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Use of detergents and high contents of organic solvents for simultaneous quantitation of ionic and nonionic drugs by electrokinetic chromatography

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Abstract

Buffers containing high percentages of organic solvents, typically 50% of acetonitrile and/or methanol, together with sodium dodecyl sulfate (SDS) are employed for the separation and quantitation by electrokinetic chromatography (EKC) of analytes found in a nasal spray. Solutes consist of benzalkonium chloride, a family of highly positive compounds, and 2-phenylethanol and beclomethasone dipropionate, which are electrically neutral and poorly soluble in aqueous buffers. It is observed that the effect of both concentration of SDS and temperature on the separation depends on the organic solvent used and the solute nature. It is also observed that SDS–solute interaction for neutral and cationic compounds are weaker in the presence of high contents of acetonitrile than in methanol. Concentration of SDS, temperature, and organic solvent nature and content, allow one to modify the selectivity of the separation when neutral and ionic species have to be simultaneously determined. The optimization of EKC conditions enables the analysis of compounds in less than 5 min. A one-step sample treatment consisting of centrifugation of the nasal spray solved in acetonitrile, together with the referenced optimum separation conditions enable the reproducible quantitation of the analytes. Relative standard deviation values of inter-day migration times lower than 2.45% are obtained (R.S.D.$_{n=12}$), while R.S.D.$_{n=12}$ values for inter-day peak areas were lower than 6.32%.

Keywords: Organic solvents; Benzalkonium chloride; 2-Phenylethanol; Beclomethasone dipropionate

1. Introduction

Micellar electrokinetic capillary chromatography (MEKC) has numerous applications in the separation of small compounds, pollutants, drugs, metabolites, etc. (for an up-to-date review see Refs. [1–4]). Introduced in 1984 [5], MEKC allows the separation of analytes without electrical charge under the conditions of separation, while improving the solubility of highly hydrophobic substances. Both effects result from the use of surfactants, mainly sodium dodecyl sulfate (SDS), which are added to the separation buffer at concentrations higher than their critical micellar concentration (cmc).

In spite of these good capabilities the use of MEKC also presents some drawbacks. For instance, the separation window is limited to a certain separation time reducing the peak capacity of the system

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while some compounds are still insoluble in aqueous buffers even containing high concentrations of detergent. The use of organic solvents (e.g., methanol, 2-propanol, etc.) added to the separation buffer seems to be a good approach toward these problems. Organic solvents can enlarge the separation window [6,7], what is mainly attributed to a change in viscosity and dielectric constant of the separation buffer as well as to a variation of zeta potential of the capillary wall [8,9]. Moreover, the use of these solvents increases the solubility of highly hydrophobic molecules in aqueous solutions [9].

Although organic solvents in MEKC have been mainly added to aqueous buffers in quantities ca. 20% or lower [6,7,10–21], the combined use of surfactants and higher contents of organic solvents has also been reported [22–29]. Thus, in 1986 Walbrohel and Jorgenson [23] carried out the separation of nonionic compounds using tetrahexylammonium salts in an aqueous medium containing 50% acetonitrile. This separation was attributed to the interaction between tetrahexylammonium moieties and analytes through what was called solvophobic association. Following this idea other authors have shown the utility of working with monomeric forms of SDS [18,24–27] or dioctyl sulfosuccinate [22] when using aqueous buffers containing ca. 50% organic solvent, mainly acetonitrile [22–28]. However, Vindevogel and Sandra [28] have extensively discussed the idea that in 40–50% acetonitrile there must be SDS micelles, that could explain the separation of neutral species, instead of the solvophobic effect as mentioned above. Recently, Seifar et al. [29] have proposed a distribution between the aqueous phase and micelle-like SDS aggregates as a separation mechanism.

Apart from these different considerations regarding the mechanism behind the capillary electrophoresis (CE) separation when using surfactants together with high contents of organic solvents, it is also of importance ''to bridge the gap between MEKC with predominantly aqueous buffer systems and MEKC with non-aqueous buffers, the latter of which has yet to be explored'', as mentioned by Ahuja and Foley [25].

The goal of this work is to carry out the simultaneous determination of two non-electrically charged substances, 2-phenylethanol and beclomethasone dipropionate, and a family of highly positive compounds, benzalkonium chloride, found in a nasal spray. The low solubility of one of the solutes in SDS–aqueous media requires the utilization of high contents of organic solvents added to the running buffer (vide infra). Optimization of their CE separation is carried out studying parameters such as type and percentage of organic modifier, type and concentration of surfactant, and separation temperature. Results on repeatability and limit of detection obtained during the quantitative analysis of real samples in such separation media are also given. From this study, some more insight on the combined use of high contents of organic solvents and surfactants in CE when employed for the simultaneous analysis of ionic and nonionic compounds is obtained.

2. Experimental

2.1. Instrumentation

Separations were carried out using a P/ACE 2000 HPCE (Beckman Instruments, Fullerton, CA, USA) electrophoresis apparatus controlled by a 486/33 MHz personal computer. Fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 27 cm (20 cm effective length, from the injection point to the detector)×50 μm I.D.×360 μm O.D. were used. External temperature of the capillaries was varied from 20 to 50°C. The injection was carried out in the anode using N_2 pressure (0.5 p.s.i.; 1 p.s.i.=6894.76 Pa). The detection took place at 214 and 254 nm. All the data were collected and analyzed using a System Gold software from Beckman running on the 486/33 MHz personal computer.

2.2. Samples and chemicals

Standards of benzalkonium chloride (BKC), 2-phenylethanol (PEA) and beclomethasone dipropionate (BDP) as well as the nasal spray Beconase were a gift from Glaxo Wellcome (Aranda de Duero, Burgos, Spain). The molecular structures of these compounds are given in Fig. 1. BKC is labeled as 1 and * since this cationic surfactant is constituted of different homologues which have been shown to distribute into two main peaks (1 and *) in CE

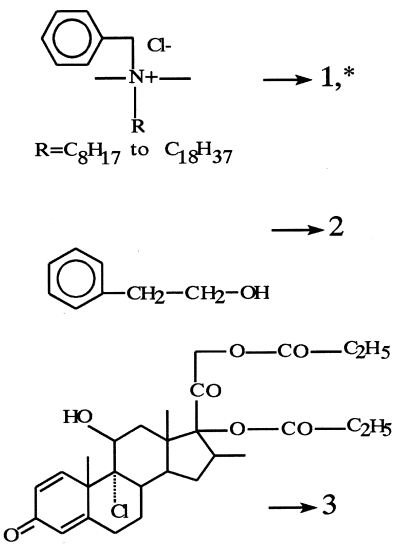

Fig. 1. Molecular structures of the different solutes from the nasal spray studied by CE in this work: benzalkonium chloride (1, *), 2-phenylethanol (2) and beclomethasone dipropionate (3).

[30,31]. Standards were solved in acetonitrile (Scharlau, Barcelona, Spain) at the concentrations indicated in each case and stored at −4°C. Acetonitrile was used to determine the electroosmotic flow (EOF). These solutions were heated at room temperature before use. SDS, Sudan III, *N*,*N*-dimethylformamide, methanol, ammonium acetate were from E. Merck (Darmstad, Germany). 2-[*N*-Cyclohexylamino]-ethanesulfonic acid (CHES) and *N*-[2-hydroxyethyl]-piperazine-*N′*-[2-ethanesulfonic acid] (HEPES) were purchased from Sigma (St. Louis, MO, USA). Boric acid and cetyltrimethylammonium chloride (CTAB) were from Aldrich (Steinheim, Germany). Milli-Q water (Millipore, Bedford, MA, USA) was used to prepare the running buffers. The quality of all reagents was for analysis or better.

2.3. Sample preparation

One g of Beconase nasal spray was weighed and acetonitrile added to a final volume of 5 ml. The mixture was shaken in a vortex for 1 min, sonicated for 1 min and centrifuged at 8000 rpm for 10 min. After samples preparation they were shortly analyzed by directly injecting the supernatant into the CE capillary.

3. Results and discussion

3.1. Solubility study

Since it was observed in preliminary experiments that PEA and BDP were insoluble in aqueous solutions, a study on the solubility of the analytes in different solvents was visually carried out. It was determined that BDP was the most insoluble compound in aqueous solutions, precipitating at concentrations higher than 0.1 mg/ml. Although, SDS micelles were then used to increase the solubility of BDP, it was observed that even at concentrations of 100 m*M* SDS, the solute BDP was not solved. Higher contents of SDS were not tested because at such high concentrations, the electric conductivity of these solutions when employed in CE running buffers would have become too high, causing high electrical currents and, therefore, heating dissipation problems.

In order to increase the solubility of BDP three different organic solvents were tested, namely methanol, acetonitrile and *N*,*N*-dimethylformamide. Mixtures of aqueous buffer and *N*,*N*-dimethylformamide provided good results in terms of BDP solubility. However, it was observed that a plastic piece of the CE apparatus (that used to close up the pressure system), in contact with the buffer containing *N*,*N*-dimethylformamide during the separation run, was destroyed after a few hours working with such a buffer. Since this part of the instrument was necessary for the normal operation of the CE apparatus, we focused on the use of methanol and acetonitrile as organic modifiers. We were able to establish that these two modifiers added to aqueous buffers in percentages of 50% (v/v) or higher provided a good solubility of the analyzed solutes.

3.2. Optimization of the separation procedure

In a first approach, good CE separations of the solutes were explored. Methanol and acetonitrile were employed as organic additives in percentages of 50% testing multiple types of aqueous buffers together with two different surfactants. Namely, 100 m*M* boric/borate at pH 8, 100 m*M* HEPES at pH 7, 50 m*M* ammonium acetate at pH 9, 20, 50 and 100 m*M* CHES at pH 10 and 50 m*M* CHES at pH 10.2

were tested as aqueous buffers. SDS and CTAB were tested as surfactants at concentrations ranging from 0 to 50 m*M* and the voltage polarity had to be reversed when CTAB was used. After testing this group of different buffers, with or without surfactant, the most suitable conditions for further optimization were the use of SDS as detergent and 100 m*M* CHES at pH 10 as aqueous buffer. SDS was chosen for providing shorter analysis times than CTAB while the CHES buffer was selected for producing simultaneously a high buffer capacity, low electrical conductivity and high EOF. Under these conditions, BDP migrates later than PEA, probably due to stronger interactions of BDP with SDS, compared to PEA–SDS interactions. This is in good agreement with the higher hydrophobicity of BDP molecules than that of PEA. The higher hydrophobicity of BDP was suspected due to its lower solubility in water and was further established by calculating the logarithm of the partition coefficients octanol–water (log *P*) for BDP and PEA using atomic parameters derived by Ghose and co-workers [32,33]. According to these calculations, the log *P* was 4.20 for BDP and 1.76 for PEA. Also, under practically all the conditions tested (vide infra) BKC compounds migrate faster than the EOF. This is in good agreement with the molecular structure of these ions bearing a net positive charge on the nitrogen atom, which makes these compounds to migrate in the same direction that the EOF. However, resolution between BDP and PEA was very low.

A better CE separation was then attempted. First, the effect of the SDS concentration on the separation selectivity was studied, testing solutions containing CHES buffer, methanol or acetonitrile and surfactant at concentrations ranging from 0 to 50 m*M*. The plots of selectivity β versus the SDS concentration obtained are shown in Fig. 2A (methanol as organic additive) and Fig. 2B (acetonitrile as organic additive). Selectivity β is defined as $\beta=2(\mu_j-\mu_i)/(\mu_j+\mu_i)$ where μ_j and μ_i are the effective electrophoretic mobility of two compounds migrating consecutively. Irrespective of the organic modifier (methanol or acetonitrile), no separation between the two neutral compounds BDP and PEA is obtained using buffers without SDS, as can be deduced from the nearly zero β value obtained in both cases. As can be seen in Fig. 2, the addition of SDS increases the β value for

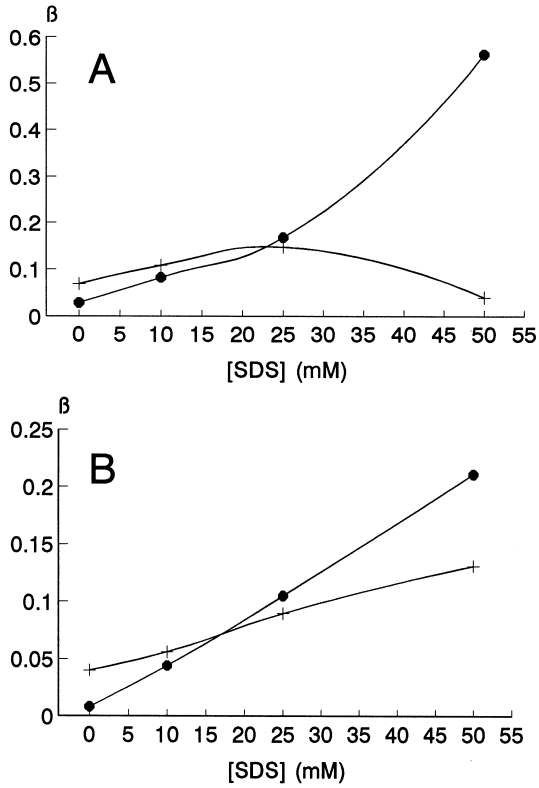

Fig. 2. Plots showing the effect of SDS addition on the selectivity β [$\beta=2(\mu_j-\mu_i)/(\mu_j+\mu_i)$] between BKC compounds (1 and *) (+) and between PEA and BDP (●), using buffers containing (A) methanol–100 m*M* CHES (pH 10) (50:50) and (B) acetonitrile–100 m*M* CHES (pH 10) (50:50). Capillary: 27 cm (20 cm effective length)×50 μm I.D. Applied voltage: 10 kV. Separation temperature: 45°C. Detection: UV at 214 nm.

these neutral compounds. Thus, the higher the surfactant concentration the higher the selectivity between these two neutral solutes irrespective of the organic additive employed. Despite this similar behavior, some interesting features, besides the well known differences in viscosity and analysis speed brought about by both organic solvents, arise when comparing solutions containing methanol with those containing acetonitrile. In the buffer with methanol as organic additive, the effective mobility of BDP at 10 m*M* and 50 m*M* SDS were $2.5\cdot10^{-9}$ and $6\cdot10^{-9}$ m^2/s V, respectively, while for the same SDS concentrations in the buffer containing acetonitrile the values were $4.3\cdot10^{-9}$ and $6.7\cdot10^{-9}$ m^2/s V, respectively. Thus, regardless of the absolute values,

controlled among other factors by the viscosity of the buffer, the ratio between both mobilities in methanol is ca. 2.5, whereas with acetonitrile it is ca. 1.5. Thus, a stronger hydrophobic interaction between neutral solutes and SDS seems to take place in methanol than in acetonitrile. This effect was further analyzed by carrying out a rough estimation of the retention factor for BDP and PEA using a buffer containing 50 m*M* SDS, 50% 100 m*M* CHES at pH 10 plus 50% acetonitrile or methanol and Sudan III as marker. By using this method and the buffer containing acetonitrile, k' values equal to 0.38 and 1.20 were obtained for PEA and BDP, respectively. When 50% methanol was employed instead of acetonitrile, the k' values obtained were 0.58 and 8.73, respectively. Therefore, the stronger hydrophobic interaction mentioned above between neutral solutes and SDS in methanol seems to be corroborated by these k' values, since higher retention factors are obtained in methanol than in acetonitrile. In spite of these consistent results, the fact that there is no certainty about Sudan III indicating the mobility of the micellar phase (if any) in such highly organic buffers [10,29] must be taken into account. Probably, using the homologous series method developed by Bushey and Jorgenson [34] more accurate k' values could have been obtained. However, using such a method additional and unavoidable errors may be introduced due to changes in the EOF, as indicated by Bushey and Jorgenson [34]. Besides, experiments with homologous series could need laborious optimization because not all homologous series can be used for all buffer systems [34]. Therefore, to carry out the mentioned rough estimation of k' values the marker method was chosen. The results obtained using this procedure are also in good agreement with those shown in Ref. [35] where it is demonstrated, using methylene selectivity, that the solute–SDS hydrophobic interaction is higher with methanol as organic additive than using acetonitrile. Moreover, the stronger interaction of neutral solutes and SDS in methanol as opposed to that in acetonitrile can be also explained using the solvophobic theory developed for high-performance liquid chromatography (HPLC) [36,37]. Due to the higher surface tension of 50% water–methanol mixture than 50% water–acetonitrile mixture, the free energy of cavity formation in methanol buffers should be higher than in acetonitrile buffers. Therefore, the SDS–solute interaction should be stronger in water–methanol than in water–acetonitrile.

Fig. 2B shows that using acetonitrile as organic additive the higher the SDS concentration the higher the β value obtained for the cationic compounds of BKC (1 and *). However, a different behavior is observed for 1 and * in methanol (Fig. 2A) where the selectivity β has a maximum value at about 25 m*M* SDS. These results can be also explained through higher solute–SDS interactions obtained in methanol compared to those obtained in acetonitrile as follows. For BKC ionic compounds, hydrophobic and/or ionic interactions with SDS must take place. Under these conditions, the higher the SDS concentration the higher the interactions between BKC and SDS. In methanol, BKC–SDS interactions are so high that they cause a progressive reduction of the positive electrical charge on BKC compounds up to the situation of zero charge at 50 m*M* SDS as could be deduced from the electrophoregrams (data not shown) where peaks 1 and * nearly co-migrate with the EOF. This co-migration is not observed at the same SDS concentration when acetonitrile was used instead of methanol. Such a different behavior could be due to weaker interactions of these ionic solutes with SDS in acetonitrile than in methanol. In conclusion, for BDP and PEA, the neutral solutes, selectivity increases with the concentration of SDS for methanol and acetonitrile. However, the selectivity for the cationic compounds BKC increases with SDS concentration when acetonitrile is used as organic modifier while it goes through a maximum when methanol is employed.

Following with the optimization procedure, the effect of temperature on solutes separation was studied. The same CHES buffer as above plus 25 m*M* SDS was utilized using methanol or acetonitrile as organic additive and varying the running temperature from 20 to 50°C in 5°C-steps. Results of resolution versus temperature are given in Fig. 3A (methanol) and Fig. 3B (acetonitrile). As can be seen in Fig. 3, in methanol as well as in acetonitrile, the higher the temperature the lower the resolution between PEA and BDP, probably as a result of the smaller buffer viscosity and the reduction of the solutes–SDS hydrophobic interaction caused by the increase in temperature [10]. However, for peaks

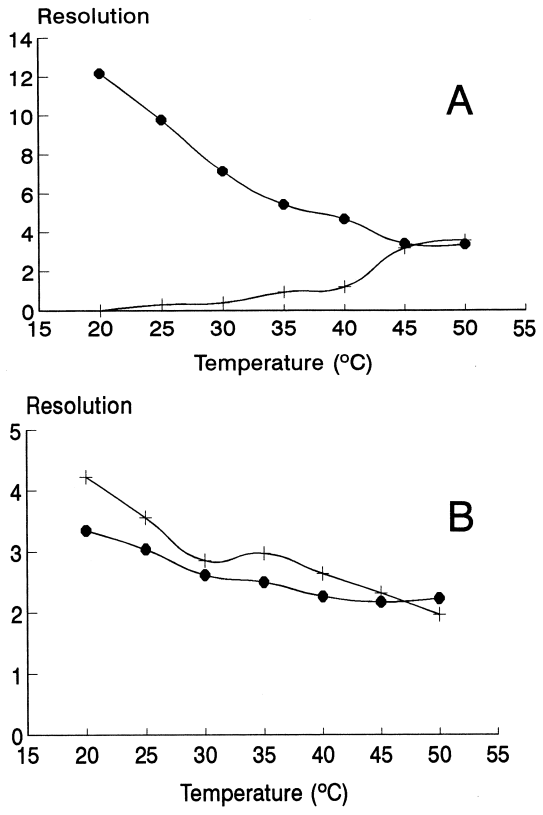

Fig. 3. Plots showing the effect of running temperature on the resolution between BKC compounds (1 and *) (+) and between PEA and BDP (●), using buffers containing (A) methanol–100 m*M* CHES (pH 10) (50:50)–25 m*M* SDS and (B) acetonitrile–100 m*M* CHES (pH 10) (50:50)–25 m*M* SDS. Other conditions as in Fig. 1.

1–* in methanol (Fig. 3A) an increase in resolution was obtained at higher temperatures. Similarly, these discrepancies in electrophoretic behavior can be explained by the lesser interactions between SDS and the different components of BKC induced by an increase in temperature. Thus, at 50% methanol and low temperatures (20–35°C) the strong BKC–SDS interactions neutralize the positive charge of BKC making all BKC compounds migrate with the EOF and causing low resolution values. At higher temperatures (40–50°C) the BKC–SDS interactions decrease and different BKC components could interact with SDS to a different extent conferring them different electrophoretic mobility, therefore increasing resolution values. On the contrary, using acetonitrile as modifier (Fig. 3B) the resolution between peaks 1–* decreases when the temperature is increased. This result could also be explained in terms of decrease of buffer viscosity and reduction of BKC–SDS interactions with temperature. Since acetonitrile causes weaker interactions with SDS than methanol, no zero charge compound is formed in this case at any temperature and, consequently, an increase in temperature only originates a decrease in BKC–SDS interactions causing a decrease in the resolution of 1 and *. However, the analysis speed, and in some cases the peak shape, improved at higher temperatures with acetonitrile or methanol. Therefore, a temperature of 45°C was chosen as suitable in terms of resolution and velocity of analysis for both types of buffers irrespective of the organic modifier added.

As demonstrated above, solute–SDS interactions are weaker with acetonitrile than with methanol. This behavior has been attributed to different solvophobic effects caused by methanol and acetonitrile [36,37]. Another possible explanation of this behavior is discussed next. The higher lipophilic nature of acetonitrile can induce the partial [29] or total [18,24] disruption of SDS micelles together with the decreasing of the SDS–solute hydrophobic interaction [26] for any of the analytes studied, whereas methanol, a typical protic solvent of little lipophilic nature, can bring about the formation of SDS aggregates [18,38–40]. These can probably interact with all the solutes more strongly than the same surfactant in acetonitrile. Moreover, acetonitrile has a higher dielectric constant than methanol (37.5 and 32.6, respectively) causing a decrease of the BKC–SDS electrostatic interactions compared to those in methanol [41]. Also, both organic additives should originate different solute–solvent interactions, mainly based on the solutes' own structural features (e.g., BDP has seven H-bond acceptor sites and one H-bond donor site, while PEA has one H-bond acceptor/donor site). However, more research is necessary to assess the role of the above interactions.

A further optimization of the running buffer composition in terms of analysis speed, resolution and detection noise was carried out by modifying the organic solvent content. Although in general slightly better values of selectivity and resolution were obtained by using methanol (see Figs. 2 and 3) than

with acetonitrile, it was observed that the analysis times provided by buffers containing acetonitrile were, as a minimum, twofold shorter than the values obtained with methanol. Therefore, we focused on the use of acetonitrile as organic co-solvent. Fig. 4 shows the effect of different quantities of this co-solvent added to the separation buffer on the separation profile and on the average noise of each electropherogram for 25 m*M* SDS. As can be seen, when 50% of acetonitrile is used (Fig. 4A) the best separation of the analytes is obtained compared to that obtained in the other three cases. However, the detection noise in Fig. 4A is almost 10-times higher than that obtained in the other electropherograms. An identical effect has been already reported [26,28,29] and it was explained through an onset of SDS nucleation due to the high content in acetonitrile of the buffer [28], or through the formation of tiny air bubbles under the influence of the electrophoretic current [29]. Similarly, as observed by the same authors [26,28,29] this effect is not detected in the other three cases in which a content of 40% of acetonitrile or less was used. Since we intended to use the optimized method to detect low concentrations of these compounds in real samples, and considering that the limit of detection of the method is directly related to the noise obtained, we gave up the use of 50% of acetonitrile. By decreasing the acetonitrile content to 40% (Fig. 4B), BDP showed a peak deformation, probably due to its low solubility. This effect was corrected by using acetonitrile–methanol (40:10) (Fig. 4C). A further increase of the

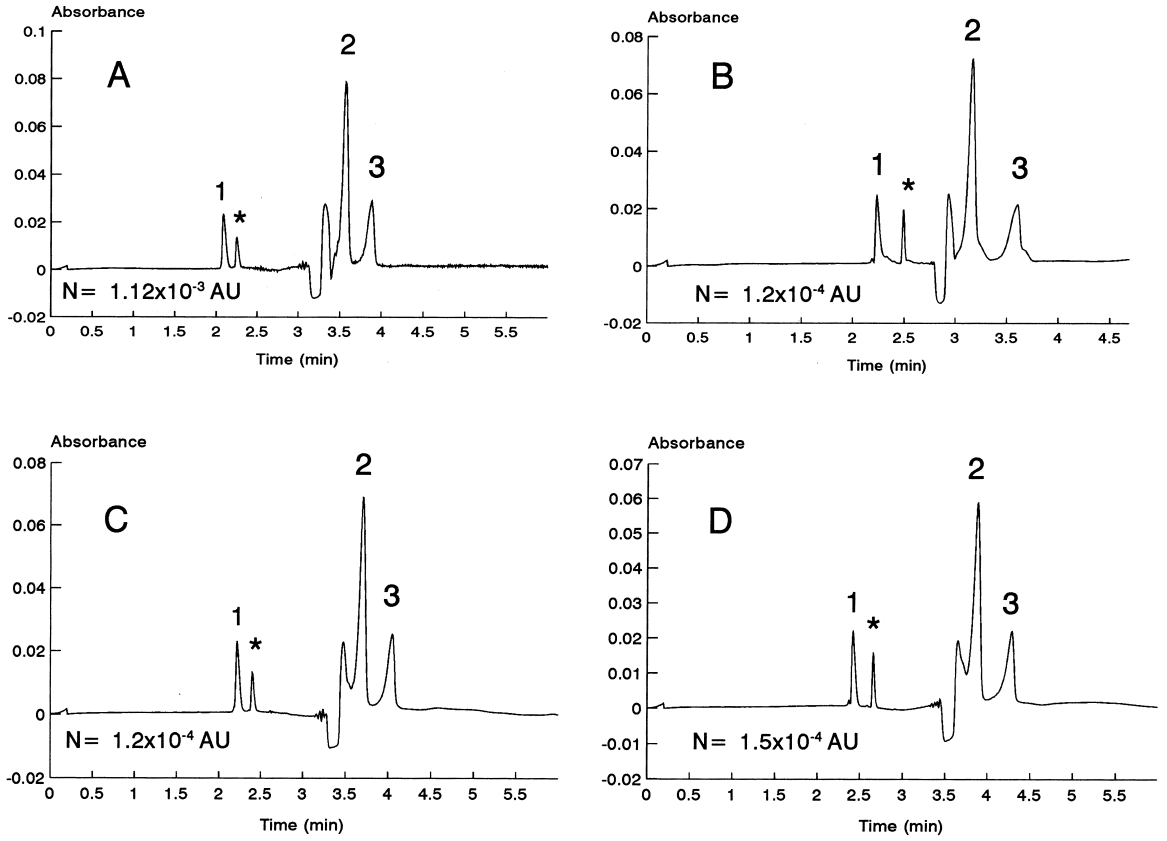

Fig. 4. Electropherograms showing the effect of addition of different organic solvents on the separation of BKC (1 and *), PEA (2) and BDP (3), using buffers containing 25 m*M* SDS and (A) 100 m*M* CHES (pH 10)–acetonitrile (50:50); (B) 100 m*M* CHES (pH 10)–acetonitrile (60:40); (C) 100 m*M* CHES (pH 10)–acetonitrile–methanol (50:40:10); and (D) 100 m*M* CHES (pH 10)–acetonitrile–methanol (50:30:20). Other conditions as in Fig. 1. N indicates the detection noise in the separation conditions.

methanol content to 20% (Fig. 4D) originated a lower resolution between peak 2 and the nearest system peak. Therefore, the conditions given in Fig. 4C were chosen for a further optimization, since the quantitation of PEA (peak 2) could appear, in these operating conditions to a slight extent inaccurate.

In Fig. 5A the separation of the compounds studied under optimized conditions [i.e., running buffer consisted of 30 m*M* SDS–methanol–acetonitrile-100 m*M* CHES, pH 10 (10:40:50)] is shown. As can be seen, by using these conditions it is possible to achieve the separation in less than 5 min with efficiencies up to 150 000 plates/m in the best case (peak * of BKC), and good resolution of the different compounds.

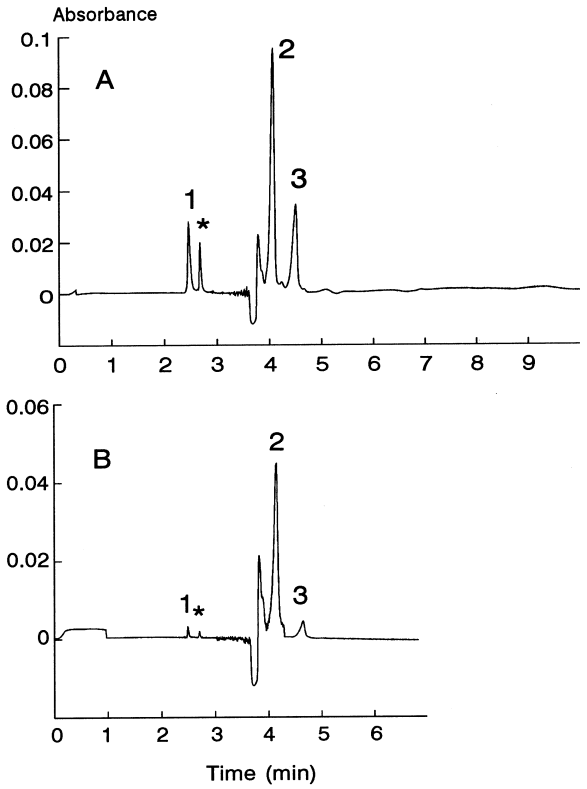

Fig. 5. Separation of BKC (1 and *), PEA (2) and BDP (3) under optimized conditions. Separation buffer: 30 m*M* SDS–methanol–acetonitrile–100 m*M* CHES (pH 10) (10:40:50). Capillary: 27 cm (20 cm effective length)×50 μm I.D. Applied voltage: 10 kV. Separation temperature: 45°C. Injection: 1 s, 0.5 p.s.i. of (A) a standard solution of BKC (1.12 mg/ml), PEA (1.22 mg/ml) and BDP (1.27 mg/ml), detection at 214 nm and (B) a nasal spray; detection at 214 nm until t_m=4.5 min, 254 nm from t_m=4.5 min to the end of analysis.

3.3. Application to real samples: quantitation of BKC, PEA and BDP found in nasal sprays

In Fig. 5B the separation of BKC, PEA and BDP found in a nasal spray is shown. As can be deduced by comparing Fig. 5A and B, the separation method developed in this work seems to be appropriate for the analysis and quantitation of these compounds in real samples. This point is addressed below.

As a first step the analysis time repeatability was studied. The results obtained for the three compounds are given in Table 1. As can be seen, relative standard deviation (R.S.D.) values lower than 1% are obtained for the same day using this procedure, while the repeatability between days was slightly worse, e.g., R.S.D. up to 2.45% was obtained for BDP. This can be due to the difficulty to obtain a reproducible inner capillary wall when bare fused-silica is employed. This point has been considered in this work and we observed that under our conditions, the better repeatability day-to-day was obtained storing the capillary overnight with acetonitrile, and making the two equilibration runs the next day, ca. 30 min of equilibration time. This negative effect has been widely studied [42–45], and it seems to be related to hysteresis phenomena [43,44] as well as the history of each capillary [45].

In Table 1 repeatability in terms of peak areas is also shown. As can be seen, R.S.D. values up to 6.32% were obtained for BKC between days (the peak area for BKC was calculated as the sum of the

Table 1
CE repeatability of analysis time and peak area of BKC, PEA and BDP found in a commercial nasal spray

	R.S.D. (%) of analysis time		R.S.D. (%) of peak area	
	Same day (n=6)	Three days (n=12)	Same day (n=6)	Three days (n=12)
BKC	0.71	0.70	5.81	6.32
PEA	0.62	2.20	1.89	2.50
BDP	0.68	2.45	3.87	0.90

Repeatability was determined for the same day and three different days and is given as relative standard deviation (R.S.D.).

areas of peak 1 and *), while the R.S.D. values for PEA and BDP were lower, i.e., 2.50 and 0.90, respectively. Problems related to the use of CE for quantitative aims [46–48] have been widely discussed, arguing that many parameters have a negative influence on the reproducibility of the injection. Thus, injection by pressure or electromigration is dramatically influenced among other factors by the temperature of the sample solution. Also, the velocity at which the sample passes the detector, which is influenced by the state of the capillary wall, affects the peak area obtained upon integration [49]. All these negative effects bring about high R.S.D. values regarding reproducibility of peak areas, e.g., these values can range from 0.37 to 14.1% depending on the analyte and the separation conditions [46]. Therefore, the relatively high R.S.D. values obtained in this work, from 0.9 to 6.32%, seem to be within the usual range when CE is employed with quantitative purposes.

The procedure developed was applied for the determination of the content of BKC, PEA and BDP found in commercial nasal sprays. First, calibration curves were obtained by injecting mixtures of the three standards at different concentrations. Good linear correlation was obtained in the range ca. 0.02 g/l to 1 g/l for the three compounds, with correlation coefficients calculated by least squared regression higher than 0.9992 ($n=5$). The equations obtained from each curve were then employed to determine the content of BKC, PEA and BDP in samples from commercial nasal sprays. Moreover, the limit of detection of this procedure, calculated in mg/l for a signal equal to two-times the detection noise was 2.3, 7.4 and 6.4 mg/l for BKC, PEA and BDP, respectively.

Three different bottles of nasal spray were tested, two from the same batch and the third from a different batch. In Table 2 the quantitative results are given as α i.e., α=(determined quantity)/(quantity according to the pharmaceutical formulation). As can be seen, a good agreement is obtained between the expected and determined values from the different sprays, that is, α values close to 1 were obtained in all the cases. However, some differences are observed mainly for PEA, e.g., an α value of 1.15 was obtained for this compound in the worst case. This can be related to the partial resolution of peak 2 from the closest system peak, as can be seen from Fig. 5B, which probably makes more difficult a precise determination of this peak area. Also, the negative effects commented above affecting the reproducibility of peak areas in CE must play a role in these determinations. According to these results, CE seems to be a suitable technique to carry out quantitation of very insoluble compounds from real samples when the adequate sample preparation and separation buffer are chosen.

Table 2
Values of α[a] obtained from three bottles of nasal spray, two from the same batch and one from a different batch

	BKC	PEA	BDP
Batch J-3-A	0.995 (5.45)[b]	1.15 (1.95)	1.09 (3.78)
Batch K-33	0.985 (4.66)	1.05 (3.10)	1.03 (4.91)
Batch K-33	1.00 (6.16)	1.07 (4.72)	1.05 (2.77)

[a] α=(Determined quantity)/(Quantity according to the pharmaceutical formulation).
[b] % R.S.D.$_{n=4}$.

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