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Determination of imidapril and imidaprilat in human plasma by high-performance liquid chromatography–electrospray ionization tandem mass spectrometry

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Abstract

A sensitive and specific assay of imidapril and its active metabolite, imidaprilat, in human plasma has been developed. This method is based on rapid isolation and high-performance liquid chromatography (HPLC)–electrospray ionization (ESI)-tandem mass spectrometry (MS–MS). Imidapril and imidaprilat were isolated from human plasma using OASIS HLB (solid-phase extraction cartridge), after deproteinization. The eluent from the cartridge was evaporated to dryness, and the residue was reconstituted in mobile phase and injected into the HPLC–ESI-MS–MS system. Each compound was separated on a semi-micro ODS column in acetonitrile–0.05% (v/v) formic acid (1:3, v/v). The selected ion monitoring using precursor→product ion combinations of m/z 406→234 and 378→206, was used for determination of imidapril and imidaprilat, respectively. The linearity was confirmed in the concentration range of 0.2 to 50 ng/ml in human plasma, and the precision of this assay, expressed as a relative standard deviation, was less than 13.2% over the entire concentration range with adequate assay accuracy. The HPLC–ESI-MS–MS method correlates well with the radioimmunoassay method, therefore, it is useful for the determination of imidapril and imidaprilat with sufficient sensitivity and specificity in clinical studies. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Imidapril; Imidaprilat

1. Introduction

Imidapril hydrochloride, (−)-(4*S*)-3-[(2*S*)-2{[(1*S*)-1-ethoxycarbonyl-3-phenyl-propyl]-amino}-propionyl] - 1 - methyl - 2 - oxo-imidazo - lidine - 4 - carboxylic acid hydrochloride (Fig. 1), is a prodrug-type angiotensin-converting enzyme (ACE) inhibitor

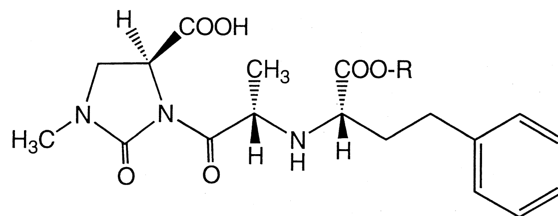

Fig. 1. Chemical structures of imidapril, imidaprilat and internal standard, R=C_2H_5; imidapril, R=H; imidaprilat, R=CH_3; internal standard.

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developed by Tanabe Seiyaku Co., Ltd. Its active metabolite, imidaprilat, in which an ethyl ester group of imidapril is hydrolyzed, shows ACE inhibiting action.

Imidapril hydrochloride is usually administered orally in a dose of 2.5–10 mg/day. Pharmacokinetic parameters of imidapril and imidaprilat in oral administration of 10 mg are C_{max}: 20–50 and 10–20 ng/ml, T_{max}: around 2 and 6 h, $T_{1/2}$: about 2 and 8 h, respectively. A sensitive and specific assay is required to determine the presence of drugs in human plasma in clinical studies, because plasma concentrations of imidapril and imidaprilat are low, less than 50 and 20 ng/ml, respectively. There are many methods for determining ACE inhibitors using radioimmunoassay (RIA) [1,2], enzyme immunoassay [3], high-performance liquid chromatography (HPLC) [4–6], gas chromatography (GC) [7], and GC–mass spectrometry (MS) [8–11]. In previous pharmacokinetic studies of imidapril and imidaprilat, plasma concentration data were determined by RIA [2] and HPLC [6]. RIA is very sensitive, but it is necessary to use the ^{125}I radioligand and some special facilities, and only imidaprilat can be determined. HPLC can determine both compounds simultaneously, but it requires a great deal of care in sample preparation and chromatographic analysis. Desorption chemical ionization (DCI)-tandem mass spectrometry (MS–MS) [12] was also developed to determine the amount of both compounds rapidly and simultaneously, but this method is not popular and requires some complicated derivatization processes.

HPLC–MS–MS methodology has recently been demonstrated to be a powerful technique for quantitative determination of drugs and metabolites in biological fluids. It is generally believed that the application of HPLC–MS–MS for the determination of drugs and metabolites in biofluids practically guarantees specificity; sample preparation may be simplified, and none or very little chromatographic separation is required. Furthermore, the high selectivity and sensitivity using MS–MS with selected reaction monitoring (SRM) make it possible to determine the amount of drugs with a small amount of plasma sample. The reduction of sampling volume reduces the stress to volunteers or patients in clinical studies.

This paper describes the rapid, selective and sensitive determination of imidapril and imidaprilat in human plasma using HPLC–electrospray ionization (ESI)-MS–MS with SRM. The successful application of this technique was demonstrated by the assay of imidapril and imidaprilat in plasma samples of clinical studies.

2. Experimental

2.1. Reagents and materials

Imidapril, imidaprilat and the methyl ester form of imidapril (Fig. 1) were supplied by Tanabe Seiyaku (Osaka, Japan). The methyl ester form was used as an internal standard (I.S.). All other reagents and solvents were of reagent grade from Wako (Osaka, Japan). OASIS HLB 3 cc (60 mg) extraction cartridges were obtained from Waters (Milford, MA, USA). Argon (99.9999%) was purchased from Taiyo Toyo Sanso (Osaka, Japan). The drug-free human heparinized plasma and Imidapril&Imidaprilat RIA KIT were obtained from Eiken (Tokyo, Japan).

2.2. Instrumentation

A TSQ 7000 tandem mass spectrometer (Finnigan MAT, San Jose, CA, USA) equipped with an ESI interface, a nitrogen generator (System Instruments, Tokyo, Japan) and a 2690 Separation Module (Waters) was used for all HPLC–ESI-MS–MS analyses.

2.3. Standard solutions

The stock standard solution of imidapril and imidaprilat (10 μg/ml) was prepared with distilled water. This solution was further diluted with distilled water to obtain the working standard solutions at given concentrations for validation and calibration. The stock I.S. solution (10 μg/ml) was also prepared in the same way as the stock standard solution with distilled water. The working I.S. solution of 100 ng/ml was prepared by dilution of the stock I.S. solution with distilled water. All stock solutions were stored at 5°C.

2.4. Sample preparation

A 200-μl aliquot of plasma sample was pipetted into a glass test tube and 100 μl of the working I.S. solution was added. The sample was deproteinized by the slow addition of 200 μl of 6% (v/v) perchloric acid with vigorous stirring. The mixture was centrifuged at 1500 *g* for 5 min, and the supernatant applied to the OASIS HLB cartridge, which was previously conditioned with 3 ml of methanol, 3 ml of distilled water, and 1 ml of 2% (v/v) perchloric acid. The cartridge was washed with 2 ml of 0.1 *M* hydrochloric acid and 1 ml of distilled water. Imidapril and imidaprilat, retained in the cartridge, were eluted with 1 ml of methanol into a disposable glass test tube, and evaporated to dryness at 50°C under a stream of nitrogen. The residue was dissolved in 100 μl of mobile phase, and 10-μl aliquot was analyzed by positive-ion HPLC–ESI-MS–MS using SRM in product ion scan mode.

2.5. HPLC–ESI-MS–MS conditions

HPLC separation was performed using a Symmetry C_{18} column (Waters), 100×2.1 mm I.D., 3.5 μm particle size, at a column temperature of 40°C. Acetonitrile–0.05% (v/v) formic acid (1:3, v/v) was used as mobile phase at flow-rate of 0.2 ml/min. The temperature of the sample cooler in autosampler was set at 10°C.

The TSQ 7000 MS–MS system was operated in the positive-ion mode under the following conditions: nitrogen (>99%) was used for the sheath gas and auxiliary gas at pressures of 70 p.s.i. and 5 units, respectively (1 p.s.i.=6894.76 Pa). The temperature of the heated capillary was maintained at 200°C, and the spray voltage of the ESI interface was set at 4.5 kV. A collision-induced dissociation (CID) was achieved using argon as the collision gas at the pressure adjusted to more than 1.8 mTorr above normal, and the applied collision offset energy was set to −20 eV (1 Torr=133.322 Pa). The electron multiplier voltage and dynode voltage were run at 1400 V and 15 kV, respectively. Data were acquired at a scan rate of 3 s for all scans. The ions for SRM analysis of imidapril, imidaprilat and I.S. were selected at *m/z* 406, 378 and 392 as the precursor ion set mass in the first quadrupole, and *m/z* 234, 206 and 220 as product ion set mass in the third quadrupole, respectively. The MS–MS system was programmed for SRM, that is, the acquisition was programmed by the unique programming language of this system for 1.5–6.0 min after the sample injection.

2.6. Validation tests

2.6.1. Linearity and calibration curve

Standards for linearity at seven concentrations (0, 0.2, 0.5, 2, 5, 20 and 50 ng/ml plasma) were prepared and assayed. To determine the precision of the slope of the calibration curve, each calibration standard at five concentrations (0, 0.2, 2, 10 and 50 ng/ml plasma) was also prepared and assayed. A linear model was fit to the concentration vs. peak-area ratio data using least-squares regression.

2.6.2. Specificity and interference

Chromatograms of the sample prepared with human blank plasma were visually inspected for peaks from endogenous sources which might correspond to the imidapril, imidaprilat and I.S. peaks.

2.6.3. Accuracy and precision

Samples at each of three concentrations (0.2, 10 and 50 ng/ml plasma, $n=5$) were prepared and assayed to determine the intra- (inter-) day accuracy expressed as relative error (R.E.), and precision as relative standard deviation (RSD).

3. Results and discussion

3.1. ESI mass spectra and MS–MS conditions

Each compound was first directly introduced to the mass spectrometer using the loop injector and ESI interface to obtain individual mass spectra. Parameters such as the temperature of the heated-capillary, spray voltage, and flow of sheath-gas and auxiliary-gas were optimized in order to obtain a much stronger intensity of the protonated molecule. Full-scan ESI-positive mass spectra of all compounds are shown in Fig. 2; the protonated molecule $[M+H]^+$ was identified at *m/z* 406, 378 and 392 for imidapril, imidaprilat and I.S., respectively. As shown in Fig. 3,

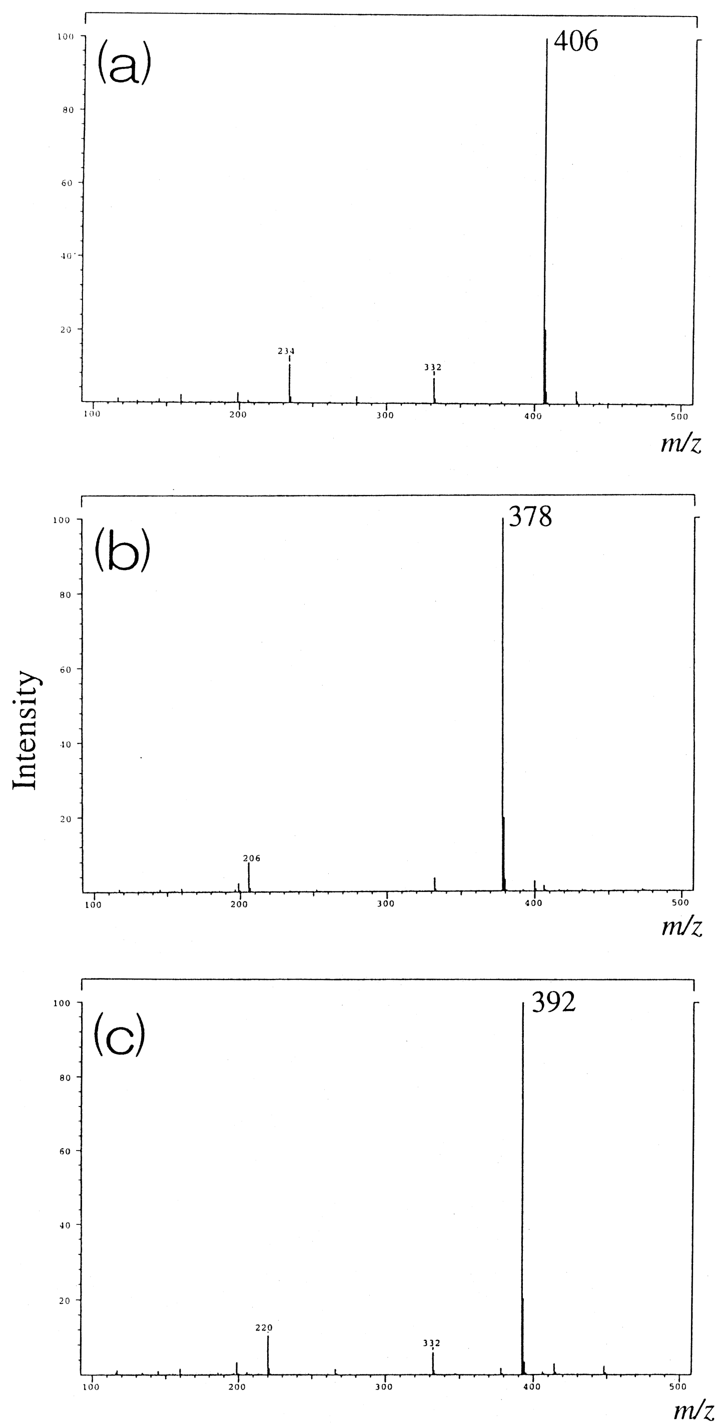

Fig. 2. ESI mass spectra of imidapril (a), imidaprilat (b), and internal standard (c), illustrating m/z 406, m/z 378 and m/z 392 as the protonated molecule, respectively.

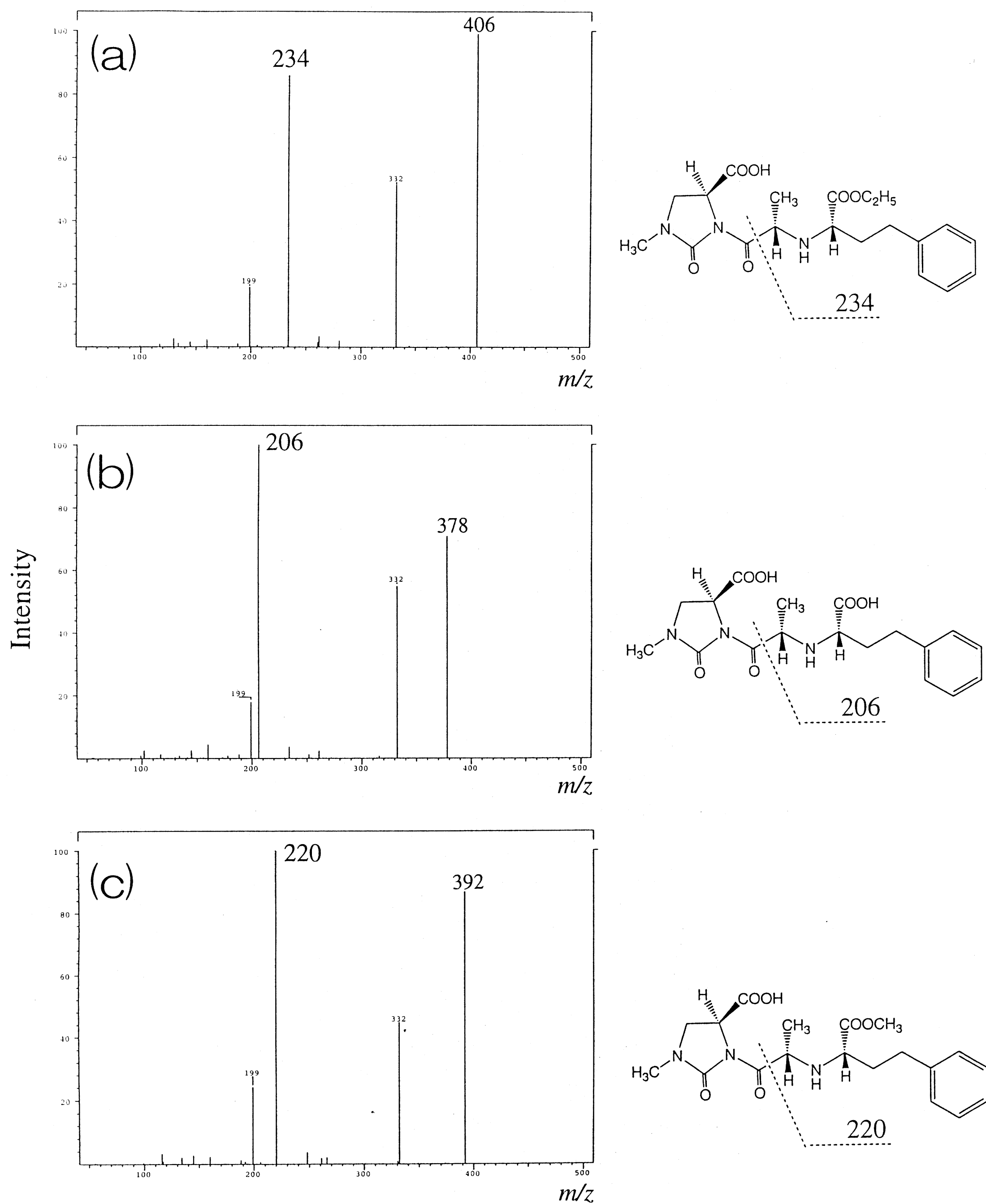

Fig. 3. Product-ion spectra of protonated molecule of imidapril (a), imidaprilat (b), and internal standard (c), illustrating m/z 406, m/z 378 and m/z 392 as the protonated molecule, respectively.

Table 1
Inter-day precision in the slope of standard curves (r=0.9995–1.0000)

Days	Imidapril	Imidaprilat
1	0.1777	0.0901
2	0.1819	0.0936
3	0.1827	0.0893
4	0.2197	0.1077
5	0.2200	0.1145
Mean	0.1964	0.0990
RSD (%)	10.9	11.5

product-ion spectra of imidapril, imidaprilat and I.S. were acquired with these protonated molecules as precursors. The most suitable collision energy and collision gas pressure were set by observing the response of the fragment ion peak. The product ion mass spectrum of imidaprilat, the active metabolite that is the main object of this assay, shows a predominant fragment ion at m/z 206. The same pattern was found in the case of imidapril and I.S; therefore, such kinds of fragment ions were selected for SRM analysis, at m/z 234, 206 and 220 for imidapril, imidaprilat and I.S., respectively.

3.2. Sample preparation

A highly sensitive and reproducible analytical method for biological samples needs suitable, sometimes tedious, pre-treatments if a low selectivity detection system was used. The technique using MS–MS has high selectivity and specificity, and is considered to simplify the preparation procedure to the greatest extent possible.

The OASIS HLB solid-phase extraction cartridge was chosen to isolate imidapril and imidaprilat from human plasma. The plasma sample deproteinized with 0.2 ml of 6% (v/v) perchloric acid was charged into the previously conditioned OASIS HLB cartridge. It is important to deproteinize the plasma sample to reduce the inferences by plasma components. After washing the cartridge, imidapril and imidaprilat were isolated by elution with methanol. These procedures make it possible to extract imidapril and imidaprilat from human plasma rapidly and quantitatively.

The stability of the sample solution in the autosampler at 10°C was assessed. Imidapril and imidaprilat in sample solution were stable for approxi-

Table 2
Intra-day precision and accuracy of imidapril and imidaprilat spiked in human plasma by LC–MS–MS

Compound	Target concentration (ng/ml)	Found (mean±SD) (ng/ml)	Precision (RSD, %)	Accuracy (error, %)
Imidapril	0.2	0.20±0.03	12.7	0.0
	10	9.6±0.9	9.5	−4.0
	50	46.3±1.9	4.0	−7.4
Imidaprilat	0.2	0.21±0.01	5.8	5.0
	10	9.7±0.7	7.4	−3.0
	50	49±2.7	5.6	−2.0

Table 3
Inter-day precision and accuracy of imidapril and imidaprilat spiked in human plasma by LC–MS–MS

Compound	Target concentration (ng/ml)	Found (mean±SD) (ng/ml)	Precision (RSD, %)	Accuracy (error %)
Imidapril	0.2	0.19±0.02	9.8	−5.0
	10	10.2±0.4	4.2	2.0
	50	46.4±1.0	2.1	−7.2
Imidaprilat	0.2	0.20±0.03	13.2	0.0
	10	10.1±0.3	3.2	1.1
	50	49.1±2.1	4.3	−1.8

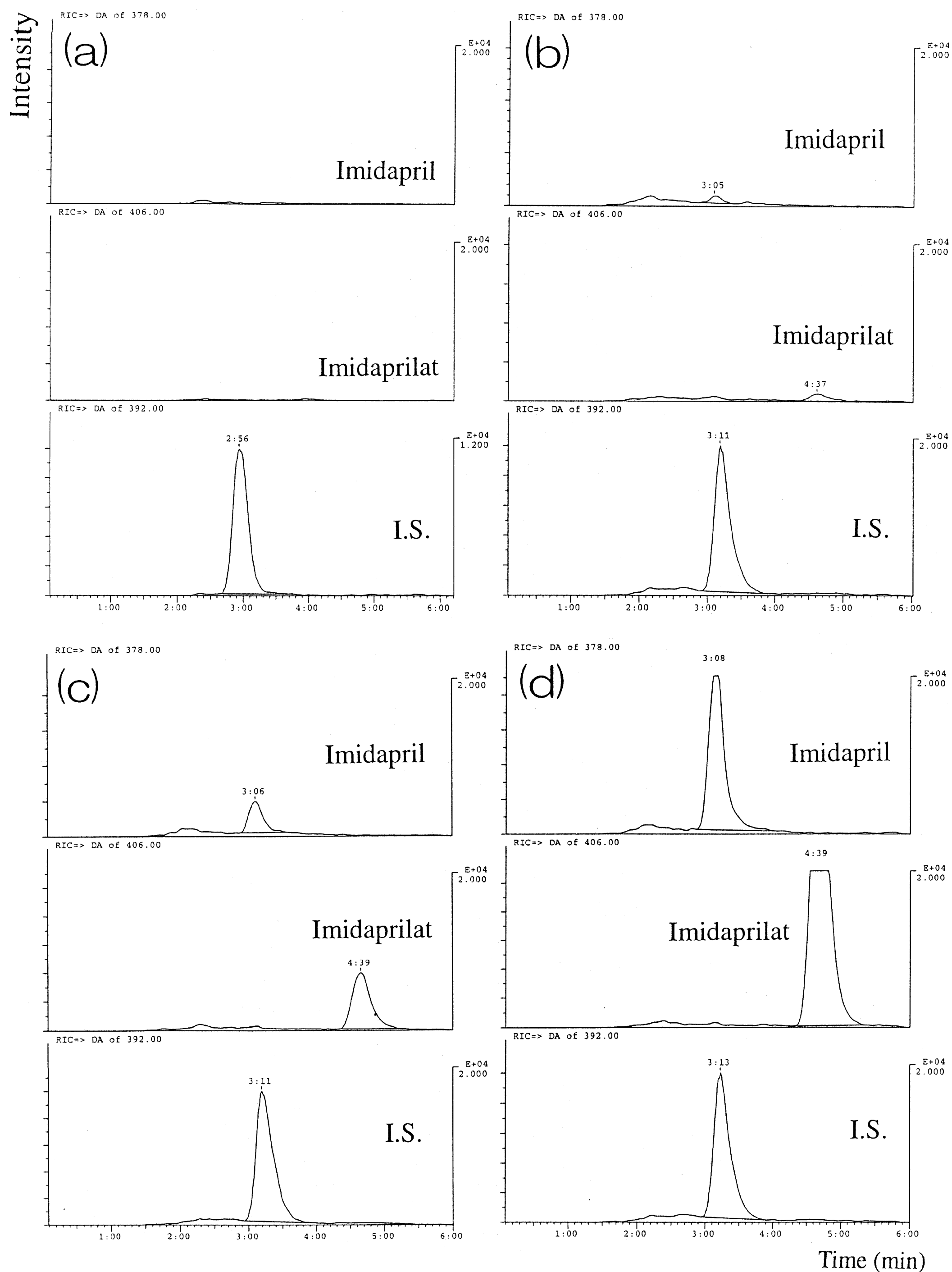

Fig. 4. Representative HPLC–ESI-MS–MS chromatograms of blank plasma (a) with I.S., and spiked plasma with 0.2 (b), 2 (c), and 10 ng/ml plasma (d) of imidapril and imidaprilat.

mately 24 h, since the found concentrations of these compounds were within 94.5–105.7% which compared with initial concentrations. The recoveries of imidapril and imidaprilat from human plasma through this extraction procedure were 94.8–112.7% and 84.9–114.9%, respectively, in the range of 0.2 to 50 ng/ml plasma.

3.3. Linearity and calibration curves

Good linearity was observed over the concentration range of 0.2 to 50 ng/ml plasma (r=0.9995–1.0000). The RSD (n=5) of the slope calculated with calibration curve data was less than 10.9% for imidapril and 11.5% for imidaprilat, showing good reproducibility (Table 1).

3.4. Selectivity and specificity

The coupling of HPLC with MS–MS in the SRM mode provides a highly selective method for the determination of drugs in biological samples. The representative SRM chromatograms of blank plasma and spiked plasma samples are shown in Fig. 4. No endogenous sources of interference were observed at the retention time of the analyte.

3.5. Accuracy and precision

The intra- and inter-day precision and accuracy are assessed in Tables 2 and 3. The RSDs of imidapril and imidaprilat ranged from 4.0 to 12.7% and 5.6 to 7.4% for intra-day, and 2.1 to 9.8% and 3.2 to 13.2% for inter-day, respectively. The R.E. of imidapril and imidaprilat ranged from −7.4 to 0.0% and −2.0 to 5.0% for intra-day, and −7.2 to 2.0% and −1.8 to 1.0% for inter-day, respectively.

4. Application

The present method was applied to the determination of imidapril and imidaprilat in plasma sample of clinical studies, which were already assayed by the RIA method. The found concentration obtained by the HPLC–ESI-MS–MS method was compared with that by the RIA method, and the correlation between the methods was demonstrated (Fig. 5).

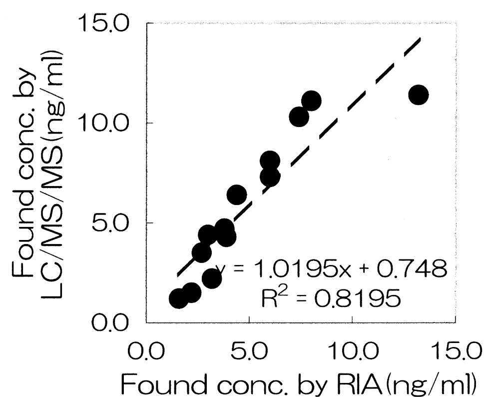

Fig. 5. Correlation between the RIA and HPLC–ESI-MS–MS methods.

5. Conclusions

The HPLC–ESI-MS–MS method described in this paper permits the rapid and simultaneous determination of imidapril and imidaprilat in human plasma with high sensitivity and specificity. The method is suitable for use in clinical studies, because it needs only a small amount of plasma sample to determine imidapril and imidaprilat.

References

[1] H. Berg, G. Resplandy, A.T. Bie, W. Floor, M. Bertrand, C.J. Arts, J. Pharm. Biomed. Anal. 9 (1991) 517–524.
[2] K. Yamanaka, S. Morikawa, K. Murata, K. Banno, T. Sato, T. Takai, T. Suzuki, M. Mizobe, M. Ito, K. Ishibashi, J. Pharm. Biomed. Anal. 14 (1996) 281–287.
[3] H. Tanaka, Y. Yoneyama, M. Sugawara, I. Umeda, Y. Ohta, J. Pharm. Sci. 76 (1987) 224–227.
[4] A. Rakhit, V. Tipnis, Clin. Chem. 30 (1984) 1237–1239.
[5] H. Kadin, H.G. Brittin, E. Ivashkiv, A.I. Cohen, J. Chromatogr. 487 (1989) 135–141.
[6] K. Tagawa, K. Hayashi, M. Mizobe, K. Noda, J. Chromatogr. 617 (1993) 95–103.
[7] V. Tipnis, A. Rakhit, J. Chromatogr. 345 (1985) 396–401.

[8] G. Kaiser, R. Ackerman, W. Dieterle, J.P. Dubois, J. Chromatogr. 419 (1987) 123–133.
[9] H. Shioya, M. Shimojo, Y. Kawahara, J. Chromatogr. 496 (1989) 129–135.
[10] H. Shioya, M. Shimojo, Y. Kawahara, Biomed. Chromatogr. 6 (1992) 59–62.
[11] M.E. Franklin, R.S. Addison, P.V. Baker, W.D. Hooper, J. Chromatogr. B 705 (1998) 47–54.
[12] S. Horimoto, M. Mabuchi, K. Banno, T. Sato, Chem. Pharm. Bull. 41 (1993) 699–702.