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Multivariate Optimization and Validation of a Capillary Electrophoresis Method for the Simultaneous Determination of Dextromethorphan Hydrobromur, Phenylephrine Hydrochloride, Paracetamol and Chlorpheniramine Maleate in a Pharmaceutical Preparation Using Response Surface Methodology

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A fast, accurate, precise and sensitive capillary electrophoresis method for the simultaneous determination of dextromethorphan hydrobromide, phenylephrine hydrochloride, paracetamol and chlorpheniramine maleate has been developed. Response surface methodology with a central composite design was used for optimization of the concentration of the buffer, pH of the buffer and applied voltage. Therefore, working with Na_2HPO_4 buffer (pH 8.00, 0.01 M) at 20 kV as an applied voltage in the capillary electrophoresis method were found to be suitable; under these optimal conditions, these four active ingredients were separated in about 7 min. This developed method was validated and successfully applied to a pharmaceutical preparation, sugar-coated tablet, and the results were compared with a high-performance liquid chromatographic method developed by us.

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Introduction

Dextromethorphan hydrobromide (DEX), (+)-3-methoxy-9a-methylmorphinan hydrobromide monohydrate (Fig. 1a), is employed as an antitussive agent. It controls cough spasms by depressing the cough center in the medulla.[1] Phenylephrine hydrochloride (PHEN), (*S*)-1-(3-hydroxyphenyl)-2-methylamino-ethanol hydrochloride (Fig. 1b), is used to maintain blood pressure, and as a nasal, scleroconjunctival and uveal decongestant; it is also used as a mydriatic agent to promote aqueous humor outflow in the treatment of open-angle glaucoma.[1] Chlorpheniramine maleate (CHL), (±)-3-(4-chlorophenyl)-*N*,*N*-dimethyl-3-(2-pyridil)propil-amine maleate (Fig. 1c), is an alkylamine antihistamine that is a common ingredient in OTC antitussive formulations.[1] Paracetamol (PAR), 4′-hydroxyacetanilide (Fig. 1d), is an effective antipyretic and analgesic that exerts its clinical effects by a mechanism similar to that of the salicylates.[1] A quaternary mixture of these drugs is widely used in diseases accompanied by cough, pain and fever, such as the common cold and other viral infections as an analgesic, antipyretic, decongestant, antihistamine and antitussive.

A response or a set of responses in an experiment are influenced by several variables also known as experimental conditions. An optimization procedure is required for developing an analytical method, as in capillary electrophoresis related to provide relevant information depending on analyzing experimental data and for optimal experimental conditions.

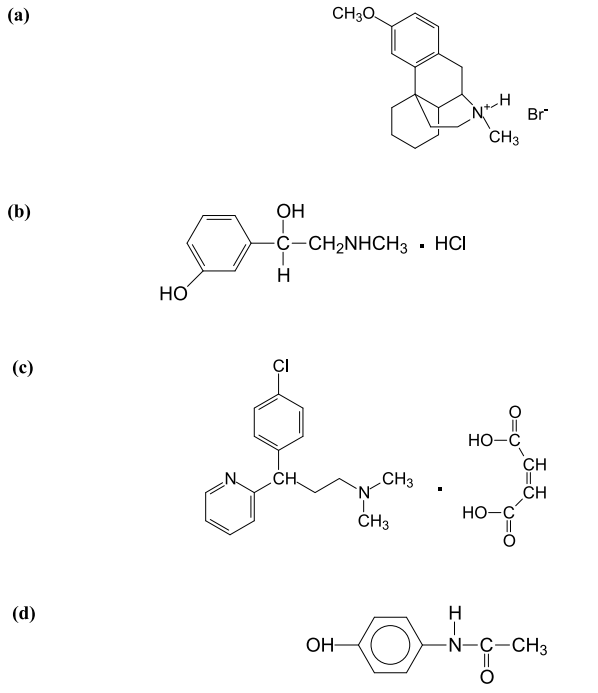


Fig. 1 Structures of dextromethorphan hydrobromide (a), phenylephrine hydrochloride (b), chlorpheniramine maleate (c) and paracetamol (d).

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Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes, interpreting the relationships between the responses and factor effects and simultaneously optimizing the levels of variables to achieve the best system performance.[2]

The determination of active ingredients in pharmaceutical preparations becomes more difficult when the number of the active components increase in the mixture, and the presence of interference from excipients in the preparations. In addition to the chromatographic techniques, capillary electrophoretic techniques have been widely used in last decades for resolving mixtures, and employed for the analysis of multi-component samples with regard to remarkable advantages, such as little sample volume, consuming few volumes of run buffer, high theoretical plates, wide working areas and cheapness.

A literature survey has revealed that there are some developed capillary electrophoretic methods for the separation of DEX, CHL and PAR with some cold medicine ingredients without any quantitative determination and pharmaceutical preparation being applied.[3-5] Besides, several capillary electrophoretic techniques have been developed for DEX and PAR with pseudoephedrine hydrochloride in a tablet,[6] for DEX and PHEN with diphenhydramine hydrochloride in a syrup,[7] for DEX and CHL with pseudoephedrine and diphenhydramine in cold medicines,[8] and PAR, DEX and CHL with pseudoephedrine hydrochloride in medicinal preparations.[9] According to our knowledge, there have been no reports concerning the simultaneous determination of DEX, PHEN, CHL and PAR in pharmaceutical preparations.

The aim of this study was to develop and optimize a simple and rapid capillary electrophoretic method for the simultaneous determination of DEX, PHEN, CHL and PAR in pharmaceutical preparations. For this purpose, the RSM technique was used for monitoring the influence of the buffer concentration, buffer pH and applied voltage, as selected experimental conditions simultaneously; method validation studies were performed. These developed and validity proved methods were successfully applied to a pharmaceutical preparation containing these active ingredients, and the obtained results were compared by the Student's *t*-test and the *F*-test with a high-performance liquid chromatographic method developed by us.[10]

Experimental

Apparatus

Capillary electrophoretic experiments were made using a PrinCE 760 system capillary electrophoresis integrated PrinCE-DAD 30/48 position autosampler with 512 diodes and a high-voltage power supply (Prince Technologies B. V., Emmen, The Netherlands) coupled to a computer processing the data using DAx-3D (Ver. 7.3) data acquisition software. Separation was carried out in a fused-silica capillary, 70 cm × 75 μm i.d. (effective length 62 cm), in the normal mode and applying a voltage of 20 kV. Sample injections were made in a hydrodynamic mode at 15 s under a pressure of 100 mbar.

A chromatographic system, Agilent Technologies HP 1100 (Germany), equipped with a G1379A Model degasser, G1311A quaternary pump, 61313A auto injector, and G1315B DAD detector were used. An ACE C18 column (Advance Chromatography Technologies, Scotland) 250 × 4.6 mm, 5 μm particle sized was used. The chromatograms were recorded and the peaks were quantified using an automatic integrator.

For pH measurements, a pH meter (Hanna Instruments, HI221, Romania) was employed. All solutions were degassed by ultrasonication (P Selecta, Barcelona, Spain), and were filtered through a 0.45-μm nylon membrane before use. A heating magnetic stirrer (Velp Scientifica, ARE, Europe) was used for stirring.

Reagents

Dextromethorphan hydrobromide, phenylephrine hydrochloride, chlorpheniramine maleate and paracetamol were kindly donated by İlsan İlaç ve Hammaddeleri San. A. Ş., Istanbul, Turkey. They were used without further purification. Phosphoric acid (H_3PO_4), sodium phosphate ($Na_2HPO_4{\cdot}12H_2O$) and deionized water were provided by Merck, Darmstadt, Germany.

WINTUS® (10 mg dextrometorphan hydrobromide, 5 mg phenylephrine hydrochloride, 250 mg paracetamol and 2 mg chlorpheniramine maleate/sugar-coated tablet) Abdi İbrahim Pharm. Ind., Turkey (batch No. 9054878) was assayed as a commercial pharmaceutical preparation.

Solutions

Solutions of DEX (400 μg mL^{-1}), PHEN (400 μg mL^{-1}), CHL (400 μg mL^{-1}) and PAR (2000 μg mL^{-1}) were prepared in $Na_2HPO_4{\cdot}12H_2O$ (pH 8, 0.1 M). For the preparation of a sodium phosphate solution as a run buffer, 35.814 g of $Na_2HPO_4{\cdot}12H_2O$ was dissolved in 980 mL of distilled water, and the pH of the resulting solution was adjusted to 8.0 with 0.1 M H_3PO_4 and then the volume was made up to 1000 mL with distilled water.

For the preparation of a pharmaceutical preparation solution, the contents of 10 sugar-coated tablets were accurately weighed and mixed in a mortar. An amount equivalent to one capsule was redispersed in 20 mL of $Na_2HPO_4{\cdot}12H_2O$ (pH 8, 0.1 M) in a 50-mL volumetric flask and diluted to volume with the same solvent. After 30 min of mechanically shaking, the solutions were filtered through 0.45 μm Whatman No. 42 filter paper in a 50-mL volumetric flask separately. Then, an appropriate amount of this filtrate was transferred to a 25-mL volumetric flask and diluted with $Na_2HPO_4{\cdot}12H_2O$ (pH 8, 0.1 M) to the volume. This solution was injected into the capillary electrophoretic system.

Response surface methodology for multivariate optimization

For interpreting the relationships between the responses and the factor effects and simultaneously optimizing the levels of variables to achieve the best system performance, response surface methodology (RSM) was used. In our study three factors were investigated: the buffer concentration, buffer pH and applied voltage. A central composite design was established by these five different levels of the factors. The obtained results were shown as equations and a contour line graph.

Method optimization

Running buffer optimization. Phosphate, borate and acetate buffer systems were carried out as a running buffer. For achieving the best migration time, peak shape and resolution, the effect of the buffer pH within the range of 7.6 – 9.0, buffer concentration within the range of 0.025 – 0.150 M and different organic modifier within the range of 2.5 – 10.0% in running buffer were investigated. During these assays, the buffer concentration, buffer pH and applied voltage were held constant as 0.1 M, 8.0 and 20 kV, respectively, while monitoring the effect of each variable on obtained responses from the capillary electrophoresis device.

Capillary electrophoresis equipment parameters optimization. During equipment optimization, the effect of applying a voltage

within the range of 18 – 22 kV, the temperature within the range of 20 – 35°C and the capillary length as the internal diameter with 50, 75 and 100 μm with different lengths were investigated. Besides, determination of the optimal conditions for injection type, time and pressure for introducing a sample to the capillary were realized.

Electrophoretic procedure

Before the first use, the capillary was conditioned by flushing with 1.0 M NaOH for 30 min, with 1.0 M NaOH for 30 min and with water for 30 min, respectively. At the beginning of each working day, the capillary was rinsed with 1 M NaOH for 20 min, with 0.1 M NaOH for 20 min, with water for 20 min and then with the running buffer for 20 min. Before each injection, the capillary was preconditioned with 1 M NaOH for 2 min, with 0.1 M NaOH for 2 min, with water for 2 min and with running buffer 6 min under 1000 mbar pressure to maintain proper reproducibility of run-to-run injections. Injection was carried out under hydrodynamic pressure at 100 mbar for 15 s. A diode-array UV detector was set at 200 nm. The capillary temperature was kept constant at 25°C and a voltage of +20 kV was applied.

System suitability test

System suitability tests including the retention factor, separation factor, resolution, asymmetry, theoretical plates and relative standard deviations of the migration times were carried out.

Method validation

Calibration curves. The calibration curves were established with seven different concentrations in the range of 4.0 – 20.0 μg mL^{-1} for DEX, 4.0 – 20.0 μg mL^{-1} for PHEN, 1.6 – 8.0 μg mL^{-1} for CHL and 50.0 – 600.0 μg mL^{-1} for PAR. Each solution was injected three times and the heights of the peaks for DEX and PHEN and the areas of the peaks for CHL and PAR, as measured at 200 nm, were integrated. Regression equations were established by plotting the ratio of the peak areas or peak heights to the concentration of each substance. The linearity was evaluated by linear-regression analysis, which was calculated by least-square regression.

Sensitiviy. The calculation method of LOQ (limit of quantitation) is based on the standard deviation (SD) of the response and the slope (m) of the calibration curve according to the following formula: LOQ = 10(SD/m).[11] The LOD (limit of detection) can be calculated based on the SD of the response and the slope (m) of the calibration curve according to the following formula: LOD = 3.3(SD/m).[11]

Accuracy and precision. The accuracy and precision were studied using three different solutions containing 4.0, 12.0, 10.4, 16.0 and 20.0 μg mL^{-1} of DEX, 4.0, 8.0, 12.0, 16.0 and 20.0 μg mL^{-1} of PHEN, 1.6, 2.8, 4.0, 6.0 and 8.0 μg mL^{-1} of CHL and 50.0, 100.0, 200.0, 300.0 and 600.0 μg mL^{-1} of PAR. Due to the stability problem of these active ingredients in $Na_2HPO_4 \cdot 12H_2O$ (pH 8, 0.1 M) as the running buffer, solutions were prepared freshly every day. Therefore, repeatability studies were not achieved on different days.

Results and Discussion

Method optimization

Running buffer optimization. For achieving the optimum conditions in terms of the migration time, peak shape and resolution, $Na_2HPO_4 \cdot 12H_2O$ as a phosphate buffer was selected after trying borate and acetate buffer.

The buffer pH has a distinctive effect on the electroosmotic flow due to a changing zeta potential. During assays, under the conditions of 7< pH and pH >9, the resolution between each active ingredients was decreased, and the peak shapes were distorted. Hence, buffers were prepared within the range of 7.6 – 9.0. Although the resolution between PHEN and CHL was improved, the migration times were decreased depending on the increasing electrophoretic mobilities, while the pH of the running buffer was increased. Therefore, a running buffer having 8.0 as the pH value was selected as being optimum.

Electroosmotic flow is decreased with increasing in the concentration of the running buffer when the capillary temperature is controlled effectively. For investigating the effect of the buffer concentration on the responses obtained from analysis, phosphate buffers were prepared within the range of 0.025 – 0.150 M. The migration time of DEX was not changed, while the analysis time was increased as a result of an increased migration time of others. Same in buffer pH trying, the resolution between PHEN and CHL was improved with increasing the buffer concentration. Hence, a running buffer having 0.100 M as the concentration value was selected as being the optimum.

The addition of an organic modifier to the running buffer effects viscosity, dielectric constant and zeta potential, depending on the type and percentage of the modifier in the running buffer. In this study, methanol and acetonitrile within the range of 2.5 – 10.0% were investigated as organic modifiers. An increase in the percentage of these in running buffer resulted in a significant increase in the migration time and, moreover, the peak shapes were not changed. For that reason, there was not any organic solvent addition to the running buffer.

Capillary electrophoresis equipment parameters optimization. During optimization studies, capillaries having different lengths and internal diameters were investigated, and a fused-silica capillary (70 cm length, 62 cm effective length and 75 μm i.d.) was selected for continuing studies.

The electrical field changed by upon applying voltage is a simple way to modify the electroosmotic flow. An increase in the applied voltage results in a decrease of the migration time. However, more potential means more current, and therefore, the Joule heating is increased. This causes a decreasing buffer viscosity, a wide peak shape, an unrepeatable migration time, denaturation of the sample and boiling of the buffer, resulting in closing the system. Taking into account this knowledge, the applied voltage within the range of 18 – 22 kV was investigated, and 20 kV was selected as being the optimum.

In capillary electrophoretic techniques, an increase in the temperature results in a decrease of the buffer viscosity; the migration time, depending on an increasing electroosmotic flow. Also, it results in a wide zone and denaturation of the sample. Temperature within the range of 20 – 35°C was attempted and 25°C was selected for optimum experimental conditions.

The injection type, time and pressure are important variables in capillary electrophoresis, owing to affecting the peak symmetry, tailing, height and area. For this reason, different values of these variables were tested, and injections were carried out under hydrodynamic pressure at 100 mbar for 15 s.

Optimal capillary electrophoretic conditions after optimization studies. It was found that good results were obtained by using a fused-silica capillary (70 cm length, 62 cm effective length and 75 μm i.d.), using $Na_2HPO_4 \cdot 12H_2O$ buffer (pH 8, 0.1 M), at 25°C and detection at 200 nm under a 20-kV potential and sample injection into the capillary under 100 mbar pressure during 15 s. Under these conditions, the migration times were

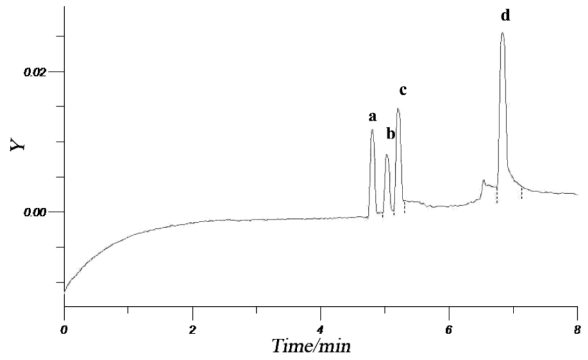


Fig. 2 Electropherogram of a) 100 μg mL^{-1} DEX, b) 100 μg mL^{-1} CHL, c) 100 μg mL^{-1} PHEN, d) 100 μg mL^{-1} PAR in $Na_2HPO_4 \cdot 12H_2O$ (pH 8, 0.1 M).

Table 1 Factor settings in the design for developed capillary electrophoretic method

Factor	$-\alpha$	−1	0	−1	$+\alpha$
Buffer pH (x_1)	7.66	7.80	8.00	8.20	8.34
Buffer concentration/M (x_2)	0.066	0.080	0.100	0.120	0.134
Applied voltage/kV (x_3)	16.64	18.00	20.00	22.00	23.36

found to be 4.78, 5.01, 5.19 and 6.80 min for DEX, CHL, PHEN and PAR, respectively. An electropherogram is shown in Fig. 2.

Response surface methodology for multivariate optimization. As defined by the ICH, the robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in the method parameters, and provides an indication of its reliability during normal usage.[11] For studying the simultaneous variation of the factors on considered responses, some designs of experimental techniques were suggested in robustness testing, including multivariate applications. The response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes, and interpreting the relationships between the responses and the factor effects.[2] An important aspect of RSM is design of THE experiment. The aim of the design of an experiment is minimizing the experiments, reducing the analyzing time and selecting of the points where the response should be interpreted. Composite designs are very efficient for a limited number of factors (<5 - 6) and include the combination of a full fractional design and an additional design, often a star design.[12] If the centers of both designs coincide, they are called central composite designs. For the number of runs, r, can be written,[13]

$$r = 2^{k-p} + 2k + n_o$$

where k, number of factors; p, number for reduction of the full design; n_o, number of experiments in the center of the design.

In this equation, 2^{k-p} is the number of experiments in the full or fractional design; $2k$ the number of experiments in the star designs; n_o the number of experiments in the center of the design. The level is (−1) and (+1) for 2^{k-p}, $\pm\alpha$ for $2k$ and (0) for n_o; α can be formulated as

$$\alpha = 2^{(k-p)/4}$$

Table 2 Experimental plan for central composite design

No.	x_1, buffer pH	x_2, buffer conc./M	x_3, applied voltage/kV
1	7.80	0.080	18.00
2	8.20	0.080	18.00
3	7.80	0.120	18.00
4	8.20	0.120	18.00
5	7.80	0.080	22.00
6	8.20	0.080	22.00
7	7.80	0.120	22.00
8	8.20	0.120	22.00
9	7.66	0.100	20.00
10	8.34	0.100	20.00
11	8.00	0.066	20.00
12	8.00	0.134	20.00
13	8.00	0.100	16.64
14	8.00	0.100	23.36
15	8.00	0.100	20.00
16	8.00	0.100	20.00
17	8.00	0.100	20.00

Table 3 System suitability test results of DEX, CHL, PHEN and PAR

Parameter	DEX	CHL	PHEN	PAR
Retention factor (k')	3.31	3.51	3.68	5.13
Separation factor (α)	1.06	1.06	1.06	1.39
Resolution (R)	1.15	1.15	1.02	8.05
Asymmetry (A_s)	0.91	0.92	0.93	1.10
Theoretical plates (N)	9139.36	10040.04	10774.44	39304.00
Relative standard deviations of migration times, %	0.15	0.12	0.14	0.14

In our study three factors were investigated: the buffer pH (x_1), buffer concentration (M) (x_2) and applied voltage (kV) (x_3); and the factor setting in the design is given in Table 1. A central composite design that was established by these levels of factors is given in Table 2. This design contains 17 experiments, including three replicates of the center point. General empirical models are second-order polynomials, where the response y is related to the variables (factors) x, as follows:

$$y = b_o + \sum_{i=1}^{k} b_i x_i + \sum_{1 \le i \le j}^{k} b_{ij} x_i x_j + \sum_{i=1}^{k} b_{ii} x_i^2$$

Here, k is the number of variables (factors) and b_o the intercept parameter, b_i, b_{ij} and b_{ii} are regression parameters for linear, interaction and quadratic factor effects.

The experimental results were computed by Microsoft Excel. The investigated response is electrophoretic mobilities (EM) for each active ingredient. The equation model for EM was as follows:

$$EM = 313.4251 + 2.9231x_1 - 9.31906x_2 + 21.56012x_3 - 9.16963x_1^2 - 21.52226x_2^2 - 18.97912x_3^2 - 2.7975x_1x_2 + 5.3175x_2x_3 - 0.7375x_1x_3 \text{ for DEX,}$$

$$EM = 299.9085 + 1.635815x_1 + 6.05571x_2 + 60.93471x_3 - 7.02165x_1^2 - 5.48225x_2^2 - 0.98479x_3^2 - 3.12125x_1x_2 + 4.01375x_2x_3 - 0.18125x_1x_3 \text{ for CHL,}$$

$$EM = 289.8761 - 8.81302x_1 - 13.2481x_2 + 16.98094x_3 - 18.6491x_1^2 - 18.8528x_2^2 + 16.73073x_3^2 - 7.4625x_1x_2 - 1.8625x_2x_3 - 5.22x_1x_3 \text{ for PHEN,}$$

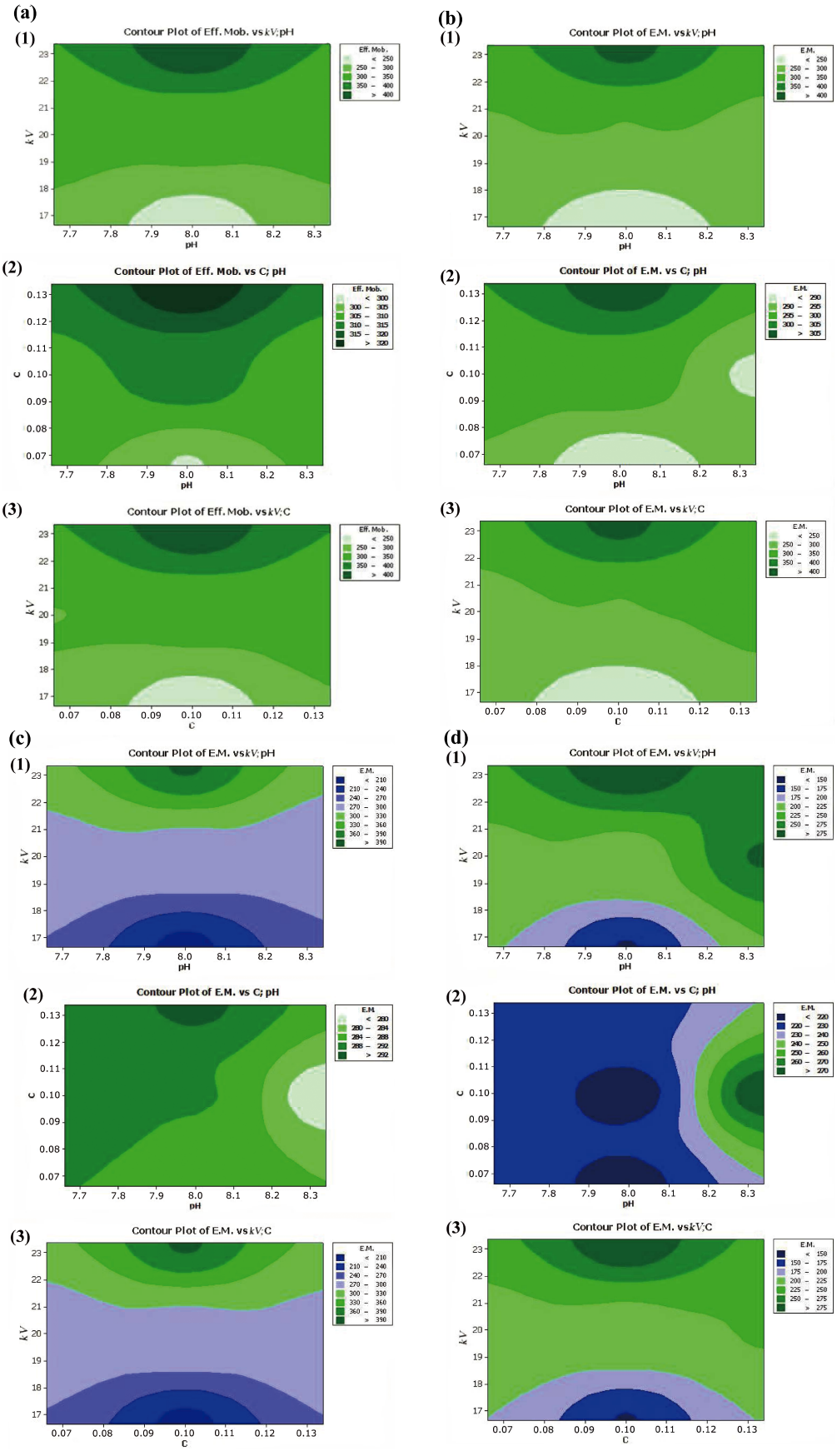


Fig. 3 Variation of the response EH for DEX (a), CHL (b), PHEN (c) and PAR (d), respectively. (1) As a function of x_1 (buffer pH) and x_3 (applied voltage, kV); fixed factor, x_2 (buffer concentration, 0.100 M), (2) as a function of x_1 (buffer pH) and x_2 (buffer concentration, M); fixed factor, x_1 (applied voltage, 20.0 kV), (3) as a function of x_2 (buffer concentration) and x_3 (applied voltage, kV); fixed factor, x_1 (buffer pH 8.0).

Table 4 Linearity and sensitivity parameters for DEX, PHEN, CHL and PAR

Parameter	DEX	PHEN	CHL	PAR
Linearity range/μg mL^{-1}	4.0 - 20.0	4.0 - 20.0	1.6 - 8.0	50.0 - 600.0
LOD/μg mL^{-1}	1.94	1.46	0.007	12.34
LOQ/μg mL^{-1}	4.00	4.00	1.60	50.00
Slope ± standard error	0.2165 ± 0.0045	0.2992 ± 0.0108	0.4522 ± 0.0035	0.0054 ± 0.0001
Intercept ± standard error	–2.9139 ± 0.0735	–3.2730 ± 0.0767	–1.7903 ± 0.0006	–1.6363 ± 0.0117
r	0.998	0.992	0.997	0.997

Table 5 Accuracy and precision data for DEX, PHEN, CHL and PAR

Added/ μg mL^{-1}		Found[a]/ μg mL^{-1}	Precision[b] (RSD%)	Accuracy[c], %
DEX	4.00	3.96 ± 0.03	1.19	98.92 ± 0.46
	12.00	11.81 ± 0.05	0.80	98.39 ± 0.68
	10.40	10.43 ± 0.05	0.89	100.35 ± 0.89
	16.00	16.09 ± 0.06	0.67	100.54 ± 0.67
	20.00	19.91 ± 0.03	0.24	99.57 ± 0.24
PHEN	4.00	4.08 ± 0.04	1.58	102.00 ± 0.93
	8.00	8.04 ± 0.03	0.56	100.54 ± 0.06
	12.00	11.98 ± 0.10	1.47	99.81 ± 1.47
	16.00	15.81 ± 0.10	1.11	98.83 ± 1.11
	20.00	20.05 ± 0.17	1.49	100.23 ± 0.86
CHL	1.60	1.62 ± 0.01	0.83	100.93 ± 0.48
	2.80	2.84 ± 0.01	0.54	101.01 ± 0.54
	4.00	3.92 ± 0.03	1.35	97.75 ± 1.35
	6.00	5.73 ± 0.01	0.30	95.50 ± 0.30
	8.00	8.01 ± 0.04	0.93	100.10 ± 0.54
PAR	50.00	49.90 ± 0.90	0.52	99.79 ± 1.79
	100.00	99.49 ± 0.84	1.46	99.49 ± 0.84
	200.00	201.43 ± 1.04	0.89	100.72 ± 0.52
	300.00	305.60 ± 1.88	1.07	101.87 ± 0.63
	600.00	596.08 ± 5.94	3.43	99.35 ± 0.99

a. Mean ± standard error.
b. RSD%: relative standard deviation.
c. Mean ± standard error.

Table 6 Recovery results obtained from standard addition method for DEX, PHEN, CHL and PAR in pharmaceutical formulation

Added/ μg mL^{-1}		Found[a]/ μg mL^{-1}	Precision[b] (RSD%)	Accuracy[c], %
DEX	6.00	6.06 ± 0.02	0.69	101.06 ± 0.40
	8.00	8.02 ± 0.03	0.56	100.21 ± 0.33
	10.00	10.21 ± 0.04	0.71	102.10 ± 0.42
PHEN	5.00	4.99 ± 0.04	1.29	99.87 ± 0.74
	8.00	7.77 ± 0.03	0.78	97.08 ± 0.44
	11.20	11.24 ± 0.03	0.49	100.33 ± 0.28
CHL	2.00	2.04 ± 0.02	0.85	102.00 ± 0.50
	2.08	2.14 ± 0.01	0.47	102.88 ± 0.28
	2.24	2.23 ± 0.02	1.19	99.55 ± 0.68
PAR	50.00	50.81 ± 0.26	0.89	101.62 ± 0.52
	100.00	99.90 ± 0.08	0.14	99.90 ± 0.08
	150.00	150.34 ± 0.03	0.03	100.23 ± 0.02

a. Mean ± standard error.
b. RSD%: relative standard deviation.
c. Mean ± standard error.

$$EM = 221.1526 + 2.346664x_1 + 1.454848x_2 + 43.73774x_3 - 5.55897x_1^2 - 4.07617x_2^2 - 3.46871x_3^2 - 1.7275x_1x_2 + 1.735x_2x_3 - 0.505x_1x_3 \text{ for PAR.}$$

Helping with these equations containing coefficients, the value of the third factor was calculated for providing the optimum experimental conditions when the other two factors were valued.

Figures 3a, 3b, 3c and 3d graphically show the relationship between the response EM and the two factors, while the third is held constant at a defined level for DEX, CHL, PHEN and PAR, respectively. Figures 3a(1), 3b(1), 3c(1) and 3d(1) are shown a contour line graph for the variation of the buffer pH (x_1) and the applied voltage (kV) (x_3), while the buffer concentration (M) (x_2) was maintained constant at its optimum of 0.1 M. An increase in these variables resulted in an increase of the electrophoretic mobilities for each active ingredient. The same contour map can be seen in Figs. 3a(2), 3b(2), 3c(2) and 3d(2) between the buffer pH (x_1) and the buffer concentration (M) (x_2), while the applied voltage (kV) (x_3) was maintained constant at its optimum of 20 kV. In these, it could also be seen that the electrophoretic mobilities increased with an increase in these variables. In Figs. 3a(3), 3b(3), 3c(3) and 3d(3), there is a contour line graph for the variation of the buffer concentration (M) (x_2) and the applied voltage (kV) (x_3), while the buffer pH (x_1) was maintained constant at its optimum of 8.0. An increase in these variables resulted in an increase of the resolution between PHEN and PAR. Minitab 14 was used for drawing these graphics.

System suitability tests

System suitability tests including retention factor, separation factor, resolution, asymmetry, theoretical plates and relative standard deviations of the migration times were carried out; the results are given in Table 3. All of these results were acceptable in their limits defined by official guidelines.[14]

Method validation

Calibration curves. The calibration curves were established as described in Experimental. The results are given in Table 4.

Sensitiviy. LOQ and LOD values were calculated for each active ingredient as described in Experimental; results are given in Table 4. Lower LOQ and LOD values indicate that this developed method has a good sensitivity.

Accuracy and precision. The accuracy and the precision were studied using three different solutions for DEX, PHEN, CHL and PAR, as described in Experimental; the results are given in Table 5 as mean recoveries and relative standard deviations (RSD) along with their standard errors. High recovery values and lower RSD's indicate that the developed method could estimate the concentration values with perfect accuracy and good precision using an electropherogram of the quaternary mixture.

Analysis of pharmaceutical preparation. According to official validation guidelines,[11] in cases where it is impossible to obtain samples of all drug product components, it may be acceptable to

Table 7 Assay results of commercial pharmaceutical preparation (WINTUS® (batch No. 9054878) labeled claim = 10 mg DEX, 5 mg PHEN, 2 mg CHL and 250 mg PAR/sugar-coated tablet)

Parameter	DEX	PHEN	CHL	PAR
Mean ± SE[a]	10.25 ± 0.02	4.86 ± 0.06	2.00 ± 0.03	260.23 ± 0.58
RSD[b], %	0.50	2.54	3.14	0.49
Variance	0.0027	0.0152	0.0039	1.667
t[c]-test	0.90	2.25	0.07	0.42
F[d]-test	1.00	4.00	3.00	1.01

a. SE: standard error.
b. RSD%: relative standard deviation.
c. Theoretical value for $t = 2.31$ (at $p = 0.05$ level, compared with a high performance liquid chromatographic method developed by us).[10]
d. Theoretical value for $F = 6.39$ (at $p = 0.05$ level, compared with a high performance liquid chromatographic method developed by us).[10]

add known quantities of the analyte to the drug product for determining the recovery. For this reason, in order to know whether the excipients in the pharmaceutical preparation show any interference with the analysis, a recovery test was performed by the standard-addition method. The recoveries for this developed method obtained after three repeated experiments are summarized in Table 6. The recovery values were close to 100% indicating that there was no interference from the excipients in the capsule. No interfering peak was observed in the electropherogram of the commercial sugar-coated tablet formulations under the described conditions. Then, sample solutions were prepared according to the *Solutions* part. Satisfactory results were obtained for each compound, and were found to be in agreement with the label claims (Table 7). The results obtained by the methods described above were compared with a high-performance liquid chromatographic method developed by us statistically,[10] and no significant difference was observed for the amount of drugs using the Student t-test and the Fisher F-test at the $p = 0.05$ level in commercial formulations.

Conclusions

A fast, accurate, precise and sensitive capillary electrophoresis method for the simultaneous determination of DEX, PHEN, CHL and PAR has been developed. During optimization studies, to calculate the method robustness, a response surface methodology was successfully applied to this analytical method. Having a short running time of about 7 min, a small sample volume, consuming few volumes of run buffer for the determination of four active ingredients is an advantage for the developed capillary electrophoretic method. Also all statistical values were within the acceptable limits. The proposed method in this article was compared with a high-performance liquid chromatographic method developed by us,[10] and no significant difference was observed for the amount of drugs between these two methods. The low RSD values, high recovery values and lower LOD and LOQ values indicate good precision, perfect accuracy and good sensitivity. This developed method has been proved to be linear, sensitive, accurate and precise to be applied in the routine analysis of these drugs.

References

1. Remington, "*The Science and Practice of Pharmacy*", 20th ed., **2000**, University of the Science in Philadelphia.
2. R. H. Myers and D. C. Montgomery, "*Response Surface Methodology: Process and Product Optimization Using Designed Experiments*", **2002**, John Wiley & Sons, Inc., Canada.
3. L. Suntornsuk, *Electrophoresis*, **2001**, *22*, 139.
4. L. Suntornsuk, O. Pipitharome, and P. Wilairat, *Scientia Pharmaceutica*, **2003**, *71*, 235.
5. P. Hasemann, M. Terbalk, L. Preu, and H. Waetzig, *Electrophoresis*, **2007**, *28*, 1779.
6. L. Zhang, Q. Hu, G. Chen, and Y. Fang, *Anal. Chim. Acta*, **2000**, *424*, 257.
7. M. R. Gomez, R. A. Oisina, L. D. Martinez, and M. F. Silva, *J. Pharm. Biomed. Anal*., **2002**, *30*, 791.
8. Y. Dong, X. Chen, Y. Chen, X. Chen, and Z. Hu, *J. Pharm. Biomed. Anal*., **2005**, *39*, 285.
9. X. Liu, L. Liu, H. Chen, and X. Chen, *J. Pharm. Biomed. Anal*., **2007**, *43*, 1700.
10. I. M. Palabiyik and F. Onur, *Chromatographia*, **2007**, *66(Supple 1)*, 93.
11. "*ICH Harmonised Tripartite Guideline: Validation of Analytical Procedure Text and Methodology Q_2 (R_1) International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use*", **2005**.
12. H. Fabre, *J. Pharm. Biomed. Anal*., **1996**, *14*, 1125.
13. M. Otto, "*Chemometrics: Statistics and Computer Application in Analytical Chemistry*", **1999**, Wiley-VCH, Weinheim, Germany.
14. "*Reviewer Guidance, Validation of Chromatographic Methods*", **1994**, Center for Drug Evaluation and Research, U. S. Food and Drug Administration, FDA, Rockville, USA.