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# The quantitative determination of several inhibitors of the angiotensin-converting enzyme by CE

S. Hillaert *, W. Van den Bossche

*Laboratory of Pharmaceutical Chemistry and Drug Analysis, Department of Pharmaceutical Analysis, Faculty of Pharmaceutical Sciences, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium*

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**Abstract**

Capillary electrophoresis (CE) was applied to the study of several inhibitors of the angiotensin-converting enzyme. Separation of the compounds was performed by means of two phosphate buffers (each 100 mM) at pH 7.0 and 6.25, respectively [S. Hillaert, W. Van den Bossche, J. Chromatogr. A, 895 (2000) 33–42.]. Due to the highest selectivity of the first mentioned running buffer, the same system has been applied for the quantification of enalapril, lisinopril, quinapril, fosinopril, perindopril and benazepril in their corresponding pharmaceutical formulation. Especially, the possibility of simultaneous identification and quantification of the active ingredient in the finished product is very attractive. Excipients do not adversely affect the results. This paper deals with the validation of some parameters of the quantitative analysis: linearity, precision, accuracy and robustness. © 2001 Elsevier Science B.V. All rights reserved.

*Keywords:* Capillary electrophoresis; ACE inhibitors; Tablets; Quantitative analysis

## 1. Introduction

The inhibitors of the angiotensin-converting enzyme (ACE inhibitors) are widely used for the treatment of mild to moderate hypertension and heart failure, either alone or in conjunction with other drugs [2]. The first developed ACE inhibitor was captopril, a thiol-containing compound. Since captopril causes some side effects and researchers believed that the thiol group was responsible for these side effects, it was preferable to develop non-thiol-containing ACE inhibitors [3].

There are three classes of new ACE inhibitors, according to the group that enhances the binding to the zinc ion of the angiontensin-converting enzyme. The first class has a second carboxyl group and lisinopril and enalaprilat (the active metabolite of enalapril maleate that normally is used as drug) are the only representatives. Fosinopril, a phosphorus-containing ACE inhibitor, forms part of the second class. It is inactive but serves as a prodrug, being completely hydrolyzed to the active diacid, fosinoprilate. The third class or all the other ACE inhibitors, viz., enalapril

* Corresponding author. Tel.: +32-9-2648101; fax: +32-9-2648193.

*E-mail address:* sandra.hillaert@rug.ac.be (S. Hillaert).

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maleate, quinapril, perindopril, and benazepril possess a carboxylic acid ethyl ester and have the common property of acting as prodrugs, being converted to the active diacid by metabolism by liver and intestinal enzymes (third class) [2,3].

Until now, high performance liquid chromatography (HPLC) has been a major technique used for the quantitative determination of the ACE inhibitors [4–19]. The same technique was also applied in the monograph about enalapril maleate, lisinopril dihydrate and ramipril in the European Pharmacopoeia [20].

Analysis by means of capillary electrophoresis (CE) has been achieved for the identification of eight ACE inhibitors [1]. Other studies have been limited to the determination and rotamer separation of enalapril maleate [21–23] and lisinopril [24]. One study has reported on the determination of fosinopril and its related impurities [25]. Another study has been limited to the determination of only four ACE inhibitors [26] while our study has investigated the separation of eight ACE inhibitors [1].

The aim of this study was to investigate if the method, able to separate a large number of ACE inhibitors, could also be used for the quantification of these compounds [1]. The system is appropriate for quantitative determination in different pharmaceutical formulations without specific sample pretreatment.

This paper deals with the validation of the most important parameters for the quantitative analysis.

Table 1
Selection of the internal standard

| Substance to be examined | Appropriate internal standard |
|---|---|
| Enalapril | Lisinopril |
| | Fosinopril |
| | Cilazapril |
| | Ramipril |
| | Quinapril |
| Lisinopril | All the other ACE inhibitors |
| Quinapril | Lisinopril |
| | Fosinopril |
| | Enalapril |
| Fosinopril | Lisinopril |
| | Quinapril |
| | Ramipril |
| | Benazepril |
| | Enalapril |
| Perindopril | Lisinopril |
| | Fosinopril |
| Benazepril | Lisinopril |
| | Fosinopril |

Table 2
Reference solutions for the quantitative determination

| Reference substance | Reference solution (mg/50 ml) | Diluted reference solution (mg/ml) |
|---|---|---|
| Enalapril maleate | ±175 | ±1.87 |
| Lisinopril dihydrate | ±250 | ±2.67 |
| Quinapril · HCl | ±60 | ±0.64 |
| Fosinopril · sodium | ±150 | ±1.60 |
| Perindopril *t*-butylamine | ±125 | ±1.33 |
| Benazepril · HCl | ±60 | ±0.64 |

## 2. Experimental

### 2.1. Instruments

The validation of the method and the experiments were performed on a Crystal CE, equipped with PC 1000 software installed on a IBM computer with OS/2 as the operating system. The capillary used was a fused-silica capillary 60 cm in total length (33 cm to the detector) and 50 μm internal diameter (I.D.). The Crystal CE can be controlled over a large temperature range and the temperature used was 25°C for the tray and 30°C for the capillary.

The sample solutions were introduced into the capillary by pressure injection (50 mbar) for 5 s. A constant voltage of 30 kV was applied and UV absorbance at 214 nm was employed for detection. The detection was by means of a variable-wavelength UV detector (Spectra FOCUS detector).

To demonstrate the ruggedness of the system, some of the work was also performed on a Waters

Table 3
Sample preparation for the quantitative determination

| | Average mass (mg) | Sample solution (mg powder/15 ml) | Internal standard solution (mg/ml) | Diluted sample solution (mg active substance/ml) |
|---|---|---|---|---|
| Enalapril [Renitec®] 20 mg — tablets | 203.9 | ±254 mg | Lisinopril · $2H_2O$: 5 mg | ±1.66 |
| Lisinopril [Zestril®] 20 mg — tablets | 226.6 | ±400 mg | Enalapril maleate: 5 mg | ±2.35 |
| Quinapril [Accupril®] 20 mg — tablets | 208.0 | ±83 mg | Lisinopril · $2H_2O$: 5 mg | ±0.53 |
| Fosinopril [Fosinil®] 20 mg — tablets | 201.2 | ±200 mg | Quinapril · HCl: 2.5 mg | ±1.33 |
| Perindopril [Coversyl®] 4 mg — tablets | 90 | ±270 mg | Lisinopril · $2H_2O$: 5 mg | ±0.80 |
| Benazepril [Cibacen®] 10 mg — tablets | 186.7 | ±150 mg | Lisinopril · $2H_2O$: 5 mg | ±0.54 |

Quanta 4000 (Millipore, Waters), equipped with a fused-silica capillary 60 cm in total length (52.5 cm to the detector) and 50 μm I.D. The data were collected on a Hewlett-Packard Integrator (HP 3396 Series II), processing both the areas and the heights of the peaks.

The sample solutions were introduced into the capillary by hydrodynamic introduction for 10 s. Hydrodynamic injections were performed by lifting the sample vial approximately 10 cm above the height of the buffer vial for 10 s. A constant voltage of 25 kV was applied and UV absorbance at 214 nm was used for detection, which was by means of an on-line fixed-wavelength UV detector with a zinc discharge lamp and a 214-nm filter.

### 2.2. Reagents

Sodium dihydrogen phosphate monohydrate and disodium hydrogen phosphate dihydrate were obtained from E. Merck (Germany). Enalapril maleate was purchased from Sigma (St. Louis, MO, USA). Lisinopril dihydrate was obtained from Zeneca, quinapril · HCl from Parke-Davis, fosinopril sodium from Bristol-Myers Squibb, perindopril *t*-butylamine from Servier and benazepril · HCl from Ciba-Geigy.

Commercially available drugs [Renitec® 20 mg (MSD), Zestril® 20 (Zeneca), Accupril® 20 mg (Parke Davis), Fosinil® (Solvay), Coversyl® (Servier) and Cibacen® (Novartis)] were used for the quantitative determination.

### 2.3. Running buffer

The sodium phosphate buffer (pH 7.0; 100 mM) was used as running buffer. It was prepared by adjusting the pH of a 100 mM disodium hydrogen phosphate solution to pH 7.0 by the addition of a 100 mM sodium dihydrogen phosphate solution.

### 2.4. Internal standard solutions

Selection of the internal standard had to be made on the basis of the substance to be examined (Table 1). Lisinopril dihydrate was chosen mostly as the internal standard because of its baseline separation with all the other ACE inhibitors and because of its availability as bulk product on the market. For the determination of lisinopril, each other ACE inhibitor can be used. An appropriate amount of the compound (Table 3) was dissolved in 20 ml running buffer and diluted to 50 ml with the same running buffer.

### 2.5. Reference solutions

Reference solutions were prepared by accurately weighing an appropriate amount of the corresponding reference substance, dissolving in

20 ml running buffer and diluting to 50.0 ml with the same buffer solution (Table 2). A volume of 8.0 ml of these solutions was mixed with 5.0 ml of the internal standard solution and diluted to 15 ml with the buffer solution.

## 2.6. Sample preparations

Minimum twenty tablets were weighed, ground, and mixed. An appropriate amount of the powder (Table 3) was mixed with 5.0 ml of the appropri-

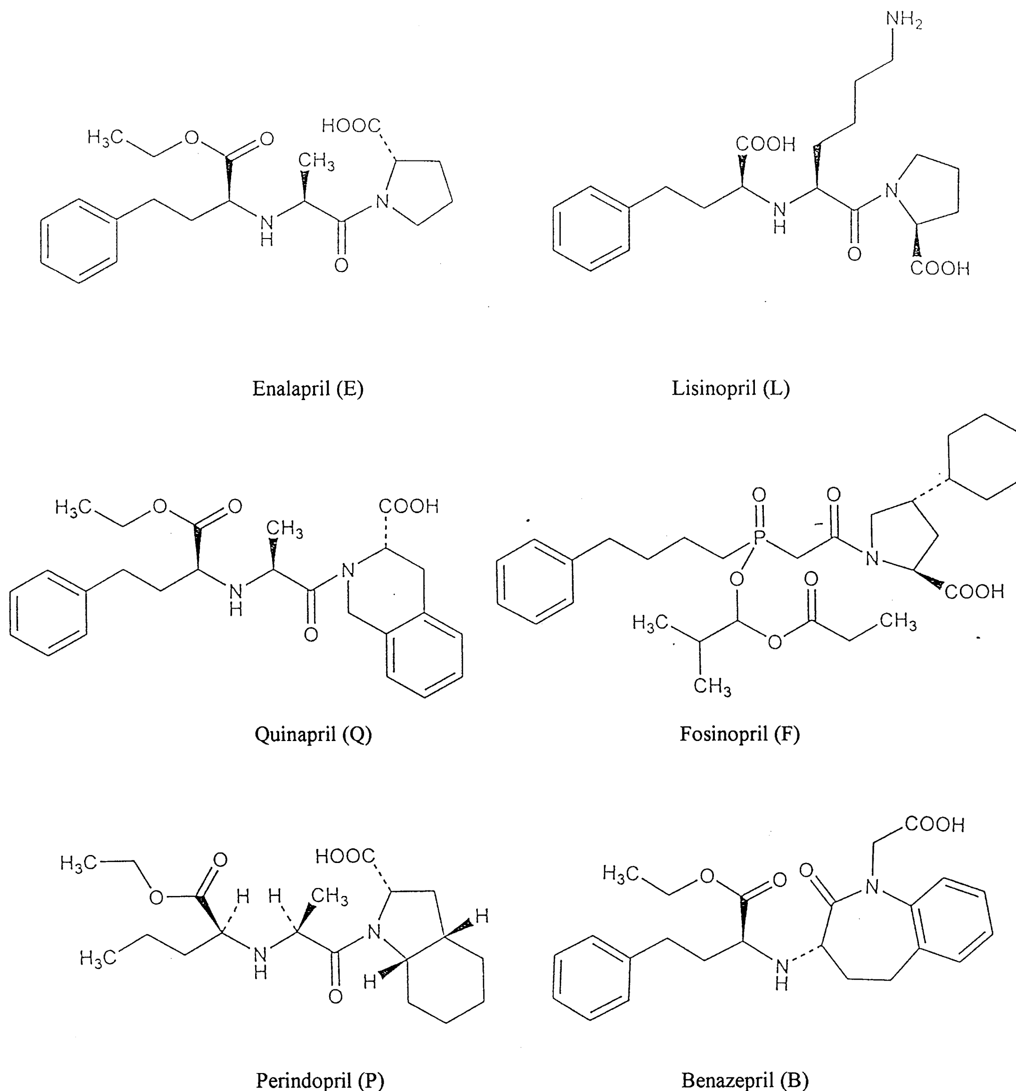


Fig. 1. Chemical structures of the ACE-inhibitors.

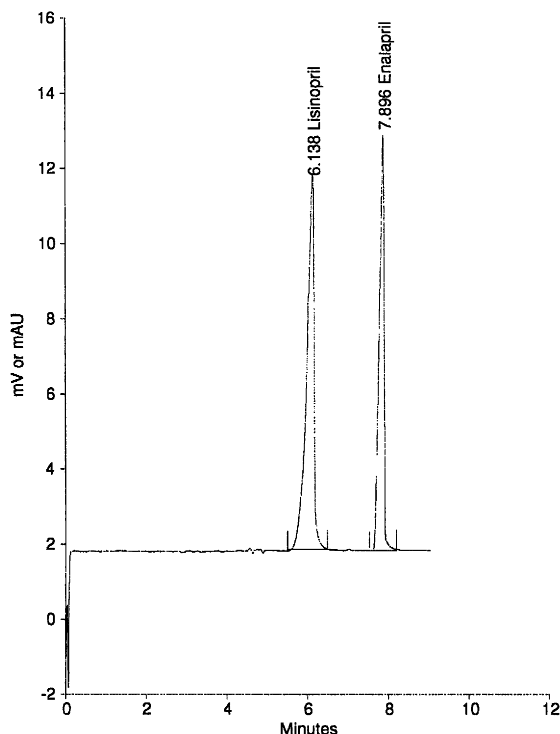


Fig. 2. Electropherogram of the quantitative determination of lisinopril [Zestril®] on a fused-silica capillary, performed on the Crystal CE. Conditions: 60 cm (33 cm to the detector) × 50 μm I.D.; sodium phosphate buffer (pH 7.0; 100 mM) as running buffer; applied voltage, 30 kV; detection at 214 nm.

ate internal standard solution (Table 3) and diluted to 15 ml with the running buffer.

All the samples and buffers were filtered through a Millipore 0.45 μm filter unit.

## 3. Results and discussion

### 3.1. Optimization of the method

Until now, the literature shows no selective method, which is able to separate and quantify several ACE inhibitors. The published studies can only be applied for the quantitative determination of one or two of these compounds [21–26].

The optimization of a selective CE separation of several ACE inhibitors was published earlier [1]. The aim of this study was to investigate if that method could also be used for the quantification of these compounds. Separation was performed by means of two phosphate buffers (each 100 mM) 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 p$K_a$-values [1]. Due to the highest selectivity of the sodium phosphate buffer (pH 7.0; 100 mM) and the good peak shapes, this system has been applied for the quantification of enalapril, lisinopril, quinapril, fosinopril, perindopril and benazepril in their corresponding formulations. The selection of the internal standard had to be made on the basis of the substance to be examined. Due to the specificity of the developed method, the possibility of simultaneous identification and quantification of the active ingredient in the finished product is very attractive.

The chemical structures of the examined ACE inhibitors are represented in Fig. 1.

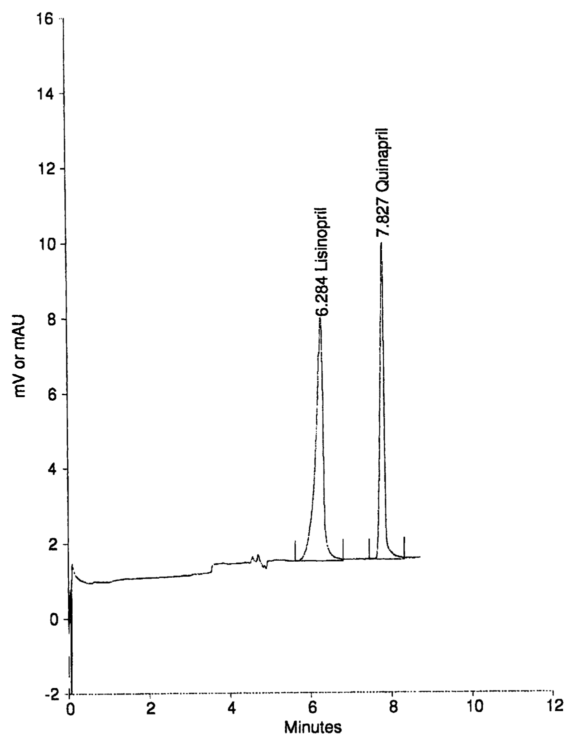


Fig. 3. Electropherogram of the quantitative determination of quinapril [Accupril®] on a fused-silica capillary, performed on the Crystal CE. Conditions: 60 cm (33 cm to the detector) × 50 μm I.D.; sodium phosphate buffer (pH 7.0; 100 mM) as running buffer; applied voltage, 30 kV; detection at 214 nm.

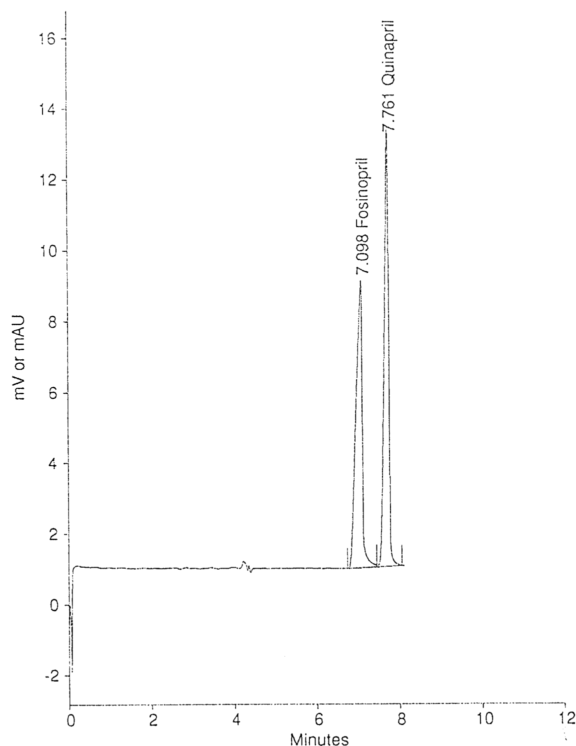


Fig. 4. Electropherogram of the quantitative determination of fosinopril [Fosinil®] on a fused-silica capillary, performed on the Crystal CE. Conditions: 60 cm (33 cm to the detector) × 50 μm I.D.; sodium phosphate buffer (pH 7.0; 100 mM) as running buffer; applied voltage, 30 kV; detection at 214 nm.

### 3.2. Quantitative determination in pharmaceutical formulations

A sodium phosphate buffer (pH 7.0; 100 mM) is appropriate for the quantitative determination of the ACE inhibitors (Figs. 2–6). By the means of different placebo mixtures it was demonstrated that the following excipients do not adversely affect the results, lactose, sodium hydrogen car bonate, maize starch, pregelatinized maize starch, mannitol, calcium hydrogen phosphate, magnesium carbonate, gelatin, polyvidone and crospovidone, microcrystalline cellulose, macrogol 400 and 8000, magnesium stearate, silicon dioxide, hypromellose and titanium dioxide.

### 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 mentioned in Table 4. The amount of the internal standard was adapted according to the used concentration range. The 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 (R.S.D.) are summarized in Table 5.

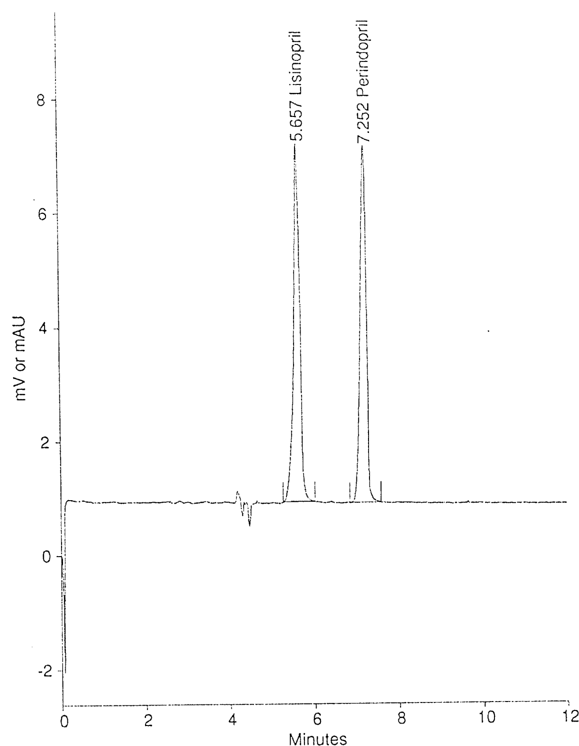


Fig. 5. Electropherogram of the quantitative determination of perindopril [Coversyl®] on a fused-silica capillary, performed on the Crystal CE. Conditions: 60 cm (33 cm to the detector) × 50 μm I.D.; sodium phosphate buffer (pH 7.0; 100 mM) as running buffer; applied voltage, 30 kV; detection at 214 nm.

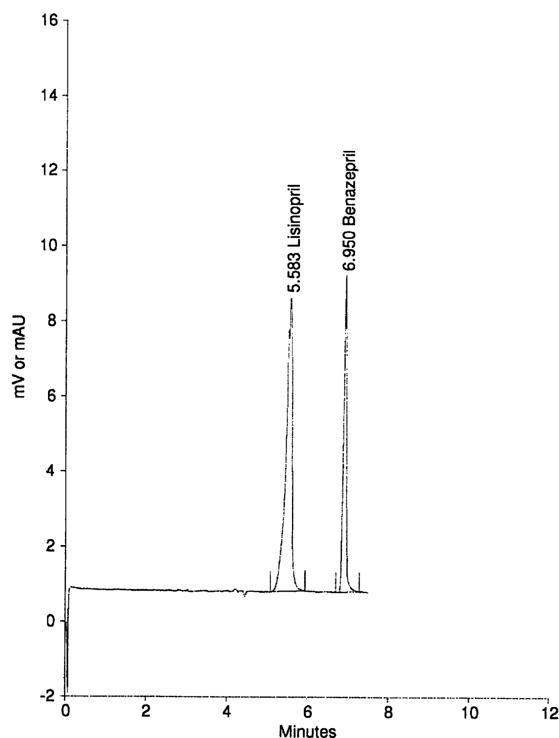


Fig. 6. Electropherogram of the quantitative determination of benazepril [Cibacen®] on a fused-silica capillary, performed on the Crystal CE. Conditions: 60 cm (33 cm to the detector) × 50 μm I.D.; sodium phosphate buffer (pH 7.0; 100 mM) as running buffer; applied voltage, 30 kV; detection at 214 nm.

Table 4
Linearity

| | Concentration range (mg/ml) | Correlation coefficient ($r^2$) |
|---|---|---|
| Enalapril maleate | 0.02–0.47 | 0.9999 |
| | 0.47–2.35 | 0.9999 |
| Lisinopril dihydrate | 0.03–0.67 | 0.9999 |
| | 0.67–3.35 | 0.9999 |
| Quinapril · HCl | 0.01–0.20 | 0.9999 |
| | 0.16–0.80 | 0.9994 |
| Fosinopril sodium | 0.02–0.36 | 0.9999 |
| | 0.40–2.00 | 0.9993 |
| Perindopril *t*-butylamine | 0.02–0.33 | 0.9996 |
| | 0.33–1.67 | 0.9998 |
| Benazepril · HCl | 0.01–0.20 | 0.9999 |
| | 0.16–0.80 | 0.9994 |

The error of the equipment, the electrophoretic separation, and the relative standard deviation were determined by performing ten consecutive injections of the same sample (Table 6). It was performed on the Waters Quanta 4000.

Table 5
Precision (repetability) of the total analysis of the six replicate samples

| Substance to be examined | Theoretical amount (mg/tablet) | Amount found | Relative standard deviation ($n=6$) |
|---|---|---|---|
| Enalapril maleate [Renitec®] | 20 mg | 19.68 mg ± 0.02 mg or 98.4% | 0.12% |
| Lisinopril · $2H_2O$ [Zestril®] | 20 mg | 20.42 mg ± 0.05 mg or 102.1% | 0.24% |
| Quinapril · HCl [Accupril®] | 20 mg | 20.23 mg ± 0.08 mg or 101.2% | 0.39% |
| Fosinopril sodium [Fosinil®] | 20 mg | 19.83 mg ± 0.11 mg or 99.2% | 0.55% |
| Perindopril *t*-butylamine [Coversyl®] | 4 mg | 3.95 mg ± 0.01 mg or 98.8% | 0.25% |
| Benazepril · HCl [Cibacen®] | 10 mg | 10.20 mg ± 0.02 mg or 102.0% | 0.20% |

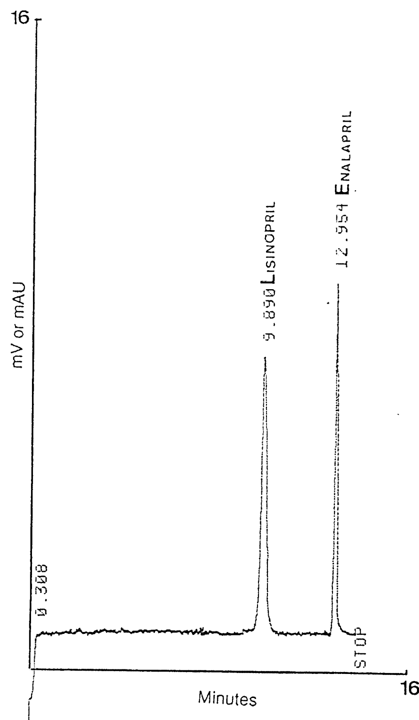


Fig. 7. Electropherogram of the quantitative determination of lisinopril [Zestril®] on a fused-silica capillary, performed on the Waters Quanta 4000. Conditions: 60 cm (52.5 cm to the detector) × 50 μm I.D.; sodium phosphate buffer (pH 7.0; 100 mM) as running buffer; applied voltage, 25 kV; detection at 214 nm.

Table 6
Repetability of ten consecutive injections of the same sample (performed on the Waters Quanta)

| Sample solution | Relative standard deviation ($n = 10$) |
|---|---|
| Enalapril maleate | 0.70% |
| Lisinopril · $2H_2O$ | 1.09% |
| Quinapril · HCl | 0.68% |
| Fosinopril sodium | 0.53% |
| Perindopril *t*-butylamine | 0.27% |
| Benazepril · HCl | 0.38% |

### 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 7).

### 3.3.4. Robustness

To demonstrate the system robustness, the quantitative determination of enalapril, lisinopril, perindopril and benazepril was also performed on a Waters Quanta 4000, equipped with a fused-silica capillary 60 cm in total length (52.5 cm to the

Table 7
Accuracy

| | Recovery placebo + 80% ($n = 3$) | Recovery placebo + 100% ($n = 3$) | Recovery placebo + 120% ($n = 3$) |
|---|---|---|---|
| Enalapril | 99.6 ± 0.1% | 100.6 ± 0.1% | 99.2 ± 0.4% |
| Lisinopril | 102.4 ± 0.2% | 100.8 ± 0.3% | 100.2 ± 0.2% |
| Quinapril | 102.4 ± 0.1% | 102.0 ± 0.2% | 102.0 ± 0.2% |
| Fosinopril | 9.9 ± 0.3% | 100.5 ± 0.2% | 100.4 ± 0.1% |
| Perindopril | 100.5 ± 0.2% | 100.5 ± 0.4% | 100.0 ± 0.2% |
| Benazepril | 101.0 ± 0.2% | 100.6 ± 0.3% | 100.1 ± 0.1% |

Table 8
Robustness

| | Waters Quanta 4000 | | Crystal CE | |
|---|---|---|---|---|
| | Amount found (mg/tablet) | RSD ($n = 6$) | Amount found (mg /tablet) | RSD ($n = 6$) |
| Enalapril [Renitec®] | 19.63 ± 0.09 mg or 98.2% | 0.46% | 19.68 ± 0.02 mg or 98.4% | 0.12% |
| Lisinopril [Zestril®] | 20.41 ± 0.06 mg or 102.1% | 0.29% | 20.42 ± 0.05 mg or 102.1% | 0.24% |
| Perindopril [Coversyl®] | 3.88 ± 0.03 mg or 97.0% | 0.69% | 3.95 ± 0.01 mg or 98.8% | 0.25% |
| Benazepril [Cibacen®] | 10.16 ± 0.06 mg or 101.6% | 0.60% | 10.20 ± 0.02 mg or 102.0% | 0.20% |

detector) and 50 μm I.D. The method conditions with the exception of the running voltage, applied on the Crystal CE could be transferred to the Waters Quanta (Figs. 2 and 7). The results of the quantitative determinations were similar to those on the Crystal CE (Table 8). The R.S.D. of the results on the Waters Quanta 4000 was higher as a result of the temperature fluctuations.

## 4. Conclusion

The determination of different ACE inhibitors by capillary electrophoresis has been achieved. The study demonstrates that CE can be successfully applied to the quantitative analysis of these compounds in pharmaceutical formulations.

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