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Simultaneous determination of hydrochlorothiazide and several inhibitors of angiotensin-converting enzyme by capillary electrophoresis

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Abstract

A capillary electrophoresis method was developed for the simultaneous determination of hydrochlorothiazide and several angiotensin-converting enzyme (ACE) inhibitors: enalapril, lisinopril, quinapril, fosinopril, ramipril, and cilazapril. The most critical parameter is the pH of the running buffer. Separation was performed on a fused-silica capillary (52 cm total length×75 μm I.D.) using a sodium phosphate buffer (pH 7.25; 100 m*M*). The method was successfully applied to the quantitative determination of these compounds in their corresponding pharmaceutical formulation. The method was validated in terms of linearity of response, reproducibility and accuracy.

Keywords: Pharmaceutical analysis; Hydrochlorothiazide; ACE inhibitors

1. Introduction

Inhibitors of angiotensin-converting enzyme (ACE) are widely used for the treatment of mild to moderate hypertension and heart failure either alone or in conjunction with hydrochlorothiazide, a thiazide diuretic [1]. Hydrochlorothiazide increases the rate of urine excretion by the kidneys, primarily through decreased tubular reabsorption of sodium and chloride and by increased osmotic transport of water to the renal tubules. Thiazide diuretics are extremely useful in the treatment of oedema associated with mild to moderate congestive heart failure. Moreover, these diuretics are also primary agents used in the control of hypertension, either alone or in combination with other drugs such as ACE inhibitors. Their hypotensive effect is believed to be due initially to the reduction of blood volume by Na^+ depletion, and later by direct relaxation of arteriolar smooth muscle [2,3].

Until now, high-performance liquid chromatography has been the major technique used for the simultaneous determination of the concentration and presence of hydrochlorothiazide (HCT) and enalapril maleate [4–6], lisinopril [7,8] and benazepril [9]. Capillary electrophoresis (CE) offers an alternative technique. Although analysis by means of CE has been achieved for both hydrochlorothiazide [10–12] and the different ACE inhibitors [13–18], these studies have usually been limited to the determination of a single component [13–16]. Only one study has reported the simultaneous determination of

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hydrochlorothiazide and enalapril maleate by CE [19]. Therefore, the aim of the present study was to develop a selective method capable of separating hydrochlorothiazide and a large number of structurally related ACE inhibitors by CE. The chemical structures of these compounds are shown in Fig. 1.

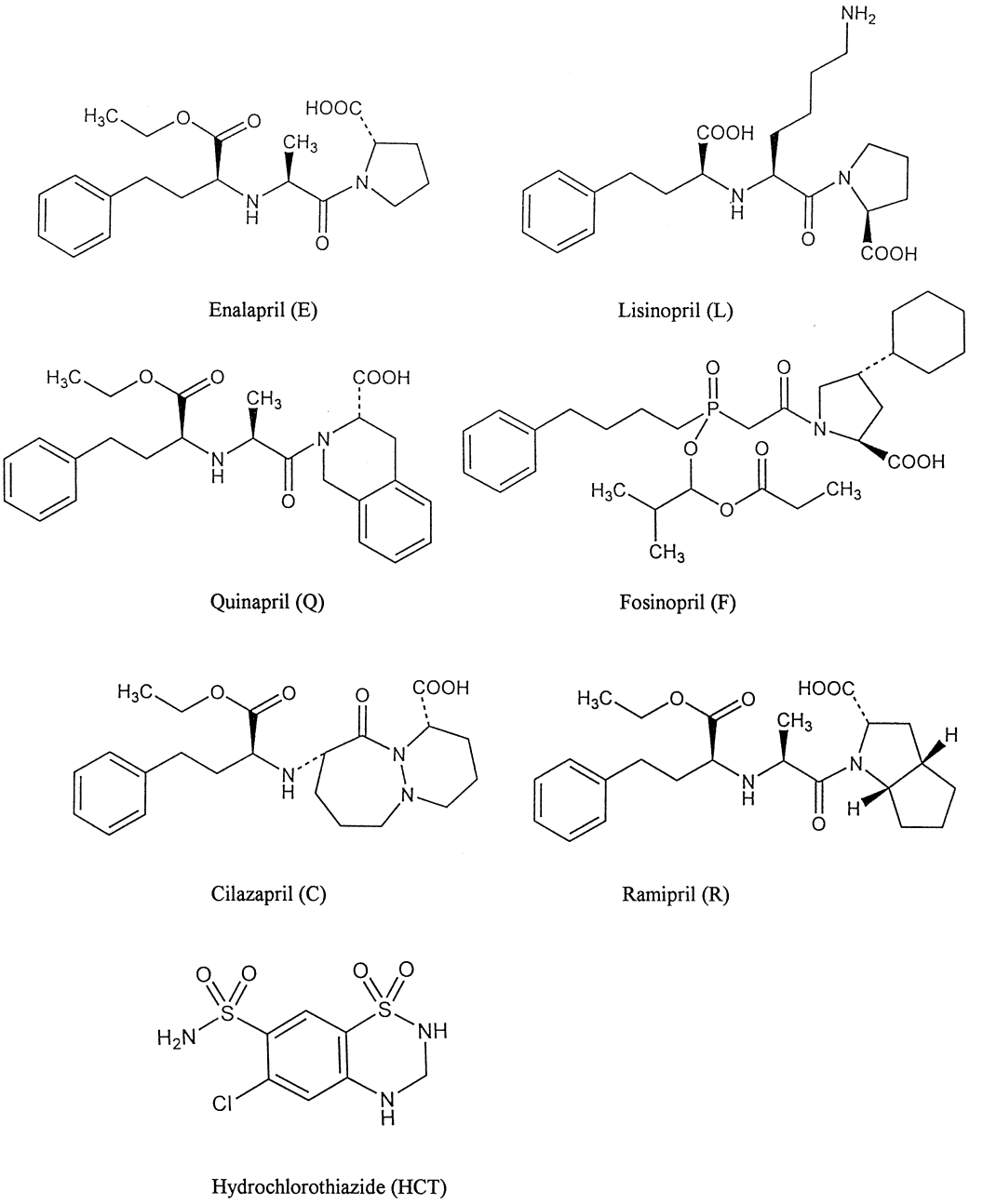

Fig. 1. Chemical structures of the ACE inhibitors and hydrochlorothiazide.

Table 1
Reference solutions for the quantitative determination

Reference substance	Reference solution (mg/100 ml)	Diluted reference solution (mg/ml)
Enalapril maleate	±60	±0.24
HCT	±38	±0.15
Lisinopril dihydrate	±80	±0.32
HCT	±50	±0.20
Quinapril·HCl (10 mg/tablet)	±60	±0.24
HCT	±70	±0.28
Quinapril·HCl (20 mg/tablet)	±60	±0.24
HCT	±35	±0.14
Fosinopril sodium	±56	±0.22
HCT	±35	±0.14
Ramipril	±17.5	±0.07
HCT	±87.5	±0.35
Cilazapril·HCl	±28	±0.11
HCT	±70	±0.28

2. Experimental

2.1. Instrumentation and electrophoresis procedure

The method was developed and subsequent experiments were performed using a Waters Quanta 4000 CE instrument (Millipore, Waters). The capillary used was a fused-silica capillary 52 cm in total length (44.5 cm to the detector) with an internal diameter of 75 μm.

Hydrostatic injections were performed by lifting the sample vial ~10 cm above the height of the buffer vial for 10 s. For detection, absorbance was measured by means of an on-line fixed-wavelength UV detector with a zinc discharge lamp and a 214-nm filter. The experiments were performed at 20 kV at room temperature (20±2°C). Data were collected on a Hewlett-Packard Integrator (HP 3396, Series II), which was also used for calculating the areas under the peaks.

2.2. Reagents

Sodium dihydrogenphosphate monohydrate and disodium hydrogenphosphate dihydrate (both analytical-reagent grade) were obtained from Merck (Darmstadt, Germany). Enalapril maleate and lisinopril dihydrate were purchased from Sigma (St. Louis, MO, USA), and hydrochlorothiazide from Profarma (Belgium). Lisinopril dihydrate was obtained from MSD and AstraZeneca, quinapril·HCl from Parke-Davis, fosinopril sodium from Bristol-Myers Squibb, ramipril from AstraZeneca, and cilazapril from Roche.

The commercially available drugs Co-Renitec (MSD), Zestoretic (AstraZeneca), Accuretic (Parke-Davis), Foside (Solvay), Tritiazide (Hoechst Marion Roussel) and Co-Inhibace (Roche) were used for quantitative determinations.

2.3. Running buffers

During the development of the method, sodium phosphate buffers of different pH values were tested as running buffers. A mixture of a sodium dihydrogenphosphate solution and a disodium hydrogenphosphate solution was used with a pH range of 6.5–8.5.

A sodium phosphate buffer (pH 7.25; 100 m*M*) was finally chosen as the running buffer. It was prepared by adjusting the pH of a 100 m*M* disodium hydrogenphosphate solution to pH 7.25 by the addition of 100 m*M* sodium dihydrogenphosphate solution.

2.4. Internal standard solutions

For the quantitative determination of HCT and one of the six ACE inhibitors, another ACE inhibitor was always used as an internal standard. Selection had to be made based on the substance to be examined. Lisinopril dihydrate was mostly chosen as the internal standard, because of its baseline separation from all the other ACE inhibitors and because of its ready availability as a bulk product on the market. For the determination of lisinopril, another ACE inhibitor must be used. In this investigation, quinapril was chosen. An appropriate amount of the compound (Table 2) was dissolved in 10 ml of methanol and diluted to 100.0 ml with water.

2.5. Choice of solvent

The running buffer cannot be used as a solvent for the preparation of reference and sample solutions because of the poor solubility of hydrochlorothiazide. Although an alkaline solution should be suitable to dissolve both the diuretic and the ACE inhibitors, this medium must be avoided, as it is unstable. Therefore, methanol was added to dissolve the active substances, and the solutions were then diluted with water.

Table 2
Sample preparation for the quantitative determination

	Average mass (mg)	Sample solution (mg powder/50 ml)	Internal standard solution (mg/ml)	Diluted sample solution (mg active substance/ml)
Enalapril (E) 20 mg HCT 12.5 mg (Co-Renitec, tablets)	200.4	±301	Lisinopril·$2H_2O$: 1	E: ±0.24 HCT: ±0.15
Lisinopril (L) 20 mg HCT 12.5 mg (Zestoretic, tablets)	226.0	±452	Quinapril·HCl: 0.5	L: ±0.32 HCT: ±0.20
Quinapril (Q) 10 mg HCT 12.5 mg (Accuretic 10/12.5, tablets)	103.5	±285	Lisinopril·$2H_2O$: 1	Q: ±0.22 HCT: ±0.28
Quinapril (Q) 20 mg HCT 12.5 mg (Accuretic 20/12.5, tablets)	207.2	±290	Lisinopril·$2H_2O$: 1	Q: ±0.22 HCT: ±0.14
Fosinopril (F) sodium 20 mg HCT 12.5 mg (Foside, tablets)	201.9	±283	Lisinopril·$2H_2O$: 0.8	F: ±0.22 HCT: ±0.14
Ramipril 5 mg HCT 25 mg (Tritazide, tablets)	102.6	±180	Lisinopril·$2H_2O$: 0.8	R: ±0.07 HCT: ±0.35
Cilazapril 5 mg HCT 12.5 mg (Co-Inhibace, tablets)	203.2	±570	Lisinopril·$2H_2O$: 0.8	C: ±0.11 HCT: ±0.28

2.6. Reference solutions

Reference solutions were prepared by weighing accurately an appropriate amount of the corresponding reference substance, dissolving it in 10 ml methanol and diluting to 100.0 ml with water (Table 1). A volume of 10.0 ml of each solution was mixed with 10.0 ml of the internal standard solution and diluted to 25 ml with a methanol–water (1:9) mixture.

2.7. Sample preparations

A minimum of 20 tablets of each compound were weighed, ground, and mixed. An appropriate amount of the powder (Table 2) was mixed with 5 ml methanol and diluted to 50.0 ml with water. Then 10 ml of the filtrate was mixed with 10.0 ml of the appropriate internal standard solution (Table 2) and diluted to 25 ml with a methanol–water (1:9) mixture.

All samples and buffers were filtered by passing them through a Millipore filter unit (0.45-μm pore size).

3. Results and discussion

3.1. Optimization of the method

The optimization of a selective capillary electrophoretic separation of several ACE inhibitors was described earlier [18]. Separation was performed by means of two phosphate buffers (each 100 m*M*) at pH 7.0 and 6.25, respectively. This combination is necessary for the selective identification of the structurally related substances because of their similar pK_a values. The addition of organic modifiers and surfactants had a negative influence on peak symmetry, and selectivity was not improved [18]. Based on these data, the pH was varied in the range of 6.5–8.5. Separation is greatly influenced by the pH of the medium (Fig. 2). Below pH 7.0, hydrochlorothiazide and the marker used (formamide) co-elute because

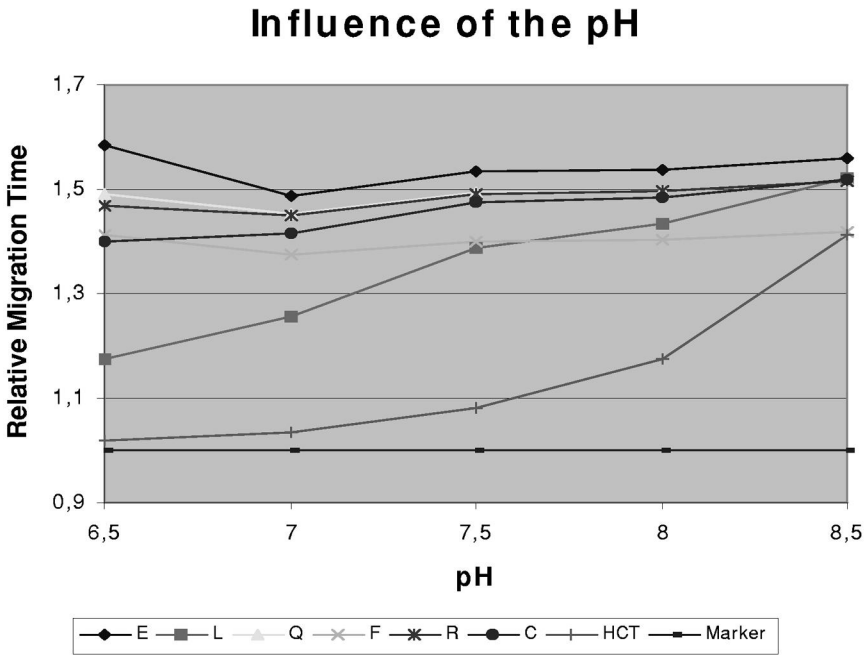

Fig. 2. Influence of pH. Experiments were carried out using a fused-silica capillary 52 cm (44.5 cm to the detector)×75 μm I.D., and sodium phosphate buffer (100 m*M*) at varying pH as the running buffer, an applied voltage of 20 kV, and a detection wavelength of 214 nm.

they are both uncharged (i.e. there is no selectivity). From pH 8.0 and higher, the separation of hydrochlorothiazide and the ACE inhibitors becomes critical because all species become negatively charged and consequently some co-elute. The optimum pH for the separation of HCT and the ACE inhibitors was in the range 7.0–8.0. The optimum conditions for the separation of HCT and the majority of the ACE inhibitors were found using a sodium phosphate buffer (pH 7.25).

As well as pH, the ionic strength of the running buffer is another parameter that controls the retention of these compounds. We varied the molarity of the sodium phosphate buffers (see above) from 50 to 100 m*M*. The selectivity of the separation was not influenced by the molarity, and only the migration times increased. Because it had the highest buffering capacity and provided acceptable migration times, a sodium phosphate buffer (100 m*M*) was chosen.

3.2. Quantitative determination in pharmaceutical formulations

The proposed system (a sodium phosphate buffer, pH 7.25, 100 m*M*) may be applied for the quantitative determination of the combination of HCT/ACE inhibitors in tablets (Figs. 3–7).

3.3. Validation of the method

3.3.1. Linearity

The detector responses were found to be linear for the different components in two concentration ranges, as described in Table 3. The amount of the internal standard was adjusted according to the concentration range used. Regression analysis data for the calibration curves were calculated using the peak areas.

3.3.2. Precision

The precision (repeatability) was determined by the total analysis of six replicate samples under the same operating conditions, by the same analyst, and on the same day. The mean value of the concentration and the relative standard deviation are summarized in Table 4.

The error of the equipment, the accuracy of electrophoretic separation, and the relative standard

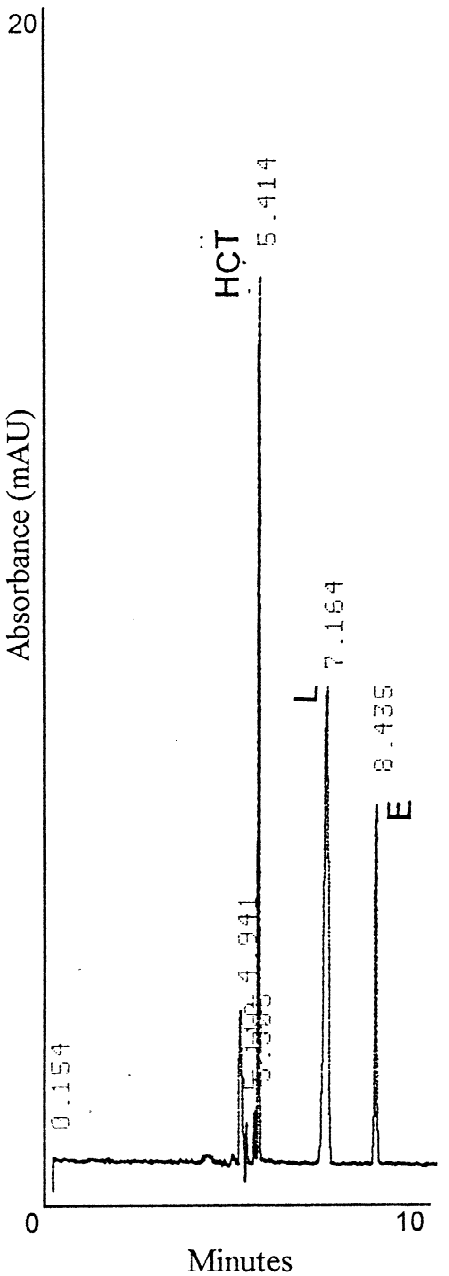

Fig. 3. Electropherogram of the quantitative determination of HCT and enalapril (Co-Renitec) on a fused-silica capillary, performed on the Waters Quanta. Conditions: 52 cm (44.5 cm to the detector)×75 μm I.D.; sodium phosphate buffer (pH 7.25; 100 m*M*) as running buffer; applied voltage, 20 kV; detection at 214 nm.

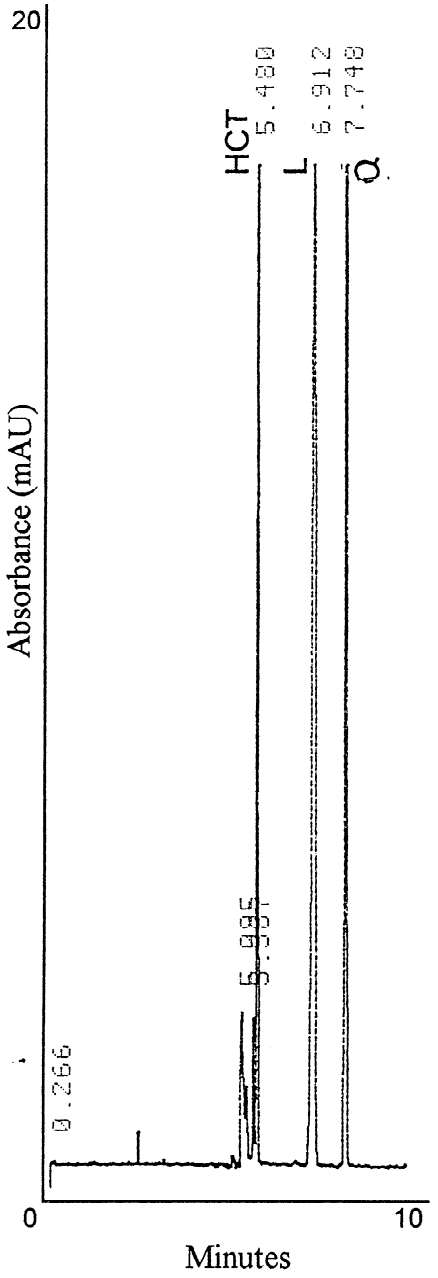

Fig. 4. Electropherogram of the quantitative determination of HCT and quinapril (Accuretic) on a fused-silica capillary, performed on the Waters Quanta. Conditions: 52 cm (44.5 cm to the detector)×75 μm I.D.; sodium phosphate buffer (pH 7.25; 100 m*M*) as running buffer; applied voltage, 20 kV; detection at 214 nm.

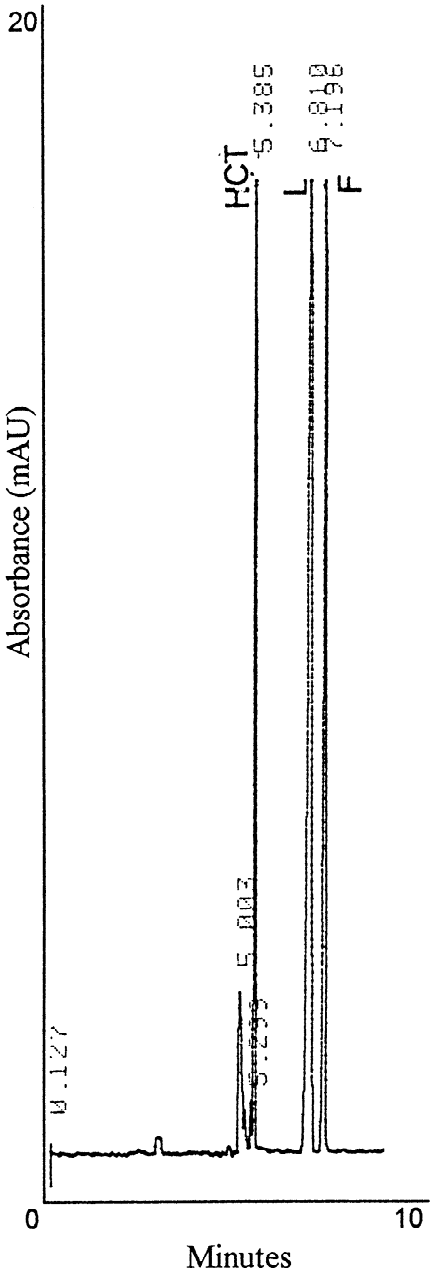

Fig. 5. Electropherogram of the quantitative determination of HCT and fosinopril (Foside) on a fused-silica capillary, performed on the Waters Quanta. Conditions: 52 cm (44.5 cm to the detector)×75 μm I.D.; sodium phosphate buffer (pH 7.25; 100 m*M*) as running buffer; applied voltage, 20 kV; detection at 214 nm.

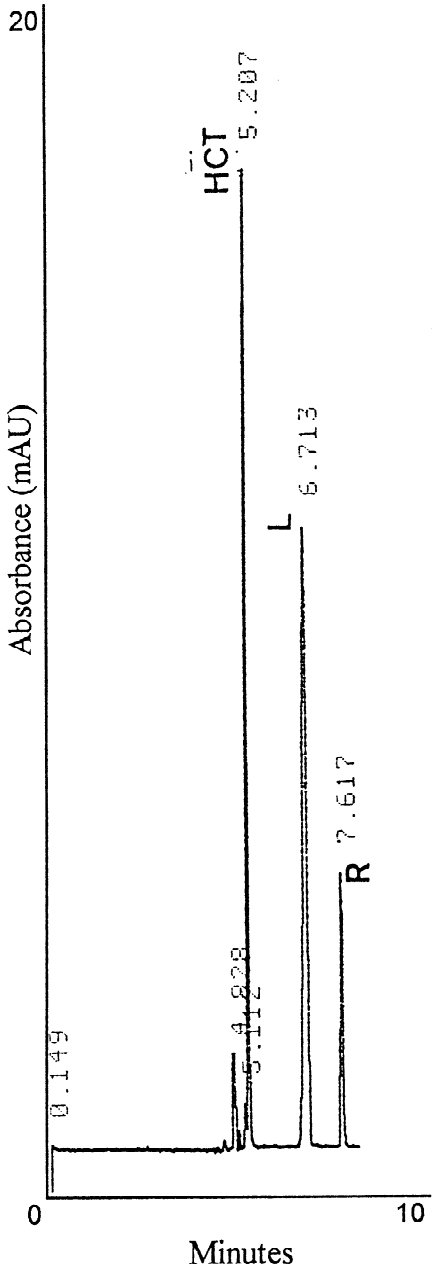

Fig. 6. Electropherogram of the quantitative determination of HCT and ramipril (Tritiazide) on a fused-silica capillary, performed on the Waters Quanta. Conditions: 52 cm (44.5 cm to the detector)×75 μm I.D.; sodium phosphate buffer (pH 7.25; 100 m*M*) as running buffer; applied voltage, 20 kV; detection at 214 nm.

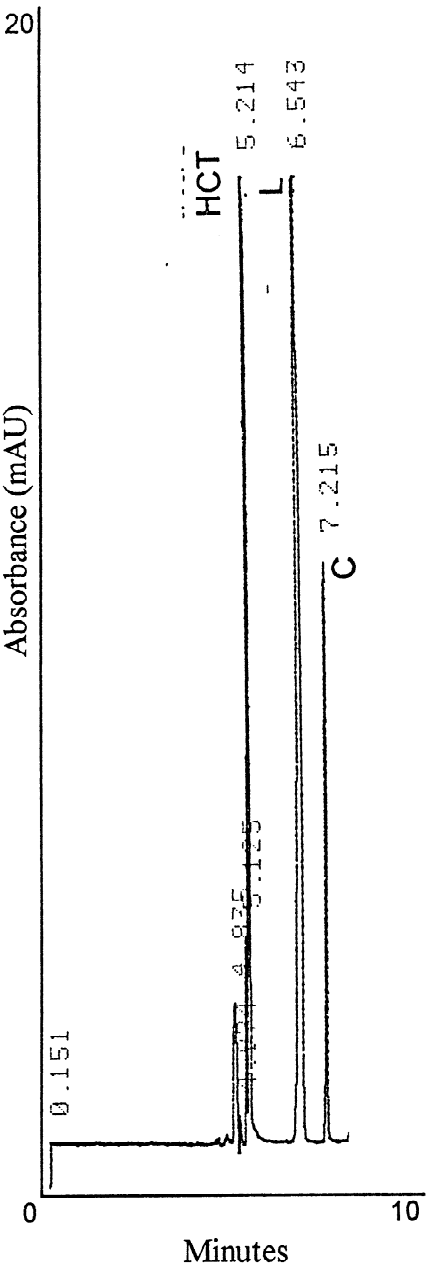

Fig. 7. Electropherogram of the quantitative determination of HCT and cilazapril (Co-Inhibace) on a fused-silica capillary, performed on the Waters Quanta. Conditions: 52 cm (44.5 cm to the detector)×75 μm I.D.; sodium phosphate buffer (pH 7.25; 100 m*M*) as running buffer; applied voltage, 20 kV; detection at 214 nm.

Table 3
Linearity

	Concentration range (mg/ml)	Correlation coefficient (r^2)
Enalapril maleate	0.026–0.320	0.9991
HCT	0.016–0.200	0.9998
Lisinopril dihydrate	0.026–0.520	0.9999
HCT	0.020–0.400	0.9995
Quinapril·HCl	0.032–0.320	0.9994
HCT	0.020–0.400	0.9997
Fosinopril sodium	0.019–0.380	0.9991
HCT	0.010–0.200	0.9992
Ramipril	0.006–0.120	0.9997
HCT	0.020–0.400	0.9997
Cilazapril	0.008–0.160	0.9995
HCT	0.020–0.400	0.9995

deviations of estimations were determined by performing ten consecutive injections of the same sample (Table 5).

3.3.3. Accuracy

The accuracy of the method was determined by investigating the recovery of each component at three levels, ranging from 80 to 120% of the theoretical concentration, from placebo mixtures spiked with the active substance (Table 6).

4. Conclusions

The above results demonstrate that CE separation of hydrochlorothiazide and one of the six ACE inhibitors can be achieved using a 100 m*M* sodium phosphate buffer at pH 7.25. This system can be

Table 4
Precision (repeatability) of the total analysis of six replicate samples

Substance to be examined	Theoretical amount (mg/tablet)	Amount found (mg)	Relative standard deviation ($n=6$) (%)
Enalapril maleate	20	20.05±0.14 or 100.2%	0.73
HCT	12.5	12.53±0.04 or 100.2%	0.30
(Co-Renitec)			
Lisinopril·2H_2O	20	20.44±0.05 or 102.2%	0.22
HCT	12.5	12.60±0.06 or 100.8%	0.48
(Zestoretic)			
Quinapril·HCl	10	10.12±0.42 or 101.2%	0.42
HCT	12.5	12.60±0.77 or 100.8%	0.77
(Accuretic 10/12.5)			
Quinapril·HCl	20	20.12±0.19 or 100.6%	0.92
HCT	12.5	12.53±0.06 or 100.2%	0.46
(Accuretic 20/12.5)			
Fosinopril sodium	20	19.47±0.10 or 97.4%	0.53
HCT	12.5	11.97±0.07 or 95.8%	0.56
(Foside)			
Ramipril	5	4.89±0.05 or 97.8%	0.92
HCT	25	24.01±0.05 or 96.0%	0.21
(Tritiazide)			
Cilazapril	5	5.23±0.02 or 104.6%	0.38
HCT	12.5	12.35±0.05 or 98.8%	0.39
(Co-Inhibace)			

Table 5
Repeatability of ten consecutive injections of the same sample

Sample solution	Relative standard deviation ($n=10$) (%)
Enalapril maleate	0.76
HCT	0.51
Lisinopril·$2H_2O$	0.56
HCT	0.52
Quinapril·HCl	0.50
HCT	0.44
Fosinopril sodium	1.08
HCT	0.47
Ramipril	1.23
HCT	0.57
Cilazapril	1.03
HCT	0.46

applied successfully to the identification and the quantitative determination of these compounds in pharmaceutical formulations.

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Table 6
Accuracy

	Recovery placebo+80% ($n=3$) (%)	Recovery placebo+100% ($n=3$) (%)	Recovery placebo+120% ($n=3$) (%)
Enalapril	100.3±0.6	99.5±0.2	100.4±0.4
HCT	99.4±0.1	101.2±0.1	99.1±0.1
Lisinopril	98.9±0.4	100.0±0.5	101.4±0.6
HCT	102.1±0.9	103.0±0.6	97.7±0.6
Quinapril 10 mg	99.5±0.5	100.5±0.6	100.9±0.4
HCT	101.3±0.2	100.1±0.5	99.8±0.5
Quinapril 20 mg	99.1±0.2	102.9±0.4	100.2±0.3
HCT	99.3±0.3	100.0±0.4	100.3±0.4
Fosinopril	103.9±0.2	101.2±0.4	102.2±0.5
HCT	98.7±0.4	101.6±0.9	99.6±0.7
Ramipril	101.0±0.3	100.4±0.6	98.6±0.4
HCT	104.2±0.4	100.5±0.6	100.5±0.5
Cilazapril	102.8±0.1	102.4±0.8	101.8±0.7
HCT	104.6±0.5	102.2±0.8	100.1±0.6

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