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Separation of alkamides from *Echinacea purpurea* extracts by cyclodextrin-modified micellar electrokinetic chromatography

Separation of nine important alkyl methylbutyl- and isobutylamides (known as alkamides) obtained from *Echinacea purpurea* extracts was investigated by using cyclodextrin-modified micellar electrokinetic chromatography (CD-MEKC). Hydrophobic alkamides interact strongly with the micelles from the most common surfactants used in MEKC and this lead to predominant partition of the analytes into the micellar phase, resulting in poor resolution. The addition of neutral CDs to the alkaline (10 mM phosphate buffer pH 8.0) micellar system of sodium dodecyl sulfate (SDS), sodium cholate (SC) and sodium deoxycholate (SDC) was found to improve the separation of the studied alkamides. Among the several combinations surfactant/CD, three different systems showed to be particularly effective: SDS/hydroxypropyl-β-CD (110 mM/100 mM) and SC/heptakis (2, 3, 6-tri-*O*-methyl)-β-CD (200 mM/40 mM) which provided a complete separation of the studied compounds, and SDC/heptakis (2, 6-di-*O*-methyl)-β-CD. The importance of appropriate surfactant *vs.* CD concentration ratio as well as that of total concentration of both surfactant and CD was considered. The optimization of the separation was performed by focussing the need for a rapid separation of nine alkamides diagnostically useful to define the fingerprint of *Echinacea* species.

Keywords: Alkamides / Bile salts / *Echinacea* extracts / Micellar electrokinetic chromatography / Neutral cyclodextrins
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1 Introduction

Alkamides are the main lipophilic components of *Echinacea purpurea* plant extracts and the structure of some of the most important compounds is reported in Fig. 1; as showed, compounds 1–3, 5, 6, 8, and 9 are typically alkyl isobutylamides while alkamides 4 and 7 are alkyl methylbutylamides [1]. Alkamides from *E. purpurea* roots possess mainly a 2,4-diene moiety with the isomeric mixture of 8/9 (dodeca-2E, 4E, 8Z, 10E-tetraenoic acid isobutylamide) as the principal alkamide constituent of both the roots and aerial parts. Extracts from *E. purpurea* are widely used in medicine [2] particularly for their immunostimulant properties and reports have shown that the lipophilic constituents, in particular alkamides, play an important immunoactive, as well as, anti-inflammatory roles *in vivo* and *in vitro*. Light-mediated antifungal activity of the alkamides fraction of *Echinacea* has been also reported [3].

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Abbreviations: DAD, diode-array detector; **DM**, dimethyl; **HP**, hydroxypropyl; **SC**, sodium cholate; **SDC**, sodium deoxycholate; **TIC**, total ion current; **TM**, trimethyl

As for the analysis of the *E. purpurea* extracts, HPLC is the most applied technique [1, 4] for the quality control purposes. In the present study, the separation of nine (compounds 1–9; Fig. 1) closely related alkamides from *E. purpurea* extracts has been differently approached by means of cyclodextrin-modified micellar electrokinetic capillary chromatography (CD-MEKC). CD-MEKC firstly proposed by Terabe and co-workers [5, 6], has been widely used for the separation of drug enantiomers [7–11] as well as isomers [12], highly hydrophobic and closely related compounds [13]. In particular, an extensive literature is concerned with the separation of environmental pollutants such as polychlorinated biphenyls and polycyclic aromatic hydrocarbon compounds [10, 11, 14–16]. The rational of CD-MEKC is founded on the use of cyclodextrins (CDs) as a "second pseudostationary phase" in an electrophoretic system where, simultaneously, are effective the aqueous solution of the electrolyte and the surfactant over the critical micelle concentration (CMC). The inclusion of analytes into the cavity of CD, reduces the capacity factor of solute/micelle allowing for highly selective separations on condition that the distribution of the solutes towards either micelle and CD does not be complete. To this regard, the choice of the appropriate couple surfactant-CD plays a significant role to perform successful separations. Due to the extended opportunity to modulate and enhance the selectivity, several applica-

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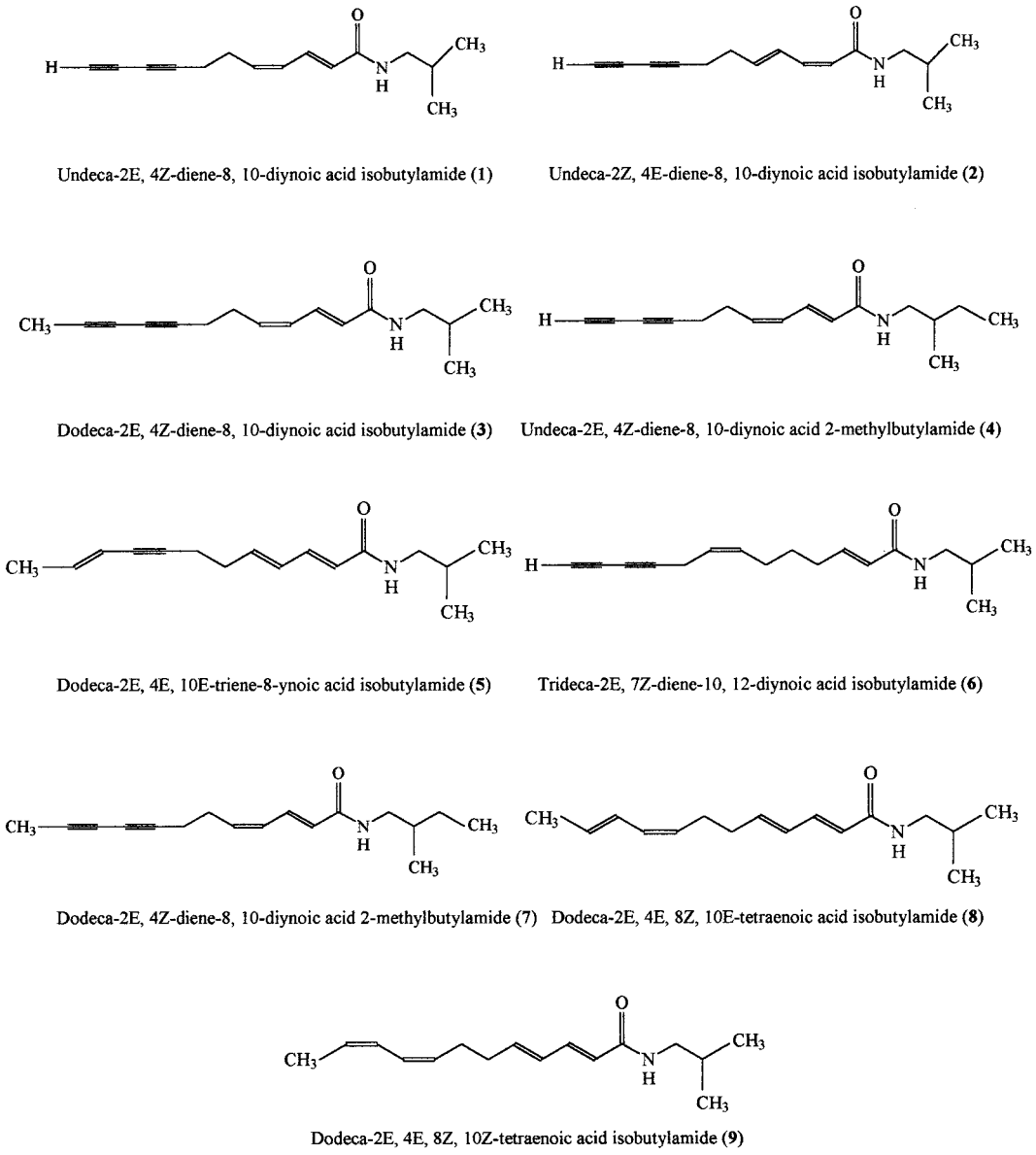

Figure 1. Structures of the studied alkamides.

tions of CD-MEKC have been reported for the analysis of multicomponent samples such as active compounds in biofluids [17] and plant extracts [18–21].

Herein, CD-MEKC systems based on the appropriate combinations of different surfactant and neutral CDs have been developed for the fast separation of alkamides 1–9 (Fig. 1) from *Echinacea* extracts. Precisely, SDS, sodium cholate (SC) and sodium deoxycholate (SDC) were used to provide the micellar phases. The effects of type and concentration of different CDs, namely β-CD, hydroxypropyl-β-CD (HP-β-CD), heptakis (2, 6-di-*O*-methyl)-β-CD (DM-β-CD) and heptakis (2, 3, 6-tri-*O*-methyl)-β-CD (TM-β-CD) have been evaluated on the selectivity of separation. Optimization of the separation was performed by evaluating the effect of different combinations CD-surfactant at different concentrations on the resolution and analysis time. From this study, the importance of an appropriate concentration ratio (surfactant *vs.* CD) has been evidenced as well as that of the total concentration of both surfactant and CD.

2 Materials and methods

2.1 Materials

Sodium phosphate used as electrolyte was from Carlo Erba Reagenti (Milan, Italy); SDS, native β-CD and HP-β-CD were purchased from Fluka (Buchs, Switzerland). DM-β-CD, TM-β-CD, SC, SDC, and phenantrene (used as a marker of migration of micelles) were from Sigma-Aldrich (Milan, Italy). Other reagents were of analytical grade and were used as received. Deionized water was obtained with a Milli-RX system (Millipore, Milford, MA, USA).

2.2 Apparatus and procedures

2.2.1 CE system

All separations were carried out using a ^{3D}CE Capillary Electrophoresis system (Agilent Technologies, Waldbronn, Germany), equipped with a diode array detector. The data were collected on a PC using the ^{3D}CE-ChemStation software Version A 06. New fused-silica "extended-path" capillaries (Agilent Technologies) 48.5 cm in length (40 cm effective length) × 50 μm ID were used after a conditioning program consisted of rinse with 1 M NaOH (10 min), 0.1 M NaOH (10 min) and finally deionized water (10 min). Prior to each run, a brief rinse (3 min) of the capillary by using the below described separation buffers provided excellent repeatability of migration times. Hydrodynamic injections were performed at 25 mbar for 3 s and the voltage was kept constant at + 20 kV; the separations were performed under controlled temperature (20°C) at a wavelength of 260 nm. However, the employed diode-array detector (DAD) allowed recording the UV signal from 200 to 400 nm.

2.2.2 HPLC system

An HPLC system equipped with a diode-array detector (Agilent Technologies) Ti series 1050 was employed. The chromatographic separation of alkamides was performed on a Phenomenex Kromasil C18, 5 μm (150 × 4.6 mm ID) column, using an elution gradient from acetonitrile/water 40/60 to 80/20 v/v in 40 min at a flow rate of 1.0 mL/min. The injection volume was 20 μL and the detection was performed at 210 and 254 nm.

2.2.3 GC-MS system

The GC-MS system consisted of a TRACE GC 2000 series (ThermoQuest CE Instruments, Austin, TX, USA) gas chromatograph equipped with a split-splitless injector (split ratio of 20:1). The column was an Rtx®-5MS (30 m × 0.25 mm, fused-silica capillary column, 0.25 μm film thickness) consisting of Crossbond® (5% diphenyl 95% dimethyl polysiloxane). Helium was the carrier gas at a flow rate of 1.0 mL/min. The GC was interfaced with a GCQ plus (Thermo-Finnigan, Austin, TX, USA) mass detector operating in the EI mode (70 eV). Temperature of the injector base, transfer line and ion source were maintained at 250, 250 and 200°C, respectively. System operation, temperature programs, qualitative and quantitative GC-MS analyses were all performed as reported in a previous paper [22].

2.3 Solutions

The running buffers were freshly prepared every day: appropriate amounts of surfactant (SDS, SC, SDC) and CD (β-CD, HP-β-CD, DM-β-CD, and TM-β-CD) were dissolved in a phosphate solution (10 mM) whose pH was adjusted to 8.0 using 0.01 M NaOH. Before the injection into the CE system, each solution (running buffer and sample solutions) was subjected to filtration through a 0.45 μm GyroDisc (Orange Scientific, Waterloo, Belgium) membrane.

2.4 Sample preparation

2.4.1 Plant materials and extraction procedure

About 1–2 kg plant materials (roots) of *E. purpurea* plants grown in the open field at Herb Garden of Casola Valsenio (Ravenna, Italy) were collected in October, 2000 at almost the full growth roots. Root materials were separated, washed, cut into small parts, air-dried, and ground into small particles (0.5 mm) just before extraction. About 1 g accurately weighed amounts of dried ground root materials were extracted, in an ultrasonic bath (for 6 min at room temperature), three times with 8 mL solvent (70% v/v methanol in water). The extracts (n = 3) were collected, filtered through 0.20 μm membrane filter and the volume was completed up to 25 mL to a final concentration equivalent to 0.04 g roots per mL. This solution was then subjected to HPLC analysis to give an eluate fraction for each component, as described below. Moreover, an aliquot of this solution was used to recover the lipophilic components (alkamides) by a liquid-liquid extraction procedure using a mixture *n*-hexane-ethylacetate 1:1 v/v. The organic extract was evaporated and the residue was dissolved in 0.3 mL of methanol. This final solution represents the sample for both GC and CE analysis. However, by applying the developed CE system, the direct injection of the crude extracts from the ultrasonic bath was allowed.

2.4.2 Identification of alkamides

Identification of the different alkamide components of the lipophilic fraction was realized by a combination of RP-HPLC and GC-MS (on nonpolar column) analyses as described by Hudaib *et al.* [22]. Briefly, the different alkamide fractions (compounds 1–9, Figs. 1, 2), separated by RP(C-18)-HPLC and identified by their UV data (DAD) and retention times [1], were individually collected and subsequently extracted by a simple liquid-liquid extraction procedure based on a *n*-hexane-ethylacetate 1:1 (v/v) mixture. The organic extract was evaporated and the residue was dissolved in 0.3 mL of methanol. This concentrated extract solution from each single fraction was simultaneously subjected to both the GC-MS [22] and the developed CD-MEKC analyses as a reference to confirm the peak identity in the total ion current (TIC)-GC-MS traces and CD-MEKC electropherograms of the tested samples, respectively. Moreover, for better confirmation of the GC-MS peaks corresponding to the smallest HPLC (and CE) peaks (minor alkamide fractions, *e.g.*, 4, 5, 6, Figs. 1 and 2), selective ion monitoring (SIM)-GC-MS chromatograms were reconstructed by monitoring the masses of the base-peak ion and other principal characteristic MS ions of these alkamides.

3 Results

The nine alkamides considered in the present study (Fig. 1, 1–9) are not commercially available and an extract from *E. purpurea*, obtained as described in Section 2.4, was employed as sample mixture. The individual alkamides were obtained by HPLC separation and their identification was performed by comparison with the HPLC retention times obtained under previously reported conditions [1]; a further confirmation about the compounds identity was carried out by GC-MS analyses of the individual HPLC fractions. The studied alkamides can be considered as compounds with a quite similar hydrophobicity and their separation was obtained by using a gradient RP-HPLC. As can be seen from the HPLC chromatogram obtained under the reported conditions (Fig. 2), the separation of the compounds 2/3 and 8/9 it seems to be critical [1]. To offer an alternative procedure to the mentioned chromatographic analyses, conventional MEKC systems using either SDS or bile acid salts (SC and SDC) as surfactants were first employed under alkaline conditions.

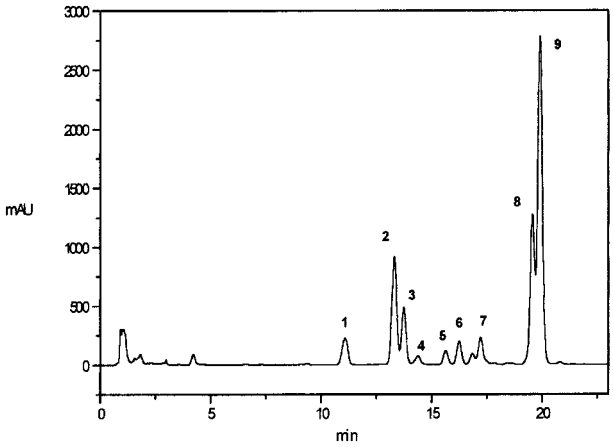

Figure 2. HPLC separation of alkamides 1–9. Chromatographic conditions: Phenomenex Kromasil C18, 5 μm (150 × 4.6 mm ID) column; mobile phase: gradient elution from acetonitrile/water 40/60 to 80/20 v/v in 40 min at a flow rate of 1.0 mL/min; injection volume, 20 μL; detection at 254 nm. Peak identification as in Fig. 1.

3.1 Separation of alkamides using SDS as surfactant

SDS is undoubtedly the most used surfactant both in conventional MEKC and CD-MEKC. In this application, however, SDS exhibited a very poor ability of resolution of the alkamides when it was used in phosphate (10 mM, pH 8.0) running buffer. In the range of the concentration of SDS within 50–100 mM, only broad peaks were obtained (Fig. 3a) and the identification of the single alkamides was not achieved. Furthermore, the migration times of the studied compounds were only slightly lower from that of phenantrene used as highly hydrophobic reference compound. These considerations suggested alternatively the use of a CD-MEKC separation, where the addition of the appropriate CD into the micelle system can selectively change the capacity factor of the analytes. Moreover, because of the necessity to provide the separation of geometric isomers (in particular compounds 8/9), the use of CDs as additional "stereoactive pseudophase" was regarded to be an adequate choice. Thus, the effect of neutral CDs supplemented to SDS solution on the separation was evaluated.

3.1.1 Choice of the suitable CD

Four different neutral CDs, namely β-CD, HP-β-CD, DM-β-CD, and TM-β-CD were added to a 50 mM SDS (10 mM phosphate, pH 8.0) solution at 20, 30 and 40 mM level for each. The addition of CDs led to a negligible reduction in the EOF compared to that obtained using a simple SDS running buffer. As expected, shortness in migration times for the analytes was observed in the presence of each considered CD in the mixed CD-MEKC system. This behavior was found to be in agreement with the competi-

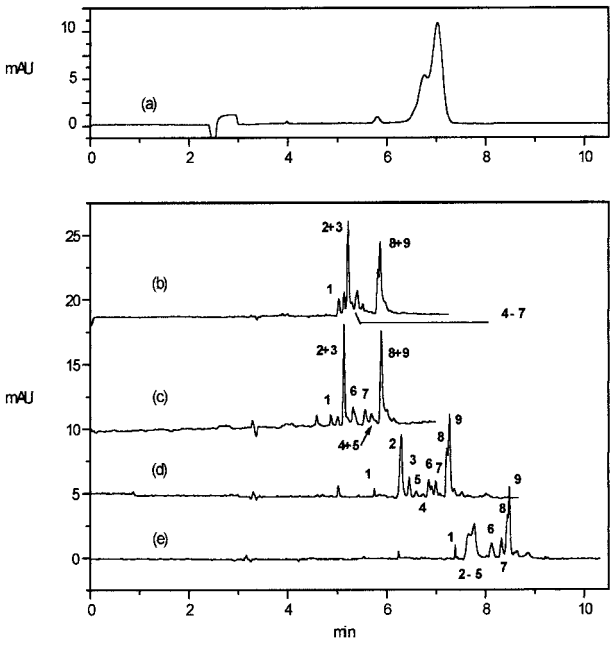

Figure 3. Electropherograms of alkamides mixture from *Echinacea purpurea.* Conditions: MEKC using (a) 50 mM SDS in 10 mM phosphate buffer, pH 8.0. CD-MEKC using 50 mM SDS in 10 mM phosphate buffer, pH 8.0 supplemented with: (b) TM-β-CD; (c) DM-β-CD; (d) HP-β-CD; and (e) β-CD, each at a concentration of 30 mM. Other conditions: fused-silica "extended path" capillary, 40 cm effective length (50 μm ID); hydrodynamic injection at 25 mbar for 3 s; constant voltage of 20 kV; temperature 20°C; detection wavelength, 260 nm. Peak identification as in Fig. 2.

tive associations of the analytes between the micelle and CD as expressed by the directly proportional relationship between the reciprocal of the apparent capacity factor and the concentration of CD suggested by Terabe *et al.* [5, 6].

A comparison of the effects of addition (30 mM) of the different CDs to the SDS solution was then performed. TM-β-CD and β-CD showed poor selectivity towards the studied alkamides (Fig. 3b and e, respectively), while DM-β-CD and HP-β-CD favorably affected the separation ability of SDS micelles. In details, by using DM-β-CD (Fig. 3c) good peak symmetry was generally shown, however the comigration of compounds 2/3, 4/5, and the complete lack of selectivity on the critical couple 8/9 did not make suitable this system for our scope. Finally, HP-β-CD (Fig. 3d) exhibited a general positive effect on the separation of all the alkamides and in particular, an initial separation of the couple 8/9 was achieved. Concluding, on the bases of these preliminary results our attention was focussed on the use of HP-β-CD.

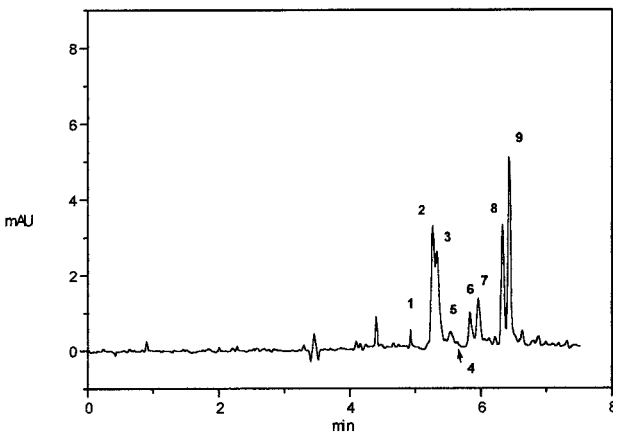

Figure 4. Electropherogram of a alkamides mixture from *E. purpurea* under CD-MEKC conditions: 40 mM of HP-β-CD and 50 mM SDS. Other conditions and peak identification as in Fig. 3.

3.1.2 Optimization of the separation with the SDS/HP-β-CD system

The optimization of the separation was performed by considering the importance of obtaining baseline resolutions in short analysis time; to this regard, given the selectivity showed by HP-β-CD, increasing amounts were supplemented to the SDS solution (50 mM). In the presence of 40 mM HP-β-CD (Fig. 4), a good resolution of the isomers 8/9 was obtained at the expense of a general worsening of separation of the other alkamides. A relatively high amount of CD should seem useful for the resolution of the couple 8/9; on the other hand, the increased partitioning into the aqueous phase significantly reduce the capacity factor of the alkamides which preferably moved driven by the EOF.

With the aim to keep the separation of compounds 1–7 (as in Fig. 3d), together with a baseline resolution of 8/9, increasing concentrations of SDS were used, allowing higher amounts of CD to be employed without loss of selectivity. By means of a systematic variation of both the concentrations of SDS and HP-β-CD it was found that starting from an SDS concentration of 90 mM in the presence of 80 mM HP-β-CD (corresponding to a ratio SDS *vs*. HP-β-CD of 1 : 0.88), the baseline resolution of alkamides 8/9 was achieved (Fig. 5a). Using the same favorable SDS *vs*. CD ratio but at higher absolute amounts of SDS and CD (100 mM and 90 mM, respectively; Fig. 5b), only a very small increase of migration times of the analytes was observed as probably due to the effect of BGE components on the viscosity. Interestingly, a further increase of the total amount of SDS and HP-β-CD (110 mM and 100 mM, respectively), provided a complete separation of the studied alkamides (Fig. 5c).

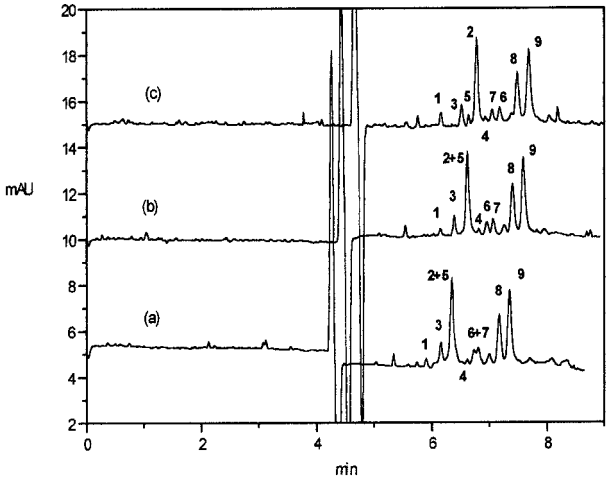

Figure 5. Effect of the absolute amounts of SDS and HP-β-CD on the separation of the studied alkamides at a constant SDS *vs.* HP-β-CD ratio of about 1:0.88. (a) SDS/HP-β-CD corresponding to 90 mM/80 mM; (b) SDS/HP-β-CD corresponding to 100 mM/90 mM; (c) SDS/HP-β-CD corresponding to 110 mM/100 mM. Other conditions and peak identification as in Fig. 3.

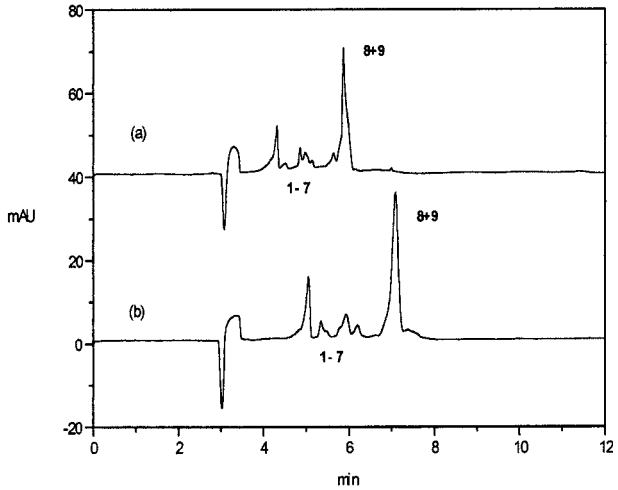

Figure 6. Electropherograms of alkamides mixture from *E. purpurea* under MEKC conditions using bile salts as surfactants: (a) 50 mM SC and (b) 50 mM SDC in 10 mM phosphate buffer, pH 8.0. Other conditions as in Fig. 3.

Moreover, in comparison with the separation pattern previously obtained (Fig. 4) it should be pointed out that the increase of both SDS and HP-β-CD concentration led also at an inversion of the migration order of compounds 2/3 (Fig. 5a) and 6/7 (Fig. 5c).

3.2 Separation of alkamides using bile acid salts as surfactants

Bile acid salts are cholesterol-related biosurfactants which are widespread used in CE; due to their chiral nature, the micelles from bile salts can allow direct MEKC enantioseparations. Furthermore, the helical spatial arrangement and the poorer hydrophobicity of bile acid salts micelle compared to SDS one could significantly affect the MEKC separations [23, 24]. For these reasons, SC and SDC were used at the concentration of 100 mM in phosphate buffer pH 8.0 (10 mM) for the separation of alkamides. In Fig. 6, the separation profiles of the mixture of alkamides obtained under conditions of a simple MEKC system based on SC (Fig. 6a) and SDC (Fig. 6b) are reported. As expected, the lower hydrophobicity of SC and SDC micelle compared to that of SDS, resulted in lower retention of the studied analytes accompanied by an increased apparent selectivity (see Fig. 3a). Nevertheless, in order to obtain efficient separations, auxiliary CDs have to be supplemented to the MEKC solution.

3.2.1 Choice of the suitable CD

Several investigations from the pharmaceutical point of view have been reported about the interaction between CDs and bile salts [25]; the evidences for inclusion interaction and complexation can strengthen the reasons for a rational use of the combination of CDs and bile salts as a "separation environment" in CE [8, 26]. The same set of neutral CDs used with SDS, was employed to design different combinations adopting SC and SDC (100 mM) as surfactants. The covered CDs concentration ranged from 10 to 30 mM and a rapid screening on the selectivity of the employed combinations was performed in analogy to the previous experiments conducted on the SDS/CD system. In the presence of SC, TM-β-CD exhibited higher ability of separation compared to the other CDs, while using SDC, DM-β-CD showed to be the most suitable for the separation of the studied alkamides.

3.2.2 Optimization of the separation with the bile salts/CD system

As for the results previously obtained with the mixture SDS/CD, an appropriate ratio bile salt *vs.* CD should be firstly found; subsequently the optimization of the absolute amount of both the "pseudophases" at a constant concentration ratio can be performed. As observed from the Fig. 7, a mixture of SC and TM-β-CD corresponding to the ratio 1:0.2 can be considered favorable for the analysis of alkamides and was chosen to evaluate the effect of variation of the absolute amount of the surfactant and CD. Under conditions corresponding to a concentration

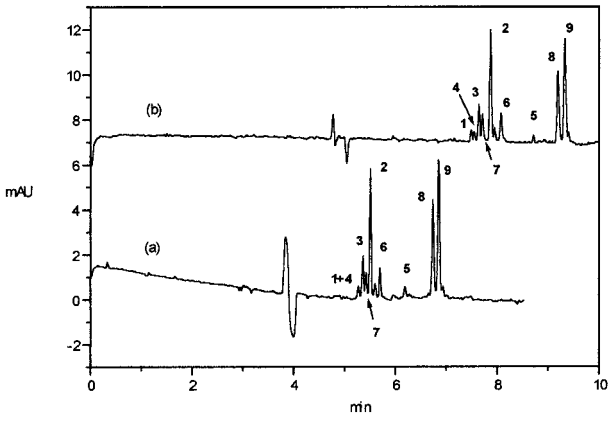

Figure 7. Electropherograms of the studied alkamides mixture obtained using SC as surfactant in the presence of TM-β-CD. Effect of the absolute amounts of SC and TM-β-CD on the separation at a constant SC *vs.* TM-β-CD ratio of 1:0.2. (a) SC/TM-β-CD corresponding to 100 mM/20 mM; (b) SC/TM-β-CD corresponding to 200 mM/40 mM. Other conditions and peak identification as in Fig. 3.

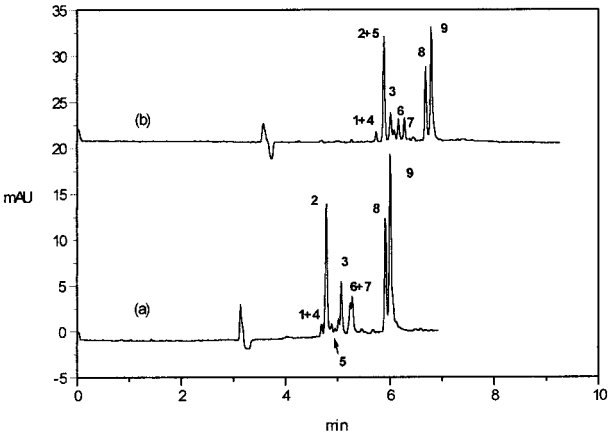

Figure 8. Electropherograms of the studied alkamides mixture obtained using SDC as surfactant in the presence of DM-β-CD. Effect of the absolute amounts of SDC and DM-β-CD on the separation at a constant SDC *vs.* DM-β-CD ratio of 1:0.2. (a) SDC/DM-β-CD corresponding to 100 mM/20 mM; (b) SDC/DM-β-CD corresponding to 200 mM/40 mM. Other conditions and peak identification as in Fig. 3.

SC/TM-β-CD of 100 mM/20 mM (Fig. 7a), a complete resolution of the analytes was obtained except for the comigration of two minor compounds 1/4 which were however resolved with a further increase in the absolute concentration of the active components of the running buffer (SC/TM-β-CD, 200 mM/40 mM; Fig. 7b).

Concerning the use of SDC, representative electropherograms obtained for the chosen SDC/DM-β-CD mixture at the relative ratio of 1:0.2 are shown in Fig. 8. The mixture

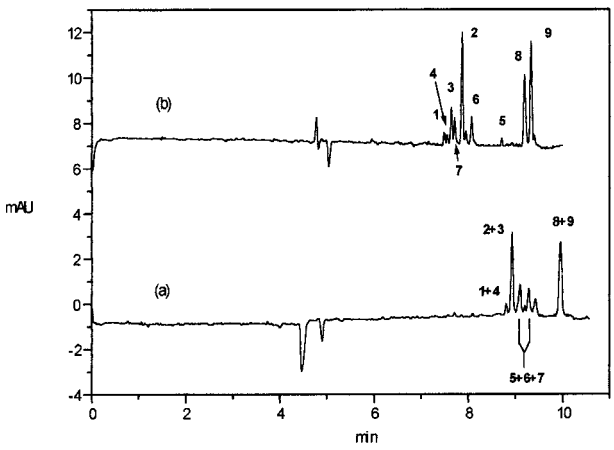

Figure 9. Electropherograms of alkamides mixture using 200 mM of (a) SDC and (b) SC as surfactant, in the presence of 40 mM TM-β-CD. Other conditions and peak identification as in Fig. 3.

corresponding to 100 mM/20 mM of SDC/DM-β-CD (Fig. 8a), proved to be useful for the separation of compound 2 from 5; by using higher concentration of the active BGE components a complete separation of alkamides 6/7 was obtained at the expense of resolution of the couple 2/5 (Fig. 8b). Further increase of bile salt and CD amounts did not improve the separation.

4 Discussion

As can be expected, strongly different patterns of separation were obtained using different combinations of surfactant-CD. By whole of the performed experiments, some considerations can be done. First of all, in the presence of bile salts the CDs can affect favorably the separation at lower concentration levels compared to the level required using SDS as surfactant. Actually, the strong association of alkamides with the highly hydrophobic SDS micelles asks for a considerably stronger competition by the aqueous pseudophase; this can be obtained with high amounts of CD, while in the presence of the relatively poor hydrophobic bile salts micelle the modulation of selectivity can be achieved using a significantly lower amount of CD.

Concerning the use of bile salts, in order to highlight the importance of their nature on the separation ability, the electropherograms obtained using the same CD (40 mM of TM-β-CD) in the presence of (a) 200 mM SDC and (b) 200 mM SC can be compared (Fig. 9). As can be seen, completely different separation profiles were obtained; the most effective CD for the SC system was TM-β-CD (Fig. 9b), which differently, under the same conditions, in

the presence of SDC completely failed to resolve the alkamide couples 2/3 and 8/9 (Fig. 9a). As a matter of fact, the evidences for a complexation of CDs and bile salts [25], do not entitle us to believe that the different profiles of separation have to be due merely to the nature of the bile salts. To further confirm that, a considerable difference between SC and SDC is represented by the opportunity of the latter to form 1:1 and 1:2 complexes with CDs, whereas SC can form only 1:1 complexes [25].

In such systems, the importance of using CDs is also evidenced by the necessity to have the separation of the isomers 8/9. Interestingly, in the presence of SDS, DM-β-CD did not show selectivity for these two compounds (Fig. 3c), while using SDC, the baseline separation was obtained at relatively low level of the same CD (Fig. 8). On the other hand, the selectivity of β-CD and HP-β-CD towards alkamides 8/9 was shown in the presence of SDS but the same CDs failed to resolve the considered two alkamides in the bile salts based systems.

From these considerations some points should be highlighted: inmost relationships between surfactant and CD have to be invoked to explain the ability of separation of the proposed mixed systems. At the same time the obtained results should support the necessity to perform systematic studies in order to choose the best combination of CD and surfactant selecting for either the nature of the components and their respective amounts. The opportunity to arrange in very simple operational modes separation systems with different selectivity can offer a powerful tool for the unambiguous identification of the active components of complex mixtures.

From the practical point of view, by considering the aspects concerning resolution and analysis time, the system based on the use of SDS allows rapid and complete separation of the studied alkamides. The separation of the compounds 1–9, was also obtained using a combination of SC and TM-β-CD (200 mM/40 mM); however, in this system the resolution of the alkamides 1/4 and 3/7 seems to be critical and the analysis time longer in comparison with that of the separation with SDS/HP-β-CD. In general, the proposed CD-MEKC systems offer the interesting advantage to allow a very fast separation in comparison with the analysis times of the validated gradient HPLC system.

5 Conclusion

The good results obtained regarding repeatability experiments on the migration time stability (RSD% 1.20, $n = 5$; related to the compound 8 using the SDS/HP-β-CD system), make the proposed CD-MEKC systems a complementary support of GC and HPLC for the analysis of structurally related alkamides. The developed methods, for their rapidity and the high selectivity, will be useful to achieve fingerprint electropherograms suitable for the rapid identification of different *Echinacea* species (*E. purpurea*, *E. pallida* and *E. angustifolia*), characterized by a specific content of alkamides. Moreover, the proposed CE systems can be applied to the determination of alkamides in real samples; in fact, it should be pointed out the absolute compatibility of the running buffers with the aqueous-methanol sample solutions directly obtained by ultrasonication or microwave extraction of *Echinacea* roots or aerial parts.

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