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MEASUREMENT OF MAPROTILINE AND OXAPROTILINE IN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF FLUORESCENT DERIVATIVES

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SUMMARY

The antidepressant maprotiline and its hydroxylated analogue oxaprotiline were assayed in plasma by solvent extraction and formation of fluorescent derivatives, which were purified by thin-layer chromatography and quantitated by high-performance liquid chromatography with fluorescence monitoring. The procedure possesses a high sensitivity, accuracy and reproducibility, and metabolites of the drugs did not interfere.

INTRODUCTION

Maprotiline (Ludiomil®) is a tetracyclic antidepressant with well documented clinical efficacy [1]. Introduction of an alcoholic hydroxy group into its side-chain leads to oxaprotiline (Fig. 1), a compound currently being tested for its therapeutic activity in depressive disorders [2, 3]. In the course of clinical studies, it is desirable to obtain information on plasma drug levels, since unsatisfactory response may be a consequence of very low as well as of extremely high levels [4].

The double radioisotope derivative method of Riess [5] does not seem suited for routine laboratory use unless very large series have to be analysed.

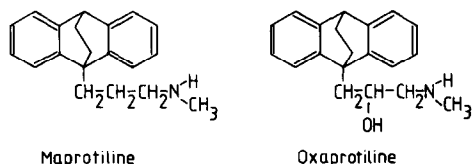


Fig. 1. Structures of maprotiline and oxaprotiline.

Whereas several gas chromatographic procedures have been described for the analysis of maprotiline in plasma [6–9], the application to oxaprotiline has only been realized using a mass spectrometer as detector [10]. The only published procedure for the determination of both compounds [11] used high-performance liquid chromatography (HPLC) of the unchanged drugs with absorbance monitoring at 214 nm. This necessitated extraction with a very unpolar solvent and implicated low recoveries. Higher percentages were recovered in a similar HPLC assay of maprotiline and desmethylomaprotiline [12], but the applicability of the method to oxaprotiline analysis was not tested. Since the latter drug produces low plasma levels, the formation of fluorescent derivatives in toluene extracts appeared promising.

EXPERIMENTAL

Materials

Blood was obtained from patients under treatment with divided doses of maprotiline (125 mg/day Ludiomil) or oxaprotiline (150 mg/day C-49802 B-Ba; Ciba-Geigy, Basel, Switzerland). Samples were drawn in the morning before ingestion of the first drug dose and placed into heparinized tubes. After centrifugation, plasma was stored at -20°C for up to eighteen months without apparent loss of drug. Blank plasma was obtained from drug-free healthy volunteers.

Reference compounds were donated by the following companies: maprotiline hydrochloride, desmethylomaprotiline methanesulphonate, oxaprotiline hydrochloride, desmethylooxaprotiline hydrochloride, desmethyl- and didesmethylmipramine hydrochloride, desmethylclomipramine hydrochloride by Ciba-Geigy; nortriptyline hydrochloride by Tropon (Cologne, F.R.G.); and *E*-10-hydroxynortriptyline by A. Jørgensen (Lundbeck, Copenhagen, Denmark). Hydroxylated maprotiline metabolites were isolated from patient urine and their structures elucidated by nuclear magnetic resonance and mass spectrometry [13].

Fluorescent derivatives of maprotiline and oxaprotiline were obtained on a preparative scale by reacting 0.2 mmol of drug hydrochloride in 1 ml of water with 100 mg of potassium bicarbonate and 0.4 mmol of 5-dimethylaminonaphthalene-1-sulphonyl chloride (dansyl chloride, Merck, Darmstadt, F.R.G.) in 2 ml of acetone. The mixture was stirred for 30 min at 45°C . The oily precipitate dissolved upon extraction with three 2-ml portions of chloroform. The residue of the chloroform extract was subjected to thin-layer chromatography (TLC) on five sheets precoated with silica gel (Polygram Sil G/UV₂₅₄, Macherey-Nagel, Düren, F.R.G.) in hexane–acetone–toluene (21:2.5:1 for dansyl maprotiline, 21:4:0.7 for dansyl oxaprotiline). The compounds were eluted from the gel using chloroform and recrystallized from chloroform–hexane. Dansyl derivatives of other reference compounds were prepared on a small scale by the procedure used for analytical work.

Plasma level assay

Principle. In the determination of maprotiline oxaprotiline (400 ng/ml) served as an internal standard, while didesmethylmipramine (30 ng/ml) was

the internal standard in the measurement of oxaprotiline. Plasma extracts were treated with dansyl chloride to form the dansyl derivatives of drug and internal standard. These were extracted and pre-purified by TLC, the conditions being chosen in a way that the two compounds had identical R_F values. Eluates of the spots were subjected to HPLC with fluorescence detection.

Procedure. To plasma (1 ml) the internal standard was added in water: 40 μ l of oxaprotiline solution (10 μ g/ml) for maprotiline analysis or 10 μ l of didesmethylimipramine solution (3 μ g/ml) for oxaprotiline analysis. After addition of 250 mg of sodium chloride, 35 μ l of 10% sodium deoxycholate solution and 100 μ l of sodium hydroxide (0.2 mol/l), the sample was extracted twice with 1.5 ml of toluene. In maprotiline analyses, the organic phase was reduced to about 1 ml in a rotary evaporator and extracted with 0.5 ml of sulphuric acid (5 mmol/l). In assays of oxaprotiline, the toluene phase was extracted without evaporation. The aqueous phase was washed with 1 ml of hexane, residual hexane being removed under a stream of nitrogen. Then it was mixed with 0.1 ml of potassium bicarbonate (0.6 mol/l) and 0.6 ml of dansyl chloride solution (1.5 mg/ml in acetone) and incubated in open tubes for 20 min at 45°C. Following addition of 0.3 ml of L-proline solution (2.5 mg/ml in water), the incubation was continued for 20 min. Residual acetone was removed by blowing a stream of nitrogen for 5–10 min at 30°C and the aqueous solution was twice extracted with 1 ml of hexane. The extract was brought to dryness under a stream of nitrogen in tapered tubes, the residue being concentrated in the tip by rinsing the tube with 0.3 ml of hexane. The extracts were sensitive to light and had to be stored frozen unless processed immediately.

For TLC the dansyl derivatives were spotted at 1.5-cm distances on 20 \times 20 cm sheets coated with silica gel (see above). In maprotiline analyses, chromatograms were run to a height of 16.5 cm above the starting line in hexane–acetone–toluene (21:4:0.7), dried for 2 min and subsequently run in ethanol–water (15:10) to a height of 7.5 cm. For the determination of oxaprotiline, running distances were 12 and 6 cm in the first and second solvent, respectively. Chromatograms had to be run and dried in the dark. The spot containing the dansyl derivatives of drug and internal standard (for R_F values see Table I) was visualized under light of 365 nm and removed after lightly spraying with water. The gel was extracted twice with 0.5 ml of a freshly prepared solution of 0.1% diethylamine in methanol. The eluate was evaporated in tapered tubes and the residue concentrated in the tip as above. For HPLC it was dissolved in 50 μ l of hexane–ethanol (4:1) and a 10- μ l aliquot was injected.

The column (300 \times 4.5 mm) contained 7- μ m silica gel particles (Nucleosil 50-7, Macherey-Nagel). The eluent was hexane–absolute ethanol (95:5, v/v) at a flow-rate of 1.8 ml/min. Fluorimetric detection was done at excitation and emission wavelengths of 365 and 420 nm, respectively, with the fluorometer (FFM 32, Kontron, Munich, F.R.G.) set at medium sensitivity for maprotiline determinations and at high sensitivity for oxaprotiline analyses. For quantitation, peak height ratios of dansylated drug and internal standard were read on a calibration curve produced by processing blank plasma samples with additions of known quantities of maprotiline or oxaprotiline.

Between-series reproducibility was examined by running control samples with each series of patient plasma. These contained 200–400 ng/ml (721–1442 nmol/l) of maprotiline or 20–50 ng/ml (68–170 nmol/l) of oxaprotiline. Absolute recovery in maprotiline analysis could be derived from a comparison of peak heights of substances obtained from plasma with peak heights of standards containing known quantities of dansyl derivatives.

A series of twelve plasma samples can be processed by one person within two days.

RESULTS

Selectivity and sensitivity

Table I presents R_F values in TLC and retention times in HPLC of dansyl derivatives of secondary and primary amines that are administered as antidepressants or produced as antidepressant drug metabolites. It can be seen that in the modification used for maprotiline analysis, secondary amines devoid of a hydroxy group (nortriptyline, desmethyylimipramine) would interfere with the determination of maprotiline, whereas all its metabolites are separated from it. Similarly, oxaprotiline differs from its demethylated metabolite in its behaviour on HPLC.

TABLE I

CHROMATOGRAPHIC BEHAVIOUR OF DANSYL DERIVATIVES OF DRUGS AND DRUG METABOLITES

Dansyl derivative of	Running distance in TLC (cm) under conditions for		Retention time in HPLC (min)
	Maprotiline	Oxaprotiline	
Maprotiline	4.1	3.3	3.8
Desmethylmaprotiline	3.0	2.5	5.2
3-Hydroxymaprotiline	1.8	1.4	7.6
3-Hydroxydesmethylmaprotiline	1.2	1.0	11.3
2-Hydroxymaprotiline	2.0	1.6	7.0
Oxaprotiline	3.5	2.9	6.8
Desmethyloxaprotiline	3.4	2.8	8.7
Desmethyylimipramine	3.9	3.0	3.8
Didesmethyylimipramine	3.2	2.6	5.4
Desmethyldesmethyylimipramine	4.2	3.3	3.9
Nortriptyline	4.5	3.6	3.8
E-10-Hydroxynortriptyline	4.5	3.6	10.3

Examples of chromatographic traces are presented in Figs. 2 and 3. No definite peaks appeared at the retention times of dansyl oxaprotiline and dansyl didesmethylimipramine when blank plasma had been subjected to the analytical procedure. A small peak was, however, present at the retention time of dansyl maprotiline, which corresponded to about 2 ng of the drug (Fig. 2A). It could be disregarded in assays of clinically relevant maprotiline concentrations, but it was a reason not to use maprotiline as an internal standard for oxaprotiline analyses which had to be more sensitive by a factor of 5.

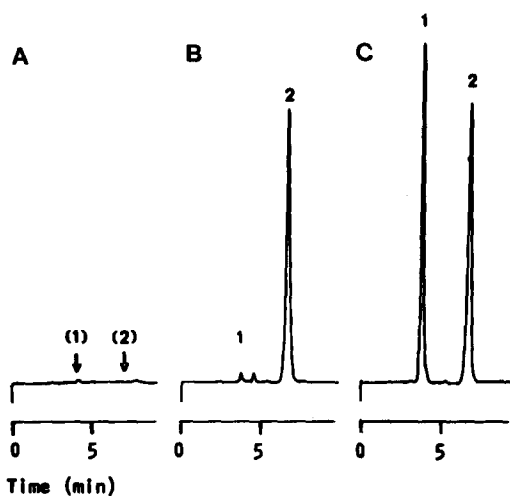


Fig. 2. HPLC fluorescence records obtained upon maprotiline analysis in plasma (1 ml). (A) Blank plasma. (B) Blank plasma with additions of 10 ng of maprotiline and 400 ng of oxaprotiline as internal standard. (C) Plasma from a patient ingesting maprotiline 150 mg per day; it was found to contain 290 ng/ml (1045 nmol/l) maprotiline. 1 = dansyl maprotiline, 2 = dansyl oxaprotiline.

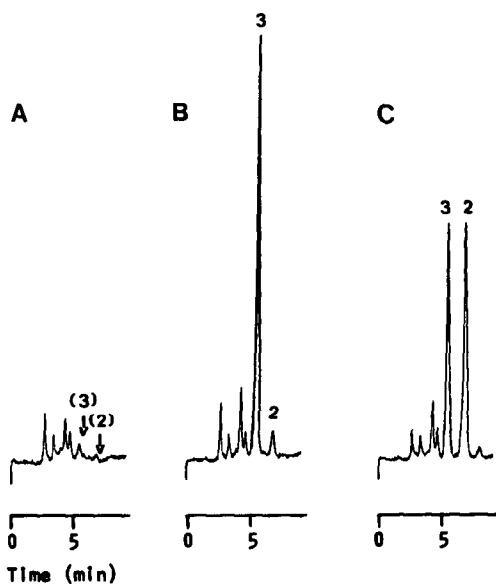


Fig. 3. HPLC fluorescence records obtained upon oxaprotiline analysis in plasma (1 ml). (A) Blank plasma. (B) Blank plasma with additions of 2 ng of oxaprotiline and 30 ng of didesmethylimipramine as internal standard; in this case a 40% aliquot was injected. (C) Plasma from a patient treated with oxaprotiline 150 mg per day; it was found to contain 32 ng/ml (109 nmol/l) oxaprotiline. 2 = dansyl oxaprotiline, 3 = dansyl didesmethylimipramine.

The detection limit of maprotiline was about 10 ng/ml (Fig. 2B) and reproducible determinations could be made at 20 ng/ml (72 nmol/l) and higher. Oxaprotiline was detected at minimal levels of 2 ng/ml (Fig. 3B) and could be quantitated at 5 ng/ml (17 nmol/l).

Accuracy and reproducibility

Calibration curves revealed a strictly linear dependence of the peak height ratio of dansylated drug and internal standard on the drug quantity added ($r = 0.994$, $n = 43$ for maprotiline 20–400 ng/ml, and $r = 0.998$, $n = 30$ for oxaprotiline 10–80 ng/ml).

Control samples analysed along with each series of patient plasma gave the results listed in Table II. Duplicate determinations of maprotiline in 48 samples from patients resulted in a mean difference of 6.1%.

The procedure for oxaprotiline determination was further controlled by a blind analysis of seven spiked plasma samples prepared by a laboratory of Ciba-Geigy at Basel. A very good accuracy becomes apparent from the results presented in Table III. At levels of 10 ng/ml (34 nmol/l) or above, the values found did not deviate by more than 4% from the theoretical ones. In addition, a series of seven pooled patient plasma samples was analysed by the present procedure and at Ciba-Geigy by gas chromatography–mass spectrometry. Maximal differences amounted to 4 ng/ml or 14% at concentrations of 4–32 ng/ml (14–109 nmol/l).

TABLE II

RESULTS OF ANALYSES OF CONTROL SAMPLES RUN IN DIFFERENT SERIES OF MAPROTILINE AND OXAPROTILINE ASSAYS

Compound	Concentration (ng/ml free base)		Percentage of theoretical value	n
	Added	Found		
Maprotiline	200	193 ± 15*	96 ± 7*	8
	300	312 ± 27	104 ± 9	9
	400	405 ± 27	101 ± 7	9
Oxaprotiline	20	18.5 ± 1.5	92 ± 7	6
	40	38.2 ± 5.5	95 ± 14	8
	50	49.6 ± 2.7	99 ± 5	11

*Mean ± S.D.

TABLE III

THEORETICAL AND EXPERIMENTAL VALUES IN A BLIND ANALYSIS OF PLASMA SAMPLES SPIKED WITH OXAPROTILINE

Sample No.	Oxaprotiline concentration (ng/ml free base)	
	Added	Found
1	1.1	0
2	10.7	10.5
3	21.7	22.5
4	32.3	33.5
5	32.3	33
6	42.4	44
7	53.1	52

Recovery

In the entire analytical procedure for maprotiline the recovery of dansyl maprotiline amounted to 58% and that of dansyl oxaprotiline to 60%.

Values in patient plasma

Maprotiline plasma levels in twenty patients ingesting 125 mg per day slowly increased during weeks 2–4 of treatment. After four weeks they varied from 34 to 225 ng/ml (128 ± 56 ng/ml or 461 ± 202 nmol/l, mean \pm S.D.) [14]. Higher concentrations are observed in single cases at a daily dose of 150 mg (Fig. 2C).

Twenty-five depressed patients ingesting oxaprotiline 150 mg per day mostly had constant plasma drug levels from two to four weeks after initiation of treatment. Interindividual variation was considerable, with steady-state values ranging from 9.5 to 74 ng/ml (34 ± 15 ng/ml or 116 ± 51 nmol/l).

DISCUSSION

The analytical procedure described here could be shown to satisfy the usual quality criteria. Its sensitivity is sufficient for measuring plasma maprotiline and oxaprotiline concentrations well below the range of values occurring upon treatment with clinically effective drug doses. While it does not allow separate determination of several antidepressants with secondary amino groups, no disturbance is caused by the corresponding primary amines and other known drug metabolites. A similar procedure not involving a purification by TLC has recently been published for the assay of metopramine [15].

As has been documented earlier [14], the range of values measured in plasma of maprotiline-treated patients agreed well with that reported by other authors. Also oxaprotiline levels are similar to those obtained by a HPLC procedure without derivatization [11].

Though the present method is somewhat laborious, this draw-back seems to be compensated for by avoiding the use of radioactive reagents or expensive instrumentation. In comparison with an alternative HPLC method [11] it has the advantage that the first extraction step can be carried out with toluene; this results in a higher recovery and thus improves the reliability of the assay.

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