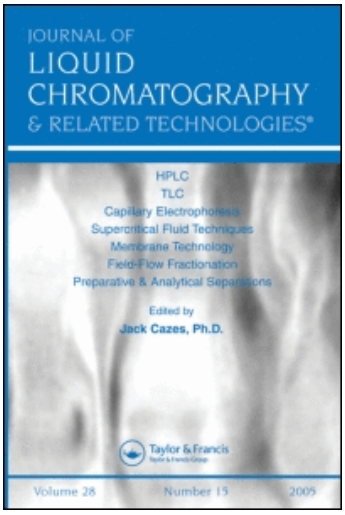

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Abstract: A capillary zone electrophoresis method is developed to achieve the separation and determination of two cardiac drugs, trandolapril and verapamil. Optimisation of the method is based on the use of a factorial design, which is applied in order to obtain more information in the factorial space and to minimise the number of experiments needed to obtain the optimum values for the pH and concentration of electrolyte buffer, the voltage applied and the effects of certain modifiers. Following these studies, the conditions selected for use were running buffer of 10 mM phosphate at pH 7.0, and a voltage of 15 kV. Under these conditions, calibration curves were constructed with good linearity ($R > 0.999$) and suitable LODs. The intra- and inter-day repeatabilities were also evaluated with RSD below 4% for trandolapril and verapamil. The proposed method was applied to the determination of verapamil and trandolapril in pharmaceutical formulations and the recoveries obtained were in agreement with the stated contents for all preparations tested.

Keywords: Factorial design, Cardiac drugs, Trandolapril, Verapamil, Capillary electrophoresis

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INTRODUCTION

Verapamil is a phenylalkylamine derivative calcium channel blocking agent. Its principal physiological action is to inhibit the transmembrane influx of extracellular calcium ions across the membranes of myocardial cells and vascular smooth muscle cells, without changing serum calcium concentrations. Verapamil is used in the management of supraventricular tachyarrhythmias, including rapid conversion of paroxysmal supraventricular tachycardias into a sinus rhythm and temporary control of a rapid ventricular rate in atrial flutter or fibrillation, as well as in the management of hypertension.[1]

Trandolapril is an angiotensin converting enzyme (ACE) inhibitor and is used in the management of mild to severe hypertension, to lower blood pressure, and to treat heart failure. Angiotensin II is a chemical substance produced in the body that causes the muscles in the walls of arteries and veins to contract, thus narrowing the arteries and veins and, consequently, raising blood pressure. Angiotensin II is formed by an enzyme called ACE. Trandolapril is an ACE inhibitor and blocks the formation of angiotensin II, thereby lowering blood pressure and reducing the amount of blood that must be pumped.[2]

Verapamil and trandolapril can be used as monotherapy or in combination for the treatment of hypertension. These drugs used as monotherapy at the recommended doses produce reductions in blood pressure. Therapy, utilising a combination of both drugs, has been shown to produce decreases in blood pressure that are greater than those brought about by any group of individual agents used alone (around twice as great as those obtained with a single drug).[3]

The combination of a calcium channel blocker and an ACE inhibitor is appealing on theoretical grounds. Although calcium antagonists exert much of their antihypertensive effect through a vasodilatory action, they also have diuretic and natriuretic properties. ACE inhibitors blind the stimulation of the rennin-angiotensin-aldosterone axis that may result from this diuretic effect. Both classes of drugs are, however, powerful vasodilators. The addition of an ACE inhibitor to dihydropyridine calcium antagonist therapy reduces the incidence of tachycardia and peripheral edema.[4]

Four fixed dose combinations of calcium channel blockers and ACE inhibitors are currently available. These combinations have yet to be proven more effective than antihypertensive combinations containing diuretics. Tarka is the brand name for the combination of verapamil and trandolapril presented with 180/2 mg, 240/1 mg, 240/2 mg, 240/4 mg amounts of the drug.[4]

Few works were found that report the determination of verapamil and trandolapril in combination. These available methods make use of techniques like high pressure liquid chromatography (HPLC)[5] and dual plate overpressured layer chromatography (OPLC).[6] In contrast, a large number of

analytical methods have been reported for the analysis of verapamil alone. These procedures include capillary electrophoresis[7–15] and HPLC, using different detection systems like UV,[5,9,16–18] fluorescence,[17,19–22] and mass spectroscopy (MS).[17,23] Others employ gas chromatography using fluorescence,[24] FID,[25] MS,[26,27] and N-P[28–33] as detectors. Thin layer chromatography[34] and gas liquid chromatography with thermoionic specific detection[35] have also been used. On the other hand, determination of trandolapril alone has been analysed less often than verapamil; indeed, only three methods involving amperometric biosensors,[36] a potentiometric enantioselective membrane electrode,[37] and liquid chromatography tandem mass spectrometric[38] have been used.

The aim of the present work was to develop a rapid CE method that allows the determination and quantification of these frequently used cardiac drugs (trandolapril and verapamil) in pharmaceutical formulations. The optimisation of the method was performed using an interpretative strategy, focused on a central composite face centered (CCF) design that supplied data to obtain a fitted polynomial model for drawing a surface response in all the variable space. Selected composition of the running buffer and conditions make the procedure easy to analyse verapamil and trandolapril alone or in combination in commercially available formulations.

EXPERIMENTAL

Instrumentation

Capillary electrophoresis runs were performed with a Beckman P/ACE System MDQ (Beckman Instruments, INC., Fullerton, CA, USA) equipped with a DAD detector. The fused silica capillaries (50 μm ID, 375 μm OD) were from Polymicro Technologies (Phoenix, AZ, USA) and the total length of the capillaries used was 40 cm, the effective length being 30 cm. The polyimide coating of the capillary was partially removed by burning at the point of detection and the uncovered portion of the capillary was aligned on the detector block. The capillary was initially conditioned by flushing for 15 min with NaOH 0.1 M followed by 15 min with water and 15 min with buffer. Between each injection, the capillary was rinsed for 2 min with water and for 3 min with electrolyte solution to ensure a consistent electroosmotic flow. The wavelength of detection was 214 nm. A PC connected to the instrument through Beckman 32 Karat software (version 5.0) was used for instrumental control and acquisition of the electrophoretic graphic data. The electrophoretic runs were performed at $25 \pm 0.1^{\circ}$C. The samples were loaded by hydrodynamic pressure at 1 psi for 10 sec. Under these conditions, the runtime was 3 min. Measurements of pH were performed with a GLP 22 from Crison (Barcelona, Spain), equipped with a

combined Ag/AgCl/glass electrode. The vortex shaker and sonification unit were from Selecta (Barcelona).

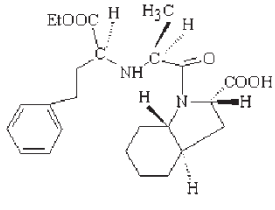
Reagents

Trandolapril was kindly provided by Abbott Laboratories (Madrid, Spain) and verapamil (Figure 1) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions containing 100 $\mu g\,mL^{-1}$ of each compound were prepared in distilled water. The solutions were suitably diluted for the analysis. Acetone (Panreac, Barcelona, Spain) was used as an electroosmotic marker.

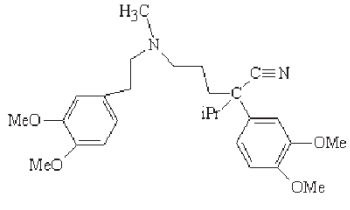
Buffer solutions were prepared with sodium dihydrogenphosphate, disodium hydrogenphosphate, and deoxycholic acid (Sigma-Aldrich). Distilled deionised water (Millipore, Billerica, MA, USA) was used throughout. Sodium hydroxide (99% purity, Merck, Darmstadt, Germany) was used to condition the capillary.

Pharmaceutical Sample Preparation

The pharmaceuticals were presented as capsules (Gopten, Tarka, Tricen) and tablets (Manidón). For the analyses, ten tablets were weighed, ground to a fine powder, and then homogenised. Several portions were taken and weighed, dissolved, and diluted to an adequate concentration with water. The capsules were treated similarly. All sample solutions were filtered into



Trandolapril



Verapamil

Figure 1. Structure of trandolapril and verapamil.

the autosampler vials through 0.45 μm nylon membranes with a diameter of 12 mm.

Computer Modelling

The SPSS program (SPSS Inc. Chicago, Illinois, USA) was used for the non-linear regression analysis of the data and to obtain the empirical mathematical model that represents the response surface. The surface plot was produced by Surfer, a contouring and 3D surface mapping software program (RockWare Europe, Cureglia, Switzerland).

RESULTS AND DISCUSSION

Screening Parameters

In order to optimise the separation conditions in a capillary zone electrophoresis method, several parameters must be considered, including capillary length, concentration and pH of running buffer, temperature, applied voltage, addition of organic modifier, and injection volume.

Preliminary results indicated that capillaries of less than 40 cm (30 or 20, for example) do not show the peak for verapamil, and lengths above 40 cm increase the analysis time of trandolapril. For these two reasons, the total capillary length was fixed at 40 cm. On the other hand, the capillary was cooled to $25 \pm 0.1^{\circ}C$ in order to dissipate heat and minimise the chances of sample decomposition, thus making it possible to use higher voltages. This temperature control improves migration times, peak height, and area reproducibilities. The injection pressure used for sample injection was maintained within the range of 1 psi for 2 to 20 s. As sensibility is not limiting in the determination of the cardiac drugs in pharmaceuticals, the use of the smallest injection lengths should be used, although at the same time the injection reproducibilities are diminished. For sample injection the best compromise between resolution, peak efficiency, and peak distortion was achieved at 1 psi for 10 sec.

The presence of an organic modifier such as acetonitrile, methanol, or propanol only reduces the electroosmotic flow (EOF) and produces the overlapping of the trandolapril and verapamil peaks. The addition of other kinds of modifiers such as SDS, β-cyclodextrin, or deoxycholic acid did not improve the resolution, the efficiency of the peaks, or reduce the analysis time; in fact they only make the process more laborious, and obviously their use was ruled out.

From other preliminary results performed for the pH and concentration of the running buffer, as well as the applied voltage, it was found that these are the factors that are most frequently affected by small changes, with respect to the responses of migration time, peak width and efficiency, resolution and analysis time for the two cardiac drugs.

Factorial Design Optimisation

A primary interest in the development of a new method for the separation and quantification of analytes of pharmaceutical interest is the amount of time and the number of trials required to implement the method, which is obviously related to costs. To minimise the experiments and to shorten method development time, a modelling strategy might well be effective. Thus, an interpretative strategy was chosen to investigate the separation of trandolapril and verapamil.

Three parameters (pH and concentration of running buffer, and voltage) have been considered to affect the peak resolution and a minimum of three levels are necessary to apply the response surface method. The experimental strategy chosen to carry out the optimisation procedure was a central composite face centered (CCF) design. This strategy consists of a combination of a factorial design and an additional design (star design) in which the centres of both designs coincide (Table 1). In this design the star points are at the centre of each face of the factorial space, so $\alpha = \pm 1$ distance. The CCF designs provide relatively high quality predictions over the entire design space. Additionally, they do not require the use of points outside the original factor range and only need three levels for each factor.

Response Surface Method

The CCF strategy was then applied to obtain a response in order to indicate the optimum conditions for screening the mixture of trandolapril and verapamil. The best conditions are determined using the response surface method, while taking into account the maximum resolution and efficiencies with the minimum analysis time.

For three factors, the CCF design requires fifteen runs. Runs of the design were carried out in a randomised sequence, and migration time and widths of peaks were measured. Replications of factor combinations were necessary to estimate the experimental error. Thus, the centre point was run five times. The parameter settings in the design given in Table 1 provide the responses in Table 2.

The resolution of peaks was calculated using Equation (1):

$$Rs = \frac{2(t_2 - t_1)}{(w_2 + w_1)} \tag{1}$$

Table 1. Factor settings in the design

CE factor	−1	0	1
Phosphate concentration (mM)	10	20	40
pH	7	8	9
Voltage (kV)	5	15	25

Table 2. Central composite face-centered design and responses obtained

Run	pH	V	Conc	CRS^{-1}
1	−	−	−	0.058
2	+	−	−	0.121
3	−	+	−	0.324
4	+	+	−	−0.008
5	−	−	+	0.065
6	+	−	+	0.056
7	−	+	+	0.294
8	+	+	+	−0.006
9	−	0	0	0.181
10	+	0	0	0.183
11	0	−	0	0.053
12	0	+	0	0.219
13	0	0	−	0.189
14	0	0	+	0.105
15	0	0	0	0.412

where w_1 and w_2 are the width at the peak base of two consecutive peaks, measured as time units. The numerator in Equation (1) describes the separation process in terms of differential migration and the denominator represents the dispersive processes acting against it.

A peak of EOF appears between the verapamil and trandolapril peaks, so it is wise to separate this peak as well. Thus, the function used as a response to evaluate the best separation is the CRS function:

$$CRS = \left\{ \sum_{i=1}^{n-1} \left[\frac{(R_{i,j+1} - R_{opt})}{(R_{i,j+1} - R_{\min})^2 \; R_{i,j+1}} \right] + \sum_{i=1}^{n-1} \frac{R_{i,j+1}^2}{(n-1)R_{av}^2} \right\} \frac{t_n}{n} \qquad (2)$$

here $R_{i,j+1}$ is the resolution between consecutive peaks, R_{av} is the average resolution of all peaks, R_{opt} is the desired resolution (in this case 1.5), $R_{\min}$ is the minimum acceptable resolution, which has a value of 1, t_n is the migration time of the last eluting solute and n is the number of compounds in the sample. The CRS considers the resolution of all solutes in the sample and incorporates three important aspects of the separation. The first term in Equation (2), called the resolution term, evaluates the resolution between all adjacent solute pairs in comparison to defined values for optimum and minimum resolution. The second term in Equation (2), which is named the distribution term, considers the relative spacing of the solute zones. The final multiplier term in Equation (2) takes into consideration the analysis time and the number of analyte peaks to be separated.

The CRS values obtained for each electrophoretic condition are shown in Table 2, together with the inverse of the CRS. The inverse of the CRS was chosen because the maximum of the function fits the optimal condition.

A response surface method was used to quantify and interpret the relationships between responses and factor effects. The general empirical model is a

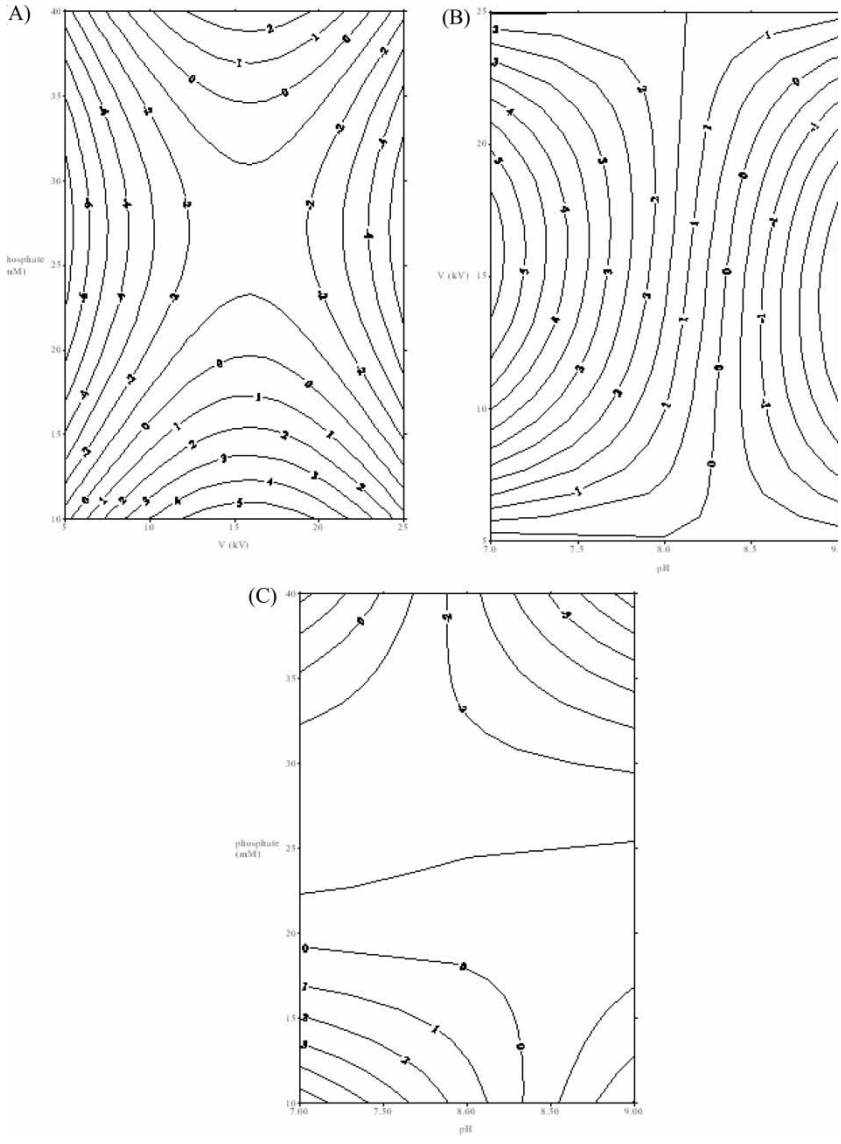


Figure 2. Response contour map obtained with the pH (A), phosphate concentration (B), and voltage (C) constant.

second order polynomial, where the response y is related to the variables (factors) x as follows:

$$y = b_0 + \sum_{i=1}^{k} b_i x_i + \sum_{1 \le j}^{k} b_{ij} x_i x_j + \sum_{i=1}^{k} b_{ii} x_i^2 \quad (3)$$

where k is the number of variables (factors), b_0 the intercept parameter, and b_i, b_{ij}, b_{ii} are regression parameters for linear, interaction and quadratic factor effects. The non-linear regression analysis of the data was carried out by the SPSS program and the model obtained was:

$$\begin{aligned} \mathrm{CRS}^{-1} = {} & 8.95 + 1.33 * \mathrm{pH} + 11.91 * \mathrm{V} - 9.46 * \mathrm{phos} - 0.30 * \mathrm{pH}^2 \\ & - 1.44 * \mathrm{pH} * \mathrm{V} + 1.11 * \mathrm{pH} * \mathrm{phos} - 0.40 * \mathrm{V}^2 - 0.0006 * \mathrm{V} * \mathrm{phos} \\ & + 0.2 * \mathrm{phos}^2 - 0.003 * \mathrm{pH}^2 * \mathrm{V} + 0.007 * \mathrm{pH}^2 * \mathrm{phos} \\ & + 0.0495 * \mathrm{pH} * \mathrm{V}^2 + 0.00008 * \mathrm{pH} * \mathrm{V} * \mathrm{phos} - 0.025 * \mathrm{pH} * \mathrm{phos}^2 \quad (4) \end{aligned}$$

According to the above mentioned description, with the desired conditions of maximum resolution and an adequate analysis time, the optimum conditions are 10 mM phosphate buffer at pH = 7.0 and V = 15 kV with a value for the response of 5.789 units. This optimum is shown in Figure 2 by three contour maps. In Figure 2A, the pH of buffer was kept constant and optimum values of phosphate concentration and voltage were determined: 10 mM and 15 kV. In Figure 2B, phosphate was constant at 10 mM and the best conditions were pH 7 and 15 kV. Finally, voltage was kept constant and phosphate concentration against pH were determined (Figure 2C).

Figures of Merit

Linearity

Calibration curves were constructed using the standard stock solution (100 μg mL^{-1}), which was taken and diluted in an appropriate ratio with running buffer to obtain six increasing concentrations (three for each concentration in the 0.5 to 20 μg mL^{-1} range). Peak areas were measured and linear

Table 3. Calibration parameters and limits of detection (*3s criterion*) in ng mL^{-1} for trandolapril and verapamil

Compound	Slope	Intercept	*r*	LOD
Trandolapril	1089.6 ± 49.0	31.0 ± 19	0,9999	25
Verapamil	733.9 ± 42.5	−41.6 ± 16	0,9997	35

Table 4. Intra- and inter-day repeatabilities (RSD %, n = 10) at three different concentrations (μg mL^{-1}): c1 = 1, c2 = 5 and c3 = 10

	Repeatability			Intermediate precision		
Compound	c1	c2	c3	c1	c2	c3
Trandolapril	3.2	0.9	2.4	4.0	1.4	2.8
Verapamil	3.4	2.5	2.2	3.5	2.5	4.2

regression calculation gives the slopes, intercepts, and regression coefficients shown in Table 3. Calibration parameters were adapted to match the requirements for the determination of trandolapril and verapamil.

Limits of Detection

The detection limit (LOD) of a method is the lowest analyte concentration that produces a response that is detectable above the noise level of the system (typically taken as being three times the noise level (3s criterion)). LODs were 25 and 35 ng mL^{-1} for trandolapril and verapamil, respectively, both of which are well below those required for analysis of the trandolapril and verapamil in pharmaceutical preparations (Table 3). Limits of quantification (10 s criterion) were 90 ng mL^{-1} for both compounds.

Precision

Intra-day (average of ten determinations performed on the same day) and inter-day repeatability (average of intra-day values taken on ten days over a

Table 5. Determination of trandolapril and verapamil in pharmaceutical preparations

Commercial name	Composition	Recovery
Gopten (abbot)	Per capsule: trandolapril (DCI) 2 mg, and excipients c.s.	103.1
Manidón 120 retard (abbot)	Per tablet: verapamil (DCI) ClH 120 mg, and excipients c.s.	94.6
Tarka (abbot)	Per capsule: verapamil hydrochloride (DCI) 180 mg, trandolapril (DCI) 2 mg, and excipients	99.1 101.2
Tricen (alter)	Per capsule: verapamil hydrochloride (DCI) 180 mg, trandolapril (DCI) 2 mg, and excipients	98.2 104.8

two-month period) were determined at three different drug concentrations (1, 5, and 10 $\mu g\,mL^{-1}$). The relative standard deviations (RSD) were always below 4% (Table 4).

Analysis of Pharmaceuticals

Once the conditions for separation and quantification were established, the CE method was applied to different pharmaceutical formulations (capsules and tablets) of trandolapril and verapamil. The pharmaceutical extract was injected into the CE system and the results are shown in Table 5. In those pharmaceuticals which contain both substances, verapamil concentration is much higher with respect to trandolapril, and thus two dilutions were analysed in order to quantify the two analytes.

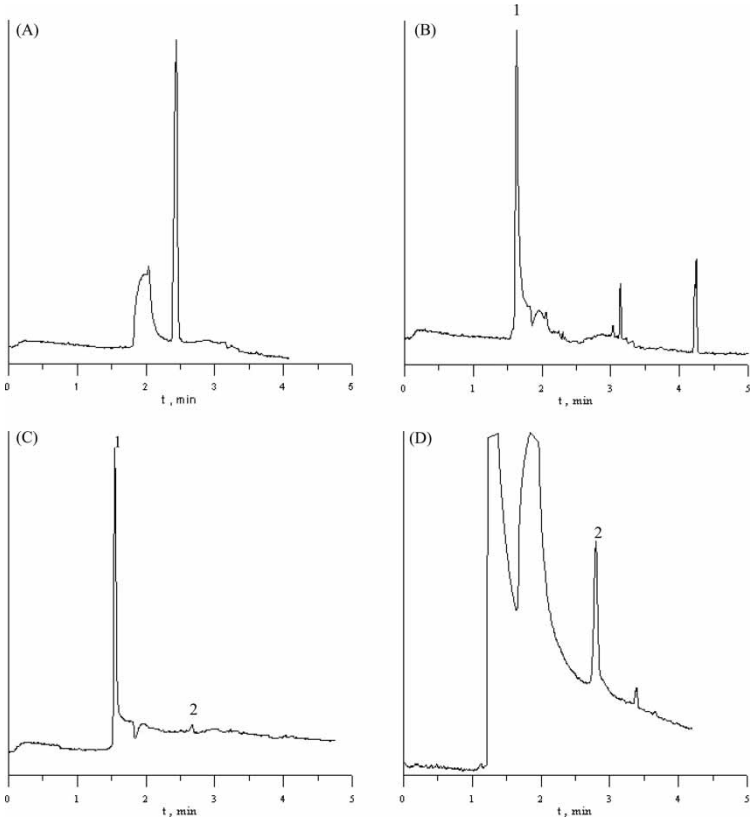


Figure 3. Electropherogram of pharmaceutical preparations: (A) Gopten; (B) Manidón; (C) Tricen (Verapamil 10 ppm; trandolapril 0.10 ppm); (D) Tricen (Verapamil 200 ppm; trandolapril 2.5 ppm). (1. verapamil; 2. trandolapril).

Figure 3 shows the electropherograms obtained in the determination of trandolapril and verapamil in the pharmaceuticals Gopten, Manidón, Tricen, and Tarka, using the optimum conditions described in this paper. The other excipients contained in the pharmaceutical preparations did not interfere with the determination of trandolapril and verapamil. The results of the analysis indicate that the optimised electrophoretic method is suitable for the assay of drugs in pharmaceuticals.

CONCLUSION

In this study a capillary zone electrophoresis method was developed using an experimental design, known as the central composite face centered design. This strategy allowed a large response surface to be obtained using only fifteen runs, while also enabling us to determine the behaviour of the electrophoretic peaks of trandolapril and verapamil over all the variable space. The response surface made it easy to determine the optimum electrophoretic conditions necessary to obtain a good resolution between the peaks with the minimum analysis time. The optimum conditions were a phosphate 10 mM at pH 7.0 running buffer and a voltage of 15 kV. Under these conditions the procedure for determining trandolapril and verapamil in pharmaceutical samples became straightforward, sensitive, simple, and fast. Thus, the method might well be suitable for quality control analyses of trandolapril and verapamil in pharmaceuticals.

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