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Note

Determination of piracetam in serum by gas chromatography

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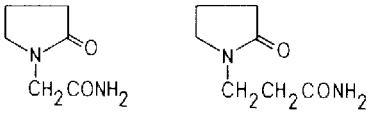
Piracetam, 2-oxopyrrolidine-1-acetamide (I, Fig. 1), is the prototype nootropic drug used to treat memory impairment, alcoholism, senile dementia or Alzheimer's disease, although little is known about the biochemical mechanism through which this drug modifies brain functions [1]. Several chromatographic methods [2–7] have been applied to determine piracetam in biological fluids (mainly serum or plasma) in order to provide pharmacokinetic data [7,8]. Published methods [2–7] were not acceptable for our investigations because of strong interferences from the biological matrix and, consequently, lack of sensitivity.

Here we describe a rapid, sensitive and reproducible gas chromatographic (GC) method for the quantification of piracetam in serum. The procedure includes acetone extraction and GC on a fused-silica capillary column with



I II

Fig. 1. Structures of piracetam (I) and the internal standard (II).

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nitrogen–phosphorus detection. This method was used to study the pharmacokinetics of a single oral dose of 800 mg of piracetam in a bioequivalence study in healthy humans.

EXPERIMENTAL

Standards and reagents

Piracetam (I, Fig. 1; Pliva A-4784016) and the internal standard, 2-oxopyrrolidinepropionamide (II, Fig. 1 [9]), were kindly donated by Pliva (Zagreb, Yugoslavia). Piracetam capsules (400 mg) were obtained from Pliva and UCB Chemie (Kerpen, F.R.G.).

Gas chromatography

A Shimadzu GC-9A gas chromatograph equipped with a flame thermionic detector, an AOC-9 autosampler and a CR3A computing integrator was used. The column used was an 8 m × 0.25 mm I.D. fused-silica SE-54 Supelco capillary, layer thickness 0.25 μm.

Operating conditions

The column oven was operated at 140°C for 1 min, followed by a temperature gradient of 10°C/min up to 160°C, then 5 min at 160°C. The injection port and detector temperatures were set at 330°C using helium as the carrier gas at a flow-rate of 2 ml/min. A 10:1 split ratio was established, and 2-μl sample aliquots were injected by an autosampler.

Extraction of piracetam from standard serum solutions

In order to establish the calibration curve and to study the recovery, linearity and reproducibility of serum piracetam extraction, a set of known piracetam concentrations (1, 5, 10, 20 and 30 μg/ml) in human serum was extracted and analysed several times ($n = 3$–5). The method developed by Hesse and Schulz [2] was adopted with some modifications. To 0.3 ml of serum containing known drug concentrations in a conical test-tube, 100 μl of 50 μg/ml II in water were added. After vortex-mixing, 6 ml of acetone were added, the test-tube was capped with a glass-ground stopper and the mixture was sonicated for 5 min in an ultrasonic bath. After shaking for another 5 min, the mixture was centrifuged at 2500 *g*. A 5-ml volume of the clear upper layer was transferred to another test-tube and evaporated to dryness. The test-tube wall was washed with 1 ml of acetone and again evaporated to dryness. The residue was dissolved in 700 μl of methanol and analysed by GC.

Sample processing

The serum samples from the bioequivalence study were stored at −20°C until analysis. They were thawed at room temperature and processed as de-

scribed for standard piracetam serum solutions. Every set of fourteen to eighteen volunteers' serum samples was extracted together with four to six standard serum piracetam solutions of different concentrations in order to control the detector response factor and corrections due to extraction recovery (calibration curve). Each extract was chromatographed twice.

Clinical application of the assay

The pharmacokinetics and bioequivalence of two oral 800-mg piracetam preparations (Pliva and UCB capsules) were studied in nine healthy volunteers (three males and six females) of 21–45 years of age with an ideal body weight ± 15%. This study was open, cross-over and randomised, and the period between two experiments was one week. Blood samples were withdrawn before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8 and 12 h post-dose. The serum was separated by centrifugation and frozen at −20°C until analysed for its piracetam content using the method described herein (see Fig. 2d).

Calculations

A two-compartment model was used for calculation of pharmacokinetic parameters. The areas under the curve (AUCs) were calculated using the trapezoid method. Statistical comparison was done using the ANOVA test.

RESULTS AND DISCUSSION

Chromatography

Chromatograms of standard piracetam (I) and 2-oxopyrrolidine-1-propionamide (internal standard, II) mixtures in methanol are shown in Fig. 2a and were used for the identification of the peaks and also for examining the reproducibility and linearity of chromatography and detector response.

A calibration curve for sample processing was established and controlled daily by extraction of a set of standard serum solutions of piracetam. Representative chromatograms are shown in Fig. 2b and c; within-day and between-day results, based on peak-area ratios, are summarised in Table I. Peak-area ratios were linear with concentrations of piracetam in the range studied (1–30 μg/ml). The calibration curve equation was $y = -0.29x + 1.03$ ($r^2 = 0.998$).

The reproducibility of the method was demonstrated by coefficients of variation (C.V.) which ranged from 2.6 to 5.2% for within-day and from 4.6 to 19.8% for between-day studies. The relatively bad between-day reproducibility is probably due to the instability of the nitrogen–phosphorus detector when used in combination with programmed temperature conditions. This was controlled and corrected by including standard serum samples into everyday analysis.

The calculated concentrations (precision) were within 4–10% of the actual concentration, except for the lowest concentration (5 μg/ml): 9–16%.

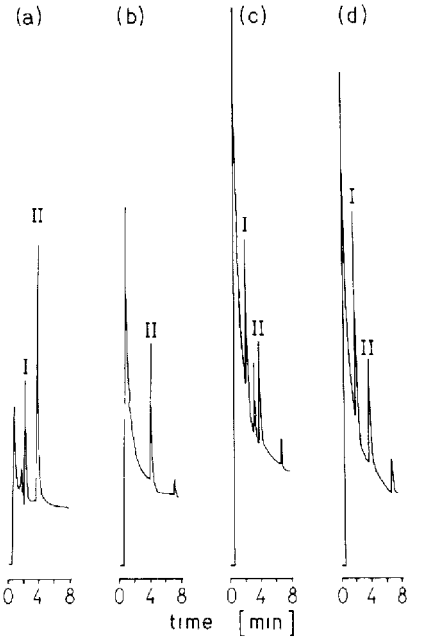

Fig. 2. Chromatograms of (a) a standard solution of piracetam (I, 5 μg/ml) and internal standard (II, 25 μg/ml) in methanol, (b) blank serum spiked with II and processed by the method described, (c) blank serum spiked with I (10 μg/ml) and processed by the method described and (d) a volunteer's serum sample (15.8 μg/ml I).

TABLE I

VALIDATION OF THE METHOD: LINEARITY, RECOVERY, WITHIN-DAY AND BETWEEN-DAY REPRODUCIBILITY

Concentration added (μg/ml)	Recovery ($n=3$)		Measured concentration			
	Mean ± S.D. (%)	C.V. (%)	Within-day ($n=3$–5)		Between-day ($n=14$–20)	
			Mean ± S.D. (μg/ml)	C.V (%)	Mean ± S.D. (μg/ml)	C V. (%)
1	78 ± 1.9	2.4	0 94 ± 0 03	2 6	1 06 ± 0.2	19 8
5	83 ± 5.6	6.8	5.10 ± 0.26	5 2	5 13 ± 0.53	10 5
10	79 ± 8.2	10.4	10 05 ± 0 49	4 9	9.5 ± 0.71	7 4
20	84 ± 9 1	10.8	19.2 ± 0.6	3.1	18.1 ± 2.6	14 4
30	85 ± 6.4	7.5	31 4 ± 1 2	3 8	30 5 + 1.4	4.6

Pharmacokinetics and bioequivalence study

The analytical method described herein was applied to study the profile of piracetam pharmacokinetics in nine healthy humans (Fig. 2d). They volunteered in a bioequivalence study of two oral 800-mg piracetam preparations (UCB and Pliva capsules). The serum concentration versus time profiles of these two preparations are presented in Fig. 3. The computed pharmacokinetic parameters are listed in Table II. No statistically significant difference in $AUC_{0-12\,h}$ is found. The relative bioavailability of Pliva piracetam capsules is more than 90% when compared to UCB piracetam. At the end of the dosage interval the concentrations are equal. The two examined preparations are not

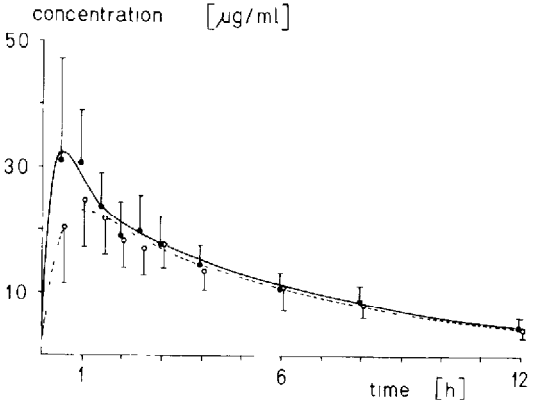

Fig. 3. Mean piracetam serum concentration versus time profiles after a single oral dose of 800 mg piracetam administered to nine human volunteers. Bars represents standard deviations. Solid line, UCB preparation; dotted line, Pliva preparation.

TABLE II

AVERAGE PHARMACOKINETIC PARAMETERS OBTAINED FROM THE SERUM CONCENTRATION VERSUS TIME PROFILES OF NINE VOLUNTEERS AFTER A SINGLE 800-mg PIRACETAM DOSE

Parameter	Value (mean ± S.D.)		
	Piracetam preparation		ANOVA
	UCB	Pliva	
C_{max} (mg/l)	37.1 ± 12.3	27.0 ± 8.0	$p < 0.05$
T_{lag} (h)	0.086 ± 0.126	0.095 ± 0.145	N.S.[a]
T_{max} (h)	0.78 ± 0.26	0.83 ± 0.35	N.S.
beta (h^{-1})	0.152 ± 0.026	0.152 ± 0.039	N.S.
$AUC_{0\,12}$ (mg h/l)	154.2 ± 34.1	138.9 ± 25.2	N.S.
r^2	0.981	0.985	

[a]Not significant.

bioequivalent as they differ in peak concentration (Table II, Fig. 3). From the therapeutic point of view the two examined preparations are expected to be equipotent, as the higher peak concentration is not ultimately advantageous in comparison to stable drug concentration in blood.

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