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Simultaneous determination of methylephedrine and pseudoephedrine in human urine by CE with electrochemiluminescence detection and its application to pharmacokeinetics

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ABSTRACT: A novel method for the determination of ephedra alkaloids (methylephedrine and pseudoephedrine) was developed by electrophoresis capillary (CE) separation and electrochemiluminesence detection (ECL). The use of ionic liquid (1-butyl-3-methylimidazolium tetrafluoroborate, $BMIMBF_4$) improved the detection sensitivity markedly. The conditions for CE separation, ECL detection and effect of ionic liquid were investigated in detail. The two ephedra alkaloids with very similar structures were well separated and detected under the optimum conditions. The limits of detection (signal-to-noise ratio = 3) in standard solution were 1.8×10^{-8} mol/L for methylephedrine (ME) and 9.2×10^{-9} mol/L for pseudoephedrine (PSE). The limits of quantitation (signal-to-noise ratio = 10) in human urine samples were 2.6×10^{-7} mol/L for ME and 3.6×10^{-7} mol/L for PSE. The recoveries of two alkaloids at three different concentration levels in human urine samples were between 81.7 and 105.0%. The proposed method was successfully applied to the determination of ME and PSE in human urine and the monitoring of pharmacokinetics for PSE. The proposed method has potential in therapeutic drug monitoring and clinical analysis. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: methylephedrine; pseudoephedrine; CE; electrochemiluminescence; pharmacokinetics

Introduction

Ephedra alkaloids, such as methylephedrine (ME) and pseudoephedrine (PSE), which are widely used in traditional Chinese medicine preparations, possess various pharmacological functions and physiological activities, for example, diaphoresis, asthma depression, cough depression, vasoconstriction, excition of nervous centralis, as well as the treatment of nasal and sinus congestion caused by the common cold, sinusitis, hay fever and other respiratory allergies. However, ME and PSE are very similar in structure; their molecular structures are shown in Fig. 1. Increasing concern on the part of both consumers and regulatory agencies as to the safety of ephedra alkaloids has led to the development of several methods to measure ME by GC (Van Eenoo *et al*., 2001) and CE (Phinney *et al*., 2005; Sander *et al*., 2005) or PSE by GC (Lee *et al*., 2006), HPLC (Aljazaf *et al*., 2003; Ishida *et al*., 2008; Karakus *et al*., 2008; Ma *et al*., 2007) and CE (Zhang *et al*., 2007). The rapid and sensitive determination and pharmacokinetics study of ME and PSE are important in biochemical and clinical analysis.

CE is a modern analytical technique that has attracted significant interest due to its short analysis time, high separation efficiency, small sample size and minimal solvent consumption (Kraly *et al*., 2006; Baena *et al*., 2005). Recently, CE has been combined with chemiluminescence (CL) because of CL's high detection sensitivity,

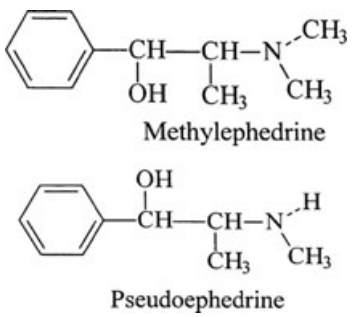

Figure 1. Molecular structures of the methylephedrine and pseudoephedrine.

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Abbreviations used: CL, chemiluminescence; ECL, electrochemiluminesence detection; IL, ionic liquid; ME, methylephedrine; PSE, pseudoephedrine.

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simple optical structure and inexpensive apparatus and used for the analysis of various analytes, such as metal ions (Ren and Huang, 2001), catecholamines (Liu *et al*., 2007), antioxidants (Tsukagoshi *et al*., 2007), amino acids (Zhao *et al*., 2005), proteins (Zhi *et al*., 2007) and antigen–antibody complexes (Wang and Ren, 2005; Liu *et al*., 2008a). Electrochemiluminescence (ECL) is a special type of CL in that the luminescence reagent is initiated by electrochemical reactions. ECL detection based on tris-(2,2′-bipyridyl)-ruthenium [$Ru(bpy)_3^{2+}$], due to its high sensitivity and selectivity, has received considerable attention (Tokel and Bard, 1972; Yin *et al*., 2004; Miao, 2008). CE coupled with ECL specially for $Ru(bpy)_3^{2+}$ system have been successfully demonstrated in analysis of drugs (Hsieh and Whang, 2006; Huang *et al*., 2007; Liu *et al*., 2008b), amino acids (Li *et al*., 2006) and proteins (Li *et al*., 2007).

Ionic liquids (ILs) are a new class of nonmolecular ionic solvents with low melting points (<100°C) at room temperature (Anderson *et al*., 2006). Compared with conventional organic solvents, ILs possess several attractive properties, for example, a wide electrochemical window, nonvolatility, good conductivity and high viscosity, high chemical and thermal stability (Zhang *et al*., 2003; Qi *et al*., 2004). As separation media, ILs have made significant contributions in recent decades in advancing research in HPLC (Laremore *et al*., 2007; Berthod *et al*., 2005), GC (Ding *et al*., 2004) and CE (Lewis *et al*., 2005; Bao *et al*., 2008; Qin *et al*., 2003).

In this work, a new method for the simultaneous determination of ME and PSE was developed utilizing CE-ECL with IL. The conditions for the CE separation, ECL detection and the effect of IL were examined. The applicability of the proposed method was illustrated in the determination of ME and PSE in human urine samples and the monitoring of pharmacokinetics for PSE in human body.

Materials and Methods

Materials and Reagents

ME and PSE were purchased from National Institute for The Control of Pharmaceutical and Biological Products (Beijing, China). PSE sustained release capsules were acquired from Zhongmei Tianjin Shike Pharmaceutical Co. Ltd (Tianjin, China). Tris(2,2′-bipyridyl) ruthenium (II) chloride hexahydrate was purchased from Alfa Aesar (A Johnson Matthey Company, Ward Hill, MA, USA). $BMIMBF_4$ was purchased from Wako (Wako Pure Chemical Industries Ltd, Japan). All chemicals and reagents were of analytical grade and used without further purification. Stock solutions, 5 mmol/L of ME and PSE were prepared by dissolving the standard alkaloids in deionized water (18.2 MΩ • cm) processed with an Ultrapure Water System (Kangning Water Treatment Solution Provider, China). A series of working standard solutions were prepared by diluting the stock solution with deionized water. All solutions were prepared with deionized water. They were stored in the refrigerator at 4°C.

Instrument and Conditions

The CE-ECL experiments were performed on a model MPI-A capillary electrophoresis electrochemiluminescence system (Xi'an Remax Electronic Science-Tech Co. Ltd, Xi'an, China). The system provided a programmable high-voltage power supply (0–20 kV), an electrochemical potentiostat, a multifunction chemiluminescence detector and a multichannel data collection analyzer.

The end-column ECL cell was composed of a 500 μm Pt disk working electrode, an Ag–AgCl reference electrode (KCl saturated) and a Pt wire counter electrode. The surface of the working electrode was polished sequentially with 0.3 and 0.05 μm α-Al_2O_3 on a piece of polishing cloth until a mirror-smooth surface appeared and then was sonicated for 10 min in water. The electrode was subjected to repeated cycling in the potential region of 0.2–1.25 V (vs Ag–AgCl) to obtain a reproducible cyclic voltammogram before each experiment. About 300 μL of $Ru(bpy)_3^{2+}$ solution was added to the cell before analysis.

Procedure

All separations were performed in a 53 cm long fused-silica capillary with 50 μm i.d. and 375 μm o.d. (Yongnian Reafine Chromatography Ltd, Hebei, China). The new capillary was rinsed sequentially with 1.0 mol/L NaOH, 1.0 mol/L HCl, H_2O and electrophoretic buffer for 30 min. At the beginning of each day, the capillary was flushed with 0.1 mol/L NaOH, water and equilibrated with the electrophoretic buffer for 10 min successively so as to maintain an active and reproducible inner surface. The capillary was rinsed sequentially with 0.1 mol/L NaOH, H_2O, and electrophoretic buffer for 2 min after each five runs. The voltage of photomultiplier tube (PMT CR105, Beijing Binsong Photonics, China) for collecting the ECL signal was set at –850 V in the process of detection. The detection potential applied at the working electrode was fixed at 1.2 V. Electrokinetic injections were performed at 10 kV for 10 s. The inlet end of the capillary was held at a positive potential and the outlet end was maintained at ground. An aliquot of 5 mmol/L $Ru(bpy)_3^{2+}$ with 50 mmol/L phosphate buffer solution (PBS) was added to the detection cell. The peak area was used for the analysis.

Urine Sample Preparation

The fresh human urine samples of two healthy male volunteers from Xinyang Normal University in the pharmacokinetics study were acquired after oral administration of 90 mg PSE sustained release capsules (a medicine for treatment of cold containing PSE). About 5 mL of urine samples were collected immediately before the oral dose, and again at 1, 2, 3, 4, 6, 8, 12 and 24 h after the oral dose. Blank urine was collected just before oral administration for the preparation of spiked samples and the calibration curve. The volunteers were asked to drink sufficient and comparable amounts of water through the collection period. Prior to analysis, urine samples were filtered through 0.22 μm cellulose acetate filters (Shanghai Xingya Purification Material Factory) and then diluted with deionized water 3-fold to decrease the interference of the ionic strength of the sample matrix. After the treatment of urine samples, they were stored in the refrigerator at 4°C.

Results and Discussion

Effects of pH and Concentration of Electrophoretic Buffer

The primary experimental results showed that ME and PSE were not well separated when electrophoretic buffer pH was below 8.9. The effect of the electrophoretic buffer pH on resolution (*Rs*) and ECL intensity was examined in the range 8.9–9.9, as shown in Fig. 2(A). The results indicated that *Rs* increased with the increase in the electrophoretic buffer pH and the highest ECL

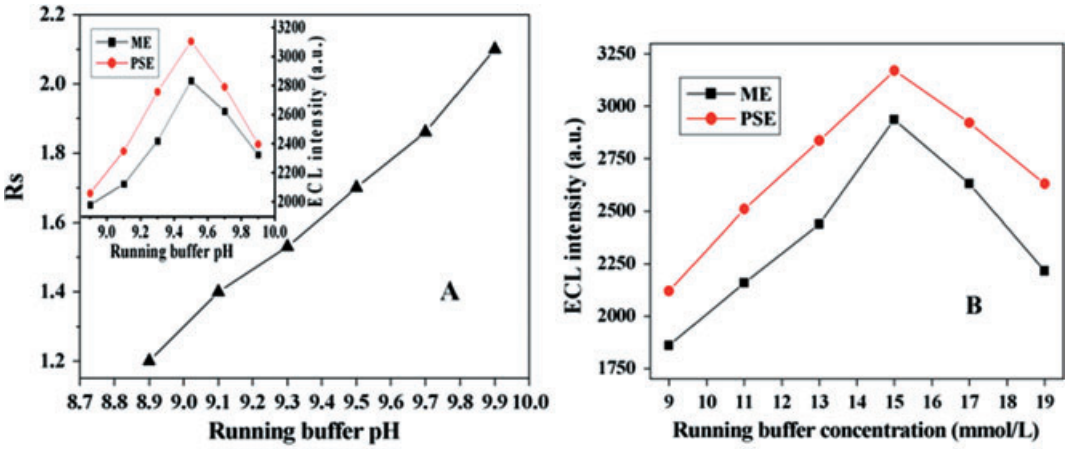

Figure 2. Optimization of the electrophoretic conditions: electrophoretic buffer, phosphate–borax containing 0.6% (v/v) $BMIMBF_4$; electrokinetic injection, 10 s × 10 kV; separation voltage, 11 kV; detection potential, 1.2 V; ECL solution, 5 mmol/L $Ru(bpy)_3^{2+}$ with 50 mmol/L PBS at pH 8.5. (–■–) 5 μmol/L ME, (–●–) 6 μmol/L PSE. (A) Buffer pH and (B) buffer concentration.

intensity [Fig. 2(A), inset] was achieved at pH 9.5 for the two analytes. The *Rs* was calculated using the following equation: $Rs = 2(t_2 - t_1)/(W_{b1} + W_{b2})$, where t_1 and t_2 are the migration times of two adjacent analytes and W_{b1} and W_{b2} are the peak widths of two adjacent analytes measured at the baseline. When the pH of buffer exceeded 9.5, the ECL responses decreased. Therefore pH 9.5 was selected.

Phosphate containing borax with the same concentration (phosphate–borax) was used as the electrophoretic buffer. The effect of the concentration of electrophoretic buffer from 9 to 19 mmol/L was evaluated, and is shown in Fig. 2(B). The highest ECL intensity of two analytes was achieved at 15 mmol/L. When the electrophoretic buffer concentration was above 15 mmol/L, the ECL intensity decreased and the migration time became longer.

Effect of Detection Potential

The detection potential has a marked effect on the ECL intensity in a CE–ECL system. The intensity of the emitted light is dependent on the rate of the light-emitting chemical reaction, and this reaction rate is dependent on the potential applied to the working electrode. The effect of the detection potential on the ECL intensity over the range from 1.05 to 1.30 V was investigated (Fig. 3). As shown in Fig. 3, the highest ECL intensity was achieved at 1.2 V for two analytes. Therefore, 1.20 V was selected.

Effect of Concentration of IL

In order to improve the sensitivity and *Rs* of analytes, IL ($BMIMBF_4$) was added to the electrophoretic buffer as additive. The effects of IL on the ECL response and *Rs* were investigated. The results showed that the sensitivities were obviously improved (see Fig. 4), while the *Rs* of ME and PSE were little improved with the use of IL. With the increase in the concentration of $BMIMBF_4$, the ECL intensity was increased. When the concentration of $BMIMBF_4$ reached 0.6% (v/v), the highest ECL intensity for the two alkaloids was obtained. The results could be attributed to the effect of the high conductivity of the IL,

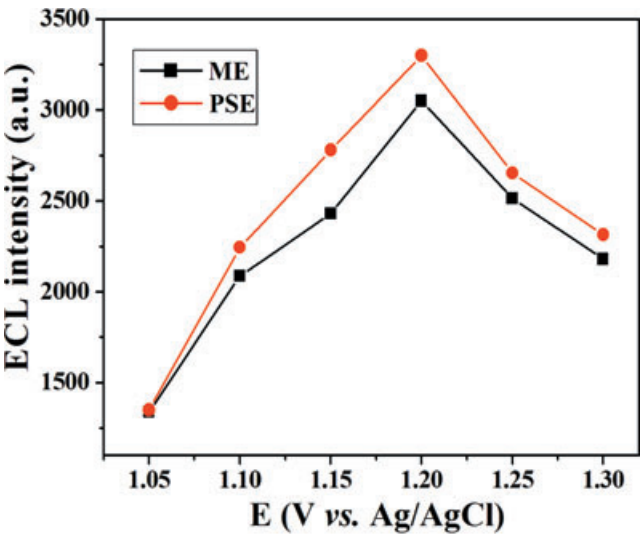

Figure 3. Effect of detection potential on ECL intensity. Other conditions are the same as in Fig. 2.

which made the resistance of the sample solution much higher than that of the electrophoretic buffer. Therefore the field-amplified sample stack effect was realized for electrokinetic injection in CE. Additionally, the addition of $BMIMBF_4$ enhanced the ionic strength and viscosity of the electrophoretic buffer, resulting in prolonged migration time of analytes. As a result, 0.6% (v/v) $BMIMBF_4$ was chosen.

Effect of Separation Voltage

The applied separation voltage controls the separation efficiency and migration time of the analytes in the CE–ECL system. The effects of separation voltage on ECL intensity and *Rs* were studied in the range 9–13 kV. As shown in Fig. 5, the ECL intensity increased and the *Rs* decreased (Fig. 5, inset) with increase in separation voltage. When the separation voltage was higher than 11 kV, the ECL intensity decreased. The reason for this could be that, in the CE–ECL system, the high separation voltage

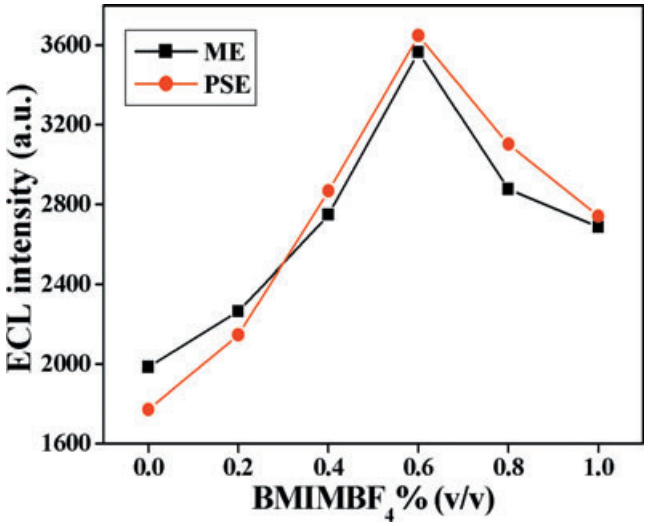

Figure 4. Effect of concentration of $BMIMBF_4$ on ECL intensity. Other conditions are the same as in Fig. 3.

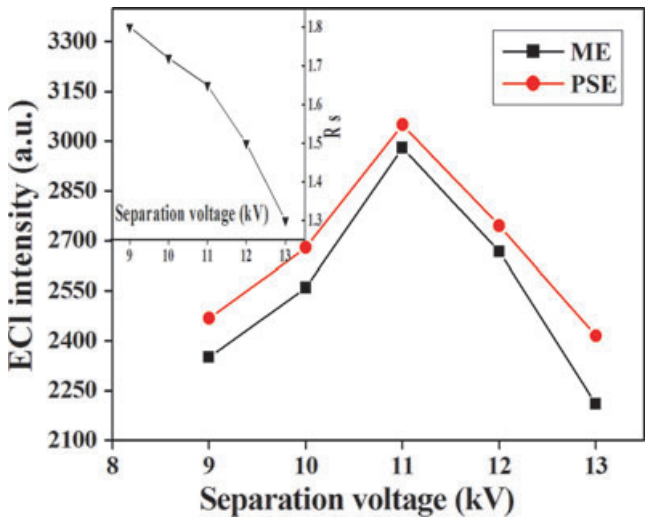

Figure 5. Effect of separation voltage on ECL intensity and *Rs*. Other conditions are the same as in Fig. 4.

caused high Joule heating within the capillary and strong flow of effluent from the capillary and thus resulted in the dilution of the concentration of $Ru(bpy)_3^{3+}$ in diffusion layer of the working electrode surface. Therefore 11 kV was chosen.

Effect of Buffer pH in Detection Cell

The effects of buffer pH in the detection cell on the ECL intensity of the two analytes were investigated with pH ranging from 8.1 to 9.1 (as shown in Fig. 6). The results indicated that the ECL intensities of the two analytes reached the maximum at pH 8.5 and then decreased with the increase in the pH. Therefore, pH 8.5 was selected.

Analytical Performance

The optimal conditions were: detection potential, 1.2 V; electrokinetic injection, 10 s for 10 kV; separation voltage, 11 kV;

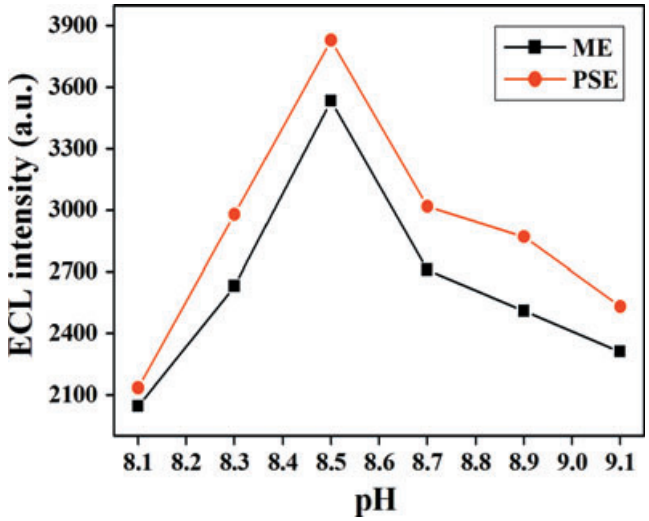

Figure 6. Effect of buffer pH in detection cell on ECL intensity. Other conditions are the same as in Fig. 5.

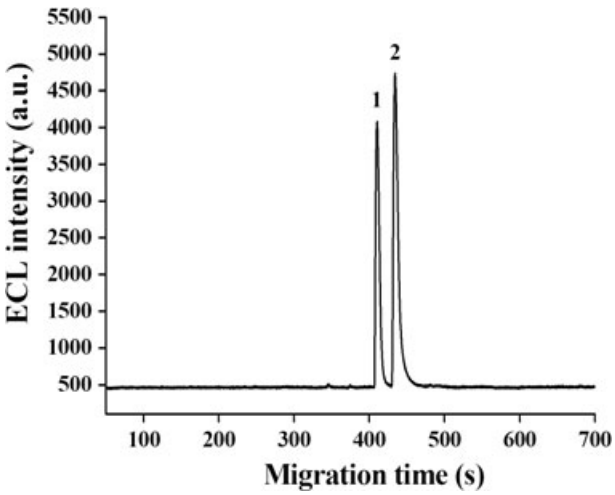

Figure 7. Electropherograms of two standard samples. Peaks: **1**, 5 μmol/L ME; **2**, 6 μmol/L PSE. Conditions: electrophoretic buffer, 15 mmol/L phosphate–borax containing 0.6% (v/v) $BMIMBF_4$ at pH 9.5; electrokinetic injection, 10 s × 10 kV; separation voltage, 11 kV; detection potential, 1.2 V; ECL solution, 5 mmol/L $Ru(bpy)_3^{2+}$ with 50 mmol/L PBS at pH 8.5.

15 mmol/L phosphate–borax electrophoretic buffer containing 0.6% (v/v) $BMIMBF_4$ at pH 9.5; 5 mmol/L $Ru(bpy)_3^{2+}$ with 50 mmol/L PBS buffer at pH 8.5 in the detection cell. The typical electropherograms of the ME and PSE were well separated within 8 min, as shown in Fig. 7. To evaluate the linearity of the established method, the calibration curves were calculated plotting the peak area values against the analyte concentrations. It can be seen from Table 1 that the regression coefficients of the calibration curves are greater than 0.999. LOD was considered the minimum analyte concentration, yielding a signal-to-noise (S/N) ratio equal to 3.

The precision (measured by relative standard deviation, RSD%) of the peak area and the migration time for five identical injections of a mixture standard solution of ME (5.0×10^{-6} mol/L) and PSE (6×10^{-6} mol/L) were 2.3–4.2 and 1.2–1.4% within 1 day, and 4.2–5.6 and 2.6–4.2% in 3 days.

Table 1. The performance characteristics of the proposed method

Analytes	Linear range (μmol/L)	Slope	Calibration curves Intercept	*r*	LOD (mol/L)
ME	0.5–100	37960.7	49803.1	0.9993	1.8×10^{-8}
PSE	0.1–50	47761.8	15584.7	0.9993	9.2×10^{-9}

Table 2. Recoveries of two analytes at three different spiked level in human urine sample

Added (μmol/L)	Found (μmol/L)	Recovery (%)	RSD (%) ($n = 5$)
ME			
20	21.0	105.0	5.9
200	191.2	95.6	5.7
400	335.2	83.8	4.5
PSE			
20	17.3	86.5	3.1
200	163.4	81.7	1.8
400	378.8	94.7	4.5

Applications

The proposed method, utilizing CE-ECL with $BMIMBF_4$, was applied to the determination of ME and PSE in human urine samples. The typical electropherograms of blank urine sample of health person and urine sample spiked with 1.0×10^{-5} mol/L ME and 1.2×10^{-5} mol/L PSE are illustrated in Fig. 8(A, B). The results indicated that the two analytes were not detected in the urine sample of a healthy person and could be well separated and detected in spiked urine samples. In addition, there also appeared several unknown peaks, which did not interfere with the separation and analysis of the ME and PSE. These may be caused by the unknown compounds existing in the urine sample matrix. The recoveries of the two analytes at three different spiked concentration levels were 83.8–105.0% for ME and 81.7–94.7% for PSE in urine samples (listed in Table 2). The RSDs of peak area were less than 5.9%. From the human urine sample analysis, the limit of quantitation (defined as the lowest analyte concentration yielding an S/N of 10) was 2.6×10^{-7} mol/L for ME, and 3.6×10^{-7} mol/L for PSE.

In the pharmacokinetics study, two healthy male volunteers received an oral administration of 90 mg of PSE sustained release capsules. The urine samples were collected and analyzed immediately before the oral dose, and again at 1, 2, 3, 4, 6, 8, 12 and 24 h after the oral dose. The results of the two volunteers are presented in the concentration–time profile in Fig. 9. As shown in Fig. 9, the maximal content of PSE in the urine samples of volunteers was achieved in about 4 h after oral dose and then decreased. The results show that the content of PSE in urine from volunteer 1 is higher than that from volunteer 2 at any time after oral administration. This might be caused by variation in the metabolizability from person to person.

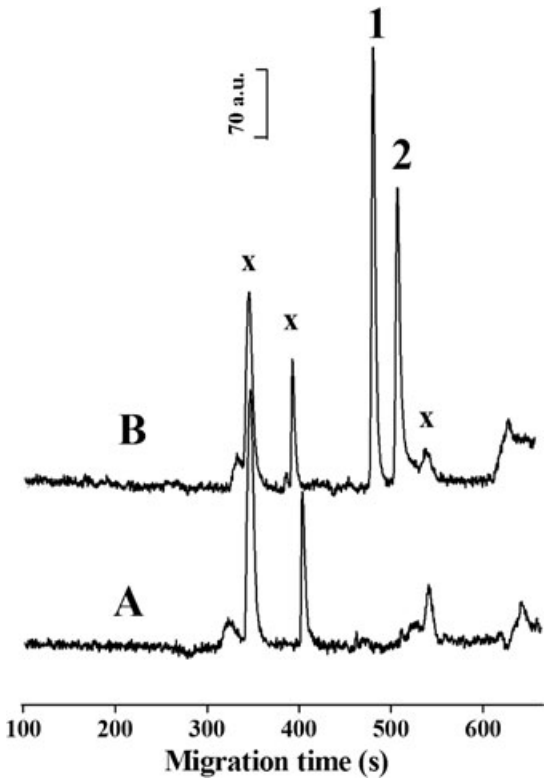

Figure 8. Electropherograms of the blank urine sample (A) and the urine sample spiked with 1.0×10^{-5} mol/L ME, 1.2×10^{-5} mol/L PSE (B). Peaks: **1**, ME; **2**, PSE; **X**, unknown compounds. Other conditions are the same as in Fig. 7.

Conclusion

A simple, rapid and sensitive CE-ECL method for the determination of ME and PSE in human urine and the monitoring of pharmacokinetics for PSE in the human body was established for the first time. The use of IL improves the detection sensitivity. The proposed method has potential in therapeutic drug monitoring and clinical analysis.

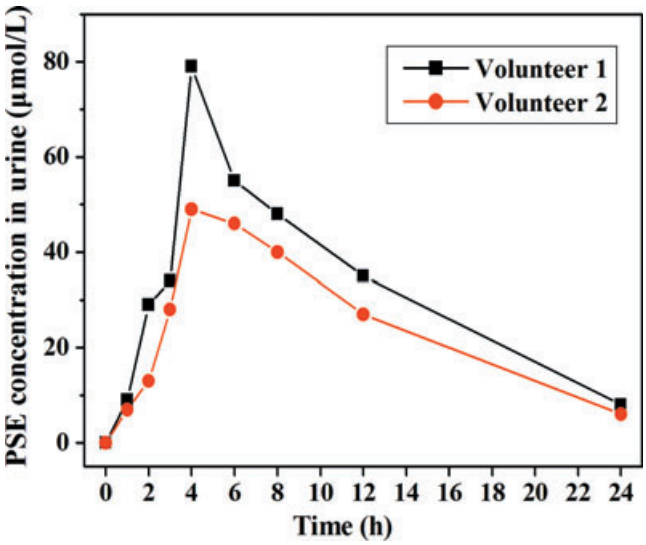

Figure 9. PSE concentration change in urine samples 0, 1, 2, 3, 4, 6, 8, 12 and 24 h after the ingestion of 90 mg of PSE sustained release capsules to the volunteers. Conditions as in Fig. 8.

Acknowledgements

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