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Research Article

Dual CD system-modified MEEKC method for the determination of clemastine and its impurities

A dual system of CDs was used for the first time in MEEKC with the aim of determining clemastine and its three main related impurities in both drug substances and tablets. The addition of methyl-β-cyclodextrin and heptakis(2,6-di-*O*-methyl)-β-cyclodextrin to the microemulsion pseudo-stationary phase was essential to increase the resolving power of the system to obtain a baseline separation among the compounds. The best microemulsion composition was identified by mixture design and the effects of the factors concentrations of CDs and voltage were investigated by a response surface study applying a Central Composite Design. In both cases, Derringer's desirability function made it possible to find the global optimum, which corresponded to the following combination: microemulsion, 89.8% 10 mM borate buffer pH 9.2, 1.5% *n*-heptane and 8.7% of SDS/*n*-butanol in 1:2 ratio; 18 mM methyl-β-cyclodextrin, 38 mM heptakis(2,6-di-*O*-methyl)-β-cyclodextrin, 17 kV. By applying these conditions, the separation was completed in about 5.5 min. The method was validated following International Conference on Harmonisation guidelines and was applied to a real sample of clemastine tablets.

Keywords:
CD-modified MEEKC / Clemastine / Dual CD system / Experimental design / Impurities
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1 Introduction

Clemastine fumarate ((2*R*)-2-[2-[(*R*)-1-(4-chlorophenyl)-1-phenylethoxy]ethyl]-1-methylpyrrolidine (*E*)-butenedioate, CLE), a monoethanolamine derivative, is an antihistamine with antimuscarinic and moderate sedative properties, used for the symptomatic relief of allergic conditions [1]. According to the drug product manufacturer (Novartis, Nyon, Switzerland), three main related substances may be present in the tablet dosage form and their structures are shown in Fig. 1.

Clemastine has been determined in bulk and in pharmaceutical formulations by spectrophotometry [2, 3], and in biological fluids by HPLC [4], HPLC-MS-MS [5, 6], GC [7, 8] and GC-MS [4, 9]. To the best of our knowledge, no method has been presented yet for the quantitation of clemastine and its impurities in pharmaceutical dosage forms. A LC method is reported in the current European Pharmacopoeia able to verify that impurity C does not exceed the specified limit of 0.3% with respect to the main component [10]. The novelty presented in this article consists in the study of the resolution power of a CD dual system in combination with MEEKC and its application to the analysis of CLE and its impurities in drug substance and drug product.

MEEKC applies microemulsion buffers to separate both charged and neutral solutes, and the complexity of the composition of the microemulsion and of the separation process allows many manipulations to be made during method development [11–14]. Pharmaceutical quality control could represent an important field of application for this technique, but up to now only a limited number of methods has been proposed [15–18].

The introduction of a CD into the microemulsion gives rise to CD-MEEKC. CDs can act as a secondary pseudo-stationary phase [19] thus facilitating the separation of similar compounds through their capacity to incorporate analytes into their cavity to different degrees based on hydrophobicity, hydrogen bonding capability and steric hindrance [20]. However, the overwhelming majority of CE methods employing CDs concern CZE, whereas the number of studies dealing with the application of CDs in MEEKC is low [19, 21–29], probably because the retention behaviour and application possibility of this new technology have not yet been elucidated in detail [30].

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Abbreviations: **CLE,** clemastine fumarate; **DMβCD,** heptakis(2,6-di-*O*-methyl)-β-cyclodextrin; **I_A,** clemastine impurity A; **I_B,** clemastine impurity B; **I_C,** clemastine impurity C; **IBU,** ibuprofen; **ICH,** International Conference on Harmonisation; **MβCD,** methyl-β-cyclodextrin

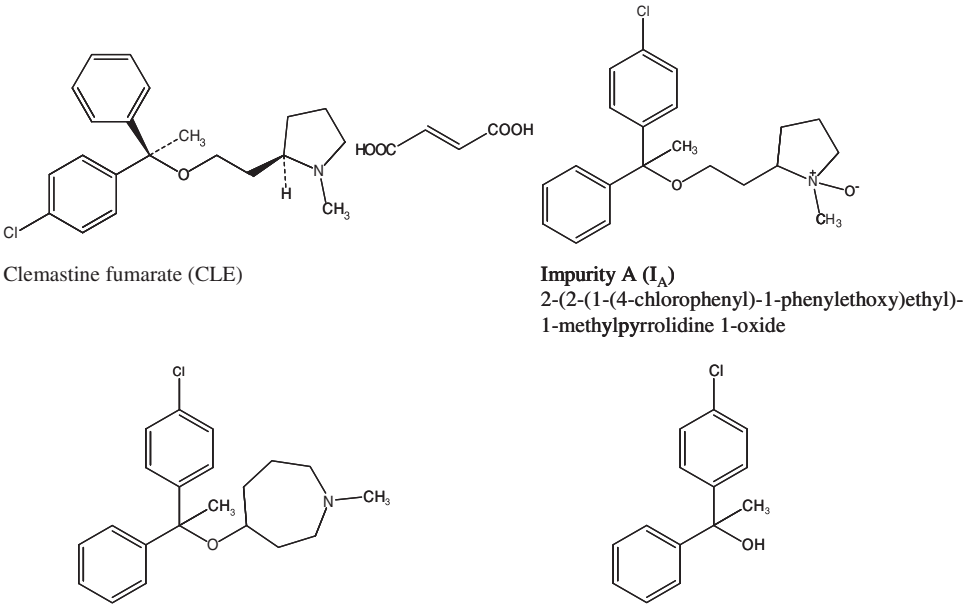

Figure 1. Structures of clemastine and related substances.

Up to now, the use of a dual mixture of CD in EKC techniques has been limited to a few cases in MEKC [31–33]. In this article, for the first time, the addition of a dual CD system to a microemulsion pseudo-stationary phase is presented. The addition of two suitable CDs was compulsory to obtain a baseline separation of the compounds.

The optimal conditions of the analysis were achieved applying a multivariate strategy [34–37]. In a first step, the microemulsion composition was optimised by applying mixture design. In a second step, the optimal values for CD concentrations and for voltage were identified using Central Composite Design. The suitability of the method for its intended use was evaluated by validation, performed following International Conference on Harmonisation (ICH) guidelines [38].

2 Materials and methods

2.1 Chemicals and reagents

The reference standards of CLE and its impurities clemastine impurity A (I_A), clemastine impurity B (I_B) and clemastine impurity C (I_C) were kindly donated by Novartis (Nyon, Switzerland), as well as the excipients maize starch, magnesium stearate, lactose, PVP and talc. Sodium borate was from BDH Laboratory Supplies (Poole, UK). Methanol (HPLC grade), *n*-heptane, SDS, methyl-β-cyclodextrin (MβCD), heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DMβCD), heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin, (2-hydroxypropyl)-α-cyclodextrin, (2-hydroxypropyl)-β-cyclodextrin, (2-hydroxypropyl)-γ-cyclodextrin, and ibuprofen (IBU, internal standard) were from Sigma-Aldrich (St. Louis, MO, USA). *n*-Butanol was obtained from Merck (Darmstadt, Germany). Tavegil® tablets (Novartis), labelled to contain 1 mg of CLE, were purchased from Petrone Group (Naples, Italy).

Ultrapure water used for the preparation of solutions and running buffers was provided by a Simplicity 185 system (Millipore, Billerica, MA, USA) after an electro-deionisation treatment using an Elix system (Millipore).

2.2 Solutions, microemulsions and sample preparation

Standard stock solutions of CLE (20 mg/mL), I_A, I_B and I_C (1 mg/mL each) and IBU (internal standard, 1 mg/mL) were prepared in methanol and stored at 4°C. Working standard solutions were prepared daily by diluting standard stock solutions with 10 mM sodium borate directly in a vial to 500 μL in order to obtain the desired final concentrations of the different compounds.

Microemulsions were prepared by sequentially mixing in a beaker proper amounts of the aqueous phase (10 mM sodium borate pH 9.2), cosurfactant (*n*-butanol), surfactant (SDS) and finally oil (*n*-heptane) in the selected ratios. Each separate addition was made only after obtaining a complete dissolution of the previously mixed compounds and keeping the mixtures under continuous stirring, in order to obtain optically transparent microemulsions. All microemulsions were filtered before use through 0.45 μm cellulose acetate syringe filters.

The percentage w/w% of the microemulsion components considered was 88.0–93.9% for the aqueous phase,

0.1–2.0% for the oil phase and 6.0–10.0% for the mixture surfactant/cosurfactant in 1:2 ratio. The optimized microemulsion consisted of 89.8% 10 mM borate buffer, 1.5% *n*-heptane and 8.7% SDS/*n*-butanol in 1:2 ratio. The final BGE system was obtained by adding 18 mM MβCD and 38 mM DMβCD to the optimized microemulsion. The BGE system, including the microemulsion, was freshly prepared daily in order to avoid possible problems of repeatability.

As regards tablets assay, 60 tablets were weighed, crushed and finely powdered. An accurately weighed amount of the powder, corresponding to about 55.5 mg of CLE, was transferred into a 25 mL beaker and diluted with 10 mL of a mixture of methanol/water 2:3, added by a manual pipette. The direct addition of the solvent was preferred to the use of a volumetric flask in order to efficiently stir the obtained suspension, due to its high concentration, and in order to obtain the expected CLE concentration in the final solution, due to the high volume of the powder. This mixture was stirred for 5 min, sonicated for 10 min and stirred again for 5 min. Then, a 1 mL portion of the mixture was centrifuged and 450 μL of the supernatant were introduced in a vial for analysis together with 50 μL of IBU 1 mg/mL. Thus, the final test concentration of CLE was about 5 mg/mL and the concentration of IBU was 0.1 mg/mL.

2.3 Apparatus and operating conditions

Solutions were sonicated by means of a 300 Ultrasonik ultrasonic bath (Ney, Bloomfield, USA). Samples were centrifuged by a microcentrifuge 5415 D (Eppendorf, Hamburg, Germany).

All experiments were carried out at 20°C on an Agilent Technologies ^{3D}CE system (Agilent Technologies, Waldbronn, Germany), equipped with an on-column UV–visible DAD and an air thermostating system. The vial carousel was at room temperature. Analytical data were collected and processed by ^{3D}CE ChemStation software (Rev. a.09.01, Agilent Technologies).

Separations were performed using a 33.0 cm (24.5 cm effective length) fused silica capillary (50 μm id, 375 μm od) from Composite Metal Services (Ilkley, UK). The capillaries were cut to this length using a Capillary Cleaving™ tool (Supelco, Bellefonte, PA, USA). The detection window was built-in by burning off the polyimide coating on the capillary using The Windowmaker™ (MicroSolv, Postnova Analytics, Landsberg/Lech, Germany). The detection wavelength was 195 nm.

Sample introduction was performed hydrodynamically by applying 50 mbar for 5 s from the inlet side. The separation was carried out in the positive polarity mode applying 17 kV. The resulting current was about 65 μA.

The new capillaries were rinsed with 1 M NaOH and water for 5 min each. At the beginning of the experiments each day, the capillary was rinsed with 0.1 M NaOH and water for 2 min each. Between CE runs, the capillary was flushed with a mixture of methanol/1 M hydrochloric acid in 95:5 ratio %v/v for 1 min, 0.1 M sodium hydroxide for 1 min, water for 1 min and finally run buffer for 4 min.

Due to the complexity of the buffer, particular care had to be given to instrument maintenance and cleaning, with special regard to capillary, electrodes and prepunchers in order to maintain good system performances.

2.4 Calibration curves, calculations and software

The corrected peak area ratios (analyte to internal standard) were plotted against the corresponding concentration of analyte in order to obtain the calibration graphs. Five different concentrations of each analyte were prepared and the solutions were analysed twice, keeping the concentration of IBU, internal standard, constant at 0.1 mg/mL.

Resolution values R were calculated on the basis of the formula

$$R = 2(t_{RB} - t_{RA}/w_B + w_A)$$

where t_{RA} and t_{RB} are the migration times and w_A and w_B the widths at the bases of adjacent peak pairs, respectively [39].

The set-up of the designs and the statistical treatment of the data were performed by NEMROD-W software [40]. The electrophoretic analyses described by the selected experimental plans were run in a randomized order analysing a test solution containing 5 mg/mL CLE, 50 μg/mL impurities and 100 μg/mL IBU.

3 Results and discussion

The target analytes presented basic (CLE, I_B) and neutral (I_A and I_C) properties, and the use of an EKC mode was necessary to obtain the desired separation. In the preliminary experiments, dedicated to the selection of a suitable CE operative mode, the concentration values of all the compounds were kept low (40 μg/mL), making it possible to better understand their migration behaviour. Instead, during application of the multivariate approach, aimed to find the optimal experimental conditions, the test concentration of the analytes was employed. The test concentration, useful for tablet analysis, corresponded to 5 mg/mL CLE and 50 μg/mL I_A, I_B and I_C (1% with respect to the main compound).

From the preliminary experiments, the major issue of the analysis regarded the separation of I_B and CLE, whose peaks were always completely overlapped, due to their high structural similarity as they differ only for the position of two methylene groups. Neither the use of plain MEKC with SDS or bile salts as surfactants nor the use of mixed MEKC was successful in obtaining I_B/CLE separation. In the latter case, the considered systems were SDS/bile salts, SDS/zwitterionic surfactants such as 3-(*N*,*N*-dimethylmyristylammonio)propanesulfonate, SDS/non-ionic surfactants

such as polyoxyethylene (23) lauryl ether (Brij 35) and polyoxyethylene (20)-sorbitan monolaurate (Tween 20).

3.1 Dual CD system-modified MEKC

In order to overcome the problems of separation between CLE and I_B, the use of two pseudo-stationary phases, one formed by SDS micelles and one involving CDs, was taken into account. Six neutral CD derivatives, reported in Section 2, were tested in a three-level concentration range (20–40 mM), keeping a constant concentration of 100 mM SDS in 10 mM borate buffer as BGE. It was noticed that the use of DMβCD at concentration values above 30 mM exclusively led to distinguish I_B and CLE peaks, demonstrating that the presence of this CD at proper concentration in the BGE was crucial for obtaining the separation.

However, the partial resolution obtained at low concentrations of the analytes was still not sufficient, considering that when analysing the test sample the CLE peak would present a much higher area than I_B peak with the real consequence of overlapping. Thus, further modifications of the BGE were required to improve the selectivity and in this sense the addition of another suitable CD was found promising. The use of a mixture of DMβCD with another CD was tested and among the CDs mentioned in Section 2 the best results were obtained adding MβCD. Thus, a good separation system was composed of 100 mM SDS in 10 mM sodium borate, to which 15 mM MβCD and 40 mM DMβCD were added. Unfortunately, when this system was effectively applied to a test sample, a complete lack of reproducibility was evidenced in the obtained electropherograms. For this reason, this system could not be retained for accurate quantitation of the compounds at the test concentrations, and was therefore discarded.

3.2 Dual CD system-modified MEEKC

Due to the very poor reproducibility of CD-MEKC analysis, it was necessary to select another separative system which could assure good reliability also at the test concentrations. In general, the use of MEEKC constitutes a valuable alternative to MEKC. Therefore, by considering the two selected CDs as essential for the separation, an oil-in-water microemulsion was tested as basis of the BGE. The composition of this standard microemulsion was 90.95% 10 mM borate buffer (aqueous phase, *W*), 1.05% *n*-heptane (oil phase, *O*) and 8.00% SDS/*n*-butanol (surfactant/cosurfactant) in 1:2 ratio, all percentages based on w/w. Using this BGE system, the separation of the peaks was quite satisfactory even at the test concentration of the analytes and this system constituted the basis for further optimization.

The migration order of the compounds was I_C, I_A, I_B and CLE, and remained the same in MEEKC with or without addition of CDs. However, the interaction of the analytes with the CDs, which are neutral and driven only by EOF, was proven by the reduction of the migration time of the analytes if compared with plain MEEKC and by the increased separation between the peaks (especially in the case of the pair I_B/CLE). This latter fact can be due to an alteration of the molar fraction of the analytes in the different phases as the analytes possess different stability constants when they form inclusion complexes with the CDs, thus different amounts of each compound are available for partitioning into the oil phase.

3.3 Selection of microemulsion composition

Optimization of the composition of the microemulsion was performed by means of mixture design, correlating the composition of the microemulsion and the characteristics of the analysis by means of a Scheffé special cubic model [36]. A 13-run experimental matrix was used to find the model coefficients. The investigated experimental domain took into consideration the following constraints for each component of the microemulsion: 10 mM sodium borate (aqueous phase, *W*), 88.0–93.9%; *n*-heptane (oil phase, *O*), 0.1–2.0%; SDS/*n*-butanol in 1.2 ratio (surfactant, *S*/cosurfactant, *CoS*), 6.0–10.0%.

The considered responses were the critical resolution values between I_C and I_A (R_1) and between I_B and CLE (R_3). Resolution between I_A and I_B (R_2) was not considered because the separation between these two peaks was always satisfactory. The experimental plan summarized in Table 1 was carried out and for each electrophoretic run the experimental responses were measured. The analyses were performed at standard values of 15 kV, 15 mM MβCD and 40 mM DMβCD.

The significance and validity of the calculated models was established by ANOVA [34], setting $\alpha = 0.05$ and estimating the experimental variance by performing three replicates at the centre of the experimental domain. Thus, it was possible to investigate the related contour plots.

Table 1. Mixture design experimental plan and responses

Exp. no.	W (%w/w)	O (%w/w)	S/CoS (%w/w)	R_1	R_3
1	93.90	0.10	6.00	0.23	0.22
2	92.00	2.00	6.00	0.01	0.47
3	89.90	0.10	10.00	0.86	0.75
4	88.00	2.00	10.00	1.05	0.89
5	92.95	1.05	6.00	0.01	0.32
6	91.90	0.10	8.00	1.23	0.69
7	90.00	2.00	8.00	1.43	0.95
8	88.95	1.05	10.00	0.70	0.71
9	90.95	1.05	8.00	1.16	1.01
10	92.43	0.58	7.00	0.94	0.62
11	91.47	1.53	7.00	0.57	1.00
12	90.43	0.58	9.00	1.86	1.06
13	89.48	1.53	9.00	1.46	0.94

Figure 2 shows the contour plots for responses R_1 and R_3. In these plots, the triangular shape of the investigated experimental domain for three components is adjusted according to the constraints imposed. The lines are called isoresponse lines and correspond to different predicted response values that are constant for each line and can be obtained in function of the different percentages of the components. As concerns R_1 (Fig. 2A), the percentage of oil resulted to not be important, whereas the best results were obtained using medium–high values of S/CoS and medium–low values of borate buffer. As concerns R_3 (Fig. 2B), the same zone for W and S/CoS was selected as optimal, but a high value of oil led to the maximization of this response.

Based on these graphs, the zone of the experimental domain where the best results for both R_1 and R_3 could be obtained was clearly identified. Derringer's desirability function (D) was then applied to practically find an experimental point inside this region which could fulfil specific requirements of resolution values [35]. In this step, the desired values of resolution for R_1 and R_3 were set at 1.3 and 0.9, respectively, and these values were introduced in the definition of the related partial desirability functions d_1 and d_2. The latter value for R_3 was chosen based on the electropherograms which showed a good separation between I_B and CLE when a value above 0.9 was registered. The total D function assumed the form $D = \sqrt{d_1 d_2}$ and made it possible to select the following optimal microemulsion: borate buffer, 89.8%; *n*-heptane, 1.5%; SDS/*n*-butanol in 1:2 ratio, 8.7%.

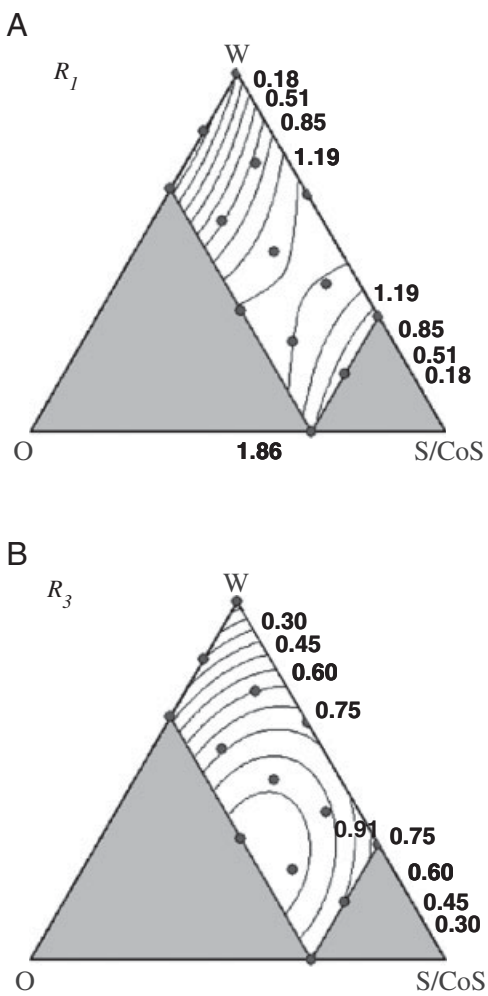

Figure 2. Contour plots for R_1 (A) and R_3 (B). Experiments are located by circles. *W*, aqueous phase; *O*, oil phase; *S/CoS*, surfactant/cosurfactant.

3.4 Optimization of independent factors by Central Composite Design

After having fixed the microemulsion composition, an experimental design for process factors was carried out with the purpose of improving the separation while keeping analysis time short. Thus, the selected responses were R_1 and R_3, to which analysis time (t) was added. The effect of the variables was evaluated in the following experimental domain: x_1, MβCD concentration ($M\beta CD$), 10–20 mM; x_2, DMβCD concentration ($DM\beta CD$), 35–45 mM; x_3, voltage (V), 12–18 kV. The experimental ranges for the CDs were chosen on the basis of the preliminary experiments for the choice of CE operative mode. In particular, high values of DMβCD concentration were necessary to obtain a sufficient separation between I_B and CLE. Values of MβCD higher than 20 mM led to a distortion of the peak shape of I_C and I_A. As regards voltage, values lower than 12 kV were not considered in order to avoid a long analysis time and values higher than 18 kV were discarded in order to keep current low and avoid undesirable drifts of the baseline.

A quadratic model was assumed to relate the factors to the responses and the coefficients were estimated by Central Composite Design [35]. The corresponding experimental plan, including also three replicates at the centre of experimental domain, is summarized in Table 2 together with the measured responses. ANOVA pointed out that the models were valid and significant for all three responses ($\alpha = 0.05$).

Then, the response surfaces were drawn and examined. R_1 (Fig. 3A) was maximised by setting low values of DMβCD and low values of voltage. R_3 (Fig. 3B) was maximised by setting a medium–high concentration of MβCD and a medium–low concentration of DMβCD. DMβCD exerted also a quadratic effect and a curvature in the response surfaces was clearly evidenced. Finally, the only significant factor on analysis time (t) was voltage (Fig. 3C), exerting as expected a negative effect.

In order to simultaneously optimise all the responses, in this case also desirability function was used. The desired value for both the resolution responses was set at 1.5 and for analysis time a fully desired value of 6 min was defined, accepting values lower than 7 min. The total desirability function was $D = \sqrt[3]{d_1 d_2 d_3}$ and its graphical representation is shown in Fig. 4. From these graphs, it is clear that there is a limited number of combinations of the variables which

Table 2. Central Composite Design experimental plan and responses

Exp. no.	*M*β*CD* (mM)	*DM*β*CD* (mM)	*V* (kV)	R_1	R_3	*t* (min)
1	12	37	13	1.78	1.22	7.35
2	18	37	13	1.95	1.33	7.80
3	12	43	13	1.39	0.96	7.68
4	18	43	13	1.33	1.11	7.75
5	12	37	17	1.54	1.35	4.90
6	18	37	17	1.70	1.44	5.00
7	12	43	17	1.15	1.11	5.02
8	18	43	17	0.78	1.02	5.08
9	10	40	15	1.36	1.05	6.17
10	20	40	15	1.52	1.59	6.39
11	15	35	15	1.89	1.35	6.42
12	15	45	15	0.96	0.94	6.31
13	15	40	12	1.70	1.24	8.07
14	15	40	18	1.31	1.54	4.39
15	15	40	15	1.31	1.32	5.89
16	15	40	15	1.36	1.43	6.66
17	15	40	15	1.69	1.56	6.38

makes it possible to gain the desired analysis performances, and among these the selected optimum was *M*β*CD*, 18 mM; *DM*β*CD*, 38 mM; *V*, 17 kV.

Applying these experimental conditions, a complete separation of the analytes was obtained in about 5.5 min with a generated current of about 65 μA. A typical electropherogram of the mixture is shown in Fig. 5. The predictivity of each considered model was verified by evaluating if the measured values for the responses fell within the confidence intervals, centred on the predicted values and defined considering the standard deviation from Central Composite Design replicates and a probability level of 95% [35]. A good agreement between predicted and observed responses was verified.

3.5 Validation

Validation of an analytical procedure for the pharmaceutical industry is regulated by ICH guidelines [38], which were followed in the development of the presented CD-MEEKC method. For assessment of the different validation parameters, a mixture made up of 5 mg/mL CLE, 50 μg/mL CLE impurities and 100 μg/mL IBU was analysed, unless otherwise described.

3.5.1 Selectivity

The baseline separation of the analytes was recognized by calculating the critical resolution values ($n = 4$, $\alpha/2 = 0.025$): R_1, 1.63 ± 0.09; R_3, 1.53 ± 0.04. As regards the dosage form, no interference in the electropherogram was caused by any of the excipients. Among the excipients, PVP showed a signal located at the beginning of the migration window, that is distant from the zone of the electropherogram related to the detection of the internal standard and the impurities.

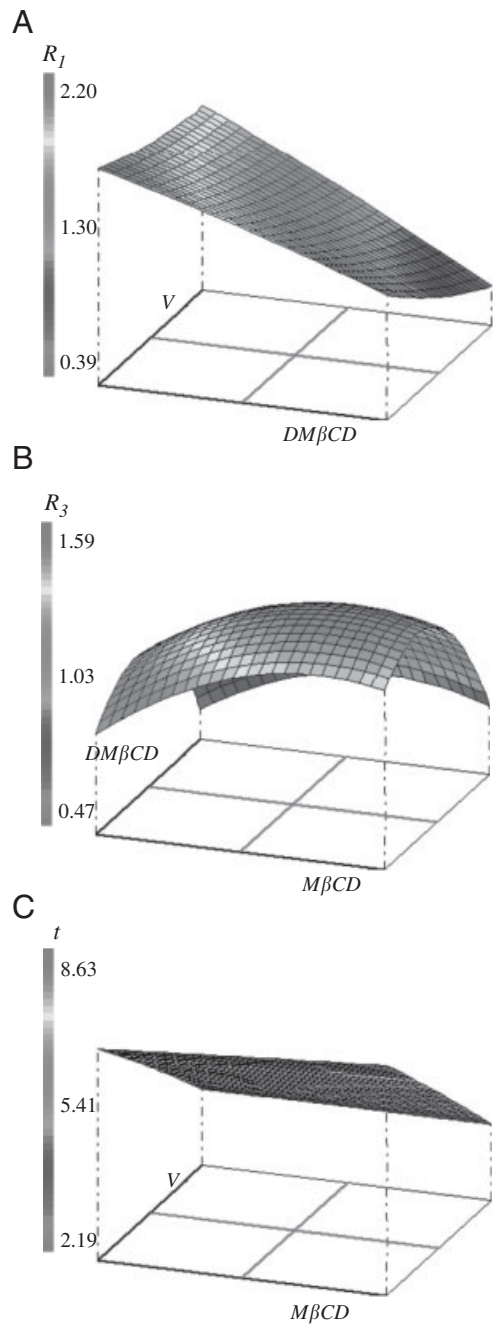

Figure 3. (A) R_1 response surface obtained by plotting DMβCD *versus* voltage; (B) R_3 response surface obtained by plotting MβCD *versus* DMβCD; (C) *t* response surface obtained by plotting MβCD *versus* voltage.

3.5.2 Migration time and peak area precision

To determine system repeatability, a total of 18 analyses were run, consisting of six replicate injections on three consecutive days. For corrected area ratios, the within-day

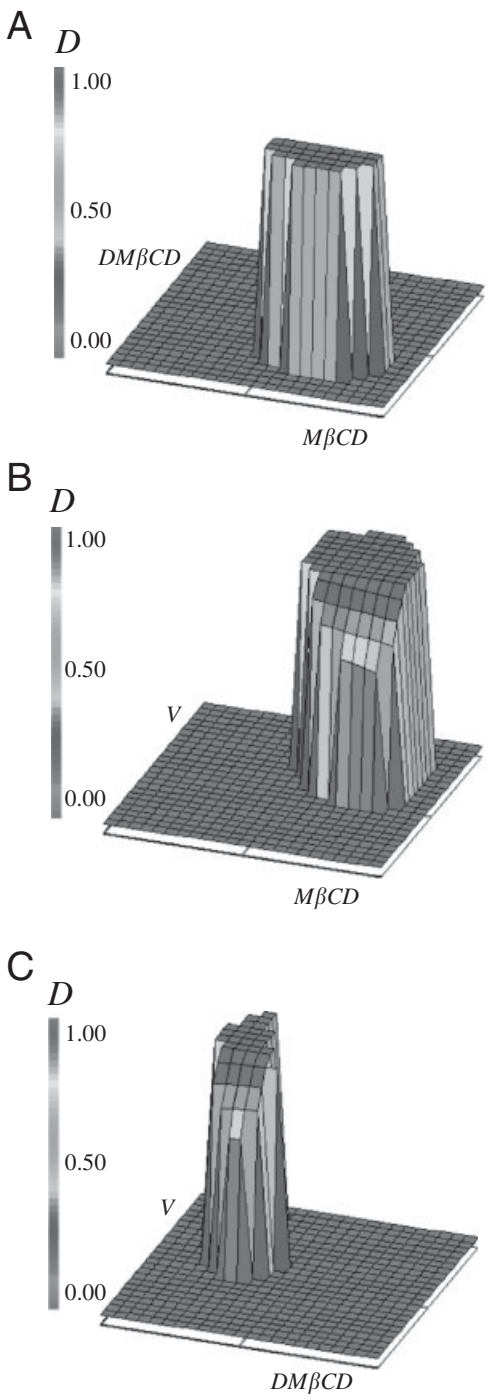

Figure 4. Desirability function three-dimensional plots obtained by plotting: (A) MβCD *versus* DMβCD; (B) MβCD *versus* voltage; (C) DMβCD *versus* voltage.

repeatability was evaluated by RSD values, which were in the range 0.3–0.8% for CLE and in the range 1.6–3.5% for CLE impurities. The between-day RSD values were 2.7% for CLE and 2.9–7.0% for CLE impurities. For analysis time, the within-day RSD ranged from 0.3 to 1.7% and between-day RSD was 2.2%.

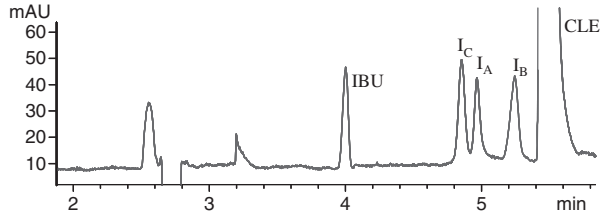

Figure 5. Electropherogram showing 5 mg/mL CLE and 50 μg/mL CLE impurities applying the optimal conditions. Voltage, 17 kV; temperature, 20°C; microemulsion: 89.8% 10 mM borate, 1.5% *n*-heptane, 8.7% SDS/*n*-butanol in 1:2 ratio; 18 mM MβCD; 38 mM DMβCD.

3.5.3 Robustness

In order to evaluate the method robustness [41] an 8-run Plackett–Burman matrix [35] was used. This design made it possible to estimate the main effects of small variations of the factors concentration of MβCD (17–19 mM), concentration of DMβCD (37–39 mM), voltage (16–18 kV) and temperature (19–21°C) on the quality of the separation, represented by the critical resolution values R_1 and R_3.

The experimental variance for each response was calculated by means of four replicates at the optimized conditions and the critical factors were identified by graphic analysis of effects [42]. As shown in Fig. 6, it can be noticed that none of the factors had a significant effect on R_1, whereas for R_3 the factor concentration of MβCD was identified as a critical parameter. Thus, the importance of accurately weighing CDs when preparing the BGE system has to be underlined.

3.5.4 LOD and LOQ

The approach for determining the limits was based on S/N, considering for LOD a ratio of 3:1 and for LOQ a ratio of 10:1. The following values of LOD were found: I_A, 2 μg/mL; I_B, 3.6 μg/mL; I_C, 2 μg/mL. The LOQ values were I_A, 3 μg/mL; I_B, 5 μg/mL; I_C, 4 μg/mL. The difference between LOD and LOQ values was quite lower than that expected. Anyway, the LOQ values were confirmed by validation, by performing eight injections and obtaining the following values of RSD for corrected areas: I_A, 5.6%; I_B, 4.6%, I_C, 5.3%.

3.5.5 Linearity, accuracy and precision

For CLE, the tested range of linearity was 40–120% of the test concentration, corresponding to 2–6 mg/mL. The equation of the curve was $y = 5.490x - 0.938$, with $R^2 = 0.999$. For CLE impurities, the considered range was from the respective LOQ to 50 μg/mL, corresponding to 1% with respect of the test concentration of CLE. The calculated R^2 were included in the range 0.996–0.998.

Accuracy (measure of systematic error) and precision as degree of repeatability (measure of random error) were established across the specified linearity range, running

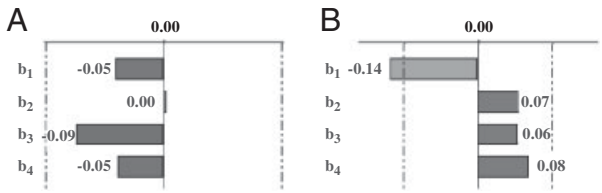

Figure 6. Robustness graphic analysis of effects: (A) response R_1; (B) response R_3. Coefficients of the linear model: b_1, *MβCD*; b_2, *DMβCD*; b_3, *T*; b_4, *V*.

three replicates at three concentration values ($n = 3$, $\alpha/2 = 0.025$). For CLE, the recovery values ranged from 98.9 ± 1.5 to $101.7 \pm 1.8\%$, with a maximum RSD of 0.7%. For CLE impurities, the range for recovery values was 98.2 ± 5.1–$102.8 \pm 8.9\%$ and maximum RSD was 4.7%.

3.5.6 System suitability

R_1 and R_3 were chosen as performance parameters to define system suitability limits. The accepted interval for these resolution values was identified on the basis of the values obtained during system repeatability study [41, 43] and corresponded to: R_1, 1.52–1.73; R_3, 1.47–1.62.

3.6 Application

The method was applied to a real sample of Tavegil® tablets, labelled to contain 1 mg CLE. Four analyses were run ($\alpha = 0.025$) and the results were in agreement with the declared content: assay, $99.5 \pm 1.3\%$; RSD, 0.8%. No CLE impurity was detected.

4 Concluding remarks

The utility of dual CD system-modified MEEKC has been demonstrated, for the first time in literature, in the development of an analytical method for the determination of clemastine and its related impurities. The apparent complexity of the BGE was justified by the quality of the obtained results. In fact, for the analysis of the target compounds a series of other CE operative modes failed to obtain a good selectivity and/or a good reproducibility. As an alternative, the addition of MβCD and DMβCD at proper concentration values to the microemulsion buffer was crucial for obtaining a baseline resolution of the compounds. Although the system was complex, it was possible to find the final optimum conditions, thanks to the use of a multivariate strategy, taking into account any possible interaction among the variables under investigation. This study confirms the power of the multivariate optimization that can be tested in the development of complex systems not easily optimized by means of a univariate strategy. The potential of this method for use in pharmaceutical quality control of clemastine tablets has been assessed by validation, performed according to ICH guidelines.

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