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Separation and simultaneous determination of the components in an analgesic tablet formulation by micellar electrokinetic chromatography

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Abstract

The separation and simultaneous determination of the active ingredients of an analgesic tablet formulation composed of caffeine, paracetamol, dextropropoxyphene, acetylsalicylic acid and chlorpheniramine was investigated by micellar electrokinetic chromatography (MEKC). As the use of sodium dodecyl sulphate could not resolve the two basic compounds, the separation was completed by using a mixture of the bile salts sodium cholate and sodium deoxycholate. The determination of the five ingredients together with salicyclic acid could be performed with acceptable accuracy and precision.

1. Introduction

Micellar electrokinetic chromatography (MEKC), one of the most important modifications of capillary electrophoresis (CE), has been proved to be an excellent alternative to LC in drug analysis. In some respects there are advantages of MEKC over LC including the ability to perform simultaneous analysis of drug combinations with different polarity and hydrophobicity [1–5], although CE is often considered less sensitive than LC owing to the minute amount of sample introduced into the capillary and the small volume of the detector cell [6]. There are many possibilities for manipulating the CE operating parameters in order to enhance the sensitivity, as suggested by Altria [7]. In this paper, the optimization of the separation of a selected analgesic tablet formulation, consisting of caffeine 36 mg, paracetamol 120 mg, acetylsalicylic acid 250 mg, dextropropoxyphene hydrochloride 30 mg and chlorpheniramine maleate 1.6 mg, is described. The conventional simultaneous determination of the active ingredients together with salicylic acid, a degradation product of acetylsalicylic acid, is then evaluated from an analytical point of view.

2. Experimental

2.1. Equipment

Electrophoresis was carried out on a P/ACE System 2100 fitted with a UV detector (Beck-

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man, Palo Alto, CA, USA). Separations were performed in a 570 mm × 0.075 mm I.D. fused-silica capillary tube (Beckman). The integration of the electropherograms was achieved by the Chromatography Software System Gold V.711 (Beckman).

2.2. Drugs, chemicals and reagents

The drug substances used in the work were of European Pharmacopoeia quality. Acetylsalicylic acid (ASA), caffeine monohydrate (C) and propyl hydroxybenzoate were purchased from Merck (Darmstadt, Germany), paracetamol (P) from Bios (Leuven, Belgium), salicylic acid (SA) from Aldrich (Bornem, Belgium) and dextropropoxyphene hydrochloride (D) and chlorpheniramine maleate (Ch) from Thissen (Br-l'Alleud, Belgium). The surfactants sodium dodecyl sulphate (SDS), sodium deoxycholate (SDC) and sodium cholate (SC) were purchased from Sigma (St. Louis, MO, USA) and used as received. All the other reagents were of analytical-reagent grade. The solvent used for preparing standard and sample solutions was 10 m*M* hydrochloric acid containing 20% (v/v) acetonitrile. The composition of the solvent mixture was intended to achieve complete dissolution and stability of the solutes. The separating buffer solutions were prepared by mixing an appropriate volume of 20 m*M* sodium tetraborate solution with 20 m*M* sodium dihydrogenphosphate solution. The water used for preparing solutions was obtained from a Seralpur Pro 90 CN purification system (Seral, Germany). All buffer solutions were filtered before use through a 0.2-μm membrane filter.

2.3. Procedure

For all experiments, injections were made hydrodynamically by pressure for 2 s and the applied voltage was 20 kV. The temperature was kept constant at 25°C. Detection was performed at 214 nm. Before each injection, separating buffer was passed through the capillary for at least 4 min.

2.4. Sample preparation

Twenty tablets were weighed and ground to a fine homogeneous powder. An accurately weighed portion of powder corresponding to one tablet was transferred to a centrifuge tube to which 50.0 ml of the solvent mixture were added. After shaking and sonicating for 15 min and centrifuging until a clear solution was obtained, 25.0 ml of the clear supernatant were transferred into a 50-ml volumetric flask to which 10.0 ml of internal standard solution (propyl hydroxybenzoate, 50 mg per 100 ml) were added. The volume was adjusted to 50.0 ml with the same solvent.

3. Results and discussion

3.1. Separation by capillary zone electrophoresis (CZE)

From some preliminary experiments carried out in the CZE mode using a buffer at pH 9, the following features were observed.

The migration order of the six compounds was as predicted from their acid–base dissociation constants. The two basic compounds Ch and D migrated first because of the combined effect of the electrophoretic mobility and the electroosmotic flow (EOF) in the direction of the cathode. Caffeine, a neutral xanthine derivative, migrated with the velocity of the EOF. The negatively charged acidic compounds, P, ASA and SA, migrated in this order with different velocities lower than that of the EOF. Although all compounds were well separated, D exhibited a distorted peak.

When performing the separation at lower pH values (down to 7), the distortion of the D peak gradually disappeared but two pairs of compounds co-eluted: P together with C and Ch together with D.

Moreover, the presence of the uncharged

compound C as test substance implied the need to add a surfactant to the separating buffer with a view to quantitative analysis.

3.2. Separation by micellar electrokinetic chromatography (MEKC)

Sodium dodecyl sulphate

Adding SDS to the buffer provided the largest effect on the migration behaviour of the two basic compounds Ch and D. In contrast to their migration in CZE, they eluted last among all the solutes but were not separated from each other in the pH range examined (7–9). Some buffer additives that were reported to improve the selectivity in MEKC, such as organic solvents [8], urea [9] and glucose [10], were investigated but none of these attempts was successful. This can be explained by the fact that both Ch and D are completely taken up by the SDS micelles owing to strong electrostatic interactions. As suggested by Nishi et al. [11,12], these basic compounds migrate at the same velocity as the micelles. Consequently, the migration time of Ch or D can be considered as the migration time of the micelles (t_{mc}). Based on this assumption, the apparent capacity factors (k') are calculated from the equation introduced by Terabe et al. [13]:

$$k' = \frac{t_r - t_0}{t_0(1 - t_r/t_{mc})} \quad (1)$$

and plotted as a function of the concentration of SDS. The results obtained at pH 7 are shown in Fig. 1. As only the k' values of the uncharged compounds C and P showed a linear relationship with the surfactant concentration, their migration can be considered as a purely chromatographic process based on the partitioning between the two phases [13]. However, in the k'-equation mentioned above a possible effect of charge on the migration is not included. Therefore, with an anionic compound it is necessary to correct for the electrophoretic mobility in order to obtain an accurate estimate of capacity factors [14]. Using the effective mobility (u_{eff}) of each of the two solutes ASA and SA calculated from

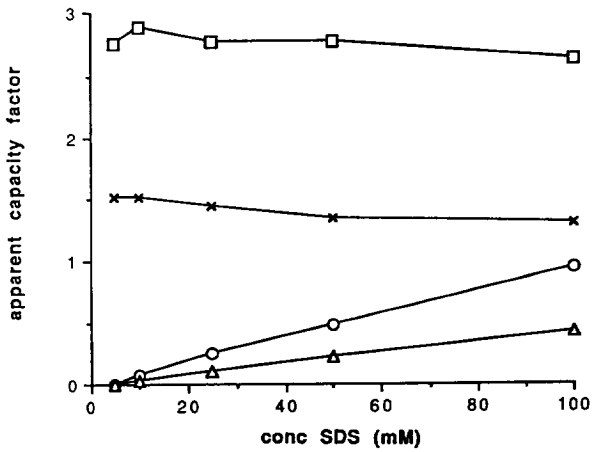

Fig. 1. Apparent capacity factors (calculated from Eq. 1) of (○) caffeine (C), (△) paracetamol (P), (×) acetylsalicylic acid (ASA) and (□) salicylic acid (SA) as a function of SDS concentration. Buffer, SDS in 20 m*M* borate–phosphate (pH 7); capillary, fused silica, 570 mm × 0.075 mm I.D.; injection time, 2 s; voltage, 20 kV, temperature, 25°C.

their migration velocities in CZE, migration times t_{ion} were calculated (at each level of SDS concentration) as would be expected in the absence of micelles [14]:

$$t_{ion} = \frac{1}{u_{eff} + u_{eof}} \cdot \frac{Ll}{V} \quad (2)$$

where u_{eof} is the mobility of the EOF observed at each SDS concentration, V is the applied voltage, and L and l are the total length and the length to the detector of the capillary, respectively. On replacing t_0 by t_{ion} in Eq. 1, the capacity factors obtained show small negative values (Table 1), which means that their migration is not affected by any partitioning with the micelles. This is not surprising, as ASA and SA are fully charged at pH 7 ($pK_a = 3.5$ and 3.0 for ASA and SA, respectively) and therefore there is no partitioning of these solutes in the micelles owing to the electrostatic repulsion of the anionic solutes from the micelles. The negative values of the capacity factors are the result of some errors according to the t_{ion} values which were calculated and expected in the absence of the surfactant and could be different from the actual t_{ion} values that would occur in the system with surfactant owing to the different factors,

Table 1
Capacity factors (k') calculated from Eq. 1 after correcting for t_0 by t_{ion} ($t_{mc} = t_m$ of Ch or D)

SDS concentration (mM)	t_{mc} (min)	ASA			SA		
		t_r (min)	t_{ion} (min)	k'	t_r (min)	t_{ion} (min)	k'
5	8.76	5.48	5.63	−0.07	6.34	6.43	−0.05
10	9.05	5.52	5.69	−0.08	6.42	6.50	−0.04
25	9.62	5.66	5.90	−0.11	6.62	6.78	−0.08
50	12.28	6.37	6.81	−0.13	7.72	8.01	−0.10
100	15.06	6.95	7.56	−0.15	8.66	9.07	−0.11

i.e., ionic strength, viscosity, charge interaction, etc.

Bile salts

Among several ways of manipulating the selectivity in MEKC, the most effective is to change the type of surfactant [15]. The fact that bile salts possess both hydrophilic and hydrophobic faces [11,12] gives rise to advantages over the conventional use of SDS for the separation of hydrophobic molecules [12,16,17] and basic compounds [11]. In this case SC and SDC were employed as surfactants, especially to separate Ch and D, together with the other solutes. With SC at a concentration of 50 mM in borate buffer (pH 9), all compounds were separated but the peak of D was distorted. Moreover, SA eluted in a region where the baseline was disturbed by some impurity in SC. With SDC used under the same conditions, however, Ch eluted together with SA in addition to ASA with propyl hydroxybenzoate (internal standard). Varying the concentration of SC and SDC from 50 to 100 mM did not improve the selectivity of the separation. Co-elution of some components still occurred. The selectivity could be optimized by mixing SC and SDC in different proportions as follows: (A) 25 mM SC + 25 mM SDC and (B) 25 mM SC + 50 mM SDC. These mixtures of the two bile salts gave a good compromise for the mobility profile of the solutes compared with that obtained from the individual bile salts (Fig. 2). Combination B provided good separation and peak shapes whereas the electropherogram with mixture A still exhibited a distorted peak of D. The reason for the distortion could be partly the effect of acetonitrile in the sample solution on the micelles in the separation buffer [18]. The fact that the distortion occurred only with D (it was also observed with Ch when the concentration of Ch in the sample solution was high) and only in the presence of SC might be due to the higher critical micellar concentration of SC compared with SDS and SDC (SC, $13 \cdot 10^{-3}$–$15 \cdot 10^{-3}$ M; SDS, $8.1 \cdot 10^{-3}$ M; SDC, $4 \cdot 10^{-3}$–$6 \cdot 10^{-3}$ M at 25°C) and also to the lower aggregation number of SC compared with SDS and SDC (SC, 2–4; SDS, 62; SDC, 4–10) [19]. Therefore SC micelles could tolerate high amounts of

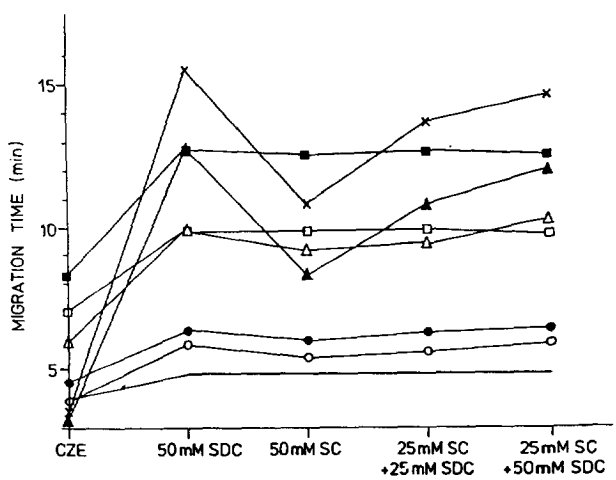

Fig. 2. Migration times of the analytes in 20 mM borate buffer (pH 9) containing different proportions of bile salts (SC and SDC). Other conditions as in Fig. 1. Line with no symbols = EOF; ○ = C; ● = P; □ = ASA; ■ = SA; △ = internal standard; ▲ = CH; × = D.

organic solvent to a lesser extent than SDS or SDC micelles. Further, D and Ch, both of which are positively charged, can be adsorbed on the negatively charged surface of the capillary.

3.3. Analysis of a tablet formulation

An analgesic tablet formulation containing the active ingredients for which the separation had been optimized was analysed by the internal standard method, which was proved to be necessary for quantitative purposes [20], using 20 m*M* borate buffer (pH 9) containing 25 m*M* SC and 50 m*M* SDC.

Calibration

Calibration lines were constructed in the concentration range covering the nominal concentration used for analysis, i.e., 10–40 mg per 100 ml for C, 25–150 mg per 100 ml for P, 100–300 mg per 100 ml for ASA, 0.8–6.5 mg per 100 ml for Ch, 12–40 mg per 100 ml for D and 1–10 mg per 100 ml for SA, together with 10 mg per 100 ml of propyl hydroxybenzoate as an internal standard. Linear regression lines of the ratios of the peak area as a function of the concentration were calculated using the least-squares method. Good linearity ($r = 0.998$–0.999) confirmed by statistical analysis was obtained for all compounds.

Precision and accuracy

The accuracy and repeatability were evaluated from a recovery experiment performed on an artificially prepared powder mixture containing all active ingredients in the concentrations present in a real sample, added to a mixture of tablet excipients composed of corn starch, microcrystalline cellulose, magnesium stearate and talc. Samples were treated as described in the procedure for sample preparation. The final concentrations of each compound expressed per 100 ml were ca. 36 mg for C, 120 mg for P, 30 mg for D, 250 mg for ASA and 1.6 mg for Ch, using 10 mg per 100 ml of propyl hydroxybenzoate as an internal standard. Six replicate samples were analysed by comparison with an external reference solution with approximately the same concentrations as the sample solution. Mean recoveries are given in Table 2 together with the relative standard deviations (R.S.D.) and 95% confidence intervals of the mean (95% C.I.). Good accuracy and acceptable precision were obtained, except for Ch, which showed a high R.S.D. owing to its very low dose.

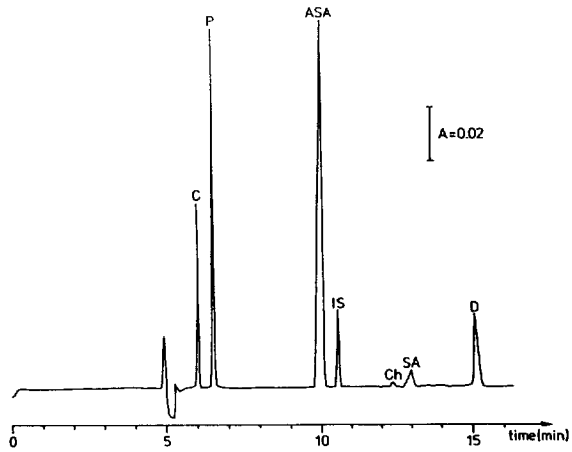

Fig. 3. Typical electropherogram for a real tablet. Buffer, 25 m*M* SC + 50 m*M* SDC in 20 m*M* borate (pH 9). Other conditions as in Fig. 1.

Table 2
Results of mean recovery ($\bar{X}$), repeatability (R.S.D.) and accuracy [95% confidence interval (95% C.I.)] from spiked powder mixture ($n = 6$)

Component	$\bar{X}$ (%)	R.S.D. (%)	95% C.I.
Caffeine	98.97	1.54	97.6–100.4
Paracetamol	100.54	0.94	99.7–101.4
Acetylsalicylic acid	99.28	0.98	98.3–100.2
Chlorpheniramine maleate	98.69	6.19	93.0–104.3
Dextropropoxyphene hydrochloride	100.43	3.92	97.3–104.6

Table 3
Results of the analysis of a real tablet formulation

Component	Label claim (mg)	Amount found (mean ±S.D.)[a]	% of label claim (mean ± S.D.)[a]
Caffeine	36	36.7 ± 0.34	101.9 ± 0.88
Paracetamol	120	121.7 ± 2.32	101.4 ± 1.79
Acetylsalicylic acid	250	251.7 ± 6.62	100.7 ± 2.65
Chlorpheniramine maleate	1.6	1.65 ± 0.09	103.2 ± 5.75
Dextropropoxyphene hydrochloride	30	29.6 ± 0.62	98.4 ± 2.08
Salicylic acid	–	4.8 ± 0.25	

[a] Means of three samples ± standard deviation.

Analysis of real samples

Determination of the active ingredients including salicylic acid, the degradation product of ASA, was performed on a real tablet with the method described above (Fig. 3). As can be seen from Table 3, the results are in agreement with the 95–105% limits during the shelf-life.

4. Conclusions

In this CE application for the determination of the active ingredients of an analgesic formulation using MEKC, it has been demonstrated that the use of bile salts is more advantageous than SDS micelles, especially for the separation of basic compounds. Optimization of the separation could be achieved by mixing sodium cholate and sodium deoxycholate in different proportions. The simultaneous determination of five active components and one degradation product in an analgesic tablet formulation could be performed accurately, although there was a large difference in the dose of the ingredients.

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