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Determination of bile acids in pharmaceutical formulations using micellar electrokinetic chromatography

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Abstract

A micellar electrokinetic chromatography method (MEKC) has been developed and validated for the determination of bile acids (BA) such as ursodeoxycholic acid (UDCA), dehydrocholic acid (DHCA) and deoxycholic acid (DCA) in pharmaceuticals for quality control purpose. The background electrolyte consisted of 20 mM borate-phosphate buffer containing 50 mM sodium dodecylsulfate (SDS), and acetonitrile as additive. UV detection was set at 185 nm. Selectivity, linearity, range, repeatability, intermediate precision and accuracy showed good results. Comparison of the values obtained by MEKC and HPLC methods were in close agreement.

Keywords: Micellar electrokinetic chromatography; Bile acids; Pharmaceuticals

1. Introduction

Analytical performance characteristics of capillary electrophoresis (CE) and its relevant advantages such as high resolution separation, speed of analysis and very low reagent consumption have made possible a rapid grow up of this technique in quality control of pharmaceuticals [1–7]. It fulfills many of the needs of the analytical requirements in quality control such as determination of main and minor inorganic and organic components and traces in simple or complex matrices. In the pharmaceutical area, batch-to-batch quantification of active components and their impurities are required for bulk drugs and finished products.

Bile acids (BA) play an important physiological role in biological systems and are also employed as therapeutic agents (Fig. 1). The most common BA used in therapy are ursodeoxycholic acid (UDCA), chenodeoxycholic acid (CDCA), dehydrocholic acid (DHCA) and deoxycholic acid (DCA). Nowadays, UDCA is widely used for the dissolution of cholesterol gallstones and in the treatment of some hepatic diseases. DHCA and DCA are used as choleretic agents associated with other components in therapy of liver disfunctions.

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By CE technique is possible not only direct detection at very low wavelength but also indirect UV detection, thus analytes with poor or non absorptive properties can be determined achieving reliable and accurate results [8–11].

Determination of BA is commonly accomplished by HPLC though these compounds have a chemical structure characterized by low absorptivity at UV. Therefore, their detectability is limited when they are analized by liquid chromatography [12,13].

At present, a CE method with indirect UV detection applied to the quality control of UDCA and related substances has been reported [14]. In this work the quantification of bile acids DCA, DHCA and UDCA by micellar electrokinetic chromatography (MEKC) in pharmaceutical solid dosage forms is proposed.

2. Experimental

2.1. Materials and reagents

Dehydrocholic acid, deoxycholic acid, ursodeoxycholic acid, thioctic acid (TA) and sodium dodecylsulfate (SDS) were obtained from Sigma Chemical Co. (St Louis, USA). Sodium borate, sodium monohydrogen phosphate, potasium dihydrogen phosphate, methanol and acetonitrile were HPLC grade and supplied by Merck (Darmstadt, Germany). Ultrapure water was obtained from an EASYpure™ RF equipment (Barnstead, USA). All other reagents were of analytical grade. Solutions and samples were filtered through a 0.45 μm nylon membrane (Micron Separations Inc. USA) and degassed before use. The excipientes povidone, silicon dioxide, sucrose, corn starch, talc, stearic acid, calcium phosphate, lactose, arabic gum were obtained from Sigma. Magnesium stearate was purchased from Aldrich (Milwakee, WI).

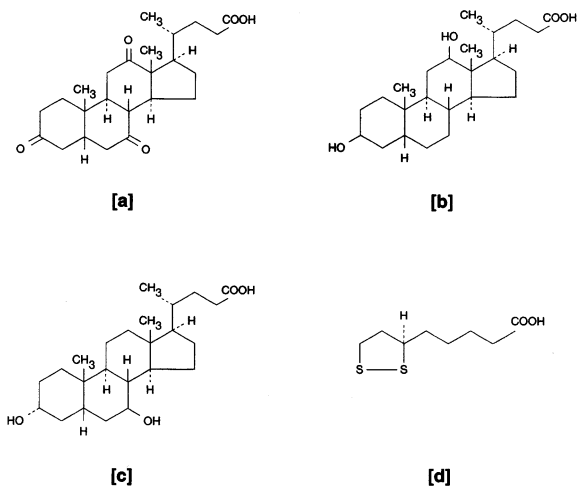

Fig. 1. Chemical structures of (A) dehydrocholic acid; (B) deoxycholic acid; (C) ursodeoxycholic acid and (D) thioctic acid.

2.2. Instrumentation

MEKC was carried out with a Capillary Ion Analizer (Water Corp., Milford, MA). Data were collected and processed by Millennium™ software (Waters Corp., Milford, MA). An uncoated fused-silica capillary of 60 cm length and 75 μm i.d. (Waters Corp., Milford, MA) was used. For HPLC analyses a liquid chromatograph Hewlett-Packard Model 1100 (Hewlett–Packard, Palo Alto, CA) with a Chemstation software was used to collect and process the data. A chromatographic column LiChrospher, RP-18, (250×4 mm I.D., 5 μm) (Merck) was employed.

2.3. CE-system

The analytical procedure by MECK was performed by employing a background electrolyte consisted of 10 mM sodium tetraborate, 10 mM sodium monohydrogen phosphate buffer, pH 9.0, with 50 mM SDS and 10% acetonitrile. Hydrostatic injection (10 cm height) for 15 s, an operating voltage of 25 kV at a temperature of 25 ± 0.1°C and UV detection at 185 nm using a mercury lamp were employed.

At the beginning of each day capillary was rinsed with 0.1 M KOH for 5 min, then washed with water for 10 min and finally with background electrolyte for 15 min. Between runs, the capillary was conditioned with running electrolyte for 3 min. At the end of the day, the capillary was flushed with 0.1 M KOH for 5 min and then with water during 10 min.

2.4. HPLC conditions

HPLC analyses of DHCA, TA and DCA were performed using a mobile phase containing acetonitrile and 75 mM potassium dihydrogen phosphate adjusted to pH 3.0 with 85% phosphoric acid (45:55, v/v). Detection was set at 208 nm. The flow rate was 1.0 ml min^{-1}, temperature was at 25°C and 10 μl was the injection volume. For UDCA the chromatographic conditions used were as those described in USP 23 monograph [15].

2.5. Stock and standard solution

Stock solutions of DHCA, DCA and TA in methanol containing 1.0 mg ml^{-1} were prepared. A standard solution was obtained by appropiate dilution of aliquots of each stock solution in 10 mM borate-10 mM phosphate buffer to obtain a final concentration of 400 μg ml^{-1} for DHCA, 200 μg ml^{-1} for DCA and 30 μg ml^{-1} for TA, respectively.

A stock solution of UDCA in methanol containing 5 mg ml^{-1} was also prepared. A standard solution of 500 μg ml^{-1} was obtained by suitable dilution of the stock solution with 10 mM borate-10 mM phosphate buffer.

2.6. Sample preparation

Formulations obtained from different companies were analyzed. A solid dosage form containing DHCA, DCA and TA was named as formulation 1. Ten tablets were weighed and the average weight was calculated. Then the tablets were finely powdered and an amount equivalent to one tablet was accurately weighed in a 50.0 ml volumetric flask and mixed with 40 ml of methanol. The mixture was introduced in an ultrasonic bath for 20 min, and then completed to 50.0 ml with methanol. Ten milliliters of the suspension were centrifuged at 4000 rpm for 10 min and an aliquot of 5.0 ml of supernatant solution were diluted in buffer and taken up to 25.0 ml in a volumetric flask.

Tablets containing UDCA were named as formulation 2 and evaluated. The average tablet weight of ten tablets was calculated. Then, the tablets were finely powdered and the analytical procedure was followed as it was described above. Finally an aliquot of 5.0 ml was diluted to 50.0 ml with buffer solution.

2.7. Recovery study

Recovery of active ingredients was measured by spiking drug substances to placebo samples at 50, 100 and 150% levels of nominal values and each level concentration was analyzed by triplicate. Each solution was injected twice.

3. Results and discussion

The aim of this work was to develop and validate a simple capillary electrophoretic method for the quantitation of bile acids in pharmaceutical dosage forms for quality control purposes.

Thioctic acid is a common component associated with BA employed as coleretic agents in pharmaceutical formulations. Therefore, capillary electrophoretic separation of this compound was optimized during the method development.

Electropherograms of standard solutions, blanks and formulations are given in Figs. 2 and 3.

During the analytical method development and optimization, the effects of several factors such as nature, concentration and pH of the running buffer, the sample solvent injection and concentrations of additives were considered in order to achieve the best separation in a short time.

In the optimization of the electrophoretic system the most suitable buffer electrolyte resulted to be 20 mM phosphate-borate because a lower noisy base line at 185 nm and an acceptable intensity current were achieved. A concentration of 10 mM was not adecuate for separation and 30 mM generated a high intensity current. A good separation of BA was obtained by using a concentration of 50 mM of SDS. This micellar agent gave the best interaction of BA with the micelles. The effect of pH was also investigated and a good selectivity was achieved between 7.0 and 9.0, although for shorter run times the latter value was chosen. The influence of the sample solvent injec-

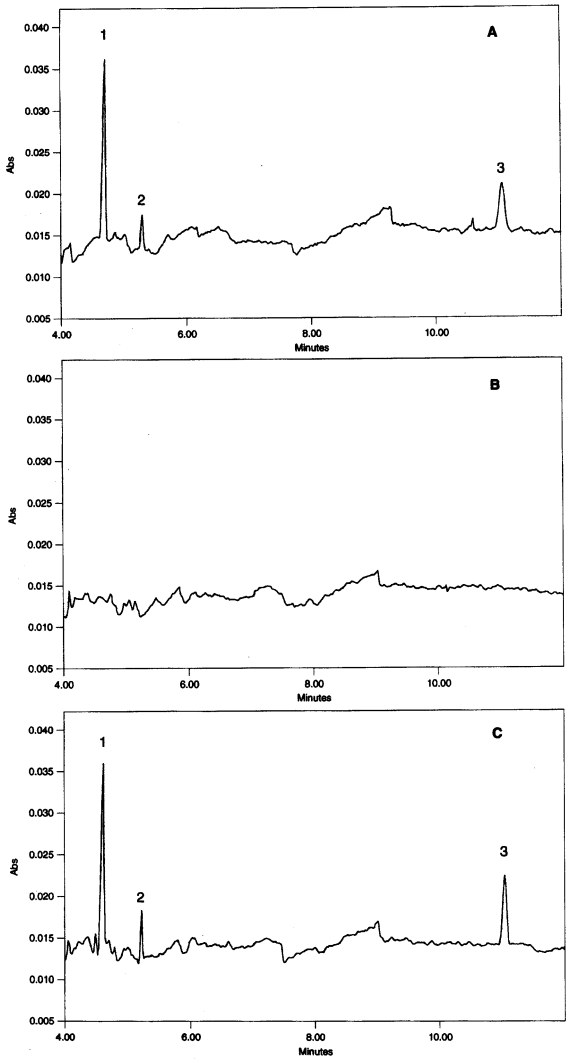

Fig. 2. Electropherograms of (A) a standard solution containing 1, dehydrocholic acid 400 μg ml^{-1} (7.6 ng); 2, thioctic acid 30 μg ml^{-1} (0.6 ng) and 3, deoxycholic acid 200 μg ml^{-1} (3.8 ng); (B) a placebo sample; (C) CE separation of active components in a solid tablet dosage form containing 1, dehydrocholic acid; 2, thioctic acid and 3, deoxycholic acid. Experimental conditions as given in the text.

tion was also examined. Addition of methanol to phosphate-borate buffer at pH 9.0 was necessary because solubility of BA in water is very limited.

Acetonitrile, used as modifier, resulted to be more effective than methanol to enhance peak shapes and electrophoretic separation. Addition of organic solvent between 5 and 20% to the running buffer was evaluated. Ten percent of acetonitrile allowed not only to improve peak shapes but also to achieve the best resolution. A higher percent of organic modifier produced troubles in the operation of the electrophoretic system.

In the validation of the method analytical figures of merit such as selectivity, linearity, range, limits of detection and quantitation, accuracy and precision were evaluated.

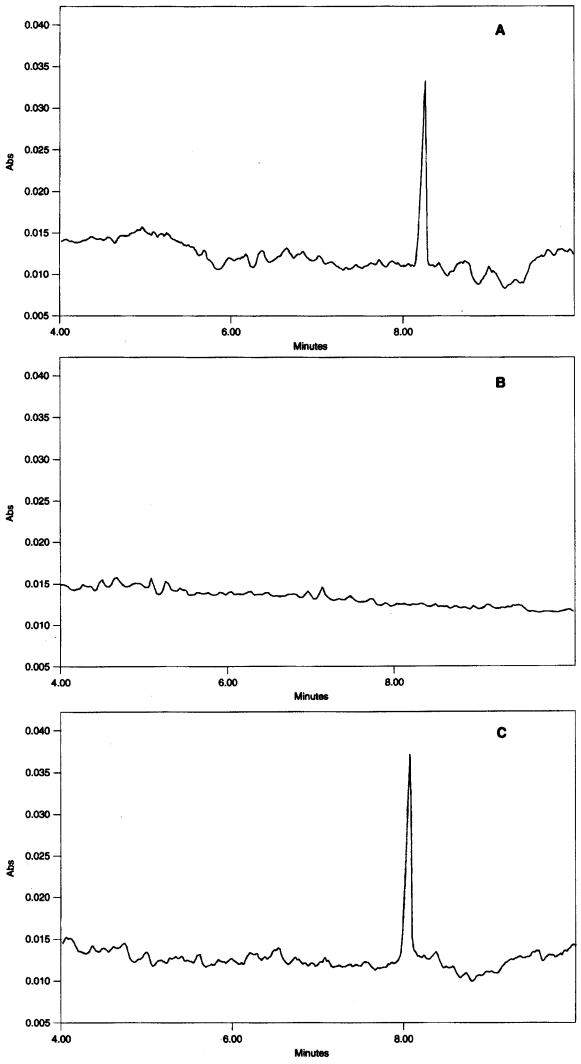

Fig. 3. Electropherograms of (A) a standard solution containing ursodeoxycholic acid 500 μg ml^{-1} (9.5 ng; (B) a placebo sample; (C) CE separation of active component in a solid tablet dosage form containing ursodeoxycholic acid. Experimental conditions as given in the text.

Table 1
Electrophoretic system precision expressed as RSD values

Component	DHCA[a]		TA[a]		DCA[a]		UDCA[b]	
	tm	Peak area/tm	tm	Peak area/tm	tm	Peak area/tm	tm	Peak area/tm
Intraday assay($n=6$)	1.5	1.2	2.0	2.0	2.0	1.8	1.0	1.8
Interday assay[c]($n=18$)	1.8	1.9	2.4	2.5	2.0	2.1	1.3	2.1

[a] Formulation 1.
[b] Formulation 2.
[c] Mean values obtained on three different days from replicate injections ($n=6$) of a standard solution.

Table 2
Linearity and detection limits

Component	LOD(μg/ml)	LOQ(μg/ml)	Linear range(μg/ml)
DHCA[a]	9.0	30.0	30.0–1000.0 $y=0.15x+3.45$; slope: S.E., 1.6; intercept: S.E., 1.8; $r=0.9992$
TA[a]	3.0	10.0	10.0–200.0 $y=0.36x-1.08$; slope: S.E., 0.7; intercept: S.E., 0.7; $r=0.9961$
DCA[a]	15.0	50.0	50.0–800.0 $y=0.15x+2.93$; slope: S.E., 0.9; intercept: S.E., 1.0; $r=0.9990$
UDCA[b]	15.0	50.0	50.0–1000.0 $y=0.27x-0.74$; slope: S.E., 3.4; intercept: S.E., 2.9; $r=0.9994$

[a] Formulation 1.
[b] Formulation 2.

Selectivity was demonstrated by examining migration times, by spiking of standard solutions to the real samples to confirm the absence of interference with analyte peaks and by estimating resolution factors. Placebo blank tests were simultaneously performed.

Repeatability and intermediate precision of the electrophoretic system were evaluated for migration times and normalized peak areas. Data is summarized in Table 1.

Linear calibration curves for each component at six different concentration levels, over the range investigated were obtained. Each solution was injected twice. By linear regression analysis equations, standard error of the slope, standard error of the intercept and correlation coefficients were calculated. Data obtained are summarized in Table 2. The limits of detection (LOD) (signal-to-noise equal to three) and quantitation (LOQ) ((signal-to-noise equal to ten) were calculated and are shown in Table 2. RSD values ($n=6$) for LOQ were 5.5% for DHCA, 6.8% for TA, 5.2% for DCA and 4.8% for UDCA.

When averaged migration times ($n=6$) of standard solutions were compared to those of the spiked placebo no significant statistical difference was observed ($P<0.05$) showing no matrix effect and therefore external calibration standards resulted to be suitable for quantitation of the active components.

Verification of the accuracy of the proposed method was evaluated by intra-day and inter-day recovery assays described in the experimental section. Precision of the entire analytical procedure was also examined and the values obtained are displayed in Table 3.

Robutness was investigated by modification of the background electrolyte with variations of $\pm$ 5% in pH values, and of $\pm$ 5% in surfactant and buffer concentrations. No significant effect on resolution between the nearest peaks were observed.

Formulation 1 contained 100 mg of DHCA, 15 mg of TA and 50 mg of DCA per tablet. Formulation 2 contained only 300 mg of UDCA per tablet.

Table 3
Method precision and accuracy

Component	Spiked levels					
	Repeatability (%)			Intermediate precision[a] (%)		
	50	100	150	50	100	150
DHCA	99.6 (1.8)	102.7 (1.0)	99.5 (0.9)	100.2 (2.0)	101.9 (1.1)	100.1 (1.2)
TA	98.0 (2.1)	98.6 (1.3)	100.1 (1.2)	99.0 (2.4)	99.3 (1.5)	99.8 (1.4)
DCA	103.2 (2.0)	102.8 (1.4)	102.1 (1.1)	102.5 (2.0)	101.6 (1.5)	101.9 (1.2)
UDCA	100.6 (1.7)	101.6 (0.9)	102.5 (1.5)	100.1 (2.0)	102.0 (1.1)	101.9 (1.8)

[a] Mean recovery values of triplicate individual samples obtained in three different days. RSD values in parenthesis.

Table 4
Analysis of pharmaceutical formulation 1 in tablets

Component	Declared mg	Found CE mg[a]	RSD	HPLC mg[a]	RSD
DHCA	100.0	100.0	2.1	100.2	1.9
TA	15.0	14.7	0.7	14.7	0.9
DCA	50.0	50.6	1.1	51.8	1.2

[a] Mean values of triplicate determinations.

Table 5
Analysis of ursodeoxycholic acid in tablet formulation 2

Laboratory	Declared mg	Found CE mg[a]	RSD	HPLC mg[a]	RSD
1	300.0	306.6	2.1	306.0	2.2
2	300.0	295.0	0.4	297.0	1.5

[a] Mean values of triplicate determinations.

The USP 23 monograph for UDCA tablets describes an assay based on RP-HPLC with differential refractive index detector. Good agreement of the results between CE and HPLC methods was obtained (Tables 4 and 5).

At present, determination of impurities of BA in bulk drugs and pharmaceutical products is in progress.

4. Conclusions

The MEKC method proposed in this work proved to be simple, rapid and useful in quality control of solid dosage forms containing bile acids. No matrix interference was observed and the method resulted to be accurate and precise. Due to the good analytical performance of MEKC method it may be proposed as an alternative to HPLC technique in the pharmaceutical laboratory.

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