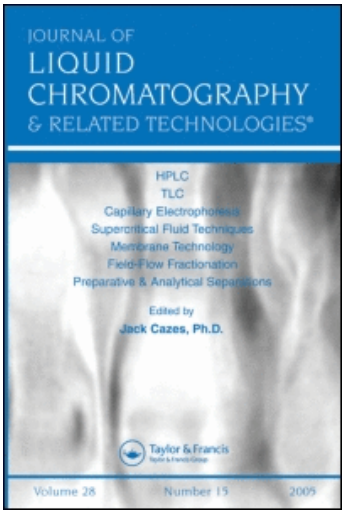

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DETERMINATION OF GENTIOPICROSIDE IN EXTRACTS OF *CENTAURIUM ERYTHREAE* AND *GENTIANA LUTEA* BY MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY

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ABSTRACT

Micellar electrokinetic capillary chromatography has been developed as a promising method for the determination of gentiopicroside in plant samples. The separation conditions have been optimised with respect to the different parameters including SDS concentration, pH, and ion strength of the background electrolyte, and temperature of capillary. A buffer consisting of 100 mM SDS in 20 mM sodium dihydrogen phosphate, 20 mM disodium tetraborate pH 8.6 was found to be the most suitable electrolyte for this separation. The applied voltage of 28 kV (positive polarity) and the temperature of capillary 20°C gave the best analysis of gentiopicroside. The linear detection range for con-

centration *versus* peak area for the assay is from 5 to 500 μg . mL^{-1} (correlation coefficient 0.9998) with a detection limit of 0.3 μg . mL^{-1}. The inter-day reproducibility of the peak area was below 2.5% and the inter-day reproducibility of the migration time was below 0.16 %.

INTRODUCTION

Secoiridoids are a group of chemically related monoterpene glucosides that were isolated from plants of the *Gentianaceae* family.[1,2] These glucosides - sweroside, swertiamarin, and gentiopicroside - possess a bitter taste (Fig. 1). The crude drugs of these plants have been used as a stomachics or stimulants of appetite. In addition, they have a variety of biological effects such as antiphlogistic,[3] fungitoxic,[4] and hepatoprotective activity.[5]

Several analytical methods have been reported for the determinations of these bitter components in the crude drugs. Previously described methods include paper[6] and thin layer[7-10] chromatographic techniques. High performance liquid chromatography has also been demonstrated to be an effective analytical method for the determination of secoiridoids in various plant samples.[11-15] In this study we report a micellar electrokinetic capillary chromatography (MEKC) for the determination of gentiopicroside, the major constituent of the plant extracts. Gentiopicroside was chosen as a marker compound because in contrast with other secoiridoids it is commercially available in adequate purity.

EXPERIMENTAL

Materials and Reagents

Gentiopicroside was obtained from Carl Roth (Karlsruhe, Germany). Plant samples were obtained from the Centre of Medicinal Plants, Faculty of

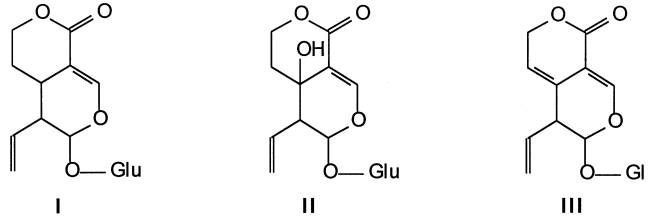

Figure 1. The chemical structures of sweroside (I), swertiamarin (II) and gentiopicroside (III).

Medicine, Masaryk University. All other chemicals and solvents were of analytical reagent grade, supplied from Fluka (Buchs, Switzerland). All solutions were prepared with Milli Q Academic water (Millipore, Milford, WA, USA) and filtrated through a 0.45 μm membrane filter.

Instrumentation

A Hewlett-Packard ^{3D}Capillary Electrophoresis System (Hewlett-Packard, 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.

A Hewlett-Packard extended light path capillary (50 μm I.D., 64.5 cm total length, 56.0 cm effective length) was used for all analyses. The capillary was washed with 0.1 M NaOH for 1 min, deionized water for 1 min and background electrolyte for 3 min before each run and washed with deionized water for 3 min after each run.

Injection was accomplished by an application of 50.0 mbar pressure to the inlet vial for 4.0 s. Separations were performed at 28 kV (positive polarity). Samples were detected using a diode-array detector at 275 nm with a bandwidth 20 nm. Spectra were also collected during the runs for peak identification.

Peak identification waFs accomplished by comparing both electrophoretic mobilities and UV spectra of suspected peaks with that of authentic standard. In addition, the identity of gentiopicroside peak was further supported by spiking samples with the standard.

Sample Preparation

A 500 mg of air dried and ground overground parts of the *Centaurium erythreae* respectively roots of the *Gentiana lutea* were placed in a 50-mL glass vial. After adding 15 mL of methanol, the vial was capped, placed in an ultrasonic bath containing water at ambient temperature and sonicated for 15 min. The extraction was repeated three times.[15] The extracts were combined and filtered through a 0.45 μm disposable filter. A 50F0 μL of the methanol extract was evaporated to dryness under vacuum. The residue was dissolved in 200 μL deionized water. The *Gentiana* extract was further diluted two times with deionized water.

RESULTS AND DISCUSSION

Preliminary experiments were conducted to determinate gentiopicroside in *Centaurium* extract using capillary zone electrophoresis (CZE) conditions. Due to the neutral nature of gentiopicroside and other secoiridoids, a borate complexing running buffer was used for the separation of these glucosides. A buffer solution of 0.05 M boric acid (pH 8.5) was applied as a background electrolyte. Under this condition, secoiridoids with similar structures - sweroside and gentiopicroside still overlapped completely (data not shown). This was confirmed by their spectral identification using a peak purity utility of ChemStation software. As shown in Fig. 1, sweroside and gentiopicroside differ only by a double bond. Poor resolution of structurally similar compounds under aqueous conditions in CZE analysis was suggested by Fujiwara and Honda[17] to be a result of hydration. The efforts to improve their separation by changing the pH values (8.5 - 10.5) and the ion strength of the background electrolyte (0.05 - 0.2 M) and the temperature of capillary (20 - 60°C) were found to be invalid. The addition of various cyclodextrins (2% final concentration) to a borate buffer was also tested.[18] No matter which cyclodextrin, α-, β- or γ-CD was added, it did not affect the poor resolution of the sweroside and gentiopicroside pair.

The technique which gave successful separations of these compounds, was micellar electrokinetic capillary chromatography. The separation mechanism is primarily based on differences in hydrophobic interactions for all of the compounds. Fig. 2 shows the effect of SDS concentration on the separation of *Centarium* extract. As it can be seen, the best separation was obtained at SDS concentration of 100 mM. In addition pH and buffer concentration and temperature of capillary were further optimized to get a suitable resolution of gentiopicroside and adjacent peaks. The electropherogram of *Centaurium* extract under optimal conditions of 100 mM SDS in 20 mM sodium dihydrogen phosphate, 20 mM disodium tetraborate (pH 8.6), 28 kV separation voltage (positive polarity) and 20°C temperature of capillary is shown in Fig. 3. As seen, the baseline separation was obtained in 8 min.

Reproducibility, linearity, and sensitivity of the method were tested by the analysis of gentiopicroside standards. The results of replicated analyses (n = 10) show good reproducibility obtained for the peak area (< 2.5%) and excellent reproducibility obtained for the migration time (< 0.16%). The calibration graph was linear over the range 5 - 500 $\mu g \cdot mL^{-1}$ of gentiopicroside with correlation coefficient better then 0.999. The detection limit was in the range 0.3 $\mu g \cdot mL^{-1}$ at a signal-to-noise ratio of 3.

The effect of methanol in the injected sample on migration time and resolution was investigated due to the possibility of direct injection of *Centaurium* extract. As reported by Ackermans *et al.*[19] the use of organic solvents to dis-

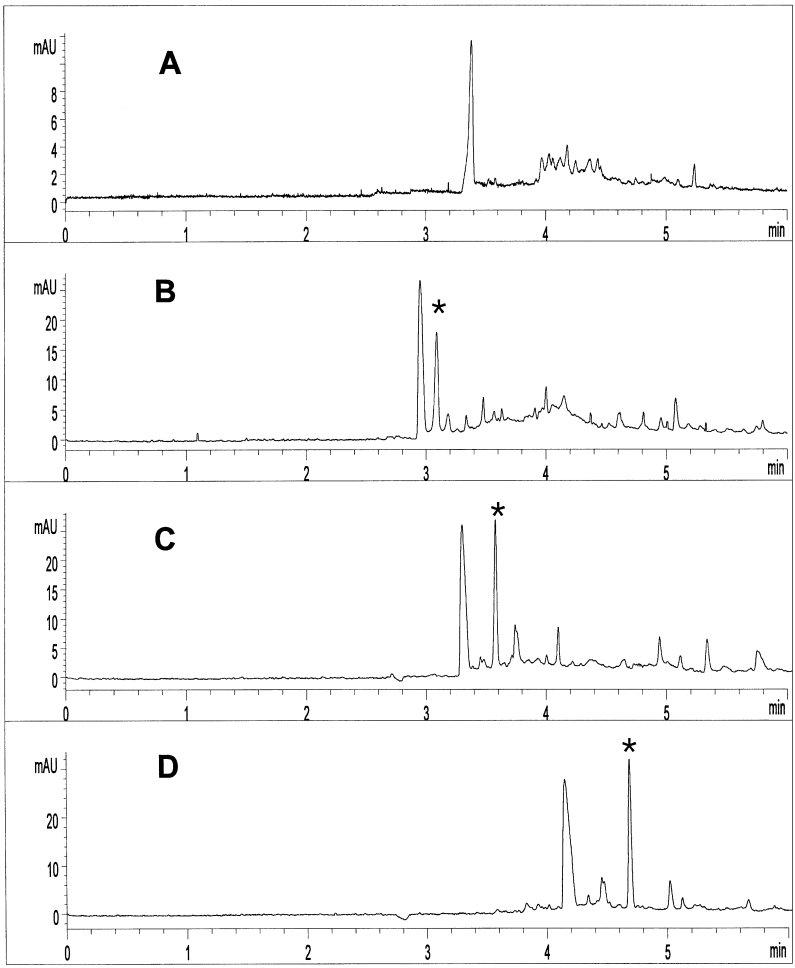

Figure 2. Effect of SDS concentration on separation of an extract of *Centaurium erythreae.* (A) 0 mM SDS; (B) 25 mM SDS; (C) 50 mM SDS or (D) 100 mM SDS was added to the separation buffer 20 mM sodium dihydrogen phosphate, 20 mM disodium tetraborate pH 8.6. Injection 50.0 mbar for 4.0 s; separation voltage 28 kV (positive polarity); detection 275 nm; temperature of capillary 30°C.

solve the sample can significantly influence the separation. The methanolic extract was evaporated to dryness and the residue was dissolved in a water solution containing different concentrations of methanol. We found that even the presence of 10% methanol in a sample has a drastic effect on the separation,

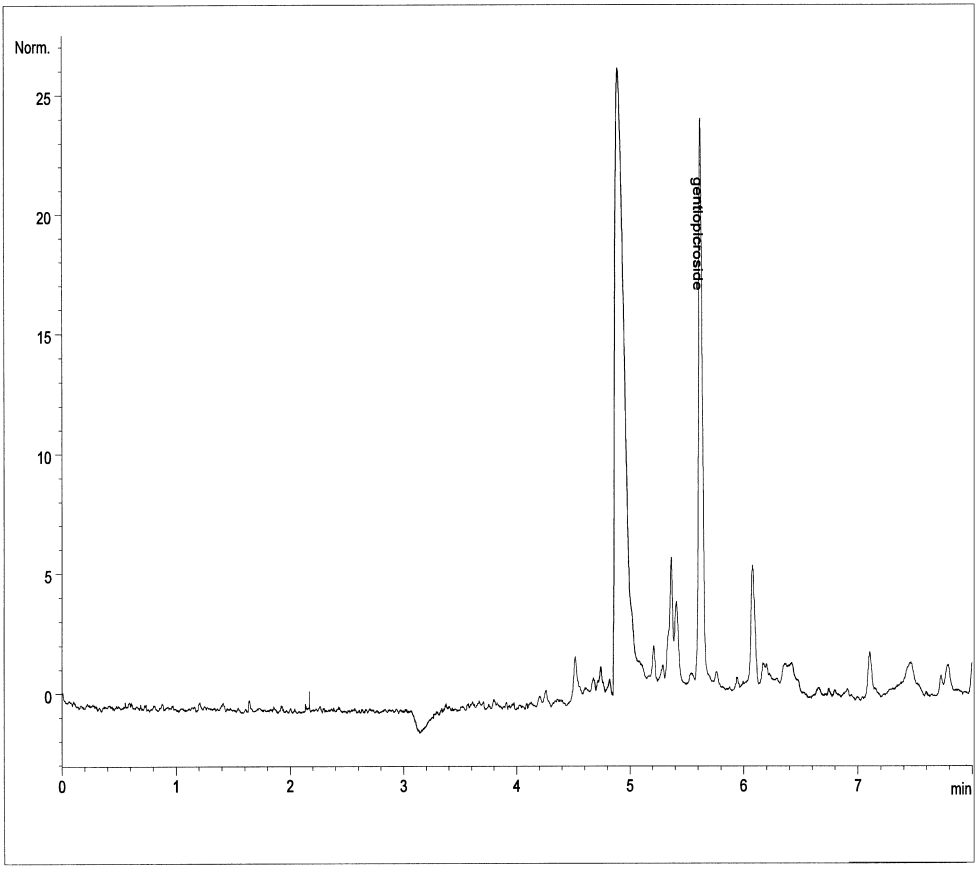

Figure 3. Electropherogram of the extract of *Centaurium erythreae* analysed by MEKC under optimal conditions: separation buffer 100 mM SDS in 20 mM sodium dihydrogen phosphate, 20 mM disodium tetraborate pH; temperature of capillary 20°C. Other conditions as in Fig. 2.

because the organic solvent modifies the interaction between micelles and analytes. These investigations show that the matrix have a strong influence on separation in MEKC especially in the case of hydrophilic components such as secoiridoids. The presence of methanol in samples thus must be eliminated prior to analysis.

The recovery of gentiopicroside by the described method was tested by adding known amounts of gentiopicroside to the methanolic extracts of

Centaurium species containing a known level of this compound. The recovery was estimated to be from 98 to 103%.

In order to demonstrate the application of the developed method for the analysis of gentiopicroside, the *Gentiana* extract was also analysed as described (Fig. 4). Table 1 lists the determined values of gentiopicroside in the oveground parts of *Centaurium erythreae* and in the roots of *Gentiana lutea*. The levels of gentiopicroside determined by MEKC in the plant extracts are in a good agreement with those determined by HPLC.[13, 15]

In conclusion, the study has shown that MEKC can be applied successfully to determine gentiopicroside in plant samples. Further the technique offers

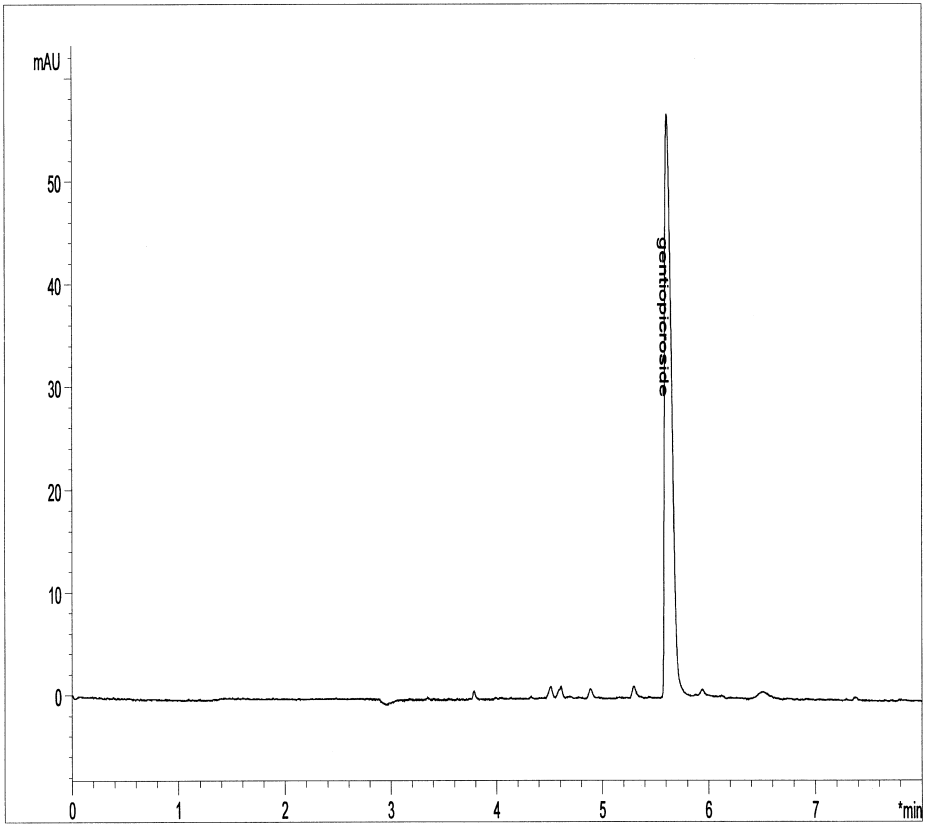

Figure 4. Electropherogram of the extract of *Gentiana lutea* analysed by MEKC. Separation conditions as in Fig. 3.

Table 1

Contents of Gentiopicroside in Plant Samples

Sample (n=5)	Concentration of Gentiopicroside (mg per g of Dried Plant Sample)
Centaurium erythrae	5.81 ± 0.145
Gentiana lutea	52.08 ± 1.30

high separation efficiencies, rapid analyses, low running costs, and is aqueous rather than organic solvent based. All these are advantages over traditional chromatographic procedures.

ACKNOWLEDGMENT

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