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Note

Fluorimetric determination of maprotiline in urine and plasma after thin-layer chromatographic separation*

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Maprotiline {Ludiomil®, Ciba-Geigy; N-methyl-[3-(9,10-ethanoanthracene-9(10H)-yl)-propyl]aminhydrochloride } is a widely used antidepressive agent. Several methods for the detection of maprotiline in plasma and urine have already been developed. Riess [1] determined maprotiline by a double-radio-isotope derivative technique. Since not every laboratory is equipped with the corresponding instrumentation, a gas—liquid chromatographic method for estimating maprotiline was suggested by Geiger et al. [2]. This procedure involves a re-extraction step and the formation of a derivative, both of which are rather time-consuming. Further gas chromatographic methods for the determination of maprotiline have been introduced by Gupta et al. [3], Sioufi and Richard [4], Charette et al. [5], and Kärkkäinen and Seppälä [6].

For compliance studies it seemed desirable to our group to find a simple and rapid procedure for the quantitative measurement of maprotiline.

The method we developed consists of four steps: (1) extraction from an alkaline sample with *n*-heptane—isopropanol (99:1); (2) derivatization with NBD chloride (7-chloro-4-nitrobenzofurazan); (3) separation of maprotiline from urine or plasma constituents by thin-layer chromatography (TLC); and (4) measuring the fluorescence of the product.

EXPERIMENTAL

Chemicals and materials

Maprotiline hydrochloride was supplied by Ciba-Geigy (Basle, Switzerland).

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Ethanolic solutions of maprotiline, containing 10 mg per 100 ml and 1 mg per 100 ml, were kept in stock.

NBD chloride as well as solvents (analytical grade) and TLC materials were obtained from E. Merck (Darmstadt, F.R.G.). NBD chloride was used as a solution containing 0.1 g in 100 ml of methylisobutyl ketone for extraction.

The TLC plates (20 × 20 cm) used were pre-coated with silica gel 60 (layer thickness 0.25 mm) without a fluorescence indicator and with a concentrating zone (2.5 × 20 cm). The plates were used without any activation prior to use.

Equipment

A chromatogram spectrophotometer KM 3 from Carl Zeiss with a Linseis recorder was used.

Procedure for assay of urine and plasma samples

Extraction. Screw-capped centrifuge glass tubes (not more than 1.2 cm in diameter) are first moistened with 1 ml of a 1 *M* sodium hydroxide solution. Then 1 ml of urine or plasma is added. The contents are then extracted for 30 min on a mechanical shaker with 6 ml of *n*-heptane—isopropanol (99:1). After centrifugation 5 ml of the organic layer are aspirated, transferred to another centrifuge tube and completely evaporated. The evaporation is carried out in a vacuum centrifuge at 30°C (Speed Vac Concentrator, Bachofer Laboratoriums-werke).

Derivatization procedure. A 0.1 *M* sodium bicarbonate solution (0.2 ml) and 0.2 ml of a 0.1% NBD chloride solution are added to the extraction residue in the centrifuge tubes. The tube is screwed up tightly and shaken on a vortex mixer for 10 sec. The reaction mixture is kept at 80°C for 3 min. After cooling to room temperature 0.1 g of sodium chloride is added. The contents are then centrifuged for 5 min. At this point it is critical that the tubes are handled carefully so that the two layers do not become mixed again.

Thin-layer chromatography. A 50-µl· volume of the upper organic layer is carefully aspirated (from the surface) with a 100-µl Hamilton syringe and applied to a TLC plate in an 8-mm strip with a Linomat III (Camag, Switzerland). Urine or plasma standards of maprotiline of four different concentrations (50, 100, 200 and 500 ng/ml) are also spotted so that a calibration line is included on each plate. The plate is developed (at room temperature) in an unlined glass tank (Desaga) containing 100 ml of chloroform—ethyl acetate (99:1). After developing for 15 cm, the plate is air dried and scanned with a spectrophotometer. On the plate maprotiline is identified by applying its fluorescent derivative without prior extraction (R_F 0.61).

Densitometric evaluation of the chromatogram. The spectrophotometer was operated in the fluorescence mode. The light source was a mercury lamp St 41, the 434-nm line being selected for excitation. Emission was filtered with a monochromatic filter having its maximum transmission at 546 nm. The slit chosen was 1 × 6 mm. The peak areas are then calculated. Results are quantified by computing the slope m and the y-intercept b of the linear regression curve. This curve links the peak areas to the amount of maprotiline. The unknown X values, representing the amounts of maprotiline, are calculated by means of the linear equation: $Y = mX + b$ (Y = value of the peak areas).

RESULTS

Recovery studies were performed by analysing spiked urine and plasma samples and comparing the peak areas with those of non-extracted standards. Mean recovery values of 99.8% for urine and 96% for plasma were obtained (mean of three determinations).

The linear behaviour was tested between 0 and 1000 ng/ml with samples containing 0, 5, 10, 20, 50, 100, 200, 300, 500, 700 and 1000 ng/ml maprotiline. For urine, the linear regression curve shows linearity between 20 and 1000 ng/ml with a correlation coefficient of 0.995. The lower limit of detection is 10 ng/ml. For plasma, linearity was tested between 0 and 700 ng/ml with samples containing 0, 5, 10, 20, 50, 100, 200, 300, 500 and 700 ng/ml with a correlation coefficient of 0.999. The lower limit of detection is 20 ng/ml.

Reproducibility experiments were carried out by investigating five samples per concentration and three different concentrations of maprotiline in urine and plasma. The results obtained are summarized in Table I.

TABLE I

SUMMARY OF REPRODUCIBILITY EXPERIMENTS

Sample	Theoretical amount of maprotiline (ng/ml)		
	100	200	300
Urine	98	204	271
	94	206	270
	101	198	295
	90	204	295
	92	201	293
Mean	95.0	202.6	284.8
C.V. (%)	4.7	1.5	4.6
Plasma	96	218	313
	97	203	306
	106	199	310
	97	202	296
	96	216	292
Mean	98.4	207.6	303.4
C.V. (%)	4.3	4.2	3.0

Drug interferences

There are no interferences from normal constituents of urine and plasma with maprotiline (see Fig. 1). No interference is observed by the major metabolite desmethylmaprotiline or by oxaprotiline.

Drugs which can not be determined simultaneously with maprotiline are, for example, desipramine and nortriptyline.

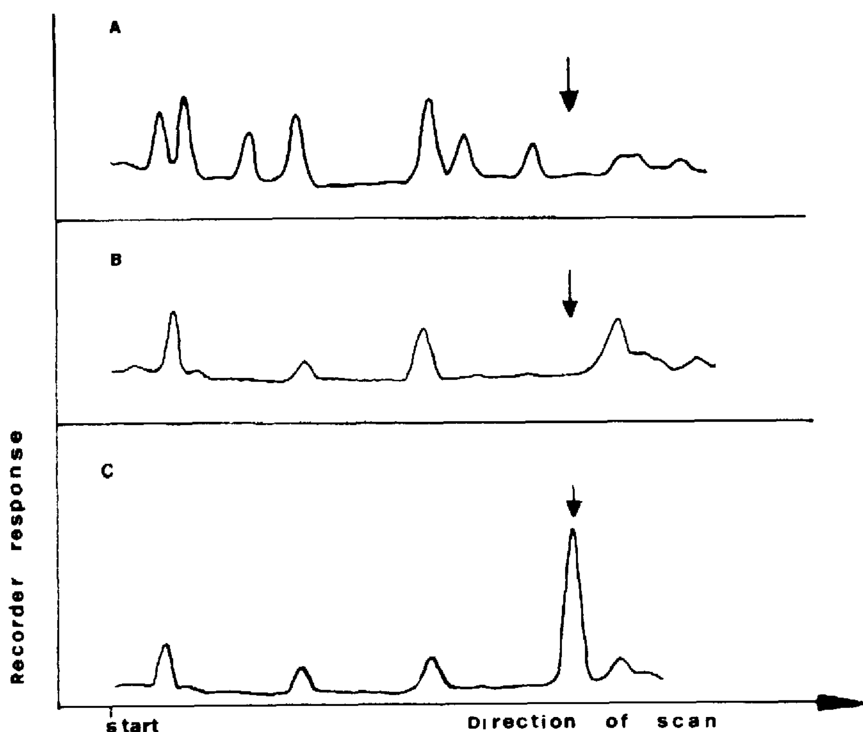

Fig. 1. Representative chromatograms of (A) blank urine, (B) blank plasma, and (C) spiked plasma (50 ng/ml).

The applicability of the method was tested by analysing seven urine samples from patients receiving maprotiline in a daily dose of three times 10—25 mg. The urine was collected and stored at −18°C until analysed. The concentrations found ranged from 122 to 913 ng/ml.

CONCLUSIONS

The procedure described in this paper allows the determination of maprotiline in a simple and rapid way. After the addition of sodium chloride and final centrifugation of the reaction mixture, attention must be paid to the fact that the two layers can easily become intermixed and thus lead to imprecise results.

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