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Determination of prednisolone, naphazoline, and phenylephrine in local pharmaceutical preparations by micellar electrokinetic chromatography

A new, rapid, and simple method is described and used to resolve and quantify mixtures of prednisolone, naphazoline, and phenylephrine. The determination was accomplished by micellar electrokinetic 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 6.6 min and the limits of quantification were about 0.4 mg/L for every component. Repeatability and reproducibility studies showed no significant differences at 95% confidence level. Application of multivariate calibration regression spectrophotometric methods (PLS-1, PLS-2, and PCR) clearly demonstrated, especially in the case of PLS-1, the high resolving powder of these techniques if all possible interferences are suppressed. MEKC has been used for quantifying these compounds in different pharmaceutical products and the method gave good results compared with spectrophotometry. The pharmaceutical preparations do not require any separation steps when analysed by the two procedures described.

Key Words: Corticosteroids; MEKC; Partial least squares; Partial component regression; Prednisolone; Naphazoline; Phenylephrine

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1 Introduction

Corticosteroids have been widely used as anti-inflammatories in medicine. Nowadays, pharmaceutical products contain corticosteroids in conjunction with antibacterials [1]. These compounds are very effective against a wide range of ocular, allergic, and cutaneous inflammatory diseases so there are numerous formulations and concentrations of corticosteroids available in various strengths for local administration. In some pharmaceutical formulations, the therapeutic action of these combinations can be supplemented by the action of decongestant agents such as Naphazoline (NAP) and Phenylephrine (PHE).

Prednisolone (PRE) is determined in combination with other natural and synthetic corticosteroids [2, 3] in pharmaceuticals with a limit of detection (LOD) around 1 mg/L and together with their metabolites [4] in biological fluids by reversed phase HPLC, by liquid chromatography-ion spray mass spectrometry [5, 6], and by MEKC in serum after prior SPE [7, 8] using phosphate-borate buffer (pH 8) with SDS and 16% acetonitrile with detection at 254 nm in 10 min with limits of quantification (LOQ) of 0.5 mg/L.

The methods described for the assay of PHE are UV spectrophotometry for amphetamines [9], HPLC for catecholamines [10], and capillary zone electrophoresis for other beta-amino alcohols [11] and amphetamine [12] using a phosphate buffer (pH 3.2) and beta-cyclodextrin to obtain an enantiomeric separation.

NAP has been determined simultaneously with PHE [13] and other imidazolines with and without derivatization by spectrofluorimetric and derivative spectrophotometric methods [14, 15] with quantitative determination. Reversed phase HPLC has been used to determine NAP in pharmaceutical [16] formulations with other corticosteroids [17] with a linearity range of 10–60 mg/L, and by capillary electrophoresis together with their degradation products [18] and other antibiotics and corticoids [19] with a LOD of 0.25 mg/L.

In this work, the separation and quantification of Prednisolone and related compounds were studied. No references

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Abbreviations: PRE, Prednisolone; PHE, Phenylephrine; NAP, Naphazoline; PLS, Partial Least Square Regression; PCR, Principal Components Regression; PRESS, Prediction Error Sum of Squares; RSMD, Root Mean Squares Difference; R^2, Squares of Correlation Coefficients; REP, Relative Error of Prediction.

Short Communication

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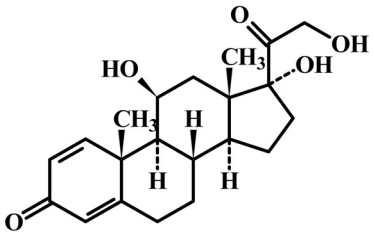
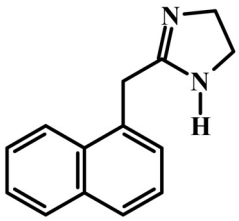
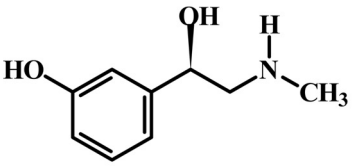


PREDNISOLONE



NAPHAZOLINE



PHENYLEPHRINE

Figure 1. Chemical structures of the mixture compounds.

were found for the association PRE-NAP-PHE in capillary electrophoresis (CE) and other techniques. This method provides a very short analysis time (7 min) for PRE and the most important decongestant agents in topical pharmaceutical applications. The LOD and level of linearity range has been reduced for NAP and PHE. Thus, our group has long been examining 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 commercial ocular and cutaneous pharmaceutical preparations [20–22]. As a result of our studies, this paper presents new, accurate, and easy MEKC, Partial Least Squared (PLS), and Principal Component Regression (PCR) methods for the determination of such mixtures in routine drug analysis. The structures of these compounds are given in **Figure 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 running 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 of 800 × 100 mm.

A Crison (Barcelona, Spain) MicropH 2002 pH meter was used for the pH measurements. Spectrophotometric measurements were performed with a Beckman (Fullerton, CA) DU-70 spectrophotometer, equipped with 1.0 cm quartz cells and connected to a computer running Beckman Data Leader software [23]. The Grams 386 Level 1, version 3.01, software package, with the PLS plus version 2.1G application software (Galactic Industries) [24] was 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 (PRE) and phenylephrine HCl (PHE) were obtained from Sigma (Deisenhofen, Germany) and naphazoline nitrate (NAP) from Fluka (Buchs, Switzerland).

A stock solution (200 mg L^{-1}) of PRE was prepared in methanol-water (50 : 50) and the NAP 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 NaOH was added to adjust the pH to the desired value. 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 three compounds were considered stable under the operating conditions.

2.3 Real sample preparation

Flogiftalmina: This is an ocular drop preparation containing PRE and PHE from the company Davi Farmacéutica (Lisboa, Portugal).

Rinovel: This is a nasal aerosol containing PRE and NAF from the company ERN (Barcelona, Spain).

Lidrone: This is a nasal aerosol containing PRE, PHE, and NAP from the company Serra Pamies (Barcelona, Spain).

Once the pharmaceutical drops and aerosols had been homogenized, different known aliquots were placed in 25 mL calibrated flasks, adding methanol (30%) and deionised water to the mark.

2.4 Procedure

2.4.1 Electrophoretic procedure

The set of separation vials was changed after each batch run (maximum of 4 separations). The capillary was condi-

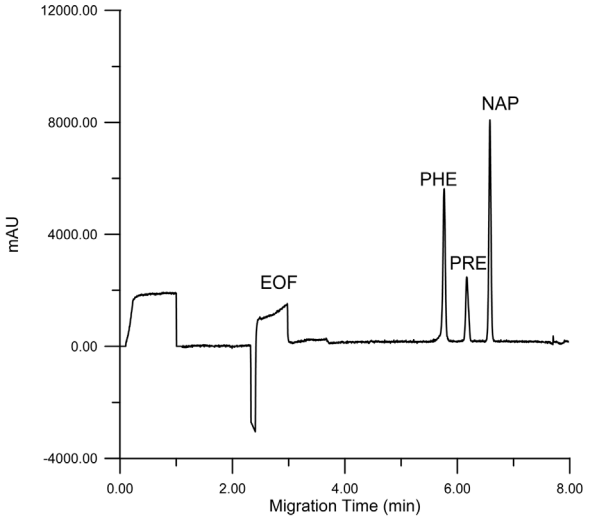


Figure 2. Electropherogram of a sample containing 20, 20, and 12 mg L^{-1} for PRE, PHE, and NAP, respectively, obtained under optimised conditions at 205 nm (5 mM phosphate-5 mM borate buffer (pH 8.2), 40 mM SDS as electrolyte solution; temperature and voltage were 25°C and 30 kV, respectively).

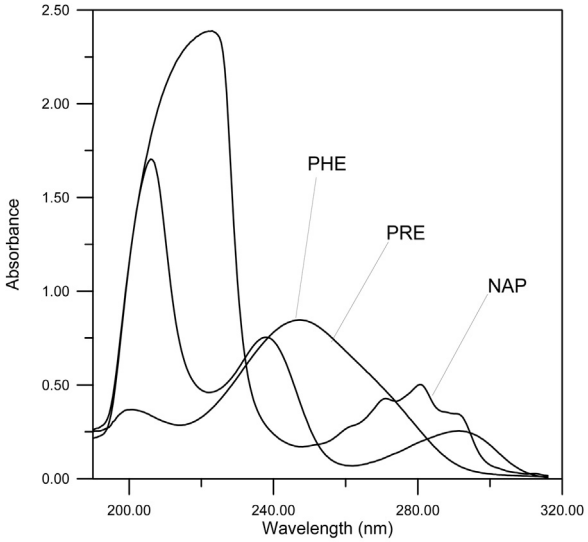


Figure 3. Absorption spectra for solutions of 20 mg L^{-1} of prednisolone, 20 mg L^{-1} of phenylephrine, 20 mg L^{-1} of naphazoline in borate buffer medium (pH 10.5) and recording against a reagent blank at a scan rate of 600 nm min^{-1}.

tioned, prior to its first use, by flushing first with 0.1 M NaOH for 20 min, and then with water for 10 min. In the optimum method (5 mM phosphate-5 mM borate buffer adjusted to pH 8.2 as electrolyte with 40 mM SDS), 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; this was followed by a 6 s hydrodynamic sample injection. Separation was performed at 30 kV for 8 min at 25°C; under the selected conditions the current was 50.0 μA. The electropherogram obtained in the separation of a synthetic sample under selected conditions is presented in **Figure 2**. Remarkably, all peaks are well resolved in a run time of 6.7 min. Corrected peak areas were used for the quantification.

2.4.2 Multivariate calibration

These approaches are useful in the resolution of band overlapping in quantitative analysis (**Figure 3**). 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 and his co-workers [27].

With the aim of verifying the analysis of these compounds, three different chemometric approaches were evaluated. Haaland and Thomas [28] compared the different multivariate calibration methods for quantitative spectral analysis. They concluded that it is very difficult to generalize whether any given method is superior to the others, because their relative performance is often dependent on the particular data set to be analysed. The best results in our particular case were obtained with the PLS-1 method.

2.4.2.1 Experimental design of calibration matrix and selection of the spectral zone for analysis

A training set of 40 standard ternary mixture samples (using a borate buffer pH 10.5 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, NAF, and PHE). The spectral region between 215–316 nm was selected as suitable for the analysis, which implied the use of 201 experimental points for each spectrum. The spectral information was selected according to the spectra of the pharmaceutical products. The range of the spectrum between 190 and 215 nm was rejected due to differences between the spectra of the artificial mixture and those of the pharmaceutical products at the same concentration. These differences could be due to other components of the pharmaceuticals such as citric acid, EDTA, phenyl mercuric nitrate, polyvinyl alcohol, benzylic alcohol, and so on.

Figure 4 depicts the experimental design and we can see the composition of the standard mixtures used in the calibration matrix.

2.4.2.2 Selection of optimum number of factors

To select the number of factors in the PLS-1 algorithm in order to model the system without overfitting the concentration data, a cross-validation method was used which left out one sample at a time [29].

The prediction error sum of squares (PRESS) is an efficiency measure for a calibration fit model. One reason-

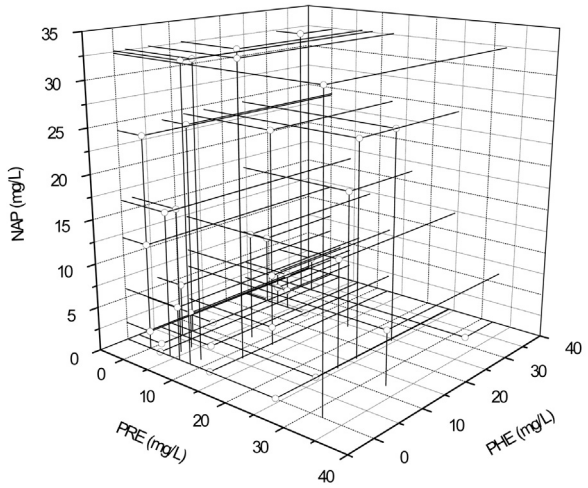


Figure 4. Experimental design of the calibration matrix presented graphically.

able choice for the optimum number of factors would be the number that yields the minimum PRESS. 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.

Table 2 shows the optimum number of factors for PRE, NAP, and PHE by means of the PLS-1, PLS-2, and PCR models. The proposed 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 Limits of detection and quantification

LOD and LOQ, respectively, 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. The LOD was obtained as the sample concentration which causes a peak that is three times as high as the baseline noise level [31] and the LOQ was calculated as being ten times as high as the baseline noise level. LODs and LOQs are shown in **Table 1** for each compound.

3.1.2 Linearity range and calibration curves

The linearity of the assay was checked by injecting the calibration solution of each drug in the range from 0.1 to 60 mg/L 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, 220, and 200 nm for PRE, NAP, and PHE respectively, versus their concentrations.

Table 1. LODs and LOQs and statistical parameters of calibration graph for each compound.

	PHE	PRE	NAF
LOD (mg L^{-1})	0.09	0.22	0.03
LOQ (mg L^{-1})	0.32	0.73	0.13
Intercepts (CAU[a])	-503 ± 330	-118 ± 59.1	-337.9 ± 176.9
Slope (CAU $\times$ L mg^{-1})	532.2 ± 9.7	124.1 ± 1.7	150.7 ± 4.2
r^2	0.9979	0.9988	0.9954
Linear range (mg L^{-1})	0.4–56.8	0.8–56.5	0.2–39.9

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

A satisfactory linear relationship ($r^2 \geq 0.998$) was obtained between the concentration and the corrected area for each component. In Table 1, 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 ($\alpha = 0.05$).

3.1.3 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 PRE, NAP, and PHE, respectively. Reproducibility was evaluated over 2 days by performing 12 replicate analyses each day.

The results showed that the repeatability for every component on each day is satisfactory (RSD $\leq$ 2.5% for each compound). In terms of reproducibility, the comparison of averages with the Snedecor test did not provide any significant difference between the two 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.

Table 2 shows the results obtained for these parameters following implementation of the three proposed chemometric approaches. We can see that R^2 values are in all cases very close to 1, which is an indication of similarity between predicted and known values. On the other hand, in general terms, the errors obtained for these statistical cross-validation parameters are the same for both multi-

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

Compound	Factor	PRESS	RMSD	R^2	REP (%)
PLS-1					
PRE	8	4.0884	0.2861	0.9996	1.831
NAF	11	131.18	0.8088	0.9939	5.208
PHE	7	8.0057	0.3266	0.9995	2.103
PLS-2					
PREA			0.3591	0.9991	2.2976
PHE	13	239.01	0.7854	0.9944	5.0554
SUL			0.2351	0.9995	1.5040
PCR					
PREA			0.2496	0.9998	1.5115
PHE	13	184.02	1.0279	0.9954	6.5258
SUL			0.3047	0.9995	1.9259

RSMD: Root mean squares difference, which is an indication of the average error in the analysis for each component.
R^2: Square of correlation coefficients, which is an indication of the quality of the straight line that fits the data.
REP: The predictive ability of each method and for each component can also be described in terms of the relative error of prediction with regard to the average value (μ).

variate calibration methods. The best statistical results were obtained by PLS-1.

3.2.1 Repeatability and reproducibility

Reproducibility of the PLS-1, PLS-2, and PCR methods was checked by recording independent series of ten samples for each compound (16 mg L^{-1} of PRE, 16 mg L^{-1} of NAP, and 16 mg L^{-1} of PHE) on two consecutive days. Repeatability studies were satisfactory, giving RSD values of 0.11, 0.66, and 0.36 for PRE, NAP, and PHE, respectively; when reproducibility studies were undertaken over the two sets of ten standards for each compound on consecutive days no significant differences were found between the two sets of ten replicates at a confidence level of 95%.

3.3 Applications

The present method was tested to determine the mentioned compounds in pharmaceutical preparations (Rinovel, Lidrone, and Flogiftalmina).

In the analysis of the commercial preparations by MEKC, the experiment was performed by comparison with standard solutions containing the concentrations expected for the pharmaceutical preparations from manufacturers' claims. The standard solution was prepared from the stock solutions after appropriate dilution. **Figure 5** shows the electropherogram of a sample of Lidrone.

In the analysis of commercial products by multivariate calibration, the spectra of the pharmaceutical 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 by the manufacturer) at a scan speed of 600 nm/min over the range 316 to 215 nm. The contents of PRE, NAF, and PHE were calculated by analysing the recorded spectra according to 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 summarized in **Table 3**, where the contents supplied by the manufacturer are also shown. The best results obtained by multivariate methods were for PLS-1.

The Table 3 displays an acceptable agreement between the results obtained by MEKC and multivariate calibration and these values are also close to those provided by the manufacturer. The results obtained by multivariate calibration show a relative error below 8%. The excipients interfere with the correct determination of these compounds in the pharmaceutical preparations especially in Flogiftalmina and Lidrone.

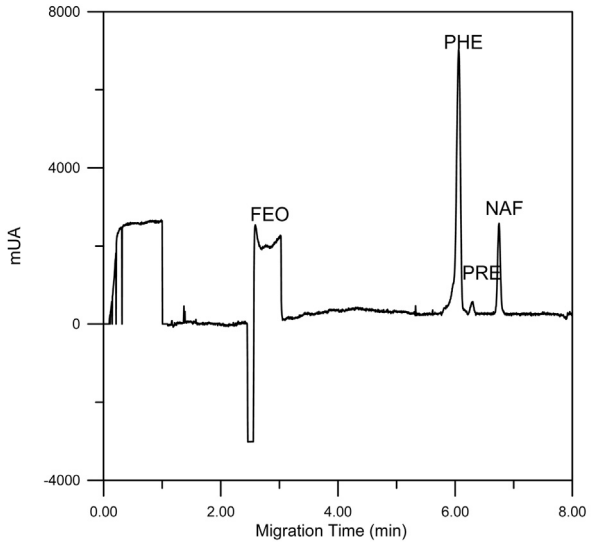


Figure 5. Electropherogram of Lidrone obtained under optimised conditions at 205 nm (5 mM phosphate-5 mM borate buffer (pH 8.2), 40 mM SDS as electrolyte solution; temperature and voltage were 25°C and 30 kV, respectively).

Table 3. Application results of MEKC and PLS-1 on different pharmaceutical preparations.

		MEKC		PLS-1	
Product	Claimed [mg/L]	Found [mg/L]	Recovery [%]	Found [mg/L]	Recovery [%]
Rinovel	PRE 7.5	7.2 ± 0.1	95.7	7.3 ± 0.1	97.3
	NAP 30.0	30.0 ± 0.2	100.1	29.0 ± 0.1	96.7
Flogiftalmina	PRE 15.6	15.3 ± 0.3	97.9	16.2 ± 0.2	103.8
	PHE 15.6	15.3 ± 0.1	97.9	17.0 ± 0.1	108.9
Lidrone	PRE 2.8	2.7 ± 0.1	97.4	3.0 ± 0.1	107.1
	NAP 3.5	3.4 ± 0.1	97.2	3.7 ± 0.1	105.7
	PHE 35.0	34.4 ± 0.3	98.4	35.5 ± 0.1	101.4

4 Conclusions

The newly-presented MEKC method for determining PRE, NAP, and PHE was readily applied to pharmaceuticals because no previous sample treatment is required, apart from dissolution of the products in water and methanol. This method proves to be as sensitive, accurate, and exact as the multivariate calibration approach for this mixture. In the multivariate calibration method, measurement is performed at the specific wavelength previously selected. However, the presence of different excipients in the formulations might cause interference to the measurement signal. Multivariate calibration was suitable only for synthetic samples.

Thanks to the high separation power of MEKC, the proposed method provides an useful tool for removing the contribution of these interferences, as well as for their detection. Thus it can be concluded that MEKC is convenient for the sufficiently exact determination of the studied compounds in the quality control of such pharmaceutical formulations.

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