ELSEVIER

Journal of Chromatography A, 916 (2001) 279–288

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Capillary zone electrophoresis applied to the determination of the angiotensin-converting enzyme inhibitor cilazapril and its active metabolite in pharmaceuticals and urine

José Angel Prieto, Urtzi Akesolo, Rosa María Jiménez*, Rosa María Alonso

Departamento de Química Analítica, Facultad de Ciencias, Universidad del País Vasco UPV/EHU, Apdo. 644, E-48080 Bilbao, Spain

Abstract

A capillary zone electrophoresis method has been developed for the quantitation of antihypertensive drug cilazapril and its active metabolite cilazaprilat in pharmaceuticals and urine. The separation of the compounds was performed in a fused-silica capillary filled with the running electrolyte, which consisted of a 60 m*M* borate buffer solution at pH 9.5. Under the optimized experimental conditions, the separation took less than 5 min. The analysis of urine samples required a previous solid-phase extraction step using C_8 cartridges. The method was successfully applied to the determination of the drug and its metabolite in urine samples obtained from three hypertensive patients (detection limits of 115 ng ml^{-1} for cilazaprilat and 125 ng ml^{-1} for cilazapril) and to pharmaceutical dosage forms. The method was validated in terms of reproducibility, linearity and accuracy. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Pharmaceutical analysis; Angiotensin-converting enzyme inhibitors; Enzyme inhibitors; Cilazapril; Cilazaprilat

1. Introduction

Cilazapril is the prodrug of the orally active angiotensin-converting enzyme (ACE) inhibitor cilazaprilat [1]. Over the past years, the ACE inhibitors have assumed great importance in the management of patients with hypertension, congestive heart failure or proteinuric renal disease, and following heart stroke [2,3]. All ACE inhibitors are well tolerated drugs and have not been shown to significantly affect quality-of-life in contrast to other kinds of antihypertensive drugs [4]. The initial success of captopril, enalapril and lisinopril encouraged the development and marketing of many other ACE inhibitors [2,5].

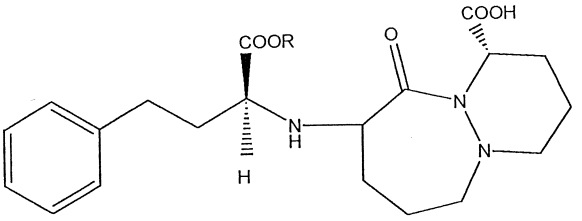
Cilazapril itself is inactive, but following oral administration it is rapidly de-esterified to the active diacid metabolite cilazaprilat. This molecule inhibits the last step of the biosynthesis of angiotensin II, a potent vasoconstrictor, and therefore it lowers blood pressure [1,6,7]. Cilazaprilat is a conformationally restricted chemical structure that allows optimal binding to the ACE [8,9].

Doses of cilazapril range from 0.5 to 5 mg per day. Bioavailability is around 57%. Cilazaprilat is the major metabolite and is primarily eliminated by the kidney. The elimination kinetic of cilazaprilat is polyphasic with a rapid elimination of the unbound drug within the first 8 h, giving an initial half-life of 1.5 to 1.8 h and a terminal half-life of 30–50 h [1,7].

*Corresponding author. Tel.: +34-94-601-5504; fax: +34-94-464-8500.

E-mail address: qapjisar@lg.ehu.es (R.M. Jiménez).

0021-9673/01/$ – see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S0021-9673(01)00565-9



R=H Cilazaprilat

$R=CH_2CH_3$ Cilazapril

Methods for the analysis of cilazapril and its metabolite are scarce. In many of the biomedical publications, the activity of these compounds is followed indirectly measuring the inhibition of the ACE, or the renine or angiotensin II levels [6–8,10]. Analytical methods include enzyme immunoassay in plasma and serum [11], HPLC with amperometric [12] and photometric detection [13] in urine and pharmaceuticals. Ultraviolet spectrophotometry [14] and derivative spectrophotometry [15] have been used for its determination in pharmaceutical formulations. Stefan et al. [16] have developed an amperometric biosensor for the enantioselective analysis of S-cilazapril, among other ACE inhibitors, in bulk drug. Cilazapril has been also included in a screening method for the analysis of standard solutions of ACE inhibitors using HPLC with photometric detection [17] and in a method using gas chromatography–mass spectrometry [18], but it was only tested with rat urine.

CE is a very rapidly growing microseparation technique for the analysis of drugs and biomolecules present in biological fluids [19,20]. However, the low detection sensitivity makes quantitative analysis in biological matrices a challenge [21].

The aim of this paper is to establish a simple and reliable capillary zone electrophoresis method for the determination of cilazapril and cilazaprilat in pharmaceuticals and urine samples after the administration of a therapeutic dose.

2. Experimental

2.1. Reagents and solutions

Cilazapril hydrate was kindly supplied by Roche (Barcelona, Spain), cilazaprilat was prepared by alkaline hydrolysis of cilazapril [12]. Hydrochlorothiazide was purchased from Sigma (St Louis, MO, USA). Quinaprilat hydrate, used as internal standard for the analysis of urine samples, was a kind gift of Parke–Davis (Barcelona, Spain). Enalaprilat was used as internal standard for the analysis of pharmaceutical formulations and was kindly provided by Merck, Sharp and Dohme (Madrid, Spain). Other ACE inhibitors were assayed as internal standards: enalapril maleate was purchased from Sigma, and the rest were kindly supplied by the pharmaceutical companies: fosinopril from Laboratorios Dr. Esteve (Barcelona, Spain), fosinoprilat from Bristol–Meyers Squibb (Princeton, NJ, USA), ramipril and ramiprilat from Hoechst (Barcelona, Spain), quinapril from Parke–Davis and lisinopril from DuPont Pharma (Madrid, Spain).

Organic solvents were HPLC grade (Lab-Scan, Dublin, Ireland). Sodium citrate dihydrate was obtained from Sigma and was electrophoresis reagent grade. The rest of the reagents were analytical-reagent grade from Merck (Darmstadt, Germany). Water was obtained from Milli-RO and Milli-Q systems (Waters).

Buffer solutions used in the clean-up procedure were made preparing 0.1 *M* phosphoric acid and 0.5 *M* boric acid, and the pH value was adjusted to 2.0 and 9.0, respectively by adding drops of 3 *M* KOH.

Buffer solutions tested as running electrolytes were made in the same way, preparing the desired concentrations of phosphoric, acetic and boric acids or sodium bicarbonate, and adjusting the pH to the required value by adding drops of 3 *M* NaOH. Citrate buffer was made by adding drops of 3 *M* HCl to a solution of sodium citrate.

Stock solutions of the drugs (200 $\mu g\,ml^{-1}$) were prepared in water in the case of cilazapril and the internal standard enalaprilat, and in 20% methanol in the case of cilazaprilat. Quinaprilat, used as internal standard, was dissolved in methanol. Hydrochlorothiazide (1000 $\mu g\,ml^{-1}$) was dissolved in 50% methanol. All solutions were stored in dark at 4°C and working solutions were prepared by appropriate dilution just before use. No degradation of the compounds was observed during this work.

Solid-phase extractions were performed using Bond Elut 1 ml/100 mg C_8 cartridges (Varian, Harbor City, CA, USA).

2.2. Apparatus and electrophoretic conditions

This work was carried out on a Waters Quanta 4000 Capillary Ion Analyser (Milford, MA. USA). Electropherograms were collected using the program Millennium^{32} Chromatography Manager (Waters).

The separation was performed in 58×75 μm I.D. fused-silica capillary tubes obtained from Composite Metal Services (Worcester, UK). The effective separation length up to the detection window was 50 cm. The capillary was kept at 35°C in the case of urine samples and at 25°C in the case of the analysis of pharmaceutical formulations. The detection wavelength was set at 214 nm. Samples were injected hydrostatically for 15 s and the running potential was +25 KV.

The electrolyte consisted of a 60 m*M* borate buffer at pH 9.5, which induced a current of 82 μA when the potential was turned on.

The capillary was conditioned every day with an initial wash cycle consisting of 1 *M* NaOH, water, and the running electrolyte for 10 min each. Between injections, the capillary was purged for 3 min with the running electrolyte. Daily after finishing the experiments, it was washed with 1 *M* NaOH and water for 5 min.

Solid-phase extractions (SPE) were performed on a vacuum manifold system (Supelco, Bellefonte, PA, USA). The extracted urine samples were evaporated to dryness under a gentle stream of nitrogen using a Zymark Turbovap LV evaporator (Hopkinton, MS, USA). pH measurements were carried out using a Radiometer pHM84 (Copenhagen, Denmark) research pHmeter equipped with a Crison 52 09 (Alella, Spain) pH electrode.

2.3. Procedure for the analysis of tablets

Five pharmaceutical formulations containing cilazapril have been analyzed throughout this work using the developed capillary zone electrophoresis (CZE) method: Inhibace (cilazapril 1 or 5 mg) and Inhibace Plus (cilazapril 5 mg and hydrochlorothiazide 12.5 mg) commercialized by Andreu-Roche (Barcelona, Spain); Inocar (Cilazapril 1 mg) and Inocar Plus (cilazapril 5 mg and hydrochlorothiazide 12.5 mg) commercialized by Nezel (Barcelona, Spain).

The preparation of the sample was the same for all formulations. Four tablets were weighed and pulverized in a mortar. A suitable amount of this powder was weighed in order to obtain 50 ml of a solution containing around 100 $\mu g\,ml^{-1}$ of the active component. The powder was dissolved in water and sonicated for 10 min. The mixture was centrifuged at 800 *g* for 5 min, and the liquid was transferred to a volumetric flask. The residual solid was washed twice with water in order to extract completely the active component. The centrifuged solutions were added to the volumetric flask, whose volume was finally made up to 50 ml with water.

Aliquots of these solutions were diluted in water and the suitable amount of the internal standard, enalaprilat, was added (final concentration in the solution 40 $\mu g\,ml^{-1}$) and injected in the electrophoretic system under the optimized conditions.

2.4. Collection of urine samples

Urine samples obtained from three hypertensive patients after ingestion of a therapeutic dose of Inhibace, cilazapril 5 mg, were collected in plastic bottles at four time intervals after the intake: 0–1 h, 1–4 h, 4–8 h and 8–24 h. The urine was transferred to 10 ml plastic tubes and frozen at −20°C.

The patients had the following features: No. 1 female, 57 years; No. 2 female, 79 years and No. 3 male, 44 years. Patient No. 2 was also under treatment with the cardiotonic digoxin, the diuretic torasemide and the benzodiazepine lormetazepam. All them had been under continuous treatment with cilazapril for a different period of time. Patients were not asked to change their habits or to be on a special diet to perform the analysis.

Blank urine samples were obtained from several healthy volunteers.

2.5. Clean-up procedure for urine samples

Deep frozen urine was thawed at room temperature. Two milliliters of human urine were alkalinized with 1 ml of 0.5 *M* borate buffer at pH 9 and 30 μl of the stock solution of quinaprilat were added as internal standard. The mixture was shaken and centrifuged at 800 g for 5 min.

Two milliliters of the buffered urine solution were applied to a C_8 cartridge, previously activated with 1 ml of methanol, 1 ml of water and 1 ml of a mixture

water–0.5 *M* borate buffer pH 9 (2:1, v/v). The sample was slowly drawn through the column at an approximate flow-rate of 0.5 ml min^{-1}. The cartridge was washed in two steps. Firstly with 1 ml of a mixture methanol–0.1 *M* phosphate buffer at pH 2 (20:80, v/v), 3 ml of water and another ml of the mixture methanol–phosphate buffer. 1 ml of hexane was added and the cartridge was let dry for 10 min at full vacuum (70 kPa). Another milliliter of hexane was passed in order to ensure that the column was free of water, and it was allowed to dry for another 5 min. Secondly, 1 ml of acetonitrile was used to complete the washing of the compounds adsorbed on the cartridge. The analytes and the internal standard were eluted with 2×1 ml of a mixture chloroform–isopropanol–water (50:50:2, v/v/v). The eluate was evaporated to dryness at 50°C under a gentle stream of nitrogen and the residue dissolved in 150 μl of water. A preconcentration factor of 8.9 times is achieved during the process.

Solutions for the calibration were made by spiking blank urine samples with known amounts of cilazapril and cilazaprilat and the internal standard, extracting them in the same way as unknown samples.

2.6. Method validation

Calibration standards for the analysis of tablets of cilazapril and hydrochlorothiazide in the range 4–50 μg ml^{-1} and 10–125 μg ml^{-1}, respectively, were made by appropriate dilution in water of the stock solutions and the addition of the internal standard enalaprilat. In order to overcome the changes of area due to variations in migration time, normalized areas were used, which is the result of dividing the peak area by the migration time [22]. Calibration plots were drawn by representing the ratio normalized area of cilazapril or hydrochlorothiazide/normalized area of enalaprilat vs. concentration.

The calibration curves for the analysis of cilazapril and cilazaprilat in urine were constructed in the range 0.2–2 μg ml^{-1} and 0.5–10 μg ml^{-1} respectively, according to the concentrations of these drugs expected in urine samples. Quinaprilat was added as internal standard to all calibration standards and urine samples. The calibration equations were obtained by least-squares linear regression of the ratio normalized area of cilazapril or cilazaprilat/normalized area of quinaprilat vs. concentration. Unknown concentrations were calculated by interpolation.

The background noise of the baseline was evaluated using a blank urine sample. The detection and quantitation limits were calculated as the analyte concentrations that produced peak heights with a signal-to-noise ratio of 3 and 10, respectively [23].

The reproducibility of the method was calculated at two concentration levels: 0.5 and 4 μg ml^{-1} for cilazaprilat and 0.5 and 2 μg ml^{-1} for cilazapril. Two spiked blank urine sample solutions were made, one containing 0.5 μg ml^{-1} of each analyte, and the other with 2 and 4 μg ml^{-1} of cilazapril and cilazaprilat respectively. They were frozen in 10 ml plastic tubes. Each day, a tube of each concentration was thawed at room temperature, the internal standard was added (concentration in urine 3 μg ml^{-1}), and three extractions of each were done. The process was repeated during 5 days. The data collected (the ratio of normalized areas of the drugs/normalized area of the internal standard) were treated using the ANOVA method (analysis of variance) [23].

Accuracy was estimated at two concentration levels, extracting urine samples spiked with cilazapril, cilazaprilat and the internal standard quinaprilat, and calculating the relative error between the prepared concentrations and the calculated using the calibration plot. Two replicates were done and the mean value used.

The recovery was calculated comparing the peak areas of cilazapril and cilazaprilat obtained after extraction with the peak areas of standard solutions of the same concentration of both drugs in water.

3. Results and discussion

3.1. Optimization of the electrophoretic system

CZE is the simplest mode of CE and, therefore, the most widely used. The separation mechanism is based on the difference in charge/mass ratio. Adjusting the pH is the most convenient and easy way to control both the electroosmotic flow (EOF) and electrophoretic mobility [24,25]. The reported pK_a values for cilazapril are 3.3 for the carboxylic acid and 6.5 for the amine group [26], the molecule can be positively or negatively charged or neutral de-

pending on the pH value of the electrolyte. At pH below 4.9, the molecule is positively charged and migrates to the negative end of the capillary where the detection window is. At pH around 4.9, which corresponds to the isoelectric point, the molecule has not a net charge, and thus the electrophoretic mobility is 0, but it is detected because it is drawn by the osmotic flow. At pH values above 4.9, the molecule is negatively charged, however, the EOF overwhelms the electrophoretic mobility and therefore cilazapril migrates towards the negative end. Cilazaprilat has an additional carboxylic acid moiety and an additional negative charge, thus it always migrates after cilazapril.

Several buffers and pH values were tested as running electrolytes at a concentration of 25 mM: carbonate at pH 10–11, borate at pH 9–10, phosphate at pH 6–8 and 3, citrate at pH 6 and acetate at pH 4–5. A potential of +25 kV was used and the capillary was maintained at 25°C. Carbonate and citrate buffers induced a high current across the capillary (carbonate buffer at pH 10: 68.2 μA, citrate buffer at pH 6: 93.2 μA) and unstable baselines. Phosphate buffers at pH 7–8 or borate buffers at pH 9–10 seemed equally good options taking into account the peak shapes and migration times. Borate buffer at pH 9.5 was finally selected because it produced a very stable baseline and induced a current of only 23 μA. Fig. 1 shows the electrophoretic mobility of cilazapril and cilazaprilat, as well as their migration times depending on the pH value. Good accordance with the expected results, taking into account the reported values of pK_a of cilazapril, was found.

The following step was the optimization of the concentration of the borate buffer, which was studied in the range 10–75 m*M*. An optimum balance in ionic strength should be used since high ionic strength generates high current and Joule heating, whereas low ionic strength may cause sample adsorption on the capillary wall. In urine samples, it was observed that the peak shape was improved when the concentration of the electrolyte was increased. 60 m*M* borate buffer was found to be optimal despite the increase of current across the capillary (68.8 μA).

The temperature of the capillary was varied in the range 25–35°C. At 35°C, higher and narrower peaks

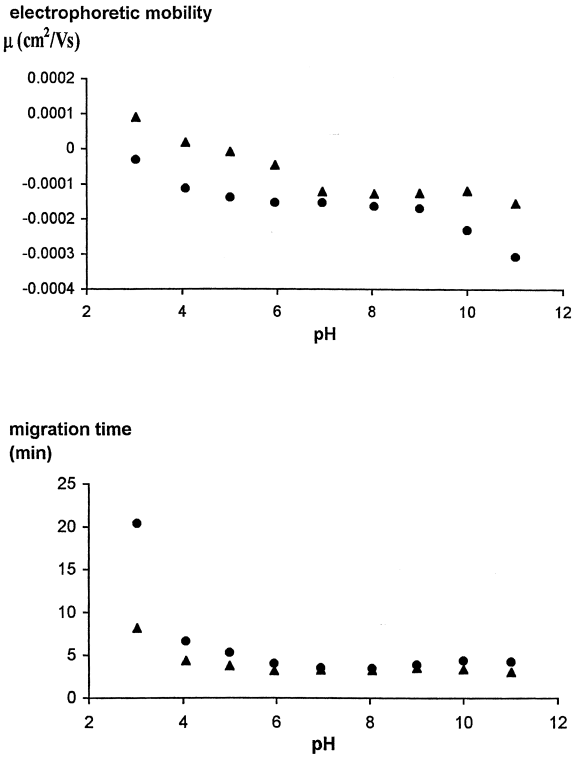


Fig. 1. Dependence of electrophoretic mobility and migration time of cilazapril (▲) and cilazaprilat (●) with pH. Conditions and types of electrolytes are described in the text.

were obtained in the case of urine samples, and their migration times were shorter (for cilazapril 3.86 and 3.04 min, and for cilazaprilat 5.00 and 4.07 min at 25 and 35°C respectively). Temperature affects the EOF and the sample volume injected since it causes a change of ca 2–3% per °C in viscosity of the buffer and the sample [21]. For the analysis of tablets, the capillary was kept at 25°C, since the peak shape was considered adequate at this temperature.

The sample was injected hydrostatically by dipping the capillary end into the sample solution and raising the vial around 10 cm. The injection time was varied from 2 to 40 s. The peak area increased proportionally with the time. However, due to peak-height saturation, the height of the peaks only increases proportionally up to a certain value. This value was 15 s in the case of urine samples.

The electrophoresis voltage is an important factor contributing to resolution. High voltage increases EOF and analysis time is therefore reduced. Addi-

tionally, high voltage results in Joule heating and, as a consequence, band broadening which decreases resolution. On the other hand, very low voltage results in high migration times and band broadening because of increase in diffusion. The voltage optimization should take into consideration that the maximum applied voltage gives a linear relationship between the voltage and the current. This value was found to be +20 kV. However, a shorter analysis time and narrower peaks were obtained at +25 kV, and finally this potential was used.

3.2. Optimization of the extraction procedure

Cilazapril and cilazaprilat do not show strong absorption in the UV–Vis spectral region. Moreover, they absorb at a very short wavelength (maximum absorbance at 196 nm), which meant that a very demanding extraction procedure had to be found in order to eliminate as much endogenous compounds from the urine matrix as possible. Solid-phase extraction was the selected option, since it allows the separation of the selected molecules from most of the interfering compounds and, at the same time, permits the preconcentration of the sample.

The clean-up procedure was the same that was previously optimized for the chromatographic method with photometric detection [13]. Summarizing, the sample was applied to an activated cartridge at pH 9 because fewer endogenous compounds were retained. Methanol–0.1 *M* phosphate buffer pH 2 (20:80, v/v) and water were used as washing solvents. After drying the cartridge passing air and hexane in order to eliminate traces of water, a second washing was needed with acetonitrile. If the cartridge were not previously dried, the drugs would be partially eluted and a poor recovery would be obtained. The analytes and the internal standard were eluted with chloroform–isopropanol–water (50:50:2 v/v/v), which provided high recoveries and the minimum amount of endogenous compounds. The eluate was evaporated to dryness.

Several other ACE inhibitors with structural similarities were assayed as internal standards (enalapril maleate, fosinopril, ramipril, quinapril and their metabolites; and lisinopril). Most of them migrated overlapping the compounds of interest, except quinaprilat and enalaprilat. Quinaprilat was selected in the case of urine samples because enalaprilat was lost during the extraction step.

Given the low sensitivity of the CE technique with UV detection due to the short pathlength, high preconcentration factors are usually necessary to overcome this problem. However, in order to obtain narrow and sharp peaks, it is necessary that the conductivity of the sample is about 10–100 times lower than the surrounding buffer, producing what is called sample stacking [24]. The optimal volume to dissolve extract was found to be 150 μl, because a smaller volume produced wide peaks, specially when high concentrations of drugs were analyzed, whereas a larger volume produced a loss of sensitivity. Fig. 2 shows the electropherograms of an extracted spiked urine sample containing 2 $\mu g\,ml^{-1}$ of cilazapril and 10 $\mu g\,ml^{-1}$ of cilazaprilat, in one case the extract was dissolved in 100 μl and in the other in 150 μl. This effect was not observed with standard solutions in water and sharp peaks were always obtained, even at the highest concentrations of the drugs that were tested (up to 150 $\mu g\,ml^{-1}$).

3.3. Quantitative determination

A calibration curve for both drugs in urine was made, using spiked blank urine samples and extracting them using the procedure described in Section 2.5. The concentration of unknown samples was calculated by interpolation. The quantitative parameters calculated for this system are collected in Table 1.

The recoveries of the extraction at two concentration levels were calculated. For cilazaprilat, the percentage of recovery and standard deviation was 75±3 and 72±2 at the levels of 0.5 and 4 $\mu g\,ml^{-1}$, respectively, and for cilazapril 86±5 and 85±2 at the levels of 0.5 and 2 $\mu g\,ml^{-1}$, respectively. The obtained results were much lower than the published using the chromatographic methods [12,13], which were ca. 90% for cilazaprilat and ca. 100% for cilazapril in all assayed concentrations. The reason for this difference could be attributed to the presence of endogenous compounds that modify the viscosity of the extract, and thus the sample volume injected could be smaller than in the case of standard solutions in water.

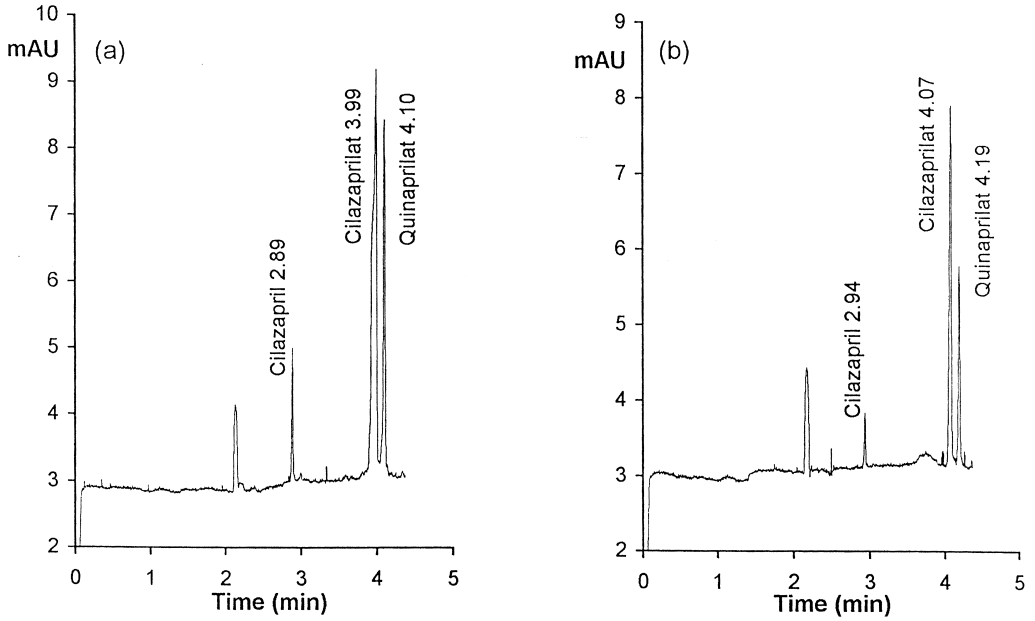


Fig. 2. Electropherograms of a blank urine sample spiked with 2 μg ml^{-1} of cilazapril, 10 μg ml^{-1} of cilazaprilat and 3 μg ml^{-1} of the internal standard quinaprilat. The extract was dissolved in 100 μl of water (a), or 150 μl (b). Capillary 50 cm×75 μm I.D.; electrolyte: 60 m*M* borate buffer at pH 9.5; potential +25 kV; temperature 35°C; injection time 15 s and detection wavelength 214 nm.

3.4. Analytical applications

Firstly, the developed method was applied to the determination of cilazapril and its combinations with hydrochlorothiazide in pharmaceutical formulations. The obtained values were in accordance with the certified by the pharmaceutical company, except for the formulation Inhibace, cilazapril 5 mg, which had already expired three years ago, and cilazaprilat was detected. Table 2 shows the results of the analysis of

Table 1
Quantitative parameters obtained for the analysis of cilazapril and cilazaprilat by CZE in spiked urine samples

	Cilazapril	Cilazaprilat
Migration time (min) (mean±SD)	2.94±0.03	3.98±0.06
Calibration range (μg ml^{-1})	0.2–2	0.5–10
Slope of the calibration curve±ts[c] (n=6)	0.170±0.007	0.22±0.01
Intercept±ts[c] (n=6)	−0.01±0.02	0.03±0.06
Regression coefficient of the calibration (r^2)	0.999	0.999
Reproducibility intra-day (RSD, %)[d]	3.5; 1.7[a]	5.9; 2.5[b]
Reproducibility inter-day (RSD, %)[d]	11.1; 10.0[a]	7.6; 8.3[b]
Accuracy (spiked; % relative error)[e]	0.49; 2.0	0.50; −11
	1.91; 2.6	5.82; −2.2
Detection limit (ng ml^{-1})	125	115
Quantitation limit (ng ml^{-1})	420	380

[a] Concentration of cilazapril 0.5 and 2 μg ml^{-1} respectively.
[b] Concentration of cilazaprilat 0.5 and 4 μg ml^{-1} respectively.
[c] Confidence level:95%.
[d] Five days, three replicates each day.
[e] Concentrations expressed in μg ml^{-1}. Found value is the mean of two replicates.

Table 2
Analysis of five pharmaceutical formulations containing cilazapril and its mixture with hydrochlorothiazide[a]

Pharmaceutical formulation	Amount certified	Found (mg)[b]
Inocar	Cilazapril 1 mg	1.01±0.01; 1.02±0.01
Inocar plus	Cilazapril 5 mg	5.04±0.01; 5.11±0.08
	Hydrochlorothiazide 12.5 mg	12.6±0.2; 12.43±0.06
Inhibace	Cilazapril 1 mg	1.02±0.02; 1.00±0.02
Inhibace plus	Cilazapril 5 mg	5.03±0.01; 5.08±0.02
	Hydrochlorothiazide 12.5 mg	12.5±0.3; 12.6±0.1
Inhibace (expired)	Cilazapril 5 mg	4.53±0.06; 4.59±0.03

[a] Two different samples, three injections of each sample.
[b] Expressed as mean value±SD.

the five formulations. The analysis was repeated twice for each formulation and each sample injected three times. The electropherogram that corresponds to the analysis of the formulation Inhibace plus, cilazapril 5 mg and hydrochlorothiazide 12.5 mg, is shown in Fig. 3.

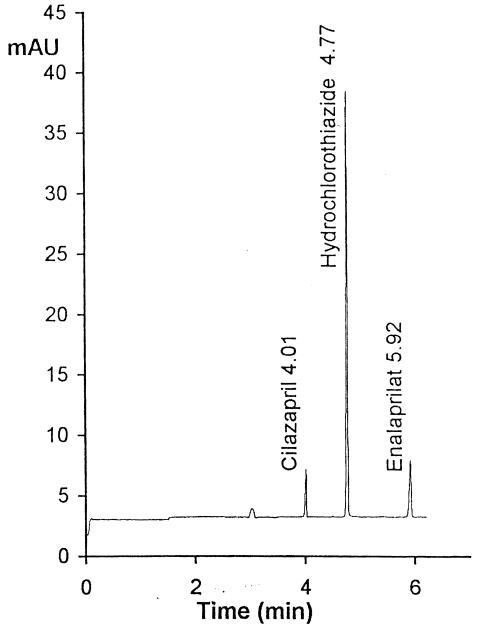


Fig. 3. Electropherogram of the pharmaceutical formulation Inhibace plus: cilazapril 5 mg and hydrochlorothiazide 12.5 mg. Concentration of the internal standard, enalaprilat, 40 μg ml^{-1}, capillary 50 cm×75 μm I.D.; electrolyte: 60 m*M* borate buffer at pH 9.5; potential +25 kV; temperature 25°C; injection time 15 s and detection wavelength 214 nm.

Secondly, the method was used for the determination of cilazapril and cilazaprilat in urine obtained from three hypertensive patients who were under treatment with a daily tablet of Inhibace, cilazapril 5 mg. The urine was collected at four time intervals after the intake. The method was sensitive enough to detect the cilazapril and cilazaprilat at least up to 24 h after the intake. Table 3 quotes the results of the analysis of these twelve samples and the amount (mg) of drug excreted. Each urine sample was analyzed by duplicate and each final extract injected twice in the electrophoretic system. Mean values are given.

As can be seen, great differences in terms of concentrations and excretion can be found among the three patients. For patient number 1, the amount of drug excreted is over 5 mg. This could be due to the excretion of drugs coming from earlier intakes, given that the patients were under continuous treatment with the drugs. Good agreement with the data obtained when using the chromatographic method [13] was obtained. In the case of patient No. 2, no other peaks were observed despite the fact that she was under treatment with other drugs. Fig. 4 shows the electropherogram of the extract of the sample obtained from patient No. 1, 1–4 h after the intake of the drug.

4. Discussion

A validated CZE assay for the determination of cilazapril and its active metabolite cilazaprilat in

Table 3
Determination of cilazapril and cilazaprilat in the urine of three hypertensive patients at four different time intervals after the ingestion of a dose of the pharmaceutical formulation Inhibace: cilazapril 5 mg[b]

Patient	Fraction (h)	Urine volume (ml)	[Cilazaprilat] ($\mu g\ ml^{-1}$)	[Cilazapril] ($\mu g\ ml^{-1}$)	Total amount excreted (mg)[a]
1	0–1	210	1.97	0.40	
	1–4	450	6.57	0.93	
	4–8	330	1.83	0.17	
	8–24	840	0.49	n. d.	7.22
2	0–1	220	n. d.	n. d.	
	1–4	180	10.7	0.70	
	4–8	280	2.05	n. d.	
	8–24	600	0.22	n. d.	2.94
3	0–1	520	n. d.	n. d.	
	1–4	600	n. d.	n. d.	
	4–8	320	6.24	1.38	
	8–24	600	1.39	n. d.	3.47

[a] Expressed as mg of cilazapril.
[b] Mean value of two extractions, two injections of each sample. (n. d. = not detected).

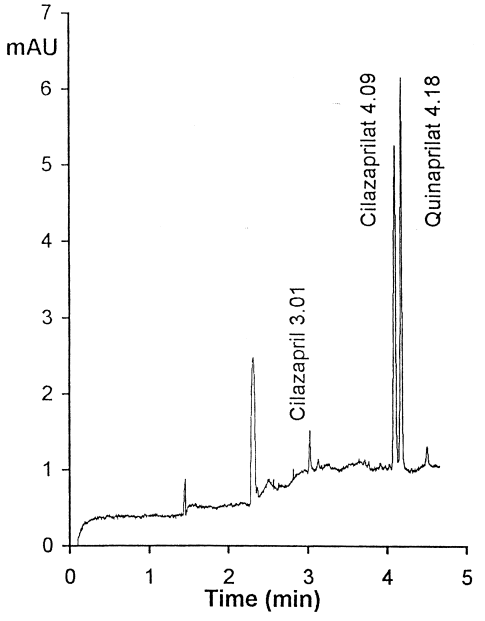


Fig. 4. Electropherograms of a urine sample obtained from patient No. 1, 1–4 h after intake of a therapeutic dose of Inhibace, cilazapril 5 mg. Concentration of cilazapril 0.93 $\mu g\ ml^{-1}$, concentration of cilazaprilat 6.57 $\mu g\ ml^{-1}$. Concentration of the internal standard, quinaprilat, 3 $\mu g\ ml^{-1}$, capillary 50 cm × 75 μm I.D.; electrolyte: 60 m*M* borate buffer at pH 9.5; potential +25 kV; temperature 35°C; injection time 15 s and detection wavelength 214 nm.

urine has been reported. In CZE, the mass/charge ratio governs the elution pattern, and the compounds have been easily separated mainly due to their high differences in charge.

In spite of the complex matrix analyzed, acceptable values of reproducibility and accuracy have been obtained, and the results of the analysis of the urine samples are in accordance with the values obtained using the HPLC method with UV detection [13].

A comparison of this method with the previously developed in our laboratories using liquid chromatography shows that the electrophoretic method has a worse inter-day reproducibility, since the reported RSD of the chromatographic method was always under 6%, and in the case of the highest concentrations below 4%. The detection limits obtained with the electrophoretic technique are also higher than those obtained by HPLC–UV detection, which were 70 $ng\ ml^{-1}$ for both compounds.

It is worth mentioning the excellent selectivity of the method, since there is almost no presence of endogenous substances of the urine in the electropherograms.

To sum up, the chromatographic system has been advantageous in terms of better quantitative parameters, specially reproducibility and detection limits, whereas the electrophoretic system seems advantageous in terms of selectivity and also if economic

factors are taken into account, given the low consumption of reagents and solvents. The analysis time was similar in both cases, 9 min in the chromatographic method and 7 min in the present work (taking into account the purge time between runs).

In the case of pharmaceutical formulations, capillary electrophoresis is a very useful technique, given that there is no problem of sensitivity. The analysis is quick, easy, cheap, and permits the simultaneous quantitation of hydrochlorothiazide. The simultaneous analysis of both drugs was not possible with the HPLC method developed in our laboratory.

Acknowledgements

The authors thank the Interministerial Commission for Science and Technology (Project PB95-0316) and the University of the Basque Country (Project UPV 171.310-EB149/99) for financial support. The pharmaceutical companies are thanked for their kind supply of ACE inhibitors. J. A. Prieto and U. Akesolo thank the Basque Country Government and the University of the Basque Country for a predoctoral grant.

References

[1] F. Deget, R.N. Brogden, Drugs 41 (1991) 799.
[2] K.R. Gerbrandt, K.C. Yedinak, PharmacoEconomic. 10 (1996) 594.
[3] J.F. Hansen, Cardiovasc. Drugs Ther. 9 (1995) 503.
[4] G.E. McVeigh, J. Flack, R. Grimm, Drugs 49 (1995) 161.
[5] J.L. Reid, Eur. Heart J. 18 (1997) E14.
[6] A.A. Ajayl, H.L. Elliot, J.L. Reid, Br. J. Clin. Pharmacol. 22 (1986) 167.
[7] T. Szucs, Drugs 41 (1991) 18.
[8] I.L. Natoff, J.S. Nixon, R.J. Francis, L.R. Klevans, M. Brewster, J. Budd, A.T. Patel, J. Wenger, E. Worth, J. Cardiovasc. Pharmacol. 7 (1985) 569.
[9] M.R. Attwood, C.H. Hassall, A. Kröhn, G. Lawton, S. Redshaw, J. Chem. Soc., Perkin Trans. I (1986) 1011.
[10] I.L. Natoff, S. Redshaw, Drugs Future 12 (1987) 475.
[11] H. Tanaka, Y. Yoneyama, M. Suwahara, I. Umeda, Y. Ohta, J. Pharm. Sci. 76 (1987) 224.
[12] J.A. Prieto, R.M. Jiménez, R.M. Alonso, J. Chromatogr. B 714 (1998) 285.
[13] J.A. Prieto, R.M. Jiménez, R.M. Alonso, E. Ortiz, J. Chromatogr. B in press.
[14] N. Erk, F. Onur, Anal. Lett. 29 (1996) 1963.
[15] N. Erk, J. Pharm. Biomed. Anal. 20 (1999) 155.
[16] R.I. Stefan, J.F. van Staden, H.Y. Aboul-Enein, Electroanalysis 11 (1999) 192.
[17] F. Barbato, P. Morrica, F. Quaglia, Farmaco 49 (1994) 457.
[18] H.H. Maurer, T. Kraemer, J.W. Arlt, Ther. Drug Monit. 20 (1998) 706.
[19] N.A. Guzman, S.S. Park, D. Schauferberger, L. Hernández, X. Paez, P. Rada, A.J. Tomlinson, S. Naylor, J. Chromatogr. B 697 (1997) 37.
[20] J.L. Brunner, J.T. Dipiro, S. Feldman, Pharmacotherapy 15 (1995) 1.
[21] N.K. Karamanos, F. Lamari, Biomed. Chromatogr. 13 (1999) 501.
[22] C. Cruces-Blanco, Electroforesis Capilar, Universidad de Almería, Servicio de Publicaciones, Almería, 1998.
[23] J.C. Miller, J.N. Miller, Statistics For Analytical Chemistry, Ellis Horwood, Chichester, 1984.
[24] S.F.Y. Li, Capillary Electrophoresis, Elsevier, Amsterdam, 1993.
[25] D.R. Baker, Capillary Electrophoresis, Wiley, New York, 1995.
[26] T. Ezawa, M. Hashimoto, M. Abe, M. Nishigata, Y. Tsuchiya, K. Sasai, Iyakuhin Kenkyu 20 (1989) 602.