ELSEVIER

Journal of Chromatography A, 945 (2002) 239–247

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Analysis of phenolic acids by micellar electrokinetic chromatography: application to *Echinacea purpurea* plant extracts

Romeo Pomponio, Roberto Gotti, Mohammad Hudaib, Vanni Cavrini*

Dipartimento di Scienze Farmaceutiche, Università di Bologna, Via Belmeloro 6, 40126 Bologna, Italy

Received 30 July 2001; received in revised form 2 November 2001; accepted 7 November 2001

Abstract

A micellar electrokinetic chromatographic (MEKC) method was developed for the separation of ten phenolic acids including cichoric acid and caftaric acids, specific marker phytochemicals of *Echinacea purpurea*. The MEKC method involved the use of 70 m*M* sodium deoxycholate (SDC) in 40 m*M* borate (pH 9.2) buffer and UV detection at 300 nm. The bile acid was used as biosurfactant able to provided a micellar system with different and more selective properties than sodium dodecyl sulfate. The effects of SDC and borate concentration and buffer pH on the analyte resolution were evaluated. The validated method was applied to the determination of cichoric acid and related compounds in *E. purpurea* root extracts, and in commercial *E. purpurea* based dried extracts and tablets.

Keywords: *Echinacea purpurea*; Pharmaceutical analysis; Plant materials; Phenolic acids; Echinaforce; Organic acids; Cichoric acid; Caftaric acid

1. Introduction

Phenolic acids are widespread secondary plant metabolites [1] and constitute an important part of the hydrophilic components of plant tissue extracts. These compounds can be responsible for the pharmacological activity ascribed to medicinal plant extracts and, therefore, the profile of their content represents a useful fingerprint to define the identity and the quality of these products (crude drug and phytopreparations). As a consequence, reliable and practical analytical methods for the determination of phenolic acids in complex natural matrices are of considerable interest. Among the chromatographic techniques, high-performance liquid chromatography (HPLC), under reversed-phase [2–14] or ion-exclusion [15,16] conditions, is commonly applied to the determination of phenolic acids in foods [4,5,14], plant sediments [6] and plant extracts [2,7–13,16], including *Echinacea* species [2,7–11].

Recently, capillary zone electrophoresis (CZE), due to its high-resolution separation and versatility, has become an effective alternative to HPLC for the separation of charged analytes. Application of CZE to the determination of phenolic acids in wines [17], soil [18] and plant extracts [19–21] have been reported. More recently, CZE with reversed electroosmotic flow (EOF) for the presence of a cationic surfactant in the buffer, has been applied to the separation of phenolic acids in soil and plant extracts [22,23]. When surfactants are added to the buffer at concentrations higher than their critical micellar

*Corresponding author. Tel.: +39-051-209-9731; fax: +39-051-209-9734.

E-mail address: vcavrini@alma.unibo.it (V. Cavrini).

0021-9673/02/$ – see front matter
PII: S0021-9673(01)01488-1

concentration, conditions are provided for micellar electrokinetic chromatography (MEKC), a technique able to separate both charged and uncharged analytes [24]. The MEKC approach has been applied to the analysis of phenolic acids in plant extracts, using sodium dodecyl sulfate (SDS) [20,25] or tetralkylammonium salts [26], as micellar systems.

In the present study, a MEKC system was developed, based on the use of sodium deoxycholate (SDC) as surfactant in pH 9.2 borate buffer, which enabled the complete separation of principal phenolic acids, including cichoric acid and caftaric acid, the typical hydrophilic components of *E. purpurea* extracts. Sodium deoxycholate (SDC) proved to be advantageous over sodium dodecyl sulfate (SDS) as source of negatively charged micelles in terms of better resolution. The optimised method, in particular, was applied to the determination of cichoric acid, known for its immunostimulatory properties, in *E. purpurea* plant roots and in commercially available *E. purpurea* based extracts and phytoproducts (tablets).

2. Experimental

2.1. Plant materials

About 2 kg entire plant materials (roots and tops) of *E. purpurea* plants grown in the open field at the Herb Garden of Casola Valsenio (Ravenna, Italy), were collected in October 2000 at almost the full growth of roots. Root materials were separated, washed, cut into small parts, air-dried and ground into small particles (0.5 mm) just before extraction. Moreover, commercial powdered *E. purpurea* extracts (1:4 ratio of the dried extract to starting crude plant roots) were obtained from Polichimica (Bologna, Italy).

2.2. Chemicals

Cichoric acid and caftaric acid were obtained from ChromaDex (Laguna Hills, CA, USA); caffeic acid, *p*-coumaric acid, ferulic acid, vanillic acid, *p*-hydroxybenzoic acid and deoxycholic acid sodium salt were purchased from Sigma (St. Louis, MO, USA); chlorogenic acid, syringic acid and 3,4-dihydroxybenzoic acid were from Fluka (Buchs, Switzerland). The echina-based commercial product (Echinaforce) was from Biohorma Italia (Pomezia, Italy). Sodium tetraborate, boric acid, methanol and all the other chemicals were purchased from Carlo Erba (Milan, Italy). The water used for preparation of the solutions and running buffers was purified by a Milli-RX apparatus (Millipore, Milford, MA, USA).

2.3. Capillary electrophoresis apparatus and conditions

Electrophoretic analyses were carried out using a Biofocus 2000 system (Bio-Rad, Hercules, CA, USA). An untreated, fused-silica capillary of total length 50 cm (effective length 43.5 cm×50 μm I.D.) was used for separation. The data were collected on a personal computer using a Biofocus System Integration Software Version 5.2. All separations were carried out at 20 °C with an optimized voltage maintained at 20 kV; hydrodynamic injections were performed at 5 p.s.i. for 2 s and the detection wavelength was 300 nm (1 p.s.i.=6894.76 Pa).

For the method optimisation, the considered concentration ranges of the background electrolyte (BGE) solution components were: sodium tetraborate (10–50 m*M*) and deoxycholic acid sodium salt (40–70 m*M*). The effect of pH value on the separation was studied within the range 8.80–9.40 adjusting the pH of sodium tetraborate with boric acid or sodium hydroxyde. Routine analyses were performed under the following conditions: 40 m*M* tetraborate (pH 9.2) and 70 m*M* deoxycholate. Prior to first use the capillary was conditioned by flushing sequentially 1 *M* sodium hydroxide, 0.1 *M* sodium hydroxide and finally water (10 min each). The capillary was equilibrated (10 min) at the beginning of the day with the running buffer. The repeatability of migration times was found to be strongly dependent on the rinsing procedure; the highest reproducibility of the migration times was obtained by flushing the capillary between the runs as follows: 1 min with methanol, 1 min with 0.5 *M* HCl, 1 min with methanol, 2 min with water and 5 min with BGE. Vials of BGE were replaced every two injections to keep the same reservoirs level of the buffer and to avoid changes of EOF due to the electrolysis of the solutions.

2.4. Calibration graphs

Stock solutions (1 mg/ml) of each phenolic acid were prepared in methanol and then diluted 1:10 with water to give the working standard solutions.

The linearity of the response was evaluated analysing standard solutions of vanillic acid (1.28–7.65 μg/ml), caffeic acid (0.760–4.58 μg/ml), chlorogenic acid (24.9–74.7 μg/ml), cichoric acid (20–40 μg/ml) and caftaric acid (5–15 μg/ml) in water–methanol (90:10, v/v), containing *p*-nitrobenzoic acid (the internal standard) at the fixed concentration of 5 μg/ml for caffeic acid and vanillic acid; 6 μg/ml for caftaric acid, 30 μg/ml for chlorogenic acid and 60 μg/ml for cichoric acid. Triplicate injections were made for each standard solution and the ratios of the corrected peak area (area/migration time) of drug to internal standard were plotted against the drug concentration to obtain the calibration graphs.

2.5. Extraction methods

2.5.1. Ultrasonic extraction

About 1 g accurately weighed amounts of dried ground root materials from plants were extracted three times in an ultrasonic bath (Bandelin Sonorex Super RK 103H, Berlin, Germany) for 5 min at room temperature, with 8 ml of 70% methanol in water. The extracts ($n=3$) were collected, filtered through 0.2 μm pore size membrane filter (GyroDisc, Orange Scientific, Waterloo, Belgium) and the volume was completed up to 25 ml to a final concentration corresponding to 0.04 g roots per ml. The same procedure was applied to about 0.250 g of commercial dried extracts and to 1 g of powdered commercial tablets.

2.5.2. Solvent-assisted microwave extraction

About 1 g accurately weighed samples of the dried *E. purpurea* roots were mixed with 20 ml volumes of 70% methanol and the final mixtures were extracted by a solvent-assisted microwave extraction (SAME) technique (Cem-Smart Microwave Extractor, USA) in closed vessels and under the following operating conditions: Temperature program was from 50 to 100 °C in 5 min and then maintained isothermal at 100 °C for 5 min. Pulsed microwave treatment was used (200 W maximum dose). After extraction, each sample was passed through 0.20 μm filter and the filtrate was completed up to 25 ml with the same extraction solvent to a final concentration equivalent to 0.04 g roots for ml. The final solutions were then subjected to capillary electrophoresis analysis.

2.6. Sample analysis

The developed MEKC method was applied to the analysis of sample solutions from: (a) *E. purpurea* roots; (b) commercial root extracts; and (c) commercial *E. purpurea*-based product (Echinaforce). All the sample solutions [prepared in methanol–water (70:30, v/v) as mentioned in Section 2.5] were appropriately diluted with water to obtain a final methanol–water ratio of 10:90. The tablets of the commercial product (Echinaforce) were subjected to the same ultrasonic extraction procedure used for the commercial dried root extract. The sample solutions were subjected to the MEKC analysis and the content of the main phenolic acid components was determined by comparison with an appropriate standard solution.

3. Results and discussion

3.1. Method development

The structures of the studied analytes (Fig. 1) would suggest a CZE under alkaline conditions as analytical procedure for their separation; in effect, all the negatively charged solutes exhibited cathodic migration due to the strong electroosmotic flow. However, using different alkaline running buffers (borate, phosphate) at various concentration no successful separation of the studied compounds was obtained. A representative separation obtained using 20 m*M* sodium tetraborate (pH 9.2) is shown in Fig. 2A. Therefore, an MEKC method based on the use of SDS as surfactant was applied; using alkaline solutions containing SDS, according to a previous report [25], the method performance was improved, but the complete separation of the examined analytes was not yet achieved (Fig. 2B). The replacement of SDS with sodium deoxycholate (SDC), a bile acid salt, resulted in a useful MEKC approach. In fact,

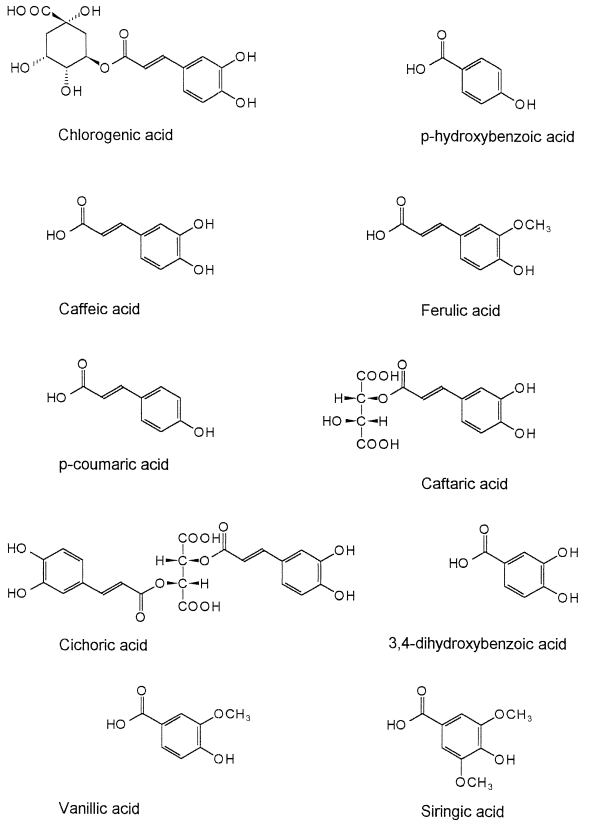

Fig. 1. Structures of the examined phenolic acids.

using SDC under the same experimental conditions, a more discriminating distribution of the anionic solutes into the negatively charged micelles, compared to SDS, was observed (Fig. 2C). Bile acids, having a large and rigid hydrophobic moiety of a steroid skeleton, in aqueous solution form small aggregates (micelles) for which various models have been proposed [27,28]. Actually, bile acids exhibit properties which considerably differs from the commonly used aliphatic surfactants (SDS); in fact, bile acids have been reported to provide different [27] or improved [29,30] selectivity than SDS in capillary electrophoresis. In the present application, the favourable effect obtained with SDC (Fig. 2B and C) could be ascribed to the hydrophilic character of the analytes whose distribution between micelles and aqueous phase is likely governed by the hydrophilic groups (two hydroxyl groups) of the surfactant [31].

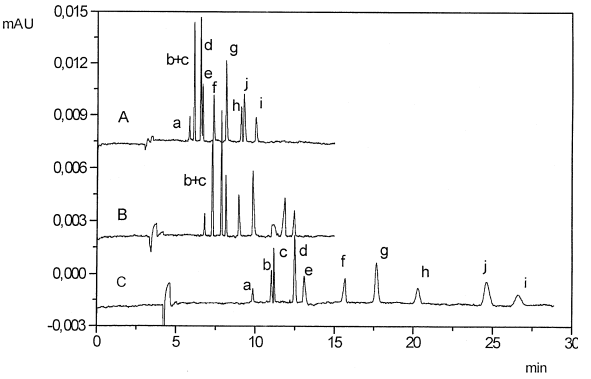

Fig. 2. Electrophoretic separation of: chlorogenic acid (a); siringic acid (b); ferulic acid (c); *p*-coumaric acid (d); vanillic acid (e); *p*-hydroxybenzoic acid (f); caffeic acid (g); cichoric acid (h); caftaric acid (i); and 3,4-dihydroxybenzoic acid (j), under different experimental conditions: A, CZE using 20 m*M* sodium tetraborate pH 9.2; B, MEKC using 20 m*M* sodium tetraborate pH 9.2 with 50 m*M* sodium dodecyl sulfate; C, MEKC using 20 m*M* sodium tetraborate pH 9.2 with 50 m*M* sodium deoxycholate. Other conditions: fused-silica capillary, total length 50 cm (effective length 43.5 cm×50 μm I.D.), injection of 5 p.s.i. for 2 s, voltage 20 kV, temperature 20 °C, detection wavelength 300 nm.

3.2. Method optimisation

The optimisation of the separation of the chosen phenolic acids was performed with the aim of developing a MEKC method of general applicability; particular attention was nevertheless focused on the specific separation of cichoric acid and caftaric acid, the marker phytochemicals of the polar fraction in the *Echinacea purpurea* extracts. In order to develop a method able to meet the previous requirements, the effects of the SDC and BGE concentration (40–70 m*M* range and 10–50 m*M* range, respectively) and of the pH value (range 8.8–9.4) on the migration times of the studied analytes were evaluated.

3.2.1. Sodium deoxycholate concentration

The surfactant concentration is an important parameter controlling the analysis selectivity. During the optimisation of the method, a crucial step was the separation between siringic acid and ferulic acid. The effect of SDC concentration was evaluated over the 40–70 m*M* concentration range, using 40 m*M* borate buffer (pH 9.2). In fact, at concentrations lower than 40 m*M* of SDC, the separation of the phenolic acids

deteriorated progressively with a general loss of resolution, while using 70 m*M* SDC, a resolution of 1.6 between siringic acid and ferulic acid was obtained. On the other hand, at concentrations higher than 70 m*M* the migration times and the measured currents significantly increased. The effect of SDC concentration on the resolution of the examined phenolic acids is illustrated in Fig. 3. For the routine analyses, 70 m*M* SDC was chosen.

3.2.2. Sodium tetraborate concentration

The role played by borate buffers in CE of polyhydroxy compounds has been investigated [18,32] and the use of this buffer, at appropriate concentration and pH values, has been found suitable to provide selective separation conditions. In the present application, sodium tetraborate showed a better behaviour compared to sodium dihydrogenphosphate. Actually, current and baseline were very stable and reproducible. The effect of sodium tetraborate concentration on the MEKC analysis was of particular interest because an increased resolution between cichoric acid and caftaric acid was obtained. In fact, under the chosen conditions (40 m*M* sodium tetraborate) caftaric acid migrated considerably later than cichoric acid (Fig. 4), with a significant improvement compared to previous data [23]. As shown in Fig. 4, with the exception of chlorogenic acid, all the dihydroxhy compounds migrated after the other studied phenolic acids. Particularly interesting was the behaviour of the cathecolic compounds

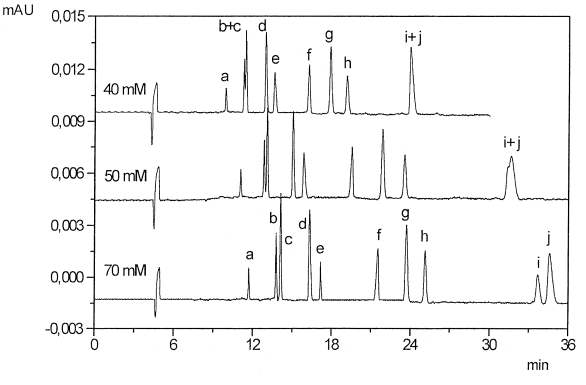

Fig. 3. Effect of the sodium deoxycholate concentration (40, 50 and 70 m*M*) in 40 m*M* (pH 9.2) borate buffer. Peak identities and other conditions as in Fig. 2.

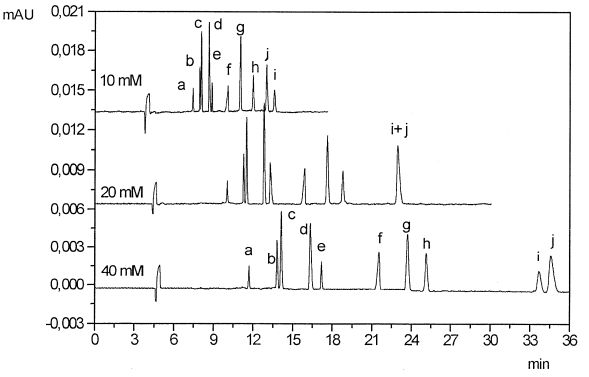

Fig. 4. Effect of the sodium tetraborate concentration using 70 m*M* SDC. Peak identities and other conditions as in Fig. 2.

3,4-dihydroxybenzoic and caffeic acid compared to the homologs *p*-hydroxybenzoic and *p*-coumaric acid, respectively. Although the cathecholic compounds exhibited an higher polarity than the corresponding monohydroxylated acids, their migration times were significantly longer. Besides, increasing the sodium tetraborate concentration from 10 to 50 m*M* an inversion of the migration times between caftaric acid and 3,4-dihydroxybenzoic acid was showed. From these observations, an active role on the separation by borate buffer can be confirmed especially regarding the BGE effects on the dihydroxy compounds behaviour. As the increase of the sodium tetraborate concentration remarkably increased the migration time values of all the analytes, with a particular effect on the *o*-dihydroxy compounds, a 40 m*M* concentration was chosen. However, for other applications lower concentrations (10 m*M*) could be used to perform more rapid analysis (Fig. 4).

3.2.3. Running buffer pH

Usually, the buffer pH is a very important parameter controlling the EOF and the ionization degree of each analyte. Anyway, due to their pK_a values (3.0–4.5), the phenolic acids were completely dissociated over the full studied pH range (8.8–9.4) and the measured EOF resulted almost constant; equally, constant migration times of dihydroxy acids were observed. Interestingly, all the other analytes (monohydroxy acids) exhibited a progressive increase of

migration with higher pH values. The observed behaviour of the monohydroxy compounds can be explained on the basis of the effects of pH value on the borate complexation [32]. This differentiated effects resulted in a variation of the resolution between various analytes. At pH 9.0, chlorogenic acid and siringic acid are overlapped, as well as *p*-coumaric acid and vanillic acid. Working at pH 9.0, siringic acid and ferulic acid were not completely separeted; besides *p*-hydroxybenzoic acid migrated as unsymmetrical peak. Using a pH above 9.4, siringic acid and ferulic acid, as well as caffeic acid and cichoric acid migrated almost overlapped. Therefore, within the studied pH range, the best results were at pH 9.2 which allowed a good compromise between the peak shapes and analysis time.

3.3. Method validation

The developed MEKC method was validated under the optimised experimental conditions (40 m*M*, pH 9.2 sodium tetraborate buffer, 70 m*M* SDC). The selectivity of the method was verified by analysing mixtures of pure and commercially available standard phenolic acids. The peak identity for the analysed samples was confirmed by migration time values and by the standard addition method. Multiple injections inter-day and intra-day of a single solution of all phenolic acids were performed to verify the repeatability of the migration times and the corrected peak area (area/migration time). The obtained RSDs of the migration times and peak area, obtained at the level of 10 μg/ml for all the analytes, are summarised in Table 1.

For quantitative applications, the response linearity was verified for the principal potential components of *E. purpurea* extracts (caffeic acid, chlorogenic acid, vanillic acid, cichoric acid and caftaric acid) using nitrobenzoic acid as the internal standard and measuring the absorbance at 300 nm. This wavelength value was selected on account of the absorption maxima of both caffeic acid derivatives (300–330 nm) and substituted benzoic acids (260–290 nm) [9,10,19]. The corrected peak area (analyte to internal standard) ratios were plotted against the corresponding analyte concentrations and the linear regression data are reported in Table 2.

The accuracy of the method was evaluated by adding known quantities of caffeic acid, caftaric acid, cichoric acid and vanillic acid to *E. purpurea* sample solutions and analysing the fortified samples with the proposed CE method. Comparing the responses from sample and fortified sample solutions, the following recovery data were obtained: 95–98% for caffeic acid, 97–99% for caftaric acid, 97–100% for cichoric acid and 96–99% for vanillic acid. Therefore, interferences by the other matrix components are not significant and the CE conditions are suitable to obtain adequate method accuracy.

The limit of detection (LOD) corresponding to a signal-to-noise ratio (S/N) of approximately 3, was evaluated for caffeic acid, vanillic acid, cichoric acid

Table 1
Intraday and inter-day precision of the migration time (t_m) and peak area (RSD, $n=5$) for the studied phenolic acids (concentration: 10 μg/ml)[a]

Analyte	Intraday precision		Interday precision	
	t_m (min) (RSD,%)	Corrected peak area (RSD,%)	t_m (min) (RSD,%)	Corrected peak area (RSD,%)
Chlorogenic acid	11.76 (0.575)	7886 (1.90)	11.69 (0.908)	7667 (2.56)
Siringic acid	13.89 (0.494)	15317 (0.642)	13.79 (1.01)	15180 (1.03)
Ferulic acid	14.18 (0.639)	27018 (1.46)	14.10 (1.01)	26682 (2.25)
p-Coumaric acid	16.35 (0.644)	35505 (1.43)	16.29 (0.928)	35359 (1.01)
Vanillic acid	17.18 (0.658)	13288 (1.42)	17.14 (0.861)	14192 (2.13)
p-Hydroxybenzoic acid	21.49 (0.731)	16634 (1.26)	21.42 (0.987)	16022 (2.69)
Caffeic acid	23.52 (0.912)	27184 (0.943)	23.59 (0.610)	26810 (1.11)
Cichoric acid	24.82 (1.07)	13803 (2.90)	24.95 (0.561)	13107 (2.35)
Caftaric acid	32.98 (1.52)	8944 (1.85)	33.42 (0.508)	8619 (1.79)
3,4-Dihydroxybenzoic acid	33.97 (1.53)	18292 (1.64)	34.40 (0.354)	18434 (1.39)

[a] Experimental conditions: 40 m*M* sodium tetraborate running buffer, pH 9.2, with 70 m*M* SDC. Fused-silica capillary (43.5 cm effective length) thermostated at 20 °C. Hydrodynamic injection (5 p.s.i.×2 s). UV detection at 300 nm. Voltage 20 kV.

Table 2
Regression curve data[a] for the studied analytes

Analyte	Concentration range (μg/ml)	*a*	*b*	r^2
Caffeic acid	0.76–4.58	0.0164 (±0.041)	424.791 (±13.668)	0.998
Caftaric acid	5–15	0.0568 (±0.036)	165.267 (±3.359)	0.999
Chlorogenic acid	24.9–74.7	0.0309 (±0.0192)	21.611 (±0.363)	0.999
Cichoric acid	20–40	0.120 (±0.002)	8.984 (±0.079)	0.999
Vanillic acid	1.28–7.65	0.0840 (±0.010)	195.724 (±1.98)	0.999

[a] Regression curve data for five calibration points. $y = ax + b$, where y is the corrected peak area (area/migration time), x is the concentration (μg/ml), a is the slope, b is the intercept and r^2 is the correlation coefficient. Experimental conditions as in Table 1.

and caftaric acid. The limit of quantification (LOQ) corresponding to a S/N value of approximately 10 was also evaluated for the same analytes (Table 3). These data support the suitability of the proposed MEKC method for its application to real samples.

3.4. Applications to *E. purpurea* extracts

The developed MEKC method was applied to the identification and quantification of phenolic acids in extracts solutions obtained from *E. purpurea* roots, collected in Casola Valsenio, in commercial extracts and products (Echinaforce). The identity of the peaks in the obtained electropherograms was confirmed by spiking experiments in which the addition of authentic analytes to the sample solutions resulted in an increase of the analyte peak without the appearance of shoulders or split peaks. In the samples obtained from collected *E. purpurea* roots, four phenolic acids were found: caffeic, caftaric, cichoric and vanillic acids, at the following concentrations: 0.073 mg/g (RSD 1.1%), 0.45 mg/g (RSD 1.21%), 1.75 mg/g (RSD 0.92%) and 0.086 mg/g (RSD 2.06%), respectively. For comparison purposes, a commercial dried extract of *E. purpurea* was also analysed; vanillic acid was not found and caffeic acid was at trace level, while caftaric acid and cichoric acid were found at the concentration of: 1.24 mg/g (RSD 0.80%) and 2.72 mg/g (RSD 1.50%), respectively. Finally, in the commercial product (Echinaforce), only caftaric acid and cichoric acid were found at the concentration level of 0.070 mg/g (RSD 2.31%) and 0.120 mg/g (RSD 3.0%), respectively. Representative electropherograms obtained from the analysed samples are reported in Fig. 5. In each sample, cichoric acid was the main component and both caftaric acid and cichoric acid were present according to the phytochemical profile of the *E. purpurea* species. The cichoric acid/caftaric acid ratio was about four in the extracts from the collected *E. purpurea* roots and about two in the commercial extracts and as well as in the commercial tablets.

Table 3
LOD values and LOQ (RSD, $n=5$) values for the four quantified analytes[a]

Analyte	LOD[b] (μg/ml)	LOQ[c] (μg/ml) (RSD,%)
Caffeic acid	0.127	0.381 (2.25)
Caftaric acid	0.625	1.875 (1.02)
Cichoric acid	0.650	1.250 (1.90)
Vanillic acid	0.320	0.956 (2.54)

[a] Experimental conditions as in Table 1.
[b] Limit of detection, as 3 S/N.
[c] Limit of quantification, as 10 S/N.

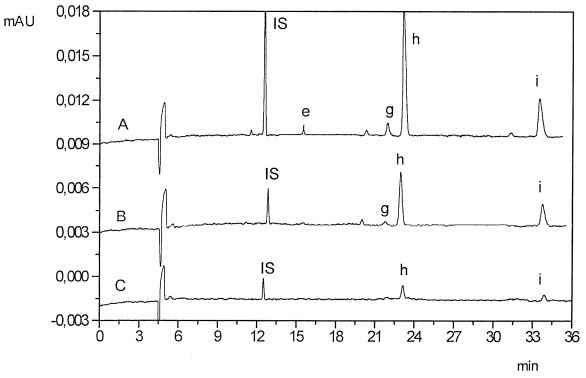

Fig. 5. Representative electropherograms obtained from: A, collected *E. purpurea* roots; B, commercial dried extract of *E. purpurea*; and C, commercial product (Echinaforce). MEKC conditions: 40 m*M* sodium tetraborate pH 9.2 with 70 m*M* sodium deoxycholate. Peak identity: *p*-nitrobenzoic acid (IS), vanillic acid (e), caffeic acid (g), cichoric acid (h) and caftaric acid (i). Other conditions as in Fig. 2.

These differences may results from enviromental and genetic variation as well as variation in plant parts used and in preservation after harvest [7,11].

As the MEKC separation was highly selective and adequately accurate, the accuracy of the whole analysis is fondamentaly depending on the extraction procedure efficiency. Its evaluation by recovery experiments is critical due to the small amounts of pure standards which are normally available. Thus, in this preliminary CE analysis the ultrasound extraction was adopted using 70% methanol, which has been reported [7] as the most efficient solvent system for cichoric acid with recovery of about 90%. In our experiments (Fig. 6) 70% methanol was confirmed to be the best methanol–water mixture for the extraction of both cichoric and caftaric acids. For comparison purposes, however, solvent-assisted microwave extraction was also applied, using the same solvent mixtures. Preliminary data suggest that the microwave extraction approach can offer an improved recovery of cichoric and caftaric acids from *E. purpurea*. Specific and wider investigations on this important aspect are in progress.

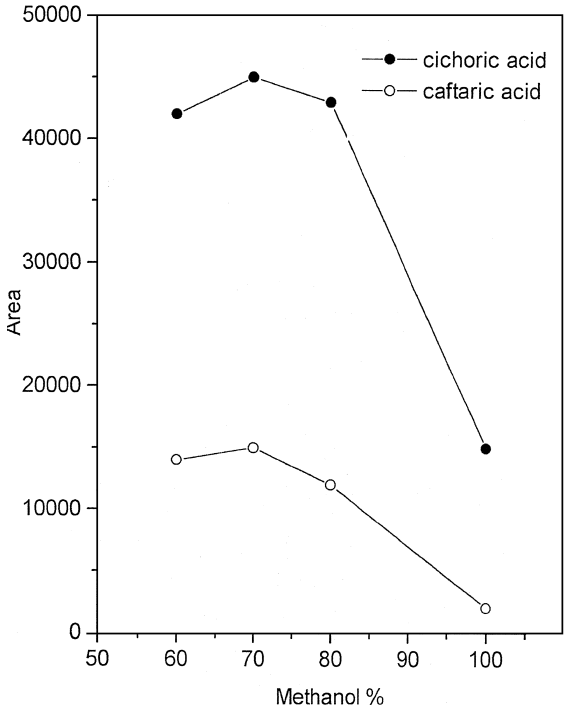

Fig. 6. Effect of the composition of the extracting solvent system (methanol–water mixtures) on the ultrasonic extraction of cichoric acid and caftaric acid from *E. purpurea* roots. MEKC conditions: 40 m*M* sodium tetraborate pH 9.2 with 70 m*M* sodium deoxycholate. Other conditions as in Fig. 2.

Acknowledgements

This work was supported by a grant from MURST (Cofin, 2000), Rome, Italy. Thanks are due to Miss Virginia Carrara for her valuable technical assistance.

References

[1] J. Bruneton, Pharmacognosy–Phytochemistry, Medicinal Plants, 2nd ed, Lavoiser Publishing, Paris, 1999.
[2] K. Glowniak, G. Zgórka, M. Kozyra, J. Chromatogr. A 730 (1996) 25.
[3] P.B. Andrade, R.M. Seabra, P. Valentao, F. Areias, J. Liq. Chromatogr. Rel. Technol. 21 (1998) 2813.
[4] E. Delage, G. Bohuon, A. Baron, J.F. Drilleau, J. Chromatogr. 555 (1991) 125.
[5] F. Buiarelli, G. Cartoni, F. Coccioli, Z. Levetsovitou, J. Chromatogr. A 695 (1995) 229.
[6] I. Lobo, A.A. Mozeto, Q.B. Cass, Chromatographia 52 (2000) 727.
[7] C. Bergeron, J.F. Livesey, D.V.C. Awang, J.T. Arnason, J. Rana, B.R. Baum, W. Letchamo, Phytochem. Anal. 11 (2000) 207.
[8] United States Pharmacopeial Convention, Pharmacopeial Forum 26 (2000) 1578–1595.
[9] R. Bauer, P. Remiger, H. Wagner, Deutsch. Apotheker Z. 128 (1988) 174.
[10] R. Bauer, S. Foster, Planta Med. 57 (1991) 447.
[11] N.B. Perry, E.J. Burgess, V.A. Glennie, J. Agric. Food Chem. 49 (2001) 1702.
[12] G. Zgórka, K. Glowniak, Phytochem. Anal. 10 (1999) 268.
[13] B. Klejdus, V. Kubán, Phytochem. Anal. 11 (2000) 375.
[14] M. Arlosio, J.D. Coïsson, A. Martelli, Chromatographia 52 (2000) 579.
[15] Z. Chen, M.A. Adams, J. Liq. Chromatogr. Rel. Technol. 22 (1999) 2595.
[16] Z. Chen, M.A. Adams, Anal. Chim. Acta 386 (1999) 249.
[17] G. Cartoni, F. Coccioli, R. Jasionowska, J. Chromatogr. A 709 (1995) 209.
[18] P. Schmitt-Kopplin, A.W. Garrison, E.M. Perdue, D. Freitag, A. Kettrup, J. Chromatogr. A 807 (1998) 101.
[19] A. Hiermann, B. Radl, J. Chromatogr. A 803 (1998) 311.
[20] S.J. Shen, C.-L. Chiech, W.-C. Weng, J. Chromatogr. A 911 (2001) 285.
[21] Y.K. Zhao, Q.E. Cao, H.T. Liu, K.T. Wang, A.X. Yan, Z.D. Hu, Chromatographia 51 (2000) 483.
[22] Z.L. Chen, G.S.R. Krishnamurti, R. Naidu, Chromatographia 53 (2001) 179.

[23] Y. Zhao, L.E. Lunte, Anal. Chem. 71 (1999) 3985.
[24] S. Terabe, K. Otsuda, T. Ando, Anal. Chem. 57 (1985) 834.
[25] P. Pietta, P. Mauri, R. Bauer, Planta Med. 64 (1998) 649.
[26] C. Biergegaad, S. Michaelsen, H. Sorensen, J. Chromatogr. 608 (1992) 403.
[27] A. Berthod, C. Garcia-Alvarez-Coque, Micellar Liquid Chromatography, Marcel Dekker, New York, 2000.
[28] A. Coello, F. Meijide, E. Rogriguez Nùñez, J. Vasquez Tato, J. Pharm. Sci. 85 (1996) 9.
[29] J.M. Herrero-Martinez, M. Fernández-Martí, E. Simó-Alfonso, G. Ramis-Ramos, Electrophoresis 22 (2001) 526.
[30] H. Nishi, T. Fukuyama, M. Matsuo, S. Terabe, J. Chromatogr. 513 (1990) 279.
[31] H. Nishi, T. Fukuyama, M. Matsuo, S. Terabe, J. Pharm. Sci. 79 (1990) 519.
[32] P. Schmitt-Kopplin, N. Hertkorn, A.W. Garrison, D. Freitag, A. Kettrup, Anal. Chem. 70 (1998) 3798.