Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 784 (2003) 39–47
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Determination of prednisolone and the most important associated compounds in ocular and cutaneous pharmaceutical preparations by micellar electrokinetic capillary chromatography

J.M. Lemus Gallego*, J. Pérez Arroyo

Department of Analytical Chemistry and Food Technology, Facultad de Ciencias Químicas, Universidad de Castilla-La Mancha, 13071 Ciudad Real, Spain

Received 1 March 2002; received in revised form 26 August 2002; accepted 20 September 2002

Abstract

A micellar electrokinetic capillary chromatographic method to separate prednisolone, prednisolone acetate, naphazoline, Zn–bacitracin, sulfacetamide and phenylefrine is described. The separation was carried out by using a fused-silica capillary (57 cm×75 μm I.D.) at 25 °C and 30 kV, using a 5 m*M* phosphate–5 m*M* borate buffer adjusted to pH 8.2, 50 m*M* sodium dodecyl sulfate (SDS) and 10% methanol–water (v/v) as background electrolyte. Under these conditions, the run time was 8 min and the limits of quantification were about 1.0 mg/l for every component. The method was applied to pharmaceutical preparations and the results provided recoveries close to 100% and the method gave good results when compared with a reference multivariate calibration spectrophotometric method.

Keywords: Prednisolone; Naphazoline; Bacitracin, Zn; Sulfacetamide; Phenylefrine

1. Introduction

Corticosteroids have been widely used as anti-inflammatory in medicine. Nowadays, pharmaceutical formulations contain corticosteroids in combination with antibacterials because corticosteroids do not solve the fundamental reason of the disease so that it can cause masking of the real disease (for example an infection) [1,2].

These compounds are very effective against a wide range of ocular, allergic and cutaneous inflammatory diseases so there are a lot of formulations (prednisolone acetate–sulfacetamide (PREA–SUL) or prednisolone–Zn–bacitracin (PRE–BAC)) and concentrations of corticosteroids in variable power for local administration. In some drug formulations, the therapeutic action of these combinations can be completed with a decongestant agent: phenylefrine (PHE) and naphazoline (NAP).

PRE and PREA have been determined in combination with other natural and synthetic corticosteroids [3,4], their metabolites [5] in pharmaceuticals with a LOD of around 1 mg/l and biological fluids by reversed-phase HPLC. PRE has been determined by liquid chromatography–ionspray mass spectrometry

*Corresponding author. Tel.: +34-926-295-300x3443; fax: +34-9-26-295318.
E-mail address: JoseMaria.Lemus@uclm.es (J.M. Lemus Gallego).

1570-0232/02/$ – see front matter
PII: S1570-0232(02)00748-1

[6,7] and by micellar electrokinetic capillary chromatography in serum with previous SPE (MEKC) [8,9] using phosphate–borate buffer (pH 8) with SDS, 16% acetonitrile with detection at 254 nm in 10 min with a LOQ of 0.5 mg/l.

SUL has been determined simultaneously with other sulfonamides in pharmaceutical preparations by liquid chromatography with spectrophotometric detection [10] with a LOD of 0.02 mg/l and by gas chromatography in animal tissues [11].

The most widely used technique for simultaneous determination of BAC A in association with the other bacitracins is HPLC, with or without derivatization [12,13] in reversed-phase mode. BAC has been determined by nonaqueous capillary electrophoresis–mass spectrometry (NACE–MS) [14] and by MEKC with UV detection [15] with phosphate buffer (pH 2.5) with propane sulfonate and Brij 35 to quantifying only BAC A at 192 nm with a LOD of 1.2 mg/l and a linearity range of 50–1000 mg/l. BAC has been determined in association with hydrocortisone and the most important associated compounds in topical pharmaceutical preparations [16] using phosphate–borate buffer (pH 8.2), SDS 10% methanol to quantifying Zn–BAC and detection at 195 nm with a LOD of 2.6 mg/l and a linearity range of 8.6–28.4 mg/l.

NAP has been determined simultaneously with PHE [17] and other imidazolines with and without derivatization by spectrofluorimetric and derivative spectrophotometric methods [18–20] with quantitative determination. Reversed-phase HPLC has been used to determine NAP in pharmaceutical [21,22] formulations with others corticosteroids [23], with a linearity range of 10–60 mg/l, and by capillary electrophoresis together with their degradation products [24] and other antibiotics and corticoids [25] with a LOD of 0.25 mg/l.

The methods described for the assay of PHE are UV spectrophotometry with amphetamines [26,27], HPLC with catecholamines [28,29] and capillary zone electrophoresis with other beta-amino alcohols [30] and amphetamine [31] using phosphate buffer (pH 3.2) and beta-cyclodextrin, to obtain an enantiomeric separation.

In this work, the separation and quantification of prednisolone, prednisolone acetate and the associated compounds was studied. No references were found for the simultaneous quantification of PREA–SUL, PRE–BAC and PRE–NAF in capillary electrophoresis and other techniques. This method provides a very short analysis time (8 min) for PRE and the most important associated compounds in topical pharmaceutical applications and enables the quantification of commercial Zn–BAC in only one peak. The LOD has been reduced for NAP and BAC and is similar for PRE, PREA and SUL. The linearity range has been reduced, specially for BAC. In this way, our group has been doing research for several years, into the possibilities offered by CE (rapid set-up of instrumentation, versatility and low cost) for the determination of corticosteroids and their drugs used in combination in ocular and cutaneous pharmaceutical commercial products [16,32,33]. As a result, regarding the routine analysis of these drugs, this paper presents a new, accurate and easy MEKC method for the simultaneous determination of the mentioned mixture. The structures of these compounds are given in Fig. 1.

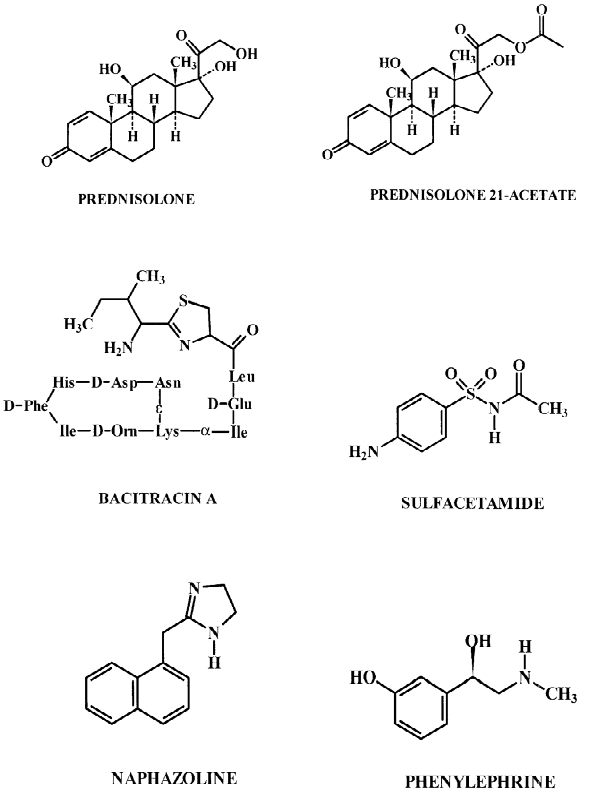

Fig. 1. Chemical structures of the mixture compounds.

2. Experimental

2.1. Apparatus

A Beckman P/ACE 5510 (Fullerton, CA, USA) 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 I.D. fused-silica capillary housed in a cartridge with a detector window 800×100 μm.

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

A Beckman (Fullerton) DU-70 spectrophotometer equipped with 1.0 cm quartz cells and connected to an IBM-PS 2 Model 30 computer, fitted with Beckman Data Leader software, was used.

2.2. Reagents and solutions

All solvents and reagents were of analytical grade unless indicated otherwise. Solutions were prepared with deionized water (Milli-Q quality). Prednisolone (PRE), prednisolone acetate (PREA), Zn–bacitracin (BAC), sulfacetamide sulphate (SUL), phenylefrine HCl (PHE) were obtained from Sigma (Deisenhofen, Germany) and naphazoline nitrate (NAP) from Fluka (Buchs, Switzerland).

Stock solutions (200 mg/l) of PRE, PREA, and BAC were prepared in methanol–water (50:50) and SUL, PHE and NAP stock solutions were prepared in water.

Buffer solutions were prepared by dissolving the adequate quantity of NaH_2PO_4 and $Na_2B_4O_7$ in deionized water and then adjusting with HCl or NaOH to the required pH. All these reagents were from Panreac (Barcelona, Spain).

2.3. Real sample preparation

2.3.1. Drops and aerosols

Blifomol. Ocular drops with SUL, PREA and PHE from Allergal (Lisboa, Portugal).

Flogiftalmina. Ocular drops with PRE and PHE from Davi Faemaceutica (Lisboa, Portugal)

Rinovel. A nasal aerosol with PRE and NAF from the ERN (Barcelona, Spain).

Lidrone. A nasal aerosol with PRE, PHE and NAP from Serra Pamies (Barcelona, Spain).

Once the pharmaceutical mixture was homogenized for drops and aerosols, different known aliquots were placed in 25 ml calibrated flasks, adding methanol (30%) and deionized water to the mark.

2.3.2. Ointments

Rinobanedfif. A nasal ointment with PRE, BAC and PHE from Roche (Madrid, Spain).

Meocil. An ocular ointment with SUL and PERA from Edol (Oeiras, Portugal).

An amount of each ointment was weighed accurately into an extraction glass. A sequential extraction was made to extract all the compounds with a total volume of 100 ml. Different volumes of 20 ml are shaken and subjected to an ultrasonic bath for 15 min, to complete to 100 ml. This total volume of extraction was filtered and different known aliquots were placed in 25 ml calibrated flasks, adding also methanol (final solution contained 30% methanol) and deionized water.

2.4. Operating conditions

Separations were performed using 4 ml glass vials. The set of separations vials was changed after each batch run (maximum 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 optimized method, the capillary was washed with 0.1 *M* NaOH under high pressure for 2 min and then filled for 2 min with the separation buffer, 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 68.0 μA.

Corrected peak area was used for quantification.

3. Results and discussion

3.1. Preliminary studies

To optimize separations, a preliminary study was carried out using a solution containing 28 mg/l of PRE and PREA, 12 mg/l of SUL and NAP and 40 and 20 mg/l of BAC and PHE mg/l, respectively. A 10 m*M* phosphate–10 m*M* borate buffer (pH 8.2)

with 40 m*M* SDS was used as electrolyte solution, the temperature was 25 °C and the voltage was 25 kV.

3.1.1. Influence of pH on the separation

Separations have been carried out at different pH values (6, 7, 8, 9, 10, 11) with and without SDS. The results show that the separation is better when the pH is 8 and when the background electrolyte contains SDS as surfactant because it gives strong peaks in a short analysis time (13 min). By these separations it could be proven that BAC, SUL, PHE and NAP are ionized under the described conditions, so they appear behind the electro-osmotic flow (EOF) (BAC very near to the EOF) when the surfactant was not added to the electrolyte; while PRE and PREA co-elute with the EOF in all those cases.

SDS was selected 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 due to the high buffer capacity of the borate (pK_a 9.2) and the high buffer capacity of the phosphate (pK_a 7.5).

3.1.2. Influence of the organic modifier

Preliminary experiences suggested addition of some kind of organic modifiers because some peaks (PRE, PERA and BAC) were not well resolved (R_s<1.5) showing overlap and shoulders. The experiments were performed using 20 m*M* phosphate–borate buffer, pH 8.2 containing 30 m*M* SDS as electrolyte. Methanol and acetonitrile were tested in concentrations from 3 to 12%. The presence of 3% acetonitrile in the electrolyte showed the most important reduction of overlapping with shortest analysis time because it modified the interaction of these compounds with the micelles, but the resolution between PREA–BAC was still <1.5.

3.1.3. Influence of phosphate–borate (1:1) buffer concentration

The phosphate–borate buffer molarity was varied from 10 to 50 m*M* using the experimental conditions mentioned above and its influence upon the migration time was studied. A 10 m*M* (5 m*M* phosphate–5 m*M* borate) concentration was considered as suitable because it gives R_s>1.5, but the peaks of BAC and PREA are not symmetric.

3.1.4. Influence of SDS

The influence of SDS in the electrolyte on the migration time is shown in Fig. 2. The results show that the SDS concentration dramatically affects the migration time of the PRE, PREA, PHE, and BAC (Fig. 2a). A concentration of 50 m*M* was selected for the experiment as to give the best resolution (R_s> 1.5) and symmetric peaks in all cases with a short analysis time (Fig. 2b). The current generated was 68.5 μA and the run time was about 10.5 min.

3.1.5. 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>1.5) and symmetric peaks in all cases in a shorter analysis time (8 min).

A temperature lower than 20 °C was not considered because the surfactant has enough solubility to form micelles only at a 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 the separation between 20 and 35 °C by employing the selected condition (5 m*M* borate–5 m*M* phosphate buffer pH 8.2; 50 m*M* SDS; 30 kV).

For a temperature higher than 35 °C, contribution of Joule heating and temperature gradient become more pronounced, giving band broadening. Therefore, 25 °C was selected as a compromise between resolution, run time, current intensity and acceptable level of baseline noise to obtain a low LOD and LOQ, because an increase of the temperature produces an increase of the current intensity and the baseline noise.

3.1.6. Optimization of rinsing and washing steps

A washing step of 2 min with 0.1 *M* sodium hydroxide, followed by a 2 min buffer wash, appeared to be adequate to restore the capillary wall surface and equilibrate the capillary between sample injections, with good repeatability.

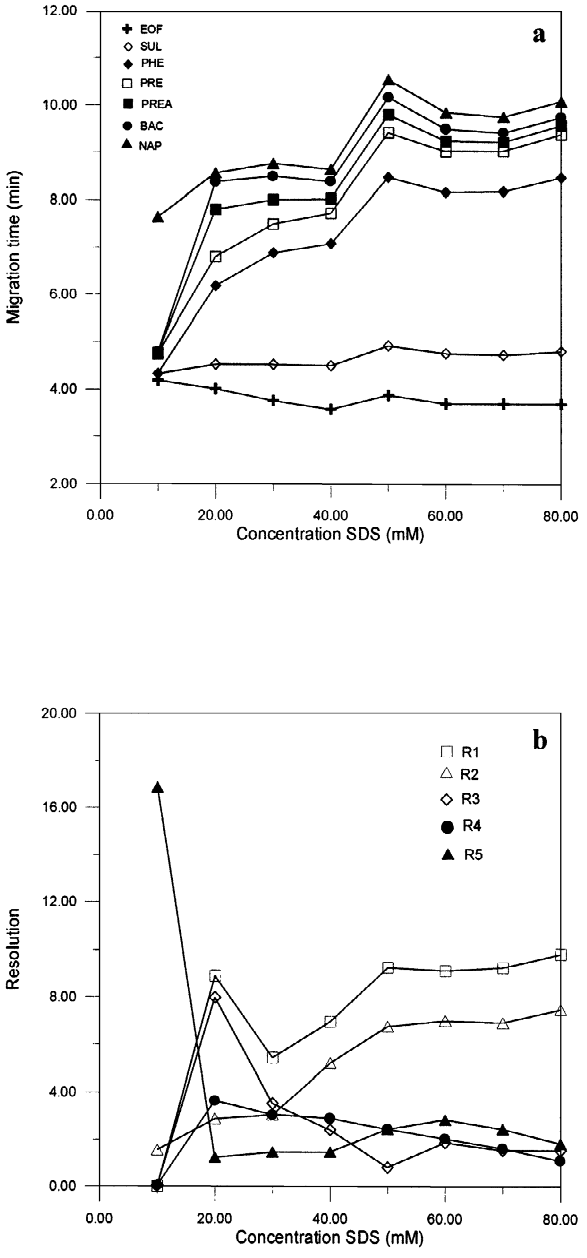

Fig. 2. Influence of the SDS concentration on the migration time (a) and on the resolution (b), using a 5 m*M* phosphate–5 m*M* borate buffer (pH 8.2) with 3% acetonitrile as electrolyte solution; temperature and voltage were 25 °C and 25 kV, respectively. $R_{1,2,3,4,5}$: resolution between SUL–PHE, PHE–PRE, PRE–PREA, PREA–BAC and BAC–NAP, respectively.

Table 1
Optimized conditions for the separation

Capillary	Fused silica (57 cm length×75 μm I.D.)
Electrolyte	5 m*M* phosphate–5 m*M* borate buffer pH=8.2; 50 m*M* SDS and 3% acetonitrile
Temperature	25 °C
Voltage	30 kV
Detector	Diode array
Window	800×100 μm

3.1.7. Selected conditions

From the studies carried out, we suggest that the procedure summarized in Table 1 is convenient to separate the mixture properly.

The electropherogram obtained in the separation under selected conditions is presented in Fig. 3. It is remarkable that all peaks have resolution higher than 1.5 in a run time of 8 min.

3.2. Performance evaluation

3.2.1. Limits of detection and quantification

Limits of detection and quantification (LOD and LOQ, respectively) were estimated in accordance to the baseline noise method. The baseline noise was evaluated by recording the detector response over a period of 10 times the peak width. The LOD was obtained as the sample concentration that caused a peak with a height three-fold the baseline noise level [34] and the LOQ was calculated as 10-fold the baseline noise level. Thus, LODs and LOQs are shown in Table 2 for each compound.

3.2.2. Linearity range and calibration curves

The linearity of the assay was checked by injecting standard solution of each drug in the range from 0.1 to 80 mg/l using 15 solutions of different concentration. In all cases, the separation was carried out by using the optimized electrophoretic procedure. The calibration curves were obtained for each component by plotting the corrected area, measured at the maximum absorption wavelength, 245 nm for PRE and PREA, 195 nm for BAC and PHE and 200 nm and 220 nm for SUL and NAP, respectively, versus their concentrations.

A good linear relationship ($r^2 \geq 0.994$ in all cases) was obtained between concentration and corrected area for each component. Different linearity ranges

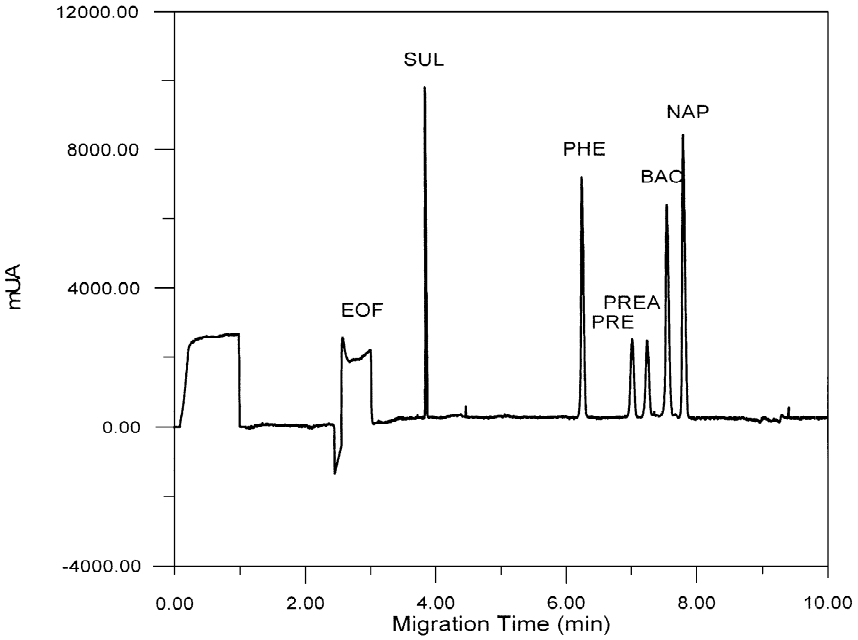

Fig. 3. Electropherogram of a sample containing 28, 28, 40, 12, 20 and 12 mg/l of PRE, PREA, BAC, SUL, PHE and NAP, respectively, obtained under the optimized conditions at 205 nm. (5 m*M* phosphate–5 m*M* borate buffer (pH 8.2), 50 m*M* SDS, 3% acetonitrile as electrolyte solution; temperature and voltage were 25 °C and 30 kV, respectively).

for each compound was obtained because of the different molar absorptivity and application concentrations. 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 by using the Student's *t*-test ($\alpha = 0.05$).

The middle point of the calibration curves were selected to study the influence of the injection time on the corrected area, from 2 to 10 s; resulting in similar linearity range conditions that were already provided.

3.2.3. Repeatability and reproducibility

Repeatability was assessed under the previously selected conditions by means of 12 replicates of a solution containing 28 mg/l of PRE and PREA, 12 mg/l of SUL and NAP and 40 and 20 mg/l of BAC and PHE mg/l, respectively. Reproducibility was evaluated over 2 days by performing 12 replicates each day.

The results showed that the repeatability (using corrected peak areas) for every component in each day was satisfactory (RSD≤2.2% in all cases). In

Table 2
Validation data

	SUL	PHE	PRE	PREA	BAC	NAP
LOD (mg/l)	0.09	0.07	0.31	0.25	1.52	0.05
LOQ (mg/l)	0.29	0.23	1.03	0.83	5.01	0.15
Intercepts (CAU[a])	−88±99	46±176	115±56	77±25	−119±67	26±141
Slope (CAU×L/mg)	304±6	444±7	87.1±2.0	83.9±1.0	113±2	619±8
r^2	0.9963	0.9974	0.9941	0.9984	0.9974	0.9987
Linear range (mg/l)	0.3–32.0	0.2–50.2	1.0–56.0	0.8–56.2	5.0–73.4	0.1–32.3

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

Table 3
Results by means of MEKC and a multivariate calibration spectrophotometric method (PLS-2) for different pharmaceutical preparations

Commercial	Claimed (mg/l)	MEKC		Spectrophotometric method (PLS-2)[a]	
		Found (mg/l)	Recovery (%)	Found (mg/l)	Recovery (%)
Blifomol	SUL 20.4	20.1±0.2	98.6	19.1±0.2	93.6
	PREA 6.5	6.3±0.1	98.2	6.9±0.1	109.5
	PHE 6.2	6.3±0.2	101.7	6.3±0.1	101.6
Rinovel	PRE 7.5	7.6±0.1	101.3	7.8±0.1	104.0
	NAP 30.0	28.9±0.8	96.8	27.8±0.4	92.6
Rinobanedif	PRE 25.7	22.2±08	91.5	24.1±0.2	93.8
	PHE 21.4	18.8±0.7	92.8	19.2±0.1	89.7
	BAC 63.8	60.5±1.9	96.8	64.5±0.5	106.6
Lidrone	PRE 4.0	3.8±0.1	96.3	3.8±0.1	95.0
	PHE 50.0	49.9±0.9	99.9	47.7±0.1	95.4
	NAP 5.0	5.1±0.1	101.8	5.4±0.4	108.0
Flogiftalmina	PRE 26.0	25.9±0.6	99.6	28.5±0.1	109.6
	PHE 26.0	26.1±0.4	100.4	23.5±0.1	90.4
Meocil	SUL 32.0	30.5±0.3	93.8	29.2.±0.2	91.3
	PRE 2.0	1.5±0.1	91.9	1.9±0.1	95.0

[a] Multivariate calibration spectrophotometric method by partial least square regression 2 (PLS-2).

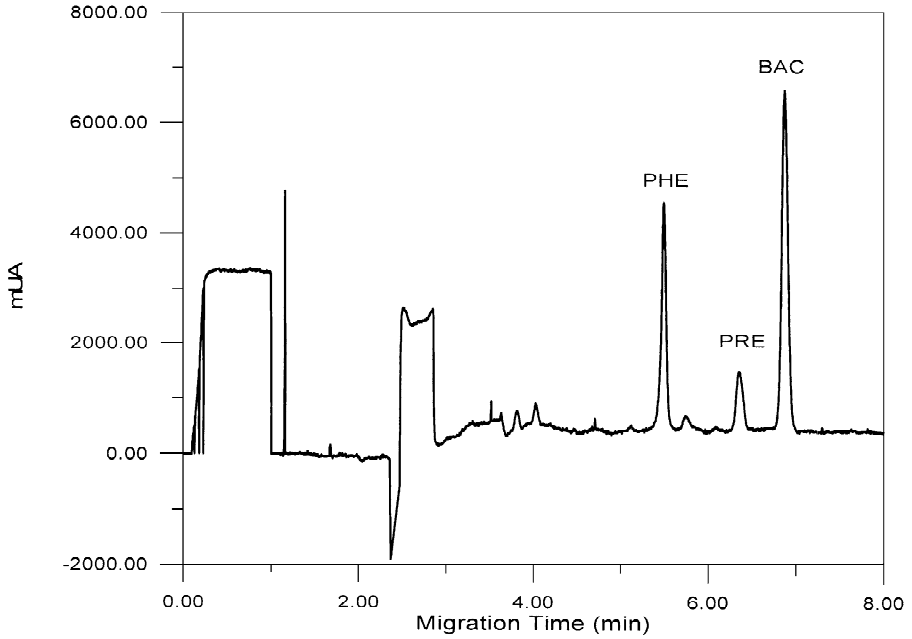

Fig. 4. Electropherogram of a sample of Rinobanedif obtained under optimized conditions at 205 nm. (5 m*M* phosphate–5 m*M* borate buffer (pH 8.2), 50 m*M* SDS, 3% acetonitrile as electrolyte solution; temperature and voltage were 25 °C and 30 kV, respectively).

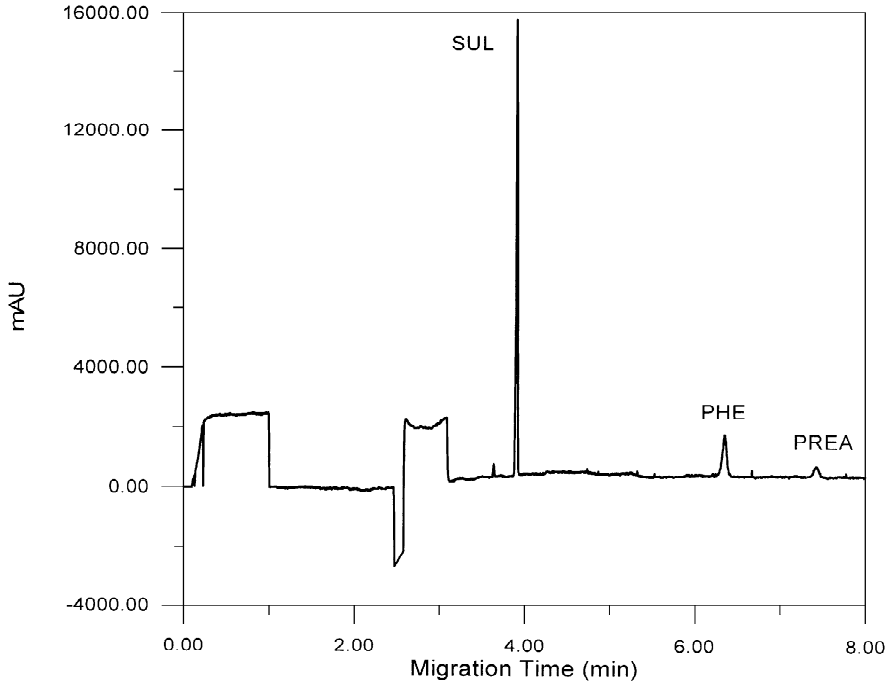

Fig. 5. Electropherogram of a sample of Blifomol obtained under optimized conditions at 205 nm. (5 m*M* phosphate–5 m*M* borate buffer (pH 8.2), 50 m*M* SDS, 3% acetonitrile as electrolyte solution; temperature and voltage were 25 °C and 30 kV, respectively).

terms of reproducibility, the comparison of averages with the Snedecor test did not provide any significant difference between both days series, for $\alpha = 0.05$ ($n = 12$) [35,36].

3.2.4. Peak purity

Peak purity was obtained for all compounds by overlapping the spectra captured at the apex, up-slope and down-slope. No differences were noted for all components.

4. Application

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

In the analysis of the commercial products, the found amounts and recoveries were achieved by comparing with standard solutions containing the same concentrations as expected for commercials, according to their claimed levels. The standard solutions were prepared from the stock solutions after convenient dilutions.

A multivariate calibration spectrophotometric method [37] by partial least square regression (PLS-2) was developed to confirm the results obtained in MEKC. For each ternary mixture a different calibration matrix with 40 standard ternary mixtures samples, selected by an arbitrary design, was used for the quantitative spectral analysis. To select the number of factors in the PLS-2 algorithm in order to model the system without overfitting the concentration data, a cross validation method leaving out one sample at a time was used [38,39]. For this model a number of optimum factor of 5 (PREA–FEN–SUL), 4 (PRE–FEN–BAC) and 13 (PRE–FEN–NAF) were selected. The results, presented in Table 3, show agreement between the claimed and found values.

In Figs. 4 and 5 we can see the electropherogram

of a sample of Rinobanedif and Blifomol under optimized conditions at 205 nm.

5. Conclusion

The results show that MEKC is a valuable technique for the simultaneous determination of prednisolone and prednisolone acetate and the most important associated compounds in ocular and cutaneous pharmaceutical applications. Assay and reproducibility results are comparable to those obtained with a spectrophotometric PLS-2 method complying with the requirements of drug quality control, in terms of reproducibility and accuracy, and it is also useful for routine analysis. In addition, it offers advantages such as simplicity of operation, flexibility and low cost.

The new-presented MEKC method with lower LOD and short analysis time (8 min), to determine PRE, PREA, BAC, SUL, PHE and NAP, was easy to apply to commercial products, because there are no previous sample treatments, only a simple extraction of the commercials with methanol and a convenient dilution of the extract is necessary.

Acknowledgements

We wish to thank the DGES of the Ministerio de Educación y Ciencia for financial support (Project PB-97-0431-C03-01).

References

[1] A. Goodman Gilman, R.W. Ruddon, P.B. Molinoff, J.G. Hardman, in: A. Goodman Gilman (Ed.), Bases Farmacológicas de la Terapéutica, Vol. 1, McGraw Hill Interamericana Publishers, 1996.
[2] M. Litter, in: M. Litter (Ed.), Farmacología Experimental y Clínica, El Ateneo Publishers, 1998.
[3] L. Valvo, A. Paris, A.L. Savella, B. Gallinela, E.C. Signoretty, J. Pharm. Biomed. Anal. 12 (1994) 805.
[4] S. Ahmed, M. Riaz, Chromatographia. 31 (1991) 67.
[5] V. Garg, W.J. Jusko, J. Chromatogr. 567 (1991) 39.
[6] H. Deng, G.J. van-Berkel, H. Tacaño, D. Gazda, M.D. Porter, Anal. Chem. 72 (2000) 2641.
[7] V. Cirimele, P. Kintz, V. Dumestre, J.P. Goulle, B. Ludes, Forensic. Sci. Int. 107 (2000) 381.
[8] S. Noe, J. Boehler, E. Keller, A.W. Frahm, J. Pharm. Biomed. Anal. 18 (1998) 911.
[9] S. Noe, J. Boehler, E. Keller, A.W. Frahm, J. Pharm. Biomed. Anal. 18 (1998) 471.
[10] A. Szymanski, W. Szczepaniak, Chem. Anal. (Warsaw) 43 (1998) 349.
[11] A.E. Mooser, H. Koch, J. AOAC Int. 76 (1993) 976.
[12] V. Pauli, M.J. Sokolic, J. Liq. Chromatogr. 27 (1990) 303.
[13] J.B. Gallagher, P.W. Love, L.L. Knotts, J. AOAC Int. 65 (1982) 1178.
[14] Q. Yang, L.M. Benson, K.L. Johnson, S. Naylor, J. Biochem. Biophys. Methods 38 (2) (1999) 103.
[15] J.W. Kang, G. de Reymaeker, A. van Schepdael, A. Roets, J. Hoogmartens, Electrophoresis 22 (7) (2001) 1356.
[16] J.M. Lemus, J. Pérez, Chromatographia 56 (2002) 455.
[17] I. Shoukrallah, Acta Pharm. Jugosl. 41 (1991) 107.
[18] S.M. Sabry, M.H. Abdel-Hay, M.H. Barary, T.S. Belal, J. Pharm. Biomed. Anal. 22 (2000) 257.
[19] G. Santoni, P. Mura, S. Pinzuati, P. Gratteri, E. La-Pota, Int. J. Pharm. 50 (1989) 75.
[20] M. Abdel-Salam, A.S. Issa, M.S. Mahrous, Anal. Lett. 19 (1986) 2207.
[21] D. De Orsi, L. Gagliardi, G. Cavazutti, M.G. Mediati, D. Tonelli, J. Liq. Chromatogr. 18 (1995) 3233.
[22] R. Bocic, C. Vallejos, A. Alvarez Lueje, F.J. Lopez, J. AOAC Int. 75 (1992) 902.
[23] J.E. Kountourellis, A. Raptouli, Anal. Lett. 21 (1988) 1361.
[24] A. Yesilada, B. Tozkoparan, N. Gokhan, L. Oner, M. Ertan, J. Liq. Chromatogr. Relat. Technol. 21 (1998) 2575.
[25] K. Raith, E. Althoff, J. Bansen, H. Neidhardt, R.H. Neubert, Electrophoresis 19 (1998) 2907.
[26] N. Erk, M. Kartal, Farmaco 53 (1998) 617.
[27] M.A. Korany, A.M. Wahbi, S. Mandour, M.A. Elsayed, Anal. Lett. 18 (1985) 21.
[28] M.E.S. Metwally, Chromatographia 50 (1999) 113.
[29] R.M. Villanueva, J.M. Sanchis, J.R. Torres, G. Ramis, Analyst 120 (1995) 1767.
[30] W. Wei, G.A. Luo, C. Yan, Z.P. Sun, J. High Resolut. Chromatogr. 21 (1998) 440.
[31] Z. Wang, Y.L. Sun, Z.P. Sun, J. Chromatogr. A 735 (1996) 295.
[32] J.M. Lemus, J. Pérez, Fresenius J. Anal. Chem. 370 (2001) 973.
[33] J.M. Lemus, J. Pérez, J. Anal. Lett. 35 (2002) 2105.
[34] K.D. Altria, Y.L. Chanter, J. Chromatogr. 652 (1993) 459.
[35] K.D. Altria, H. Fabre, Chromatographia 40 (1995) 313.
[36] J.C. Millar, J.N. Miller, Statistics For Analytical Chemistry, Ellis Horwood Limited, London, 1993.
[37] J.M. Lemus, J. Pérez, Determination of Prednisolone and associated compounds in pharmaceuticals preparations by univariate and multivariate regression methods (in preparation).
[38] M. Stones, Statistic Soc. 36 (1974) 111.
[39] D.M. Haaland, E.V. Thomas, Anal. Chem. 60 (1988) 1193.