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Note

Determination of lofepramine and desipramine using high-performance liquid chromatography and electrochemical detection

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Lofepramine is a tricyclic antidepressant structurally related to imipramine. Its major metabolite is desipramine (desmethyl imipramine, DMI) [1] (Fig 1)

Attempts to measure lofepramine in biological fluids have been hindered by its instability in plasma or in aqueous solution where it readily undergoes basic hydrolysis to DMI. Attempts to extract it from basic or neutral solutions lead to extensive loss of the compound The only available method to quantify this

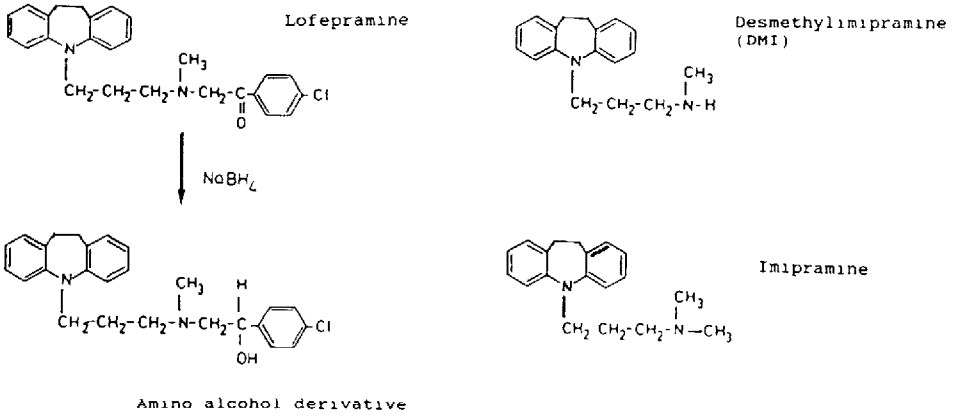

Fig 1 Structures of lofepramine, desipramine (DMI) and imipramine and the chemical reduction of lofepramine to yield its amino alcohol derivative

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drug relies on a complex gas chromatographic (GC) method involving the reduction of lofepramine to the corresponding amino alcohol (Fig. 1). This is then treated with sodium periodate to effect the oxidative cleavage of the *p*-chlorobenzoylmethyl side–chain The resulting *p*-chlorobenzaldehyde is then distilled off and subsequently extracted with hexane which is injected for GC. The *p*-chlorobenzaldehyde is quantified using either electron-capture detection [2] or mass fragmentography [3].

The lofepramine molecule contains two tertiary amino groups and it is therefore potentially amenable to electrochemical oxidation [4]. This property, together with the stabilisation afforded to the lofepramine molecule by its reduction to the amino alcohol derivative, provided the basis for the development of a rapid and sensitive method for the quantitation of lofepramine and its major metabolite DMI using high-performance liquid chromatography (HPLC) and electrochemical detection.

EXPERIMENTAL

Reagents and materials

Acetonitrile and methanol were HPLC grade (Rathburn, Walkerburn, U K.). Potassium dihydrogenorthophosphate, sodium hydroxide, sodium acetate, acetic acid, hydrochloric acid, sodium bicarbonate and sodium borohydride were Analar or Aristar grade (BDH, Poole, U.K.). All extractions were carried out in Eppendorf 3810 plastic tubes. Glassware was silanised by treatment with 10% (w/v) dichlorodimethylsilane in hexane.

Instrumentation and chromatographic conditions

The equipment used for this assay was a Hewlett-Packard 1081B high-performance liquid chromatograph, equipped with an oven and a variable-volume injector. A 25 cm × 4.5 mm I D Apex Cyano (5 μm particle size) column was used (Jones Chromatography, Llanbradach, U.K.). The detector was an LCA15 electrochemical detector (EDT Research, London, U.K.) equipped with a glassy carbon electrode. The potential was set at +1.00 V versus a reference electrode (Ag/AgCl) and the sensitivity was set at 10 nA full scale The response signals were recorded on a Yokogawa 3047 chart recorder and integrated on a Hewlett-Packard 3392A integrator.

The mobile phase was an acetonitrile–methanol–buffer mixture (55 5 40, v/v) The buffer used was a 0.02 *M* phosphate buffer pH 6.8, prepared with potassium dihydrogenorthophosphate and adjusted with 5 *M* sodium hydroxide solution. The flow-rate was set at 1.8 ml/min and the oven temperature was kept at 30°C. The solvent was recycled when no injections were being made

Preparation of standards and sample handling

Lofepramine standards were prepared by dissolving the hydrochloride salt in methanol to a concentration of 1 mg/ml of free base. This solution was then diluted with 0.01 *M* hydrochloric acid to the required concentrations Pooled drug-free human plasma was then spiked and lofepramine reduced to the amino alcohol derivative as described below. Silanised glassware was used throughout this procedure

Due to the instability of lofepramine, certain handling precautions had to be taken. Blood samples were collected in heparinised glass tubes and the plasma was immediately separated and stored in plain glass tubes at −40°C until analysed

Extraction

Plasma (400 μl) was placed in an Eppendorf tube and treated with a large excess of sodium borohydride in a 0.02 *M* sodium hydroxide solution (10 mg/ml, 50 μl). After 15 min, acetate buffer pH 5.3 (200 μl), internal standard (imipramine 500 ng/ml, 20 μl) and the extracting solvent methyl *tert.*-butyl ether (0.5 ml) were added. The tube was vortex-mixed for 1 min and centrifuged (2000 *g*, room temperature) for 2 min. The organic phase was separated and evaporated under a stream of nitrogen. The residue was reconstituted in the mobile phase (150 μl) and transferred to a vial for automatic injection (80 μl).

For lofepramine concentrations estimated to be greater than 50 ng/ml, a smaller plasma sample was taken and diluted to 400 μl with drug-free plasma

RESULTS AND DISCUSSION

The method described gave good separation between the lofepramine derivative, DMI and the internal standard imipramine (Fig 2a) A relatively high and therefore less selective operating potential of 1 00 V was chosen in order to provide an optimum overall response from all the compounds under investigation while maintaining a minimum of baseline noise The limits of detection were calculated for a signal-to-noise ratio of 3.

Initially, an attempt was made to measure lofepramine directly Although the electrochemical response was good and sensitivity could be lowered below 1 ng/ml detection, some hydrolysis to DMI during the extraction and injection procedure could not be avoided even with careful handling, so the method was deemed unreliable From the previously reported GC method it was known that the amino alcohol derivative showed much greater stability and it was therefore decided to try this route for lofepramine quantification

Extraction of the amino alcohol was optimum at pH 5.3 (>90%). At this pH imipramine extraction was not at an optimum but it was adequate and reproducible whereas DMI extracted poorly and only plasma levels down to 5

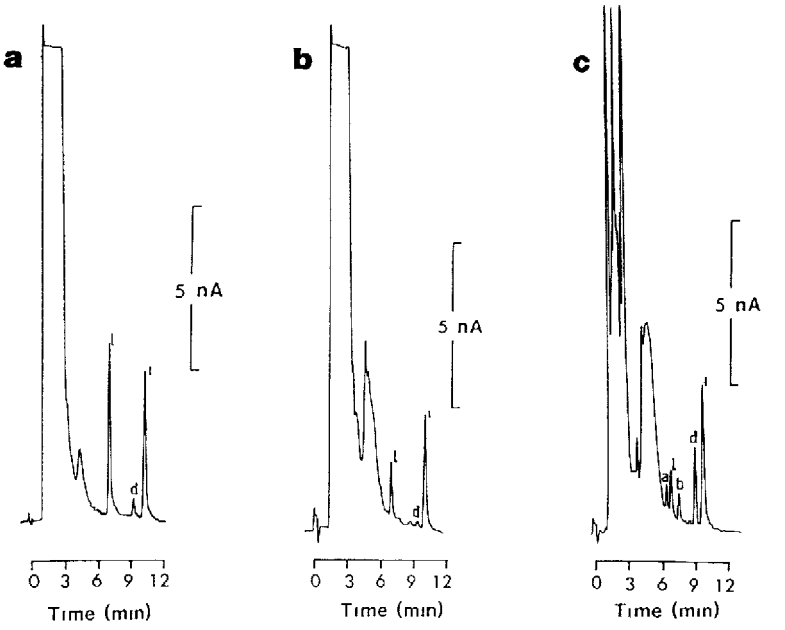

Fig 2 (a) Chromatogram of a plasma sample spiked to a concentration of 20 ng/ml of both lofepramine (l) and desipramine (d) extracted at pH 5 3 with imipramine (i) as internal standard (b) Chromatogram of a plasma sample taken 5 h after ingestion of a single 70-mg lofepramine tablet extracted at pH 5 3, peaks l=lofepramine (5 5 ng/ml), d=desipramine (5 0 ng/ml), i=imipramine (25 ng/ml) (c) Same sample as (b) but extracted at pH 9 3, peaks a=2-dehydroxydesipramine, l=lofepramine derivative, b=desmethyldesipramine, d=desipramine, i=internal standard (imipramine)

ng/ml were measurable. An identical extraction using a bicarbonate buffer pH 9.3 was required to quantify DMI below this level. The internal standard used was also imipramine (300 ng/ml, 20 μl). At the higher pH other metabolites also extracted· 2-hydroxydesipramine, desmethyldesipramine and desmethyllofepramine. These were identified by comparison against standards from the pure compounds. None of these interfered with DMI quantification (Fig 2b and c) For routine extractions where the lofepramine concentration is expected to be greater than 2 ng/ml, quantification of both lofepramine and desipramine can be done with a single extraction at pH 9.3.

The limit of detection for plasma concentration of both lofepramine and DMI was 0.5 ng/ml, although levels below this could be achieved with larger plasma samples.

Separate calibration lines for the two different pH values used were drawn up using spiked plasma samples at concentrations of 1, 2.5, 5, 10, 25 and 50 ng/ml of both lofepramine and DMI The detector response was linear over this range and a correlation coefficient (r) of 0.999 was obtained consistently

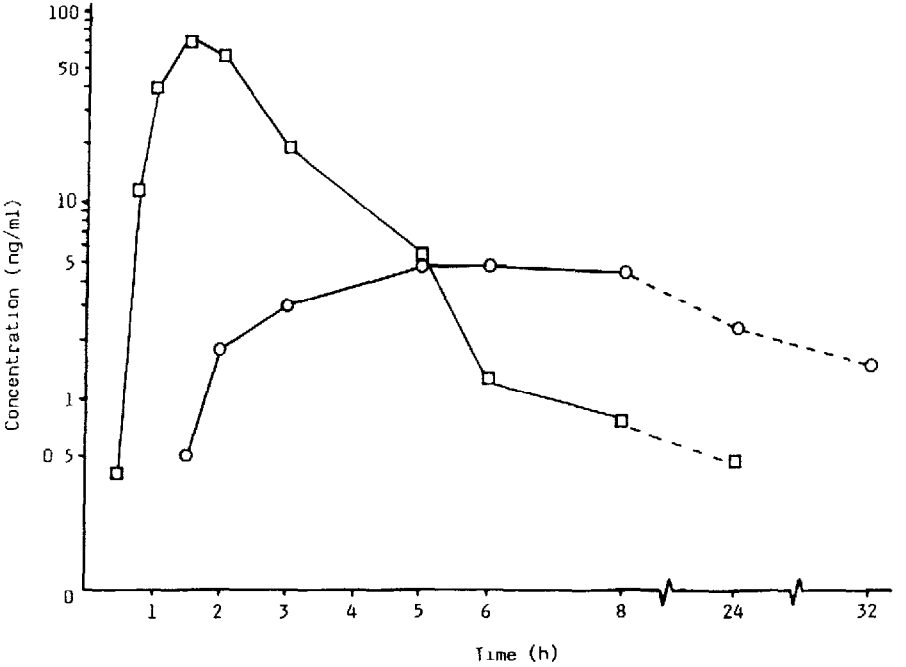

Fig 3 Plasma concentration versus time curve for lofepramine (□) and desipramine (○) following ingestion of a 70-mg lofepramine oral dose

for the calibration lines. The equations for the calibration lines were $y=0.26+14.2x$ for lofepramine (at pH 5.3) and $y=0.33+16\ 3x$ for desipramine (at pH 9.3).

The inter-assay coefficient of variation for lofepramine extracted at pH 5 3 was 1.7% at 50 ng/ml ($n=12$), 3.4% at 25 ng/ml ($n=12$) and 4 5% at 2.5 ng/ml ($n=12$).

The method was tested in a human volunteer by giving a healthy male a 70-mg lofepramine oral dose Blood samples were taken at twelve different time points, the plasma was quickly separated and stored at $-40°C$ until analysis. The plasma concentration versus time curve is shown in Fig. 3. Because of low plasma concentrations, DMI had to be extracted with pH 9.3 buffer. No interfering peaks were found in any of the samples

ACKNOWLEDGEMENT

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