Development and Validation of a Capillary Electrophoretic Method for the Determination of Enalapril

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A simple, rapid and sensitive capillary electrophoresis (CE) technique for the determination of Enalapril was developed and validated. The influence of buffer pH, buffer concentration, capillary temperature, applied voltage and injection time was investigated in a fused silica capillary (50 cm x 50 μm ID). Detection wavelength was set at 214 nm. Optimum results were found with 0.067 M phosphate buffer at pH 7.0, capillary temperature 25°C and applied voltage 25 kV. The samples were injected hydrodynamically for 10 s at 35 mbar. The proposed method was validated by testing linearity, precision, accuracy, recovery, detection limit and quantification limit. The method presented a good linearity in the concentration range 10 - 100 μg/mL and the correlation coefficient was r = 0.9994. The relative standard deviation (RSD) for the precision system was 0.3864 %. The RSD value for the within-day and between-day precision was 1.7880 % and 1.8590 % respectively. The limit of detection (LOD) was 2.43 μg/mL and the limit quantification (LOQ) was 7.38 μg/mL. The percentage of recovery of the Enalapril was 100.91 %. The method was successfully applied to the quantitative determination of Enalapril from pharmaceutical forms.

Keywords: Enalapril, capillary electrophoresis, validation

Many new inhibitors of angiotensin-converting enzime (ACE inhibitors) are widely used for the treatment of mild to moderate hypertension and heart failure [1]. There are different types of ACE inhibitors, some of them being prodrugs that are more easily absorbed than the active compound [2]. Enalapril (E) is the most widely used ACE inhibitor in hypertension and it acts as a prodrug. After hydrolysis it form the diacid Enalaprilat and inhibits the ACE thus lowering blood pressure [3]. The Enalapril official drug is the maleate salt of Enalapril, that is chemically described as 1-[N-[(S)-1-carboxy-3-phenyl-propyl]-L-alanyl]-L-proline 1'-ethyl ester maleate [4].

In literature the most used techniques include the spectrophotometric methods [5-8] and high - performance liquid chromatography [9 - 12].

Capillary electrophoresis (CE) has emerged in recent years as a powerful analytical technique for a large variety of substances with large and small molecules. Due to its high rate of analysis, high efficiency and low solvent and sample consumption, this technique has gained momentum in pharmaceutical research laboratories [13].

CE has become an official method in European Pharmacopoeia 6th edition for the determination of active pharmaceutical ingredients [14].

In literature are reported a few methods for the determination of Enalapril with CE techniques from a mix of ACE inhibitors [15, 16, 17, 18, 19]; other studies were focused on the determination and separation of cis and trans rotamers of Enalapril [20] and on the determination of Enalapril from pharmaceutical forms [21, 22].

The aim of the present study was the development and validation of a capillary electrophoresis method for the determination of Enalapril. For this purpose, the influence of buffer type, buffer *p*H, buffer concentration was systemically investigated. Studies regarding validation of this method were performed according to the development and validation parameters of ICH Guidelines [23-27].

Experimental part

Materials and method

This work was performed on a Beckman Coulter P/ACE Capillary Electrophoretic system MDQ (Fullerton, CA, USA), using PC 32 Karat software, equipped with a diode array detector (DAD). Analysis was carried out in a fused-silica capillary (Beckman Coulter, USA, internal diameter 50 μm, total lenght 67 cm) with the detection window at 50 cm. For *p*H measurements, a pH meter (InoLab Level 1) calibrated with standard buffers was employed.

Enalapril maleate (EM) pur drug (purity 99.9%) as a raw material is provided from, Zhejiang Huahai Pharmaceutical co., China. All the chemicals used have been of analytical reagent grade: monopotassium phosphate (KH_2PO_4) - Fluka AG, disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4.12H_2O$) - Fluka AG, monosodium phosphate monohydrate ($NaH_2PO_4.H_2O$) - Fluka AG, orto-phosphoric acid (85 %, w/w; H_3PO_4) - Lach-ner and sodium hydroxide (NaOH) - Fluka AG.

For electrophoretic method was used a buffer system *p*H 7.0, of concentration 0.067 M, in accordance with European Pharmacopoeia 6th edition [14]. In order to obtain the recommended buffer two solutions were prepared: solution I: 0.908 g of KH_2PO_4 were dissolved in 100 mL water and solution II: 2.38 g of $Na_2HPO_4.12H_2O$ were dissolved in 100 mL water. 38.9 mL from solution I were mixed with 61.1 mL from solution II

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Stock solution of Enalapril (1000 μg/mL) was prepared with buffer solution *p*H 7.0 of concentration 0.067 M and kept in the dark at 4 °C temperature for 7 days.

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Working solutions in concentration range 10 - 100μg/mL was prepared daily from stock solution by dilution with the same buffer solution *p*H 7.0 of concentration 0.067 M. The study examined two types of tablets, one with a concentration of enalapril maleate of 10 mg/tablet and one with 20 mg/tablet.

For each type, were taken ten tablets and were accurately weighed and finely powdered and mixed. From each type of tablets, a portion of the powder equivalent to 5 mg Enalapril was transferred into a 100 mL volumetric flask and 50 mL buffer solution *p*H 7.0 of concentration 0.067 M were added. The content of the flask was sonicated for 15 min, then solution was centrifuged for 15 min at 5000 rpm to separate out the insoluble excipients. All solution were filtered through a 0.45 μm syringe filter and were made at 100 mL with the same buffer system *p*H 7.0, before injection to the CE system.

Before the first use, the capillary was conditioned by washing with 0.1 M NaOH for 20 min, then with water for 20 min. At the beginning of each working day, the capillary rinsed with 0.1 M NaOH for 15 min, water for 10 min and then the running buffer for 10 min. Because sample components can become absorbed onto the capillary surface and change the effective charge on the wall, before each injection, the capillary was preconditioned with 0.1 M NaOH (2 min), water (2 min) and running buffer (4 min).

Hydrostatic injections were performed by lifting the sample vial approximately 10 cm above the height of the buffer vial for 10 s. For detection, the absorbance was measured by means of an on-line fixed-wavelength UV detector a 214 nm filter. The experiments were performed at 25 kV at capillary temperature of 25°C.

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the following formulae: $LOD = 3.3 \cdot SD \cdot Slope^{-1}$ and $LOQ = 10 \cdot SD \cdot Slope^{-1}$; were :

SD = standard deviation of the intercept;

slope = the slope of the calibration curve equation.

Method precision was evaluated through repeatability and reproducibility. Standard addition method was used to evaluate the accuracy of the method.

Results and discussions

The electrophoretic parameters were preliminary optimized to develop a capillary electrophoresis method for determination of Enalapril with short analysis time and acceptable resolution.

Optimization of the electrophoretic conditions

The most important parameters that affect the electrophoretic process , were examined in the following order:

Influence of *p*H

The *p*H of the running buffer is the principal parameter which influences the ionization of the drug and the magnitude of the electroosmotic flow. Because Enalapril maleate have an amphoteric character, and the pK_a value of amino group is 5.36 and the pK_a value for group carboxylic is 2.97, this offers the possibility of using an acidic or an alkaline running buffer [4, 28]. At *p*H 4.0 Enalapril become zwitterion with a net charge of zero (neutral compound) and consequently the time of migration become too long. Based on the literature, it is known that at higher *p*H, Enalapril degrades increasingly, for the *p*H 12 Enalapril that remains is 1.5% [29].

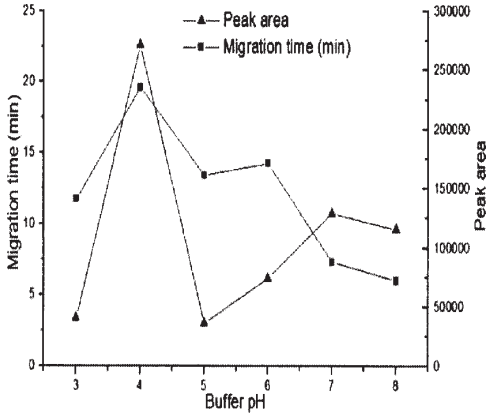

Fig. 1. Effect of buffer *p*H on migration time and peak area of Enalapril

The effect of the buffer *p*H was investigated within the range of 3.0 - 8.0 at a 0.02 M phosphate buffers concentration. In the range of 3.0 to 5.0, a mixture of a phosphoric acid solutions and disodium hydrogenphosphate solutions was used while in the *p*H range of 6.0 to 8.0, a mixture of a sodium dihydrogenphosphate solutions and a disodium hydrogenphosphate solutions was used. The solutions of Enalapril (100 μg/mL) were prepared with buffer solutions of different *p*H of 3.0 to 8.0.

In figure 1, are presented the effect of buffer *p*H on the migration time and peak area of Enalapril.

At *p*H 3.0, the shape of the peak become less symmetrical and the migration time increased. At *p*H 4.0 Enalapril become zwitterion with a net charge of zero (neutral compound) consequently the migration time was too long. At *p*H 5.0 and 6.0 the shape of the peaks are improved, but migration time is too long. The peak symmetry and migration time is acceptable at *p*H 7.0. Although at *p*H 8.0 the migration time is much better, the baseline creates problems and Enalapril solution is unstable. The *p*H optimum for determination of Enalapril was found to be 7.0.

Influence of molarity

As well as *p*H, the ionic strength of the running buffer is another parameter that controls the migration time of Enalapril. The effect of phosphate buffer was examined by varying the concentration from 0.025 M to 0.1 M at *p*H 7.0. All solutions were made in accordance with the European Pharmacopoeia [14]. The solutions of Enalapril (100 μg/mL) were prepared with phosphate buffer solutions of different concentrations. As shown in table 1, when phosphate buffer concentration was increased, the migration time and the peak area of Enalapril increased. A concentration of 0.067 M phosphate buffer at *p*H 7.0 was selected as optimum for running buffer since it maintains

Table 1

EFFECT OF BUFFER CONCENTRATION ON MIGRATION TIME AND PEAK AREA OF ENALAPRIL

Buffers pH 7.0	Buffer concentration (M)	Migration time (min)	Peak area
KH_2PO_4 $Na_2HPO_4.12H_2O$	0.1	19.417	257810
KH_2PO_4 $Na_2HPO_4.12H_2O$	0.067	11.813	204946
Na_2HPO_4 anh. $NaH_2PO_4.H_2O$	0.063	11.088	187682
KH_2PO_4 $Na_2HPO_4.12H_2O$	0.05	9.483	176092
Na_2HPO_4 anh. $NaH_2PO_4.H_2O$	0.025	8.171	143757

a good peak shape, a good migration time, low peak width and higher efficiency.

Under these optimized conditions, the migration time of Enalapril was 11.9.

Effect of applied voltage

The applied voltage was gradually increased from 15 to 30 kV. The best resolution was observed when applying a voltage of 25 kV. At 15 kV, no signals were detected. Further increase in the applied voltage of more than 25 kV resulted in a decreased resolution.

Effect of injection time

Injection time effects on the peak width and peak height. In order to improve sensitivity, sample solutions were hydro-dynamically injected at 35 mbar while the injection time was varied from 5 to 15 s. The peak area increased with increasing injection time. After 10 s, the peak shapes of Enalapril was deformed, so 10 s was selected as the optimum injection time. The pressure used for injection was always 35 mbar.

Stability in solution

Investigation of the stability was established for 50μg/mL solution of Enalapril in phosphate buffer *p*H 7.0 of concentration 0.067 M, kept in the dark at 4 °C for 7 days. The results presented in figure 2, shows that the solution of Enalapril was stable under these conditions. Additional peaks were not found in the electrophoregram throughout the analysis time, indicating the stability of drug in the solution.

From these optimization studies, the following electrophoretic conditions were selected as optimal:

- running buffer: 0.067 M phosphate buffer at *p*H 7.0;
- capillary: fused silica capillary (total length 67cm and effective length 50 cm, 50 μm ID)

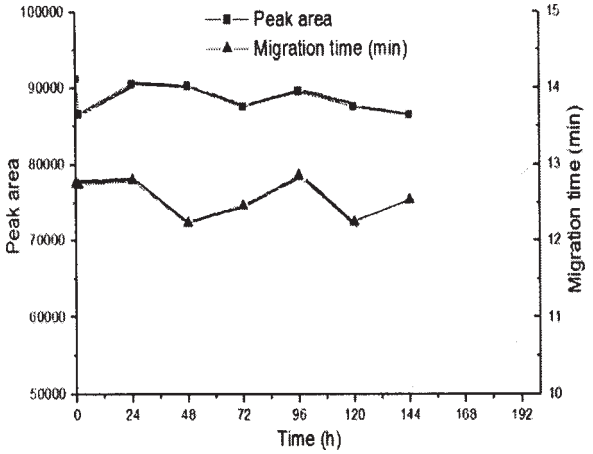

Fig. 2. Study of Enalapril stability in running buffer

- injection: hydrodynamically, 10 s at 35 mbar;
- voltage: 25kV;
- temperature: 25 °C;
- detection wavelength: 214 nm

Validation of the method

The main objective of validation of an analytical procedure is to demonstrate that the procedure is suitable for its intended purpose. In this study, the capillary electrophoresis method for determination of Enalapril was validated according to the ICH guidelines for specificity, linearity, range, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ).

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. These might include impurities, degradation product and excipients. The electropherogram in figure 3, demonstrates the specificity of the method.

It is observed at electrophoregram the existence of a single peak which is allocated to Enalapril.

Linearity range

Under the optimum analysis conditions, linearity was studied in the concentration range of 10 - 100 μg/mL for Enalapril. Calibration curve was constructed with 10 different Enalapril concentration. Each point of the calibration graph corresponded to the mean value obtained from 5 independent measurements and the calibration curve obtained is shown in figure 4.

In literature, the presented methods have a much larger area of concentration 80-640 g/mL, but from a higher concentration than the one obtained in this research [22].

The parameters obtained for the validation of the method are summarized in table 2.

Precision

In order to evaluate the precision of the system (while keeping the operating conditions identical), ten consecutive injection were made with a standard solution containing 50μg/mL Enalapril and the results were evaluated by considering migration time and peak area. The RSD value for repeatability of the system is 0.3864 % and indicated that the system is precisely.

The precision of a method is defined as the closeness of agreement between independent test results obtained under optimum conditions. Three different concentration of Enalapril (40, 50 and 60 μg/mL) were analyzed in three independent series in the same day (intra-day precision) and the same concentration of Enalapril in three replication were injected next day (inter-day precision). The precision of the method was determined by calculating the relative

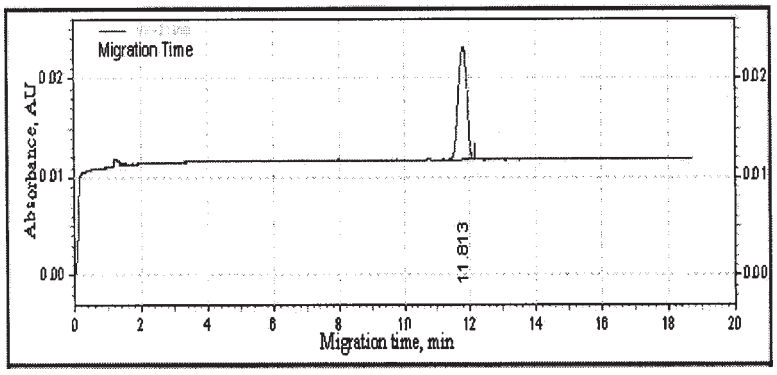

Fig. 3. The electrophoregram of 50 μg/mL Enalapril in 0.067 M phosphate buffer *p*H 7.0

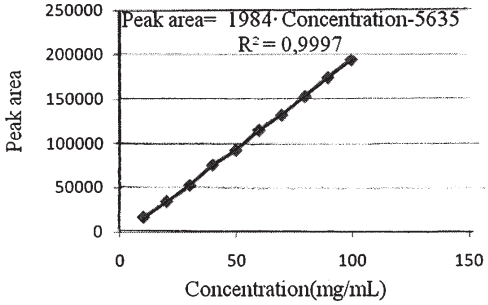

Fig. 4. Calibration curve

Parameters	Values
Linearity range (μg/mL)	10 - 100
Regression equation	$Peak\ area = 1984 \cdot Concentration + 5635$
Correlation coefficient (r)	0.9994
Regression coefficient (r^2)	0.9997
Slope	1984.24
Intercept	-5635.16
Standard error (SE)	1466.33
LOD (μg/mL)	2.43
LOQ (μg/mL)	7.38

Table 2
VALIDATION OF PARAMETERS DETERMINAED BY PROPOSED METHOD

Concentration (μg/mL)	Intra-day precision			Inter-day precision		
	Peak area	Concentration found (μg/mL)	Recovery (%)	Peak area	Concentration found (μg/mL)	Recovery (%)
40	73468	39.87	99.66	73968	40.12	100.29
	74710	40.49	101.23	74510	40.39	100.98
	75997	41.14	102.85	76597	41.44	103.61
50	92780	49.60	99.20	92680	49.55	99.0
	91554	48.98	97.96	91214	48.81	97.62
	93948	50.19	100.37	94248	50.34	100.68
60	116387	61.50	102.49	115387	60.99	101.65
	115356	60.98	101.63	117356	61.98	103.31
	117356	61.98	103.31	114356	60.47	100.79
Statistical data	Mean = 100.97 SD = 1.8053 RSD = 1.7880			Mean = 100.89 SD = 1.8756 RSD = 1.8590		

Table 3
THE RESULTS OF PRECISION VALUE FOR PROPOSED METHOD

Theoretical concentration (μg/mL)	Peak area	Calculated concentration (μg/mL)	Recovery (%)
40	74168	40.22	100.55
	75510	40.90	102.25
	76297	41.30	103.24
50	93180	49.81	99.61
	92814	49.62	99.24
	94148	50.29	100.59
60	113007	59.80	99.67
	116956	61.79	102.98
	113496	60.05	100.08
Statistical data	Mean	100.91	
	Min	99.24	
	Max	103.24	

Table 4
THE RESULTS OF ACCURACY VALUE FOR PROPOSED METHOD

Concentration declarated/tablet (mg)	Peak area	Concentration calculated (μg/mL)	Enalapril found /tablet (mg/T)	Recovery ± RSD[a] (%)	Acceptance value (± 7.5 %)
10	93225	49.83	9.96	99.6	9.25 - 10.75
	92945	49.69	9.94	99.4	
	94158	50.29	10.06	100.6	
Average value		49.94	9.99	99.87 ± 0.64	
20	94012	50.22	20.09	100.45	18.5 - 21.5
	93752	50.09	20.04	100.2	
	93255	49.84	19.94	99.7	
Average value		50.05	20.02	100.12 ± 0.38	

mean ± RSD for three determinations

Table 5
ANALYSIS OF ENALAPRIL IN TABLETS BY THE PROPOSED METHOD

standard deviation (RSD %). The RDS values of intra-day and inter-day studied varied from 1.7880 to 1.8590 and showed that the precision method was satisfactory. The analytical results obtained from this investigation were summarized in table 3.

Accuracy

Accuracy has been determination by the addition method and has been evaluated as percentage relative error between the measured and theoretical concentration of Enalapril. The results for the accuracy of the proposed method are presented in table 4. These values proved that the proposed method was accurate.

Analysis from tablets

The CE method was applied to the determination of Enalapril in tablets. The amounts of Enalapril in tablets were calculated using calibration curve method. The obtained percentage recoveries and relative standard deviations based on the average of three replicate measurements were found and the results are presented in table 5.

The results show that the commonly used excipients in the preparation of tablets (such as starch, lactose, talc and magnesium stearate) were found not to interfere in the analysis. All the results from uncoated tablets are within permissible deviation presented in European Pharmacopoeia [14].

Conclusions

The proposed method has the advantage of a simple processing of the running buffer and samples, so it can become fast, inexpensive, precise and non-time-consuming for the determination of Enalapril. The small value of the detection limit shows that the method is sensitive and the 100.91 % value of recovery indicate the high accuracy of the method. In addition, the proposed

method can be applied to the quality control analysis of Enalapril in pharmaceutical forms.

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