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Note

Determination of tiapride in plasma by high-performance liquid chromatography

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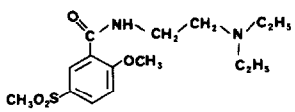
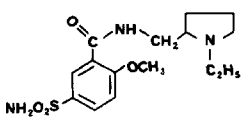
Tiapride [N-(diethylaminoethyl)-2-methoxy-5-(methylsulphonyl)benzamide hydrochloride, Fig. 1] is a substituted benzamide possessing properties of a dopamine receptor antagonist, but without the ability to block dopamine-stimulated adenylate cyclase [1]. It increases dopamine turnover in certain brain regions without binding potently to dopamine receptors [1, 2]. Tiapride has been found to be useful in reducing dyskinesias associated with Parkinson's and Huntington's diseases [3, 4].

In order to study the pharmacokinetics of tiapride in patients with Huntington's disease a method for measurement of the drug in plasma was developed based on reversed-phase high-performance liquid chromatography



Tiapride



Sulpiride

Fig 1 Structures of tiapride and sulpiride (internal standard)

(HPLC). A previously published method based on HPLC, although seemingly adequate for this purpose, was not fully described [5]

EXPERIMENTAL

Drugs and reagents

All chemicals were of analytical-reagent grade Ethyl acetate and acetonitrile (HPLC grade) were purchased from Waters Assoc. (Milford, MA, U.S.A.), ethanol (AR grade) from E Merck (Darmstadt, F R G.) Tiapride, sulpiride, compounds 2724 (desethyltiapride) and 2109 (tiapride-N-oxide) were supplied by Delagrange (Paris, France) and used as received.

Stock solutions of sulpiride and tiapride to give a concentration of 100 mg/l of the free base in ethanol were prepared. These solutions are stable at 4°C for at least one month. Tiapride plasma standards containing 100, 250, 500, 700 and 1000 μg/l were prepared by adding the appropriate volume of stock solution to drug-free plasma. The standards were stored frozen at - 20°C as 2-ml aliquots in plastic tubes until required Plasma standards were stable for at least six months.

Glassware

All glassware used in the assay was washed with laboratory detergent (Lipsol, Lip Equipment and Services, Shipley, U.K), rinsed once with tap water, twice with distilled water and allowed to drain dry overnight

Extraction procedure

Sulpiride, the internal standard (100 μl equivalent to 1 μg), was added to 1 ml of plasma in a 30-ml stoppered glass tube. The plasma was alkalinised with 1 ml of 0.1 *M* sodium hydroxide and the contents of the tube were mixed thoroughly Ethyl acetate (5 ml) was added to the tube and the mixture shaken on a reciprocating shaker for 10—15 min The tubes were centrifuged at 400 *g* for 10 min and the organic layer transferred to a clean set of tubes The plasma was further extracted with 5 ml of ethyl acetate, centrifuged and the organic layer separated. The two ethyl acetate extracts were combined and shaken with 1 ml of 0.1 *M* hydrochloric acid for 15 min and the tubes centrifuged at 400 *g* for 5—10 min. The aqueous phase was transferred to a set of 10-ml tubes and alkalinised with 0 3 ml of 5 *M* sodium hydroxide, extracted with 2 ml of ethyl acetate on a Matburn wheel for 10 min and allowed to stand for 10—15 min. The organic layer was separated into a clean set of tubes and evaporated to dryness in a water bath at 60°C in a stream of nitrogen. The aqueous layer was extracted with 2 ml of ethyl acetate as before, allowed to stand, separated and added to the same set of tubes and evaporated to dryness The extract was reconstituted in 10 μl of ethanol and as much as possible injected into the chromatograph

Apparatus

A Waters Assoc. high-performance liquid chromatograph consisting of a Model 45 solvent delivery system, Model U6K universal injector, a Model 441 fixed-wavelength absorbance detector and a Model 730 data module was used.

Separations were effected at 20°C with a 10 cm × 0.8 cm radial compression column packed with 10 μm diameter cyanopropyl silane-coated silica beads (C_{18} Rad-Pak, Waters Assoc) in a radial compression Z module The column was eluted with a solvent system consisting of acetonitrile—phosphate buffer (0 01 *M* KH_2PO_4 adjusted to pH 3 0 with phosphoric acid) (10 90, v/v) at a flow-rate of 2 0 ml/min Absorbance of the eluate was measured at 214 nm at a detector sensitivity of 0.02 a u f s. with a chart speed of 0 25 cm/min on the 730 data module. The peak-height ratio of tiapride to sulpiride was calculated for each sample and standard curves were constructed using linear regression analysis The calculated regression equation was peak-height ratio = $7.23 \cdot 10^{-4}$ concentration + 0.0275 (r = 0 9993).

Precision studies

Drug-free plasma (Blood Bank) was used to prepare standards containing 200 μg/l and 600 μg/l tiapride. These were used to evaluate the within-day and day-to-day precision of the assay.

Single-dose studies

Three patients with Huntington's disease each received a single 100-mg tablet of tiapride Blood samples were collected over 12 h from an indwelling heparinized cannula and a sample 24 h after the dose was drawn by venipuncture. Plasma was separated by centrifugation and stored at −20°C until analysed The half-life of elimination was calculated by linear regression analysis of the terminal plasma concentrations

RESULTS AND DISCUSSION

Sulpiride was chosen as the internal standard for the assay because of its close structural similarity to tiapride (see Fig. 1)

A three-step extraction procedure was found to be necessary for the assay to provide samples free of interfering peaks in the chromatograms A one-step extraction was not satisfactory in this regard. Chromatograms of an extracted plasma blank sample and a plasma standard are shown in Fig. 2 A small peak near the retention time of tiapride was noted but did not interfere with the results, since no significant deviation from linearity was noted in the standard curves ($r^2 > 0.95$ over 25 curves) Using ethyl acetate for the extractions gave a satisfactory recovery of tiapride and sulpiride (about 60–70%) but the desethyl metabolite of tiapride did not extract satisfactorily (less than 10% was recovered). The N-oxide did not extract at all under the conditions of the assay as described here. Only a small proportion of tiapride is converted to these metabolites, even after multiple-dose administration [6] The sensitivity of the assay for tiapride was less than 25 μg/l. The intra- and inter-assay variability is shown in Table I.

The assay is suitable for the measurement of plasma concentrations of tiapride following single doses as demonstrated by the data in Fig 3. Following a single 100-mg dose in three Huntington's disease patients a mean peak plasma concentration of 670 μg/l was attained at 3 h post dose. The mean plasma elimination half-life was 5 1 h. This elimination half-life is somewhat longer

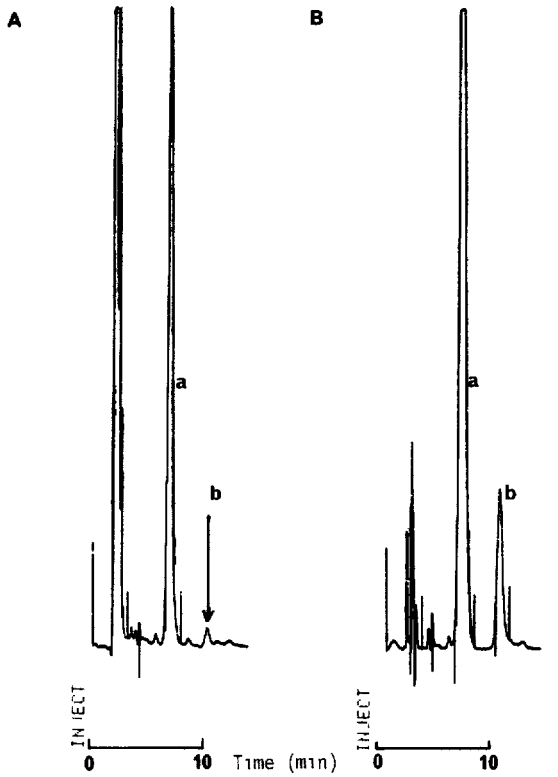


Fig 2 Chromatograms of extracted plasma samples (A) Blank plasma, (B) plasma standard containing 100 μg/l tiapride Chromatographic conditions as noted in text under *Apparatus* Peaks a = sulpiride (retention time, t_R = 6 8 min), b = tiapride (t_R = 10 1 min) N-Desethyl tiapride had a t_R of 6 3 min and the N-oxide a t_R of 14 4 min

TABLE I

PRECISION OF TIAPRIDE ASSAY

Concentration (μg/l)		*n*	Coefficient of variation (%)
Expected	Found		
Intra-assay			
100	97	9	9
200	200	8	11
600	580	8	4
1000	992	9	4
Inter-assay			
200	190	9	17
600	560	9	8

than the 3.2 h measured in eight healthy volunteers who each received 200 mg of tiapride as a single oral dose [6]. It is difficult to draw any meaningful conclusions at this time about any pharmacokinetic differences between patients and volunteers, since data from too few patients have been collected. A more

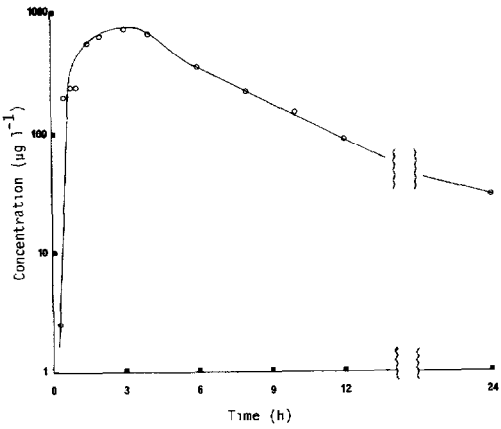


Fig 3 Mean plasma concentrations of tiapride in three patients with Huntington's disease who each received a single oral dose of 100 mg Plasma concentrations were measured in duplicate which agreed to within 10%

detailed pharmacokinetic analysis will be presented elsewhere. The method as described is simple to perform, has the required sensitivity for single-dose kinetic studies and is reproducible over the range of concentrations required for steady-state data

ACKNOWLEDGEMENTS

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