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QUANTITATIVE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY FOR THE DETERMINATION OF MAPROTILINE AND OXAPROTILINE IN HUMAN PLASMA

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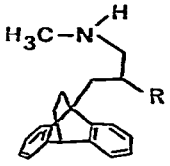
SUMMARY

A procedure is described which permits the determination of maprotiline and oxaprotiline in human blood or plasma at therapeutic concentrations. The biological fluids are extracted with base and the extract is injected into a high-performance liquid chromatograph. The substances are detected by a UV detector operating at 214 nm, amitriptyline being used as internal standard. Concentrations as low as 2 ng per sample can be measured.

INTRODUCTION

Maprotiline (Ludiomilz, Ciba-Geigy, Basel, Switzerland) is an antidepressant drug, the main action of which is thought to be the strong inhibition of the noradrenaline uptake into neurons[1]. In pharmacological experiments[2] the side-chain hydroxy derivative of maprotiline (oxaprotiline, Fig. 1) inhibited the neuronal uptake of noradrenaline by a factor of 10.



R = H maprotiline
R = OH oxaprotiline

Fig. 1. Structural formulae of maprotiline and oxaprotiline.

Some methods concerning the quantitative determination of maprotiline have been published[3–10], most of which involve gas chromatographic separations. As detectors, the electron-capture detector[3], the nitrogen-selective flame-ionization detector and the mass spectrometer[8,9] can be used. In 1974 a double radioisotope derivatization technique, which made use of a thin-layer separation, was described[7]. Recently, the first high-performance liquid chromatographic (HPLC) assay was published[10], in which a derivatization step is also necessary. However, the most frequently used

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HPLC detector with a wavelength of 254 nm is not suitable for the determination of maprotiline and its derivatives because of their very low absorption in this region. Only below 220 nm is the extinction of the UV-radiation high enough to allow the estimation of these drugs in the therapeutic range.

In this paper, an HPLC method for the determination of maprotiline and oxaprotiline without derivatization is described. Hundreds of quantitative determinations of both these compounds have been carried out in our laboratory, thus establishing the method as a routine determination.

EXPERIMENTAL

The simple high-performance liquid chromatograph consisted of an Altex A 110 pump, a Rheodyne 7120 injection valve, a commercially available Nucleosil C_{18} column and an LDC UV-monitor III with a zinc source operating at 214 nm. The mobile phase was a mixture of 35% acetonitrile and 65% of a 0.05 *M* phosphate buffer, pH 2.7. It was vacuum-filtered and then degassed ultrasonically. All water used was double-distilled. The acetonitrile (Burdick & Jackson Labs., Muskegon, MI, U.S.A.) had an UV cut-off of 190 nm. Reagents for the extraction were of analytical grade.

In the morning before the first drug dose, blood from patients was drawn into collection tubes containing lithium heparinate. The plasma was separated and stored frozen in polypropylene tubes until analysis. Each tube contained exactly 1 ml and the contents were used directly for extraction.

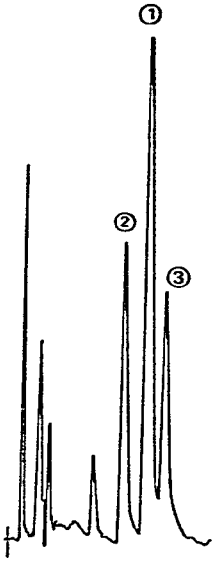
The extraction procedure is summarized in Table I. After thawing the plasma, 1 ml 2 *M* $NaHCO_3$ solution and 100 ng amitriptyline were added. The alkaline solution was mixed with 6 ml hexane and centrifuged for 10 min. After separating the

TABLE I

EXTRACTION PROCEDURE

1 ml plasma
+ 100 ng amitriptyline
+ 1 ml 2 *M* $NaHCO_3$ solution
+ 6 ml hexane ← shake and centrifuge
↓
+ 1 ml 0.1 *N* H_2SO_4 ← shake and centrifuge
↓
+ 0.1 ml 5 *N* NaOH solution
+ 2 ml hexane ← shake and centrifuge
↓
evaporate to dryness
solve in 0.02 ml eluent
inject all
↓
HPLC
↓
integrator → result

hexane layer with a pasteur pipette it was mixed with 1 ml 0.1 *N* H_2SO_4 for 10 min and centrifuged for another 10 min. Then the hexane layer was aspirated. A 0.1-ml volume of 5 *N* NaOH solution was added to the aqueous phase foliowed by 2 ml hexane. The solution was mixed for 10 min and then centrifuged. The hexane was evaporated in a vacuum and the residue dissolved in 20 μl of the eluent. As much as possible of this solution was then transferred to the injection valve of the high-performance liquid chromatograph. For automatic estimation of the results an integrator was used. Fig. 2 shows the chromatogram of a plasma extract from a patient receiving 150 mg maprotiline for therapy. It can be seen that both maprotiline and desmethylmaprotiline can be determined. Fig. 3 shows a similar chromatogram for oxaprotiline. For these substances the lower limit of detection is 2 ng/ml using 1 ml plasma for the extraction.



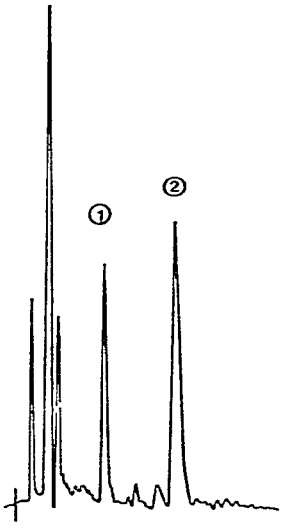


Fig. 2. HPLC of plasma extract from a patient receiving 150 mg maprotiline. Mobile phase, 35% acetonitrile and 65% 0.05 *M* phosphate buffer, pH 2.7. Detection at 214 nm at 0.08 a.u.f.s. Peaks: 1 = maprotiline, 2 = desmethylmaprotiline, 3 = amitriptyline.

Fig. 3. HPLC of plasma extract from a patient receiving 150 mg oxaprotiline. Conditions as in Fig. 2. Peaks: 1 = oxaprotiline, 2 = amitriptyline.

RESULTS

The range of concentrations found is shown in Table II. The values are in accord with other studies of maprotiline in plasma. So far, no measurements of oxaprotiline concentrations in plasma have been published.

One possible explanation for the lower concentration range of oxaprotiline as compared with maprotiline is the direct glucuronidation of the hydroxy group. In our determination the desmethyl derivatives are also included. The pharmacological importance of these metabolites is unknown, but the measured plasma concentration is so high that an action on the nervous system cannot be excluded. We established a

TABLE II

PLASMA CONCENTRATIONS (ng/ml) FOUND

Twenty patients were sampled in each case.

Dosage	*Maprotiline*		*Desmethylmaprotiline*		*Oxaprotiline*	
	Found	*Mean*	*Found*	*Mean*	*Found*	*Mean*
150 mg maprotiline per day	37–208	107	13–111	40		
150 mg oxaprotiline per day					5–48	25

negative correlation at the 2% level of the therapeutic effect and the plasma level of maprotiline, and a negative correlation at the 0.1% level with the concentration of desmethylmaprotiline. No correlation could be found for the plasma level of oxaprotiline and the patients' improvement[11].

In Fig. 4 the pharmacokinetics of oxaprotiline in human blood is shown. It can be seen that the method can also be used to analyze the low concentrations obtained after a single dose.

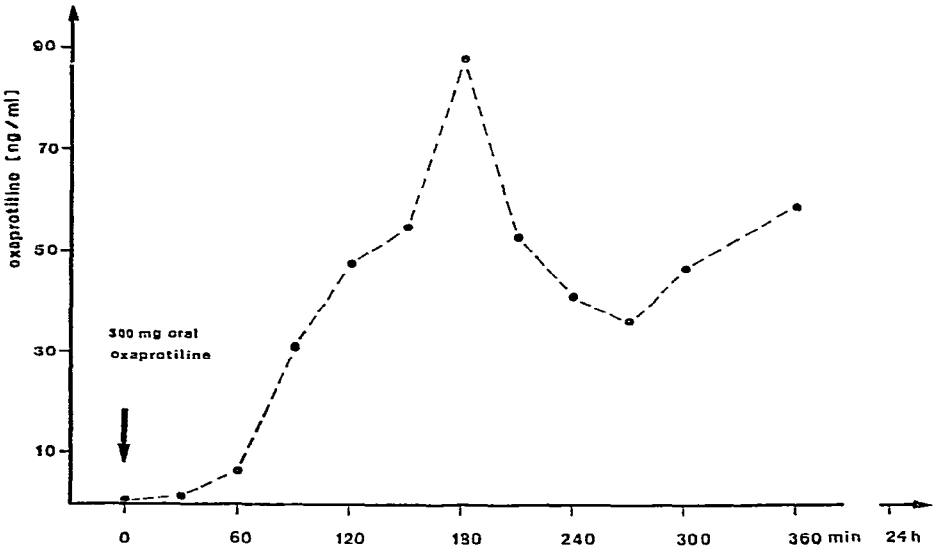


Fig. 4. Pharmacokinetics of oxaprotiline in human blood.

DISCUSSION

Gas chromatography–mass spectrometry is the best tool for analyzing the metabolic fate of drugs in the body, but is very expansive. For routine analysis in a clinical laboratory, HPLC is the most valuable method for determining the concentration of drugs in human blood during therapy. Some manufactures are also developing immuno-assays, but these are not as specific as chromatographic analyses. The quantitative separation of the parent compound and its active metabolites is the principal requirement (*cf.* ref. 12) for progress in clinical psychopharmacology.

Using a 214-nm detector with a zinc source we could achieve a 20-fold increase

in sensitivity, as compared to the usual 254-nm detector, for the detection of maprotiline and oxaprotiline. This high sensitivity allowed us to analyze plasma concentrations of these two substances in the therapeutic range. For the extraction we used a very common method including two back-extraction steps. The mean recovery was 50% or lower. Each day a recovery curve was constructed from spiked plasma samples. All the plasma concentrations of maprotiline, desmethylmaprotiline and oxaprotiline were corrected for recovery. The concentrations of desmethyloxaprotiline were in the range of the detection limit.

An important problem in plasma level monitoring is the presence of more than one drug. In our assay some of the tricyclic antidespressants and of the benzodiazepines can result in interferences. Monitoring the effluent of the column at 214 and 254 nm allows detection of these interferences. If there is no interference the 254-nm detector will not show any signal at the retention time of the drugs determined When there is any doubt regarding the identification of the peaks, the effluent can be injected into a gas chromatograph for further identification. Our values are in good agreement with the measurements of Gupta *et al.*[6] using the only assay procedure, which included the concentration of desmethylmaprotiline. These workers found a mean concentration of 117 ng/ml for maprotiline (range 40–237) and 52 ng/ml for desmethylmaprotiline (range 20–132); for comparison see Table II.

Some authors prefer the extraction of whole blood. We use blood concentration measurements only for pharmacokinetic purposes (Fig. 4). For therapeutic drug monitoring we prefer plasma, because most studies dealing with concentrations and the therapeutic effect make use of plasma extractions.

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