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# Separation and determination of lignans from seeds of *Schisandra* species by micellar electrokinetic capillary chromatography using ionic liquid as modifier

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**Abstract**

In this paper, a micellar electrokinetic chromatographic (MEKC) method using ionic liquid as modifier for the quantification of the active components of lignans found in the medicinal herbs *Schisandra* species was developed for the first time. Preliminary investigations employing sodium dodecyl sulfate (SDS) as surfactant did not lead to the necessary resolution of the studied compounds, the addition of ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate (BMIM-$BF_4$) to the SDS micellar system resulted in the complete separation of all the compounds. The effects on the separation by several parameters such as BMIM-$BF_4$ and SDS concentration, applied voltage, background electrolyte pH and concentration, were evaluated. Under the optimal conditions (5 mM borate-5 mM phosphate buffer in the presence of 20 mM SDS and 10 mM BMIM-$BF_4$, pH 9.2, applied voltage 25 kV and detection at 254 nm), the method successfully applied to the determination of lignans in extracts of *Schisandra chinensis* (Turcz.) Baill. and *Schisandra henryi* C.B. Clarke in less than 13 min. The separation mechanism was also discussed.


*Keywords:* Micellar electrokinetic capillary chromatography; *Schisandra* species; Lignans; Ionic liquid

## 1. Introduction

*Schisandra* species [1] are famous medicinal plants native to East Asia. Their fruits and seeds are widely used in oriental medicine. The biologically active compounds are the lignans with a uncommon structure derived from dibenzo[a,c]cyclooctadiene (Fig. 1). The lignans prevent liver injuries [2–4], stimulate liver regeneration [5], and also inhibit hepatocarcinogenesis [6,7] and lipid peroxidation [3,8]. Moreover, several reports indicate that the lignans have effects on human intellectual activity [9] and physical performance [10]. Recently, some dibenzo[a,c]cyclooctadiene lignans were identified as potent anti-human immunodeficiency virus agent [11].

Previously described methods for the determinations of the lignans in *Schisandra* species plants include thin-layer [12], gas [13] and high-performance liquid chromatographic [14–16] techniques. Capillary electrochromatography using polymer-based monolithic stationary phase has been developed and successfully applied to analyse and quantify the lignans from seeds of *Schisandra chinensis* Baill. However, the preparation of the capillary was laborious and the overall separation time was long [17]. Recently, micellar electrokinetic capillary chromatography using acetonitrile as additive also developed for the separation of the lignans. However, organic solvent acetonitrile is highly volatile and harmful to the environment [18]. In addition, the analysis of lignans in *Schisandra henryi* using CE has not been reported before. In another work, 12 lignan compounds originating from *Phyllanthus* plants were separate by MEKC, because of the high lipophilicity of the lignan analytes, tetrahydrofuran was

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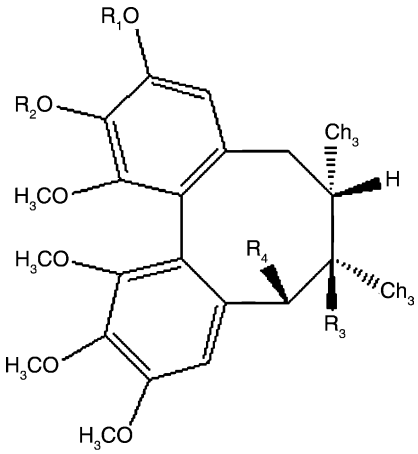


1, $R_1 = R_2 = CH_3$ $R_3 = OH$ $R_4 = H$
2, $R_1R_2 = CH_2$ $R_3 = OH$ $R_4$ =β-O-Benzoyl
3, $R_1 = R_2 = CH_3$ $R_3 = R_4$=H
4, $R_1R_2 = CH_2$ $R_3 = R_4$=H

Fig. 1. Molecular structures of the lignans: (1) schisandrin, (2) schisantherin A, (3) deoxyschisandrin (schisandrin A, wuweizisu A), (4) γ-schisandrin (schisandrin B, wuweizisu B).

added to the SDS micellar system to increase its separating ability [19].

Capillary electrophoresis (CE) is a very powerful technique for the separation of charged analytes [20]. With the introduction of micellar electrokinetic capillary chromatography [21], the high separation efficiency of CE became accessible for the separation of neutral compounds. The most commonly used surfactant for MEKC is sodium dodecyl sulfate (SDS). However, the nucleus of the SDS micelles is strongly hydrophobic, for very hydrophobic compounds, MEKC with SDS is often insufficiently selective because all compounds tend to be absorbed virtually completely into the micelles and migrates with the velocity of the micelles [22]. Several methods have been proposed to expand the application range of MEKC to more hydrophobic compounds: the use of surfactants with a hydrophobicity lower than SDS such as cholic acid or derivatives [23], or the addition of compounds to the background electrolyte (BGE) that interact with the analytes in the aqueous phase such as cyclodextrins [24,25]. Also, a frequently applied procedure is to change the solvent strength of the aqueous phase by the addition of organic modifiers such as methanol [26,27], acetonitrile [28,29], 2-propanol [30] or urea to the BGE [31].

Room-temperature ionic liquids (RTILs) are those compounds which are liquids at room temperature or whose melting points are slightly higher than ambient temperature. In the past several years, there has been growing interest in ionic liquids for their potential in different chemical processes, such as liquid–liquid extraction [32], organic synthesis [33], electrochemistry [34,35], catalysis for clean technology [36], ultralow volatility liquid matrixes for matrix-assisted laser desorption/ionisation (MALDI) mass spectrometry [37]. The application of ionic liquids for the separation of various classes of compounds in CE and chromatography has also been recently recognized. In the work of Armstrong et al., they were employed as stationary phase in gas chromatography (GC) because they can dissolve a number of complex organic molecules [38]. Yanes et al. [39] developed a CE method for the separation of polyphenols found in grape seed extracts using only 1-alkyl-3-methylimidazolium-based ionic liquids as the background electrolyte. Qin et al. [40] reported that the use of 1,3-dialkylimidazolium-based room-temperature ionic liquids as background electrolyte and coating material in aqueous capillary electrophoresis. In another publication, Vaher et al. [41] reported that the use of 1,3-dialkylimidazolium-based ionic liquids as background electrolyte for nonaquous capillary electrophoresis.

To the best of our knowledge, there is only one report in the literature on the using of ionic liquids as modifiers in MEKC [42]. There are several advantages for using ionic liquids over organic solvents as modifiers. Ionic liquids are soluble in water, have a good electrical conductivity, nonflammable and act as good electrolytes in CE either when used independently or when mixed with other buffers. In addition, ionic liquids are less volatile and are referred to as “green solvents”, meaning they are environmentally friendly, with increasing environmental concerns about volatile organic carbon (VOC), the ionic liquids are considered attractive alternatives (given their lack of vapor pressure) to organic solvents. In contrast, organic solvents are poor conductors of electricity, and high concentrations of organic solvents in the buffer cause current breakdowns in CE. Moreover, most organic solvents are highly volatile and harmful to the environment.

The ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate (BMIM-$BF_4$) was reported to be the most air and water stable and conductive, liquid at room temperature and was highly miscible in water [43,44]. Thus, it was expected that it would have the ability to assist in the MEKC separation of hydrophobic mixtures. The purpose of the present study was to investigate the potential application of ionic liquid as modifier in MEKC to the separation of lignans found in the seeds of *Schisandra* species. Optimization of the separation was performed by evaluating the effect of SDS and BMIM-$BF_4$ concentration, applied voltage, background electrolyte pH and concentration on the resolution and analysis time of the lignans. The separation mechanism was also discussed.

## 2. Experimental

### 2.1. Apparatus and procedures

All separations were carried out with a Waters Quanta 4000 capillary electrophoresis system (Milford, MA, USA), equipped with a UV detector. Data acquisition was carried out with a Maxima 820 chromatography workstation. Fused-silica capillary of 60 cm (52.5 cm effective length) $\times 50\,\mu m$ I.D. (Yongnian Photoconductive Fiber Factory, Hebei Province, China) was used. The temperature was maintained at $23.5 \pm 0.5\,^{\circ}C$. Samples were introduced from anodic end

of the capillary by hydrodynamic injection where the samples vial was raised by 10 cm for 5 s and the detection wavelength was 254 nm. Methanol was the marker of electroosmotic flow (EOF). A PHS-10A acidity meter (Xiaoshan Science Instrumentation Factory, Zhejiang, China) was used for the pH measurement. The pH was adjusted with 0.2 M NaOH or HCl. All data reported reflect at least three replicate separations recorded for each different composition of the running buffer.

Newly cut capillary was washed successively with 1 M NaOH for 10 min, water for 10 min, 0.1 M NaOH for 10 min and water for 10 min. The capillary was equilibrated (15 min) at the beginning of the day with the running buffer. The capillary rinsed between the runs as follows: 2 min with water, 3 min with 0.1 M NaOH, 2 min with water and 3 min with BGE.

### 2.2. Materials

Seeds of *S. chinensis* (Liaoning Province, China) and *S. henryi* (Sichuan Province, China) were purchased from Zhongyou drugstore, Lanzhou, China. Standards of lignans derivatives schisandrin, deoxyschisandrin, γ-schisandrin and schisantherin A obtained from the National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China. SDS was purchased from the Peking Xizhong Chemical Factory, Peking, China. β-CD was purchased from China Medicine Group, Shanghai Chemical Reagent Company, Shanghai, China. Phosphate and borate were purchased from Tianjin Chemical Reagent Factory, Tianjin, China. The ionic liquid BMIM-$BF_4$ was a gift to the authors from National Key Laboratory of Applied Organic Chemistry, Lanzhou, China. All chemicals and solvent were of analytical regent grade and were used without further purification.

### 2.3. Solutions and sample preparation

The stock solutions were prepared by dissolving precisely weighed standards into 5 mL methanol and stored at −4 °C. The running buffers were prepared daily by mixing appropriate volumes of 0.1 M $Na_2HB_4O_7{\cdot}10H_2O$, 0.1 M borax, 0.2 M SDS and 50 mM BMIM-$BF_4$. All solutions were filtered through a 0.45 μm filter.

The sample treatment was prepared according to the published procedure [45]. A 2 g sample of dried and pulverized seeds of *S. chinensis* was immersed in 10 mL *n*-hexane at room temperature for 12 h and then extracted for 30 min in an ultrasonic bath. The extraction was repeated with 10 mL *n*-hexane for three times. The extracts were combined, evaporated to dryness, and then the residue was dissolved in 5 mL methanol as the stock solution. A 2 g of powdered seeds of *S. henryi* was extracted, respectively, using the same procedure as that for *S. chinensis*. The solutions were passed through a 0.45 μm filter and injection directly into the capillary electrophoresis system.

## 3. Results and discussion

### 3.1. Method development

In preliminary measurements, it was found that studied compounds could not be separated by ordinary CZE using BMIM-$BF_4$ or β-CD as modifier, all selected analytes have no electrophoretic mobility and migrated with the electroosmotic velocity (results not shown). The above experiments would suggest that one perform a MEKC. In this experiment, however, under normal MEKC conditions where there were no modifiers added to the buffer, in the range of the concentration of SDS within 15–100 mM, borate–phosphate (1:1) 10–60 mM and pH 7.0–11.0, no successful separation of the studied compounds was obtained. The representative electropherogram was shown in Fig. 2. The elution order was determined by spiking a small amount of each analyte into the mixture, and the peaks were numbered according to the analyte notation in Fig. 1. As can be seen from Fig. 2, the first and second eluted compounds schisandrin and schisantherin A were well resolved, unfortunately, the last two compounds deoxyschisandrin and γ-schisandrin completely overlapped one another.

The separation of neutral solutes in MEKC is mainly due to their partitioning between an aqueous phase and a micellar phase. In any case, with SDS surfactant, the smaller the aqueous phase solubility and/or the stronger the degree of solute association with the micelle, the longer the migration time. Accordingly, the migration order is that of decreasing aqueous phase solubility. In this study, the elution order for these lignans was apparently from the least hydrophobic compound schisandrin to the most hydrophobic compounds γ-schisandrin and deoxyschisandrin. Since schisandrin and schisantherin A have an additional hydroxyl group compared with the other two compounds, thus, it was expected that they may be solubilized in aqueous phase more than the other

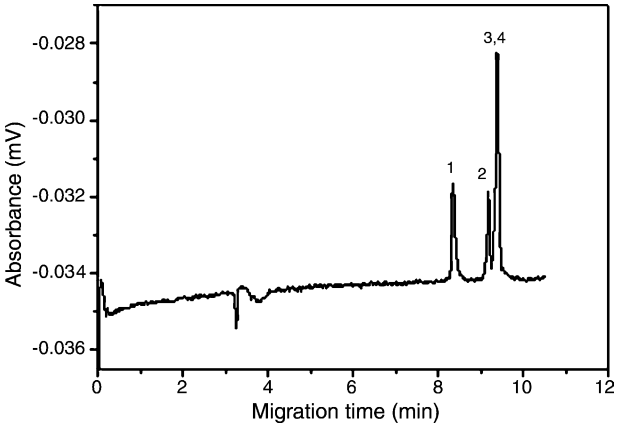


Fig. 2. The typical electropherograms of the standards mixture solution under normal MEKC. Electrophoretic conditions: buffer, 5 mM borate–5 mM phosphate containing 20 mM SDS, pH 9.2; $I$ = 29 μA; uncoated fused-silica capillary, total length 60 cm (52.5 cm effective length) × 50 μm I.D.; applied voltage, 25 kV; temperature, 23.5 ± 0.5 °C detection, 254 nm.

compounds. Simultaneous addition of a hydroxyl group and benzoyloxy group decreased migration time, indicating that the hydroxyl group produced a larger increase in aqueous phase solubility than addition of the benzoyloxy group does to change affinity for the micelle. Deoxyschisandrin and γ-schisandrin are highly hydrophobic, neutral species, which tend to have similar, large partition coefficients in the micelle phase because of their small aqueous solubility, resulting in long migration time and poor resolution.

Because deoxyschisandrin and γ-schisandrin were difficult to completely separate under normal MEKC conditions, efforts were shifted towards the used of modifiers. β-CD and urea were initially used independently or mixed as modifiers in the separation, however, separation was still incomplete with deoxyschisandrin continuing to comigrate with γ-schisandrin, the obtained electropherograms were similar to that provided under normal MEKC conditions. BMIM-$BF_4$ was then added to the buffer to enhance the separation, the addition of BMIM-$BF_4$ to the SDS micellar system provided the complete separation of the studied compounds. On the bases of these preliminary results, our attention was focused on the use of BMIM-$BF_4$ as modifier.

### 3.2. Method optimization

The optimization of the separation of the lignans was aimed to resolve separated lignans from each other and also from the interferefering matrix ingredient in the real samples. The method optimization was carried out also considering the general requirements of method suitable for the quality control of the medicinal plants: selectivity, reproducibility and short analysis time.

#### 3.2.1. Running buffer pH

To verify the effect of running buffer pH on migration behavior, experiments were performed with pH ranging from 8.0 to 10.0 and 7.5 mM BMIM-$BF_4$, other conditions were same as in Fig. 2. It was found that the electroosmotic velocity was slowly reduced and electrophoretic velocities of the lignans also decreased concurrently with the decrease of the pH, this change was rather sharp at low pH and slightly more gradual over the pH 9.20. The migration times increased excessively when the pH was lower than 9.20 and only slight resolution of a pair of compounds deoxyschisandrin and γ-schisandrin was achieved when the pH was above 9.20. Within the studied pH range, the best results were obtained at pH 9.20 and it was used in the subsequent work.

#### 3.2.2. SDS concentration

The effect of SDS concentration on the separation of lignans was investigated with 15–30 mM SDS and 7.5 mM BMIM-$BF_4$, other conditions were same as in Fig. 2. It was found that the migration behavior or the selectivity of the analytes was influenced significantly by the SDS concentration. The time window between the first and the last migrating analytes was decreased with increasing SDS concentration, revealing the highly hydrophobic character of the analytes and the low selectivity of SDS for these compounds. An increase in the SDS concentration from 20 to 30 mM caused a clear deteriorated in the separation of the studied compounds deoxyschisandrin and γ-schisandrin with a general loss of resolution, this can be explained in terms of the solubilization of these analytes by the micelle. Since lignans are highly hydrophobic, neutral species, the solubilization into the micelle or interaction with the micelle of the analytes will be probably increased with an increase of SDS concentration and this lead to increasing partition of the analytes into the micellar phase, resulting in degradation of the quality of the separation as reflected by poor resolution and longer migration times. When SDS concentration was lower than 20 mM, the analytes migrated as unsymmetrical peaks. Mikkers et al. have suggested that unsymmetrical peaks were usually generated when the electrophoretic mobilities of a solute and of a buffer constituent were quite different [46]. In this study, it was also found that peak symmetry deteriorated with decreasing SDS concentration due to the increasing mobility mismatch between analytes and buffer constituent.

Because better peak shapes and resolution of all the compounds were observed at 20 mM SDS and thus it was chosen for the further experiments.

#### 3.2.3. BMIM-$BF_4$ concentration

As for this MEKC separation, BMIM-$BF_4$ concentration was considered an important parameter controlling the analysis selectivity. Fig. 3 illustrated the effect of BMIM-$BF_4$ concentration on the separation of the lignans mixture. As apparent from the chromatograms, selectivity and resolution were greatly improved with the increased concentration of BMIM-$BF_4$. The two compounds deoxyschisandrin and γ-schisandrin that coeluted under simple MEKC conditions became baseline resolved with the addition of 10 mM

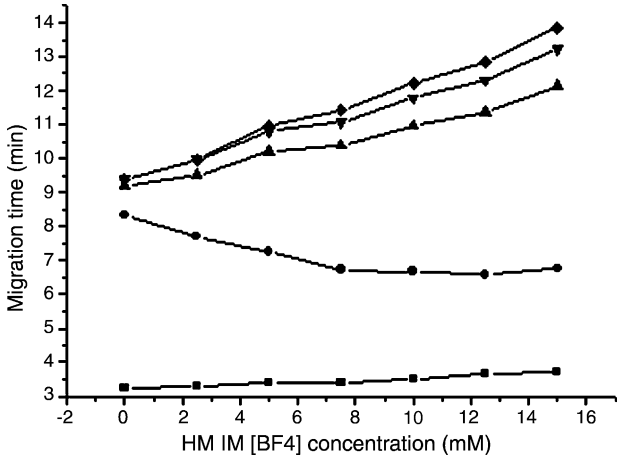


Fig. 3. Effect of BMIM-$BF_4$ concentration on the migration times of the analytes. Electrophoretic conditions: buffer, 5 mM borate–5 mM phosphate containing 20 mM SDS and 0–15 mM BMIM-$BF_4$, pH 9.2; peak identification symbols: EOF (■), 1 (●), 2 (▲), 3 (▼), 4 (♦). Other conditions as in Fig. 2.

BMIM-$BF_4$ to the buffer. A further increase in BMIM-$BF_4$ concentration resolved the analytes more than necessary for an adequate separation and only served to increase the analysis time, but dramatically illustrated the capabilities of the technique for selectivity manipulation. In Fig. 3, it also could be found that the migration time of schisandrin decreased with the increase of the BMIM-$BF_4$ concentration. This indicated that the retention of schisandrin decreased with the addition of BMIM-$BF_4$ to running buffer.

These data here showed that the addition of BMIM-$BF_4$ to typical anionic surfactant systems could dramatically affect MEKC separations. SDS micelle solutions with only sodium counterions present, these positively charged imidazolium cations could be electrostatically attracted to the negatively charged SDS micelle surface, which neutralized the effective head group charge and reduced electrostatic repulsion between the charged hydrophilic headgroups of the surfactant molecules, thus affecting the size and shape of the micelles formed and thereby altering the separation. As indicated in ref. [47], it has proved that lower the electrostatic repulsion between the charged hydrophilic headgroups of ionic surfactants caused a remarkable decrease in critical micelle concentration (CMC) as compared to that in pure water (8.1 mM). In this study, separation of the compounds could still be achieved even with 5 mM SDS (data not show), which below the CMC of SDS, so one possible explanation for the separation was the presence of some micellar aggregate. This result proved the binding ability of imidazolium cations on SDS.

At 10 mM BMIM-$BF_4$, lignans were well resolved and removed interference from other coexisting constituents in the real samples, so the optimized BMIM-$BF_4$ concentration was chosen as 10 mM.

### *3.2.4. Effect of buffer concentration and applied voltage*

The influence of the concentration of borate-phosphate (1:1) buffer in the range of 5–30 mM and applied voltage 20–30 kV on the separation were examined. Take account of the resolution and analytical time, 5 mM borate–5 mM phosphate buffer and 25 kV separation voltage were chosen as the ideal selected for the sample separation. Under these conditions, the recorded current was lower than 40 μA.

From the above results, the best conditions were obtained with running electrolyte containing 5 mM borate–5 mM phosphate, 20 mM SDS and 10 mM of BMIM-$BF_4$ at pH 9.2. Under the optimum conditions, all the four analytes were well separated within 13 min with symmetrical peaks. The typical electropherogram for a standard mixture was shown in Fig. 4A.

## *3.3. Determination of the lignans in extracts of Schisandra species*

### *3.3.1. Reproducibility, linearity and detection limit*

Under the optimum conditions, the linear relationship between the concentration of the four lignans 1–4 and the corresponding peak areas were shown in Table 1. The calibration curves exhibited excellent linear behavior over the concentration ranges. The reproducibility of the method was determined with a standard mixture solution at the level of

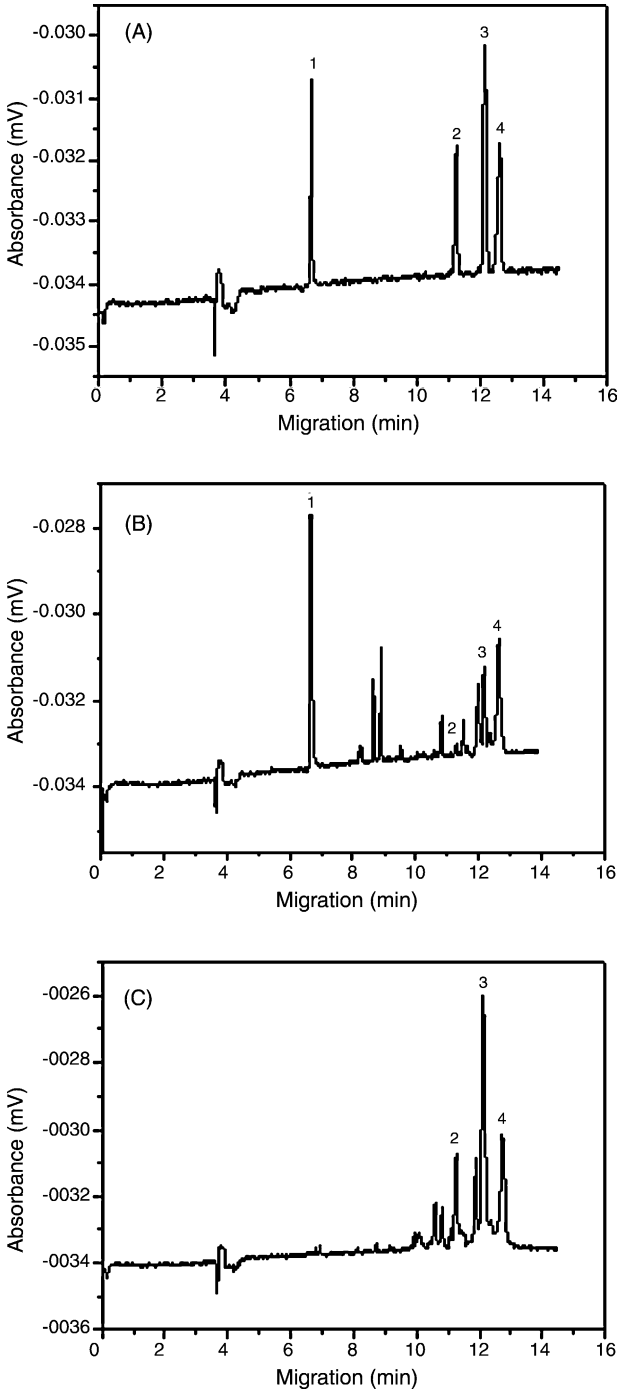


Fig. 4. The typical electropherograms of the standards mixture solution and the real samples under the optimum conditions. (A) The standards mixture; (B) *Schisandra chinensis*; (C) *Schisandra henryi*; the concentrations of the standards were 400 μg/mL for all analytes, respectively; electrophoretic conditions: buffer, 5 mM borate–5 mM phosphate containing 20 mM SDS and 10 mM BMIM-$BF_4$; pH 9.2; $I$ = 38 μA; peaks identification: (1) schisandrin, (2) schisantherin, (3) deoxyschisandrin, (4) γ-schisandrin. Other conditions as in Fig. 2.

Table 1
The results of regression analysis on calibration curves and the detection limits

| Compound | Calibration curves $Y = a + bx$[a] | Correlation coefficient | Linear range (μg/mL) | Detection limit (μg/mL)[b] |
|---|---|---|---|---|
| 1 | $y = -202.30 + 33.60x$ | 0.9994 | 5.0–1000 | 0.6 |
| 2 | $y = 265.52 + 27.78x$ | 0.9995 | 12.0–700 | 0.7 |
| 3 | $y = 979.39 + 51.13x$ | 0.9996 | 6.0–1000 | 0.4 |
| 4 | $y = 1122.50 + 36.17x$ | 0.9992 | 7.5–800 | 0.6 |

[a] $y$ and $x$ stand for the peak area and the concentration (μg/mL) of the analytes, respectively.
[b] The detection limits corresponding to concentrations giving signal-to-noise ratio of 3.

Table 2
Contents of the four lignans in real samples and RSD ($n = 5$)

| Sample | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| *Schisandra chinensis*[a] | 0.179 (1.23)[b] | 0.006 (3.12) | 0.029 (1.22) | 0.135 (2.38) |
| *Schisandra henryi* | –[c] | 0.120 (1.25) | 0.224 (0.82) | 0.157 (1.56) |

[a] % of dry mass.
[b] The data in parentheses refer to the RSD
[c] Not found.

100 μg/mL for all analytes. The RSD values ($n = 6$) of the migration times and peak areas were 0.8–1.3, 1.6–2.1% (intra-day), and 1.2–1.7, 1.9–2.7% (inter-day, for a 5-day period), respectively. All the RSDs were shown as less than 3%, which demonstrated that this method was of good repeatability and accurate for all analytes. The limits of detection for the analytes (S/N = 3) were 0.4–0.7 μg/mL.

### 3.3.2. Application and recovery

The optimum conditions were applied to determination of lignans in the extracts of *S. chinensis* and *S. henryi*. The peaks were identified by spiking a small amount of each analyte into the sample solutions, and also by comparing their migration times with the migration time of the standards. The typical electropherograms were shown in Fig. 4B and C. The contents of the four lignans found in the seeds of *Schisandra* species together with RSDs ($n = 5$) were given in Table 2.

The recoveries of the method were determined with the standard addition method for lignans 1–4 in the extracts of *S. chinensis* and *S. henryi* with results of 103.70 and 97.01% for 1, 97.46 and 102.79 for 2, 98.23 and 105.87% for 3, 102.91 and 104.83% for 4, respectively. These data supported the suitability of the proposed MEKC method for its application to real samples. It was found that schisandrin did not present in the seeds of *S. henryi*. This result was confirmed by qualitative analysis using HPLC [45]. The results differs form that of Song et al. [45], which was most probably due to the different collection time or the different growing period of the plants.

## 4. Conclusion

In this study, ionic liquid was used as modifier in MEKC to separate lignans found in the seeds of *Schisandra* species. BMIM-$BF_4$ was found to be effective additive for improving the separation of lignans. The results demonstrated that the developed method, for their rapidity and the high selectivity, was very suitable for the fast determination of different *Schisandra* species. This technique was shown to provide a complementary method to MEKC for the separation of members of this class of compounds using capillary electrophoresis.

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