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Note

Gas—liquid chromatographic determination of etoperidone in plasma, serum, and urine

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Etoperidone, 2-[3-(4-(*m*-chlorophenyl)-1-piperazinyl)-propyl]-4,5-diethyl-2,4-dihydro-3H-1,2,4-triazol-3-one monohydrochloride (Fig. 1a), is a psychotropic drug with antidepressant activity, and no cardiotoxic or cholinergic effects. The drug is potentially of particular utility in geriatric patients, since it is well tolerated, and appears to have a beneficial effect on certain of the psychosomatic parameters of ageing.

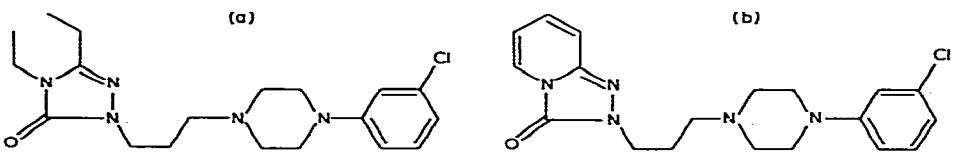

Fig. 1. Structures of etoperidone (a) and trazodone, the internal standard (b).

For metabolic and pharmacokinetic studies on this drug it was necessary to have available a method for its measurement in biological fluids. A similar compound, trazodone, has previously been determined by fluorimetry [1], gas—liquid chromatography (GLC) [2, 3], and high-performance liquid chromatography (HPLC) [4]. GLC with flame ionization detection has previously been used for the determination of etoperidone during animal studies with a sensitivity of 200 ng/ml [5]. In view of the low absorption of this compound in the near ultraviolet region, we felt that separation and detection by HPLC would not yield a method of adequate sensitivity; however, by using the extra selectivity which a nitrogen—phosphorus detector offers, a method has been developed with a sensitivity of 20 ng/ml in plasma or serum, and 5 ng/ml in urine.

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EXPERIMENTAL

Materials

Analytical-grade reagents were used throughout, and inorganic reagents were prepared using glass distilled water. Etoperidone as the hydrochloride was supplied by Sigma-Tau Research Labs. (Domezia, Rome, Italy). Trazodone as the hydrochloride (2-[3-(4-(*m*-chlorophenyl)-1-piperazinyl)-propyl]-*s*-triazole-[4,3-*a*]-pyridine-3-(2H)-one monohydrochloride) (internal standard, see Fig. 1b) was supplied by the same company.

Extraction procedure

Serum or plasma samples (1 ml) were transferred to 10-ml centrifuge tubes, spiked with internal standard solution (50 μl; 10 μg/ml of trazodone in methanol), and made alkaline with 5 *M* potassium hydroxide solution (200 μl). This mixture was extracted twice, each time with 5 ml of diethyl ether—petroleum ether (b.p. 40°—60°C) (1:1, v/v). Phases were separated by centrifuging for 5 min, the organic layers were combined in a clean tube, and reduced to low volume on a water bath. The remaining solvent was evaporated in a stream of nitrogen at room temperature, and the residue taken up in methanol (200 μl).

Urine samples (5 ml) were spiked with internal standard (50 μl; 10 μg/ml), and made alkaline with 5 *M* potassium hydroxide solution (500 μl). The sample was extracted twice with 5 ml of the ether mixture, separating the phases as above. The combined organic layers were extracted twice with 0.5 *M* hydrochloric acid (2 × 2.5 ml). These combined aqueous layers were made alkaline by the addition of 5 *M* potassium hydroxide solution (1 ml), and then extracted with the ether mixture (2 × 5 ml). The ether extract was evaporated and redissolved as described above.

Calibration procedures

Samples of blank serum were spiked with etoperidone at concentrations of 50—300 ng/ml, and carried through the extraction procedure described above. For urine, solutions of the drug in water were extracted as above. Water was used in place of urine as no significant difference was observed between the two, but water produced a much cleaner chromatogram. The standard curve for urine was prepared over the range 50- 500 ng/ml, although the parent drug has only shown concentrations up to 100 ng/ml.

GLC conditions

Chromatography was carried out on a Sigma 3 gas chromatograph (Perkin-Elmer, Beaconsfield, Great Britain) fitted with a nitrogen—phosphorus detector. The output was taken via a CRS308 computing integrator (Infotronics, Stone, Great Britain) to a Perkin-Elmer Model 56 chart recorder. The column used was glass 1 m × 4 mm I.D., packed with 3% OV-17 on Chromosorb W HP 100—120 mesh, maintained at 290°C. The carrier gas was nitrogen at a flow-rate of 40 ml/min, and the injector and detector blocks were maintained at 350°C. Flow-rates of the combustion gases were optimised according to the manufacturer's instructions.

Linearity, sensitivity, and recovery

The detector gave a linear response from 2.5 to 600 ng of etoperidone injected on column. Under the operating conditions described, the method is capable of routinely detecting down to 20 ng/ml of etoperidone in plasma or serum and 5 ng/ml of etoperidone in urine. Total recovery is about 84% because of extraction and transfer losses.

RESULTS AND DISCUSSION

The calibration curve was constructed from six replicate measurements of concentration over the working range. The plot of peak area ratios against concentration was linear ($y = 0.002926x - 0.002$, correlation coefficient $r = 0.999$). The value of the intercept was not significantly different from zero.

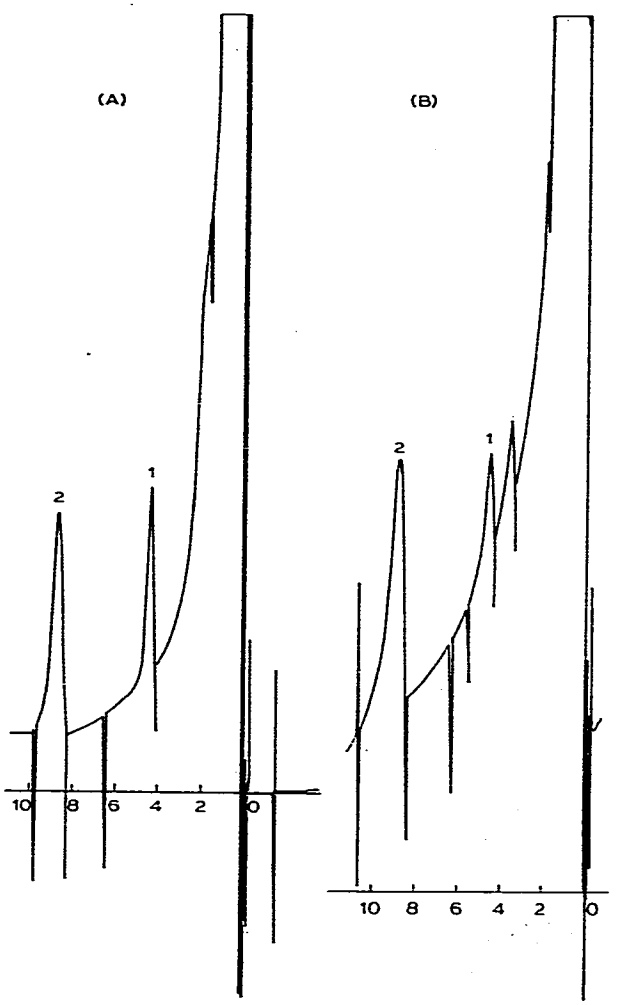

Fig. 2. Chromatograms from (A) a plasma sample containing 75 ng/ml of etoperidone, and (B) from the 0.5-h post-dose serum sample. Peaks: 1 = etoperidone, 2 = trazodone.

This method has been used for the analysis of samples collected after dosing a volunteer with a 50-mg capsule of etoperidone. The peak concentration in blood occurred between 0.5 and 1 h after dosing, and the concentration at 24 h was below the limit of detection (Table I). Insufficient samples were taken to examine the descending portion of the plasma concentration curve in detail, but it appears that the half-life is of the order of 1.5 h. Results from plasma and serum were similar for the lower concentrations of the drug, but the peak level values observed in plasma were noticeably lower than those from serum. No explanation is offered for this difference; however, the chromatogram resulting from serum has less interfering peaks than that from plasma (Fig. 2).

Results from urine were more complex, and are still under investigation. An amount of 8.2 μg of unchanged drug was found in urine collected between 0 and 6 h post-dose, and a further 0.7 μg up to 24 h. However, at least three metabolites were observed in the traces (Fig. 3). These compounds have not yet

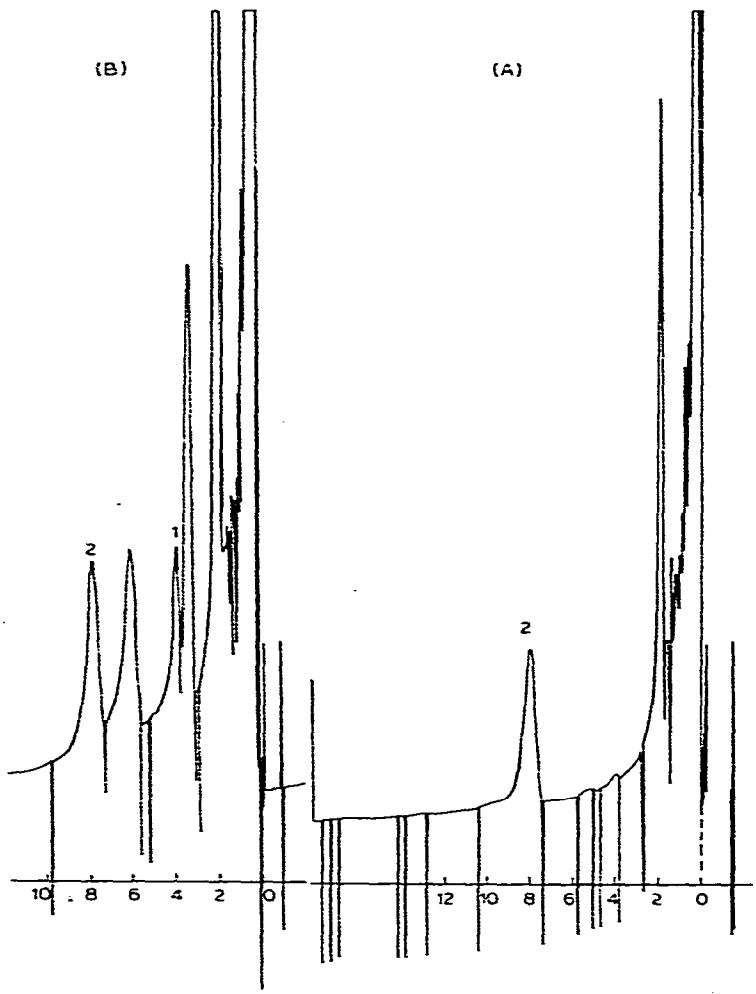

Fig. 3. Chromatograms from urine samples taken pre-dose (A), and 2 h post-dose (B). Peaks: 1 = etoperidone, 2 = trazodone.

TABLE I

CONCENTRATIONS OF UNCHANGED DRUG IN SERUM AND PLASMA, AFTER ADMINISTRATION OF A SINGLE ORAL DOSE OF 50 mg OF ETOPERIDONE

Time (h)	Concentration of etoperidone (ng/ml)			
	Serum		Plasma	
	Mean*	Coefficient of variation (%)	Mean*	Coefficient of variation (%)
0.5	305	0	199	2.5
1	321	3.1	221	6.6
1.5	129	7.3	103	3.4
2	88	4.0	75	0
3	50	2.0	58	1 sample
5.5	23	9.7	21	7.5
8	no sample		21	1 sample
24	none detected		none detected	

*Mean of two samples.

been identified, but all show larger peak areas than the etoperidone, and all are present for up to 36 h.

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