Journal of Chromatography, 232 (1982) 203—206
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1359

Note

Determination of 2-oxo-pyrrolidine-1-acetamide (piracetam) in human plasma using high-performance liquid chromatography

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(First received March 9th, 1982; revised manuscript received April 6th, 1982)

The high-performance liquid chromatographic (HPLC) determination of piracetam proved to be desirable in the course of a pharmacokinetic study [1].

Research into the gas chromatographic detection of piracetam can be found in the literature [2, 3]; however, until recently, there was no mention at all of a method for determining piracetam using HPLC [4, 5].

The method described below is distinguished by its simplicity, selectivity and sensitivity. Preliminary results concerning the pharmacokinetics of piracetam in man using this assay are also given.

EXPERIMENTAL

Chemicals

Piracetam was kindly provided by the UCB Company (Batch No. 4070), the methanol for chromatography was supplied by Merck.

Instruments and chromatographic conditions

The analyses were carried out isocratically in an HPLC system consisting of a Kontron LC pump (Model 410), Kontron autosampler (Model MSI 660), Kontron ultraviolet (UV) detector (Model Uvikon 720LC), and a Spectra-Physics integrator (Model SP 4100).

A LiChrosorb RP-18 column (particle size 10 μm, 25 cm × 4.9 mm I.D.) manufactured by Kontron was used. The flow-rate of the methanol saturated with helium was 1 ml/min. A 20-μl volume of the prepared sample solution was used for each injection, piracetam being detected at 206 nm.

The external standard method was chosen for calibration.

Sample preparation

Venous blood was taken (containing no piracetam) and left to stand in a heparinized test tube for 1 h at room temperature and subsequently centrifuged at 3000 *g* for 10 min. The stock solution was obtained by adding 71.0 mg of piracetam to 5 ml of the plasma, mixing for 1 h in a reciprocating impeller agitator (IKA-Vibrax VXR) and then removing samples of 250, 150, 50, 25, and 10 μl from the solution, pouring them into small measuring flasks and filling to 1 ml with pure plasma. (Plasma concentrations: 3.55, 2.13, 0.71, 0.355, and 0.142 mg/ml.) Then 50 μl were taken from each of the five solutions and 1 ml of methanol was added to each 50-μl sample.

After centrifugation (Eppendorf Centrifuge, Model 3200) for 3 × 2 min at 17,000 *g*, the remaining liquid was filtered through a methanol-preconditioned C_{18} Sep-Pak cartridge (Waters Assoc.) and the eluate was collected in a 5-ml measuring flask.

The centrifuged residue was extracted twice with 1 ml of methanol each time and the extracts filtered once again through the C_{18} Sep-Pak cartridge. Finally, the cartridge was rinsed with 1 ml of methanol and the measuring flask was filled to a final volume of 5 ml with methanol.

The patients' plasma samples were processed using the same method.

Each sample was injected four times.

RESULTS

The calibration curve of piracetam from plasma was linear in the concentration range concerned (Table I).

The detection limit of piracetam with plasma components is approximately 0.7 μg/ml, corresponding to approximately 14 ng per injection, and without plasma components approximately 0.3 μg/ml, corresponding to 6 ng per injection.

The typical chromatographic behaviour of piracetam on the LiChrosorb RP-18 column is shown in Fig. 1.

The recovery rate for piracetam in the concentration range 3.55 and 0.142 mg/ml is 99.8% (relative S.D. = 10.5%) (average value from 20 injections).

In contrast to the paper of Daldrup et al. [4], the piracetam detection presented here is distinguished by the fact that neither a gradient system nor expensive acetonitrile need be used. Furthermore, the decisive advantage of our

TABLE I

CALIBRATION CURVE FOR PIRACETAM

Plasma concentration (mg/ml)	Area (arbitrary units)	Relative S.D. (%)
3.55	1.679	2.004
2.13	1.000	1.719
0.71	0.336	1.037
0.355	0.139	2.073
0.142	0.052	5.050

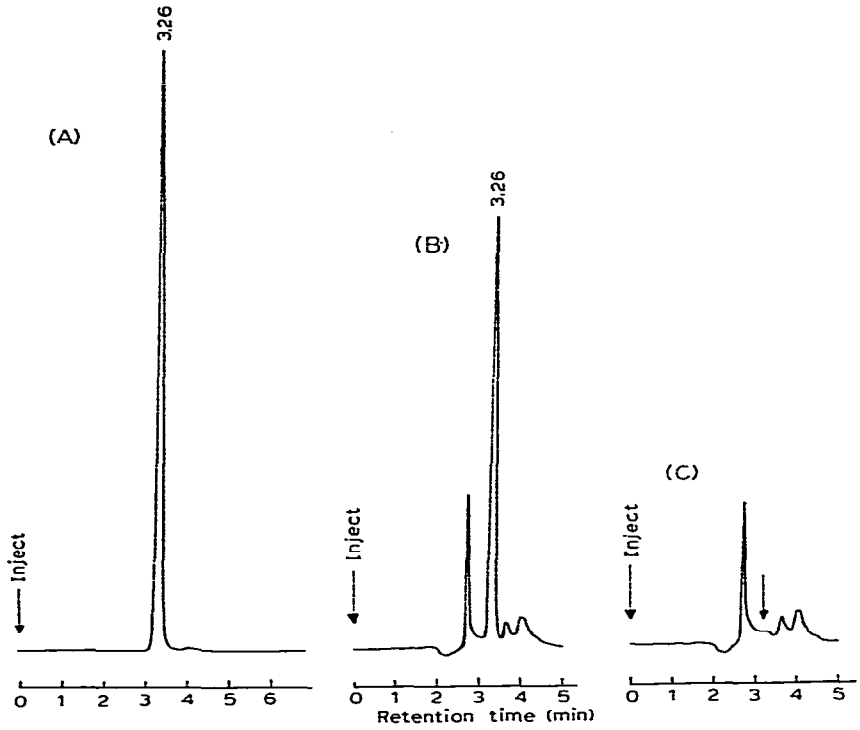

Fig. 1. Chromatograms of piracetam (A) without plasma components, (B) with plasma components, (C) blank plasma. For chromatographic conditions see text.

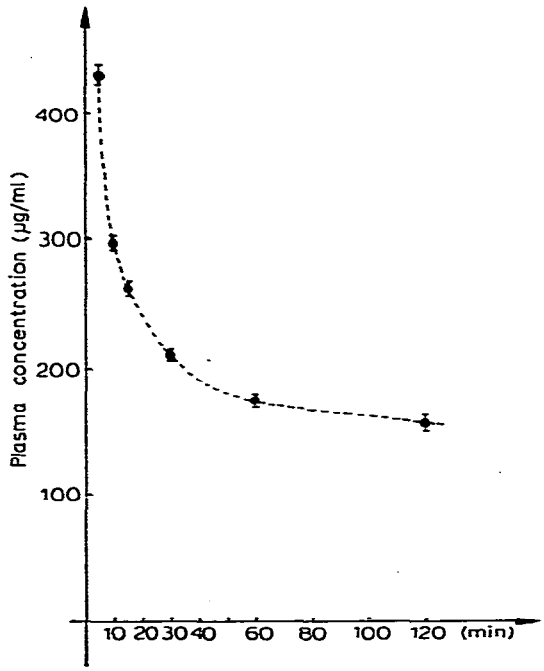

Fig. 2. Profile of piracetam plasma concentration versus time after an intravenous injection of 6 g. Each point is the mean of the plasma concentration of three geriatric patients.

method is that UV detection of piracetam at 206 nm can be increased by a factor of approximately four in comparison to that at a wavelength of 220 nm.

The time course of the plasma concentration of piracetam after a single intravenous injection in each of three geriatric patients with coronary heart disease and cardiac insufficiency is shown in Fig. 2.

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