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Determination of prednisolone acetate, sulfacetamide and phenylefrine in local pharmaceutical preparations by micellar electrokinetic chromatography

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Abstract

A new, rapid and simple method is described and applied to resolve and quantify mixtures of prednisolone acetate, sulfacetamide and phenylefrine. The determination was accomplished by micellar electrokinetic capillary chromatography (MEKC) using a fused silica capillary (57 cm × 75 μm ID). The separation was carried out at 25 °C and 30 kV, using a 5 mM phosphate–5 mM borate buffer adjusted to pH 8.2, 40 mM sodium dodecylsulfate (SDS) as background electrolyte. Under these conditions, the run time was 6.5 min and the limits of quantification were about 0.5 mg l^{-1} for every component. Repeatability and reproducibility studies achieved were showing no significant differences at 95% confidence level. Then, multivariate calibration regression spectrophotometric methods (PLS-1, PLS-2 and PCR) were applied providing, especially PLS-1, a clear example of the high resolving power of these techniques. The MEKC method has been applied for quantifying these compounds in different commercial pharmaceuticals products and the method gave good results when it is compared with the spectrophotometric method. The pharmaceutical preparations do not require any separation step by the two described procedures.

Keywords: Corticosteroids; MEKC; Partial least-squares; Partial component regression; Prednisolone; Sulfacetamide; Phenylefrine

1. Introduction

Corticosteroids have been widely used as anti-inflammatories in medicine. Nowadays, pharmaceuticals products contain corticosteroids in conjunction with antibacterials since corticosteroids do not cure the fundamental cause of the disease by themselves, and a result of that it can cause masking of the real disease (e.g. a infection) [1,2].

These compounds are very efficient over a wide range of ocular, allergic and cutaneous inflammatory diseases; so there are a lot of formulations and concentrations of corticosteroids in variable power for local administration. In some commercials, the therapeutic action of these combinations can be

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completed with a decongestant agent, phenylefrine (PHE).

Prednisolone (PRE) is determined in combination with other natural and synthetic corticosteroids [3,4], their metabolites [5] in pharmaceuticals with limits of detection (LOD) around 1 mg l^{-1} and biological fluids by means of reversed-phase HPLC, by means of liquid chromatography–ionspray mass spectrometry [6,7] and by means of micellar electrokinetic capillary chromatography (MEKC) in serum with previous SPE [8,9] using phosphate–borate buffer (pH 8) with sodium dodecylsulfate (SDS), 16% acetonitrile with detection at 254 nm in 10 min with limits of quantification (LOQ) 0.5 mg l^{-1}.

Sulfacetamide sulfate (SUL) is determined simultaneously with other sulfonamides in pharmaceutical preparations by liquid chromatography with spectrophotometric detection [10] with LOD of 0.02 mg l^{-1} and by gas chromatography in animal tissues [11].

The methods described for the assay of PHE are UV spectrophotometry with amphetamines [12,13], HPLC with catecholamines [14,15] and capillary zone electrophoresis with other beta-amino alcohols [16] and amphetamine [17] using a phosphate buffer (pH 3.2) and beta-cyclodextrin to obtain an enantiomeric separation.

In this work, the separations and quantification of prednisolone acetate (PREA) and these related compounds were studied. No references were found for the association PREA–SUL–PHE in capillary electrophoresis (CE) and other techniques. This method provides a very short analysis time (6 min) for PRE and the most important associated compounds in topical pharmaceutical applications. The LOD and level of linearity range has been reduced. Thus, our group has been doing research, for a long time, into the possibilities offered by CE (rapid set-up of instrumentation, versatility and low cost) and multivariate calibration for the determination of corticosteroids and their most important related compounds in ocular and cutaneous pharmaceutical commercials [18–21]. As a result, regarding the routine analysis of these drugs, this paper presents new, accurate and easy MEKC, partial least-squared (PLS) and principal component regression (PCR) methods for the determination of this mixture. The structures of these compounds are given in Fig. 1.

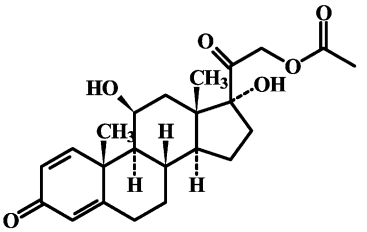
2. Experimental

2.1. Apparatus

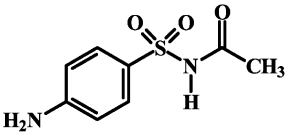
A Beckman P/ACE 5510 (Fullerton, CA) capillary electrophoresis system equipped with a diode-array detector was used. The system was controlled by a Dell DIMENSION™ P133V with P/ACE Station Software. Separation was carried out on a 57 cm (50 cm to the detector) × 75 μm ID fused silica capillary housed in a cartridge with a detector window 800 × 100 mm^2.

A Crison (Barcelona, Spain) MicropH 2002 pH meter was used for the pH measurements.

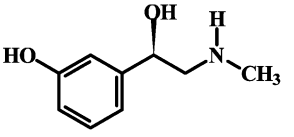
A Beckman (Fullerton, CA) DU-70 spectrophotometer, equipped with 1.0 cm quartz cells and connected to a computer, fitted with Beckman Data Leader software [22] was used. The Grams



PREDNISOLONE 21-ACETATE



SULFACETAMIDE



PHENYLEPHRINE

Fig. 1. Chemical structures of the mixture compounds.

386 Level 1, version 3.01, software package, with the PLS plus version 2.1G application software (Galactic Industries) [23] were used for statistical treatment of the data and for the application of PLS and PCR methods.

2.2. Reagents and solutions

All the solvents and reagents were of analytical grade unless indicated otherwise. Solutions were prepared with deionised water (Milli-Q quality). Prednisolone acetate (PREA), sulfacetamide sulfate (SUL) and phenylefrine HCl (PHE) were supplied by Sigma, Germany.

A stock solution (200 mg l^{-1}) of PREA was prepared in methanol–water (50:50) and the SUL and PHE stock solutions were prepared in water.

The buffer solutions were prepared with NaH_2PO_4, $Na_2B_4O_7$ and water and then with NaOH to adjust the required pH. All these reagents were from Panreac (Barcelona, Spain).

The spectra of all the compounds were recorded at a concentration level of 20 mg l^{-1} between 190 and 316 nm at a scan speed of 600 nm min^{-1}. All the three compounds were assessed to be stable under the operating conditions.

2.3. Procedure

2.3.1. Electrophoretic procedure

The set of separations vials was changed after each batch run (maximum of four separations). The capillary was conditioned, prior to its first use, by flushing first with 0.1 M NaOH for 20 min, then with water for 10 min. In the optimum method, the capillary was washed with 0.1 M NaOH under high pressure for 2 min, then filled for 2 min with the separation buffer, and followed by a 6 s hydrodynamic sample injection. The separation was performed at 30 kV for 8 min at 25 °C; under the selected conditions, the current was 50 μA. Corrected peak area was used for the quantification.

2.3.2. Multivariate calibration

Under computer-controlled instrumentation, multivariate calibrations methods are playing a very important role in the multicomponent analysis of mixtures by UV molecular absorption spectrophotometry [24]. These approaches are useful in the resolution of band overlapping in quantitative analysis (Fig. 2). The advantage of multicomponent analysis using multivariate calibration is the speed of the determination of the components in a mixture, avoiding a preliminary separation step. The application of quantitative chemometric methods, particularly PCR and PLS to multivariate method needs a calibration step where the relationship between the spectra and the component concentration is deduced from a set of reference samples, followed by prediction step in which the results of the calibration are used to determine the component concentration from the sample spectrum. The basic concept of PLS regression was originally developed by Wold [25,26], and the use of the PLS method for chemical applications was also pioneered by Wold et al. [27].

With the aim of verifying the analysis for these compounds, three different chemometric approaches were evaluated. Haaland and Thomas [28] made a comparison of different multivariate calibration methods for quantitative spectral analysis. They conclude that it is very difficult to generalise about the superiority of one method over each other, because their relative performance is often dependent on the particular data set to analyse. The best result in our particular case was for the PLS-2 method.

2.3.2.1. Experimental design of the calibration matrix and selection of the spectral zone for the analysis. A training set of 40 standard ternary mixture samples (using a phosphate buffer of pH 4.5 as optimum in the multivariate calibration), selected taking into account the relation between compounds in the pharmaceutical preparations, was taken as a calibration matrix (0.0–32.0 mg l^{-1} of PREA, SUL and PHE). The spectral region between 215 and 316 nm was selected as suitable for the analysis, which implied the use of 201 experimental points for each spectrum. The selection of spectral information was made according to the spectra of the pharmaceutical products. The range of the spectrum between 190 and 215 nm was rejected owing to differences between the

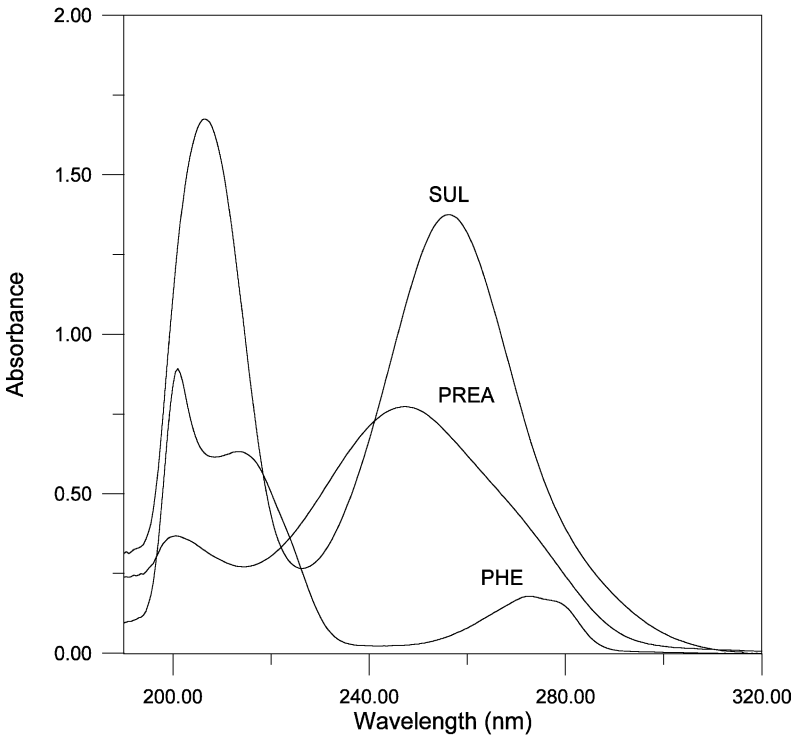


Fig. 2. Absorption spectra for solutions of 20 mg l^{-1} of PREA, 20 mg l^{-1} of PHE, 20 mg l^{-1} of sulfacetamide in phosphate buffer medium (pH 4.5) and recording against a reagent blank with a scan of 600 nm min^{-1}.

artificial mixture spectra and the pharmaceutical spectra products at the same concentration. These differences could be owing to other components of the pharmaceuticals as the excipients such as citric acid, EDTA, phenyl mercuric nitrate, polyvinyl alcohol, bencilic alcohol, and so on.

In Fig. 3, the experimental design is given graphically and we can see the composition of the standard mixtures used in the calibration matrix.

2.3.2.2. Selection of optimum number of factors. To select a number of factors in the PLS-1 algorithm in order to model the system without overfitting the concentration data, a cross-validation method which left out one sample at a time was used [29]. The process was repeated 40 times for each tested number of factor until each calibration standard had been left out once ($n = 40$, number of calibration samples). The predicted concentration (X) of the compounds in each sample was compared with the concentration already known (x) and the prediction error sum of squares (PRESS) was calculated by each number of factors:

$$\text{PRESS} = \sum_{i=1}^{n} (x_i - X_i)^2.$$

This parameter is an efficiency measure for a calibration fit model. The maximum number of factors used to calculate the optimum PRESS was selected as 21 (half the number standards plus one). One reasonable choice for the optimum number of factors would be the number that yields the minimum PRESS. However, using the number of factors that yield a minimum PRESS usually leads to some overfitting. A better criterion for calculating the optimum number of factors involves the comparison of PRESS for models with

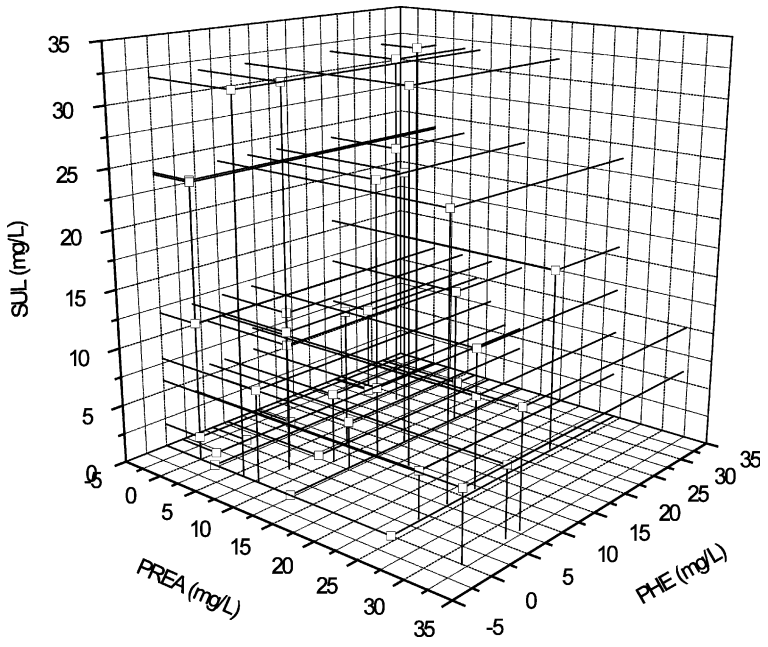


Fig. 3. Experimental design of the calibration matrix given graphically.

fewer than factors. The model selected is not significantly greater than PRESS from the model with h^* factors. Haaland and Thomas [30] empirically determined that an F-ratio probability of 0.75 is an appropriate choice. The number whose F-ratio probability drops below 0.75 was selected as optimum.

In our particular case, a number of six, five and four factors were obtained as optima for PREA, SUL and PHE components, respectively, by means of the PLS-1 method. Also, the PLS-2 model was optimised by using the same set of standard samples and finding as optimum a number of five factors for this model and the same for PCR model finding as optimum a number of seven factors for this model.

The proposed PLS and PCR calibration models were evaluated by internal validation (prediction of compounds concentration in its own designed training set of calibration) obtaining, in general terms, recoveries ranging from 96.8 to 103.5%.

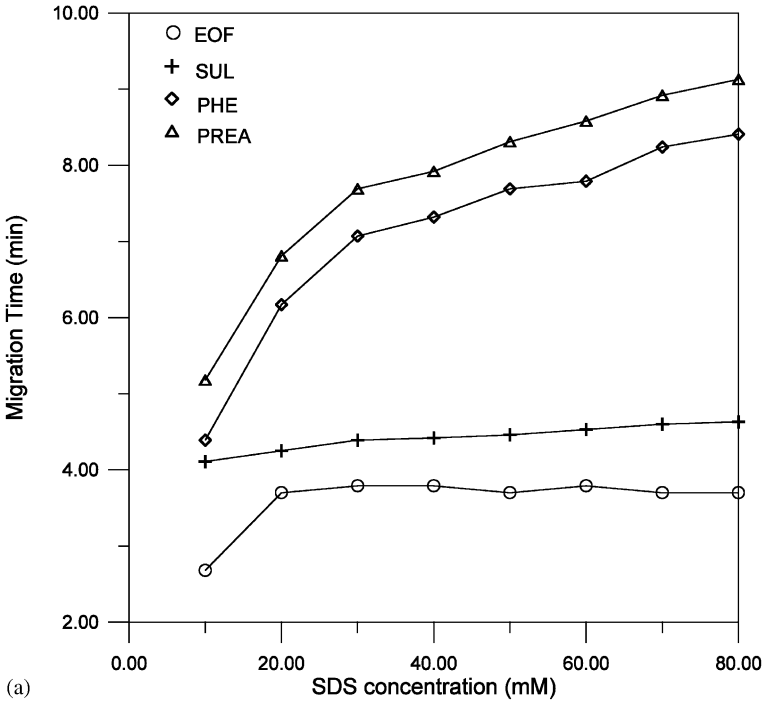
3. Results and discussion

3.1. Electrophoretic procedure

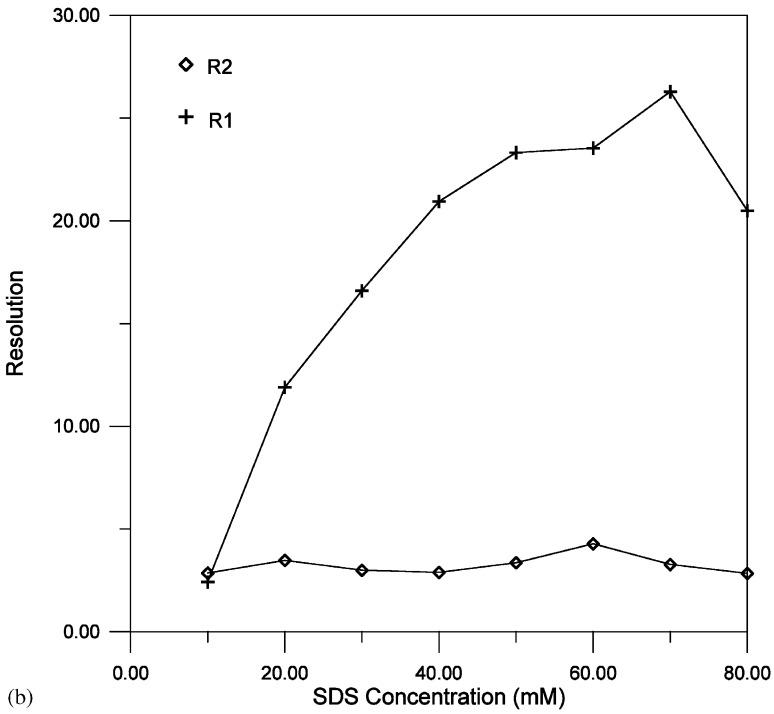
3.1.1. Preliminary studies

To optimise separations, a preliminary study was carried out using a solution containing 28, 12 and 20 mg l^{-1} of PREA, SUL and PHE, respectively. Using a 10 mM phosphate–10 mM borate buffer (pH 8.2) with 40 mM SDS as electrolyte solution, temperature and voltage were 25 °C and 25 kV, respectively.

3.1.1.1. Influence of pH on separation. Separations have been carried out at different pH values (6, 7, 8, 9, 10, 11) with and without SDS. The results demonstrate that the separation is better when pH is 8 because the peaks are stronger and when the background electrolyte contains SDS as surfactant. By these separation, it could be proved that SUL and PHE are ionic forms under the described



(a)



(b)

Fig. 4. Influence of SDS concentration on (a) migration time and (b) on resolution (5 mM phosphate–5 mM borate buffer (pH 8.2) as electrolyte solution; temperature and voltage were 25 °C and 25 kV, respectively).

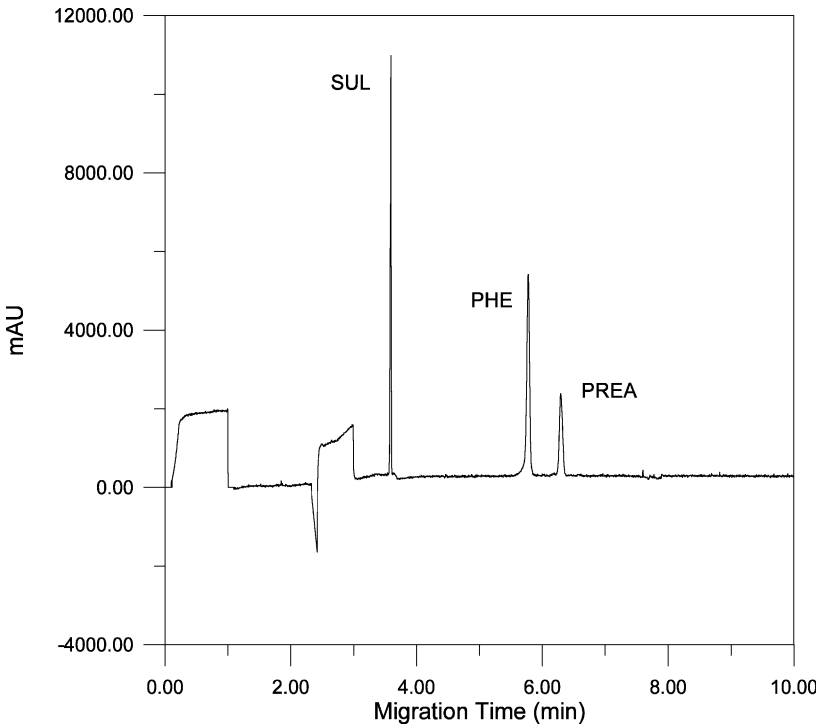


Fig. 5. Electropherogram of a sample containing 20, 20 and 12 mg l^{-1} for PREA, PHE and SUL, respectively, obtained under optimised conditions at 205 nm (5 mM phosphate–5 mM borate buffer (pH 8.2), 40 mM SDS as electrolyte solution; temperature and voltage were 25 °C and 30 kV, respectively).

conditions, and so they appears away from the electro-osmotic flow (EOF) when the surfactant was not added to the electrolyte, while PREA eluents with the EOF in all those cases.

SDS was selected as micellar additive in the electrolyte as it is the most common surfactant used in MEKC. A phosphate–borate (1:1) buffer at pH 8.2 was chosen in our study owing to the high buffer ability of borate ($pK_a = 9.2$) and phosphate ($pK_a = 7.5$).

Table 1
Optimised conditions for separation

Capillary	Fused silica (57 cm length × 75 μm inner diameter)
Electrolyte	5 mM phosphate–5 mM borate buffer, pH 8.2; 40 mM SDS
Temperature	25 °C
Voltage	30 kV
Detector	Diode array
Window	800 × 100 μm^2

3.1.1.2. Influence of phosphate–borate (1:1) buffer concentration. The phosphate–borate buffer molarity was varied from 10 to 50 mM using the experimental conditions mentioned above and its influence upon the migration time was studied. A 10 mM (5 mM phosphate–5 mM borate) concentration was considered as suitable for its satisfac-

Table 2
LODs and LOQs and statistical parameters of calibration graph for each compound

	SUL	PHE	PREA
LOD (mg l^{-1})	0.09	0.09	0.34
LOQ (mg l^{-1})	0.29	0.32	1.21
Intercepts (CAU[a])	62.3 ± 26.1	−503 ± 330	−32.1 ± 20.9
Slope (CAU × l mg^{-1})	310.8 ± 1.2	532.2 ± 9.7	100.7 ± 0.6
R^2	0.9999	0.9979	0.9997
Linear range (mg l^{-1})	0.3–40.0	0.3–56.8	1.2–55.3

Linear regression calibration curves.
[a] CAU, correct area unit.

tory resolution ($R_s > 1.5$) and peak slope, whereas higher concentrations resulted in peak broaden.

3.1.1.3. Influence of SDS. The influence of SDS in electrolyte on migration time is given in Fig. 4. The results demonstrate that the SDS concentration dramatically affects the mobility of PREA and PHE (Fig. 4a). A concentration of 40 mM was selected for the experiment as to give the best resolution ($R_s > 1.5$) without broadened and deformed peaks (Fig. 4b) with the shortest analysis time. The current generated and the run time was 49.5 μA and 8 min, respectively.

3.1.1.4. Influence of running voltage and temperature. Running voltages in the range 5–30 kV were tested by using the above experimental conditions. As expected, decreasing migration times were obtained with increasing applied voltages. A potential of 30 kV can be selected as optimum because it gives the best resolution ($R_s > 1.5$) and symmetric peaks in all cases in a shorter analysis time (6 min).

Temperature lower than 20 °C was not considered because the surfactant has enough solubility to form micelles only at temperature above the Kraft point (16 °C for SDS), and temperature regulation with the instrument is efficient only until 4 °C below room temperature. We investigated the effect of temperature on separation between 20 and 35 °C by employing the selected condition (5 mM borate–5 mM phosphate buffer, pH 8.2; 40 mM SDS; 30 kV).

For temperature higher than 35 °C, contribution of Joule heating and temperature gradient become more pronounced, giving band broadening. 25 °C was selected as a compromise between resolution, run time, current intensity and acceptable level of baseline noise.

3.1.1.5. Selected conditions. From the studies carried out before, we suggest that the procedure summarised below is convenient to separate the mixture properly (Table 1).

The electropherogram obtained in the separation under selected conditions is presented in Fig. 5. It is remarkable that all peaks have good relation in a run time of 6.5 min.

3.1.2. Performance evaluation

3.1.2.1. Limits of detection and quantification. LOD and LOQ were estimated in accordance with the baseline noise method. The baseline noise was evaluated by recording the detector response over a period as much as 10 times the peak width. LOD was obtained as the sample concentration that causes a peak with three times as high as the baseline noise level [31] and LOQ was calculated as being 10 times as high as the baseline noise level. Thus, LOD and LOQ are shown in Table 2 for each compound.

Table 3
Statistical parameters of cross-validation process for PLS-1, PLS-2 and PCR

Compound	Factor	PRESS	RMSD	R^2	REP (%)
PLS-1					
PREA	6	2.519	0.2509	0.9994	1.587
PHE	4	71.696	1.3388	0.9802	8.467
SUL	5	139.612	1.8682	0.9703	11.816
PLS-2					
PREA	5	254.996	0.3135	0.9990	1.9831
PHE			1.4059	0.9782	8.8917
SUL			2.0736	0.9633	13.1149
PCR					
PREA	7	261.595	0.2968	0.9992	1.8777
PHE			1.8528	0.9639	11.7079
SUL			2.0579	0.9666	13.0149

3.1.2.2. Linearity range and calibration curves. The linearity of the assay was checked by injecting the calibration solution of each drug in the range from 0.3 to 60 mg l^{-1} using 15 standard solutions. In all cases, the separation was carried out by using the optimised electrophoretic procedure. The calibration curves were obtained for each component by plotting the correct area, measured at the maximum absorption wavelength, 245, 200 and 195 nm for PERA, PHE and SUL, respectively, versus their concentrations.

A satisfactory linear relationship ($R^2 \geq 0.998$) was obtained between the concentration and the corrected area for each component. In Table 2, the slopes, intercepts, R^2 and linearity ranges for the calibration curves are presented. In all cases, the intercepts were estimated as negligible using Student's t-test ($\alpha = 0.05$).

3.1.2.3. Repeatability and reproducibility. Repeatability was assessed under the previously selected conditions by means of 12 replicates of a solution containing 28, 12 and 20 mg l^{-1} of PREA, SUL and PHE, respectively. Reproducibility was evaluated over 2 days by performing 12 replicates each day.

The results showed that the repeatability for every component in each day is satisfactory (RSD ≤ 2.5 in each compound). In terms of reproducibility, the comparison of averages with the Snedecor test did not provide any significant difference between both days' series, for $\alpha = 0.05$ ($n = 12$) [32,33].

3.2. Multivariate calibration studies

Three multivariate calibration methods were developed by authors in order to check the MEKC method and as well as confirming the electrophoretic results in pharmaceutical mixtures. PLS and PCR methods were evaluated and a comparative study of the prediction capabilities of all the three chemometric approaches in our particular work was undertaken.

3.2.1. Statistical parameters of cross-validation method

Using the cross-validation method, the following statistical parameters have been obtained:

a) The values of root mean squares difference (RSMD), which is an indication of the average error in the analysis for each component.
b) The square of correlation coefficients (R^2), which is an indication of the quality of the straight line that fits the data.
c) The predictive ability of each method and for each component can also be described in terms of the relative error of prediction (REP) with regard to the average value (μ).
d) The standard error of calibration (SEC/SEP).

In Table 3, the results obtained for these parameters following the implementation of the three proposed chemometric approaches are shown. We can see that R^2 values in all cases are very close to 1, which is an indication of similarity between predicted and known values. On the other hand, in general terms, the obtained errors for these statistical cross-validation parameters are the same for both multivariate calibration methods. The best statistic results were obtained by PLS-1.

3.2.2. Repeatability and reproducibility

Reproducibility of the PLS-1, PLS-2 and PCR methods was checked by recording independent series of 10 samples for each compound (16 mg l^{-1} of PREA, 16 mg l^{-1} of PHE and 16 mg l^{-1} of SUL) in 2 consecutive days. Repeatability studies were satisfactory obtaining RSD values of 0.31, 0.09 and 0.35 for PREA, PHE and SUL, respectively; when reproducibility studies were achieved over the two sets of 10 standards for each compound in consecutive days, no significant differences between the two sets of 10 replicates at a confidence level of 95% were found.

3.3. Applications

The present method was tested to determine the mentioned compounds in pharmaceutical preparations. The pharmaceutical industry has different commercial formulations containing PREA, SUL and PHE at present.

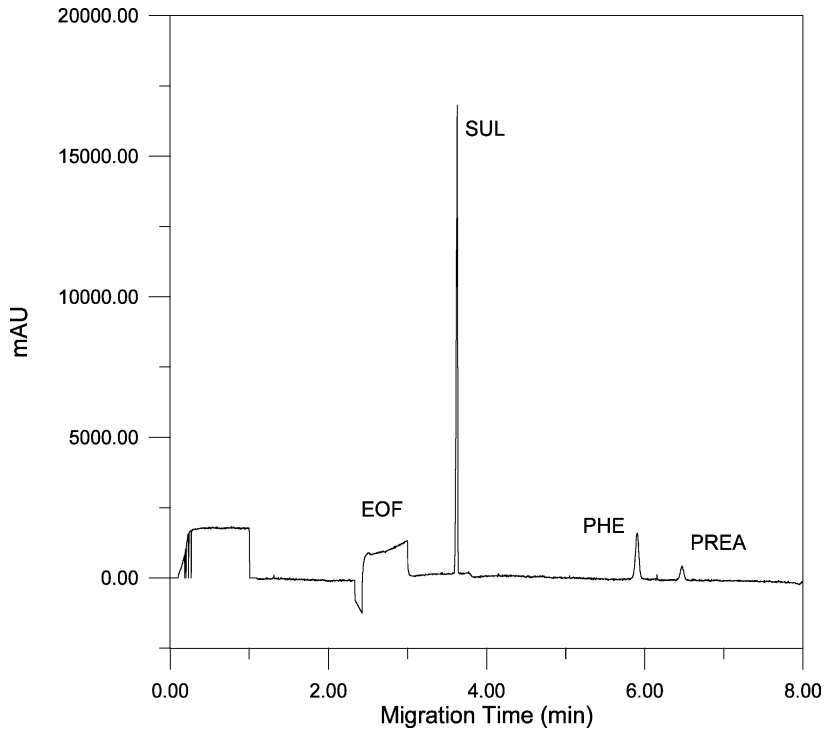


Fig. 6. Electropherogram of blifomol obtained under optimised conditions at 205 nm (5 mM phosphate–5 mM borate buffer (pH 8.2), 40 mM SDS as electrolyte solution; temperature and voltage were 25 °C and 30 kV, respectively).

3.4. Drops

3.4.1. Blifomol

This is an ocular drops with SUL, PREA and PHE from the enterprise Allergal S.A.

Once the pharmaceutical mixture was homogenised for drops and aerosols, different known aliquots were placed in 25 ml calibrated flasks, adding methanol (30%) and deionised water to the mark for MEKC analysis and 5 ml of phosphate buffer 0.1 M, pH 4.5, methanol (30%) and water to the mark for multivariate methods.

3.5. Ointments

3.5.1. Meocil

This is an ocular ointment with SUL and PERA from the enterprise Edol.

An amount of each ointment was weighed accurately inside an extraction glass. A se-

Table 4
Application results of MEKC and PLS-1 on different pharmaceutical preparations

Commercial	Claimed (mg l^{-1})	MEKC		PLS-1	
		Found (mg l^{-1})	Recovery (%)	Found (mg l^{-1})	Recovery (%)
Blifomol	SUL 20.4	19.6±0.2	96.8	19.1±0.2	93.6
	PERA 6.5	6.2±0.1	96.1	6.9±0.1	109.5
	PHE 6.2	5.9±0.2	96.2	6.3±0.1	101.6
Meocil	SUL 32.0	30.5±0.3	93.8	29.2.±0.2	91.3
	PREA 2.0	1.5±0.1	91.9	1.9±0.1	95.0

quential extraction was made to extract all the compounds with a total volume of 100 ml. Volumes of 20 ml were shaken and then subjected to an ultrasonic bath for 15 min, to complete 100 ml. This total volume of the extraction was filtered and different known aliquots were placed in a 25 ml calibrated flask, adding also methanol (the final solution contained 30% methanol) and deionised water for MEKC analysis and 5 ml of phosphate buffer 0.1 M, pH 4.5, methanol (30%) and water to the mark for multivariate methods.

In the analysis of the commercials by MEKC, the experiment was achieved by comparison with standard solutions containing the same concentrations as expected for commercials, according to their claimed levels. The standard solution was prepared from the stock solutions after convenient dilution. In Fig. 6 is shown the electropherogram of a sample of Blifomol.

In the analysis of the commercials by multivariate calibration, the spectra of the commercial samples thus prepared were recorded against a reagent blank (the same as that of the samples without the compounds to be determined and with some of the excipients indicated to the enterprise), with a scan speed of 600 nm min^{-1} ranging from 316 to 215 nm. The contents of PREA, PHE and SUL were calculated by analysing the recorded spectra with the PLS-1, PLS-2 and PCR chemometric approaches. The predicted concentrations expressed as mass/volume ratio (mg l^{-1} in the commercial product) are summarised in Table 4, where the contents supplied by the manufacturer are also shown. The best results by multivariate methods were for PLS-1.

Table 4 displays an acceptable agreement between the results obtained by MEKC and multivariate calibration, and these values are also close to the ones provided by the manufacturer. The results obtained by multivariate calibration show a relative error below 8%. The excipients interfere in the correct determination of these compounds in the pharmaceuticals preparations. In the ointment, the extraction must be another negative reason in the correct determination.

4. Conclusions

The newly presented MEKC method to determine PREA, SUL and PHE proved to be easy to apply in pharmaceuticals because there are no previous sample treatments, apart from the dissolution of the commercials in water and methanol or a simple extraction of the commercials with methanol and a convenient dilution of the extract if it is necessary. This method proves to be as sensitive, accurate and exact as the multivariate calibration one for this mixture. In this way, by means of multivariate calibration method, the measurement is performed at the specific wavelength previously selected. However, the presence of different excipients in the formulations might cause interferences on the measurement signal. The multivariate calibration was suitable only for synthetic samples.

The new proposed method, owing to the high separation power of MEKC, provides an useful tool for removing the contribution of these interferences, as well as for their detection. So, it can be concluded that the MEKC method is convenient for the determination of the studied compounds with appropriate exactness in the quality control of these kinds of pharmaceutical formulations.

Acknowledgements

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