Determination of Enalapril in Human Plasma by High Performance Liquid Chromatography-Electrospray Ionization Mass Spectrometry

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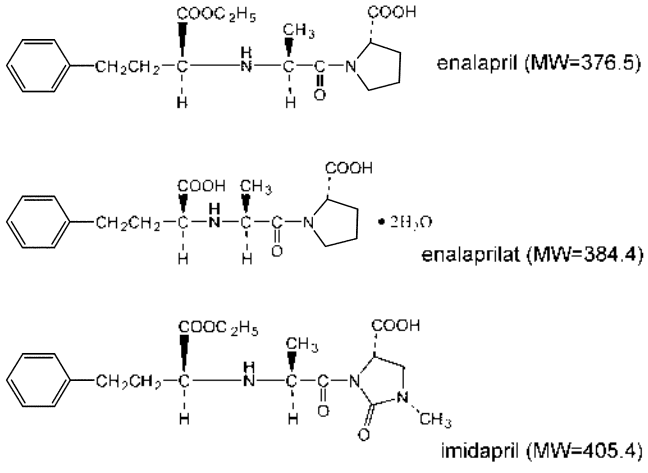
Revered-phase LC-electrospray ionization mass spectrometry was used to selectively determine enalapril from plasma with minimal sample preparation. Detection limit of the method was 1 ng/mL. Precision (within day and between days) and accuracy of the method at various concentrations were acceptable. The analytical technique was used for pharmacokinetic studies after administration of enalapril to human test subjects.

Key Words : Enalapril, LC-ESI MS, Pharmacokinetic study

Introduction

Enalapril maleate ((S)-1-{N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl}-L-proline, (Z)-2-butenedioate (1 : 1) salt is a salt of enalapril and maleic acid. Enalapril is hydrolyzed in the body to enalaprilat, which acts as an inhibitor of angiotensin-converting enzyme (ACE) in the renin-angiotensin aldosterone system. ACE is a peptidyl dipeptidase that catalyzes the conversion of angiotensin I to the vasoconstrictor substance, angiotensin II, which stimulates aldosterone secretion by the adrenal cortex. Inhibition of ACE results in decreased plasma angiotensin II, which leads to decreased vasopressor activity and to decreased aldosterone secretion.[1] Thus it is used to treat high blood pressure and heart failure.



In treating high blood pressure or heart failure it is desirable to maintain a certain level of enalapril in the blood. Therefore, when investigating the efficacy of enalapril or its synthetic equivalent, it is necessary to determine the concentration of residual enalapril in the plasma.

Analytical methods for enalapril in plasma reported in the literature include high performance liquid chromatography (HPLC) with UV detection,[2] capillary electrophoresis,[3,4] and flow injection analysis based on the formation of a copper complex.[5]

Recently, HPLC coupled with highly selective mass spectrometric detection (MSD) has been widely used for pharmaceutical analysis. For example, LC-MS or LC-MS/MS is a method of choice for analysis of amplodipine, a dihydropyridine calcium channel blocker.[6-8] LC-MS analysis of ramipril in the plasma using enalapril maleate as the internal standard has been reported.[9] In this paper, we demonstrate that the LC-MS technique based on reversed-phase separation and electrospray ionization mass spectrometry can be used with minimal sample pre-treatment to selectively determine and monitor enalapril in human plasma.

Experimental Section

Chemicals. Standard enalapril and imidapril (internal standard) were from Sigma (Oakville, Ont., Canada) and Tanabe Seiyaku (Osaka, Japan), respectively. HPLC grade acetonitrile was from Burdick & Jackson (Muskegon, USA). All other reagents were from Wako (Osaka, Japan).

Calibration. 100 μg/mL enalapril solution in 10 mM ammonium formate (pH 2.4) was stored frozen. It was diluted with blank plasma to 1, 2.5, 5, 10, 25, 50, 150, and 300 ng/mL concentrations. To 200 μL of each plasma sample, 20 μL imidapril solution (3 μg/mL) and 20 μL 60% (w/v) perchloric acid were added, mixed with a vortex mixer for 10 s and centrifuged for 10 min at 14,000 rpm. 100 μL of the supernatant was mixed with 100 μL 0.2 M ammonium formate (pH 6.6 adjusted with formic acid), and 20 μL of the mixture was injected into the LC-MSD system. Calibration curve was constructed using the enalapril peak area relative to the imidapril peak. Five analyses a day were repeated for five consecutive days to establish reproducibility of the method.

Sample Preparation. The blood sample was withdrawn from 24 human test subjects immediately before oral intake of enalapril and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, and 9 h

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afterwards (selected based on predetermined half-life of enalapril in the blood, 2.9 h). The blood sample was centrifuged for 10 min at 3,000 rpm. Plasma was withdrawn from the blood and stored at -70 °C until analysis.

For analysis of enalapril, 20 μL of 3 ppm imidapril solution and another 20 μL of 60% (w/v) perchloric acid were added to 200 μL of the plasma. The mixture was vortex-mixed for several seconds and centrifuged for 5 min at 14,000 rpm to remove the precipitated proteins. Before injection into the chromatograph, the pH of the supernatant was increased from 0.7 to 2 using 0.2 M ammonium formate buffer (pH 6.6 adjusted with formic acid).

LC-MS. Agilent (Wilmington, DE, USA) 1100 HPLC system with Agilent 1946B mass selective detector (MSD) was used. The Restek Ultra C18 guard cartridge (3.5 μm, 2.0 × 10 mm) and Waters Xterra C18 column (3.5 μm, 2.1 × 100 mm) were maintained at 55 °C. Mobile phase was deionized water : acetonitrile (67 : 33, v/v) containing 2 mM ammonium formate buffer (pH 2.4 adjusted with formic acid). Flow rate was 0.3 mL/min. The pH of the moblie phase and the column temperature were determined after the extensive study of those factors on the peak shape and splitting of enalapril maleate by Salamoun and Slais.[10]

Positive ion selective ion monitoring (SIM) mode was used to detect *m/z* 377.1 (enalapril + H^+) and *m/z* 406.1 (imidapril − H^-) ions simultaneously. Electrospray ionization was performed using nitrogen as nebulizing gas at 12 L/min flow rate, 30 psig nebulizing pressure, and 350 °C drying gas temperature. Capillary voltage was set at 3,000 V, and fragment voltage was set at 70 V. Gain was set at 1.0 for both signals. Agilent Chemstation was used for data management.

The accuracy, precision, and detection limit of the method were studied by performing five separate analyses per day for five days at six enalapril concentrations.

Results and Discussion

Mass Selective Detection. Under this chromatographic conditions, enalapril and imidapril were almost coeluted at 1.33 min and 1.34 min, respectively. However, the mass selective detector could detect enalapril and imidapril independently. The chromatogram obtained from plasma sample spiked with 250 ng/mL imidapril by monitoring at *m/z* 377.1 showed no signal around 1.33 min (Fig. 1) and was practically identical with that from blank plasma. The chromatogram from the same run obtained at *m/z* 406.1 showed a strong peak at 1.34 min. Similarly, plasma sample spiked with 150 ng/mL enalapril showed no signal when monitored at *m/z* 406.1 and a strong peak at *m/z* 377.1 (data not shown).

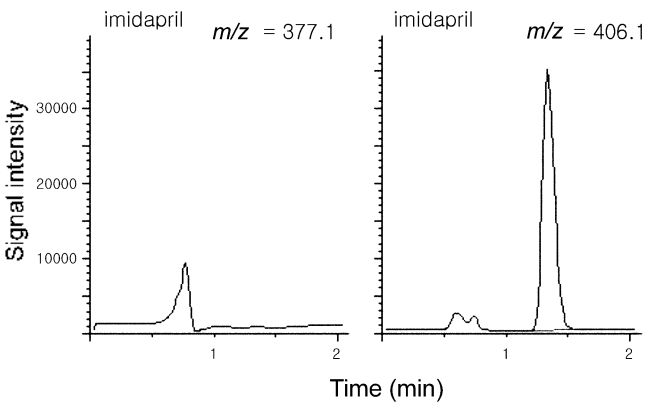

Figure 1. Plasma spiked with imidapril monitored at *m/z* 377.1 and 406.1

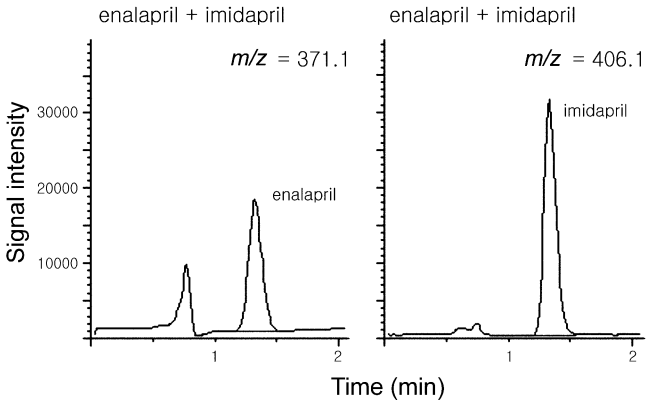

Figure 2. Plasma spiked with both enalapril and imidapril monitored at *m/z* 377.1 and 406.1.

Figure 2 shows that enalapril and imidapril can be analyzed separately from a plasma sample spiked with both enalapril and imidapril by monitoring at *m/z* 377.1 and 406.1 simultaneously. Chromatograms obtained from objects administered with enalapril were practically identical with those in Figure 2 except that the signal intensity for enalapril changed over time.

Since detection was highly selective, sample preparation was simple and straightforward. The sample was ready for injection after precipitation of proteins with perchloric acid, centrifugation, and pH adjustment. Chromatogram with UV detection was crowded with many peaks from other components and, therefore, was not useful for analysis of enalapril (data not shown).

Calibration. The calibration curve was obtained by comparing the area for enalapril against the area for a fixed amount of the internal standard. It was linear (R^2 = 0.99999)

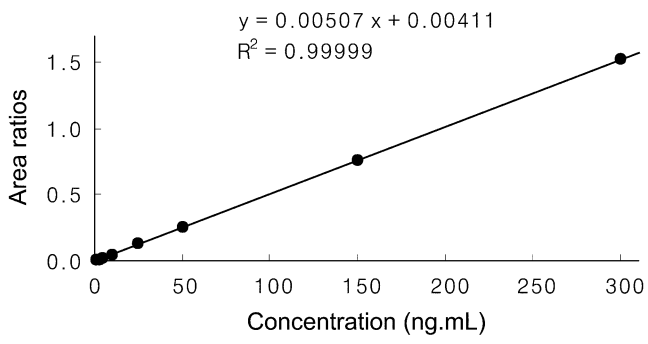

Figure 3. Calibration curve for enalapril in plasma.

Table 1. Precision and accuracy of the LC-MSD method

Concentration (ng/mL)	Precision (C.V., %) within day (n = 5)	Precision (C.V., %) between days (n = 5)	Concentration Found (ng/mL) and Accuracy (%, n = 9)
1	5.10	7.78	0.95 (95.1)
2.5	4.62	8.10	2.35 (94.1)
5	5.87	3.20	4.68 (93.6)
10	2.18	1.89	9.68 (96.8)
25	0.56	2.49	25.4 (101.6)
50	5.37	2.24	50.4 (100.9)
150	0.44	0.88	150.2 (100.1)
300	0.86	0.95	299.8 (99.9)

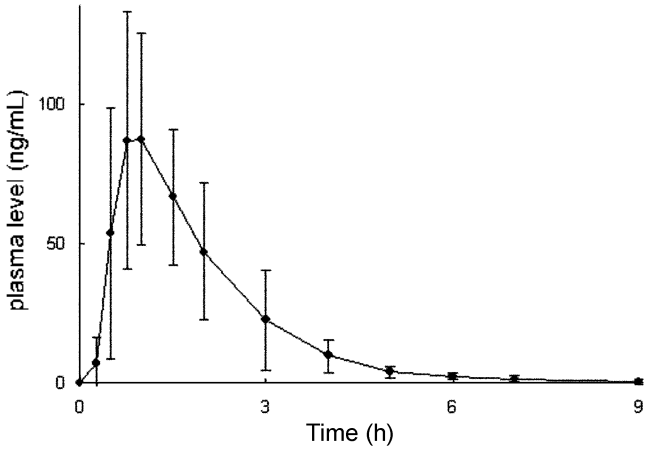

Figure 4. Change in the plasma level of enalapril determined by the LC-MSD method (n = 24).

over the range tested (1-300 ng enalapril/mL plasma) (Fig. 3).

Lower limit quantitation of the method was 1 ng/mL (S/N = 10). Precision (within day and between days) and accuracy of the method at various concentrations are summarized in Table 1.

Pharmacokinetic Profile. Having established the validity of the analytical method, the LC-MSD method was applied to determination of residual enalapril in the plasma of test subjects. The LC-MSD chromatograms obtained from test subjects were practically identical with that in Figure 2 from spiked plasma. Figure 4 shows a rapid increase after intake and a gradual decline in the enalapril level in plasma. Similar pharmacokinetic profiles were obtained from 24 subjects over the 9 h test period. The results were considered to further validate the analytical method for enalapril. The average value of the maximum level of enalapril was 102 (± 39) ng/mL. The maximum level was reached after 0.96 (± 0.36) h.

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