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Analysis of clindamycin by micellar electrokinetic chromatography with a mixed micellar system

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Abstract

This study details the development and validation of an optimized method with micellar electrokinetic chromatography for the analysis of clindamycin. The method uses a mixed micellar phase containing anionic sodium dodecylsulfate (SDS) and non ionic Brij 35 on an untreated fused-silica capillary. The influences of buffer concentration, pH, SDS, Brij 35 and organic modifier were investigated. Special attention was given to the role of the non ionic Brij 35 in the mixed micellar system. Optimization with a central composite design resulted in optimal separation conditions: background electrolyte containing 25 m*M* sodium tetraborate pH 7.75, 90 m*M* SDS, 14 m*M* Brij 35 and 21% acetonitrile. The applied voltage was 15 kV and the capillary temperature 15°C. The method was robust and gave good linearity and repeatability. The limits of detection and quantitation were 0.05 and 0.15%, respectively, relative to a 2.5 mg/ml clindamycin solution. Two commercial bulk products were analysed with this system.

Keywords: Clindamycin; Antibiotics

1. Introduction

Clindamycin is an antibiotic effective against Gram-positive aerobes and both Gram-negative and Gram-positive anaerobic pathogens, especially *B. fragilis* [1,2]. It is a lincosaminide and is synthesized by chemical reaction of lincomycin with thionyl chloride. This results in the replacement of the 7*R*-hydroxyl group to form 7*S*-chloro-7-deoxylincomycin [3].

The following impurities are commonly found in clindamycin bulk drug: clindamycin B, 7-epiclindamycin, lincomycin, lincomycin B and 7-epilincomycin [4]. Structures are shown in Fig. 1. A TLC method [5], a GLC method [6,7] and several LC methods [4,8,9] were described for the analysis of clindamycin. No CE method has been reported. The most recently developed LC method [9] separates lincomycin B, lincomycin, 7-epilincomycin, clindamycin B, 7-epiclindamycin and clindamycin within 20 min. A disadvantage of this method is the elution of lincomycin B near the solvent front [9].

The popularity of mixed micelles has increased over the past few years. Different combinations of surfactants have been reported in micellar electrokinetic chromatography (MEKC): anionic/non ionic, anionic/cationic, anionic/zwitterionic, cationic/cat-

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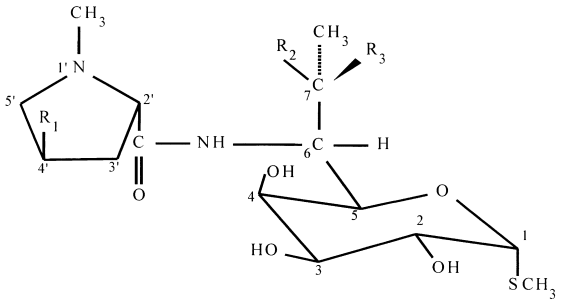

	R_1	R_2	R_3
Lincomycin	CH_3-CH_2-CH_2-	OH	H
Lincomycin B	CH_3-CH_2-	OH	H
7-Epilincomycin	CH_3-CH_2-CH_2-	H	OH
Clindamycin	CH_3-CH_2-CH_2-	H	Cl
Clindamycin B	CH_3-CH_2-	H	Cl
7-Epiclindamycin	CH_3-CH_2-CH_2-	Cl	H

Fig. 1. Clindamycin and related substances.

ionic, cationic/zwitterionic, bile salt/anionic, bile salt/bile salt, fluorocarbon/anionic and non ionic/non ionic (for charged solutes) [10]. The system described in this paper uses a mixed micellar phase of anionic sodium dodecylsulfate (SDS) and non ionic Brij 35. The addition of Brij 35 to SDS micelles leads to a narrower elution window and changes the selectivity [11]. Increasing the concentration of the non ionic Brij 35 decreases the effective charge and therefore the electrophoretic mobility of the mixed micelles [11]. Brij 35 also has a capillary wall coating effect and an increased concentration of Brij 35 in the buffer increases the viscosity. Both effects lead to a decrease in electroosmotic flow (EOF) [12].

One major advantage of the mixed anionic–non ionic system is that there is no increase in the current with increasing concentration of non ionic surfactant [13]. Therefore, Brij 35 may be added to the micellar phase without an increase in Joule heating [14]. One of the problems with the SDS–Brij 35 micellar phase however, is the capillary wall coating effect of Brij 35. This can significantly alter solute retention if many runs are done in sequence [15].

This paper describes the development and validation of a MEKC mixed micellar system with SDS–Brij 35 for the analysis of clindamycin.

2. Materials and methods

2.1. Apparatus

CE was performed on Spectraphoresis 1000 equipment (Thermo Separation Products, Fremont, CA, USA). Clindamycin and related substances were detected by UV absorption at 200 nm. Injection was done hydrodynamically for 4 s. The untreated fused-silica capillary of 43 cm (35 cm to detection window) (75 μm I.D.) was from Polymicro Technologies (Phoenix, AZ, USA). Buffer pH was measured with a Consort C-831 pH meter (Turnhout, Belgium). The applied voltage was 15 kV and the temperature of the capillary was 15°C.

2.2. Chemicals, reagents, samples and solutions

Acetonitrile (HPLC-S grade) was from Biosolve (Valkenswaard, Netherlands), methanol (HPLC grade) and sodium hydroxide from BDH (Poole, UK), 2-propanol from Riedel-de Haën (Seelze, Germany), 2-methyl-2-propanol, boric acid and dimethyl sulfoxide from Merck Eurolab (Leuven, Belgium). Sodium tetraborate decahydrate, sodium dodecylsulfate and Brij 35 (suggested molecular mass= 1199.56) were purchased from Acros Organics (Geel, Belgium). Sudan III was from Geigy (Switzerland). Water was purified using a Milli-Q water purification system (Millipore, Milford, MA, USA).

Clindamycin hydrochloride used for method development was obtained from Alpha Pharma (Zwevegem, Belgium) and reference samples of 7-epiclindamycin hydrochloride, lincomycin hydrochloride, lincomycin B hydrochloride and 7-epilincomycin hydrochloride from Pharmacia and Upjohn (Kalamazoo, MI, USA). Commercial samples were from Alpha Pharma and Medera Pharma (Braine-l'Alleud, Belgium).

Samples were dissolved in water at a concentration of 2.5 mg/ml. Lincomycin B was spiked at a concentration of 0.5% or 0.0125 mg/ml. All solutions were filtered through 0.2-μm nylon filters (Euroscientific, Lint, Belgium).

The pH was adjusted with a saturated boric acid solution. Stock solutions of sodium tetraborate buffer (100 m*M* and adjusted with boric acid to the right

pH), SDS (400 m*M*) and Brij 35 (100 m*M*) were made in order to prepare the final buffer.

2.3. Procedure

Before use, a new capillary was treated by washing it with 1 *M* NaOH at 50°C for 5 min, water at 15°C for 2 min and with running buffer at 15°C for 30 min. The capillary was conditioned at the beginning of each day by washing it with 0.1 *M* NaOH for 5 min and water for 5 min. Before every analysis the capillary was washed for 1 min with 0.1 *M* NaOH and for 3 min with running buffer.

2.4. Software

Experimental design and optimization were performed using Modde 4.0 software (Umetri, Umeå, Sweden).

3. Results and discussion

3.1. Development of the method

Preliminary experiments with different buffers showed that capillary zone electrophoresis was unable to separate the different components. Therefore it was necessary to use a MEKC system. A buffer containing 10 m*M* sodium tetraborate pH 8.5 and 20 m*M* SDS gave separation of lincomycin B, epilincomycin, lincomycin and clindamycin. The addition of acetonitrile was necessary to separate clindamycin B and epiclindamycin from clindamycin. Although this system gave good separation of all components, there was a problem with the shape of the main peak. The peak was broad and in some runs distortion, shoulders or splitting of the peak were observed (Fig. 2A). This was not due to overloading because lowering the amount of clindamycin injected could not solve the problem. Changing the organic modifier to methanol, 2-propanol or 2-methyl-2-propanol gave the same separation but the shape of the main peak did not improve. Increasing the concentration of SDS to 100 m*M* could improve the shape of the main peak only when the concentration of clindamycin was lowered to 0.5 mg/ml. This resulted in problems of sensitivity and high values for the limits of detection (LOD) and quantitation (LOQ).

Further increase in SDS was impossible because of the high current (>160 μA) and the long analysis time (>40 min). Therefore a non ionic surfactant, Brij 35, was added. Addition of Brij 35 to the buffer improved the shape of the main peak (Fig. 2B,C). The optimal ratio of the two surfactants had to be

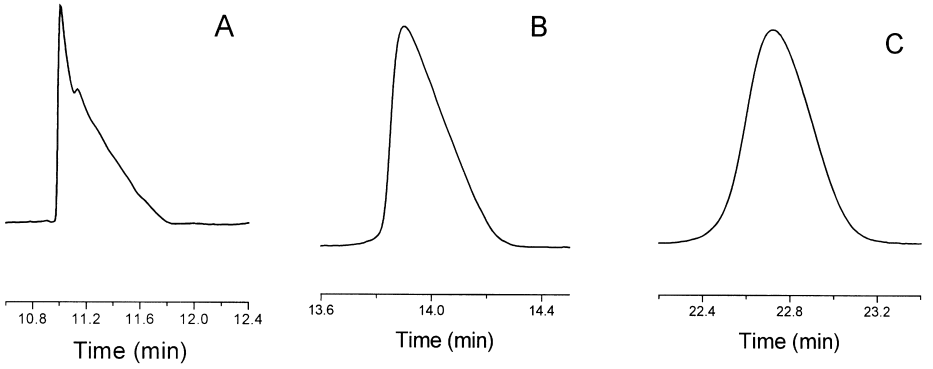

Fig. 2. SDS (20 m*M*) in the buffer resulted in poor peak shape (A). Improvement was made by increasing SDS concentration to 100 m*M* (B). Good peak shape could only be achieved by addition of Brij 35 (C). The three conditions gave good selectivity. In every condition, 1 mg/ml clindamycin was injected hydrodynamically for 4 s. (A) Buffer: 10 m*M* sodium tetraborate pH 8.5, 20 m*M* SDS, 15% acetonitrile; 15 kV; 20°C. (B) Buffer: 10 m*M* sodium tetraborate pH 8.0, 100 m*M* SDS, 10% acetonitrile; 18 kV; 20°C. (C) Buffer: 25 m*M* sodium tetraborate pH 7.75, 90 m*M* SDS, 14 m*M* Brij 35, 21% acetonitrile; 15 kV; 15°C. Other parameters like pH and % acetonitrile were slightly adapted to obtain good selectivity.

Table 1
Influence of an increasing concentration of Brij 35 on the EOF (t_0), the elution window and the current

Concentration of Brij 35 in buffer (m*M*)	t_0 (min)	Elution window (t_{mc}/t_0)	Current (μA)
8	6.423	7.76	118.5
14	6.583	4.85	109.5
25	7.023	2.38	98.9
50	7.376	1.56	82.1

The buffer contains 25 m*M* sodium tetraborate pH 7.75, 90 m*M* SDS, 21% acetonitrile and Brij 35 as indicated. EOF was measured with DMSO and t_{mc} with Sudan III ($n=3$).

found. When more Brij 35 was added, separation became too fast and selectivity was lost. This can be explained by the narrow elution window. Table 1 shows how an increase in Brij 35 concentration in the buffer leads to a narrow elution window. Dimethyl sulfoxide (DMSO) was used as a marker for the EOF and Sudan III as a marker for the micelles. The t_0 increases because of the decreasing EOF. Another observation is that increasing Brij 35 concentration decreases the current. This may be caused by three factors. Adding Brij 35 will result in mixed micelles with a lower charge compared to SDS micelles. These micelles will have a lower effective mobility and thus conductivity will decrease [12]. A second factor could be the shielding of the negatively charged surface of the SDS micelles by the poly-(oxyethylene) chains of Brij 35 [11]. In addition, adding Brij 35 increases viscosity which in turn will result in a lower conductivity. To decrease the EOF and broaden the elution window with the same ratio of micelle forming agents, the sodium tetraborate buffer concentration was increased from 10 to 25 m*M*, without obtaining too high a current. The optimal SDS–Brij 35 ratio was found to be 6:1.

This ratio of surfactants was found to give good selectivity, similar to the one in the SDS system without Brij 35, and an improved peak shape of the main peak, without shoulders or splitting of the peak. The other parameters were slightly changed to obtain an improved separation. The pH of the tetraborate buffer was adjusted to pH 7.75 with boric acid to separate clindamycin B and epiclindamycin. The amount of acetonitrile was increased to separate epiclindamycin and a small impurity of unknown identity (unknown III) from the main peak. Two very small impurities (unknown I and unknown II) were eluted between lincomycin and clindamycin B. Using a system, containing 25 m*M* sodium tetraborate pH 7.75, 90 m*M* SDS, 15 m*M* Brij 35 and 21% acetonitrile, 2.5 mg/ml of clindamycin was injected without problems of main peak distortion.

3.2. Optimization and robustness

In order to determine if these conditions were optimal, an optimization of the method was performed. Four variables at two levels and five responses were examined in an experimental design. With this design, a relatively low number of experiments has to be performed to obtain the optimal conditions [16]. The robustness of the analytical method was also verified with this experimental design. Variables and their ranges studied are summarized in Table 2. The five responses examined correspond with the selectivities of lincomycin B–epilincomycin (S1), epilincomycin–lincomycin (S2), clindamycin B–epiclindamycin (S3), epiclindamycin–unknown III (S4) and unknown III–clindamycin (S5). Selectivities were calculated by ratioing the migration time of the second peak to the migration time of the first peak.

The most critical pairs were S2, S3 and S5. A central composite design was used for the purpose of this study. This experimental design needed 27 experiments in total (2^k+2k+3, k=number of variables). All experiments were performed twice and the mean values were used. To determine if the data fitted well with the model, the response of the model had to be checked. The values for R^2 and Q^2 were over 0.94 and 0.70 for S2 and S5, and over 0.96 and 0.80 for S1, S3 and S4. These values show that the data fit well with the model.

Fig. 3 shows the normalized regression coefficient plots for responses S2, S3 and S5. The error bar over the coefficient corresponds with the 95% confidence

Table 2
Variables and their ranges

Parameter	Low	Center	High
pH	7.50	7.75	8.00
SDS (m*M*)	80	90	100
Brij 35 (m*M*)	13	15	17
Acetonitrile (%)	19	21	23

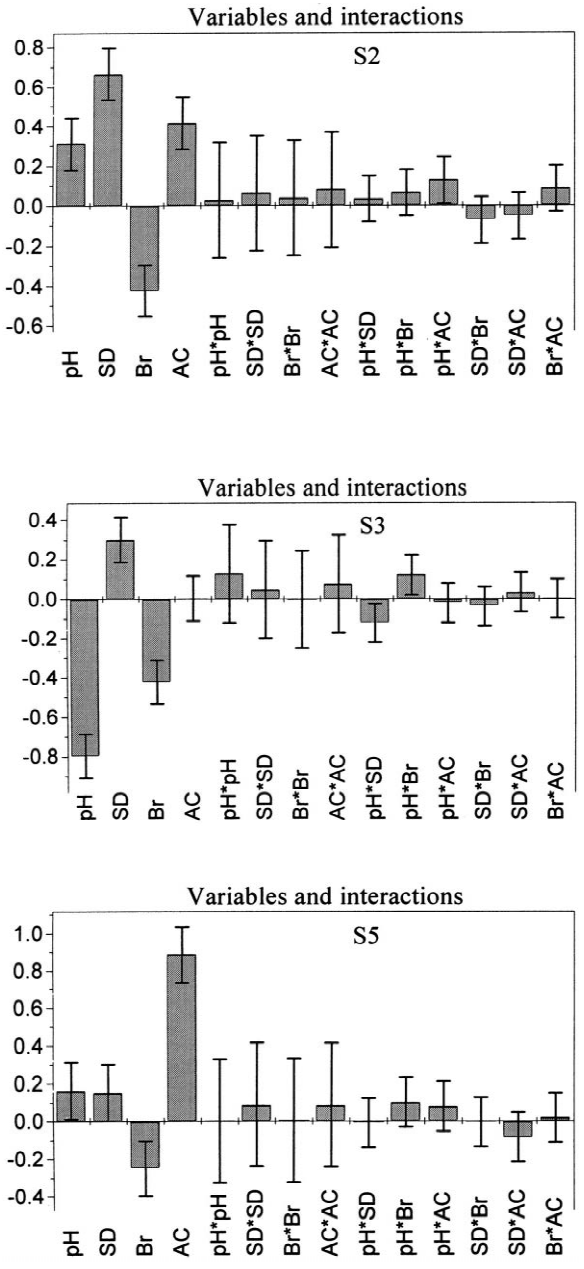

Fig. 3. Normalized regression plots for responses S2, S3 and S5. Variables pH, SD (SDS), Br (Brij 35) and AC (acetonitrile) and the interactions of the variables are shown.

interval. If the coefficient is smaller than the interval, changing the variable in the range examined has no significant effect on the selectivity. These plots show that the buffer pH has a significant positive effect on S2 and S5. Buffer pH has a significant negative effect on S3. Acetonitrile has no significant effect on S3. Increasing the acetonitrile content significantly improves selectivities S2 and S5, but it also decreases the EOF and gives long analysis times.

Increasing SDS improves selectivities S2 and S3 but it increases the current and analysis time as well. Therefore the center point (90 m*M*) was retained. Brij 35 has a significant negative effect on all selectivities. When the concentration was lowered from 15 to 14 m*M*, the unknown III and the main component (S5) were baseline separated. This is an important improvement for quantitative work. The analysis time increased only a little and the peak shape of the main peak was not affected by this small change.

The same conclusions can be deduced from the response surface plots. These plots show selectivity as a function of important variables. Fig. 4A shows selectivities S2, S3 and S5 as a function of pH and acetonitrile concentration. The optimal pH for S2 and S5 is found at high pH while for S3 it is found at low pH. The planes of the corresponding pairs cross each other. Therefore the center point (pH 7.75) was taken as the optimum for all pairs. The plots also show that an increasing concentration of acetonitrile improves selectivities S2 and S5, while it has no significant effect on S3. The center point (21%) was taken as a good compromise between selectivity and analysis time. Fig. 4B again illustrates the choice of 14 m*M* concentration of Brij 35.

The results of the optimization prove the robustness of the method. No problems of selectivity were seen in the extreme conditions.

Fig. 5 shows a typical electropherogram of clindamycin with the final optimized method. The buffer contains 25 m*M* sodium tetraborate buffer pH 7.75 (adjusted with boric acid), 90 m*M* SDS, 14 m*M* Brij 35 and 21% acetonitrile. All known components of clindamycin are separated in an acceptable analysis time of 23 min (27 min with washing cycle). The small peak after the main peak occurs very occasionally and is not related to the sample. In comparison with the published LC method [9], this CE method

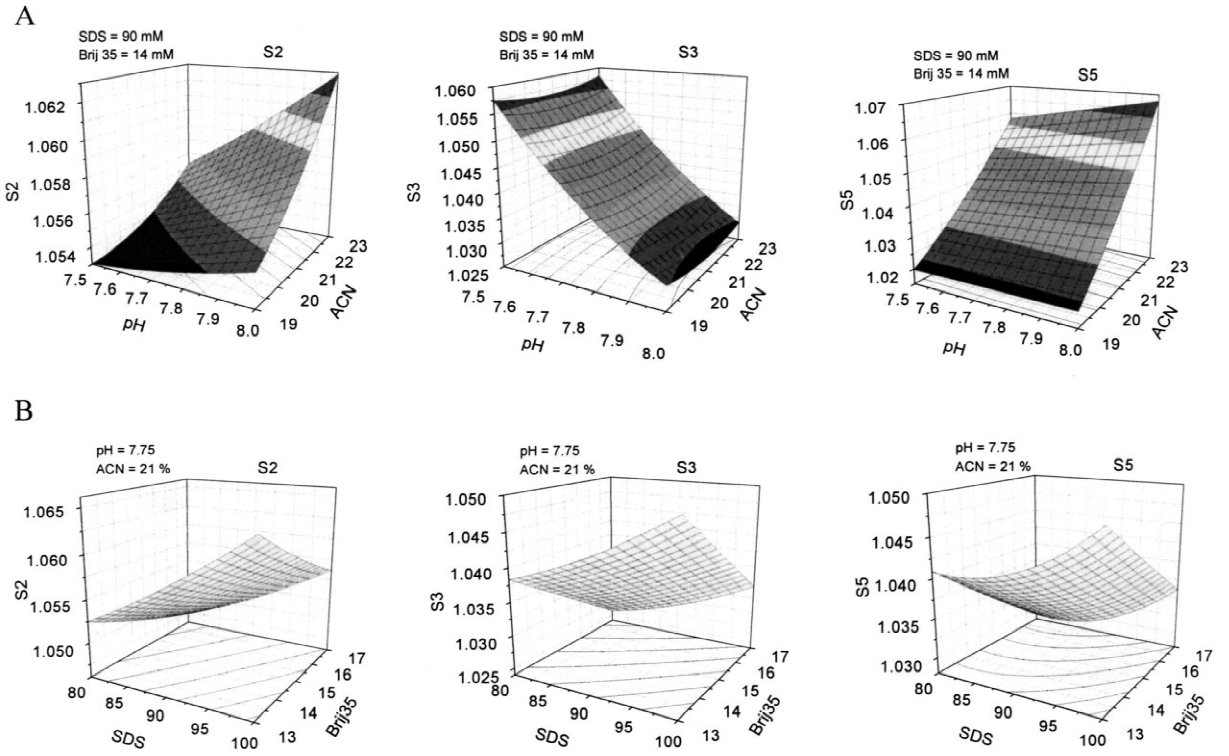

Fig. 4. Response surface plots for responses S2, S3 and S5. Selectivity is shown as a function of two variables: (A) pH combined with concentration of acetonitrile (%, ACN) and (B) SDS combined with Brij 35.

has a 6-min longer analysis time but lincomycin B is better separated.

3.3. Quantitative analysis

The results of the quantitative analysis are shown in Table 3. Within-day repeatability was examined by six replicate injections on the same day. RSD values on the mean of the surface area and the migration time of the main peak are given. Between-day repeatability was examined by three injections every day during 6 days. The RSD values on the mean of the surface area and the migration time of the main peak from these 18 injections are given. The rather high RSD of 2.8% on the migration time can be explained by the coating effect of the Brij 35. Migration times fluctuate between different runs but selectivity is never lost. Nevertheless this can be seen as a drawback of Brij 35. A series of analyte concentrations corresponding to 0.25, 0.75, 1.75, 2.50 and 2.75 mg/ml were injected ($n=3$) to determine linearity. The data were subjected to linear regression analysis. The correlation coefficient of 0.9998 shows the good linear relationship between the concentration injected and the surface area. The LOD ($S/N=3$) and the LOQ ($S/N=10$) of clindamycin were obtained by injection of diluted solutions and were found to be 0.05 and 0.15%, respectively, relative to a 2.5 mg/ml solution. This is similar to the LC system [9]. LODs and LOQs of the impurities were determined and found to be equal to (lincomycin B and lincomycin) or slightly higher (7-epilincomycin and 7-epiclindamycin, LOD: 0.08% and LOQ: 0.22%) than for the main component. Results for clindamycin B are not available because of lack of a pure sample.

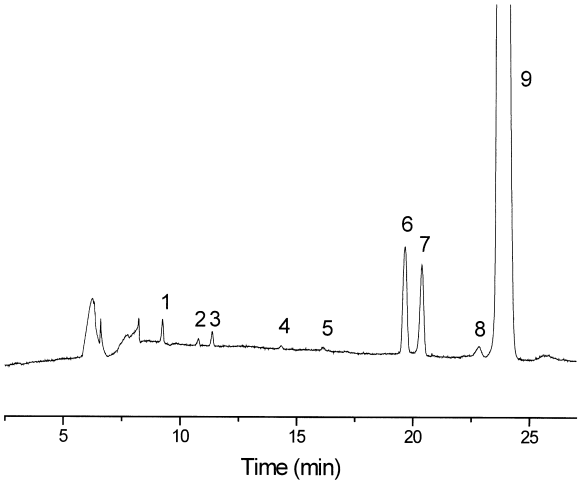

Fig. 5. A typical electropherogram of a commercial sample of clindamycin hydrochloride (2.5 mg/ml) spiked with lincomycin B (0.5%). 1, lincomycin B; 2, 7-epilincomycin; 3, lincomycin; 4, unknown I; 5, unknown II; 6, clindamycin B; 7, 7-epiclindamycin; 8, unknown III; 9, clindamycin. Buffer: 25 m*M* sodium tetraborate pH 7.75, 90 m*M* SDS, 14 m*M* Brij 35, 21% acetonitrile; capillary temperature 15°C; applied voltage 15 kV (current=110 μA); UV detection 200 nm; hydrodynamic injection, 4 s.

3.4. Commercial samples

Two commercial bulk products were investigated with this method. Results are given in Table 4. The percentage amounts are expressed as clindamycin base, calculated with reference to the European Pharmacopoeia reference sample (86.4% clindamycin). In the two samples, the amount of lincomycin B, unknown I and unknown II was below the detection limit (0.05%).

Table 4
Analysis of commercial samples

	Sample 1 (%)	Sample 2 (%)
Lincomycin B	<0.05	<0.05
7-Epilincomycin	0.59	0.19
Lincomycin	0.17	0.06 (<LOQ)
Unknown I	<0.05	<0.05
Unknown II	<0.05	<0.05
Clindamycin B	1.74	1.52
7-Epiclindamycin	0.91	0.08 (<LOQ)
Unknown III	0.20	0.08 (<LOQ)
Clindamycin	82.3 (RSD=1.7%)	84.1 (RSD=2.2%)

Percentages expressed as clindamycin base (n=3).

4. Conclusion

This method shows the advantage of a mixed micellar system for the separation of clindamycin and its impurities. The use of a mixed micellar system improved the shape of the main peak while a very good selectivity was obtained. All known impurities of clindamycin were separated and three unknown peaks were detected. The analysis time was acceptable. A disadvantage of the use of Brij 35 was

Table 3
Quantitative analysis

Parameter	Value
Within-day repeatability (n=6)	
Corrected area	RSD=1.9%
Migration time	RSD=1.5%
Between-day repeatability (n=18)	
Corrected area	RSD=2.3%
Migration time	RSD=2.8%
Linearity	y=37 746x+173.5
Range (mg/ml)	0.25–2.75; r=0.9998
y=corrected area	$S_{y,x}$=993
x=clindamycin concentration (mg/ml)	
LOD (S/N=3)	0.05%
LOQ (S/N=10)	0.15% (RSD=12.8%, n=6)

the somewhat fluctuating migration times when many runs were done in sequence. Nevertheless selectivity was not lost. The method was robust and showed good linearity and repeatability. LOD and LOQ were similar to the LC method.

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