive sophisticated instrumentation is not readily available in all laboratories. For these reasons, an improved GLC procedure employing an AFID, which is simple, sensitive, specific and rapid, has been developed in our laboratories.

CONCLUSION

The described analytical procedure has been applied successfully to the determination of maprotiline from plasma with a detection limit of 2 ng/ml.

The method is applicable to therapeutic monitoring of the drug.

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