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Micellar Electrokinetic Chromatographic/ Ultraviolet Diode Array Analysis of *Eleutherococcus senticosus*

P. G. Pietta†*, P. L. Mauri‡, C. Gardana† and L. Zini†

† Università degli Studi di Milano, Via Celoria, 2-20133 Milan, Italy
‡ ITBA-CNR, Via Ampere, 56-20131 Milan, Italy

The main components of the roots of *Eleutherococcus senticosus* have been separated by capillary electrophoresis in the micellar mode. The best separation was achieved using a fused silica capillary tube and a 25 mM sodium borate buffer (pH 8.25) containing sodium dodecyl sulphate (SDS; 50 mM) at a constant voltage of 280 V/cm. The quantitative determination of eleutheroside B and E in *E. senticosus* extracts and formulations demonstrates the applicability of this method.

Keywords: Capillary electrophoresis; *Eleutherococcus senticosus*; Araliaceae; eleutherosides.

INTRODUCTION

Capillary electrophoresis (CE) is a powerful analytical separation technique which has become an important tool in peptide and protein chemistry (Deyl and Struzinsky, 1991). In addition to the special selectivity, the CE method provides extreme sensitivity, short separation times, small sample requirements and practically no waste production. Moreover, CE in the micellar mode (MEKC) (Terabe *et al.*, 1985) provides an alternative to reverse phase-high pressure liquid chromatography (RP-HPLC) as the analytes separate under the influence of a high-voltage potential (10–20 KV) as a result of differences in partitioning into and out of the hydrophobic core of the micelles. Therefore, it can be considered as an ideal complement to RP-HPLC. In spite of these advantages, a limited number of papers have been published so far (Pietta *et al.*, 1991; Seitz *et al.*, 1992; Stuppner *et al.*, 1992) and only recently MEKC combined with diode array detection (DAD) (Pietta *et al.*, 1992) has been proposed for the analysis of natural plant constituents.

Eleutherococcus senticosus Rupr. et Maxim. (syn. *Acanthopanax senticosus* Rupr. et Maxim.) Harms (Araliaceae), also known as 'Siberian Ginseng', has been reported as an 'adaptogen' and immunostimulant remedy (Farnsworth *et al.*, 1985; Sprecher, 1989). Thin layer chromatographic (TLC) and HPLC analysis of *E. senticosus* extracts have been described (Bladt *et al.*, 1990; Slacanin *et al.*, 1991), and eleutherosides B, E and chlorogenic acid (Fig. 1) have been detected as the main components, while other phenol carboxylic acids and coumarin derivatives were found in low amounts. Owing to the potential of MEKC as a complementary technique to RP-HPLC, different samples of *E. senticosus* extracts and formulations have been evaluated by means of MEKC/DAD and the results are reported in this paper.

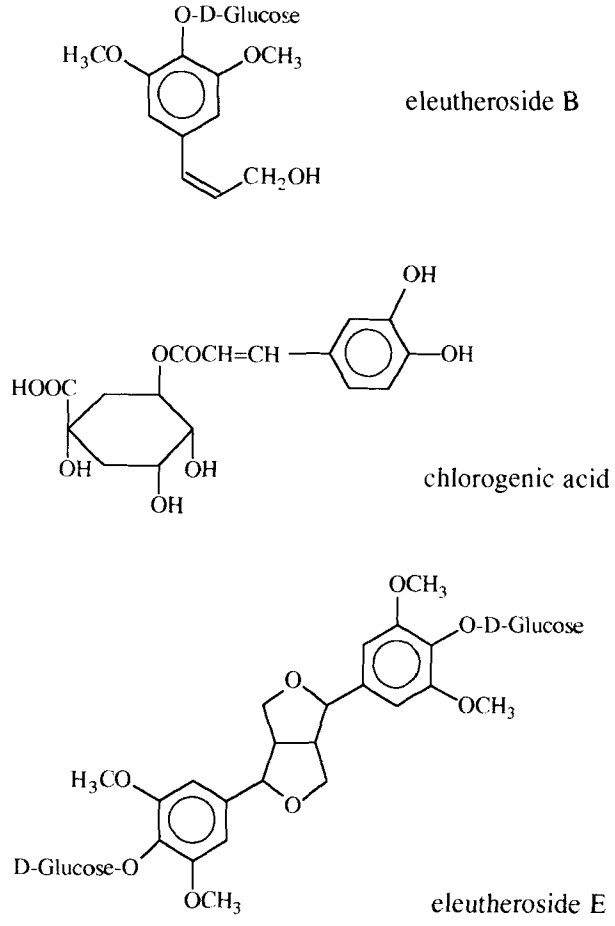

Figure 1. Structures of the main components in extracts of *Eleuthercoccus senticosus*.

EXPERIMENTAL

Micellar electrokinetic chromatography. MEKC was performed on an Eureka 2000 CE-DAD apparatus (Kontron Instruments, Milan, Italy) and on a 270A CE apparatus (Applied Biosystems S.r.l., Milano, Italy) equipped with a

* Author to whom correspondence should be addressed.

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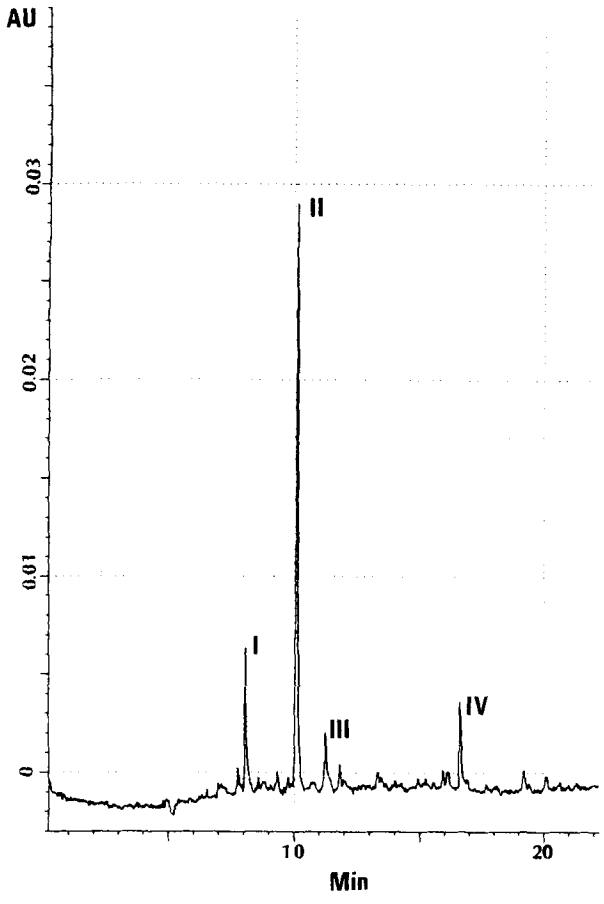

Figure 2. Electropherogram of a crude extract of *Eleuthercoccus senticosus*. Peak identity: I, eleutheroside B; II, chlorogenic acid; III, unknown phenolic acid derivative; IV, eleutheroside E. (For chromatographic protocol see Experimental section).

65 cm × 50 μm i.d. fused-silica capillary. The running buffer was 25 mM sodium borate (pH 8.25) containing sodium dodecyl sulphate (SDS; 50 mM). The voltage was 280 V/cm; the injection volume (by gravity in the 2000 CE-DAD apparatus and by aspiration in the 270A CE apparatus) was 4 nL, and the temperature was 25 °C.

Materials. Eleutheroside B and E were isolated from *E. senticosus*. Chlorogenic acid was purchased from Extrasynthese (Genay, France). *E. senticosus* extracts and formulations were obtained from various commercial sources (Galke, Gittelde/Harz, Germany; Giuliani SA Farmaceutici, Lugano, Switzerland; Frau s.r.l., Milano, Italy; NCE, Milano, Italy).

Sep-pak C_{18} cartridges (Millipore Waters, Milford, MA, USA) were used for MEKC sample preparation. Petroleum ether (b.p. 40–60 °C; analytical reagent grade) was purchased from J. T. Baker Chemicals (Deventer, Holland).

Sample preparation. *E. senticosus extracts.* An aliquot (40 mg) of extract was dissolved in 400 μL of 30% methanol and diluted to 1.5 mL with water. A 1 mL volume was applied to a previously activated Sep-pak C_{18} cartridge. After washing with water (4 mL) and 10% methanol (4 mL), the main fraction was eluted with 50% methanol (4 mL). The cartridge was then washed with 100% methanol (5 mL). Each eluate was evaporated to dryness under vacuum and the residue was dissolved in 300 μL of 30% methanol.

Capsules. An accurately weighed amount from five capsules corresponding to 40 mg of *E. senticosus* extract was introduced into a centrifuge tube and extracted with petroleum ether (3 × 5 mL). The resulting powder was suspended in 400 μL of 30% methanol and diluted to 1.5 mL with water. The resulting sample was processed as described for *E. senticosus* extracts.

Fluids. A 0.5 mL volume of fluid (syrup, tonic, elixir) was

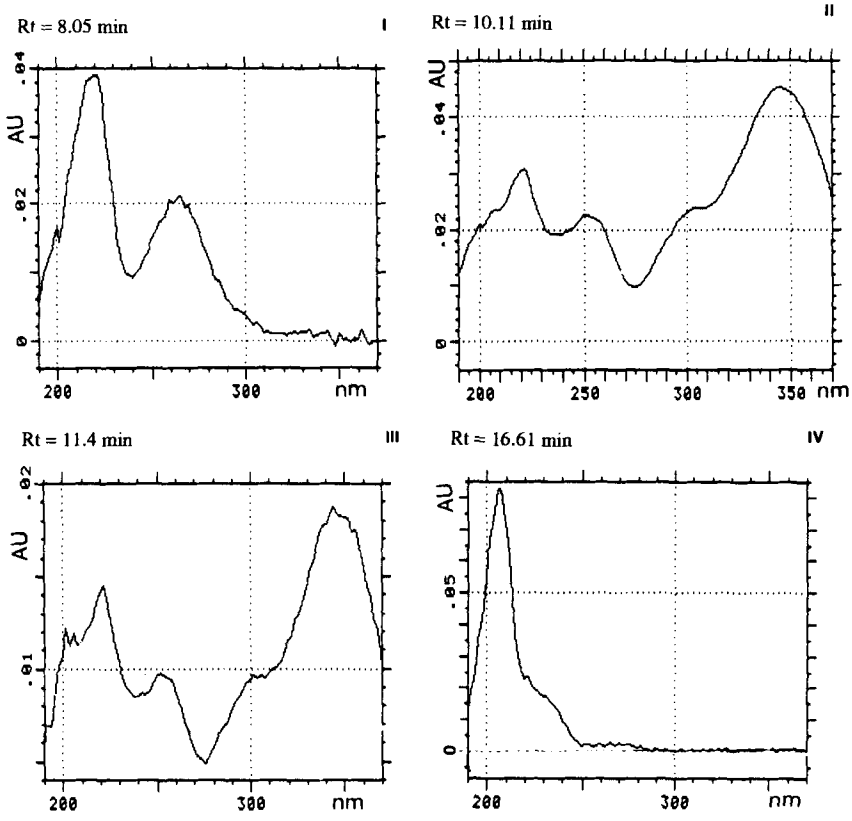

Figure 3. 'On-line' UV spectra of components of a crude extract of *Eleuthercoccus senticosus*. Peak identity and chromatographic detail are as in Fig. 2.

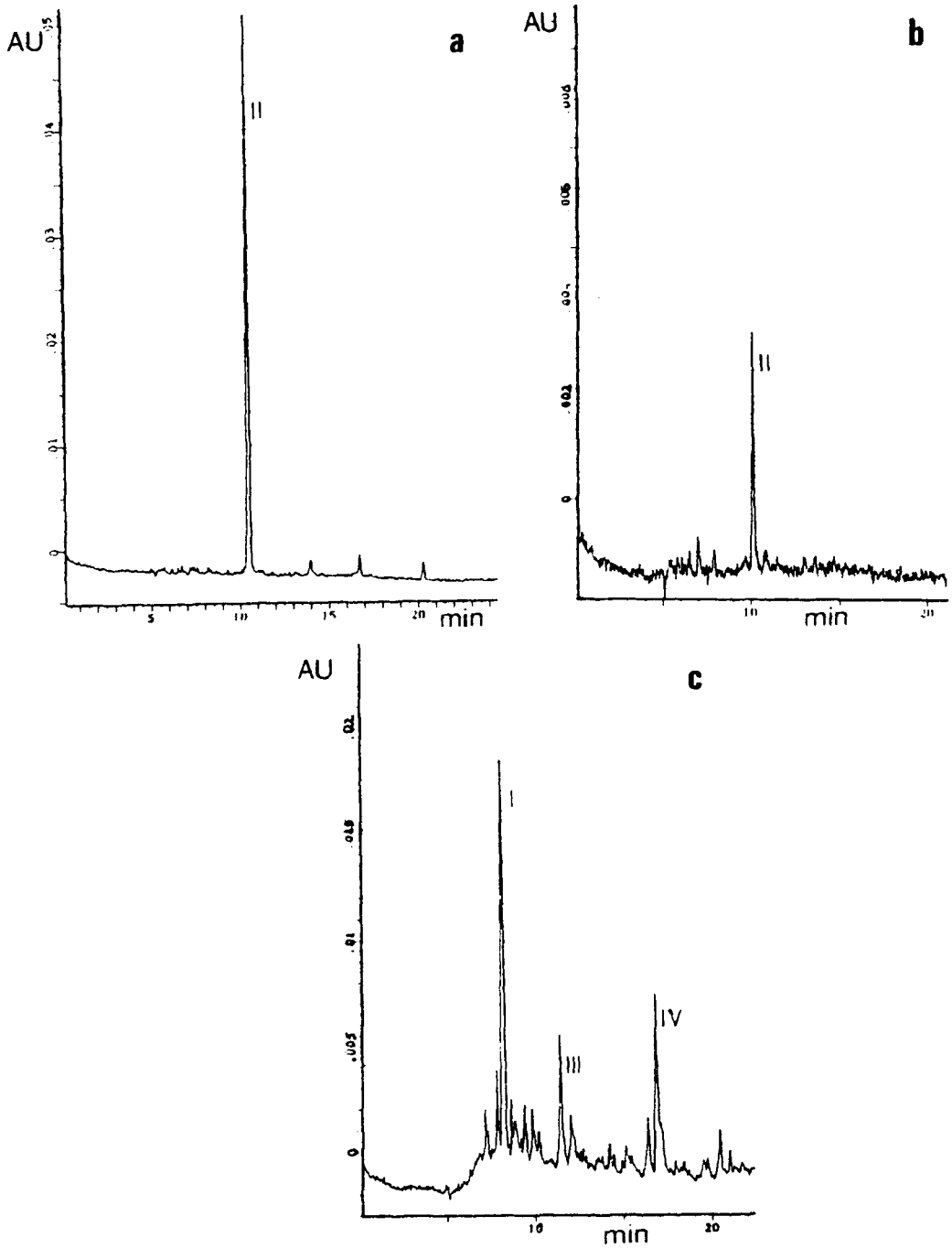

Figure 4. Purification of extract of *Eleuthercoccus senticosus* by C_{18} SPE cartridge. (a) Aqueous fraction; (b) 10% methanolic fraction; (c) 50% methanolic fraction. (For separation protocol see Experimental section: for peak identity see Fig. 2).

diluted to 3 mL with 10% methanol. A 1.5 mL volume was applied to a previously activated Sep-pak C_{18} cartridge. The resulting sample was treated as described for *E. senticosus* extracts.

Calibration graphs. Standard solutions of eleutherosides B and E each at a concentration of 1 mg/mL were prepared, and appropriate aliquots of these solutions were diluted with the same solvent to obtain reference solutions containing 0.04–0.2 mg/mL. Aliquots (4 nL) were injected into the CE. The peak areas were integrated against the corresponding masses of standard injected.

RESULTS AND DISCUSSION

A typical electropherogram of a crude extract from *E. senticosus* shows four main peaks (Fig. 2). Chlorogenic acid (peak II) is the major component, while eleutherosides B (peak I) and E (peak IV) are present in low amounts. The identities of these peaks has been ascertained by co-electrophoresis and comparison with the UV spectra of standards (Fig. 3). An unknown phenolic acid derivative (as suggested from the UV spectrum) is represented by peak III.

Owing to the large amount of chlorogenic acid in the crude extract, the sample was purified using a C_{18} SPE

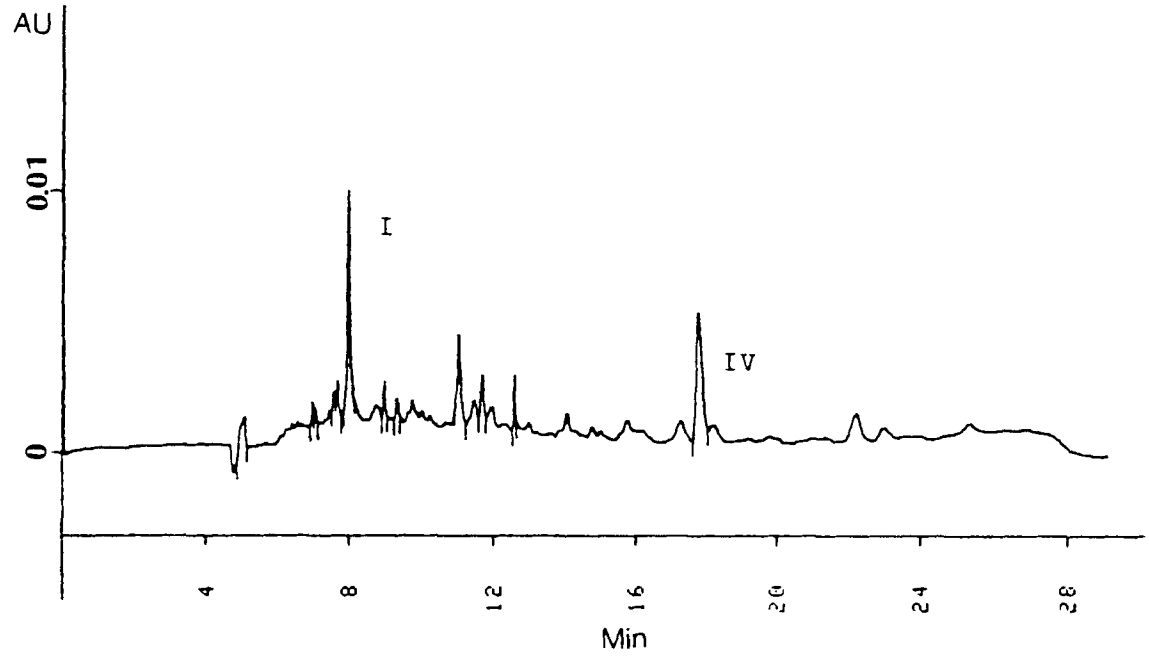

Figure 5. Typical electropherogram of the 50% methanolic fraction obtained from a commercial formulation of *Eleuthercoccus senticosus* (For fractionation and chromatographic protocols see Experimental section: for peak identity see Fig. 2).

Table 1. Percentages of eleutherosides B and E in commercial samples of *Eleutherococcus senticosus*

Samples	Eleutheroside B	Eleutheroside E	Total
A	0.023%	0.102%	0.125%
B	0.056%	0.149%	0.205%
C	0.004%	0.279%	0.282%
D	0.028%	0.091%	0.118%

cartridge. The aqueous and 10% methanolic fractions (Fig. 4a,b) contained only chlorogenic acid (peak II). The fraction eluted with 50% methanol (Fig. 4c) consisted mainly of eleutherosides B and E. The recovery of these components was almost quantitative as indicated by their absence in the 100% methanolic fraction.

Commercial samples of *E. senticosus* formulations were analysed by MEKC/DAD, and Fig. 5 shows a typical electropherogram of the 50% methanolic fraction. Eleutherosides B and E were quantified using external standardization. The calibration curves were linear in the range investigated (0.04–0.2 mg/mL). The linear regression equations are: $Y = 3.7X - 0.09$ ($R = 0.998$) for eleutheroside B, and $Y = 3.5X + 0.01$ ($R = 0.998$) for eleutheroside E. The minimum detectable amount was 0.01 ng for both the standards. As shown in Table 1, eleutheroside E is the main component in the investigated samples, while eleutheroside B is detected only in low amounts.

From these results it may be concluded that MEKC/DAD represents a valuable alternative to HPLC for the routine analysis of *E. senticosus* extracts and formulations.

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