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Short Communication

Rapid method for the sensitive determination of Piracetam in plasma by high-performance liquid chromatography

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Introduction

Piracetam (2-oxo-1-pyrrolidineacetamide; Fig. 1) is a CNS-active substance, which was developed from GABA, an endogen active neurotransmitter substance. Piracetam influences interhemispherical exchange and protects the polyribosomal organism against oxygen-deficit states.

Various analytical approaches based on gas chromatography [1] and high-performance liquid chromatography (HPLC) [2, 3] have been published; generally they lack sensitivity.

This paper describes a new and reliable HPLC method for the analysis of Piracetam, especially applicable to routine work. The analyses are performed by direct injection of plasma samples after deproteinization and employs UV-detection at 200 nm.

Experimental

Reagents, chemicals and standards

All reagents used were of analytical grade or better and were purchased from E. Merck (Darmstadt, FRG). All the solvents used were purified in the authors' laboratory.

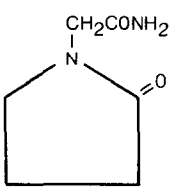

Figure 1
Chemical structure of Piracetam.

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Stock solutions (200 mg/20 ml) of Piracetam were prepared in 20% methanolic solution and kept at +4°C. Standard solutions for determination of the linearity of detector response and in the spiking experiments were then prepared from this stock by dilution with water. Blank plasma samples (10 ml) were spiked with Piracetam in the concentration range of 1–64 μg ml^{-1} suitable for single-dose pharmacokinetic studies.

Instruments and equipment

An isocratic HPLC system consisting of the following components was used: a HP 1090 liquid chromatograph with Diode Array Detector, 25-μl sample loop and a HP Chem Station (Hewlett–Packard, Palo Alto, CA).

Chromatographic system

Piracetam was separated on a stainless steel column filled with Spherisorb S5 NH_2 125 × 4 mm, i.d. (SRD, Vienna, Austria) using a mobile phase consisting of 0.02 M perchloric acid.

Preparation of plasma samples

Prior to analysis, plasma samples were stored at −30°C. After thawing, 1.0 ml of plasma (patient, blank or spiked plasma) was mixed with 0.15 ml 20% perchloric acid for deproteinization and vortexed for 20 s. After centrifugation at approx. 3000 ***g*** for 4 min the supernatants were used for analysis.

Results and Discussion

Chromatographic separation of Piracetam

Since Piracetam possesses insignificant UV absorption at wavelengths higher than 230 nm, the analysis was performed using a detector wavelength of 200 nm. Using an amino-bonded column and 0.02 M perchloric acid as mobile phase, Piracetam is quickly and efficiently separated from plasma matrix components as shown in Fig. 2. Other chromatographic systems using C18-columns and different aqueous mobile phases give rise to inferior separation of Piracetam from endogen substances. When columns filled with, for example, Polygosil C18 or RoSil ODS3 were used, no separation from endogen substances could be achieved. For plasma levels of about 100 μg Piracetam ml^{-1} an eluent containing 0.02 M perchloric acid gave the best peak shape and least interferences when the plasma supernatant is injected. Due to the high hydrophilic non-ionic character of Piracetam, pre-column enrichment or extraction was not possible. Therefore, the direct injection of deproteinized plasma samples was chosen for the assay. Plasma deproteinization with perchloric acid is not accompanied by a significant loss of Piracetam from the supernatant.

Calibration curves

Standard curves were prepared over the concentration range 1–64 μg ml^{-1}. Data from a typical calibration curve are shown in Table 1. The calibration data from five standard curves for Piracetam fit linear equations with correlation coefficients ranging from 0.99995 to 0.99998. The relative standard deviation (RSD) of the slopes of the five calibration lines ranged from 0.34 to 0.45%. Based on a signal-to-noise ratio of 2:1 the limit of detection was found to be 0.08–0.20 μg ml^{-1} calculated from the calibration curves, but in practice a determination limit of 0.4 μg ml^{-1} was chosen.

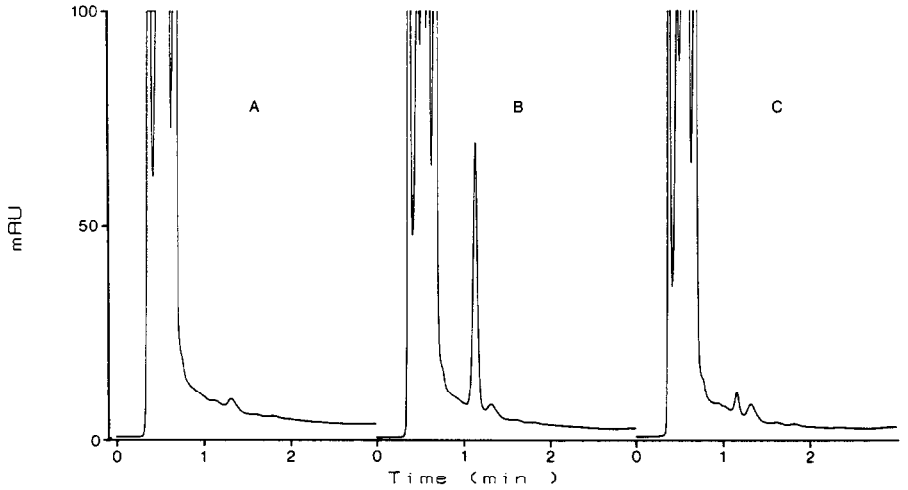

Figure 2
Chromatographic determination of Piracetam after direct injection of deproteinized plasma samples. Conditions: Spherisorb S5 NH_2, 125 × 4 mm, i.d. Eluent: 0.02 M perchloric acid. Flow rate: 2.0 ml min^{-1}; UV detection at 200 nm. (A) "pre-dose" patient plasma; (B) 45 min after a single dose application; retention time 1.2 min (plasma level 19.5 μg ml^{-1}); (C) 15 h after a single dose application (1.3 μg ml^{-1}).

Table 1
Typical standard curves for Piracetam in plasma: linear regression of the spiked plasma concentration (X) versus the peak area (Y); number of replicates (n) = 3; mean values ± standard deviation

Intercept	Slope	R
−1.4750 ± 0.4313	15.0843 ± 0.0522	1.000

Table 2
Day-to-day reproducibility and accuracy of the determination of Piracetam in spiked human plasma

Spiked value (μg ml^{-1})	n	Assay value (μg ml^{-1}) Mean value ± SD	RSD (%)	Accuracy (%)
Day-to-day	5			
1		0.953 ± 0.063	6.62	95.3
2		1.961 ± 0.068	3.48	98.0
4		3.985 ± 0.037	0.93	99.6
8		7.949 ± 0.114	1.44	99.4
16		15.900 ± 0.134	0.84	99.4
32		32.014 ± 0.658	2.05	100.0
64		64.380 ± 0.569	0.88	100.6
Within day	3			
1		1.025 ± 0.039	3.89	102.5
2		2.027 ± 0.009	0.45	101.4
4		4.029 ± 0.016	0.39	100.7
8		7.949 ± 0.014	0.18	99.4
16		16.007 ± 0.047	0.29	100.0
32		32.766 ± 0.126	0.39	102.4
64		65.055 ± 0.063	0.10	101.6

Table 3
Recovery of the drug for plasma deproteinization

Spiked value ($\mu g\ ml^{-1}$)	Peak area		
	Water	Plasma	Recovery (%)
2	28.75 ± 1.16	26.98 ± 0.21	94
10	142.19 ± 4.66	133.72 ± 3.27	94
30	400.14 ± 14.79	417.58 ± 3.35	104

Day-to-day precision

Plasma samples containing Piracetam at seven different levels of concentration were analysed on five consecutive days. The concentration of Piracetam ranged from 1 to 64 $\mu g\ ml^{-1}$. The mean accuracy [(conc. found/conc. added) × 100%] was 98.6% (RSD 1.78%). More detailed results are presented in Table 2. The recovery of the drug from the plasma using deproteinization with perchloric acid was specified at three different concentrations and was compared to water samples (Table 3).

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