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Short communication

Determination of piracetam in human plasma by capillary electrophoresis

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Abstract

A capillary electrophoresis (CE) procedure has been developed for the determination of piracetam in human plasma. Analyses were performed on an uncoated silica capillary using borax buffer modified with the addition of α-cyclodextrin. The detection was UV, operated at 200 nm. The detection limit of the authentic samples was 1 μg/ml. The calibration curve was linear over a range of 4 to 24 μg/ml (r=0.997). Inter-assay R.S.D. was below 9.3%. The described method has been successfully applied to the quantitative determination of piracetam in human plasma and should be useful for clinical and bioavailability investigations.

Keywords: Piracetam

1. Introduction

Piracetam (2-oxopyrrolidine-1-acetamide) (Fig. 1) is a nootropic drug used to treat memory impairment, alcoholism, senile dementia and Alzheimer's disease. Several chromatographic methods, including GC [1–3] and HPLC [4–7] have been applied in order to determine piracetam in human serum or plasma, and thus provide useful pharmacokinetic data. Some of the earlier published methods [2,4] are not specific enough because of strong interferences from the biological matrix. Moreover, GC methods require an extensive extraction procedure and programmed temperature gradient. This causes drift of the base line and subsequently produces a difficulties with quantification. On the other hand, application of HPLC methods are strongly limited by UV-absorption properties of piracetam, which requires a short wavelength detection (approx. 210 nm), in which most of the commonly used organic modifiers of the mobile phase absorbs UV light as well. Therefore, an capillary electrophoresis method seems to be worth considering.

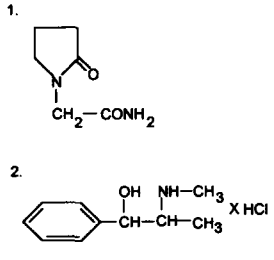

Fig. 1. Structures of piracetam (1) and ephedrine hydrochloride (2).

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2. Experimental

2.1. Reagents

Piracetam was provided by UCB (Kerpen, Germany), ephedrine hydrochloride by Pharmaceutical Enterprise "Polfa" (Starogard, Poland), acetonitrile was supplied by Merck (Darmstadt, Germany), α-cyclodextrin was purchased from Sigma (St. Louis, MO, USA) and sodium tetraborate decahydrate from POCh (Gliwice, Poland). Acetonitrile was purified by double distillation from above anhydrous $CaCl_2$. All aqueous solutions were prepared in triple distilled water and were filtered through 1.5-μm membrane prior to use.

2.2. Apparatus

Analyses were performed with a Beckman P/ACE 2100 electrophoresis apparatus. The resultant electropherograms were monitored at 200 nm, with a fixed-wavelength detector, using Beckman System Gold. The voltage was maintained at 25 kV, which gave a current of 16 μA. All experiments were performed at 25°C, using an unmodified silica capillary 58 cm (separation distance 51 cm)×50 μm I.D.. The buffer solution was composed of 10 m*M* borax (pH 9.36) with the addition 40 m*M* of α-cyclodextrin. Analytes were introduced into the capillary at the anode via a 7 s (0.5 p.s.i. (1 p.s.i.=6894.76 Pa)) argon pressure injection, whereas the detector was set on the cathode end of the capillary. Prior to use, the capillary was rinsed with 0.1 *M* sodium hydroxide for 20 min in order to rehydrate the fused-silica material on the internal capillary wall, then with distilled water for 10 min and finally with buffer solution for 10 min. Between analyses, the capillary was regenerated by treatment with 0.1 *M* hydrochloric acid (0.5 min), for removing adsorbed plasma components, then with 0.1 *M* sodium hydroxide (2 min) and finally with distilled water (2 min). Prior to each run, the capillary was flushed with running buffer for 2 min. Stock solutions of standards were prepared in buffer solution (10 m*M* borax) at a concentration of 1 mg/ml. From these solutions, appropriate injection solutions were prepared by mixing the required volume of the stock solution with water.

2.3. Sample preparation

The plasma samples (0.5 ml) were transferred to 10-ml centrifuge tubes and spiked with 15 μl of ephedrine hydrochloride (internal standard) at a concentration 1 mg/ml. Subsequently, the samples were deproteinized with 3 ml of acetonitrile. The samples were agitated on a rotary mixer for 10 min and centrifuged at 2000 *g* for 10 min. The supernatant (acetonitrile phase) was transferred to a clean test tube and evaporated to dryness. The residue was suspended in 0.5 ml of buffer solution, transferred into a 1.5 ml microfuge tube and centrifuged at 2000 *g* for 5 min. The supernatant was stored at −16°C until needed.

2.4. Calibration procedure

Using the standard solutions of piracetam and ephedrine hydrochloride, samples of blank control plasma (0.5 ml) were spiked with both compounds at concentrations ranged from 4 to 24 μg/ml for piracetam and with a fixed concentration of internal standard (30 μg/ml). All the samples were prepared and analysed using the same procedures which were described in Section 2.3 above. Calibration curve based on the peak-height ratios of piracetam to I.S. was constructed using six different concentrations of piracetam analysed six times for each concentration. The data were subjected to linear-regression analysis in order to achieve the appropriate calibration factors.

2.5. Calculations

Ultimate sample concentrations were calculated by determining the peak-height ratios of piracetam related to the I.S. and comparing these ratios with the standard curves obtained after analysis of the calibration samples.

3. Results and discussion

Fig. 2a–c show electropherograms of a standard mixture, plasma blank and plasma sample, respectively. As can be seen, in each case, it was possible to achieve base line separation between piracetam

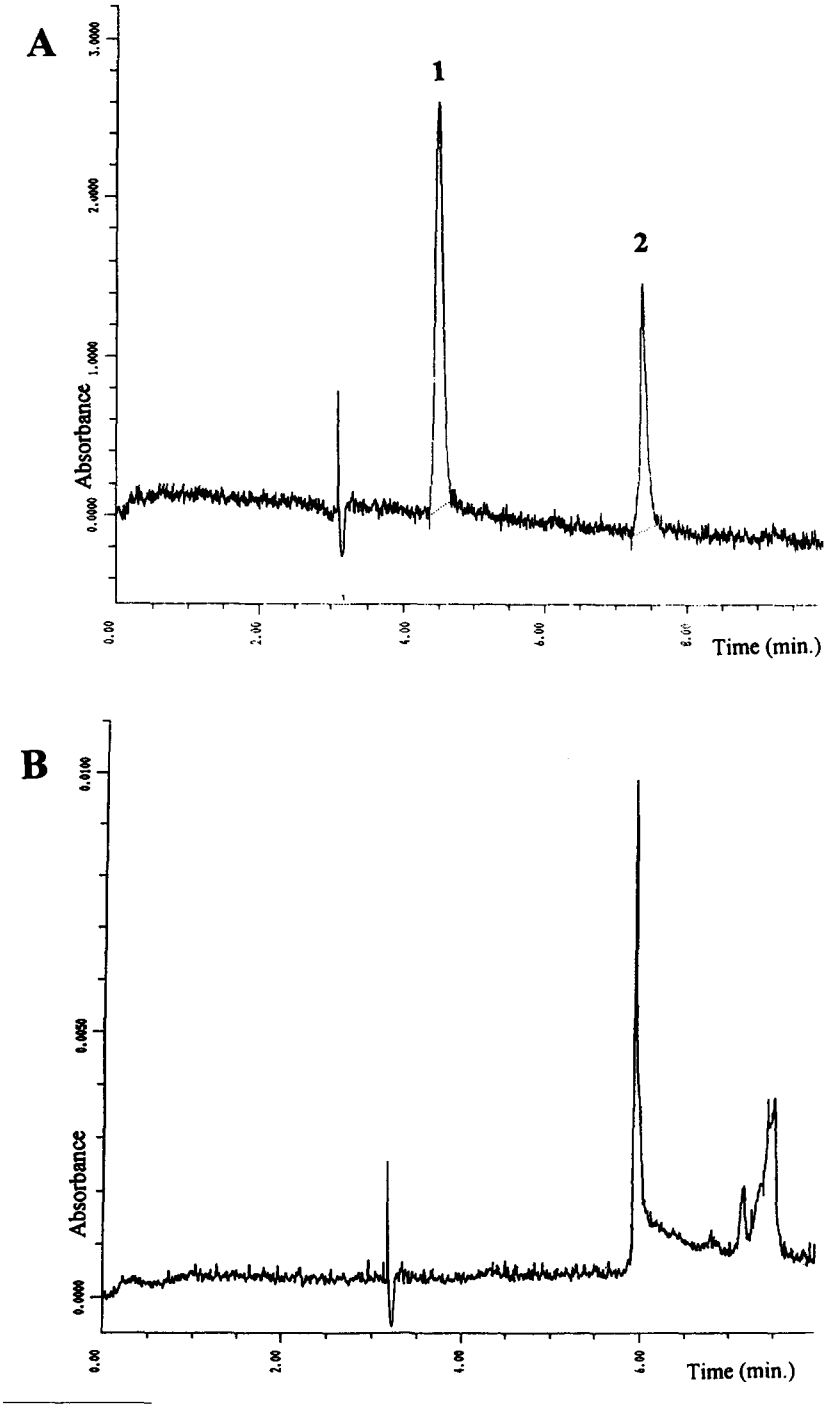

Fig. 2. (A) Electropherogram of a standard solution containing (1) ephedrine hydrochloride (internal standard) at 100 μg/ml and (2) piracetam at 16 μg/ml. (B) Electropherogram of drug-free plasma. (C) Electropherogram of a plasma containing (1) ephedrine hydrochloride at 30 μg/ml and (2) piracetam at 16 μg/ml. Conditions: applied voltage 25 kV; 7 s pneumatic injection; unmodified silica capillary (57 cm×50 μm I.D.) at 25°C; buffer solution 40 m*M* α-cyclodextrin in 10 m*M* sodium tetraborate decahydrate; UV detection at 200 nm.

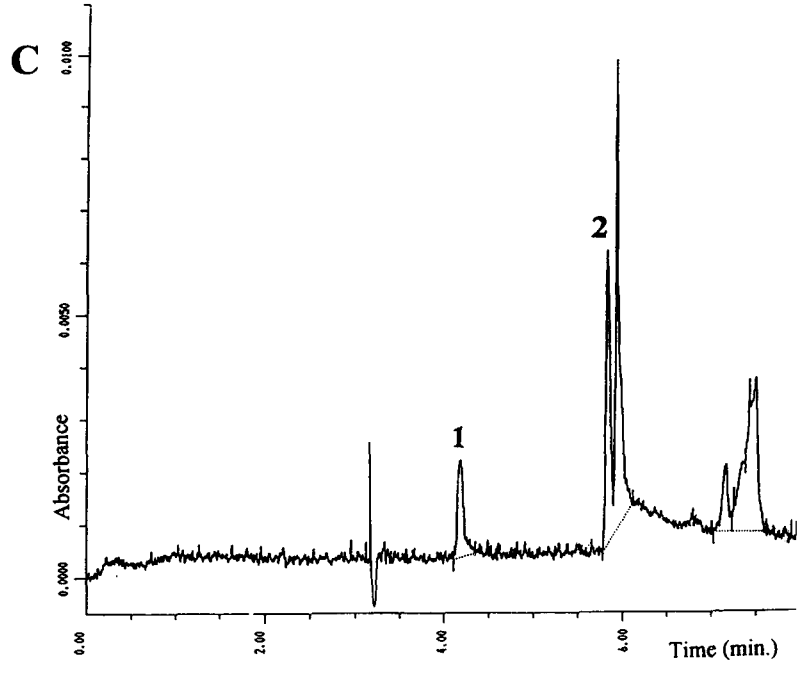

Fig. 2. (*continued*)

and internal standard in a relatively short migration time. The electroosmotic flow-time (t_{eo}) was 3.6 min and the migration time of the internal standard was 4.2 min. The addition of cyclodextrin allows for the separation of the piracetam peak from plasma impurities. Presumably, in this case an inclusion complex of piracetam with cyclodextrin is formed, which has shorter migration time than uncomplexed impurities. Furthermore, the influence of different concentrations of α-cyclodextrin (10–40 m*M*) on the electrophoretic separation was studied. The best results were obtained using a buffer solution composed of 10 m*M* borax (pH 9.36) modified by the addition of 40 m*M* α-cyclodextrin.

Linearity of the standard curves with accuracy below 10% was found in the range from 4 to 24 μg/ml. The mean correlation coefficient was 0.997. The limit of detection of piracetam, which gave a peak three times that of the baseline noise, was 1 μg/ml, whereas quantitative limit was 2 μg/ml. Precision of the assay calculated as a relative standard deviation for intra-assay variability ranged from 7.9% for 24 μg/ml to 10.2% for 4 μg/ml. The numerical data are displayed in Tables 1 and 2.

Table 1
Numerical data for calibration graph for piracetam using the CE method with ephedrine hydrochloride as internal standard

Concentration of piracetam (x) (μg/ml)	Peak-height ratio (y)
4	0.979
8	1.866
12	2.373
16	3.148
20	3.848
24	4.774

Regression equation: $y=0.140(\pm0.09)+0.191(\pm0.006)x$, $r=0.9974$.

Table 2
Intra-assay precision for the CE method

Amount added (μg/ml)	Amount found (mean±S.D.)[a] (μg/ml)	R.S.D.[a] (%)
4	4.398±0.4	10.2
8	9.056±0.9	9.7
12	11.714±1.2	9.9
16	15.781±1.5	9.5
20	19.454±1.7	8.6
24	24.399±1.9	7.9

[a] $n=6$.

This method was tested in a limited bioavailability study on two groups of 10 healthy volunteers, aged 18–26 years and not taking concurrent medication. The concentration–time data course of piracetam in plasma after a single oral administration of 400 mg capsule of the drug was found to be in almost the same therapeutic range as those suggested by other investigators [5–7]. The basic pharmacokinetic parameters were as follow; $t_{max}=1.5$ h, $C_{max}=9.63$ μg/ml and AUC=103.92 mg/l per h.

The main advantages of the application of CE for the determination of piracetam are short analysis time, low cost of the reagents used as the mobile phase and a simple procedure for sample preparation. Moreover, because water buffer is used as a mobile phase, it is possible to use short-wavelength UV detection.

The described method is specific, precise, sensitive and accurate enough for therapeutic drug monitoring and pharmacokinetic studies.

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