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Note

Determination of maprotiline and desmethylmaprotiline in plasma and urine by gas chromatography with nitrogen–phosphorus detection

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The tetracyclic drug maprotiline has become an important component of the physician's armamentarium of antidepressants. Maprotiline is a secondary amine which undergoes metabolic N-desmethylation in the body, and this N-desmethyl metabolite of maprotiline appears in plasma in significant concentrations at steady state [1]. In the present report we describe a rapid and simple procedure for the simultaneous determination of maprotiline and its desmethyl metabolite utilizing acetylation under aqueous conditions followed by separation and quantitation using gas chromatography (GC) with nitrogen–phosphorus detection.

EXPERIMENTAL

Reagents and glassware

The ethyl acetate and toluene used were distilled in glass and were obtained from BDH (Toronto, Canada) and Caledon Labs. (Georgetown, Canada), respectively. The acetic anhydride (reagent grade) was purchased from Caledon Labs., and the sodium bicarbonate was obtained from Fisher Scientific (Nepean, Canada).

All glassware was sonicated in a diluted Contrad 70 (Canlab) solution for a total of 60 min and was then vigorously rinsed and dried before use.

Standards

Maprotiline was a gift from Ciba-Geigy (Mississauga, Canada). The desmethylmaprotiline methane sulfonate was kindly provided Dr. D.F. LeGatt, De-

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partment of Laboratory Medicine, University of Alberta Hospitals. Desipramine hydrochloride (the internal standard) was purchased from Sigma (St. Louis, MO, U.S.A.).

Stock solutions of each drug were prepared in distilled deionized water at a concentration of 1 mg/ml, aliquoted and stored at −20°C. They were diluted appropriately with distilled deionized water for preparation of the calibration standards.

Apparatus and chromatographic parameters

The analytic instrument used was a Hewlett-Packard Model 5890 gas chromatograph equipped with a nitrogen–phosphorus detector and a Hewlett-Packard 3392A integrator. A fused-silica capillary column (25 m × 0.31 mm I.D.) coated with of 0.5 μm film thickness of 5% phenylmethylsilicone (Hewlett-Packard, Palo Alto, CA, U.S.A.) was employed. The carrier gas was pure helium (Linde, Union Carbide, Medigas Alberta, Edmonton, Canada) at a flow-rate of 30 ml/min. The detector was purged with pure hydrogen (Linde, Union Carbide) at 3.5 ml/min mixed with dry air (Linde, Union Carbide) at 80 ml/min.

Operating temperatures were: injection port, 200°C; initial column, 105°C; final column, 295°C; and detector, 320°C. The oven temperature was held at 105°C for 0.5 min following injection and then was raised at a rate of 25°C/min.

Patients

The patients were diagnosed as having Major Depressive Disorder according to DSM-III Criteria [2]. In addition to the clinical interview given by the psychiatrist, the patients were interviewed using the Diagnostic Interview Schedule (DIS) [3] and the severity of depression was assessed using the Hamilton Depression Rating Scale [4].

Extraction procedure for samples

To 1 ml of patient plasma, diluted with 2 ml of distilled deionized water, desipramine was added as internal standard. Sufficient solid sodium bicarbonate was added to basify the solution. This was followed by acetic anhydride (150 μl), resulting in a bubbling reaction mixture. Extra sodium bicarbonate was added to maintain a small residue at the bottom of the reaction tube. At the completion of this reaction, marked by a disappearance of effervescence at approximately 15–20 min, ethyl acetate (6 ml) was added directly to the reaction tube. The samples were shaken vigorously for 10 min. Solid sodium chloride was then added to the reaction tube to aid in the separation of phases and the samples were centrifuged briefly. The organic phase was carefully transferred to a smaller evaporation tube. The samples were evaporated to dryness under a stream of nitrogen and reconstituted by the addition of toluene (300 μl). A portion (1 μl) of this solution was injected into the gas chromatograph for analysis.

For the analysis of urine, the reaction mixture consisted of a 0.50-ml urine sample diluted with 0.5 ml deionized distilled water. Only 3 ml of ethyl acetate were required for extraction of the acetylated drugs. Sodium chloride was not necessary for the separation of the phases.

Calibration

The ratio between the peak height of the derivatized drugs and that of the derivatized internal standard was calculated and plotted against the concentration of the drugs. A calibration curve for urine encompassing the range 125 ng to 10 μg and for plasma covering the range 25 ng to 1 μg (in samples diluted as described in the text) was adequate for study of the samples as prepared above. Calibration curves were prepared and run in parallel with every assay.

TABLE I

ABSOLUTE AND RELATIVE RETENTION TIMES OF THE DRUGS UNDER INVESTIGATION

Drug (derivatized)	Retention time (min)	Retention time relative to derivatized desipramine
Desipramine	10.38	1.00
Desmethylmaprotiline	10.80	1.04
Maprotiline	11.11	1.07

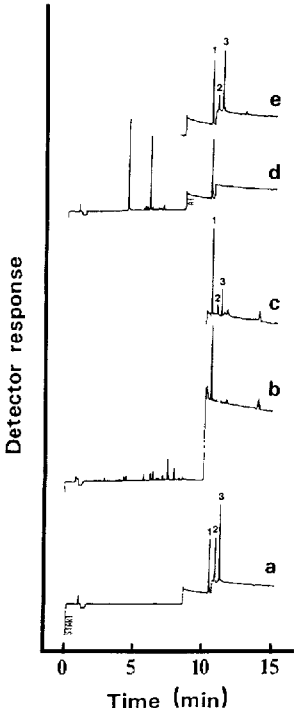

Fig. 1. Typical GC traces from urine and plasma samples carried through the procedure described in the text. (a) Authentic standards (1000 ng); (b) and (c) plasma samples obtained from a patient pretreatment and after treatment with maprotiline for three weeks, respectively; (d) and (e) urine samples obtained from a patient pretreatment and after treatment with maprotiline for three weeks, respectively. Peaks: 1 = derivatized desmethylimipramine (internal standard): 2 = desmethylmaprotiline; 3 = maprotiline. Attenuation changes were programmed in just before peak 1 in all cases and before peak 2 in a, d and e.

RESULTS

The procedure results in the formation of the monoacetyl derivatives of desmethylimipramine, maprotiline and desmethylmaprotiline [structures confirmed using GC–electron-impact mass spectrometry (MS)]. The absolute and relative retention times of the derivatives on the gas chromatogram are shown in Table I. Typical chromatograms obtained from the analysis of a sample patient urines and plasma are shown in Fig. 1.

Good linear correlations (mean r^2 values of 0.999138 and 0.999053 for maprotiline and desmethylmaprotiline, respectively) were obtained between peak-height ratios and concentration over the ranges used for maprotiline and N-desmethylmaprotiline. The limits of quantitation under the conditions described were < 10 ng per original sample for both urine and plasma. Recoveries from urine samples spiked with 100 ng of drug and metabolite were essentially quantitative, while for plasma the mean recoveries were 87.8 and 86.2%, respectively.

DISCUSSION

A number of techniques have been utilized for the analysis of maprotiline. These include a double radioisotope derivative technique [5], GC [6–11], GC–MS [12–16] and high-performance liquid chromatography [1, 17–22]. In some cases, the assays also permit simultaneous analysis of the N-desmethylated metabolite.

Maprotiline and its desmethyl metabolite can be accurately measured simultaneously in plasma and urine at therapeutic concentrations by the procedure described in the present report. The procedure is rapid and relatively simple because the acetylation under aqueous conditions provides extractive derivatization of the parent drug, the desmethylated metabolite and the internal standard simultaneously. Acetylation under slightly basic aqueous conditions has been used extensively for extractive derivatization of a number of biogenic arylalkylamines and their metabolites [23–31]. Gupta et al. [10] and Sioufi and Richard [11] also formed the N-acetyl derivative of maprotiline in their procedures for analysis of this drug, but they extracted maprotiline first with an organic solvent and then derivatized under anhydrous conditions, resulting in a more time-consuming method.

A good linear correlation between peak-height ratio and concentration is possible over a wide range of concentrations and makes this procedures suitable for therapeutic monitoring as well as for analytical purposes in cases of overdose. The reproducibility is good, with coefficients of variation of 4.3 and 3.4% being obtained for maprotiline and desmethylmaprotiline, respectively, at the 100-ng level and 4.8 and 4.1% at the 500-ng level in spiked urine samples.

The entire derivatization and extraction procedure, including a standard curve and 24 patient samples, could be completed in 2 h. The chromatography time of 15 min per sample also makes this procedure readily applicable to both routine and emergency analyses.

Other studies have measured plasma/serum levels of maprotiline and desmethylmaprotiline simultaneously and these data are summarized in Table II. Our

TABLE II

PLASMA OR SERUM LEVELS OF MAPROTILINE AND DESMETHYLMAPROTILINE REPORTED IN THE LITERATURE

n	Daily dose of maprotiline (mg)	Concentration (ng/ml)				Reference
		Maprotiline		Desmethylmaprotiline		
		Mean	Range	Mean	Range	
4	75	230	100–310	128	80–180	32
13	150	117	40–237	52	20–132	10
20	150	107	37–208	40	13–111	22
7	150–250	291	72–494	96	42–222	1
3	50–150	201	169–261	136	37–220	17

TABLE III

PLASMA LEVELS AND URINARY EXCRETION OF MAPROTILINE (MAP) AND DESMETHYLMAPROTLINE (DMMAP) IN THREE DEPRESSED PATIENTS

Maprotiline was taken for three weeks in each case. Urine samples (24 h) were collected in receptacles containing 20 ml of 2% EDTA solution. Venous blood samples used for preparation of plasma were taken in the morning before breakfast in each case.

Patient code	Age (years)	Sex	Daily dose of maprotiline (mg)	Plasma level (ng/ml)		Urinary level (unconjugated) in 24-h sample (μg/g of creatinine)	
				MAP	DMMAP	MAP	DMMAP
ID018	20	F	200	166	70	1486	778
OD020	50	M	300	322	218	1734	599
ID021	44	F	150	168	99	774	355

findings in plasma and urine samples from three patients taking maprotiline are shown in Table III.

In summary, the procedure described here represents a rapid and convenient method of analyzing maprotiline and its N-desmethyl metabolite simultaneously. The assay could be readily adopted by laboratories interested in therapeutic and toxicological monitoring of maprotiline and structurally related drugs.

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