Arch Pharm Res Vol 28, No 4, 463-468, 2005

Archives of Pharmacal Research
http://apr.psk.or.kr

LC-MS Determination and Bioavailability Study of Imidapril Hydrochloride after the Oral Administration of Imidapril Tablets in Human Volunteers

Ji Hye Yun, Ja Hye Myung, Hye Jin Kim, Sibeum Lee, Jong-Sei Park[1], Won Kim[1], Eun-Hee Lee[2], Cheol Jin Moon[2], and Sung-Joo Hwang

NRL of Pharmaceutical Technology, College of Pharmacy, Chungnam National University, 220 Gung-dong, Yuseong-gu, Daejeon 305-764, Korea, [1]Lab Frontier, Co., ltd., KSBC Bldg, #Mt, 11 1-8, Lui-dong, Paldal-ku, Suwon, Kyonggi-do 442-270, Korea, and [2]Green Cross Reference Laboratory, 164-10 Poyi-dong, Kangnam-ku, Seoul 135-260, Korea

(Received July 30, 2004)

The purpose of the present study was to develop a standard protocol for imidapril hydrochloride bioequivalence testing. For this reason, a specific LC-MS method was developed and validated for the determination of imidapril in human plasma. A solid-phase extraction cartridge, Sep-pak® C18, was used to extract imidapril and ramipril (an internal standard) from deproteinized plasma. The compounds were separated using a XTerra MS® C18 column (3.5 μm, 2.1×150 mm) and acetonitrile-0.1% formic acid (67:33, v/v) adjusted to pH 2.4 by 2 mmol/L ammonium formic acid, as mobile phase at 0.3 mL/min. Imidapril was detected as *m/z* 406 at a retention time of ca. 2.3 min, and ramipril as *m/z* 417 at ca. 3.6 min. The described method showed acceptable specificity, linearity from 0.5 to 100 ng/mL, precision (expressed as a relative standard deviation of less than 15%), accuracy, and stability. The plasma concentration-versus-time curves of eight healthy male volunteers administered a single dose of imidapril (10 mg), gave an AUC_{12hr} of imidapril of 121.48 ± 35.81 ng mL^{-1} h, and C_{max} and T_{max} values of 32.59 ± 9.76 ng/mL and 1.75 ± 0.27 h. The developed method should be useful for the determination of imidapril in plasma with sufficient sensitivity and specificity in bioequivalence study.

Key words: Imidapril hydrochloride, Quadruple mass spectrometry, Bioavailability, Human plasma, SPE

INTRODUCTION

Imidapril (IMP) hydrochloride (Fig. 1), (-)-(4*S*)-3-[(2*S*)-2-[[(1*S*)-1-ethoxycarbonyl-3-phenylpropyl]amino]-propionyl]-1-methyl-2-oxoimidazolidine-4-carboxylic acid hydrochloride, is a prodrug-type in the angiotensin-converting enzyme (ACE) inhibitor without the S-H group (Matsuoka *et al.*, 1992). Its metabolite, imidaprilat (Fig. 1), in which an ethyl ester group of imidapril is hydrolyzed, shows ACE inhibitory action. Imidapril hydrochloride is usually administered at a dose of 2.5-10 mg/day enterally. The pharmacokinetic parameters of imidapril at 10 mg are; C_{max}: 39.3±10.1 ng/mL, T_{max}: around 2 h, and $T_{1/2}$: 1.8 ± 0.9 h, respectively (Harder *et al.*, 1998).

A sensitive and specific assay is required to determine the present levels of drugs in human plasma in clinical studies. Because the plasma concentrations of imidapril are very low (i.e. less than 50 ng/mL), there are many methods for determining ACE inhibitors, e.g., radioimmunoassay (RIA) (Yamanaka *et al.*, 1996), enzyme immunoassay (Tanaka *et al.*, 1987), high-performance liquid chromatography (HPLC) (Tagawa *et al.*, 1993; Bonazzi, *et al.*, 1997), gas chromatography (GC) (Tipnis and Rakhit, 1985), and GC-mass spectrometry (GC-MS) (Matsuoka *et al.*, 1992). Despite a number of methods available, the plasma concentrations of imidapril and of its active metabolite (imidaprilat) are determinable only by RIA (Yamanaka *et al.*, 1996) and HPLC (Tagawa *et al.*, 1993). The RIA technique used is very sensitive, but it is necessary to use the ^{125}I radio ligand and special

Correspondence to: Sung-Joo Hwang, National Research Laboratory of Pharmaceutical Technology, College of Pharmacy, Chungnam National University, 220 Gung-dong, Yuseong-gu, Daejeon 305-764, Korea
Tel: 82-42-821-5922, Fax: 82-42-823-3078
E-mail: sjhwang@cnu.ac.kr

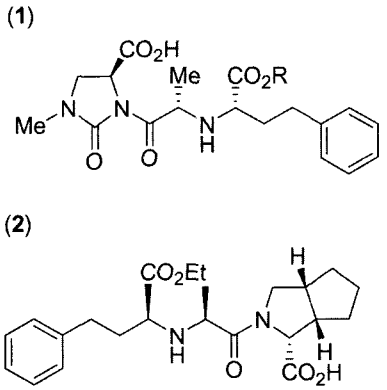


Fig. 1. Chemical structures of (1) imidapril (R=C_2H_5, $C_{20}H_{27}N_3O_6HCl$ =441.9, monoisotopic exact mass=405) and imidaprilat (active metabolite, R=H, $C_{18}H_{23}N_3O_6$), (2) ramipril ($C_{23}H_{32}N_2O_5$=416.5, monoisotopic exact mass=416).

facilities; moreover, only imidaprilat can be determined. The other technique is HPLC, but it requires a great deal of care during sample preparation and chromatographic analysis (Tagawa *et al.*, 1993; Bonazzi *et al.*, 1997). Thus desorption chemical ionization (DCI)-tandem mass spectrometry (MS-MS) was developed to determine the amounts of these compounds rapidly and simultaneously, but the technique is not popular and requires a complicated derivatization (Zhu *et al.*, 2002). Therefore, no practical method is available for the rapid analysis of large numbers of biological samples (Mabuchi *et al.*, 1999). LC-MS or LC-MS-MS methodology has recently been demonstrated to be a powerful technique when applied to the quantitative analysis of drugs and metabolite in biological fluids. It is generally believed that the application of LC-MS for the determination of drugs and metabolites in biological fluids practically guarantees specificity. In addition, sample preparation is generally straightforward, and little or no chromatographic separation is required. Furthermore, the low sample volumes required can reduce the stress to volunteers or patients in clinical studies.

The purpose of the present study was to develop a standard protocol for imidapril hydrochloride bioequivalence testing. For this purpose, a LC/MS method involving the detection of the m/z of imidapril at 406 and that of ramipril at 417 (used as an internal standard) was developed and validated for the determination of imidapril in human plasma. After the validation stage, a bioavailability study was performed by administering a Tanatril® tablet (10 mg as imidapril hydrochloride, Dong-A Pharm. Co.) orally once to 8 healthy male volunteers and then determining the pharmacokinetic parameters. Based on the pharmacokinetic data, bioequivalence test conditions for imidapril hydrochloride, i.e., sampling time, washout period, and the detected analytical method, were determined and the final bioequivalence test guideline for imidapril hydrochloride was prepared.

EXPERIMENTAL

Materials and reagents

Imidapril hydrochloride and ramipril internal standard (IS), (Belal *et al.*, 2001) were purchased from Sigma (St. Lous, MO, USA). Tanatril® tablets (10 mg as imidapril hydrochloride, Lot. No. 2032) were purchased from Dong-A Pharmaceutical Co., Ltd. (Korea). One dose of Tanatril® was administrated enterally to eight healthy male volunteers. Methanol and acetonitrile were of HPLC grade from Burdick & Jackson (Muskegon, MI, USA). All other reagents were of analytical grade. Helium (99.9999%) as a collision gas and nitrogen (>99%) as a sheath and auxiliary gas were purchased from the Jung-ang Industrial Company (Daejeon, Korea).

Instrumentation

The LC-MS system used was an Agilent 1100 HPLC system equipped with a degasser, binary pump, autosampler, thermostatic column compartment, and 1946D LC-MS selective trap detector (Agilent, USA). It was also equipped with an electrospray ionization-mass spectrometry (ESI-MS) interface, which was operated in positive mode. The nitrogen generator (System Instruments, Daejeon, Korea) and the Agilent Chemstation and Bruker data analysis were used for all LC-MS analyses. The column was an XTerra® MS C18 (3.5 μm, 2.1×150 mm) from Waters (USA). A Sep-pak® 6 cc (500 mg) C18 cartridge was purchased from Waters (Milford, MA, USA) for solid phase extraction of imidapril hydrochloride and ramipril.

Preparation of stock solutions and standard calibration

The stock standard solution of imidapril (10 μg/mL) was prepared with acetonitrile. Spiking standard solutions at concentrations of 5, 20, 50, 100, 200, 500, and 1000 ng/mL were prepared by diluting the stock solution with acetonitrile. The working solution of ramipril (10 μg/mL) as an internal standard was also prepared in the same way as the stock solution of imidapril with acetonitrile. Subsequently, the ramipril stock solution was diluted to 200 ng/mL with acetonitrile. All stock solutions were stored at 4°C when not in use. Standard calibration for imidapril and ramipril was performed by adding 50 μL of stock solutions into 450 μL of the human plasma to produce concentrations of 0.5, 2, 5, 10, 20, 50, and 100 ng/mL with control plasma, which was obtained from healthy and non-smoking volunteers who had abstained from drinking coffee.

Sample preparation and extraction procedure

Blood samples were taken at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, and 12 h after imidapril administration from eight healthy male volunteers. The samples were immediately centrifuged at 4000 rpm for 10 min, and the separated plasma samples were stored under -20°C. Subsequently, 900 μL aliquots of plasma samples were pipetted into glass test tubes and 100 μL of ramipril (IS) solution was added to each tube. The plasma samples were then applied to a Sep-pak® C18 cartridge, which had been previously conditioned with 3 mL of methanol and 3 mL of distilled water, and then washed with 2 mL of 0.1 M hydrochloric acid and 1 mL of distilled water. Imidapril retained in the cartridge was eluted with 2 mL of methanol into a disposable glass test tube, and evaporated to dryness at 45°C under a stream of nitrogen. The residue was dissolved in 50 μL of methanol, and a 10 μL aliquot was analyzed by positive-ion LC–MS in product ion scan mode.

LC-MS conditions

HPLC separation was performed using a XTerra® MS column C18 (3.5 μm, 2.1×150 mm, Waters, USA), at a column temperature of 40°C, using acetonitrile-0.1%(v/v) formic acid in distilled water (67:33, v/v) as mobile phase at 0.2 mL/min. The LC/MSD trap system was operated in positive-ion mode under the following conditions: nitrogen (>99%) was used as the sheath gas and auxiliary gas at a pressure of 40 psi (1 psi=56894.76 Pa). The temperature of the heated capillary was maintained at 350°C, and the spray voltage of the ESI interface was set at 4.5 kV. A collision-induced dissociation (CID) was achieved using helium as the collision gas at a pressure of >1.8 mTorr; the applied collision offset energy was set at 220 eV (1 Torr=5133.322 Pa). Data were acquired at a scan rate of 5 s for all scans. The molecular ions of imidapril and ramipril were selected at *m/z* 406 and *m/z* 417, respectively.

Validation tests

Specificity

Specificity was assessed by extracting samples in six different sources of blank plasma, and comparing plasma samples spiked with imidapril and ramipril (IS). The concentrations of imidapril hydrochloride ranged from 0.5 ng/mL to 100 ng/mL. Chromatograms were also visually inspected for interfering chromatographic peaks from endogenous substances.

Linearity

To examine the linearity of imidapril, calibration standards at eight concentrations (0, 0.5, 2, 5, 10, 20, 50 and 100 ng/mL in plasma) were prepared and assayed. A linear model was fitted to the concentration-versus-peak area ratio data by least-squares regression.

Precision and accuracy

To determine the precision of the method at these eight concentrations (0, 0.5, 2, 5, 10, 20, 50, and 100 ng/mL) of imidapril in human plasma, inter-day and intra-day precisions were investigated. Intra-day precision and accuracy were determined by repeating six times during one day, and inter-day precision was determined by repeating this testing once a day for six consecutive days. Samples were prepared at each concentration and assayed to determine intra-/ inter- day accuracies, which were expressed precision as relative standard deviation (RSD).

Stability

In this study, the stability of imidapril was tested by (1) Short-term temperature stability, (2) Long-term storage stability, (3) Freeze-thaw stability, (4) Stock solution stability, and (5) Processed stability. To test the short- and long-term and freeze-thaw stability of imidapril in plasma, two samples (0.5, 100 ng/mL) were stored under different conditions. The short and long-term stability tests were performed at room temperature for 6 hours and at -20°C for 10 days.

Freeze-thaw stability testing was performed for three frozen and thawed cycles. "Freezing" was performed at -20°C for 24 h and "thawing" at room temperature. The results of freeze-thaw, and short and long -term stability tests were compared with the average of intra-day calibration curves.

To test the stock solution stability of imidapril and ramipril (IS), stock standard solution of imidapril (10 μg/mL) and ramipril (IS, 10 μg/mL) were performed at room temperature for 6 h.

Processed sample stability test was performed by analyzing first intra-day calibration curve samples after intra-day analysis.

Pharmacokinetic analysis

The maximum plasma concentration (C_{max}) of imidapril and the time to reach C_{max} (T_{max}) were determined from its determined plasma concentrations. The area under the plasma concentration-time curve (AUC) was calculated using K-BE® Test 2002 (Lee *et al.*, 2002). The elimination half-life ($T_{1/2}$) was calculated by log linear regression of declining plasma concentrations against time after administration.

RESULTS AND DISCUSSION

Specificity and selectivity (n=6)

The coupling of HPLC and MS provides a highly selective method for the determination of drugs in biological samples. Representative chromatograms of blank plasma and spiked plasma samples are shown in

Fig. 2 and Fig. 3. No endogenous sources of interference were observed at the retention times of the analytes. The retention times of imidapril and ramipril (IS) were 2.9 min and 3.9 min, respectively.

Linearity and limit of quantitation (n=6)

The calculation was based on the peak area ratio of analyte versus its internal standard. The calibration curves

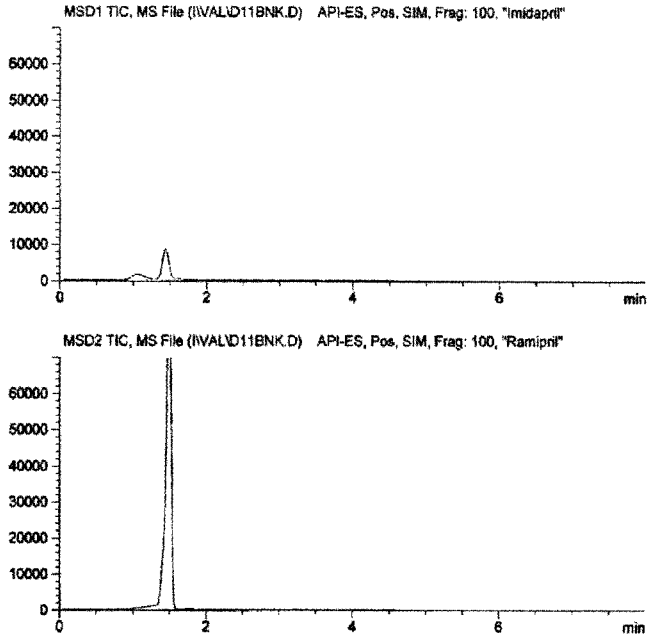
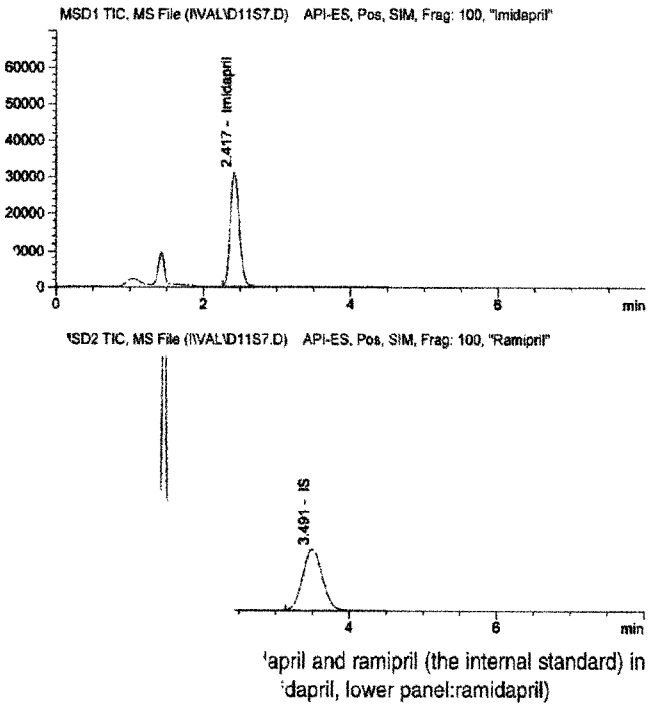


Fig. 2. Chromatogram of blank plasma (upper panel:imidapril, lower panel:ramidapril)



april and ramipril (the internal standard) in
dapril, lower panel:ramidapril)

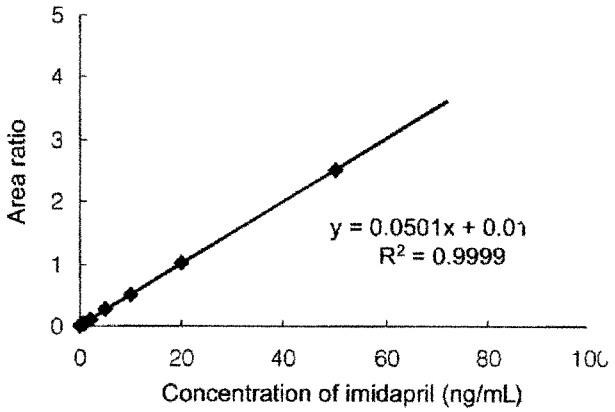


Fig. 4. Typical calibration curve of imidapril

were linear in the concentration range of 0.5-100 ng/mL for imidapril. The mean equation of a typical calibration curve consisting of eight points was $y = 0.0501x + 0.0115$ with a correlation coefficient = 0.9999, where y represents the ratio of imidapril/ramipril peak area and x represents the ratio of the imidapril/ramipril (IS) concentration (Fig. 4). The limit of quantitation (LOQ) for imidapril in human plasma was 0.5 ng/mL.

Precision and accuracy (n≥6)

The intra- and inter-day precision (n=6) and accuracy (n=11) are summarized in Table I. The precision of imidapril was determined to be in the range from 2.79 to 11.88% for intra-day and from 3.14 to 9.77% for inter-day. The intra-and inter-day precisions for imidapril samples at its LOQ level were 10.94 and 9.77%. Accuracy ranged from 100.4 to 112.5% for imidapril. The accuracy for samples at the LOQ level was 105.6% for imidapril. These results showed that this method has good precision and accuracy.

Stability

To determine short- and long-term stabilities, two sets of

Table I. Intra- and inter-day precision accuracy of imidapril

Concentration (ng/mL)	Precision (RSD, %)		Accuracy (%, n=11)
	Intra-day (n=6, day 1)	Inter-day (n=6, day 6)	
0.5	10.94	9.77	105.6
1	11.88	4.55	100.7
2	4.70	9.30	107.2
5	11.02	6.48	102.6
10	6.62	7.43	110.9
20	2.79	3.99	112.5
50	3.76	3.14	105.9
100	5.55	4.24	

Table II. Data of short-term, long-term and freeze/thaw stabilities of imidapril

Stability	Mean area ratio of Imidapril in plasma (ng/ml)					
	0.5 ng/mL			100.0 ng/mL		
	Standard	Test sample	Difference (%)	Standard	Test sample	Difference (%)
Short-term	0.0279	0.0275	-1.46	5.0469	5.1198	1.65
Long-term	0.0279	0.0276	-1.2	5.0469	4.8450	-4.0
Freeze/thaw	0.0279	0.0285	2.12	5.0469	5.1023	1.10

samples (0.5, 100 ng/mL) were stored at room temperature for 6 h and at -20°C during 10 days. The samples were then analyzed using freshly prepared calibration samples. The results are shown in Table II. The samples were found to be stable at -20°C for 10 days.

To test freeze and thaw stability, spiked plasma samples containing 0.5 and 100 ng/mL of imidapril were prepared. The samples were then frozen and thawed three times. The results are shown in Table II. Difference percent were less than 3% for imidapril, showing good stability.

Standard solution stability testing was performed at room temperature for 6 h using a stock solution of imidapril (10 μg/mL) and ramipril (IS, 10 μg/mL). Differences percent for imidapril and ramipril were -1.03 and 4.12% (Table III).

Processes sample stability testing was performed by analyzing first intra-day calibration curve samples after intra-day analysis. The error (%) of imidapril ranged from 0.16 to 7.86% (Table IV).

From the results, spiked plasma samples are has a showed good stability.

Table III. Standard solution stability of imidapril and ramipril (IS)

Material	Mean area		
	0 h	6 h	Difference (%)
Imidapril	2807121.0	2778196.5	-1.03
Ramipril (IS)	775867.2	807834.1	4.12

Table IV. Processed sample stability

Concentration (ng/mL)	Area ratio		Error (%)
	Standard	Test sample	
0.5	0.0348	0.0333	4.70
1	0.0477	0.0476	0.16
2	0.0943	0.1000	5.67
5	0.2340	0.2433	3.80
10	0.4325	0.4909	6.04
20	0.9320	0.9620	3.12
50	2.3965	2.6010	7.86
100	4.6905	4.6462	0.95

Pharmacokinetic study

The developed method was used determine imidapril in plasma samples. One Tanatril® tablet (imidapril hydrochloride, 10 mg) was administered enterally to eight healthy male volunteers. And blood samples were collected from each volunteer over a period of 12 h. Fig. 5 shows in an mean plasma concentration versus time of imidapril in human plasma.

The plasma level of imidapril reached a maximum about 1.75 ± 0.27 h after the administration and then slowly decreased. The average C_{max} was about 32.59 ± 9.76 ng/ml and the AUC was about 121.48 ± 35.81 ng mL^{-1} h of imidapril. Our values are similar to the previously published data (Harder *et al.*, 1998).

CONCLUSIONS

The purpose of the present study was to develop a standard protocol for imidapril hydrochloride bioequivalence testing. For this reason, a specific LC-MS method using a single quadrupole mass spectrometer was developed and validated for the determination of imidapril in human plasma. The validated method allows the determination of imidapril in the 0.5-100 ng/mL range. The method provides excellent specificity and linearity with a limit of quantitation of 0.5 ng/mL for imidapril. The precision, accuracy,

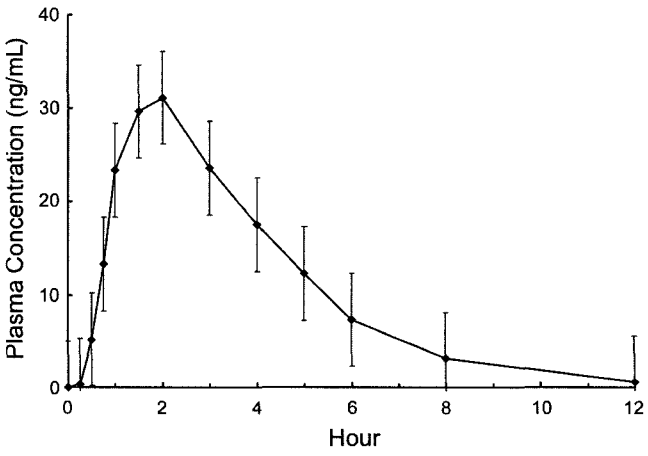


Fig. 5. Mean plasma concentrations (±S.D.) of imidapril after a 10 mg single oral dose (8 healthy volunteers)

and stability were good within the limits of the bioequivalence study. The developed method was successfully applied to a pharmacokinetic study.

ACKNOWLEDGEMENTS

The study was supported by KFDA (Korea Food and Drug Administration) and the Korean Ministry of Science and Technology through the National Research Laboratory Program (contract number:M1-0302-00-0016). The authors would like to thank the KFDA and Korean Ministry of Science and Technology for their continued support.

REFERENCES

Belal, F., Al -Zaagi, I. A., Gadkariem, E. A., and Abounassif, M. A., A stability-indicating LC method for the simultaneous determination of ramipril and hydrochlorothiazide in dosage forms. *J. Pharm. Biomed. Anal.*, 24, 335-342 (2001).

Bonazzi, D., Gotti, R., Andrisano, V., and Cavrini, V., Analysis of ACE inhibitors in pharmaceutical dosage forms by derivative UV spectroscopy and liquid chromatography (HPLC). *J. Pharm. Biomed. Anal.*, 16, 431-438 (1997).

Harder, S., Thurmann, P. A., and Ungethum, W., Single dose and steady state pharmacokinetics and pharmacodynamics of the ACE-inhibitor imidapril in hypertensive patients. *Br. J. Clin. Pharmacol.*, 45, 377-380 (1998).

Lee, Y. J., Jung, S. J., and Shim, C. K., K-BE Test 2002 for window, Version 1.2.1. (2002).

Mabuchi, M., Kano, Y., Fukuyama, T., and Kondo, T., Determination of imidapril and imidaprilat in human plasma by high-performance liquid chromatography-electrospray ionization tandem mass spectrometry. *J. Chromatogr. B*, 734, 145 -153 (1999).

Tanaka, H. Y., Yoneyama, Sugawara, M., Umeda, I., and Ohta, Y., Enzyme immunoassay discrimination of a new angiotensin-converting enzyme (ACE) inhibitor, cilazapril, and its active metabolite. *J. Pharmaceutical Science,* 76, 224 -227 (1987).

Tipnis, V. and Rakhit, A., Determination of pentopril, an angiotensin converting enzyme inhibitor, and its active metabolite in urine. *J. Chromatogr.:Biomedical Application*, 345, 396-401 (1985).

Yamanaka, K., Morikawa, S., Murata, K., Bano, K., Sato, T., Takai, T., Suzuki, T., Mizobe, M., Ito, M., and Ishibashi, K., Radioimmunoassay for imidapril, a new angiotensin-converting enzyme inhibitor, and imidaprilat, its active metabolite, in human plasma and urine. *J. Pharm. Biomed. Anal.,* 14, 281 -287 (1996).

Yamanaka, K., Takehara, N., Murata, K., Banno, K., and Sato, T., Pharmacokinetic and pharmacodynamic study of imidaprilat, and active metabolite of imidapril, a new angiotensin-coverting enzyme inhibitor, in spontaneously hypertensive rats. *J. Pharm. Biomed. Anal.,* 15, 1851-1859 (1997).

Zhu, Z., Vachareau, A., and Neirinck, L., Liquid chromatography-mass spectrometry method for determination of ramipril and its active metabolite ramiprilat in human plasma. *J. Chromatogr. B*, 779, 297-306 (2002).