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Application of capillary electrochromatography using macroporous polyacrylamide columns for the analysis of lignans from seeds of *Schisandra chinensis*

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Abstract

Capillary electrochromatography (CEC) using polymer-based monolithic stationary phase has been developed as a promising method for the determination of lignans of *Schisandra chinensis*. The columns were prepared by in situ copolymerisation of acrylamide, *N,N′*-methylenebisacrylamide, vinylsulfonic acid and lauryl acrylate in presence of poly(ethylene glycol) as a porogenic agent. The columns [33 cm (24.5 cm effective length)×75 μm I.D.] were successfully used to analyse and quantify the major lignans in extract of the seeds of *Schisandra chinensis*. Good separations were achieved in less than 35 min. The calibration graphs were linear in the range 0.025–1.0 mg/ml of given lignan with correlation coefficients between 0.9951 and 0.9996. The inter-day reproducibility of the peak area were below 3.9% and the inter-day reproducibility of the migration time were below 4.2%. The results of quantitative CEC analyses were compared with those obtained by reversed-phase HPLC, the levels of schizandrin, gomisin A, gomisin N and wuweizisu C determined by CEC were in a good agreement with those determined by HPLC.

Keywords: Electrochomatography; *Schisandra chinensis*; Plant materials; Stationary phases, electrochromatography; Lignans

1. Introduction

Schisandra chinensis Baill. (Schisandraceae/Magnoliaceae) [1] is a famous medicinal plant native to East Asia. It grows wild mainly in the most Eastern parts of Russia, the Kuril Islands, southern Sachalin and also north-eastern China, Korea and Japan. The biologically active compounds are the lignans with an 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].

The lignans are present in different amount in all parts of the plant. The highest concentration about 4% dry weight has been found in seeds, up to 2% is present in fruits. Several analytical methods have

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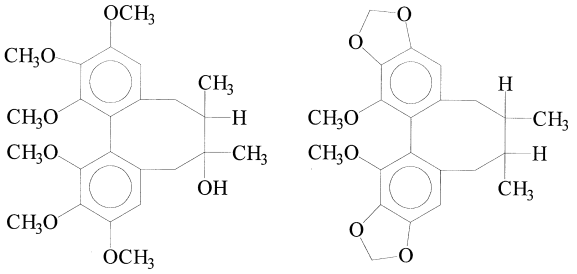

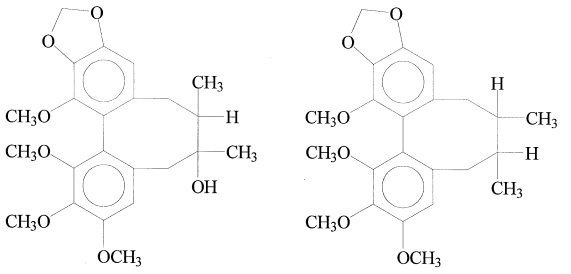

Fig. 1. Structure of the major lignans in *Schisandra* extract.

been reported for the determinations of the lignans in *Schisandra* plant. Previously described methods include thin-layer [12] and high-performance liquid chromatographic [13,14] techniques. Gas [15] and high-performance liquid [16] chromatography in combination with mass spectrometry have also been demonstrated to be effective analytical method for the determination of the lignans in various samples.

Capillary electrochromatography (CEC) is a hybrid separation technique that combines the benefits of both capillary zone electrophoresis and high-performance liquid chromatography. The high efficiency and resolution of CEC offers an alternative to pressure-driven chromatography systems. Most of the reported work concerning CEC separation and analysis so far has been focused on the use of particle packed capillary column. Several methods for CEC columns packing have been used, some of them similar to procedures used in micro liquid chromatography (μLC) [17–19]. Also new ways have been developed employing supercritical fluids [20] and electrokinetic packing [21,22]. All these procedures are laborious and need high experimental skill for reproducible columns packing and especially retaining frits preparation. Moreover the problems associated with gas bubble formation and heterogeneity of electric field have to be eliminated as well. Recently introduced polymer-based monolithic capillary columns quickly become well-established stationary phases in CEC [23–30] as they eliminated the need of frits and packing procedures. The large variety of available chemistries make these columns an attractive alternative to columns packed with particulate materials.

Several applications on CEC in polymer-based monolithic capillary columns have been reported. However, most studies have considered measurements of standard solutions and only few have included real samples [31,32]. In this study we report CEC using macroporous polyacrylamide columns for the determination of several lignans — schizandrin, gomisin A, gomisin N and wuweizisu C, the major compounds of the *Schisandra* extracts. The reproducibility of migration time and peak area and linearity of detection were also investigated. The results of CEC analyses were compared with those obtained by HPLC.

2. Experimental

2.1. Materials and reagents

The standard compounds, schizandrin, gomisin A, gomisin N and wuweizisu C, were isolated at the Department of Biochemistry, Faculty of Medicine, Masaryk University, Brno, Czech Republic. The lignans were unambiguously identified by ^{1}H NMR and electron impact ionization (EI) MS spectra and by comparison of spectral data, melting points and optical rotation with those originally reported [33–35]. Plant samples were obtained from the Centre of Medicinal Plants, Faculty of Medicine, Masaryk University, Brno, Czech Republic.

Acrylamide, *N*,*N*′-methylenebisacrylamide, ammonium persulfate and poly(ethylene glycol) M_r 10 000 were supplied by Sigma (St. Louis, MO, USA). *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), *N*-methylformamide, acetonitrile, vinylsulfonic acid (sodium salt) and lauryl acrylate were purchased from Aldrich (Milwaukee, MI, USA). All

other chemicals and solvents were of analytical reagent grade and were used without further purification. All solutions were prepared with Milli-Q Academic water (Millipore, Milford, WA, USA) and filtered through a 0.45 μm membrane filter.

2.2. Sample preparation

2.2.1. Lignan stock solutions

Lignan standards were dissolved in methanol to make each stock solution (5 mg/ml) and stored at −4°C.

2.2.2. Plant extract

Supercritical carbon dioxide was used to extract lignans from seeds of *Schisandra chinensis* [36,37]. A 0.5-g sample of pulverised seeds was extracted with supercritical CO_2 (temperature of extraction 50°C, pressure 40 MPa, the flow-rate of CO_2 250 ml/min) for 60 min. The trapping device consisted of a thermostated vial filled with 6 ml of dichloromethane. The extract was evaporated to dryness, dissolved in 1 ml of methanol and applied onto a column containing 500 mg of silica gel Separon SGX C_{18} (Tessek, Prague, Czech Republic) for removal of highly nonpolar compounds such as lipids. The lignans were eluted with 10 ml of methanol. The methanolic solution was diluted with mobile phase (1:1) before CEC or HPLC analysis.

2.3. Column preparation

The columns were prepared according the slightly modified procedure described by Que et al. [32] (see below). Fused-silica tubing (Polymicro Technologies, Phoenix, AZ, USA) with an inner diameter of 75 μm was used as the separation capillaries.

2.3.1. Activation of capillary tubing

The inner wall of the capillary was statically treated with 1 *M* sodium hydroxide for 2×15 min, then with 0.1 *M* hydrochloric acid for 30 min and finally rinsed with water for 30 min. Thereafter 50% 3-methacryloxypropyltrimethoxysilane (solution in acetone) was introduced and left inside the capillary for 2×30 min. Finally the capillary was rinsed with acetone and water.

2.3.2. Polymerisation procedure

Acrylamide (30.2 mg), 60 mg *N*,*N*′-methylenebisacrylamide, 24.8 μl solution of vinylsulfonic acid (25% solution in water), 60 mg poly(ethylene glycol) and 24.5 μl lauryl acrylate were dissolved in 1.85 ml of *N*-methylformamide and 100 μl 200 m*M* Tris–300 m*M* boric acid (pH 8.2). A freshly prepared solution was filtrated and deoxygenated by purging with nitrogen for 30 min. Following the addition of 2 μl of TEMED and 5 μl of 30% ammonium persulfate to 250 μl of the above monomer solution and the rapid mixing, the solution was introduced into the freshly activated capillary under water in bath at 20°C. The capillary was filled up completely. The polymerisation proceeded overnight at temperature 20°C. The polyimide coating in the place of the detection window was removed by a razor blade.

Before use, 1 cm sections at the capillary ends were removed and the column was subsequently washed with water and mobile phase by applying a pressure and finally conditioned at increasing field strengths (50–850 V/cm) for 5 h. Care must be taken not to expose the capillary ends to air, since the air bubbles destroy the gel matrix.

2.4. Instrumentation

2.4.1. Capillary electrochromatography

A Hewlett-Packard ^{3D}Capillary Electrophoresis system (Waldbronn, Germany) with a diode-array UV–Vis detector was used to carry out all separations. Data were collected on HP Vectra VL 5 166 MHz personal computer using the Hewlett-Packard ^{3D}CE ChemStation Software.

Injections were accomplished by an application of 5 kV (positive polarity) for 2.0 s. Separations were performed at 22.5 kV (positive polarity) and the temperature of column 25°C. Samples were detected using a diode-array detector at 200 nm with a bandwidth 10 nm using on-gel approach.

2.4.2. High-performance liquid chromatography

The HPLC system consisted of a high-pressure pump HPP 5001 and a UV detector LCD 2040 (Laboratorní Přístroje Prague, Czech Republic). A Rheodyne 7125 sampling valve (Cotati, CA, USA) equipped with a sample loop of 20 μl was used for sample injection. A separation column Separon SGX

C_{18} 5 μm (150×3 mm I.D.) from Tessek was used for all analyses. The mobile phase consisted of methanol–deionised water (75:25). The flow-rate was 0.3 ml/min, detection was performed at 254 nm. Data were evaluated using CSW Chromatography Station (DataApex, Prague, Czech Republic).

2.5. Peak identification

Peak identifications were accomplished by comparing both migration times and UV spectra of suspected peaks with those of authentic standards. In addition, spiking samples with the standards further supported the identities of lignan peaks.

3. Results and discussion

The macroporous polyacrylamide columns for CEC were prepared according to the procedure developed by Que et al. [32]. In order to improve the reproducibility of the column preparation several parameters affecting the polymerisation were studied: (i) the presence of dissolved oxygen in polymerisation mixture; (ii) the concentrations of catalyst and initiator; and (iii) the temperature of polymerisation. The best reproducibility in the column characteristics was achieved when the oxygen was removed by purging the monomer and ammonium persulfate solutions by nitrogen for 30 min, using lower concentration of ammonium persulfate and by keeping the temperature during the capillary filling and during the polymerisation on 20°C.

The overall performance of the columns was tested by analysing the samples of alkyl phenones (acetophenone, propiophenone and butyrophenone) and thiourea as an electroosmotic flow (EOF) marker, with both acetonitrile and methanol used as a mobile phase modifier. Higher efficiency up to 300 000 theoretical plates/meter for acetophenone was achieved using acetonitrile, also the peak symmetries were better with the values between 0.8 and 1.1 (data not shown). A partial explanation could be found in 2 times higher linear velocity of EOF in presence of acetonitrile in contrast with methanol (Fig. 2). The same behaviour was observed with particle-packed capillary columns [38]. Moreover, acetonitrile has also lower viscosity and UV absorbance and consequently it was chosen as a mobile phase modifier for separation of *Schisandra* extract.

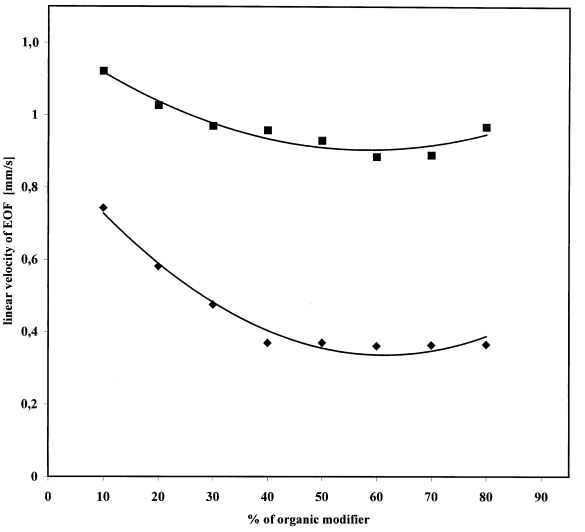

Fig. 2. Effect of organic modifier on the linear velocity of EOF. Experimental conditions: mobile phase: given concentration of acetonitrile (■) or methanol (♦) in 10 m*M* Tris–15 m*M* borate pH (8.2). Thiourea was used as a EOF marker. Other conditions as described in Section 2.

A preliminary investigation was performed varying the concentration of acetonitrile in mobile phase to optimise the separation. Fig. 3 shows that the increasing concentration of the organic modifier resulted in shorter migration times. In addition higher peaks and higher separation efficiency were achieved due to the smaller peak widths. These also confirmed that the separation mechanism is primarily based on differences in hydrophobic interactions for all of the compounds. Because of suitable resolution of lignans and adjacent peaks at the concentration of acetonitrile in mobile phase of 30%, no further optimisation of separation parameters was performed.

The typical electrochromatogram of the extract of *Schisandra chinensis* seeds under conditions of the mobile phase containing 30% of acetonitrile in 10 m*M* Tris–15 m*M* borate (pH 8.2), 22.5 kV separation voltage (positive polarity) and temperature of column 25°C is shown in Fig. 4. The same sample was also analysed by reversed-phase HPLC (Fig. 5) As seen from these figures, higher efficiency of CEC

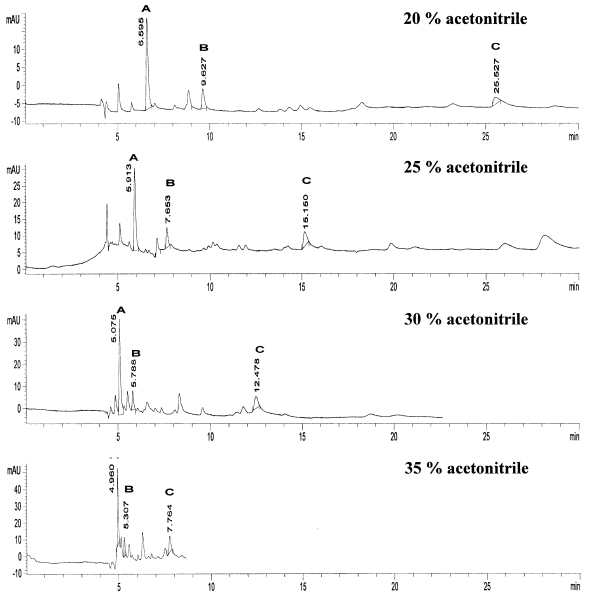

Fig. 3. Effect of acetonitrile concentration on CEC separation of *Schisandra* extract. Experimental conditions: macroporous polyacrylamide column 33 cm (24.5 cm effective length)×75 μm I.D., 375 μm O.D.; mobile phase given concentration of acetonitrile in 10 m*M* Tris–15 m*M* borate (pH 8.2); electrokinetic injection, 5 kV (positive polarity) for 2.0 s; separation voltage, 22.5 kV (positive polarity); temperature of column, 25°C; detection at 200 nm with a bandwidth of 10 nm. Peak identifications: (A) schizandrin, (B) gomisin A and (C) deoxyschizandrin.

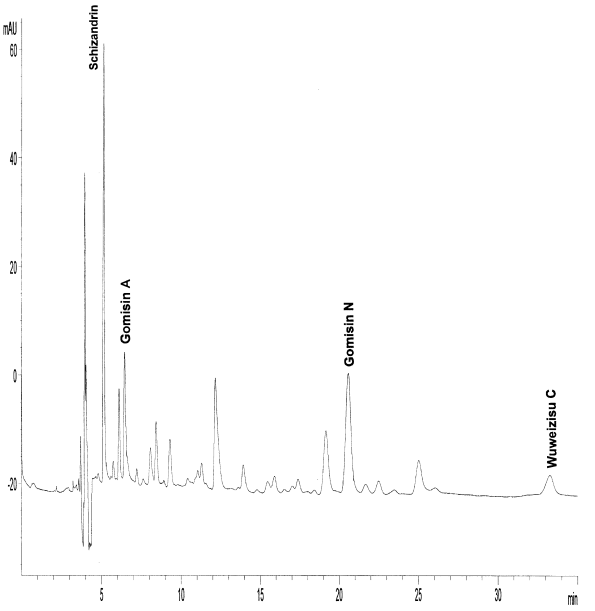

Fig. 4. Typical electrochromatogram of *Schisandra* extract. Conditions as in Fig. 3, except that the acetonitrile in the mobile phase has been changed to 30%.

allowed baseline resolution all of lignans whereas by HPLC only wuweizisu C was fully resolved.

Reproducibility and linearity of the CEC method were tested by the analysis of pure lignans. Table 1 shows good inter-day reproducibility obtained for the peak area and the migration time. The calibration graphs were linear over the studied concentration range of 0.025–1.0 mg/ml of given lignan with correlation coefficients between 0.9951 and 0.9996 (Table 1).

Finally, to demonstrate the applicability of the developed CEC method for quantitative analysis, both CEC and HPLC were used to determinate the concentrations of the lignans in extract of *Schisandra* seeds. The levels of schizandrin, gomisin A, gomisin N and wuweizisu C in the extract determined by CEC were in a good agreement with those determined by HPLC (Table 2) and with the literature data [36].

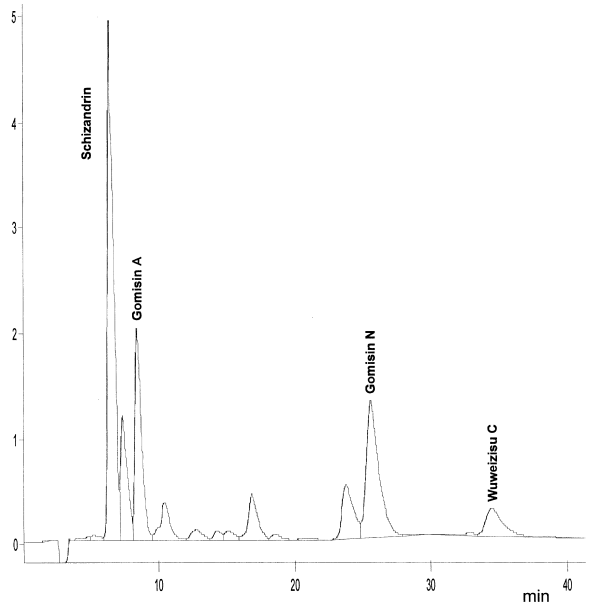

Fig. 5. HPLC analysis of *Schisandra* extract. Experimental conditions: column, Separon SGX C_{18} 5 μm, 150×3 mm I.D.; mobile phase, methanol–deionised water (75:25); flow-rate, 0.3 ml/min; detection at 254 nm. Twenty μl of *Schisandra* extract were loaded on the column.

Table 1
Parameters of the developed method[a]

Parameter	Schizandrin	Gomisin A	Gomisin N	Wuweizisu C
Migration time (min)	5.13	6.45	20.57	33.26
Migration time reproducibility (%, $n=10$)	1.43%	1.92%	4.10%	2.29%
Peak area reproducibility (%, $n=10$)	3.18%	2.05%	3.81%	2.30%
Linearity (mg/ml)	0.025–1.00	0.025–1.00	0.025–1.00	0.025–1.00
Correlation coefficient	0.9951	0.9965	0.9971	0.9996

[a] Samples: lignan standards dissolved in mobile phase. Separation conditions: macroporous polyacrylamide column, 33 cm (24.5 cm effective length)×75 μm I.D., 375 μm O.D.; mobile phase, 30% of acetonitrile in 10 m*M* Tris–15 m*M* borate (pH 8.2); electrokinetic injection, 5 kV (positive polarity) for 2.0 s; separation voltage, 22.5 kV (positive polarity); temperature of column, 25°C; detection at 200 nm with a bandwidth of 10 nm.

4. Conclusion

The CEC method using macroporous polyacrylamide monolithic columns was developed for the determination of the lignans in *Schisandra chinensis*. The study has shown that CEC on polymer columns prepared in laboratory can be applied successfully to analyse and quantify the lignans in plant seeds. Further the technique offers high separation efficiency and resolution, good precision and reduced operation cost. All these are advantages over traditional chromatographic procedures. The polymer monolithic stationary phases thus represent perspective separation media for capillary electrochromatography as the tedious packing of particles into narrow capillaries and the fabrication of frits are avoided.

Acknowledgements

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Table 2
Contents of the lignans in the *Schisandra chinensis* seeds determined by CEC and HPLC

Method	Content (% of dry mass)			
	Schizandrin	Gomisin A	Gomisin N	Wuweizisu C
CEC	1.03±0.3	0.53±0.01	0.75±0.03	0.21±0.01
HPLC	1.29±0.05	0.61±0.02	0.88±0.04	0.25±0.01

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