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Simultaneous determination of dexpanthenol, lidocaine hydrochloride, and mepyramine maleate in combined pharmaceutical gel by capillary electrophoresis

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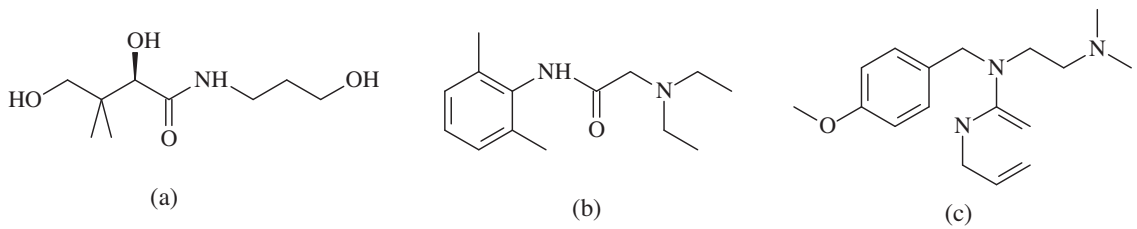
Abstract: A new capillary electrophoresis method was developed for the simultaneous determination of dexpanthenol (DEX), lidocaine (LID), and mepyramine (MEP) in pharmaceutical preparations. The best results were obtained using 20 mM (pH 3.0) phosphate buffer as the background electrolyte. Separation was obtained using a fused-silica capillary (75 μm internal diameter, 50 cm total length, 41 cm effective length) and a potential of +30 kV at 20 °C. Standards and samples were injected using a pressure injection at 50 mbar for 5 s and the analytes were monitored at a detection wavelength of 200 nm. Under optimum conditions, migration times were 2.457 min for MEP, 3.520 min for LID, and 7.363 min for DEX. The method was linear over the ranges of 25–200 μg mL^{-1} for DEX, 7.5–60 μg mL^{-1} for MEP, and 7.5–60 μg mL^{-1} for LID. Limit of detection (LOD) values were 0.8 μg mL^{-1} for MEP, 1.8 μg mL^{-1} for LID, and 3.1 μg mL^{-1} for DEX. The developed method is accurate, precise, sensitive, selective, and repeatable.

Key words: Capillary electrophoresis, dexpanthenol, lidocaine, mepyramine, pharmaceutical gel

1. Introduction

Dexpanthenol (DEX), (R)-2,4-dihydroxy-N-(3-hydroxy propyl)-3.3-dimethylbutyramide, is the alcohol analogue of pantothenic acid (vitamin B_5) and is a provitamin of B_5. DEX is a racemic mixture, but only the D form is biologically active. Lidocaine (LID), 2-diethylaminoaceto-2',6'-xylidide, is a common local anesthetic and antiarrhythmic drug. LID is used topically to relieve itching, burning, and pain, and is injected as a local anesthetic for minor surgery. Mepyramine (MEP), N-(4-methoxybenzyl)-N',N'-dimethyl-N-pyridin-2-ylethane-1,2-diamine, is a first generation antihistamine. Chemical structures of DEX, LID, and MEP are shown in the Scheme.



(a) (b) (c)

Scheme. Chemical structures of dexpanthenol (a), lidocaine (b), and mepyramine (c).

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The combination of DEX, LID, and MEP in gels is widely used because of its effective antiallergic, antiinflammatory, antipruritic, anesthetic, and antiseptic features.

Several analytical methods have been reported for the determination of DEX, LID, and MEP. Most of the assays have been reported for LID. An analytical method has been described using partial least squares multivariate calibration with UV-Vis spectroscopy for the simultaneous determination of DEX, MEP, and LID in gel form.[1] To the best of our knowledge, only one HPLC method has been reported for the determination of MEP in dosage forms.[2] Moreover, for DEX, an HPLC method[3] and a reversed-phase HPLC method[4] have been developed.

Reversed-phase HPLC methods have mostly been used for the determination of LID.[5–9] HPLC with UV[10] and MS detection[11] has been reported besides other chromatographic techniques.[12–14] Furthermore, capillary electrophoresis (CE) with direct UV detection[15] and CE with electrochemiluminescence detection have been reported for the determination of LID.[16,17]

However, a rapid, inexpensive, and simple method for the determination of DEX, MEP, and LID has not been developed yet. CE was used in this study due to its advantages over HPLC and other chromatographic techniques such as short analysis time, simple procedures before analysis, and low consumption of solvents.

In the present study, a simple, economical, accurate, reproducible, and fully validated analytical method with good detection ranges for simultaneous determination of DEX, LID, and MEP in pure forms, synthetic mixture, and pharmaceutical form was developed. In the proposed CE method, there is no need for an extraction step for DEX, MEP, and LID from the excipient matrix of pharmaceutical form, thereby decreasing inaccuracy in quantitation.

2. Results and discussion

2.1. Choice of BGE and effect of its pH

Different electrolyte solutions were investigated to optimize response in the separation and determination of DEX, MEP, and LID. In order to determine the suitable background electrolyte solution, first pH was studied between 3.0 and 9.0 using acetate, phosphate, and borate buffer solutions. Acetate and borate buffer solutions did not provide a baseline resolution for all analytes; the best separations were obtained with phosphate buffer at pH 3.0. The pKa values of DEX, MEP, and LID are 7.9, 13.03, and 8.8, respectively. For this reason, in the BGE solution all substances are cationic forms. After optimization of pH and type of buffer, the concentration of phosphate buffer was studied in the range of 10–40 mM and 20 mM phosphate concentration (pH 3.0), which provided better resolution, and a stable baseline was selected.

2.2. Optimization of electrophoretic conditions

In order to study the effects of injection time, the injection pressure was fixed at 50 mbar and injection times of 3 s, 5 s, and 10 s were studied for the best results. Electropherograms showed that 5 s gave sharper peaks with better peak symmetry as shown in Figure 1. Effects of different injection pressures were investigated and 50 mbar was chosen for the best results and shortest analysis time.

The applied voltage was varied between 10 and 30 kV under the previously optimized conditions. It is known that lower voltages cause higher migration times and broad peaks, while higher voltages can reduce the total analysis time due to rapid migration; thus +30 kV was chosen as the separation voltage. The results at this value showed that analysis time was reduced with a satisfactory resolution (Figure 2).

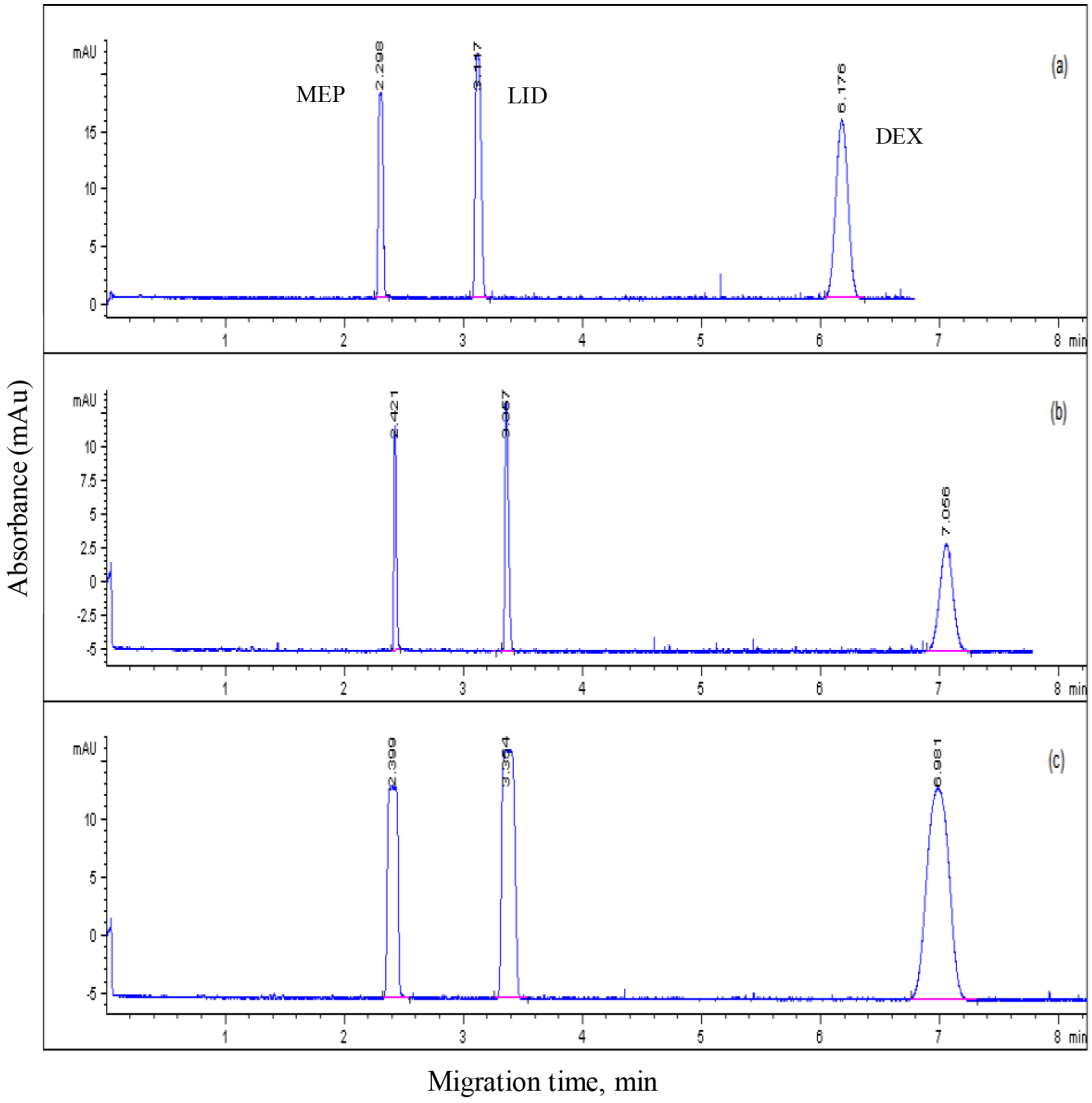

Figure 1. Effect of injection time. (a) 5 s, (b) 3 s, and (c) 10 s. Conditions: 20 mM phosphate buffer (pH 3.0), P_{inj} = 50 mbar, V = 30 kV, λ = 200 nm. Concentrations of MEP and LID: 30 μg mL^{-1}, DEX: 100 μg mL^{-1}.

Finally, the capillary temperature was studied between 20 and 30 °C. The results obtained showed that there was no significant difference between these temperatures. Therefore, temperature was set at 20 °C for further studies.

2.3. Method validation

Calibration curves of DEX, LID, and MEP were plotted under the optimized experimental conditions described above. The equations corresponding to least squares regression analysis for calibration curves and analytical performance characteristics are shown in Table 1. The obtained coefficient of determination (R^2) values proved that DEX, LID, and MEP signals gave a linear relationship for the studied concentration ranges. Limit of detection (LOD) and limit of quantification (LOQ) values were calculated using a signal to noise ratio of 3 and 10, respectively.

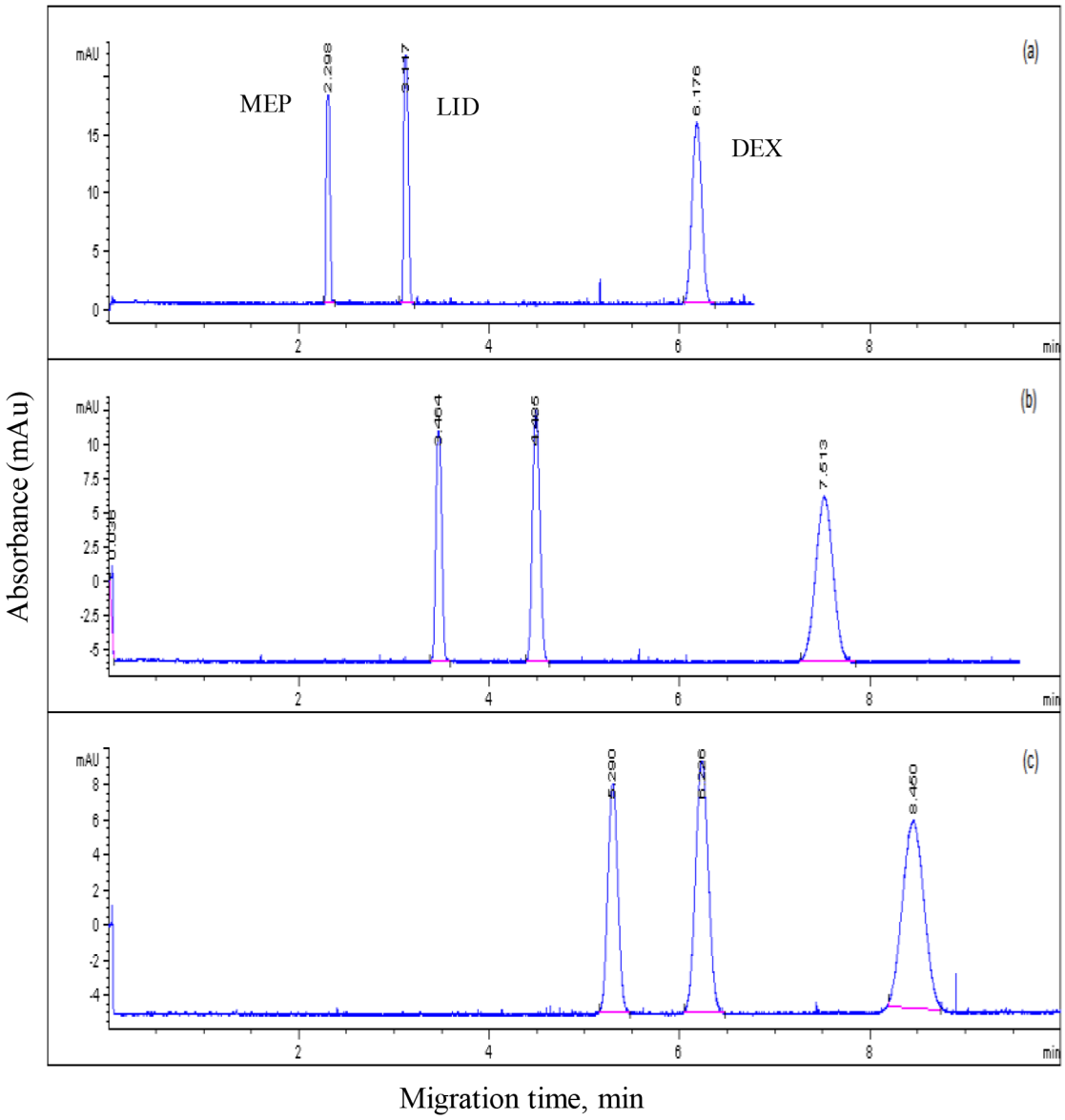

Figure 2. Effect of applied voltage. (a) 30 kV, (b) 20 kV, and (c) 10 kV. Conditions: 20 mM phosphate buffer (pH 3.0), P_{inj} = 50 mbar, t_{inj} = 5 s, λ = 200 nm. Concentrations of MEP and LID: 30 μg mL^{-1}, DEX: 100 μg mL^{-1}.

Table 1. Analytical parameters of the proposed method.

	DEX	MEP	LID
Slope	1.47 ± 0.038	1.94 ± 0.034	2.75 ± 0.033
Intercept	–12 ± 3.98	–1.66 ± 1.06	–3.10 ± 1.05
Coefficient of determination (R^2)	0.9982	0.9986	0.9994
Linear range (μg mL^{-1})	25–200	7.5–60	7.5–60
Limit of detection (μg mL^{-1})	3.1	0.8	1.8
Limit of quantification (μg mL^{-1})	10.21	2.53	5.84

Working standard solutions containing 100 μg mL^{-1} DEX, 30 μg mL^{-1} MEP, and 30 μg mL^{-1} LID were injected. Peak area, peak height, and migration time values were evaluated for precision. The relative standard deviations of MEP, LID, and DEX were 0.68, 0.34, and 0.48 for the peak areas and 1.22, 1.42, and 2.44 for the retention times, respectively. Peak areas were used to quantitate the analytes in the samples.

The method developed was applied for the determination of DEX, MEP, and LID in Stilex gel. This pharmaceutical formulation contains 50.0 mg of DEX, 15.0 mg of MEP, and 15.0 mg of LID per gram as labeled. The target analytes were analyzed for the target analytes in 5 parallel samples. Electropherograms of BGE solution; standard mixture solution of DEX, MEP, and LID; and Stilex gel solution are shown in Figure 3. The compounds formed well-shaped and symmetrical single peaks that were well separated from the background electrolyte front. The amounts found in the gel formulation and the labeled amounts are shown in Table 2. As shown in Figure 3 and Table 2, the sample matrix did not interfere with the analytes and the results found by external calibration method were significantly similar to the labeled values.

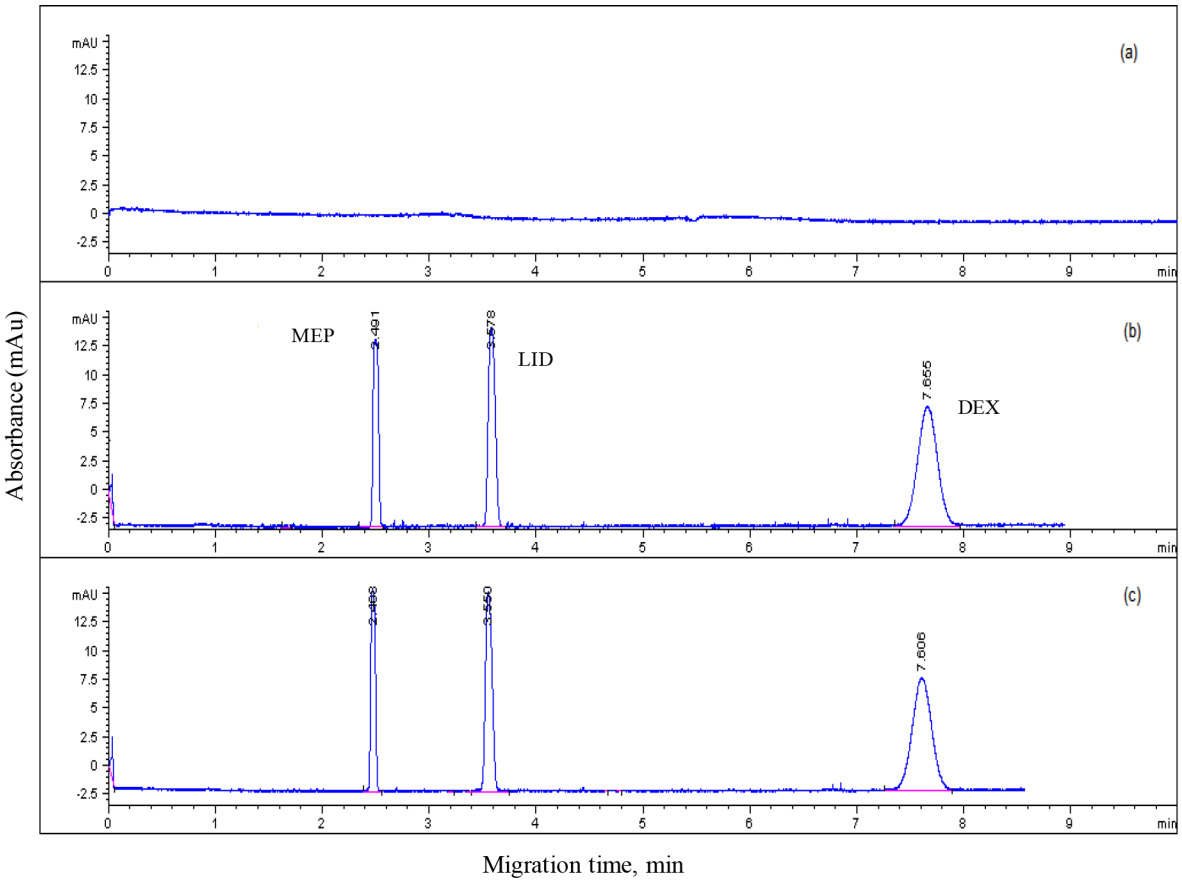

Figure 3. Electropherograms of (a) buffer solution; (b) standard mixture solution of MEP, LID, and DEX; (c) the solution of pharmaceutical gel MEP: 30 μg mL^{-1}, LID: 30 μg mL^{-1}, DEX: 100 μg mL^{-1}. Conditions: 20 mM phosphate buffer (pH 3.0), t_{inj} = 5 s, P_{inj} = 50 mbar, V = 30 kV, 20 °C, λ = 200 nm.

Recovery studies were performed with gel samples to check the accuracy of the method. Standard solutions of DEX, MEP, and LID were spiked into analyzed gel sample solution and the recovery results were obtained in the range of 97.9%–101.8% (Table 3). High recovery data showed that the developed method was accurate and free from interference by excipients used in the formulations.

Table 2. Determination of MEP, LID, and DEX in gel.

Sample	MEP found (mg) (labeled amount, 15 mg/1 g gel)	LID found (mg) (labeled amount, 15 mg/1 g gel)	DEX found (mg) (labeled amount, 50 mg/1 g gel)
1	15.05	14.82	49.90
2	14.74	15.28	50.40
3	15.10	15.10	47.90
4	14.74	14.59	50.35
5	15.15	14.46	47.75
	SD = 0.20 RSD% = 1.33 CL = 15.21–14.71 (P = 0.05)	SD = 0.34 RSD% = 2.28 CL = 15.27–14.43 (P = 0.05)	SD = 1.32 RSD% = 2.69 CL = 50.9–47.62 (P = 0.05)

CL: Confidence level

Table 3. Recovery analysis of spiked MEP, LID, and DEX in gel.

Sample	MEP%	LID%	DEX%
1	100.6	101.8	100.9
2	101.1	99.4	97.9
3	101.6	98.2	97.9
	SD = 0.50 RSD% = 0.49 CL = 102.3–99.9 (P = 0.05)	SD = 1.83 RSD% = 1.83 CL = 104.3–95.3 (P = 0.05)	SD = 1.73 RSD% = 1.75 CL = 103.2–94.6 (P = 0.05)

System suitability tests were done using migration time, peak symmetry, and peak width for the target analytes for ensuring the performance of the analytical method. For efficiency, plate numbers (N), and resolution factors (R) were calculated and satisfactory results were obtained (Table 4).

Table 4. System suitability test results.

	MEP	LID	DEX
Retention time (min)	2.457	3.520	7.363
Peak symmetry	0.99	0.80	0.97
Peak width (min)	0.056	0.070	0.195
Efficiency (plate number)	10766	14307	8143
Area (mA s)	58.5	81.1	134.4
Height (mA)	16.3	17.6	10.4
Resolution (R)	10.03		17.37
Selectivity (α)	1.43		2.10

The intraday and interday precision values of MEP, LID, and DEX were also studied. Analytes in standard mixture solution were determined 3 times a day and for 3 days. The RSD% values for the obtained peak areas were less than 1.3% as shown in Table 5 for both intraday and interday peak area values.

Table 5. Intraday and interday analysis of MEP, LID, and DEX standard solutions.

			Intraday			Interday		
Analyte	Day	Peak area	$\bar{x}$	SD	RSD%	$\bar{x}$	SD	RSD%
MEP (30 μg mL^{-1})	Day 1	58.7	58,4	0.40	0.69	59.5	0.25	0.42
		58.7						
		58.0						
	Day 2	61.7	60.9	0.75	1.23			
		60.9						
		60.2						
	Day 3	59.0	59.2	0.40	0.68			
		59.0						
		59.7						
LID (30 μg mL^{-1})	Day 1	81.9	81.4	0.45	0.55	82.9	0.23	0.28
		81.0						
		81.3						
	Day 2	84.9	84.5	0.29	0.34			
		84.4						
		84.4						
	Day 3	82.7	83.0	0.52	0.62			
		82.7						
		83.6						
DEX (100 μg mL^{-1})	Day 1	133.2	133.4	0.64	0.48	133.0	0.80	0.60
		133.0						
		134.2						
	Day 2	129.5	128.3	1.10	0.85			
		127.3						
		128.2						
	Day 3	137.9	137.4	1.11	0.81			
		136.2						
		138.3						

3. Experimental

3.1. Instrument

The analyses were performed using an Agilent 3D capillary electrophoresis system equipped with a UV-diode array detector. The separation was carried out on a 75 μm id $\times$ 50 cm (41 cm effective length) uncoated fused silica capillary. An ultrasonic water bath (Sonorex Bondelin Electronic, Germany) was used for dissolution of the gel samples. pH of the solutions was measured using an Orion 720 A plus pH/ionmeter.

3.2. Chemicals and reagents

All chemicals and solvents were of analytical grade. DEX, LID, and MEP were supplied by Refik Saydam National Public Health Agency (Ankara, Turkey). Phosphoric acid, sodium hydroxide, hydrochloric acid, and methanol were obtained from Merck (Darmstadt, Germany). The Stilex gel was kindly provided by Abdi İbrahim Pharmaceuticals (Ankara, Turkey).

Deionized water (18.2 MΩ cm) treated with the Millipore (Simplicity, 185 water purification system) Milli-Q water purification apparatus was used for all aqueous solutions.

3.3. Electrophoretic conditions and optimization

New capillaries were conditioned with 1.0 M NaOH for 15 min, deionized water for 15 min, and the background electrolyte (BGE) for 15 min. Between runs, the capillary was flushed with 0.1 M NaOH (2 min), deionized water (2 min), and BGE solution (20 mM, pH 3.0 phosphate buffer) (2 min). At the end of the day, 0.1 M NaOH for 10 min and deionized water for 10 min were used for flushing the capillary.

For optimization studies, the effects of type of BGE solution, pH and concentration of the buffer, and other instrumental parameters were investigated. These parameters included: injection time, injection pressure, applied voltage, and temperature of the capillary. During the optimization studies, the concentrations were chosen as 30 μg mL^{-1} for MEP and LID and 100 μg mL^{-1} for DEX.

3.4. Preparation of buffer and standard solutions

Stock solutions of MEP and LID with a concentration of 1500 μg mL^{-1} were prepared by dissolving 15.0 mg of pure standard material in deionized water and the volume was made up to 10.0 mL. Stock solution of DEX with a concentration of 5000 μg mL^{-1} was prepared by dissolving 50.0 mg in deionized water and the volume was made up to 10.0 mL. Stock solutions were kept at 4 °C in the dark and the working standard solutions were daily prepared by diluting stock solutions with BGE.

BGE solutions at different pH values were prepared using acetate, phosphate, and borate buffer solutions. pH of buffer solutions was adjusted to the desired value by adding 1.0 M NaOH or 1.0 M HCl.

3.5. Preparation of sample solutions

First, 1.0 g of Stilex gel was weighed and dissolved in deionized water and the volume was completed to 10.0 mL. This solution was agitated in an ultrasonic bath for 15 min and centrifuged at 5000 rpm for 10 min. Then 200 μL of this solution was transferred into a 10.0-mL volumetric flask and diluted to the final volume with BGE solution. Final concentrations were expected to be 100 μg mL^{-1} DEX and 30 μg mL^{-1} MEP and LID.

3.6. Recovery studies from gel and synthetic mixture

Recovery studies were carried out by the standard addition method to study the accuracy of the proposed method and to check the interference from excipients used in the dosage form. For this purpose, known amounts of standard DEX, LID, and MEP were added to pre-analyzed gel.

In order to investigate the interference of the compounds with each other, a laboratory-made mixture was prepared. The recovery values from this solution were calculated for each compound.

3.7. Validation procedures of the method

The validation parameters were linearity, range, selectivity, accuracy, precision, LOD, and LOQ. Intraday and interday precision parameters were calculated for 30 μg mL^{-1} MEP and LID and 100 μg mL^{-1} DEX on the same day and on 3 separate days to obtain relative standard deviations (RSD%). Accuracy values were determined by recovery studies. Percent recovery values of drugs were calculated by comparing the peak area before and after the addition of the standard solutions.

System suitability for the CE method was evaluated. Plate numbers (N), resolution factors (R), and selectivity (α) values were calculated by using migration time, peak symmetry, and peak width values.

4. Conclusions

The CE method developed in the present work is suitable for the simultaneous determination of DEX, MEP, and LID in pharmaceutical gel samples. The results showed that the proposed method was accurate, precise, selective, and repeatable. In addition, the developed method is very simple and the determination of DEX, MEP, and LID from gel matrix was achieved without any extraction procedure.

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