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Multiple response optimization applied to the development of a capillary electrophoretic method for pharmaceutical analysis

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Abstract

Multiple response simultaneous optimization by using the desirability function was used for the development of a capillary electrophoresis method for the simultaneous determination of four active ingredients in pharmaceutical preparations: vitamins B_6 and B_{12}, dexamethasone and lidocaine hydrochloride. Five responses were simultaneously optimized: the three resolutions, the analysis time and the capillary current. This latter response was taken into account in order to improve the quality of the separations. The separation was carried out by using capillary zone electrophoresis (CZE) with a silica capillary and UV detection (240 nm). The optimum conditions were: 57.0 mmol l^{-1} sodium phosphate buffer solution, pH 7.0 and voltage = 17.2 kV. Good results concerning precision (CV lower than 2%), accuracy (recoveries ranged between 98.5 and 102.6%) and selectivity were obtained in the concentration range studied for the four compounds. These results are comparable to those provided by the reference high performance liquid chromatography (HPLC) technique.

Keywords: Multiple response optimization; Desirability function; Capillary electrophoresis; Lidocaine; Dexamethasone; Vitamins B_6 and B_{12}

1. Introduction

The analysis of complex preparations in the pharmaceutical field calls for very powerful separation techniques. The simultaneous determination of a large number of components can be performed by high performance liquid chromatography (HPLC). On the other end, due to its high efficiency, capillary electrophoresis (CE) appears as an appropriate technique for the analysis of complex formulations, as demonstrated in several published papers in this area, in which CE has been shown as a valuable alternative technique for their separation [1–4].

In developing a CE method, optimization is usually applied to reduce the analysis time, without losing the resolution between the peaks originated by the analyte migration. The need of taking into account different aspects of the analysis at the same time calls for the use of multicriteria optimization. In order to carry out the latter type of optimization, experimental design is a valuable tool, specifically surface response analysis [5]. In addition, when different objective functions have to be optimized, the so-called Derringer's desirability function is a valuable tool [6]. This function requires to define which results are acceptable for each individual response and which results are not be acceptable at all. Then a continuous function between these two conditions should be fitted. Remarkably, though this methodology presents considerable advantages in chemical analyses, few applications can be found in the literature [7–11].

The mixture of vitamin B_6 (pyridoxine hydrochloride), vitamin B_{12} (hydroxocobalamin), dexamethasone and lidocaine hydrochloride is usually present in several pharmaceutical preparations (injection and tablets). This association is used in human medicine as analgesic, anti-inflammatory, myorelaxant and antineuritic. It produces a rapid and effective response in the treatment of many diseases such as articular rheumatism, post-traumatism of the local motion system, osteoarthritis, ankylosing spondylitis, musculoeskeletal disorders, postpartum pain and sport injuries. The most important side effects that have been reported are of gastrointestinal origin (ulcer, bleeding ulcers, etc.) [12–14]. Fig. 1 shows the structure of the four studied analytes.

Different monographs were introduced in the USP XXVI for the separate determination of dexamethasone, lidocaine, vitamin B_6 and B_{12} in injections, by using high performance

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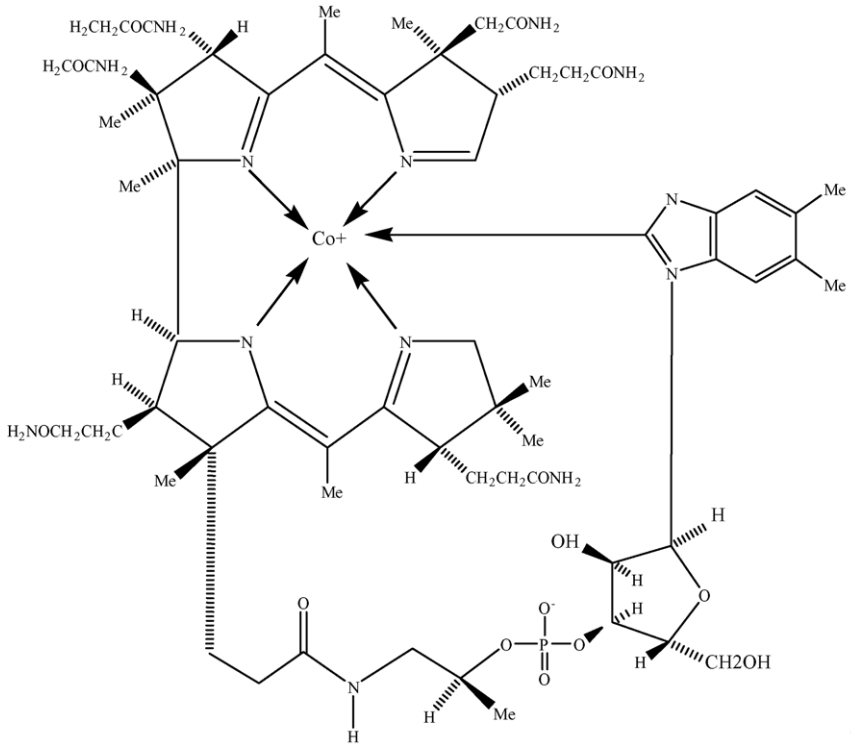
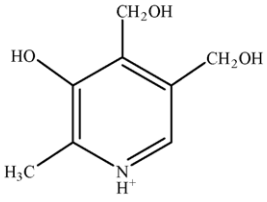
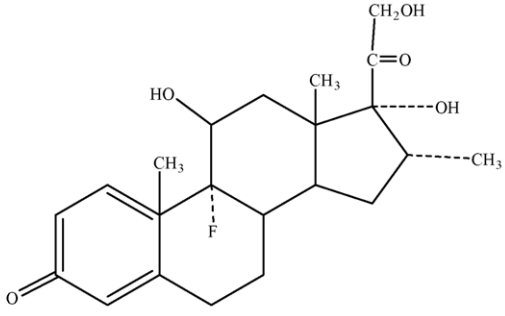


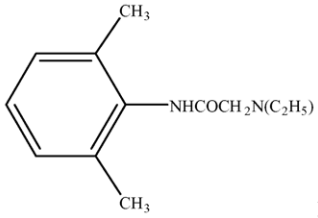
Vitamin B12



Vitamin B_6



Dexametasone



Lidocaine

Fig. 1. Structures of the four studied analytes.

liquid chromatography (HPLC) and UV detection [15]. However, owing to the presence of interferences, the determination of these analytes in samples containing mixtures of several of them is not possible if the cited monographs are followed. Therefore, the routine quality assurance of products containing a mixture of these active components represents a difficult analytical task to be accomplished. On the other hand, few methods have been published for the simultaneous determination of some of these compounds in pharmaceutical preparations. They are based on techniques such as UV spectrophotometry [16,17], spectrofluorimetry [18], HPLC [19–21] and micellar electrokinetic chromatography (MEKC) [16]. Nevertheless, to the best of our knowledge, there seems to be no reports concerning methods for the simultaneous determination of vitamins B_6 and B_{12}, dexamethasone and lidocaine in such different proportions as those presented in the samples herein analyzed.

In this work, a CE method was developed, optimized and validated for the determination of vitamins B_6 and B_{12}, dexamethasone and lidocaine, all of them present in commercial preparations (injections and tablets). The multiple response criteria was successfully used to optimize the separation of the four analytes, allowing the reduction of the analysis time with excellent peak resolutions.

2. Experimental

2.1. Apparatus

All experiments were performed using a Spectraphoresis 100-Thermo Separation Products equipped with a UV detector. A PC Athlon 2.2 microcomputer was used for instrument control and data handling. The pH of the buffers was adjusted by means of an Orion 9165 BN model 710[a] with Ag/ClAg,KCl electrode. UV spectra were obtained with a Perkin-Elmer spectrometer Lambda 20, using quartz cells of 1.00 cm of path length.

2.2. Software

Data acquisition and integration was made by using a homemade software. The electropherograms were then saved in ASCII format for subsequent graphic representation with the software Origin 6.1. Experimental design, data analysis and desirability function calculations were performed by using the software Stat-Ease Design-Expert trial Version 6.0.10.

2.3. Reagents

Standards of pyridoxine hydrochloride (vitamin B_6), hydroxocobalamin (vitamin B_{12}), dexamethasone, and lidocaine hydrochloride were provided by Sidus S.A. laboratories (Argentine). All the reagents were of analytical-reagent grade. They were preserved at $-4\,^{\circ}C$ in darkness during the experiments. Milli-Q quality water was used in all the CE experiments. Sodium tetraborate, sodium phosphate and sodium hydroxide were obtained from Merk. All the buffers were filtered through a 0.20 μm cellulose acetate membrane (Sartorius—Germany) and degasified before use. Flexicamin B_{12} was obtained from Sidus S.A. (Buenos Aires, Argentina) and the pharmaceuticals Dexabion (Merk Química, Argentina), Flogiatrin (Sanofi Winthrop, Argentina) and Sindrolen (Temis Lostalo, Argentina) were obtained in the local market.

2.4. Standard solutions and commercial samples

A stock solution was prepared for the optimization procedure containing the four analytes at the same concentration that in the investigated pharmaceutical formulation. It consisted in a water solution mixture of vitamin B_6 ($82.88\,g\,l^{-1}$), vitamin B_{12} ($3.26\,g\,l^{-1}$), dexamethasone ($0.69\,g\,l^{-1}$) and lidocaine HCl ($8.42\,g\,l^{-1}$). This later stock solution was also used for the precision study. On the other hand, a synthetic sample, similar to the commercial product (see below), was prepared to be used in the accuracy study.

The studied pharmaceutical formulation contains two vials that should be joined before application: (a) a powder consisting of piroxicam, 20 mg and trometamine (excipient), 240 mg, and (b) 3.0 ml of a solution of vitamin B_6, 250 mg, dexamethasone, 2 mg, vitamin B_{12}, 10 mg, lidocaine HCl, 25 mg and sodium acetate 32.08 mg in distilled water. Taking into account that the objective of the present work was the determination of the four analytes contained in the liquid preparation, this solution was diluted 400 μl in 5.00 ml with Milli-Q water before determinations. All the solutions were filtered through a 0.20 μm cellulose acetate membrane before use.

2.5. Electrophoretic procedure

Electrophoretic separations were carried out with uncoated fused silica capillaries having 75 μm internal diameter and 70 cm length (47 cm to the detector). Before use, the capillary was washed successively with basic solutions (5 min with $1\,mol\,l^{-1}$ NaOH followed by $0.1\,mol\,l^{-1}$ NaOH during 5 min), water and running buffer. After each injection, the capillary was washed with running buffer for 1 min.

The applied voltage varied between 15.0 and 20.0 kV and UV detection was performed at 240 nm. This wavelength was selected in order to increase the sensitivity for the less concentrated compound (i.e., dexamethasone). Sample injections were performed using the hydrodynamic mode for 1 s. The capillary was maintained at constant temperature of 20 °C. The analyte migration order was found by injecting diluted standard solutions of each compound and subsequently comparing the peak shapes and migration time.

3. Results and discussion

3.1. Screening phase

Developing a separation method involves demonstrating specificity, i.e., the ability of the method to accurately measure the analyte response in the presence of all potential sample components. For finding out the best conditions for the correct separation of the four studied analytes, a screening phase was carried out in the present work. Two buffers were evaluated

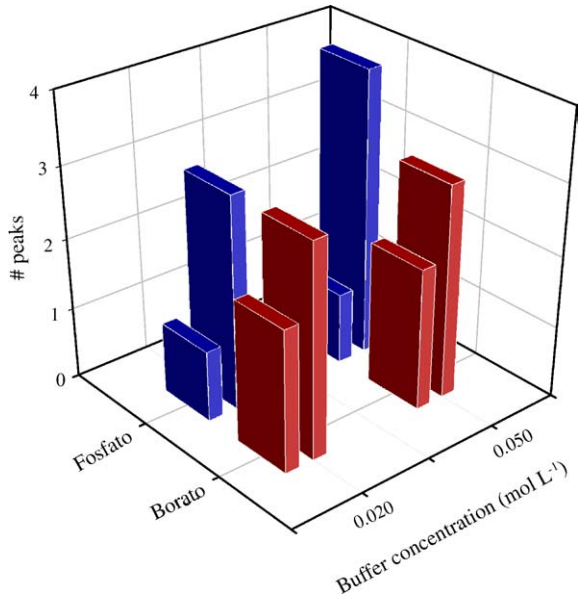


Fig. 2. Schematic representation of the screening experiment. The *x*-coordinate corresponds to the buffer composition (i.e., phosphate and borate). The *y*-coordinate corresponds to the concentration of the buffer solution (0.020 and 0.050 mol l^{-1}). The number of peaks obtained is represented for the high of the cubes.

(borate and phosphate) at two concentration levels (0.020 and 0.050 mol l^{-1}). On the other hand, two pH values were considered for each buffer at each concentration level: 5.0 and 7.0 for phosphate, and 7.0 and 9.0 for borate. These pHs were selected according to the pK_as of the buffering compounds. The evaluation consisted in analyzing the stock standard solution (see Section 2), diluted 1/10 in all the cited conditions. In each case, the number, shape and resolution of the electrophoretic peaks were evaluated. Fig. 2 shows the number of peaks obtained in the eight experiments performed. As can be seen, four peaks were obtained only for phosphate at pH 7 and 0.050 mol l^{-1}. In addition, both the shape (without appreciable deformations) and resolution were satisfactory. These experiments were performed at a fixed voltage of 18 kV and 20 °C of temperature. Consequently, a buffer phosphate 0.050 mol l^{-1} (pH 7.00) was selected, as it provides a good separation of all the four analytes: lidocaine, vitamin B_{12}, vitamin B_6 and dexamethasone, in the latter migration order. The presence of SDS was not studied, owing to the good results obtained in its absence. In a previously published work [16], the authors postulate the use of SDS for accomplishing the separation of three of the compounds herein studied (vitamins B_{12} and B_6 and dexamethasone) plus piroxicam. Fortunately, when piroxicam is absent, SDS seems to be not necessary, making the experiment simpler.

Considering that the separation order corresponds to a neutral pH, one can analyze if the order matches the mass/charge ratio. Table 1 shows the pK_as, molecular weight, charge at pH 7 and migration order of the active ingredients. As can be seen, two compounds are positively charged (lidocaine and vitamin B_{12}), but the molecular weight of vitamin B_{12} is almost four times larger than that for lidocaine, and consequently this compound migrates more slowly, appearing in the second place. On the other hand, vitamin B_6 is neutral, and therefore, it migrates in the third order with the electroendoosmotic flow (EOF). Finally, dexamethasone, holding a negative charge, is the last compound reaching the detector.

Table 1
Migration order of the analytes

Compound	Molecular weight (g mol^{-1})	pK_a[a]	Charge at pH 7.0	Migration order
Lidocaine	234.34	7.9	Positive	First
Vitamin B_{12}	1346.37	–	Positive	Second
Vitamin B_6	169.18	5.0 and 9.0	Neutral	Third
Dexamethasone	600.44	–	Negative	Fourth

[a] (–) No data were found in the literature.

3.2. Response surface design

Once the conditions that ensure the analyte separation were established, an optimization procedure was applied in order to find out the exact values of the most important factors for a correct separation and a rapid analysis. Experimental design allows a large number of factors to be tested simultaneously and precludes the use of a huge number of independent runs when the traditional step-by-step approach is used. Systematic optimization procedures are carried out by selecting an objective function, finding the most important factors and investigating the relationship between responses and factors by the so-called response surface methods (RSM).

In order to carry out a quadratic regression on the model coefficients, each design variable has to be studied at least at three distinct levels, and consequently the central composite design is often used to provide estimation of a second-order equation. Among the standard designs used in response surface methodology, the latter one represents a good choice because of its high efficiency with respect to the number of required runs and also because it is built considering five levels of the factors being studied. Important variables to be tested during the optimization process are voltage, pH and buffer solution concentration. Taking into account the screening experiments, pH 7 is a critical value that should not be varied to produce good electropherograms. Therefore, voltage and buffer solution concentration were the variables considered in the present study. Table 2 shows the levels of each variable studied for finding out the optimum values and responses. As can be seen in this table, the ranges used were: voltage (14.9–20.0 kV) and buffer concentration (0.050–0.101 mol l^{-1}). These ranges were selected by taking into consideration previous studies, as the one mentioned above for pH, as well as certain instrumental limitations. All experiments were performed in randomised order to minimize the effects of uncontrolled factors that may introduce a bias on the measurements. This design allowed us to obtain the surface response fitting the data to the following polynomial model:

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \varepsilon_i \quad (1)$$

Table 2
Central composite design used for the multiple response optimization procedure

Run	Buffer concentration (mol $l^{-1} \times 10^3$)	Voltage (kV)	R1	R2	R3	Analysis time (min)	Current (μA)
1	50.34	17.5	2.86	1.23	10.03	19.54	66
2	56.70	15.7	2.40	1.10	10.28	18.19	66
3	56.70	19.3	1.78	0.88	8.35	12.57	90
4	75.52	14.9	3.18	1.36	12.09	20.05	80
5	75.52	17.5	2.32	0.95	8.00	14.97	110
6	75.52	17.5	2.40	0.92	7.22	14.44	110
7	75.52	17.5	2.11	0.88	7.34	14.59	110
8	75.52	17.5	2.38	1.08	8.47	14.4	110
9	75.52	17.5	2.56	1.15	8.54	14.47	110
10	75.52	20.0	1.58	0.79	6.44	10.64	150
11	93.64	15.7	3.32	1.50	11.31	17.79	125
12	93.64	19.3	2.00	0.99	7.17	10.99	205
13	100.69	17.5	2.72	1.34	9.54	13.47	175

where Y_i represents the optimized responses, X_1 and X_2 the analyzed factors (voltage and buffer concentration, respectively), β_i the model coefficients and ε_i is the experimental error.

In Table 2, five responses (objective functions) are shown. They are: (a) R1, resolution for lidocaine and vitamin B_{12}, (b) R2, resolution for vitamin B_{12} and vitamin B_6, (c) R3, resolution for vitamin B_6 and dexamethasone, (d) total analysis time (min) and (e) capillary current intensity (μA). The resolution can be defined according to Eq. (2):

$$R = 2\frac{(t_{m2} - t_{m1})}{(w_1 + w_2)} \tag{2}$$

where t_{m1} and t_{m2} are the migration times and w_1 and w_2 are the peak electrophoretic widths. When the resolution is higher than l.5, the two species are considered to be resolved at the baseline [22]. Moreover, time is an important factor to be optimized, since speed is one of the most important advantages of a CE method over other separative techniques. On the other hand, the capillary current is a very significant factor since high current values might produce peak spreading by increasing the temperature due to Joule heating effects [23]. Table 3 shows the statistical parameters corresponding to the fitting for all the five responses. As can be seen, the lack of fit is only significant for total analysis time.

When a simple response is being analyzed, the model analysis indicates areas in the design region where the process is likely to give desirable results, which is a relatively easy task. However, the *desirability* is a function of more than one response. Overlaying contour plots obtained in separate surface analysis is often sufficient for solving the problem, but in the present case, in which five responses are simultaneously analyzed, the methodology suggested by Derringer and Suich [6] becomes an interesting tool. The method proposes a desirability function which includes the researchers priorities and desires on building the optimization procedure. One- or two-sided functions are used, depending on whether each of the m responses has to be maximized or minimized, or has an allotted target value. The procedure involves creating a function for each individual response d_i and finally obtaining a global function D that should be maximized choosing the best conditions of the designed variables.

For goal maximum, the desirability curve is defined by the Eq. (3):

$$d_i = \left[\frac{Y_i - \mathrm{Low}_i}{\mathrm{High}_i - \mathrm{Low}_i}\right]^{\mathrm{wt}_i} \qquad \mathrm{Low}_i < Y_i < \mathrm{High}_i \tag{3}$$

where Y_i is the predicted response using the fitted model (Eq. (1)), High_i and Low_i are the highest and the lowest values obtained for the response i, respectively, and wt_i is the weight. Weights give emphasis to upper or lower bounds, or to a target value. With a weight of 1, the d_i will vary from 0 to 1 in a linear way while approaching to the desired value. Weights greater than 1 give more emphasis to the goal, whereas weights lower than 1 give less emphasis to the goal (in both cases, d_i varies in a non linear way).

Table 3
ANOVA of lack of fit and regression of the selected models

Response	Model	r^2	Model		Lack of fit	
			p	Conclusion[a]	p	Conclusion[b]
Resolution 1	Linear	0.7518	0.0009	Significant	0.0945	No significant
Resolution 2	Quadratic	0.8612	0.0065	Significant	0.6145	No significant
Resolution 3	Quadratic	0.9397	0.0004	Significant	0.6854	No significant
Analysis time (min)	Quadratic	0.9211	0.001	Significant	0.0012	Significant
Current (μA)	Quadratic	0.997	<0.001	Significant	>0.1	No significant

[a] Considered significant when $p < 0.05$.
[b] Considered significant when $p > 0.05$.

Table 4
Criteria for the optimization of the individual responses

Response	Goal	Lower limit	Upper limit
Resolution 1	Minimize	1.58	3.32
Resolution 2	Target value	1.0	1.5
Resolution 3	Minimize	6.44	12.09
Analysis time (min)	Minimize	10.64	20.05
Current (μA)	Range	66	100

For a goal of minimum, the desirability is defined as Eq. (4):

$$d_i = \left[\frac{\text{High}_i - Y_i}{\text{High}_i - \text{Low}_i}\right]^{\text{wt}_i} \quad (4)$$

On the other hand, for goal as a target, the desirability ramps are created like a maximum on the way up, and a minimum on the way down. Finally, for a goal within a range, the desirability will be defined by the following Eqs.:

$$d_i = 0, \quad \text{for } Y_i = \text{Low}_i \quad (5)$$

$$d_i = 1, \quad \text{for Low}_i < Y_i < \text{High}_i \quad (6)$$

In the present report we chose weights equal to 1 for all the five responses.

The function D varies from 0 (value totally undesirable) to 1 (all responses are in a desirable range simultaneously), and it is defined by the Eq. (7):

$$D = \{d_1 \times d_2 \times d_3 \times \cdots \times d_m\}^{1/m} \quad (7)$$

where $d_1, \ldots, d_m$ correspond to the individual desirability function for each response being optimized.

Table 4 shows the criteria for the optimization of each individual response. As can be seen, three responses were minimized: analysis time, R1 and R3. These resolutions corresponded to peaks with excellent separation, and which can be minimized in order to shorten the analysis time. On the other hand, R2 was adjusted to a fixed value (1.125). This was due to the fact that we could see good separation up to a resolution value of 1.0. It is important to remark that less than 1.0 can originate superimposition of the peaks. Finally, the capillary current was set in a range in which no significant Joule effects were expected.

Following the conditions and restrictions previously discussed, the optimization procedure was carried out. The response surface obtained for the global desirability function is presented in Fig. 3. The coordinates producing the maximum desirability value ($D = 0.443$) are buffer concentration of 0.057 mol l^{-1} and voltage of 17.23 kV. The individual response values corresponding to the latter value of D are: R1 = 2.4 cm, R2 = 1.1 cm, R3 = 9.1 cm, time = 17.5 min and current = 73.0 μA. According to the fitting performed, the obtained desirability is highly acceptable, taking into account the large number of responses being simultaneously optimized. The suggested values during the optimization procedure were experimentally corroborated, and the corresponding electropherogram is shown in Fig. 4.‘

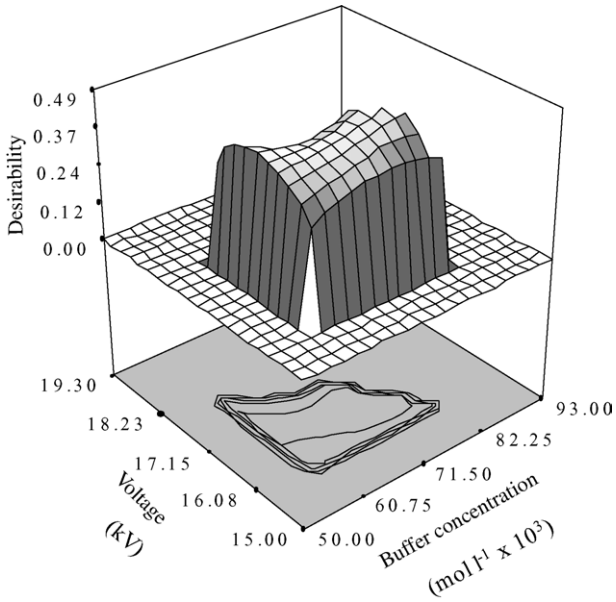


Fig. 3. Response surface corresponding to the desirability function when the voltage and buffer concentration were optimized analyzing five responses simultaneously.

3.3. Performance

3.3.1. Linearity

In order to verify the method linearity within a concentration range of 50–l50% of the target analyte concentration [24], three replicates were prepared at five concentration levels (see Table 5). A least square fitting was performed with the data obtained and the results are shown in Table 6. Coefficients of determination (r^2) greater than 0.999 were obtained in all the cases except for vitamin B_6 ($r^2 = 0.968$). ANOVA of lack of fit test was performed in all cases showing that linearity exists for three components within the range studied (vitamin B_{12}, lidocaine and dexamethasone) [25]. On the other hand, the cal-

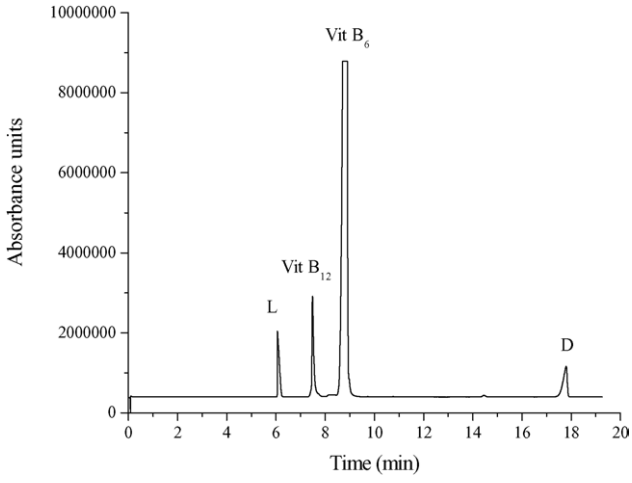


Fig. 4. Electropherogram corresponding to a synthetic mixture of the four analytes. The analytes concentrations are: 0.808 g l^{-1} of lidocaine (L), 0.294 g l^{-1} of vitamin B_{12} (Vit. B_{12}), 8.043 g l^{-1} of vitamin B_6 (Vit. B_6) and 0.0637 g l^{-1} of dexamethasone (D).

Table 5
Standard solutions used in the linearity study

Standard	Lidocaine ($g\,l^{-1}$)	Vitamin B_{12} ($g\,l^{-1}$)	Vitamin B_6 ($g\,l^{-1}$)	Dexamethasone ($g\,l^{-1}$)
1	0.337	0.134	3.315	0.026
2	0.505	0.202	4.881	0.039
3	0.674	0.267	6.638	0.053
4	0.825	0.339	8.298	0.067
5	0.994	0.423	9.958	0.080

Table 6
Linearity of the method

Regression parameter	Lidocaine	Vitamin B_{12}	Vitamin B_6	Dexamethasone
a[a]	5.03×10^7 (2)	2.18×10^8 (2)	5.8×10^7 (4)	6.00×10^8 (5)
b[a]	2.0×10^6 (1)	8×10^5 (5)	3.1×10^8 (3)	5×10^5 (2)
r^2	0.9998	0.9996	0.9680	0.9994
F (lack of fit test)	0.19	0.90	28.41	1.86
F ($p = 0.05$)	3.71	3.71	5.14	4.46
Concentration levels (%)	50–150	50–150	75–125	50–125

[a] Values between parenthesis correspond to standard deviation.

ibration plot for vitamin B_6 does not present linear behavior owing to the fact that this component is present at higher concentration than the other analytes. Therefore, a calibration graph should be made for vitamin B_6 each time the analysis is performed.

3.3.2. Precision

The intra-assay precision (repeatability) was determined by total analysis of six replicates samples (see Experimental), under the same conditions, by the same analyst, and on the same day. The coefficient of variation (CV%) values obtained when computing the concentrations are shown in Table 7. As can be seen, these values are lower than 2% as recommended by regulatory agencies [15], indicating an excellent precision and the capability of the present method.

The precision of migration time was evaluated by determining the migration time of the four analytes on the same sample. The CV% values were estimated from six replicates and are shown in Table 7. Excellent results (CVs lower than 1%) were obtained for all the studied analytes.

Table 7
Results obtained in both the intra-assay precision and accuracy studies

	Lidocaine	Vitamin B_{12}	Vitamin B_6	Dexamethasone
Repeatability (CV%)	0.78	0.24	0.48	1.12
Time of migration (min)[a]	6.24 (3)	7.55 (3)	8.77 (1)	17.7 (1)
Time of migration (CV%)	0.56	0.51	0.54	0.96
Recovery % (level: 80%)	101.5	102.1	98.5	102.6
Recovery % (level: 100%)	99.8	100.9	98.9	101.2
Recovery % (level: 120%)	99.5	98.6	97.7	99.0

[a] Values between parenthesis correspond to standard deviation.

3.3.3. Accuracy

Three concentration levels (80, 100 and 120% of the commercial sample) were evaluated after performing the corresponding dilution. Determinations were made in triplicate and the average recovery values are shown in Table 7. These results show the high accuracy obtained in the three concentration levels studied.

3.3.4. Selectivity

The response (migration time and peak shape) of the four analytes in test mixtures containing the analytes and potential sample components (excipients and degradation products) was compared with the response of solutions containing only the analyte with acceptable results. The presence of degradation products was investigated by analyzing commercial samples which were out of the expiration date. In these cases, no peak shape alterations were observed, although new small peaks were present in the electropherograms. It is important to consider that excipients do not interfere in the determination of the four active ingredients since the samples used to evaluate recovery were prepared with excipients.

3.3.5. Analysis of commercial pharmaceutical preparation

A commercial sample containing the four components (Flexicamin B_{12}) was analysed performing five replicates with similar qualitative and quantitative results to those obtained for synthetic samples. Fig. 5 shows the electropherogram obtained. The results achieved when analyzing this particular sample were: 24.7 (4) mg of lidocaine, 248 (3) mg of vitamin B_6, 10.2 (2) mg of vitamin B_{12} and 1.91 (6) mg of dexamethasone per 3 mL vial, with the values within parenthesis being the standard deviation of the five replicates. The corresponding computed coefficient of variations are: 1.62, 1.21, 1.96 and 3.14%, respectively.

Other commercial samples containing one or more of the four studied analytes were analyzed: Dexabion (injection), Flogiatrin (tablet) and Sindrolen (injection). In these pharmaceutical formulations, four or less of the studied active ingredients were present, but they contained different excipients to those taken

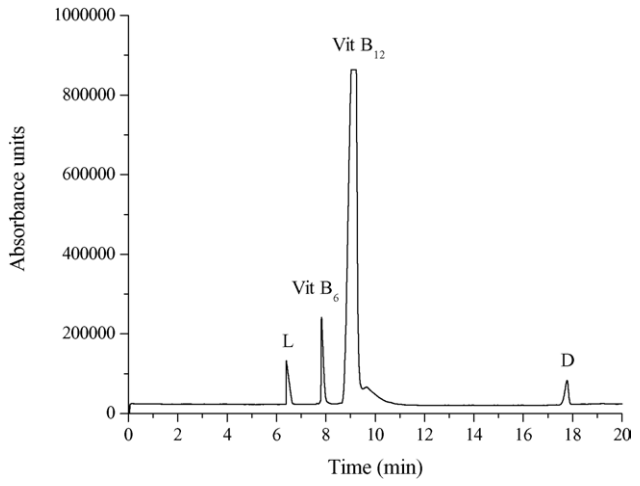


Fig. 5. Electropherogram corresponding to a real sample (Flexicamim B_{12}). The analytes concentrations are: 0.666 g l^{-1} of lidocaíne (L), 0.266 g l^{-1} of vitamin B_{12} (Vit. B_{12}), 6.666 g l^{-1} of vitamin B_6 (Vit. B_6) and 0.053 g $l^{-1}$of dexamethasone.

into account in the present study. Despite the mentioned fact, good resolutions between the active ingredients were obtained.

4. Conclusions

The active ingredients vitamin B_6, vitamin B_{12}, lidocaine and dexamethasone can be simultaneously analyzed in pharmaceutical formulations (injections and tablets) by using capillary electrophoresis. Time of analysis, resolutions and quality of the peaks were simultaneously optimized by handling a useful tool: multiple response optimization. The use of experimental design and response surface methodology enhanced by the application of the desirability function allows for a rapid solution of analytical tasks like the one studied in the present work.

Good results with respect to precision, accuracy and selectivity were obtained in the concentration range studied for the four compounds, and these results are similar to those achieved when the reference high performance liquid chromatography technique is applied in pharmaceutical analysis.

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