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Short communication

Determination of piracetam in human plasma and urine by liquid chromatography

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Abstract

A method for the determination of piracetam in human plasma and urine by liquid chromatography with absorbance detection at 206 nm and isocratic elution is proposed. The assay involved a liquid–liquid extraction into hexane–2-propanol at pH 9.2. The calibration graphs were linear in the range 3–40 mg/l in plasma and 100–2000 mg/l in urine. Bias was negligible and coefficients of variation were less than 10% throughout the working range except at 100 mg/l in urine. The limits of quantification were 3 mg/l in plasma and 100 mg/l in urine. The assay was reliably used for pharmacokinetic studies in humans after administration of 800 mg of piracetam per os.

1. Introduction

The determination of piracetam (2-oxopyrrolidine-1-acetamide) in biological fluids is required for the determination of its pharmacokinetic parameters. Previous methods used gas chromatographic assays [1,2] or liquid chromatography [3–8]. One required solid-phase extraction [4] while the others used extraction of plasma with solvents or acids. Isocratic elution was employed in all but one case [3]. However, all these methods were applied to serum or plasma and were not useful for urine because of strong interferences with endogenous compounds. In this paper, a thoroughly validated method is described for the determination of piracetam in both human plasma and urine by liquid chromatography using an isocratic system with UV detection.

2. Experimental

2.1. Reagents

Piracetam was obtained from Parke-Davis Labs. (Courbevoie, France; batch No. 23468). Human plasma was obtained from the Centre de Transfusion Sanguine (Bobigny, France) and urine from a healthy volunteer. All other reagents were of analytical-reagent grade and were purchased from local suppliers.

2.2. Apparatus

The liquid chromatograph consisted of a

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Shimadzu LC-6A pump, a Shimadzu SIL-6A automatic injector, a Shimadzu SPD-10A ultraviolet detector and a Shimadzu CR-5A integrator (Touzart et Matignon, Vitry, France). A Hibar LiChrosorb RP-18 column (250 × 4.0 mm I.D., 5-μm particle size, batch No. 322141) manufactured by Merck (Darmstadt, Germany) was used.

2.3. Chromatographic conditions

The mobile phase was methanol–water (5:95, v/v), pumped at 1 ml/min. The UV absorption was monitored at 206 nm. The volume injected on to the column was 20 μl (plasma) or 5 μl (urine).

2.4. Preparation of standard solutions

For plasma, a 1000 mg/l piracetam stock standard solution in distilled water was prepared and stored at 4°C. Working standard solutions of 200 mg/l were prepared in distilled water each week and stored at 4°C. A five-point calibration graph (3, 5, 10, 20 and 30 mg/l) and a blank were obtained every day. Three quality control solutions at 3, 15 and 40 mg/l were prepared and kept frozen at −20°C for at most 2 months.

For urine, the procedure was similar but only a 400 mg/l stock standard solution was used, the points for the calibration graph were at 0, 100, 250, 500, 1000 and 2000 mg/l and the quality control solutions were 100, 500, 1000 and 2000 mg/l.

2.5. Plasma extraction procedure

Samples (1 ml) were added 100 μl of a 0.1 *M* ammonia–ammonium buffer (pH 9.2) and 5 ml of chloroform–2-propanol (70:30, v/v). After capping, the samples were transferred to a reciprocating shaker and agitated for 10 min, then centrifuged for 10 min at 1000 *g*. The upper aqueous phase was discarded and 3 ml of the lower organic phase were transferred into glass tubes. The samples were evaporated to dryness under air with mild heating (40°C). The residue was dissolved in 0.5 ml of mobile phase.

2.6. Urine extraction procedure

Samples (1 ml) were first washed with 1 ml of toluene–hexane (50:50, v/v) by agitation for 10 min in a reciprocating shaker. After centrifugation for 10 min at 1000 *g*, the upper organic layer was discarded. The samples were then treated in the same way as plasma. In the final step, the dry residue was dissolved in 1 ml of mobile phase.

2.7. Regression analysis of the calibration lines

Linear regression of peak heights versus piracetam concentration was performed by weighted last-squares without taking the blank into account. The weights were the inverse of the experimental variances of the calibrators, obtained from the initial validation of the assay on six calibration lines.

3. Results and discussion

Fig. 1 shows the chromatograms of drug-free plasma and plasma containing 5 mg/l of piracetam. Fig. 2 shows the same analysis of drug-free urine and urine samples containing 200 mg/l of piracetam.

3.1. Precision and accuracy

Tables 1 and 2 show the inter-assay reproducibility and the accuracy of piracetam determinations measured as a function of the quality controls in plasma and urine, respectively. Bias was found to be negligible and the reproducibility, expressed as the coefficient of variation (C.V.), was less than 10% throughout the working range except at 100 mg/l in urine. The C.V. of the slope of the calibration graph was 1.1% for plasma and 3.2% for urine.

3.2. Extraction efficiency

Comparison of a five-point plasma or urine calibration graph with an aqueous calibration graph showed average recoveries of 83.5% and 41% for piracetam in plasma and urine, respec-

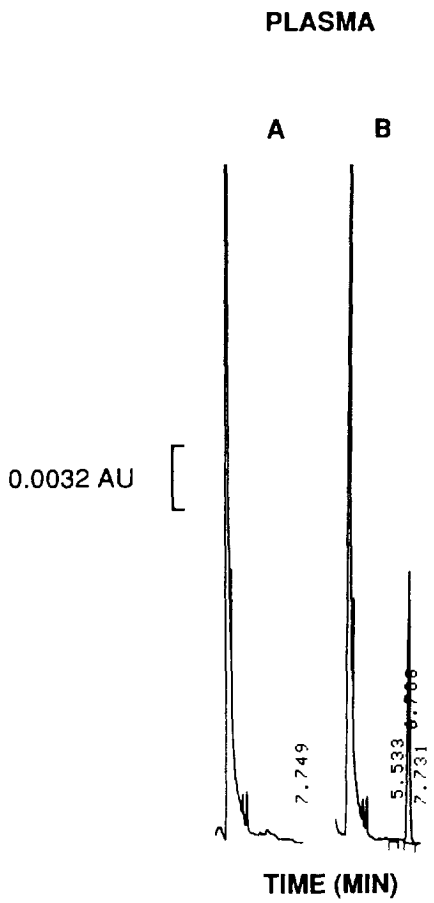

Fig. 1. Chromatograms of (A) a drug-free human plasma sample and (B) 5 mg/l piracetam in human plasma.

tively, over the calibration range. In these calculations, a correction was applied to account for the fact that only 60% of the organic phase was taken. Attempts were made to improve the extraction yield by using a less polar solvent than chloroform–2-propanol (7:3), i.e. chloroform + 0, 1, 2, 5 and 10% of butanol. The highest yield (91%) was obtained with chloroform–butanol (90:10) but the evaporation duration was almost double that with chloroform–2-propanol. Replacement of chloroform with dichloromethane in butanol mixtures was also evaluated; the extraction yields were comparable but some interferents were co-extracted with piracetam when dichloromethane was used. Finally, chloroform–2-propanol was adopted as the best

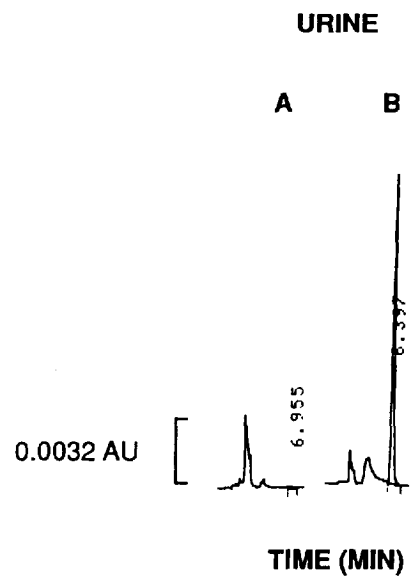

Fig. 2. Chromatograms of (A) a drug-free urine plasma sample and (B) 200 mg/l of piracetam in human urine.

compromise in terms of selectivity, evaporation time and extraction yield. The much lower yield observed in piracetam extraction from urine could be explained by some loss during the washing procedure with toluene–hexane. However, despite this low extraction yield, the reproducibility of the assay was sufficient in the working range.

3.3. *Quantification and detection limit*

The limit of detection, defined as the concentration of piracetam in biological fluid giving

Table 1
Reproducibility and accuracy of piracetam determination in plasma

Parameter	Value		
	3 mg/l	15 mg/l	40 mg/l
Number of samples	12	12	12
Mean (mg/l)	3.20	15.1	39.5
Bias (%)	+6.6	+0.6	−1.2
Standard deviation (mg/l)	0.19	0.89	1.20
Coefficient of variation (%)	5.9	5.9	3.1

Table 2
Reproducibility and accuracy of piracetam determination in urine

Parameter	Value			
	100 mg/l	500 mg/l	1000 mg/l	2000 mg/l
Number of samples	12	6	6	12
Mean (mg/l)	105	504	989	2018
Bias (%)	+5.0	+0.9	-1.1	+0.9
Standard deviation (mg/l)	21.3	24.4	97.6	126
Coefficient of variation (%)	20.3	4.8	9.8	6.2

a signal-to-noise ratio of 3, was 0.5 mg/l in plasma (mean of three determinations) and 8 mg/l in urine. The limit of quantification (LOQ), defined as the lowest concentration measured with a C.V. less than 20% and a bias less than 10%, was 100 mg/l in urine. In plasma, the lowest quality control (3 mg/l), corresponding to the lowest point on the calibration graph, had a C.V. of only 5.9%. The LOQ was therefore fixed at 3 mg/l, which was sufficient for pharmacokinetic studies with piracetam given at a dose of 800 mg per os, but a lower LOQ could probably be obtained by changing the concentrations of the calibrators.

3.4. Linearity

A χ^2 test was used to assess the linearity of the calibration line, as recommended by the Analytical Methods Committee [9]. The weighted sum of squares of the mean calibration graph ($n = 6$) was 18.98 and 2.60 for the determination of piracetam in plasma and urine, respectively. This value should be compared with the χ^2 value at the 5% level for three degrees of freedom, i.e., 7.81. Therefore, linearity had to be rejected in plasma. However, the weighted sum of squares could also have been overestimated by underestimation of the variances of the calibrators. As no significant bias was found in the quality controls (see Table 1), the calibration graph was considered to be linear in the range 3–40 mg/l. The mean (±S.D.) calibration graph equations, were $y = 1721\ (\pm 19)x - 816\ (\pm 260)$ with $r = 0.9991\ (\pm 0.0005)$ in plasma and $y = 115\ (\pm 3.7)x - 1053\ (\pm 972)$ with $r = 0.9995\ (\pm 0.0005)$ in urine, where y = peak height (mV) and x = concentration (mg/l).

3.5. Specificity

Fifteen plasma samples from hospitalized patients were assayed to search for possible interferences in piracetam determination. These patients received 6–10 drugs each. No interference at the retention time of piracetam was found. Urine from twelve healthy volunteers was also assayed. Many interferences with endogenous compounds were seen when urine was treated in the same way as plasma. Washing urine with hexane–toluene largely reduced these interferences. Further washing of the chloroform–2-propanol extract with 0.5 ml of water or 1 m*M* acetic acid resulted in inconsistent removal of the interferences and a lower extraction yield. Therefore, this approach was not considered further.

3.6. Application to a pharmacokinetic study

Piracetam (Nootropyl) was given to twelve healthy volunteers (800 mg, single dose per os) and blood and urine samples were drawn for 24 h. The mean concentration–time plots are shown in Fig. 3. The mean ± S.D. area under the plasma piracetam curve was 158 ± 47 h mg/l, the maximum concentration was 27.4 ± 5.5 mg/l, the corresponding time was 0.69 ± 0.16 h, the terminal half-life was 6.6 ± 1.7 h and the piracetam recovery in urine was 538 ± 165 mg at 24 h.

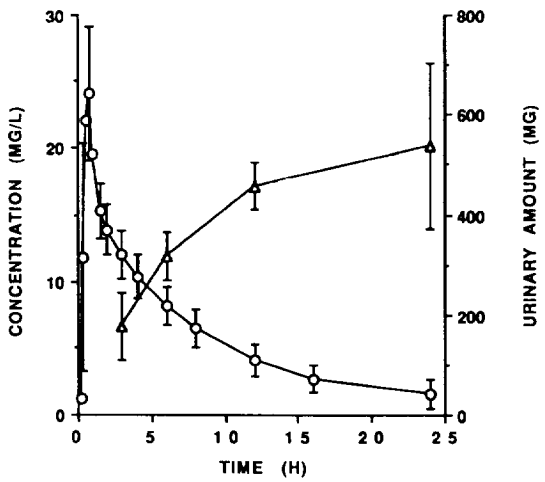

Fig. 3. Mean ± S.D. piracetam plasma concentration (○) and urine cumulative amount (△) versus time after administration of 800 mg per os to twelve healthy volunteers.

4. Conclusions

A simple, rapid and specific assay was found for the determination of piracetam in biological fluids. Compared with other methods, this assay allows the determination of piracetam in urine and does not require gradient elution. It was found suitable for pharmacokinetic studies of piracetam given orally at the usual dosage in humans.

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