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# Rapid determination of piracetam in human plasma and cerebrospinal fluid by micellar electrokinetic chromatography with sample direct injection

Hsin-Hua Yeh [a,d], Yuan-Han Yang [b], Ju-Yun Ko [c], Su-Hwei Chen [a,d,*]

[a] *Graduate Institute of Pharmaceutical Sciences, Kaohsiung Medical University, Kaohsiung 807, Taiwan*
[b] *Department of Neurology, Kaohsiung Medical University, Chung-Ho Memorial Hospital, Kaohsiung, Taiwan*
[c] *School of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*
[d] *Department of Pharmacy, Kaohsiung Medical University, Chung-Ho Memorial Hospital, Kaohsiung, Taiwan*

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**Abstract**

A simple micellar electrokinetic chromatography (MEKC) method with UV detection at 200 nm for analysis of piracetam in plasma and in cerebrospinal fluid (CSF) by direct injection without any sample pretreatment is described. The separation of piracetam from biological matrix was performed at 25 °C using a background electrolyte consisting of Tris buffer with sodium dodecyl sulfate (SDS) as the electrolyte solution. Several parameters affecting the separation of the drug from biological matrix were studied, including the pH and concentrations of the Tris buffer and SDS. Under optimal MEKC condition, good separation with high efficiency and short analyses time is achieved. Using imidazole as an internal standard (IS), the linear ranges of the method for the determination of piracetam in plasma and in CSF were all between 5 and 500 μg/mL; the detection limit of the drug in plasma and in CSF (signal-to-noise ratio = 3; injection 0.5 psi, 5 s) was 1.0 μg/mL. The applicability of the proposed method for determination of piracetam in plasma and CSF collected after intravenous administration of 3 g piracetam every 6 h and oral administration 1.2 g every 6 h in encephalopathy patients with aphasia was demonstrated.


*Keywords:* Piracetam; MEKC-direct injection; Plasma and cerebrospinal fluid

## 1. Introduction

Piracetam(2-oxopyrrolidine-1-acetamide) (Fig. 1) a low molecular weight derivative of γ-aminobutyric acid (GABA), is a nootropic agent that improves cognitive functions such as learning and memory. In general, it shows no affinity for most important central receptors, but it is able to modulate the action of most central neurotransmitters [1–5]. It has been shown to increase the fluidity of neuronal membrane and protect the nerve cell. Piracetam exerts its effects not only at neuronal level but also on the microcirculation through effects on red cell deformability and decreases platelet aggregation and capillary vasospasm [6]. These properties lead to decreased plasma and whole blood viscosity, to improvement in compromised cerebral blood flow, and increase cerebral perfusion. It has shown improvement in motor function, level of consciousness [7–10], and has confirmed the beneficial effect in aphasia [11–14]. Moreover, clinical insights have shown that long-term and high dose treatment with piracetam may slow down the progression of several clinical features of Alzheimer's disease [15]. Consequently the determination of piracetam in biological fluids is required for determination of its pharmacokinetic parameters.

Several chromatographic methods are available for the determination of piracetam including gas chromatography (GC) [16] and high performance liquid chromatographic (HPLC) methods [17–20]. Capillary electrophoresis [21] for the determination of piracetam in biological samples has been published and thus provides useful pharmacokinetic data. These analytical methods developed for determining piracetam in biological fluids involve various pretreatment procedures prior to analysis.

* Corresponding author at: School of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan. Tel.: +886 7 3121101x2253; fax: +886 7 3210683.
*E-mail address:* suhwch@kmu.edu.tw (S.-H. Chen).

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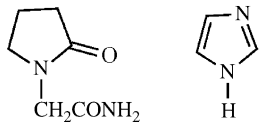


Piracetam Imidazole

Fig. 1. Chemical structures of piracetam and imidazole (IS).

These pretreatment procedures include solid phase extraction, liquid–liquid extraction and deproteination by organic solvents. These analytical methods including the CE method [21] require a more complicated procedure and are time-consuming for sample cleanup and the measurements of protein-bound and unbound piracetam in biological samples. However, the free or unbound drug, is in equilibrium with the receptor site and is the pharmacologically active moiety.

Capillary electrophoresis (CE) is a powerful separation technique for the determination of ionic and neutral components. With the advantage of a small sample size and running buffer needed, the CE method has became the major analytical tool to determine chemicals, especially in biological samples such as CSF. So far, no CE method with direct injection without any sample pretreatment for determination of unbound piracetam in human plasma and in CSF has been reported.

In this paper, we demonstrate a simple, rapid, accurate MEKC method with direct sample injection for determination of unbound piracetam, pharmacologically active moiety, in plasma and in CSF. Application of the proposed method to analyze the piracetam in patients with aphasia was evaluated and it proved to be satisfactory.

## 2. Experimental

### 2.1. Instrumentation

The Beckman P/ACE MDQ system (Fullerton, CA, USA) equipped with UV detection and a liquid-cooling device was used. MEKC was performed in an uncoated fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 40.2 cm (effective length 30 cm) × 50 μm I.D. The temperature of the separation was controlled at 25 °C by immersion of the capillary in a cooling liquid circulating in the cartridge. The temperature of sample tray was at room temperature. Detection was carried out by the on-column measurement of UV absorption at 200 nm (cathode at the detection side). The plasma and CSF samples were injected by pressure (0.5 psi) for 5 s (about 7.41 nL), keeping the separation voltage at 11 kV (anode at the injection end). The Beckman P/ACE MDQ Microsoft Software system was used for data processing.

### 2.2. Chemicals

Piracetam(2-oxopyrrolidine-1-acetamide) was kindly provided by UCB Pharmaceutical Sector (Belgium). Imidazole (internal standard, IS), sodium hydroxide (NaOH), tris(hydroxymethyl)-aminomethane (Tris), sodium dodecyl sulfate (SDS) and phosphoric acid ($H_3PO_4$, 85%) were supplied by Merck (Darmstadt, Germany). Other agents were of analytical-reagent grade. Milli-Q (Millipore, Bedford, MA, USA) treated water was used for the preparation of buffer and related drugs.

### 2.3. Preparation of background electrolyte solutions

The stock solution of 60 mM Tris buffer was prepared by dissolving 0.7269 g of Tris in 100 mL volumetric flask with 50 mL deionized water and diluting to volume. Solutions of various Tris buffers at different pH levels were prepared by neutralizing Tris solution with $H_3PO_4$. Solutions of Tris–SDS buffer at various levels of SDS were obtained by dissolving different amounts of SDS in Tris (30 mM, pH 9.5) followed by dilution with Tris buffer (30 mM, pH 9.5) as a background electrolyte. The final electrolyte solutions containing Tris buffer (30 mM, pH 9.5) with 280 mM SDS were used for plasma and CSF samples analysis. Capillary conditioning before startup was undertaken with methanol for 10 min, 1 M HCl solution for 10 min, deionized water for 2 min, 1 M NaOH for 10 min and deionized water for 2 min. The conditioning between runs was rinsed with 1 M NaOH (3 min), deionized water (2 min), and background electrolyte solution (5 min) under positive pressure applied at the injection end. The buffer was renewed after every two runs for good reproducibility.

### 2.4. Sample preparations and method validation

Drug-free human plasma samples were obtained from five normal volunteers (22–24 years old) used as controls. However, due to the limited supply of human CSF, simulated CSF was prepared for the standard curve and optimized MEKC conditions studied. Simulated CSF was prepared by mixing human serum with the isotonic 20 mM ammonium phosphate buffer at a ratio of one part serum to 99 parts buffer, so the protein and salt content would be comparable to human CSF [22]. A 180 μL aliquot of human plasma or simulated CSF was pipetted into a 1.5 mL Eppendorf vial, and 20 μL of aqueous solution containing various concentrations of piracetam was added to each tube to prepare the final piracetam concentrations in plasma and in simulated CSF samples over the range of 5.0–500.0 μg/mL following the addition of 20 μL 600 μg/mL of imidazole (IS). The samples were mixed for 10 s. An aliquot of 30 μL of spiked drugs in biological samples was transferred to a 0.2 mL mini-vial that could be placed into the sample tray (at room temperature) in a Beckman P/ACE MDQ system for CE analyses. The calibration graphs of piracetam in biological matrix were established with the peak area ratio of piracetam to imidazole (IS) as ordinate ($y$) versus the concentration of piracetam in μg/mL as abscissa ($x$). The precision and accuracy of piracetam assay in biological matrix was tested by analyzing three concentration levels at 50, 300 and 500 μg/mL for plasma and for simulated CSF. The precision and accuracy of the method were estimated from back-calculated standard concentration. The intra-day of mean precision was defined by relative standard deviation (RSD) and relative error (RE) from analyses on the same days. The inter-day precision and accuracy were calculated from repeated analyses

of identical samples on 5 consecutive days for these concentrations of piracetam and expressed also as RSD and RE. The limit of quantitation (LOQ) is the minimum injected amount that gives precise measurements. The LOQs in plasma and CSF were defined as the sample concentrations generating a peak height ten times the level of the baseline noise. The limits of detection (LOD) were calculated on the basis of the baseline noise, which was defined as the sample concentration generating a peak of height three times the level of the baseline noise (signal-to-noise ratio of 3).

### 2.5. Application

One encephalitis patient with aphasia received intravenous infusion of 3 g piracetam (Nootropil®, UCB) every 6 h, another patient with aphasia received an oral dosage of 1.2 g piracetam (Nootropil®, UCB) every 6 h at the Department of Neurology intensive care ward; venous blood sample and CSF were withdrawn at 6 h after dosing and plasma fraction was separated immediately. Plasma and CSF samples were stored at −40 °C until analysis.

## 3. Results and discussion

A large number of individual compounds are present in body fluids, leading to difficulty in resolving the analytes of interest from the complicated matrix and the presence of components that can modify the chromatographic column. Therefore, the majority of bioanalytical methods have to use one or more sample pretreatment steps, which progressively isolate analytes from sample matrix. These techniques suffer from the need of intensive time-consuming clean-up procedures and the concentration step. There has been considerable interest in performing single-step analyses with direct injection of body fluids on-column in CE without any sample pretreatment [23]. This is quite often feasible in CE because the open capillary columns are less prone to irreversible modification by sample matrix components.

### 3.1. Optimization of the separation buffer

In a preliminary test of piracetam in human plasma by capillary zone electrophoresis (CZE) were briefly studied at 11 kV with Tris buffer under various pHs in the absence of surfactants, the interference peaks were observed in our study as shown as Fig. 2A. MEKC is the most general approach because it permits the simultaneous determination of acidic, neutral and basic drugs. Moreover, chemical substances can be separated easily from serum albumin in the MEKC analysis. The drug should be eluted in front of the solubilized serum proteins. In MEKC, two distinct phases—the aqueous and micellar phases—are used. These two phases are established by employing buffer-containing surfactants that are added above their critical micellar concentrations. Separation efficiency depends on the hydrophobicity of the solutes in MEKC. The very commonly used anionic surfactant, SDS, as a micellar source with Tris buffer was used to study the resolution of the analyzed substance. The SDS at various concentrations in Tris buffer in the pH range 8.0–10.0 was tested to separate the analytes in plasma. After MEKC separation of piracetam in biological matrix using Tris buffer, the eluted compounds were monitored at 200 nm.

Ionic strength or concentrations of buffer have significant effects on solute mobilities, separation efficiency and sensitivity. The retention behavior of piracetam and imidazole (IS) in plasma and in simulated CSF using 280 mM SDS and Tris buffer (pH 9.5) at concentrations within the range of 10–50 mM was studied. MEKC of the piracetam and imidazole (IS) in plasma

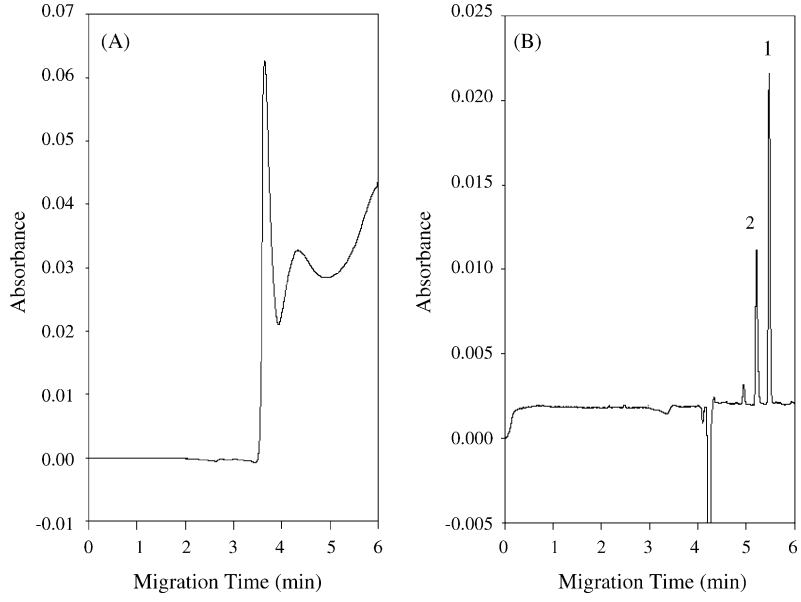


Fig. 2. Electropherograms of analyses of piracetam in spiked plasma by (A) CZE (Tris 30 mM, pH 9.5); (B) MEKC (Tris 30 mM, pH 9.5 + 280 mM SDS). Peaks: 1 and 2 for piracetam and imidazole (IS) at 180 and 60 μg/mL, respectively. CE conditions: applied voltage, 11 kV (detector at cathode side); uncoated fused-silica capillary, 40.2 cm (effective length 30 cm) × 50 μm I.D; sample size, 0.5 psi, 5 s; wavelength, 200 nm.

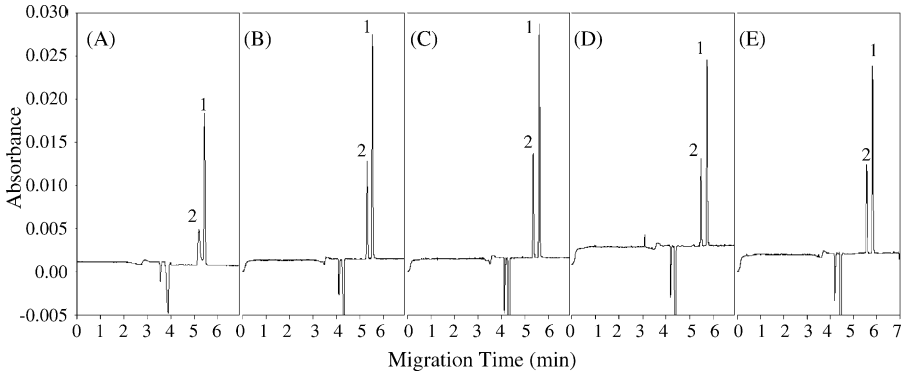


Fig. 3. Effect of Tris concentrations (10–50 mM) in SDS (280 mM) on the migration of piracetam and imidazole at 180 and 60 μg/mL, respectively. Peaks: (1) piracetam and (2) imidazole (IS). (A) 10 mM; (B) 20 mM; (C) 30 mM; (D) 40 mM and (E) 50 mM. Other CE conditions as in Fig. 2.

and in simulated CSF under the 20–30 mM Tris buffer with 280 mM SDS can give good separation efficiency and resolution for piracetam and imidazole from plasma proteins as shown as Fig. 3. The peak shape of the piracetam slightly change at various Tris concentrations, but a significantly broad peak width of imidazole was obtained at concentration of Tris ≤ 10 mM. Comparing concentrations of Tris from 10 to 50 mM on the effect of the sensitivity for piracetam, 20–30 mM of Tris buffers gives a better response than any other. The sensitivity ratios are 0.78, 1.0, 1.0, 0.83 and 0.83 at 10, 20, 30, 40 and 50 mM, respectively. The Tris buffer concentrations for plasma and CSF determination are all set at 30 mM. The effects of SDS concentration range of 150–300 mM SDS in Tris buffer (30 mM; pH 9.5) for plasma matrix and simulated CSF separation of piracetam were studied. Human protein in the biological samples interacted with hydrophobic SDS. This phenomenon made the protein highly soluble in micellar phase and then slow moving in capillary. Therefore, the higher the concentrations of SDS, the less the interference for the tested drug determination in biological matrix. The concentration of SDS effect on migration of the drug in human plasma is shown in Fig. 4. Fig. 4A–E represent 150, 200, 250, 280 and 300 mM of SDS in Tris buffer as a surfactant, respectively, affecting the separation resolution of piracetam from plasma matrix. For plasma, serious interference at 40 mM

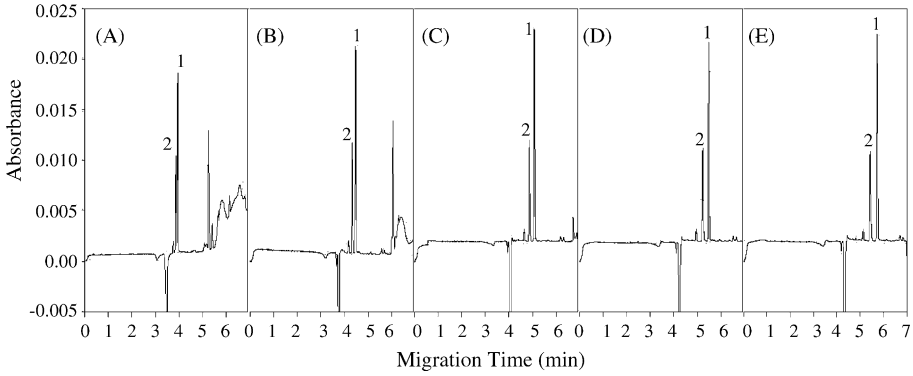


Fig. 4. Effect of SDS concentrations (150–300 mM) in Tris buffer (30 mM, pH 9.5) on the migration of piracetam and imidazole in plasma at 180 and 60 μg/mL, respectively. Peaks: (1) piracetam and (2) imidazole (IS). (A) 150 mM; (B) 200 mM; (C) 250 mM; (D) 280 mM and (E) 300 mM. Other CE conditions as in Fig. 2.

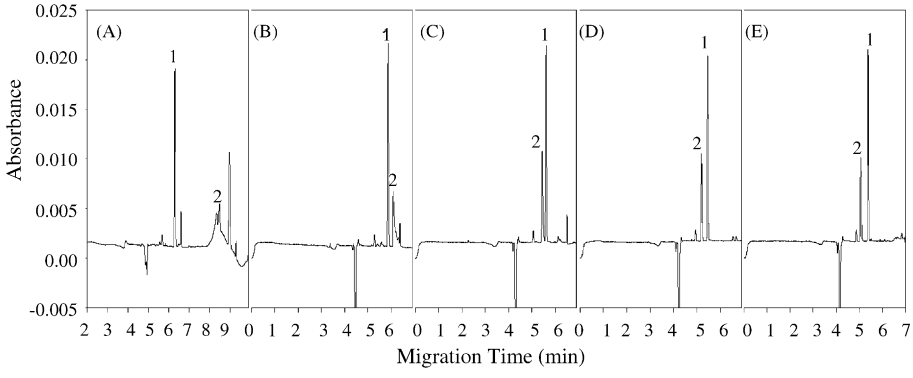


Fig. 5. Effect of pH values of Tris buffer (30 mM) with 280 mM SDS on the migration of piracetam and imidazole in plasma at 180 and 60 μg/mL, respectively. Peaks: (1) piracetam and (2) imidazole (IS). (A) pH 8.0; (B) pH 8.5; (C) pH 9.0; (D) pH 9.5 and (E) pH 10.0. Other CE conditions as in Fig. 2.

SDS and partial overlapping of piracetam with interference were observed at 80 mM SDS (data not shown). When SDS concentration was increased, the peak of interferences slowed down and completely resolved the tested drug from serum protein at 150 mM but partial overlapped with imidazole (IS). With the concentration of SDS ≥ 200 mM in 30 mM Tris buffer (pH 9.5), a base-line resolution of drug from plasma matrix and IS was observed. The resolution values of piracetam with imidazole (IS) are 0.82, 1.41, 1.67, 1.79 and 2.15 at 150, 200, 250, 280 and 300 mM SDS, respectively. The effects of SDS for the drug in simulated CSF were also studied. The same resolution values of piracetam with imidazole (IS) in CSF are obtained. Therefore, a higher concentration of SDS, 280 mM, was selected for further study of analysis of piracetam in plasma and in CSF. The 30 mM Tris buffers with SDS (280 mM) at different pH (8.0, 8.5, 9.0, 9.5 and 10.0) for plasma and CSF were studied. In MEKC, the pH of the buffer is one of the experimental parameters to find the optimum experimental condition for the baseline resolution of the analytes. The dissociation degree of charged/chargeable analytes and the electroosmotic flow are pH dependent. The movement of analyzed compounds is influenced not only by the EOF and interaction with micelle but also by their effective mobility. In this study, piracetam has no significant change at various pH values owing to its structure has neutral property. However, the p$K$a$_1$ and p$K$a$_2$ values of imidazole (IS) are 7 and 14.5, respectively, effectively influencing the peak shape and the migration mobility by pH of the background electrolyte. A broader peak of imidazole below pH 8.5 and partial overlapping of piracetam with imidazole were observed at pH 9 as shown as Fig. 5. The pH effects on resolution and migration time of piracetam and imidazole in plasma are shown in Fig. 6. Migration times of piracetam and imidazole (IS) in CSF are the same as in plasma. Therefore, pH 9.5 of Tris buffer was the choice for optimal pH for determination of piracetam. The 30 mM of Tris buffer (pH 9.5) with 280 mM SDS was the optimal choice for buffer concentration, pH and concentration of surfactant for determination of piracetam in plasma and in CSF without any sample pretreatment by MEKC. Different applied voltages, including 8, 10 and 11 kV, were investigated regarding the effectiveness of the separation. A shorter migration time and higher theoretical plate was observed at 11 kV (data not shown); the resulting current was 60 μA. Fig. 7B and A presents the typical electropherograms of MEKC separation of human plasma blank, and piracetam and IS spiked in plasma under optimized conditions. Fig. 8A and B presents the typical electropherograms of MEKC separation of human simulated CSF blank and piracetam and IS spiked in simulated CSF. The migration times of piracetam,

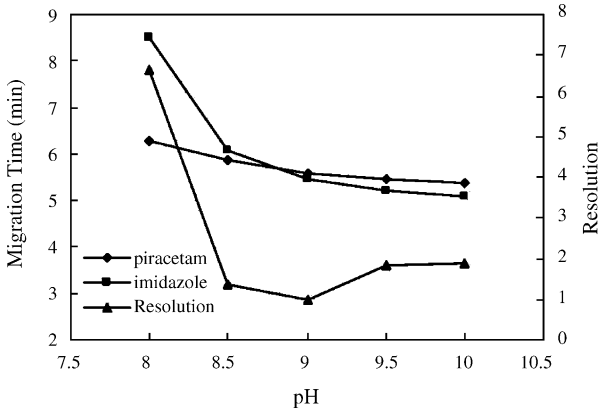


Fig. 6. Effect of pH values on the migration and resolution of piracetam and imidazole (IS). CE conditions as in Fig. 2.

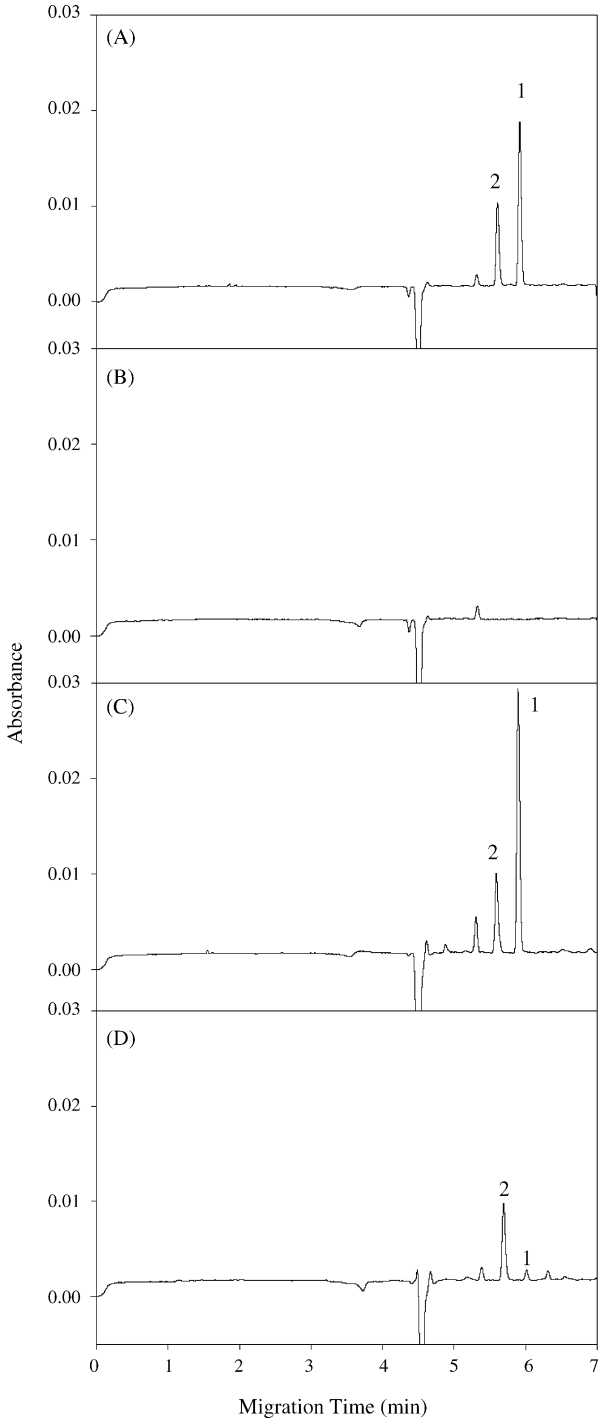


Fig. 7. Electropherograms of piracetam in plasma samples determination. (A) Plasma spiked piracetam and imidazole at 180 and 60 μg/mL; (B) plasma blank, respectively; (C) IV dosing after 6 h later and (D) oral dosing after 6 h later. Peaks: (1) piracetam and (2) imidazole (IS). CE conditions as in Fig. 2.

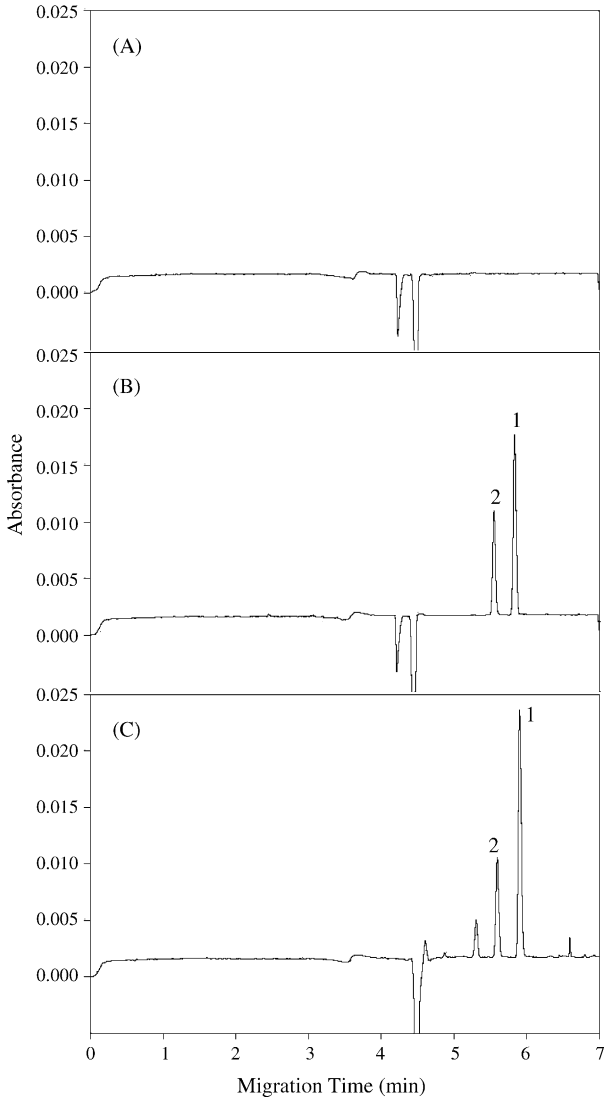


Fig. 8. Electropherograms of piracetam in CSF samples determination. (A) Simulated CSF blank; (B) simulated CSF spiked piracetam and imidazole at 180 and 60 μg/mL, respectively and (C) IV dosing after 6 h later. Peaks: (1) piracetam and (2) imidazole (IS). CE conditions as in Fig. 2.

imidazole and EOF were 5.83, 5.61 and 4.42 min, respectively. The apparent mobility (μA) was calculated according to the equation: $\mu A = \mu E + \mu EOF = (lL/tV)$ where $l$ is the length along the capillary (cm) to detector, $V$ the voltage, $t$ the migration time (s) and $L$ is the total length (cm) of the capillary [24]. The methanol is used for EOF determination. In optimized CE conditions, the apparent mobility values of piracetam, imidazole and EOF are $3.13 \times 10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$, $3.26 \times 10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$ and $4.13 \times 10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$, respectively. According to the equation, $\mu A = \mu E + \mu EOF$, the electrophoretic mobility values (μE) of piracetam and imidazole are $-1.00 \times 10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$ and $-0.87 \times 10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$, respectively. Reproducibility of migration velocity of piracetam and imidazole in simulated CSF was investigated, and observed migration times were $5.83 \pm 0.03$ min and $5.61 \pm 0.03$ min for piracetam and imidazole in CSF, respectively. A high accuracy on migration time for

Table 1
Regression analyses for determination of spiked piracetam in biological samples

| | Regression equation | Correlation coefficient ($r$) |
|---|---|---|
| Plasma | | |
| Intra-day[a] | | |
| 5–500 μg/mL | $Y = (0.0104 \pm 0.0002)X + (0.0304 \pm 0.0181)$ | 0.999 |
| Inter-day[a] | | |
| 5–500 μg/mL | $Y = (0.0106 \pm 0.0002)X + (0.0048 \pm 0.0145)$ | 0.999 |
| CSF[b] | | |
| Intra-day[a] | | |
| 5–500 μg/mL | $Y = (0.0099 \pm 0.0002)X + (0.0057 \pm 0.0139)$ | 0.999 |
| Inter-day[a] | | |
| 5–500 μg/mL | $Y = (0.0100 \pm 0.0002)X + (0.0208 \pm 0.0145)$ | 0.999 |

[a] Intra-day data were based on five replicate analyses and inter-day were based on results from 5 consecutive days.
[b] CSF, cerebrospinal fluid.

piracetam and imidazole ($n = 20$) was found with RSDs of 0.52 and 0.56%, respectively.

### 3.2. Validation of piracetam spiked in biological samples

To evaluate the quantitative applicability of the methods, five different concentrations of piracetam (5, 50, 100, 300 and 500 μg/mL) were analyzed using imidazole (60 μg/mL) as an internal standard. The linear regression equations in biological samples are listed in Table 1. The straight lines obtained from five separate experiments had good correlation coefficients of 0.999. The data indicate high linearity of this method for the intra- and inter-day assays. Repeatability was determined by RSD of the slope of the linear regression equations. For plasma, the RSDs of intra- and inter-day average slope of the equations were all below 1.9%. For CSF, the RSDs of intra- and inter-day average slopes of the equations were all below 2.0%. The precision of the proposed method for spiked samples was studied. The results in Table 2 show that the intra- and inter-day variances at the concentrations were all below 3.4%. The relative recoveries of piracetam were obtained from the calibration graph constructed from plasma spiked with different amounts of piracetam at low, medium and high concentration levels. Table 2 shows all of recoveries were >95%. The LOQ is the minimum injected amount that gives precise measurements. The LOQs in plasma and CSF were all 5 μg/mL and the LODs of the proposed method for piracetam in plasma and in simulated CSF (0.5 psi, 5 s) were found to be 1.0 μg/mL. Effect of storage time on the stability of the sample was also tested. Frozen at −40 °C it was stable for at least 30 days. The selectivity of the proposed method was briefly tested on the separation of piracetam with other structural or pharmacological similar of drugs including levetiracetam, donepezil and rivastigmine. Under present MEKC conditions, a complete separation of piracetam and other nootropic drugs was obtained as shown in Fig. 9. Peaks 1–3 represent piracetam, imidazole and levetiracetam, respectively,

Table 2
Precision and accuracy for the recovery of spiked piracetam in biological samples

| Concentration known (μg/mL) | Concentration found (μg/mL) | RSD (%) | RE (%) |
|---|---|---|---|
| Plasma | | | |
| Intra-day[a] ($n=6$) | | | |
| 50.0 μg/mL | 47.9 ± 0.2 | 0.4 | −4.2 |
| 300.0 μg/mL | 307.8 ± 5.3 | 1.7 | 2.6 |
| 500.0 μg/mL | 492.3 ± 10.6 | 2.2 | −1.5 |
| Inter-day[a] ($n=5$) | | | |
| 50.0 μg/mL | 49.1 ± 0.4 | 0.9 | −1.8 |
| 300.0 μg/mL | 302.4 ± 3.4 | 1.1 | 0.8 |
| 500.0 μg/mL | 498.6 ± 9.2 | 1.8 | −0.3 |
| CSF[b] | | | |
| Intra-day[a] ($n=6$) | | | |
| 50.0 μg/mL | 50.2 ± 1.4 | 2.8 | 0.4 |
| 300.0 μg/mL | 298.8 ± 3.9 | 1.3 | −0.4 |
| 500.0 μg/mL | 503.1 ± 16.4 | 3.3 | 0.6 |
| Inter-day[a] ($n=5$) | | | |
| 50 μg/mL | 49.2 ± 0.6 | 1.2 | −1.6 |
| 300 μg/mL | 300.7 ± 2.9 | 0.9 | 0.2 |
| 500 μg/mL | 497.5 ± 16.8 | 3.4 | −0.5 |

[a] Intra-day data were based on five replicate analyses and inter-day data were from three replications on 5 consecutive days.
[b] CSF, cerebrospinal fluid.

donepezil and rivastigmine were not observed under this MEKC condition.

### 3.3. Application

After one encephalitis with aphasia patient received 3 g piracetam intravenously and another aphasia patient received 1.2 g from oral administration, the plasma and CSF concentrations of piracetam were measured 6 h later by the MEKC method with sample direct injection described above. The electropherograms of plasma after infusion or oral of the drug are shown in Fig. 7C and D, respectively. The concentrations of piracetam in patients' plasma at 6 h were 282.75 and 14.55 μg/mL for IV infusion and oral of the drug, respectively. The electropherograms of CSF after infusing the drug are shown in Fig. 8 C. The concentrations of piracetam in patients' CSF at 6 h were 250.1 μg/mL. The characteristics of the small amount sample volume needed and no necessity for sample pretreatment of this MEKC analytical method for quantitation of piracetam in plasma and in CSF make it very useful in clinical use.

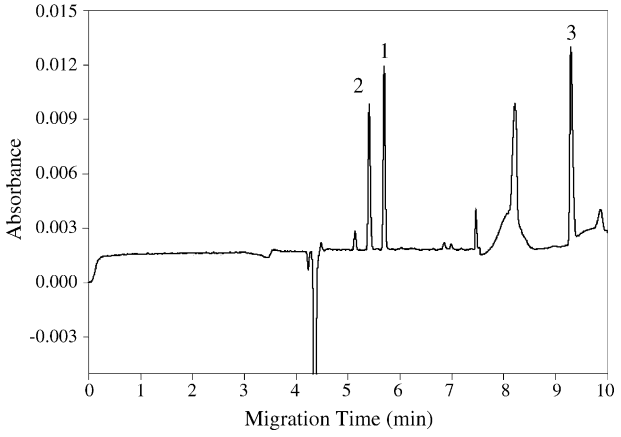


Fig. 9. Electropherogram of the selectivity study in human plasma each at 180 μg/mL. Peaks: (1) piracetam; (2) imidazole (IS) and (3) levetiracetam. CE conditions as in Fig. 2.

## 4. Conclusions

The new MEKC method with sample direct injection without any sample pretreatment for determination of piracetam in plasma and in CSF described here represents a rapid, sensitive, and efficient analytical method. The analytical characteristics of the proposed method are satisfactory for pharmacokinetic and clinical use. Validation of the method for quantitation of piracetam in plasma and in CSF showed that the method has a high accuracy. The piracetam in biological matrix was stable for at least 30 days when frozen at −40 °C. Therefore, the method was suitable for the analysis of piracetam in plasma and CSF collected during pharmacokinetic investigations in humans.

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