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Determination of captopril and its degradation products by capillary electrophoresis

S. Hillaert *, W. Van den Bossche

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

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Abstract

Captopril, an antihypertensive agent, and its degradation products have been quantified in pharmaceutical formulations by capillary zone electrophoresis (CZE). A method using cetyltrimethylammonium bromide (CTAB) added to a sodium phosphate buffer (pH 5.5; 100 mM) as running buffer and using *N*-acetyl-L-tyrosine as an internal standard has been developed and validated for the quantitative determination of captopril in tablets. The method is an indicator of compound stability and can also be applied to the purity control of the raw material and for the determination of the degradation products. For this purpose, salicylic acid is used as an internal standard.

Keywords: Captopril; Capillary electrophoresis; Tablets; Purity control

1. Introduction

Captopril, 1-{3-mercapto-2-(S)-methyl-1-oxo-propyl}-(S)-proline (Fig. 1) is an inhibitor of the angiotensin-converting enzyme and is widely used for the treatment of hypertension and congestive heart failure [1]. The literature shows that high-performance liquid chromatography (HPLC) is a major technique used for the determination of captopril in pharmaceutical formulations [2–7]. Analysis of underivatized and derivatized thiols using capillary electrophoresis has been achieved. However, the aim of those studies was to separate several biologically and pharmacologically important thiol-containing compounds, including captopril [8–10]. The conditions of these existing methods have been directed to the separation of a complex mixture of thiols and not to the determination of captopril and its degradation products.

This study was especially directed towards captopril. Thus, other conditions were investigated to determine captopril, without derivatization and specific sample pretreatment. The commercial products used as samples were dissolved directly in the running buffer. The addition of sodium metabisulphite prevents the formation of oxidation products. The determination of captopril and related substances was performed from the

* Corresponding author. Tel.: +32-9264-8101; fax: +32-9264-8193.

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

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cathode to the anode on a fused-silica capillary with and without addition of cetyltrimethylammonium bromide (CTAB) to a sodium phosphate buffer (pH 5.5; 100 mM) and also on a coated capillary. Good quantitation was obtained in short analysis times.

2. Experimental

2.1. Instrumentation and electrophoresis procedure

The development and validation of the method were mainly performed on a Waters Quanta 4000 (Millipore, Waters, US), equipped with a fused-silica capillary 60 cm in total length (52.5 cm to the detector) and 75 μm internal diameter (ID). The data were collected on a Hewlett-Packard Integrator (HP 3396 Series II) (US), 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 ~10 cm above the height of the buffer vial for 10 s. A constant voltage of −15 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.

To demonstrate the ruggedness of the system, some of the work was also 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 75 μm ID. 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 −25 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).

2.2. Chemicals and reagents

Captopril was purchased from Sigma (St Louis, MO, USA). It was tested as prescribed in the monograph of the European Pharmacopoeia (third ed.) [7] and contains 99.0% of (2S)-1-{(2S)-3-mercapto-2-methylpropanoyl}pyrrolidine-2-carboxylic acid, calculated with reference to the dried substance. It was used as a standard. Capoten tablets (25, 50 and 100 mg potency) manufactured by Bristol-Myers Squibb (Brussels, Belgium) were used for the quantitative determination. Sodium dihydrogen phosphate monohydrate and disodium hydrogen phosphate dihydrate were purchased from Merck (Darmstadt, Germany), CTAB (99%) and salicylic acid (99%) from Janssen Chimica (Beerse, Belgium), sodium metabisulphite from UCB (Leuven, Belgium) and *N*-acetyl-L-tyrosine from Sigma.

2.3. Stock, standard, sample and electrolyte preparations

The sodium phosphate buffer (pH 5.5; 100 mM) was prepared by adjusting the pH of a 100 mM sodium dihydrogen phosphate solution to pH 5.5 by the addition of a 100 mM disodium

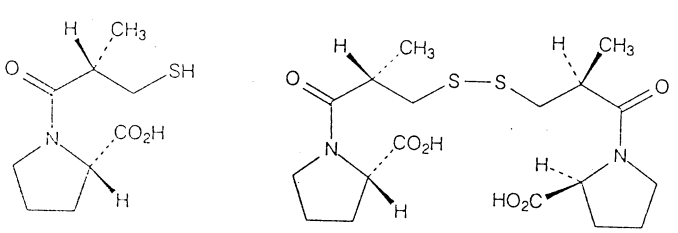

Fig. 1. Chemical structure of captopril and captopril-disulphide.

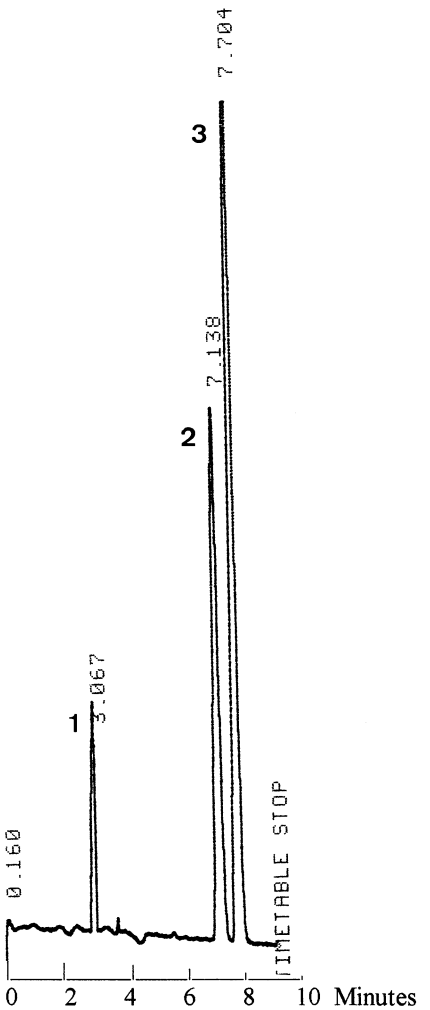

Fig. 2. Electropherogram of a mixture of the antioxidant sodium metabisulphite (1), captopril (2) and *N*-acetyl-L-tyrosine (3) on a coated capillary, performed on the Waters Quanta 4000. Conditions: 34.5 cm (27 cm to the detector) × 75 μm internal diameter Celect-N coated capillary; sodium phosphate buffer (pH 5.5;100 mM) as running buffer; applied voltage, −13 kV; detection at 214 nm.

hydrogen phosphate solution. The sodium phosphate buffer at pH 5.5 was used as a solvent for the preparation of stock, standard and sample solutions containing 2 mg sodium metabisulphite per ml. The running buffer contained 0.025 or 0.045 mM CTAB, respectively, depending on the instrument (Waters Quanta 4000 or Crystal CE).

N-acetyl-L-tyrosine can be used as an internal standard solution for the determination of captopril at a concentration of 0.3 mg ml^{-1} running buffer (solution I). A solution containing about 0.07 mg salicylic acid per ml was used as an internal standard solution (solution II) for the determination of the degradation products.

A stock solution of captopril was prepared by dissolving about 50 mg of the reference standard in the solvent and diluting to 100.0 ml with the same solvent. Appropiate dilutions were made with the solvent to a final concentration of 80–400 μg ml^{-1} for the quantitative determination of captopril and to a final concentration of 10–80 μg ml^{-1} for the purity control. Each dilution contained 120 μg *N*-acetyl-L-tyrosine or 28 μg salicylic acid ml^{-1}, respectively.

Sample solutions of Capoten tablets were prepared by sonicating for 5 min an amount of powder of ground tablets, equivalent to 15 mg captopril in a mixture of 20.0 ml internal standard solution (solution I) and about 20 ml solvent. This was diluted to 50 ml with the same solvent. The sample was filtered.

For the purity control, 100 mg of the substance to be examined (raw material) was dissolved in a mixture of 10.0 ml internal standard solution (solution II) and 10 ml solvent. This was diluted to 25 ml with the same solvent.

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

Table 1
Inter-day variation

	25 mg Capoten (mg)	50 mg Capoten (mg)	100 mg Capoten (mg)
Day 1	24.9 ± 0.03	50.0 ± 0.36	102.9 ± 0.77
Day 2	25.4 ± 0.21	48.9 ± 0.16	103.4 ± 0.11
Day 3	25.3 ± 0.34	48.8 ± 0.25	103.3 ± 0.22

Table 2
Recovery of captopril added to a placebo (accuracy)

Placebo (g)	Captopril (mg)	Theoretical concentration (mg captopril g^{-1})	Amount found (mg captopril g^{-1})	Recovery (%)	RSD (%) ($n=3$)
3.2052	804.1	200.6	204.2 ± 1.06	101.8	0.52
3.0646	1020.2	249.8	252.7 ± 0.91	101.2	0.36
2.8029	1207.6	301.1	300.3 ± 0.63	99.7	0.21

3. Results and discussion

3.1. Stability of captopril

The literature shows that captopril in aqueous solution undergoes oxidative degradation at its thiol function to yield captopril-disulphide (Fig. 1) [11,12]. Maximum stability is found in acidic solutions below pH 4. The presence of antioxidants has significant effects on the reaction mechanism of oxidation and some water-soluble antioxidants (sodium sulphite, sodium metabisulphite and ascorbic acid) were investigated. Sodium metabisulphite gave the best results and 2 mg ml^{-1} buffer was found to offer sufficient protection for 1 day.

3.2. Internal standards

Two different internal standards were used: *N*-acetyl-L-tyrosine for the assay of captopril in pharmaceutical formulations and salicylic acid for the purity control of the raw material. At pH 5.5, proline is present as a zwitterion and cannot be used as an internal standard. Acetylated derivatives of the amino acids proline (*N*-acetyl-L-proline) and tyrosine (*N*-acetyl-L-tyrosine) migrate before and after captopril, respectively. *N*-acetyl-L-proline is less suitable because of the interference with captopril-disulphide. For the purity control of captopril, a more concentrated solution (100 mg 25 ml^{-1}) was used. The peak of captopril is consequently very large so an overlapping of both peaks (captopril and *N*-acetyl-L-tyrosine) occurred.

Another internal standard was necessary and our choice was salicylic acid. It migrates before captopril so the large peak of captopril cannot interfere with those of salicylic acid and there is a baseline separation with the degradation products of captopril.

3.3. Optimization of the method

By analogy with the methods in the literature, the injection took place at the cathode [8–10]. Because the conditions of these methods are directed to the separation of thiols, they are not perfect for the determination of captopril (migration time > 15 min, asymmetric peak, insufficient buffer capacity). The aim of this study was to optimize the conditions for the quantitative determination of captopril in tablets.

Initially, standard solutions of captopril were investigated at room temperature. Several capillary zone electrophoresis (CZE) conditions (different buffers, buffer concentration, pH, applied voltage etc.) were investigated. It was found that the best conditions (baseline separation between captopril and its degradation products, symmetry of the peaks, acceptable migration times) for the determination of captopril are provided by a sodium phosphate buffer (pH 5.5; 100 mM). Captopril has pKa values of 3.7 (carboxyl group) and 9.8 (thiol group) so captopril is mainly negatively charged under the above-mentioned conditions.

In this system, captopril moves from the cathode to the anode and the electro-osmotic flow (EOF) moves from the anode to the cathode. The EOF has a retraining influence on the migration times. For this reason, only by using a short capillary (34.5 cm total length, 27 cm to the detector) did this method give good and usable results. This experiment was performed on the Waters Quanta 4000, equipped with the above-mentioned capillary and a running voltage of -13 kV.

In CZE, the migration time of an analyte is determined by the apparent or observed electrophoretic mobility, which is the sum of the analyte electrophoretic mobility and the electrophoretic mobility contribution of the EOF [13]. When the EOF becomes zero, the determining factor is the analyte electrophoretic mobility. The EOF can be eliminated by using a coated capillary. With a coated capillary, the migration times were shorter and the results were analogous with those on a short, uncoated fused-silica capillary (Fig. 2). Another 'problem' occurred: there were

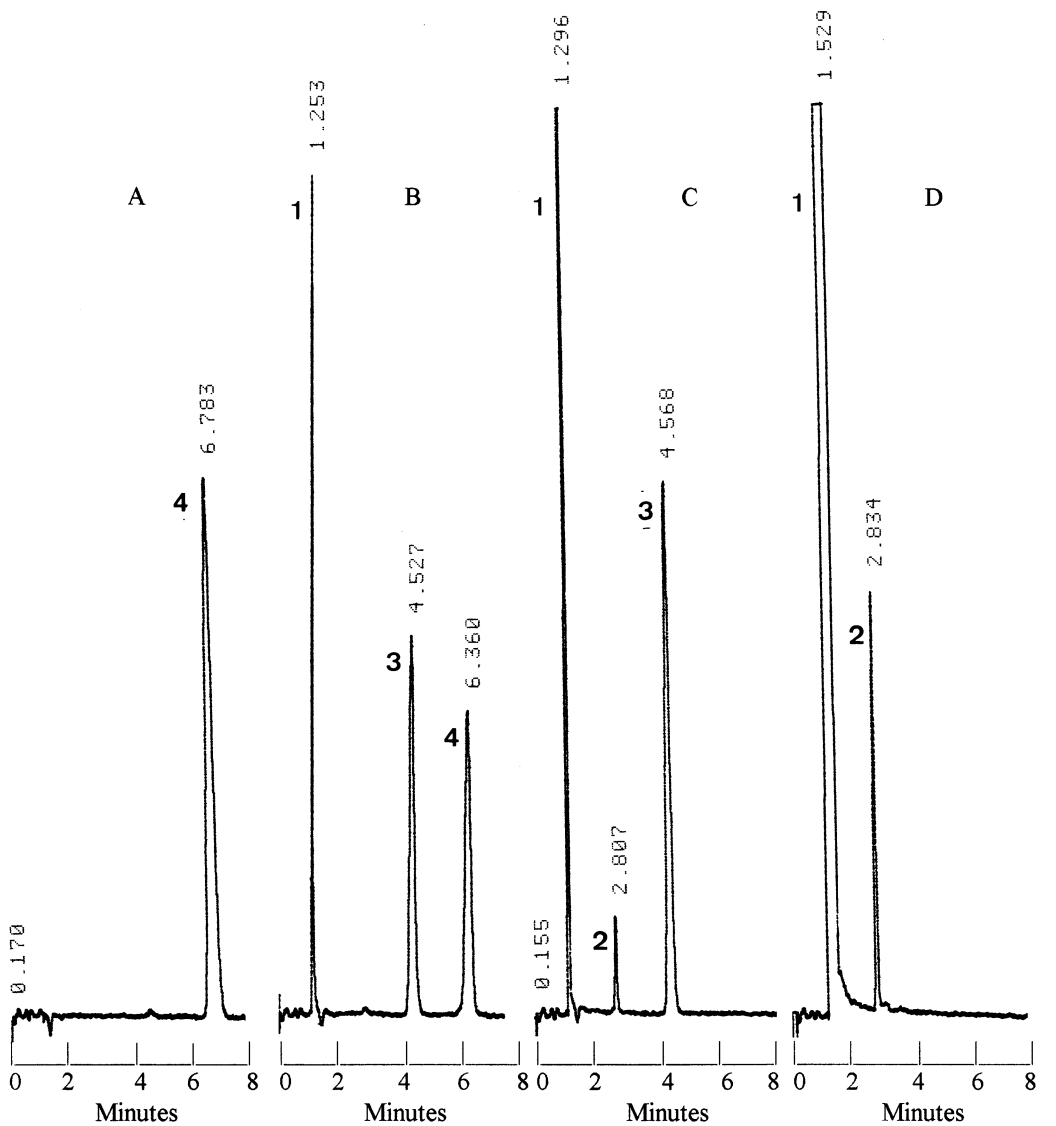

Fig. 3. Illustration of the selectivity of the method. Electropherogram of a solution of captopril to which iodine was added in three different concentrations, performed on the Waters Quanta 4000. Conditions: 60 cm (52.5 cm to the detector) × 75 μm internal diameter fused-silica capillary; sodium phosphate buffer (pH 5.5;100 mM) containing 0.025 mM cetyltrimethylammonium bromide (CTAB) as running buffer; applied voltage, −15 kV; detection at 214 nm. Peaks: (1) iodide; (2) second degradation product (probably captopril-sulphonate); (3) captopril-disulphide and (4) captopril.

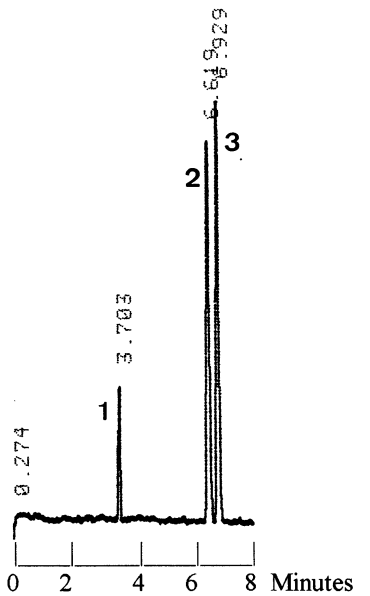

Fig. 4. Electropherogram of a mixture of the antioxidant sodium metabisulphite (1), captopril (2), and *N*-acetyl-L-tyrosine (3) on a fused-silica capillary, performed on the Waters Quanta 4000. Conditions: 60 cm (52.5 cm to the detector) × 75 μm internal diameter; sodium phosphate buffer (pH 5.5;100 mM) containing 0.025 mM cetyltrimethylammonium bromide (CTAB) as running buffer; applied voltage, −15 kV; detection at 214 nm.

fluctuations in the baseline, so the integration was not always perfect. Another solution had to be found to eliminate the EOF.

The literature shows the possibility of eliminating the EOF or even of moving the EOF in the other direction (from cathode to anode) by using an electrolyte such as CTAB, depending on the concentration. At a concentration higher than ~0.35 mM, the EOF reverses in the opposite direction [14]. Several concentrations of CTAB were investigated and the critical factor was in achieving good peak resolution and baseline separation between captopril and the internal standard, namely *N*-acetyl-L-tyrosine. The optimal concentration depended not only on the length of the capillary between inlet and detector, but also on the length between inlet and outlet, and is consequently different for the two instruments. Below 0.025 mM, CTAB had no effect and above 0.045 mM neither chemical (captopril and *N*-acetyl-L-tyrosine) was baseline separated on the two instruments. On the Waters Quanta 4000, the best separation was obtained with 0.025 mM CTAB in sodium phosphate buffer (pH 5.5; 100 mM) using a fused-silica capillary with a total length of 60 cm and a length of 52.5 cm between inlet and detector.

The Crystal CE system gave the same separation using a 0.045 mM CTAB solution on a fused-silica capillary with the same total length, but with a length of only 33 cm from the inlet to the detector.

3.4. Validation of the method

3.4.1. Linearity

The detector responses were found to be linear

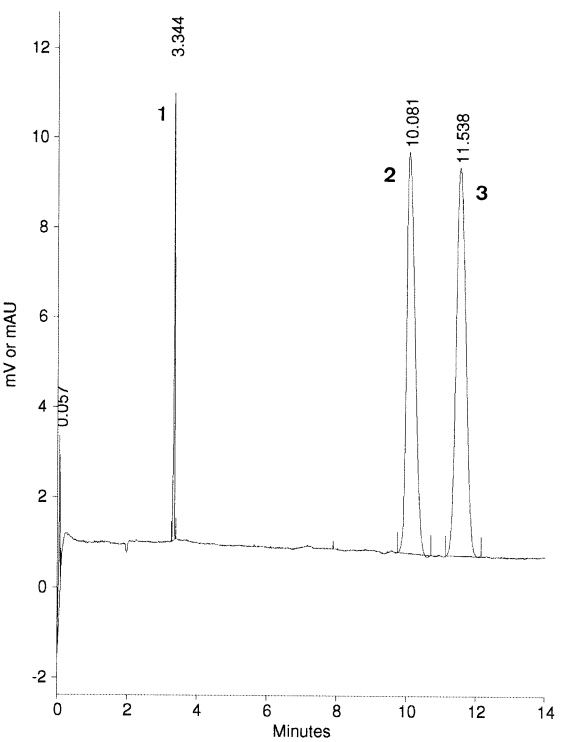

Fig. 5. Electropherogram of a mixture of the antioxidant sodium metabisulphite (1), captopril (2) and *N*-acetyl-L-tyrosine (3) on a fused-silica capillary, performed on the Crystal CE. Conditions: 60 cm (33 cm to the detector) × 75 μm internal diameter; sodium phosphate buffer (pH 5.5;100 mM) containing 0.045 mM cetyltrimethylammonium bromide (CTAB) as running buffer; applied voltage, −25 kV; detection at 214 nm.

Table 3
Quantitative determination of Capoten tablets

	Waters Quanta 4000			Crystal CE		
	Amount found (mg captopril per tablet)	%	RSD (%) ($n=3$)	Amount found (mg captopril per tablet)	%	RSD (%) ($n=3$)
25 mg Capoten	24.9 ± 0.03	99.6	0.12	25.3 ± 0.14	101.2	0.55
50 mg Capoten	50.0 ± 0.36	100.0	0.72	51.1 ± 0.12	102.2	0.24
100 mg Capoten	102.9 ± 0.77	102.9	0.75	100.6 ± 0.70	100.6	0.70

for captopril in the concentration range of 10–80 μg ml^{-1} (purity control) and 80–400 μg ml^{-1} (quantitative determination) by either peak area or peak height measurement. The correlation coefficients (r^2) were 0.9994 and 0.9993, respectively, for the low concentration range and 0.9998 and 0.9997, respectively, for the high concentration range.

3.4.2. Repeatability

The repeatability was determined on the same day by the total analysis of six replicate samples, containing about 0.30 mg captopril ml^{-1}. The mean value obtained was 103.6 ± 1.47 mg so the RSD was 1.42%. The Relative Standard Deviation (RDS) for six consecutive injections of the same standard solution (0.246 mg captopril ml^{-1}) was 0.73% for peak height and 0.43% for peak area. The inter-day variation is summarized in Table 1. The values mentioned are the mean of three consecutive injections.

3.4.3. Accuracy

The analytical procedure was applied to mixtures of the excipients of the placebo (microcrystalline cellulose 3.20 g, maize starch 0.56 g, lactose 2.00 g and stearic acid 0.24 g) to which a known quantity of captopril was added. The accuracy of the method was determined at three levels ranging from 80 to 120% of the theoretical concentration (0.30 mg ml^{-1}) from placebo, spiked with captopril. The results indicate recoveries ranging from 99.7 to 101.8%, as shown in Table 2.

3.4.4. Selectivity

The method selectivity is demonstrated by the good separation of captopril from its two degradation products, as shown in Fig. 3. For the determination of the related substances in the monograph of captopril in the European Pharmacopoeia, the principal degradation product or captopril-disulphide is formed in situ by the addition of 0.05 M iodine to a solution of the substance to be examined. Under these circumstances only a part of the active substance can be oxidized [Fig. 3A (pure captopril) and Fig. 3B (partially oxidized)]. Other impurities were not mentioned in the monograph, but the sum is limited to 2%, calculated as captopril.

From a complementary study of the stability of captopril under stress conditions in the presence of a large excess of iodine (yellow solution), we observed that the peak of captopril disappeared, the peak of the disulphide increased, and a supplementary peak appeared (Fig. 3C). When an excess of iodine was added and the solution was boiled, the captopril-disulphide peak disappeared in favour of the second-formed degradation product (Fig. 3D). This substance was probably formed by oxidation of the thiol function to a sulphonate by analogy with the iodometric determination of benzylpenicillin [15]. Under normal storage conditions it is hardly likely that this product would be formed. The possibility of separating the three substances (captopril, captopril-disulphide and the second degradation product) proves once more the selectivity of the proposed method. Under the same CZE conditions, captopril, captopril-disulphide and the second degradation product (probably captopril sulphonate) migrate at 6.7, 4.5 and 2.8 min, respectively.

3.4.5. Robustness

To demonstrate the system robustness, the method was also performed on a Crystal CE, equipped with a fused-silica capillary 60 cm in total length (33 cm to the detector) and 75 μm ID. The method conditions, applied on the Waters Quanta could be transferred to the Crystal CE if the critical factor (concentration of CTAB) was adapted. Only a 0.045 mM CTAB solution may be used for a baseline separation of captopril and *N*-acetyl-L-tyrosine. The reason for this adaptation is the difference in the length of the capillary between inlet and outlet and between detector and outlet and also the difference in construction of the instruments [13].

All the quantitiative determinations were repeated for this equipment and the results were similar to those on the Waters (Figs. 4 and 5).

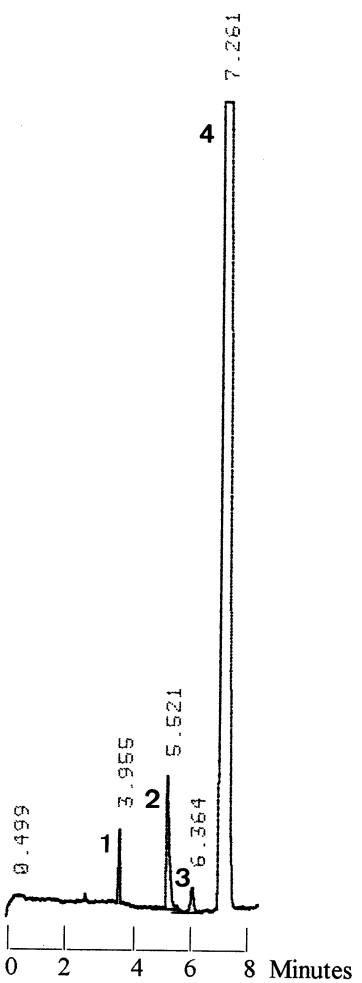

Fig. 6. Electropherogram of the purity control of the raw material (captopril) on a fused-silica capillary, performed on the Waters Quanta 4000. Conditions: 60 cm (52.5 cm to the detector) × 75 μm internal diameter; sodium phosphate buffer (pH 5.5;100 mM) containing 0.025 mM cetyltrimethylammonium bromide (CTAB) as running buffer; applied voltage, −15 kV; detection at 214 nm. Peaks: (1) sodium metabisulphite; (2) salicylic acid (internal standard); (3) captopril-disulphide and (4) captopril.

4. Applications

4.1. Quantitative determination of Capoten tablets

Twenty tablets of each potency of Capoten were ground and the resultant powder was used for the assay test. Quantitative determination was performed on both instruments. The results are summarized in Table 3.

4.2. Purity control of the raw material

The same method as described for the assay of captopril in pharmaceutical preparations can be used for the quantification of the degradation products (Fig. 6). As mentioned previously, the internal standard or *N*-acetyl-L-tyrosine must be replaced by salicylic acid owing to interference with the captopril peak. As in the monograph on captopril in the European Pharmacopoeia, the content of the related substances was expressed as captopril. Therefore, we needed a calibration curve in the lower concentration range (between 10 and 80 μg ml^{-1}). An amount of raw material, equivalent to 100 mg was dissolved in 10.0 ml internal standard solution (solution II) and diluted to 25 ml with solvent. The percentage content of captopril-disulphide, expressed as captopril, was 0.39%. The minimum amount of captopril-disulphide that could be quantified (LOQ) was 0.25% and the limit of detection (LOD) was ~0.15%. Using the HPLC method as described in the European Pharmacopoeia and a spectrophotometer set at the same wavelength (214 nm) as the detector, the percentage content of captopril-disulphide was 0.44%. The second degradation product could not be detected.

5. Conclusions

The determination of captopril and its degradation products by CZE has been achieved. This study demonstrates that CZE can be successfully applied to the determination of captopril and related substances in pharmaceutical preparations and raw material. It offers a good alternative to conventional HPLC methods.

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