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Rapid Determination of a New Angiotensin-Converting Enzyme Inhibitor, Imidapril, and Its Active Metabolite in Human Plasma by Negative-Ion Desorption Chemical Ionization–Tandem Mass Spectrometry (MS/MS)

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A very rapid and highly sensitive method using desorption chemical ionization (DCI)–tandem mass spectrometry (MS/MS) with selected reaction monitoring is reported for the simultaneous determination of imidapril and its active metabolite (M1) in human plasma. Imidapril and M1 in plasma were extracted by a C_{18} solid phase extraction cartridge after deproteinization, and derivatized with pentafluorobenzyl bromide. One μl of prepared sample was applied to the DCI filament and analyzed by DCI/MS/MS within a few minutes. The limits of determination of imidapril and M1 were 0.2 and 0.5 ng/ml in human plasma, respectively. The features of this method make it appropriate for use in pharmacokinetic studies with human plasma after oral administration of imidapril.

Keywords imidapril; desorption chemical ionization; tandem mass spectrometry; MS/MS; selected reaction monitoring; human plasma

Imidapril hydrochloride, (4*S*)-1-methyl-3-(2*S*)-[*N*-[(1*S*)-1-ethoxycarbonyl-3-phenylpropyl]amino]propionyl-2-oxo-imidazolidine-4-carboxylic acid hydrochloride, is a new prodrug type angiotensin converting enzyme (ACE) inhibitor developed by Tanabe Seiyaku Co., Ltd. Its active metabolite (M1), which an ethyl ester group of imidapril is hydrolyzed, shows ACE inhibiting action.

A sensitive assay is required to determine the presence of imidapril and M1 in plasma and urine in pharmacokinetic studies of human, because a clinical dose of imidapril can be as low as 5 or 10 mg. There are methods for determining ACE inhibitor and its metabolites using inhibitor binding assay (IBA),[1] radio immunoassay (RIA),[2] high-performance liquid chromatography (HPLC),[3] and gas chromatography (GC).[4,5] The IBA and RIA methods are very sensitive, but it is difficult to evaluate several kinds of compounds simultaneously; the HPLC and GC methods are specific, but no more sensitive than the others. Recently, a means of determining compounds having a carboxylic group was developed using HPLC with a fluorescent labeling reagent, 9-anthryldiazomethane (ADAM). Figure 1 shows the chemical structure of imidapril and M1. Both compounds have a carboxylic group, so the HPLC method using ADAM was applied,[6] but required a great deal of time for both sample preparation and measurement. GC/MS was used to determine ACE inhibitor,[7,8] but we found that this means did not have the rapidity and sensitivity necessary. This paper describes the rapid, selective and sensitive determination of imidapril and M1 in human plasma using desorption chemical ionization (DCI)–tandem mass spectrometry (MS/MS) with selected reaction monitoring (SRM). The successful application of this technique was demonstrated by the assay of imidapril and M1 in the plasma samples of a healthy volunteer orally administrated 10 mg of imidapril.

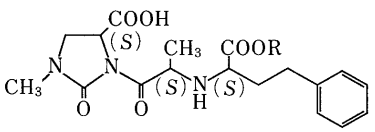

Fig. 1. Structures of Imidapril, Active Metabolite and I.S.
R = C_2H_5, imidapril; R = H, active metabolite; R = CH_3, I.S.

Experimental

Materials and Reagents Authentic specimens of imidapril and M1 were synthesized by Hayashi *et al.*[9] Methyl ester of imidapril was used as an internal standard (I.S., Fig. 1). Pentafluorobenzyl (PFB) bromide was purchased from Tokyo Kasei (Tokyo, Japan). All other reagents and solvents were of reagent grade from Katayama Chemicals (Osaka, Japan). Bond Elut C_{18} solid phase extraction cartridges (1 ml) were obtained from Varian (Harbor City, CA, U.S.A.).

Heparinized blood samples were taken from a healthy male volunteer prior to, then 1, 2, 4, 6, 8, 10, 14 and 24 h after administration of a tablet containing 10 mg of imidapril, and centrifuged at 1500*g* for 5 min to obtain the plasma. The plasma samples were stored at −20 °C until analysis.

Sample Preparation The Bond Elut C_{18} solid phase extraction cartridge was conditioned in advance with 3 ml of methanol, 0.5 ml of 0.045% (v/v) hydrochloric acid–ethanol solution, 3 ml of distilled water and 1 ml of 0.1 M hydrochloric acid. To 1 ml of plasma sample containing I.S. was slowly added 1 ml of 12% (v/v) perchloric acid under vigorous stirring. The mixture was centrifuged at 1500*g* for 5 min, and its supernatant was applied to the previously conditioned C_{18} cartridge. Then, the C_{18} cartridge was washed with 1 ml of 0.1 M hydrochloric acid. Imidapril and M1 retained in the C_{18} cartridge were eluted with 0.5 ml of 0.045% (v/v) hydrochloric acid–ethanol solution into a reaction vial. The eluent was evaporated to dryness at 40 °C under a stream of nitrogen, and the residue was dissolved in 90 μl of ethyl acetate. After adding 5 μl

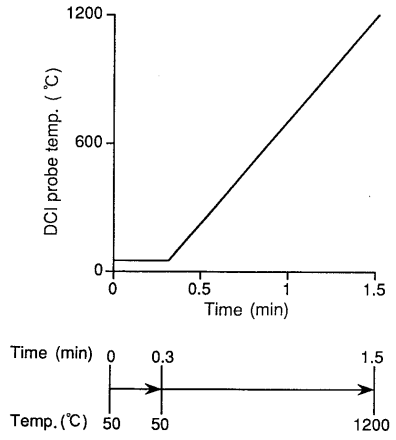

Fig. 2. Measurement Conditions of the DCI Probe

of diisopropyl ethylamine, the solution was supersonicated (45 kHz) for 3 min, and then reacted with 5 μl of PFB bromide at 60 °C for 30 min. The reaction mixture was evaporated to dryness at 60 °C under a stream of nitrogen, and the residue was dissolved with 200 μl of toluene.

Instruments and Conditions A Finnigan MAT triple-stage quadrupole mass spectrometer, TSQ700 was used in this study. Electron energy of 70 eV and electron current of 200 μA were used for analysis. The temperature of the ion source and the manifold were set at 150 °C and 70 °C, respectively. The instrument was operated in the negative-ion chemical ionization mode with methane as the reagent gas at the source pressure of 0.9—1.0 Torr. A collision-induced dissociation (CID) was achieved using argon as the collision gas at the pressure adjusted to more than 1.0 mTorr above the pressure without the collision gas. The applied collision offset energy was set at 20 eV. The electron multiplier and dynode voltage were run at 1.8 and 15 kV, respectively. SRM was used to confirm the presence of imidapril and M1. The ions for SRM analysis of imidapril, M1 and I.S. were selected at *m/z* 404, 556 and 390 as parent set masses in the first quadrupole (Q1) respectively, and *m/z* 143 as a daughter set mass in third quadrupole (Q3). One μl of sample applied to DCI filament was analyzed by negative-ion DCI/MS/MS with SRM in the daughter scan mode. The temperature of DCI filament was increased from 50 to 1200 °C within 1 min for analysis (Fig. 2).

Calibration Curves The plasma samples spiked with known amounts of imidapril and M1 (0.5, 1, 3, 10, and 25 ng) were prepared and analyzed by the procedure described above. The calibration curve was obtained by plotting the peak area ratio of the PFB derivative of imidapril or M1 to that of I.S. against the concentration.

Results and Discussion

DCI/MS/MS An assay method of imidapril and M1 using HPLC with ADAM was developed by Tagawa *et al.*,[6] but required much time in tedious sample preparation and chromatographic analysis. A rapid and sensitive method was tested using DCI/MS/MS for determination of imidapril and M1, and the time required for analysis was much less than that by HPLC and GC. Since the temperature of the DCI filament can be raised to 1200 °C within a few minutes, samples on the filament are evaporated and analyzed quickly; further, no degradation product of the samples appeared during analysis.

The pressure of CI and CID gas was adjusted to maximize the appropriate ion intensities and the temperature of DCI filament was controlled to obtain useful peaks for determination. The DCI mass spectra of the PFB derivatives of imidapril, M1 and I.S. are shown in Fig. 3; the quasi-molecular ion, $[M-H]^-$, is identified at *m/z* 584 (imidapril), *m/z* 736 (M1), *m/z* 570 (I.S.), and the major fragment ion, $[M-PFB]^-$, at *m/z* 404 (imidapril), *m/z* 556 (M1), *m/z* 390 (I.S.), respectively. Figure 4 shows the daughter ion mass spectra of PFB derivatives of imidapril, M1 and I.S. when each $[M-PFB]^-$ ion is selected as a parent set in Q1. All daughter ion mass spectra show the highest intensity signal at *m/z* 143, so, these abundant ions of imidapril, M1 and I.S. were selected for SRM analysis.

Sample Preparation A highly sensitive and reproducible analytical method for biological samples is based on the selective separation and extraction of only the object compounds from the admixture. The technique using MS/MS has high selectivity and specificity and is considered to simplify the preparation procedure to the greatest extent possible.

The Bond Elut C_{18} solid phase extraction cartridge was chosen to isolate imidapril and M1 from human

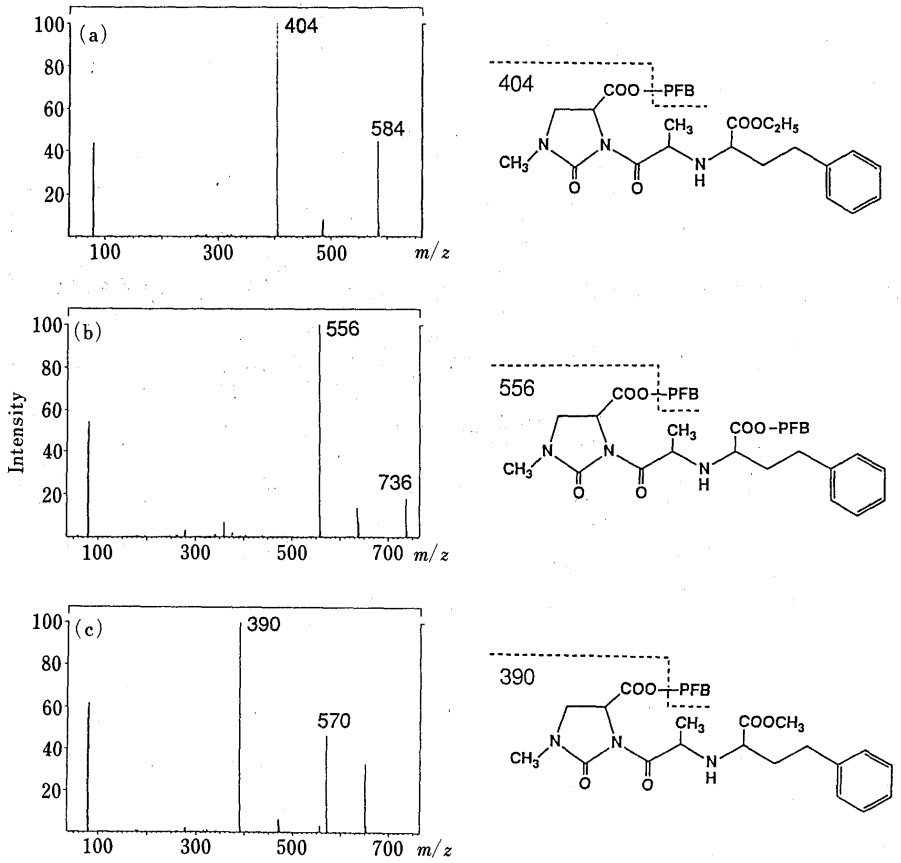

Fig. 3. DCI MS of the PFB Derivatives of Imidapril, Active Metabolite and I.S.
(a) imidapril, (b) active metabolite (M1), (c) internal standard.

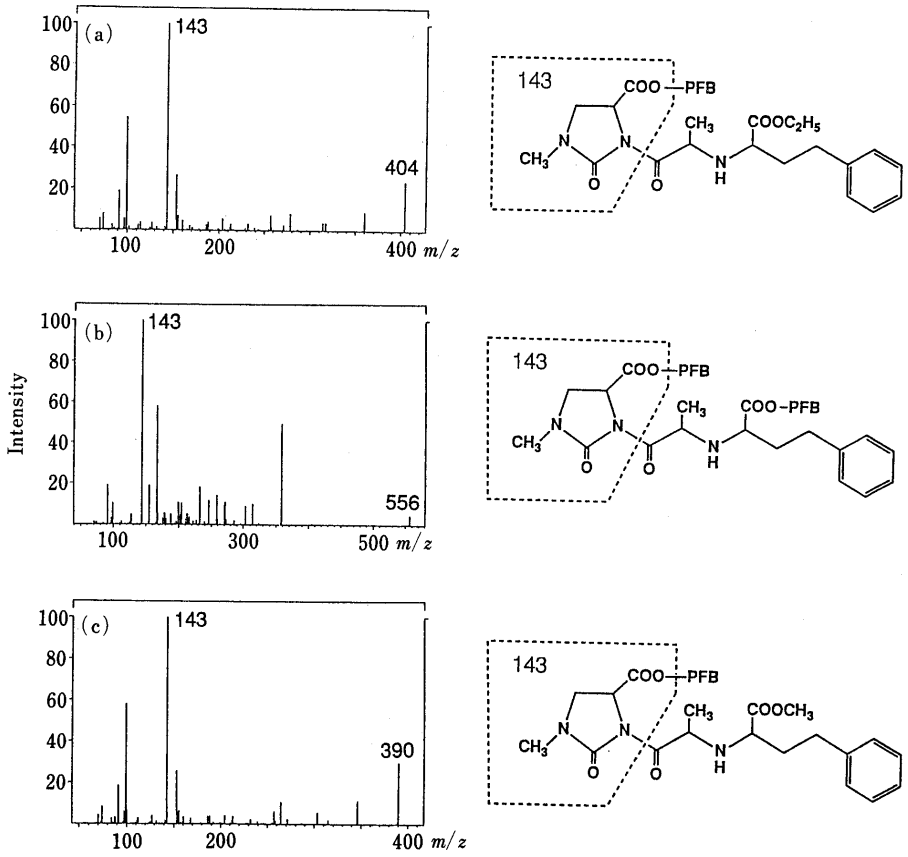

Fig. 4. Daughter Ion MS of the PFB Derivatives of Imidapril, Active Metabolite and I.S.
(a) imidapril, (b) active metabolite (M1), (c) internal standard.

plasma. The plasma sample deproteinized with 1 ml of 12% (v/v) perchloric acid was charged into the previously conditioned C_{18} cartridge. After washing the cartridge, imidapril and M1 were isolated by elution with acidic ethanol. These procedures make it possible to extract imidapril and M1 from plasma completely and quantitatively.

Using DCI/MS/MS, the derivatization of samples was not generally required. In the case of imidapril and M1, however, the derivatization was required because of low sensitivity and tailing of the peak of non-derivative compounds. Therefore, several means of derivatization for the carboxylic and amino groups of imidapril and M1 were investigated; silylation, methylation, isopropylation and 3,5-bis-(trifluoromethyl)benzyl esterification and so on. These reactions proceeded quantitatively, but they could not be used for the determination owing to a low sensitivity and an interference of excess reagents. The derivatization into PFB ester which facilitated the highly sensitive detection in the negative-ion mode was thus chosen for imidapril and M1. A complete evaporation of excess reagents and solvents at room temperature is needed when 1 μl of sample is put on the DCI filament. General catalysts for the reaction of PFB bromide caused the formation of by-products that remained on the DCI filament. Diisopropyl ethylamine[10] was used as catalyst, and the residue after dryness was dissolved with toluene, so that there was no residue on the DCI filament when a sample applied. This derivatization method was therefore suitable for the microanalysis of imidapril and M1 using DCI/MS/MS.

TABLE I. Reproducibility of Imidapril and M1 Spiked in Human Plasma ($n=3$)

Target conc. (ng/ml)		Found (ng/ml)			Mean (ng/ml)	Recovery (%)	C.V. (%)
		Rep. 1	Rep. 2	Rep.3			
Imidapril	1	0.96	1.05	0.97	0.99	99.0	5.0
	5	4.81	4.53	4.67	4.67	93.4	3.0
M1	1	0.76	0.68	0.77	0.74	74.0	6.7
	5	3.37	3.65	3.67	3.56	71.2	4.7

Linearity and Reproducibility The linear calibration curves for imidapril and M1 were obtained in the concentration range from 0.5 to 25 ng/ml plasma with regression equations of $y=0.0170x$ and $y=0.00149x$, and with correlation coefficients of 0.9992 and 0.9959, respectively. The determination limits of imidapril and M1 were 0.2 and 0.5 ng/ml, respectively.

The recovery test was performed with known amounts of imidapril and M1 spiked in human plasma. The recovery of imidapril from plasma was quantitatively found to be 90—100%, and that of M1 was approximately 70%. As shown in Table I, the coefficients of variation (C.V.) indicated a good reproducibility of this method. Table II shows the between-days variation; the C.V. was 1.0—7.1% for imidapril, and 5.0—18.2% for M1. Correlation coefficients of between-days were constant in the concentration range from 1 to 30 ng/ml, indicating the precision of this method.

TABLE II. Concentration of Imidapril and M1 Found in the Between-Day Plasma Quality Control Samples ($n=3$)

Target conc. (ng/ml)		Found (ng/ml)			Mean (ng/ml)	C.V. (%)
		Day 1	Day 2	Day 3		
Imidapril	30	27.8	27.4	27.3	27.5	1.0
	10	10.3	9.7	9.0	9.7	6.7
	5	5.1	5.1	4.5	4.9	7.1
	1	1.4	1.3	1.4	1.4	4.2
Correlation		0.9996	0.9999	0.9998		
M1	30	23.6	22.1	24.4	23.4	5.0
	10	6.8	7.1	7.8	7.2	7.1
	5	3.8	3.2	3.3	3.4	9.4
	1	0.5	0.7	0.7	0.6	18.2
Correlation		0.9990	0.9998	0.9996		

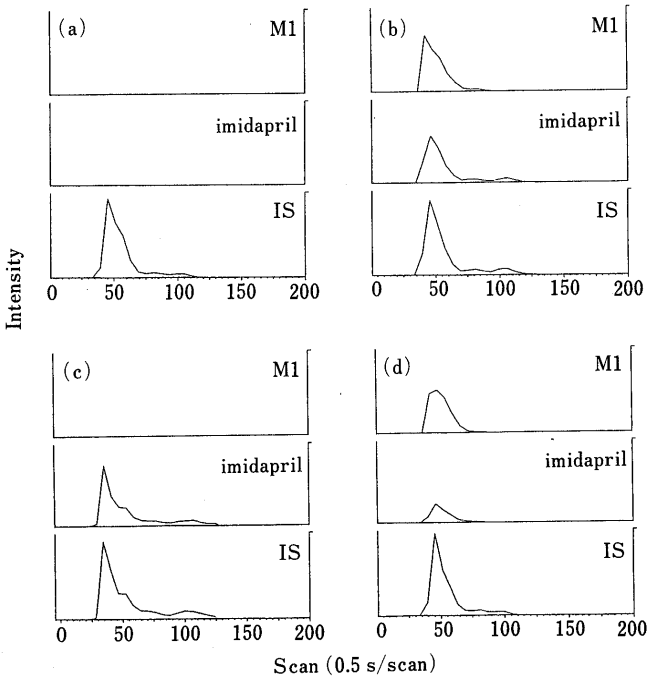

Fig. 5. Typical Mass Fragmentograms of Imidapril, Active Metabolite and I.S. Using SRM

(a) blank; (b) spiked at 10 ng/ml; (c) 2 h after administration; (d) 10 h after administration.

Application The proposed method was applied to the determination of imidapril and M1 in plasma of a healthy male volunteer orally administrated 10 mg of imidapril. Typical mass fragmentograms are shown in Fig. 5. The plasma concentration–time profiles of imidapril and M1 obtained by DCI/MS/MS were compared with those obtained by HPLC (Fig. 6). The difference between two values was not significant, and the profiles were similar. The graphs demonstrate that the prodrug, imidapril was rapidly absorbed and extensively metabolized to the pharmacologically active metabolite.

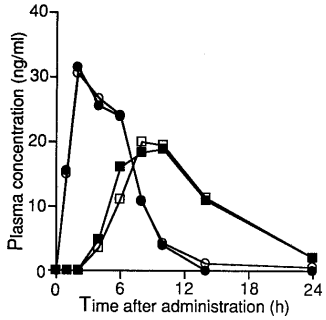

Fig. 6. Typical Plasma Concentration of Imidapril and M1 after Oral Administration of 10 mg to a Healthy Volunteer

●, imidapril by HPLC method; ○, imidapril by DCI/MS/MS method; ■, M1 by HPLC method; □, M1 by DCI/MS/MS method.

Conclusions

The DCI/MS/MS method described in this paper allows the rapid and simultaneous determination of imidapril and its active metabolite in human plasma with high sensitivity and specificity. The method is suitable for use in pharmacokinetic and bioavailability studies with a new prodrug type ACE inhibitor, imidapril.

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