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Determination and rotamer separation of enalapril maleate by capillary electrophoresis

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ABSTRACT

Capillary electrophoresis (CE) was applied to the study of enalapril maleate, an inhibitor of angiotensin-converting enzyme. By the use of capillary zone electrophoresis, a method using sodium borate or sodium phosphate buffers (pH 8.25–9.7) as an electrolyte and using an external standard was developed for the determination of enalapril maleate at room temperature. At a method concentration of 0.2 mg/ml, the injection precision (R.S.D. = 0.62%, n = 10) and linearity of detector responses (range 50–150% of the method concentration, R^2 = 0.999) were satisfactory. The method precision was also satisfactory as demonstrated by the content uniformity assays of ten tablets of Vasotec (20 mg potency). The method is stability indicating and specific as enalapril can be well separated from its two degradates, enalaprilat (a hydrolysis product) and its diketopiperazine (a cyclization product). By the use of micellar elektrokinetic capillary chromatography, in which 40–100 m*M* sodium dodecyl sulfate were added to the electrolyte buffers to form micelles in capillary, the *cis* and *trans* rotamers of enalapril was separated at or below 20°C. As the temperature increased, the two peaks spread and coalesced to one peak above 40°C. The study demonstrates that CE can be effectively applied to both the determination and conformer separation of drugs in pharmaceutical research and development.

INTRODUCTION

Enalapril maleate {N-[(*S*)-1-ethoxycarbonyl-3-phenylpropyl]-L-alanyl-L-proline (*Z*)-2-butenedioate} (**1**) is an orally active inhibitor of angiotensin converting enzyme, widely used for treating hypertension [1]. The rotation around the proline amide bond in enalapril is hindered at room temperature. In the solid state, enalapril was found to be exclusively in the *trans* form around the amide bond [2,3]. In solution [4], enalapril can exist as *cis* and *trans* rotamers. The *cis–trans* interconversion of enalapril in solution, like that of other proline dipeptides, has a relaxation time of the order of minutes [5,6].

High-performance liquid chromatography (HPLC) is a major technique used for the determination of enalapril maleate [7]. The retention times of enalapril observed in reversed-phase HPLC are of the order of minutes similar to the relaxation time of *cis–trans* interconversion. At room temperature, peak spreading and distortion could occur owing to the slow *cis–trans* interconversion. Higher column temperatures (*ca.* 60–80°C) are generally employed in HPLC assays in order to obtain sharp peaks of enalapril for quantitative analysis. It is reported here that the determination of enalapril maleate in tablets at room temperature can be achieved by capillary zone electrophoresis (CZE), a separation technique first introduced in the early 1980s [8–10]. Recently, it has been recognized that CZE possesses great potential in pharmaceutical applications [11–13]. This study provides a good example of the applicability of CZE to pharmaceutical analysis.

HPLC has also been successfully used for confor-

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mational and kinetic studies of drugs [14]. For example, the *cis–trans* rotamers of enalapril have been separated by HPLC at 10°C [7]. These studies are important, as *cis–trans* interconversion of enalapril and of other proline-containing drugs may have significance in pharmaceutical development. Several groups have reported that the *trans* rotamer is the active form of the proline-containing drugs [15–17]. In this paper, it is shown that a modified capillary electrophoresis (CE) technique, micellar electrokinetic capillary chromatography (MECC) [18], is well suited for the separation of rotamers of enalapril and of other proline-containing drugs. Using this technique, the separation of cis and *trans* rotamers of enalapril was observed at or below 20°C. This study demonstrates that CE is as effective as HPLC in the determination and separation of conformers.

EXPERIMENTAL

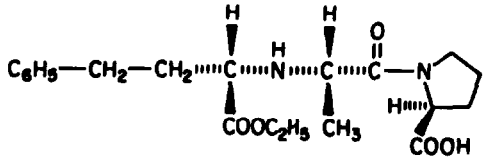
Instrumentation and electrophoresis procedure

The development and validation work was mainly performed on a Beckman P/ACE 2100 equipped with System Gold software, a fused-silica capillary 57 cm in total length (50 cm to the detector) and 75 μm I.D. and a liquid-cooled capillary cartridge that can be controlled over the temperature range 20–50°C. The sample solutions were introduced into the capillary by pressure injection (0.034 bar) with a typical injection for 8 s. A constant potential of 16.1 kV was applied for determinations. UV absorbance at 200 nm was employed for detection. The data were collected and analyzed by the System Gold software program installed in an IBM PS/2 computer. Some of the work to demonstrate the method ruggedness was performed on a Dionex capillary electrophoresis system equipped with a fused-silica capillary 60 cm in length (50 cm to the detector) and 75 μm I.D. Gravity injection at a 60 mm height for 30 s was employed for sample injection. UV absorbance at 215 nm was used for detection. A constant potential of 20 kV was used for analyses.

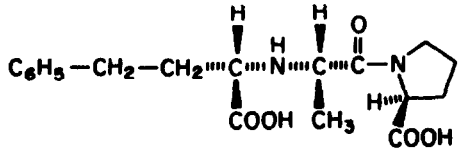
Before analysis, fused-silica capillaries were prepared by successive washing with 0.1 *M* sodium hydroxide solution, distilled water and finally the running electrolyte. Between each run the capillary was always flushed with 0.1 *M* sodium hydroxide solution and then the running electrolyte.

Chemicals

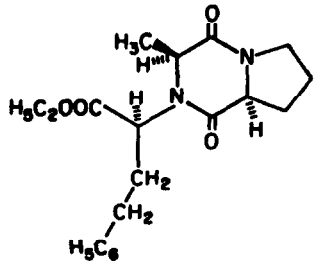
Enalapril maleate (**1**) and its two main degradates [7], enalaprilat {N-[(*S*)-1-carboxyl-3-phenylpropyl]-L-alanyl-L-proline} (**2**) and its diketopiperazine



Enalapril (SSS)
(1)



Enalaprilat (SSS)
(2)



Diketopiperazine
(3)

(DKP) (3) of pharmaceutical grade manufactured by Merck Research Labs. (Rahway, NJ, USA) were used as standards. Enalapril has three chiral centers and consequently eight stereoisomers. The isomer used in this study is a pure *SSS*-isomer. Vasotec tablets (20 mg potency) manufactured in Merck Research Labs. (West Point, PA, USA) were used for content uniformity study. Sodium dodecyl sulfate (SDS) (99%) was purchased from Sigma (St. Louis, MO, USA), maleic acid (99%) from Aldrich (Milwaukee, WI, USA) and methanol (Optima grade), sodium hydroxide (0.1 *M* solution), phosphoric acid (85%, HPLC grade) and sodium tetraborate (electrophoresis grade) from Fisher Scientific (Philadelphia, PA, USA). All solvents and reagents were used as received. Deionized water with at least 18 MΩ resistance purified with a Milli-Q system (Millipore, Bedford, MA, USA) system was used for electrolyte, sample and standard preparations.

Standard, sample and electrolyte preparations

A standard solution of enalapril maleate or enalaprilat was prepared by dissolving about 20 mg of the corresponding reference standard in 100 ml of water (0.2 mg/ml solution) or 200 ml of water (0.1 mg/ml solution) by sonication for 2 min. A standard solution of diketopiperazine was made by dissolving 20 mg of reference standard in 200 ml of water–methanol (60:40, v/v). Sample solutions of Vasotec tablets were prepared by dissolving a tablet into 100 ml water by sonicating for 2 min.

The running electrolyte for CZE was 80 m*M* sodium borate buffer or 50 m*M* sodium phosphate buffer. The pH of the buffers was adjusted in the range 8.25–9.7 by addition of 0.1 *M* sodium hydroxide solution.

The electrolyte used for MECC was prepared by dissolving 40–100 m*M* SDS in the above-mentioned buffers. Typical electrolytes used were 80 m*M* sodium borate–100 m*M* SDS buffer (pH 8.5) and 50 m*M* sodium phosphate–40 m*M* SDS buffer (pH 8.25).

All samples and buffers were filtered through a Millipore 0.22-μm filter unit.

RESULTS AND DISCUSSION

Determination of enalapril maleate

Method development. Initially, solutions of enalapril maleate standard were investigated at room temperature. Several CZE conditions (different buffers, buffer concentration, pH, applied voltage, etc.) were investigated. Enalapril has pK_a values of 2.97 and 5.35 at 25°C [7]. A fairly wide pH range (8.0–9.7) and buffer concentration (80–100 m*M* sodium borate and 25–50 m*M* sodium phosphate) were suitable for the determination of enalapril maleate.

In contrast to HPLC, in which the enalapril peaks were severely distorted at room temperature, the electropherogram of enalapril, shown in Fig. 1, is a sharp peak migrating at about 6.7 min. The electrolyte used was 50 m*M* sodium phosphate (pH 8.25). A solution of maleic acid (concentration 0.27 mg/ml) was examined under the same conditions. Maleic acid did not elute within 10 min, confirming the assignment of the enalapril peak. The different observation of enalapril peaks in CZE can be understood by the separation mechanisms of CZE [8–10]. In CZE, the migration time of an analyte is determined by the apparent electrophoretic mobility, which is the sum of the analyte electrophoretic mobility and the electrophoretic mobility contribution from the electroosmotic flow. The electroosmotic flow contribution is the same for *cis* and *trans* rotamers of enalapril. The determining factor is therefore the analyte electrophoretic mobility. As

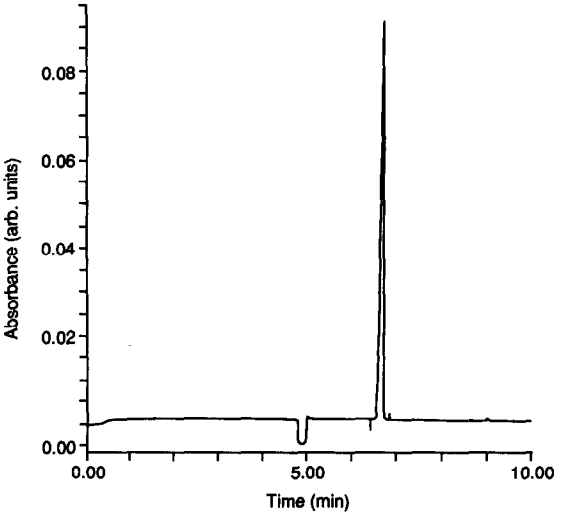


Fig. 1. Electropherogram of enalapril (0.2 mg/ml) recorded at room temperature. Conditions: capillary, fused silica [57 cm (50 cm to detector) × 75 μm I.D.]; electrolyte, 50 m*M* sodium phosphate (pH 8.25); applied voltage, 16.1 kV.

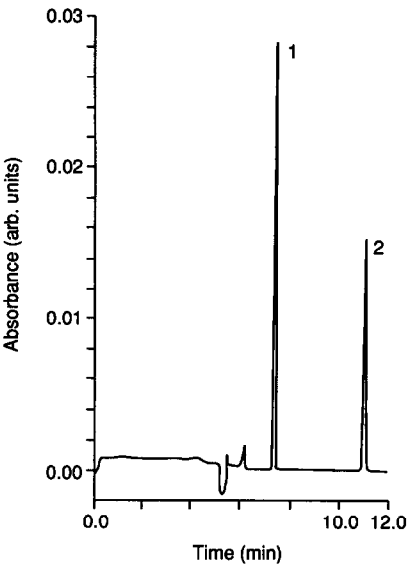


Fig. 2. Electropherogram of (1) enalapril and (2) enalaprilat recorded at room temperature. Conditions: capillary, fused silica [57 cm (50 cm to detector) × 75 μm I.D.]; electrolyte, 80 m*M* sodium borate (pH 9.7); applied voltage, 16.1 kV.

the *cis* and *trans* rotamers of enalapril have the same charge to mass ratio and hence the same electrophoretic mobility, they migrate at the same rate. Consequently, re-equilibration from the original conditions did not occur during the course of the CE experiments, and a single sharp peak was observed.

Although a pH range between 8.0 and 9.7 could be used for the determination of enalapril maleate, it may not be totally suitable for the determination of enalaprilat, a hydrolysis degradate of enalapril. Enalaprilat has two carboxyl groups with pK_{a1} and pK_{a2} values of 3.3 and 7.6, respectively, at 25°C. At pH 8.25, the peak of enalaprilat showed considerable fronting, probably owing to a large difference in mobilities between enalaprilat and the buffer component [19,20]. Therefore, 80 m*M* sodium borate (pH 9.7) was chosen as the electrolyte in further method development. Under these more basic conditions, enalapril and enalaprilat migrate at about 7.3 and 11.0 min, respectively, and their peaks are both sharp, as illustrated in Fig. 2. In addition to the enalapril and enalaprilat peaks, a small peak migrating at about 6.2 min could be observed in the electropherogram shown in Fig. 2. This peak appears to bear no relation to the enalapril peak as the intensity of the peak ($2 \cdot 10^{-3}$ absorbance units) did not change when the concentration of the enalapril maleate solution was reduced from 0.2 to 0.002 mg/ml (a 100-fold difference). This peak seems to be related to the higher pH. No further identification of the peak was attempted.

Method validation. The injection precision of the method (80 m*M* sodium borate buffer, pH 9.7) was examined at three different concentrations of enalapril maleate solutions, *i.e.*, 0.203 mg/ml (standard), 0.0999 mg/ml (50% standard) and 0.00203 mg/ml (1% standard). The average migration time, average peak area and relative standard deviation (R.S.D.) values obtained are summarized in Table I. The injection precisions were satisfactory (nearly as good as those of HPLC). Peak-area measurements were therefore adopted for the determination of enalapril maleate in this method.

The detector responses in a range of 50–150% of the standard concentration were determined to be linear with $R^2 = 0.999$. Duplicate injections were used at each of the five concentrations.

TABLE I

INJECTION PRECISION OF ENALAPRIL MALEATE ($n = 10$)

Parameter	Sample concentration (mg/ml)		
	0.205	0.0999	$2.03 \cdot 10^{-3}$
Average migration time (min)	7.477	7.707	7.717
Precision of migration time (R.S.D., %)	0.37	1.7	0.42
Average peak area	8.198	4.030	0.0844
Precision of peak area (R.S.D., %)	0.62	1.59	6.50

The method specificity was demonstrated by the good separation of enalapril from its hydrolysis degradate enalaprilat. As already shown in Fig. 2, under the same CZE conditions, enalapril and enalaprilat migrate at about 7.3 and 11.0 min, respectively, which gives a relative migration time of 1.5. Enalapril is also well separated from another degradate, diketopiperazine. Under the same conditions as described in Fig. 2, diketopiperazine migrates at about 5.2 min as a neutral compound (relative migration time 0.7). The peak is broad. The determination of enalaprilat was good, and its detection limit was determined to be less than 0.2%. However, the determination of diketopiperazine was difficult under the present conditions owing to the possible co-elution of other neutral components. An MECC method is now under development. The method was also specific against components of the placebo formulations for Vasotec. As the enalapril peak is well separated from its degradates, and the main hydrolysis degradate, enalaprilat, can be determined, this method is stability indicating.

The validation was extended to the method precision as shown in the following content uniformity study of Vasotec tablets.

Content uniformity of Vasotec tablets. Ten Vasotec tablets (20 mg potency) were used in the content uniformity assay. The results were 19.46, 19.29, 19.16, 19.56, 19.08, 19.77, 19.21, 19.57, 19.37 and 19.35 mg per tablet, yielding an average of 19.38 mg per tablet with an R.S.D. of 1.1%, in reasonably good agreement with the results obtained by the HPLC method (19.84 mg per tablet, R.S.D. = 3.2%).

The CZE method for determining enalapril maleate in Vasotec tablets was reproducible. The content uniformity study of ten tablets was repeated using a Dionex capillary electrophoresis system, which resulted in an average of 19.50 mg per tablet with an R.S.D. of 3.5%, again in good agreement with the results obtained by the HPLC method.

Separation of rotamers of enalapril

Separation of cis and trans rotamers by MECC. The CZE method used for the determination of enalapril maleate showed high separation efficiency and resolution, but could not be applied to the separation of rotamers of enalapril. Under all the CZE conditions investigated, a single sharp peak contributed by both *cis* and *trans* rotamers was always observed.

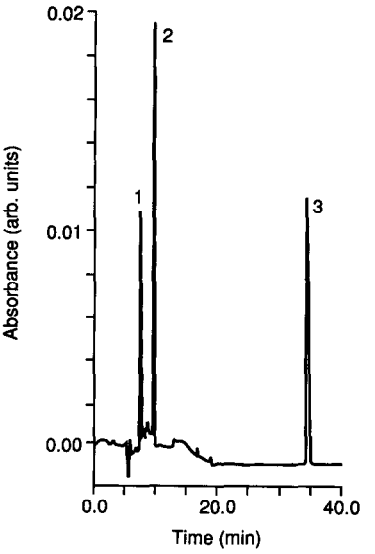


Fig. 3. Electropherogram of enalapril (0.1 mg/ml) recorded at room temperature. Peaks 1 and 2 were assigned to the enalapril rotamers and peak 3 to maleic acid. Conditions: capillary, fused silica [57 cm (50 cm to detector) × 75 μm I.D.]; electrolyte, 80 m*M* sodium borate (pH 8.5)–100 m*M* SDS; applied voltage, 16.1 kV.

The separation of *cis* and *trans* rotamers was attempted by MECC. The same instrument as used for CZE was conveniently used for MECC, the electrolyte being modified by the addition of about 40–100 m*M* of SDS. At these concentrations, SDS forms micelles in the electrolytes [21].

Using the electrolyte [80 m*M* of sodium borate buffer–100 m*M* SDS (pH = 8.5)], a pair of peaks of enalapril with migration times of about 8 and 10 min were observed at room temperature, as illustrated in Fig. 3 for an enalapril maleate standard solution of 0.1 mg/ml. This pair of peaks were assigned to the *cis* and *trans* rotamers of enalapril. The third peak in Fig. 3 eluting at 34 min was assigned to maleic acid. This assignment was confirmed by examining a maleic acid solution (0.27 mg/ml) under the same MECC conditions, in which the only peak observed was the late eluting peak due to maleic acid. As the enalapril used in this study was a pure isomer (the *SSS* isomer), the two enalapril peaks could not be assigned to different

isomers, and must be assigned to the enalapril rotamers.

In order to confirm the peak assignment, a solution of 0.2 mg/ml of enalapril maleate was examined under the same MECC conditions. Again, a pair of peaks were observed at room temperature. The peak height (or peak area) ratio (peak at 8 min/peak at 10 min $\approx$ 1.8) of the two peaks was about the same as the ratio (*ca.* 1.9) observed at an enalapril maleate concentration of 0.1 mg/ml. This observation indicates the close relationship of these two peaks, in agreement with the rotamer assignment.

The separation of *cis* and *trans* rotamers of enalapril by MECC may be understood by the MECC separation mechanism. At pH 8.5 as in the MECC conditions, enalapril forms the corresponding carboxylic anion, which moves in the capillary according to the electrophoretic mechanism. However, enalapril also has a large hydrophobic group at the other side of the molecule, which can partition between the aqueous solution and the SDS micelles. Because of the difference in hydrophobicity of the *cis* and *trans* rotamers of proline-containing compounds [22], their partitioning between the aqueous solution and micelles exhibits a different selectivity. As a result, enalapril rotamers were separated by MECC as by HPLC. The distributions of enalapril molecules between the sample solution and the electrolyte makes a negligible contribution to the separation of rotamers because under the CZE conditions in which such distributions also took place only one peak was observed.

The migration sequence of the two rotamer peaks was assigned as *trans* at 8 min and *cis* at 10 min based on two considerations. First, as the *cis* rotamer has a larger hydrophobic surface area than the *trans* rotamer [22], it should bind with the SDS micelles more strongly and, as a result, have a longer migration time than the *trans* rotamer. Second, molecular mechanics calculations [23,24] predicted that for the enalapril anion, the *cis* rotamer is thermodynamically more stable than the *trans* rotamer, the calculated difference being 1.4 kcal mol^{-1} for the optimized structures of the two rotamers [25]. Hence the peak at 10 min having the larger peak area is assigned to the *cis* rotamer.

Temperature effect on the separation of cis and trans rotamers. The MECC conditions for the separation of enalapril rotamers were rugged. In addition to the sodium borate buffer mentioned above, a sodium phosphate buffer [50 m*M* sodium phosphate–40 m*M* SDS (pH 8.25)] was also used as an electrolyte. Excellent separation of *cis* and *trans* rotamers was again obtained at room temperature. Using this buffer, the effect of temperature on the separation of *cis* and *trans* rotamers was as depicted in Fig. 4. The pair of peaks from the *cis* and *trans* rotamers were clearly separated at 20 and 25°C. However, the two peaks gradually spread as the temperature increased, and coalesced above 40°C, this observation being similar to that in the previous HPLC studies of rotamers [6,22]. The temperature dependence of the two peaks gives further strong support to the rotamer assignment. Using the earlier mentioned buffer [80 m*M* sodium borate–100 m*M* SDS (pH 8.5)] as electrolyte, a similar temperature effect on the *cis* and *trans* rotamers was also observed.

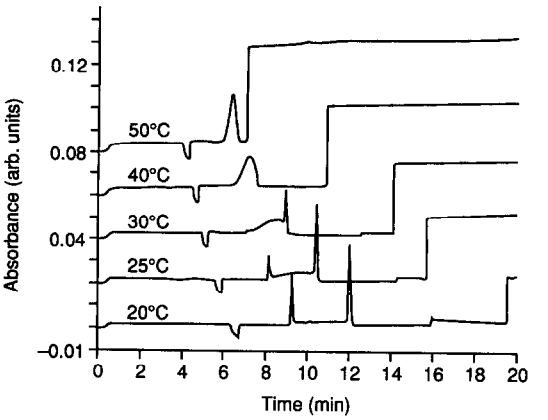


Fig. 4. Electropherogram of enalapril (0.2 mg/ml) recorded at 20, 25, 30, 40 and 50°C, showing the effect of temperature on the enalapril rotamer separations. Conditions: capillary, fused silica [57 cm (50 cm to detector) × 75 μm I.D.]; electrolyte, 50 m*M* sodium phosphate (pH 8.25)–40 m*M* SDS; applied voltage, 16.1 kV.

It is noteworthy that Fig. 4 shows sudden absorption increases in the baseline of the electropherograms after the enalapril peaks. As this did not interfere with the observation of enalapril peaks, its exact cause was not pursued.

It is well established that the influence of conformational changes on peak shapes in HPLC is governed by the relative rate of interconversion with

respect to the time scale of the HPLC elution [26]. If this general rule can be applied to CE, the observed temperature effect described above could be attributed to two contributing factors. First, when the temperature increases, the rate of interconversion is expected to increase, which would lead to the coalescence of the rotamer peaks. Second, when the temperature increases, the electroosmotic flow increases, which should result in more dispersion of the rotamer peaks, as illustrated by the effect of the flow-rate on the shape of the rotamer peaks [22]. However, the interconversion rate increased much faster than the electroosmotic flow as the temperature increased. Therefore, at 40°C, the *cis–trans* interconversion rate became sufficiently fast with respect to the CE time scale, and the rotamer peaks coalesced into one peak.

Clearly, the temperature effect observed contains valuable kinetic information on the rotamers. In HPLC, Hanai and Wada [27] developed the "elution-band relaxation method" for reversible isomerization kinetics. Horváth and co-workers [6,22,26] developed two procedures to analyze isomerization kinetics, one to evaluate the plate-height contribution due to isomerization and the other to simulate chromatograms by solving differential equations numerically. However, there is no kinetic model for MECC. In order to develop procedures to analyze the isomerization kinetics in MECC, a systematic study of the enalapril rotamers and rotamers of other proline-containing drugs (*e.g.*, captopril) is in progress, including the investigation of the effects of pH, micellar concentration and applied voltage.

CONCLUSIONS

The determination of enalapril maleate by the use of an external standard by CZE and the separation of *cis* and *trans* rotamers of enalapril at 20°C by MECC have been achieved. These studies demonstrate that CE can be successfully applied to both the determination and conformer separation of drugs in pharmaceutical research and development.

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