Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 856 (2007) 190–197
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Quantitative determination of olmesartan in human plasma and urine by liquid chromatography coupled to tandem mass spectrometry

Dongyang Liu [a], Pei Hu [a], Nobuko Matsushima [b], Xiaoming Li [a], Li Li [a], Ji Jiang [a,*]

[a] *Clinical Pharmacology Research Center, Peking Union Medical College Hospital and Chinese Academy of Medical Sciences, Beijing 100730, China*
[b] *Clinical Pharmacology and Biostatistics Department, Sankyo Co. Ltd., Tokyo 140-8710, Japan*

Received 21 December 2006; accepted 25 May 2007
Available online 16 June 2007

Abstract

A specific, sensitive and fast method based on high performance liquid chromatography coupled to tandem mass spectrometry (HPLC–MS/MS) was developed for the determination of olmesartan in human plasma and urine. Solid-phase extraction (SPE) was used to isolate the compounds from biological matrix followed by injection of the extracts onto a C_{18} column with isocratic elution. The method was validated over the concentration range of 0.2–1000 and 5–10,000 ng/mL for olmesartan in human plasma and urine, respectively. The method was applied to the pharmacokinetic study of olmesartan medoxomil in healthy Chinese male and female subjects.

Keywords: Olmesartan; Urine; Plasma; Antihypertensive

1. Introduction

Olmesartan medoxomil (5-methyl-2-oxo-1, 3-dioxolen-4-yl) methoxy-4-(1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(tetrazol-5-yl)-phenyl] phenyl} methylimidazol-5-carboxylate), is a potent and selective angiotensin AT1 receptor blocker [1] which has been approved for the treatment of hypertension in the United States, Japan and European countries. The drug contains a medoxomil ester moiety and is cleaved rapidly by an endogenous esterase to release the active metabolite olmesartan [2]. Due to the fact that hydrolysis of olmesartan medoxomil in human plasma is extremely rapid [3], determination of olmesartan in plasma is the only choice for the study of pharmacokinetic profile of olmesartan medoxomil. Up to date, olmesartan has been determined in plasma and other biological fluids using high performance liquid chromatography (HPLC) coupled to fluorescent detection [4,5]. However, these methods have obvious drawbacks that they either need prolonged operating time or they are lack of a complete validation procedure. Here, we present a fast, robust and specific HPLC–MS/MS method to fulfill the pharmacokinetic study.

* Corresponding author. Tel.: +86 10 6529 6573; fax: +86 10 6529 6573.
E-mail address: pk.frosh@gmail.com (J. Jiang).

1570-0232/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2007.05.049

2. Experimental

2.1. Chemicals

Olmesartan (purity 95.5%) and internal standard (IS) RNH-6272 (Fig. 1), an analog with olmesartan, were provided by Sankyo Co. Ltd. (Japan). Methanol (HPLC grade) was purchased from Fisher (Fair Lawn, NJ, USA). Formic acid and ammonium acetate were both of analytical grades and purchased from Peking Chemical Plant (Beijing, China). Drug-free human plasma (anticoagulant: heparin lithium) used in the research was supplied by Peking Union Medical College Hospital Blood Bank. Drug-free human urine used in the research was supplied by six different healthy subjects dosing no drugs in the lately 2 weeks. Distilled water was prepared by a Milli-Q water purifying system (Millipore, Bedford, USA).

2.2. Calibration standard (CS) and quality control (QC) samples in human plasma and urine

Stock solution of olmesartan for CS and QC were prepared separately in methanol after correction for purity. Stock solution of RNH-6272 was also prepared in methanol. The concentrations of stock solution of olmesartan and RNH-6272 were both of

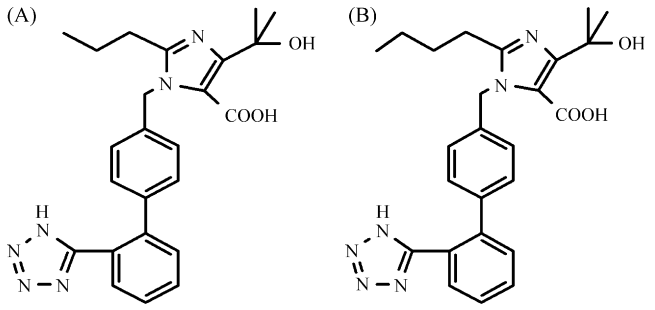

Fig. 1. Structure of olmesartan (A, MW 446) and internal standard RNH-6272 (B, MW 460).

1 mg/mL. They were further diluted with methanol to obtain working solutions at several concentration levels.

Calibration standard and QC samples in plasma and urine were prepared by diluting corresponding working solutions with drug-free human plasma and urine, respectively. The final concentrations of calibration standard in plasma and urine were 0.200, 0.500, 1.00, 5.00, 10.0, 50.0, 100, 500, 1000 ng/mL and 5.00, 10.0, 50.0, 100, 500, 1000, 5000, 10,000 ng/mL, respectively. The final concentrations of QC in plasma and urine were 0.480, 8.00, 80.0, 800 and 15.0, 60.0, 600, 7500 ng/mL, respectively. Internal standard working solution was prepared with methanol.

All the plasma and urine samples were stored at −30 °C. All preparation steps were done in subdued red light.

2.3. Extraction procedure

Calibration standard samples, QC samples, and clinical plasma samples were extracted employing a SPE technique. 0.4 mL of a 10 ng/mL internal standard working solution was added to 0.2 mL of plasma treated with heparin. After vortex and centrifugation at 13,000 rpm for 10 min, the supernatant was separated and mixed with 0.8 mL of 100 mmol/L ammonium acetate (pH 4.6). Following vortex, the mixture was loaded to Waters Oasis HLB SPE column (30 mg) which was pretreated with 1 mL of methanol first and followed by 1 mL of 100 mmol/L ammonium acetate (pH 4.6). SPE columns were washed with 1 mL of 100 mmol/L ammonium acetate (pH 4.6) followed by 1 mL of methanol/water (20/80, v/v). The column was vacuumed to dryness and the analytes were eluted with 1 mL of methanol/water (90/10, v/v). The elute was collected, and evaporated to dryness under nitrogen stream at 35 °C, and reconstituted by 0.2 mL of mobile phase.

Fifty microliters of 100 ng/mL internal standard working solution and 0.8 mL of 100 mmol/L ammonium acetate (pH 4.6) were added to 0.2 mL of urine sample. Following preparation steps were same as above. Finally, the residues were reconstituted by 0.5 mL of mobile phase after evaporated to dryness.

All preparation steps were processed in subdued red light.

2.4. Liquid chromatography/mass spectrometry

HPLC was performed on Waters Alliance 2695 HPLC system. Chromatography separation was carried out on Thermo BDS Hypersil C_{18} column (50 mm × 4.6 mm, 3 μm) protected by Thermo BDS Hypersil C_{18} guard column (4 mm × 4.6 mm, 3 μm) at 40 °C. The mobile phase was composed of formic acid/methanol/water (0.5/70/30, v/v/v). The flow rate was 0.6 mL/min and splitting ratio was set at 3:4. The injection volume was 15 μL.

Mass chromatograms were recorded using an API 3000 triple-quadrupole mass spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA, USA) equipped with Turbo IonSpray interface. Analysis was performed with an ionizing voltage of 5000 V. Ion source temperature was set at 350 °C with ultrahigh-purity nitrogen as curtain gas (9 L/h), nebulizer gas (14 L/h) and auxiliary gas (8 L/h). Other mass-dependent parameters such as orifice plate voltage (OR), focusing ring voltage (RNG), Q2 rod offset voltage (RO_2), RF-stubbies voltage (ST_3), and Q2 rod offset voltage (RO_3) for each compound were determined in positive mode using standard solutions. Multiple reaction monitoring (MRM) was carried out using nitrogen as collision gas (9 L/h), and with a dwell time of 200 ms for each transition. The analytes were detected by monitoring the transitions m/z 447.3 → 206.9 and 461.4 → 206.9 with the collision energy 35 and 36 eV for olmesartan and RNH-6272, respectively. The analytical time for each run was 4 min in total.

2.5. Method validation

The method was validated for selectivity, matrix effect, precision, accuracy, linearity, sensitivity, recovery, and stability according to the US Food and Drug Administration (FDA) [6] and Chinese State Food and Drug Administration (SFDA) guidelines [7] for the validation of bioanalytical method.

The specificity of this method was investigated by analyzing six individual human blank blood samples and urine samples. Each blank sample was tested for interference using the proposed extraction procedure and HPLC–MS/MS conditions and compared with spiked sample whose concentration of olmesartan was lower limit of quantitation (LLOQ) in plasma or urine.

Calibration standard samples in human plasma and urine were prepared for three separate days. The lower limit of quantitation for olmesartan in plasma and urine were all set at the lowest concentration of non-zero calibration standard (S/N ≥ 10:1). Intra- and inter-day precision and accuracy were determined by determining the concentrations of olmesartan in plasma and urine in five replicates of QC samples at four different concentrations for three separate days.

The extraction recovery of olmesartan was determined by comparing quantitative results of extracted QC samples at four concentrations to unextracted QC samples at the same concentration.

The matrix effect was investigated by comparing the areas of analytes in spiked QC sample with or without biological matrix from five drug-free volunteers, plasma and urine, at four concentrations. During the preparation of QCs at same concentration level, each individual's biological matrix was used only once. The corresponding peak areas of compounds in spiked QCs in plasma or urine (A) were then compared to those of the aqueous standards in the mobile phase (B) at equivalent concentrations.

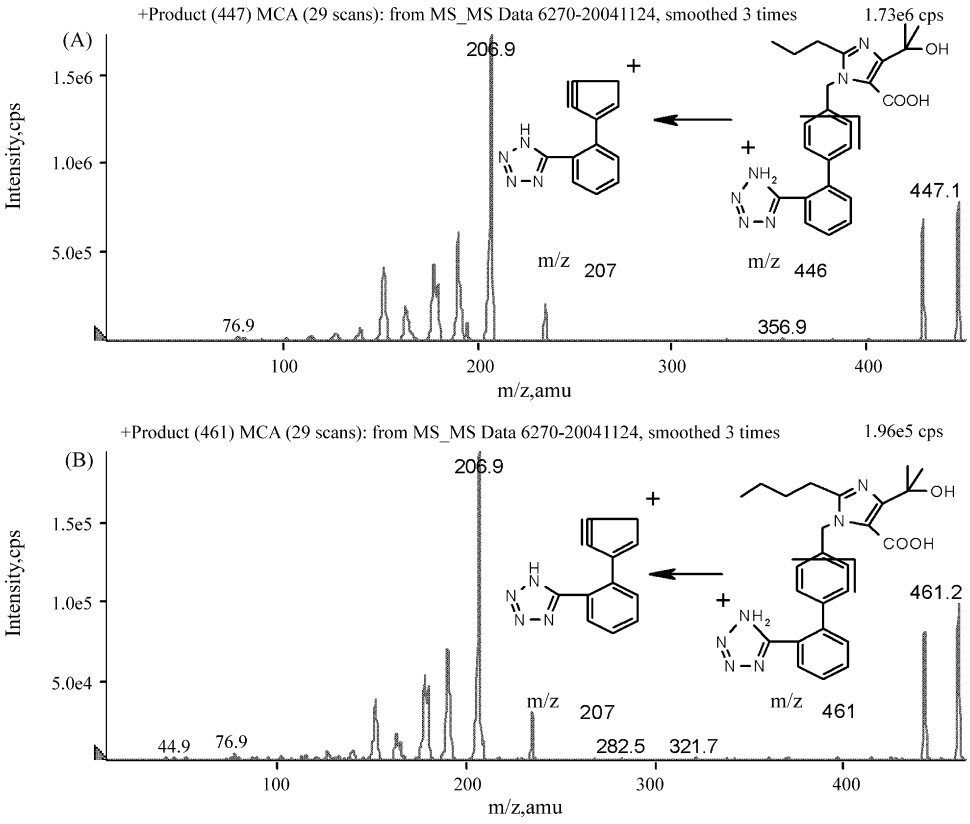

Fig. 2. Product ion spectrum of olmesartan (A) and RNH-6272 (IS, B).

The ratio ($A/B \times 100$) is defined as the matrix effect. The inter-subject variability of matrix effect at every concentration level should be less than 15% [8].

The stabilities of olmesartan and/or internal standard (IS) in biological matrix and in working solution at different storage condition were evaluated as follows and the results were expressed as percentage recoveries. The stabilities of olmesartan and IS working solutions were tested for 7 h at room temperature (subdued red light) The stabilities of olmesartan in plasma and urine sample at four concentrations were examined under different study conditions; i.e. standing at room temperature for 24 h (subdued red light) and storing at −30 °C for 3 months. The stabilities of olmesartan in plasma and urine extracts were also tested by sitting samples at room temperature for 24 h (away light). Freeze/thaw stability was determined after freezing (−30 °C) and thawing QC samples for three cycles.

3. Result and discussion

3.1. HPLC–MS/MS optimization

An HPLC–MS/MS method for the detection of olmesartan in plasma and urine was firstly investigated. The analyte was introduced into the mass spectrometer using the electrospary interface, and the parameters such as IS, OR and RNG were optimized to obtain protonated molecular ion $[M+H]^+$ or deprotonated molecular ion $[M-H]^-$. After comparing the response of two scan modes, $[M+H]^+$ ion was chosen to detect the analyte. In order to ensure high specificity of the method, MRM scan mode was selected to assay the analytes and the most suitable collision energy was determined when observing the maximum response for fragment ions. The product ion mass spectra of the compounds are depicted in Fig. 2 where $[M+H]^+$ of each compound was selected as precursor ion, and the most abundant fragment ion was chosen as the product ion in the MRM acquisition with the optimized collision energy for each compound.

For HPLC separation, the Hypersil C_{18} column was selected to retain the analyte because of its low carbon content (9%), which can provide suitable retention time for compounds and

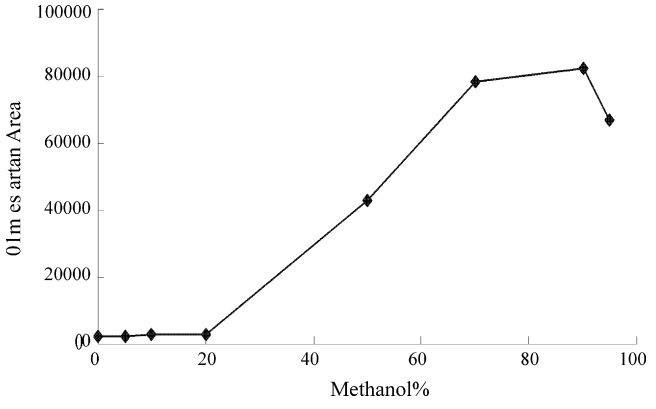

Fig. 3. The peak area–methanol% in water as elution solvent curve of olmesartan in plasma.

non-tailed peaks. 0.5% formic acid was chosen because of its highest response.

3.2. Extraction procedure optimization

The methods of sample preparation were studied by using precipitating protein and solid phase extraction (SPE). If the SPE procedure was employed directly, the high extraction recovery will not be obtained since 99% of olmesartan in plasma is combined with protein and may not be separated by SPE procedure (poor peak shapes and response, data not shown). Therefore, the procedure of precipitating protein coupled with SPE was selected to give a high recovery and good mass chromatograms. Furthermore, the analytes were extracted more efficiently from plasma and urine samples under acidic condition. Also, the percentage of methanol in water as elution

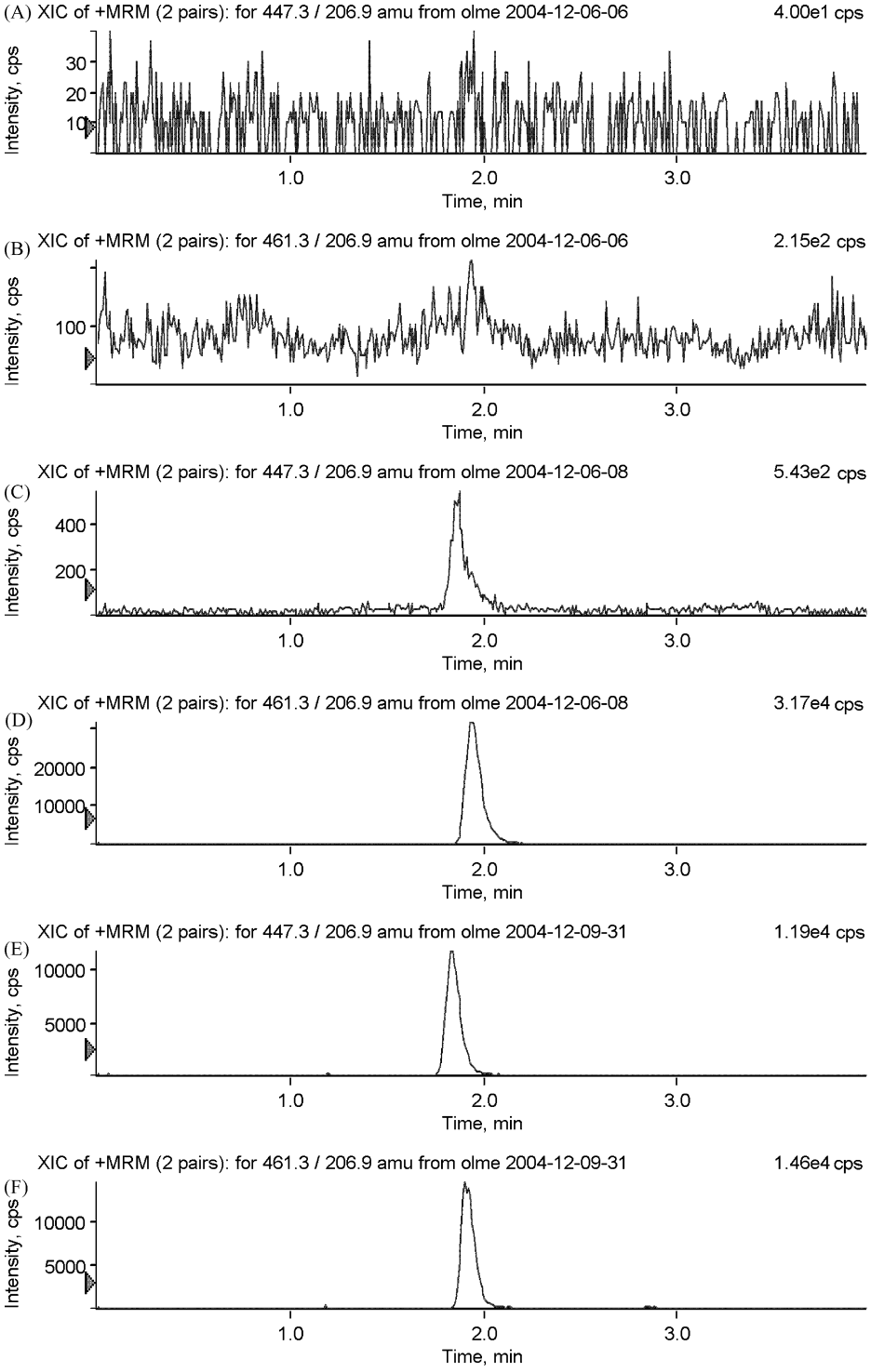

Fig. 4. MRM chromatograms of olmesartan and RNH-6272 (IS) and in blank plasma (A and B), LLOQ (C and D) and plasma from a subject's sample 24 h post dose of 20 mg olmesartan medoxomil (E and F).

solvent was investigated and the result is presented in Fig. 3. It was found that if the samples were washed by methanol/water (20/80, v/v) and eluted by methanol/water in (90/10, v/v) during the SPE procedure, the highest extraction efficiency could be obtained. Because the analytes were sensitive to light, the samples were prepared in faint light.

3.3. Validation steps

3.3.1. Specificity and sensitivity

No endogenous source of interference was observed at the retention times of the analytes, which was approximately 1.70 and 1.75 min for olmesartan and RNH-6272, respectively. Typi-

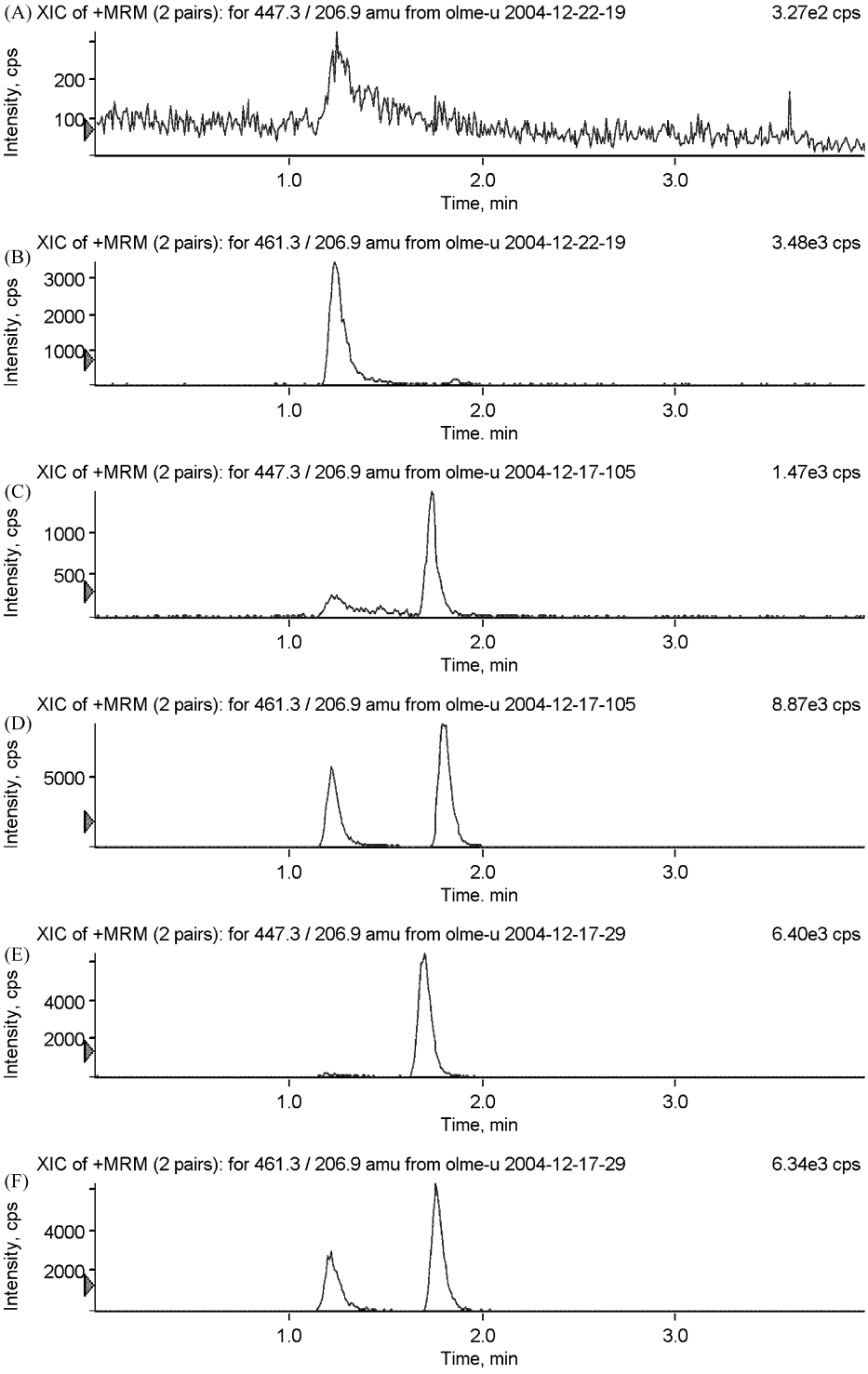

Fig. 5. MRM chromatograms of olmesartan and RNH-6272 (IS) in blank urine (A and B), LLOQ (C and D) and urine of a subject at 24–48 h after dosing 20 mg olmesartan medoxomil (E and F).

Table 1
Results of extraction recovery of olmesartan in plasma ($n = 5$)

Concentration (ng/mL)	Recovery (%)	CV (%)
0.480	93.9	7.3
8.00	80.0	3.2
80.0	83.2	5
800	77.9	7.9

cal chromatograms obtained from blank plasma, plasma sample containing 0.2 ng/mL of olmesartan (LLOQ) and a subject's sample 24 h post dose of 20 mg olmesartan medoxomil are presented in Fig. 4. Typical chromatograms of blank urine, a urine sample containing 5 ng/mL of olmesartan (LLOQ) and a subject urine sample are shown in Fig. 5. Because the only notable metabolite of olmesartan medoxomil in plasma and urine is olmesartan, the interference from other metabolites of pre-drug can be ignored.

3.3.2. Linearity

A calibration curve was established on each validation day. The calibration curve was linear over the concentration range of 0.2–1000 ng/mL for plasma and 5–10,000 ng/mL for urine with coefficient of correlation (r) > 0.99. A weighting factor of $1/x^2$ for olmesartan in both plasma and urine was chosen. Linearity was found to be quite satisfactory.

3.3.3. Recovery

The recoveries of the extraction method from plasma and urine observed (value and CV %, $n = 5$) are shown in Table 1 and Table 2, respectively. Recoveries were more than 77.9% at different concentrations for both plasma and urine with little variability.

Table 2
Results of extraction recovery of olmesartan in urine ($n = 5$)

Concentration (ng/mL)	Recovery (%)	CV (%)
15.0	79.1	8.7
60.0	96.0	6.4
600	99.5	1.8
7500	100.0	3.7

3.3.4. Matrix effect

Matrix effects and inter-subject variability data from plasma and urine of individual subjects who not receiving olmesartan are summarized in Table 3. The inter-subject variabilities were observed up to 9.4% in both plasma and urine. It indicated that the analytical method could be kept free of endogenous substance in human plasma and urine, and was judged to be useful for subject samples.

3.3.5. Precision and accuracy

Five quality control samples at each concentration level (0.48, 8, 80, 800 ng/mL for plasma and 15, 60, 600, 7500 ng/mL for urine) were processed and calculated each batch of five for three batches to provide precision (CV %) and accuracy of this method. The intra- and inter-day precision and accuracy data are summarized in Table 4 for plasma and Table 5 for urine. In both plasma and urine, intra-day precisions were ranged from 2.3% to 10.4%, and accuracies ranged from 91.3% to 105.3%; inter-day precisions ranged from 2.0% to 13.1% and accuracies ranged from 88.0% to 109.9%.

3.3.6. Stability

The stability tests of the analytes were designed to cover expected conditions of handling of clinical samples. The stabilities of the analytes in human plasma and urine were investigated under a variety of storage and processing conditions. Briefly, three freeze/thaw cycles and ambient temperature (subdued red light) storage of the QC samples up to 24 h appeared to have no effect on results of quantification of olmesartan in plasma and urine. QC samples stored in a freezer at or below −30 °C remained stable for at least 3 months. Processed samples were allowed to stand at room temperature (away light) in reconstituted solution for 24 h prior to analysis, with no observed effect on results of quantification. When working solution of olmesartan in methanol was stored at room temperature (subdued red light) for 7 h, the analyte were found to be stable.

Table 3
Results of matrix effect and inter-subject variability for olmesartan and RNH-6272 in plasma and urine

	Nominal concentration (ng/mL)	Matrix effect (mean ± SD, %)	Inter-subject variability[a] (%)
Plasma			
Olmesartan ($n = 5$)	0.480	90.2 ± 6.3	6.9
	8.00	95.5 ± 1.7	1.8
	80.0	82.8 ± 6.1	7.4
	800	87.1 ± 6.0	6.9
RNH-6270 ($n = 5$)	10.0	84.7 ± 8.0	9.4
Urine			
Olmesartan ($n = 5$)	15.0	103.7 ± 6.7	6.4
	60.0	98.1 ± 3.0	3.1
	600	98.8 ± 5.1	5.1
	7500	98.0 ± 5.3	5.4
RNH-6270 ($n = 5$)	100	92.1 ± 2.7	2.9

[a] Expressed as CV%.

Table 4
Accuracy and inter, intra-precision for the detection of olmesartan in plasma

	Q1	Q2	Q3	Q4
Intra-batch				
Nominal concentration (ng/mL)	0.480	8.00	80.0	800
Mean	0.527	8.25	85.0	832
SD ($n = 5$)	0.020	0.200	2.44	17.0
Accuracy (%)	109.9	103.1	106.2	104.0
Precision (%)	3.4	2.4	2.9	2.0
Inter-batch				
Nominal concentration (ng/mL)	0.480	8.00	80.0	800
Mean	0.506	8.19	82.6	833
SD ($n = 3$)	0.030	0.260	2.55	18.9
Accuracy (%)	105.3	102.4	103.3	104.1
Precision (%)	6.3	3.1	3.1	2.3

Table 5
Accuracy and inter, intra-precision for the detection of olmesartan in urine

	QC1	QC2	QC3	QC4
Intra-batch				
Nominal concentration (ng/mL)	15	60	600	7500
Mean	13.7	57.6	576	7130
SD ($n = 3$)	0.8	2.18	59.6	327
Accuracy (%)	91.3	96	96	95.1
Precision (%)	5.8	3.8	10.4	4.6
Inter-batch				
Nominal concentration (ng/mL)	15	60	600	7500
Mean	13.2	58.5	612	7110
SD ($n = 5$)	0.82	2.71	80.3	218
Accuracy (%)	88	97.5	101.9	94.8
Precision (%)	6.2	4.6	13.1	3.1

3.3.7. Carryover test

The MRM chromatogram of double blank (free of olmesartan and internal standard) analyzed by following the samples with the highest concentration of olmesartan (1000 ng/mL for plasma and 10,000 ng/mL for urine) had showed that there was no carryover in the condition of the present method.

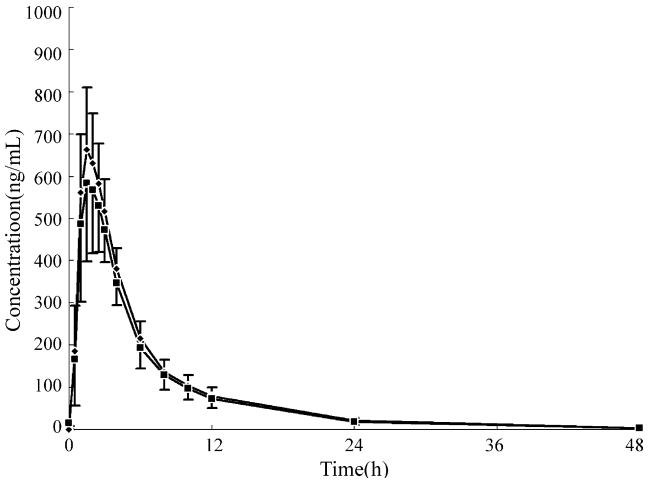

Fig. 6. The average concentration–time curves of olmesartan in plasma from Chinese subject after single (♦) and repeat (■) doses of olmesartan medoxomil (Mean ± SD, $n = 14$).

3.4. Application of the method in pharmacokinetic studies

The HPLC–MS/MS method described in this paper was used to investigate the plasma and urine pharmacokinetic profiles of olmesartan in healthy Chinese subjects after single and multiple doses of 20 mg olmesartan medoxomil ($n = 14$). The mean plasma concentration–time curve of olmesartan after single and repeat dose is shown in Fig. 6. The cumulative amount of olme-

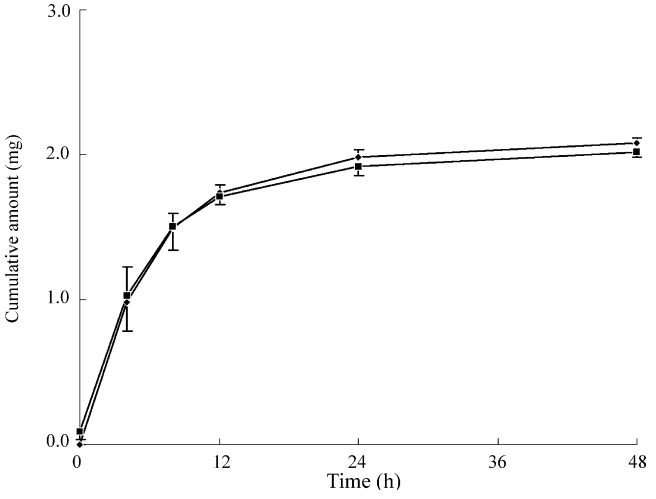

Fig. 7. The cumulative amount–time curves in urine from Chinese subjects after single (♦) and repeat (■) doses of olmesartan medoxomil (Mean ± SD, $n = 14$).

sartan excreted in urine from the same subjects after single and repeat dose is shown in Fig. 7.

4. Conclusion

A fast, sensitive and specific HPLC–MS/MS method based on the procedure of precipitating protein coupled with SPE has been developed and validated for the determination of olmesartan in plasma and urine of Chinese subjects. The extraction procedure and HPLC–MS/MS conditions were optimized in order to improve the sensitivity and robustness of the method. The procedure was fully validated to meet the requirements of State Food and Drug Administration and GLP Guidelines for Industry. This procedure was successfully applied to the determination of olmesartan in human plasma and urine.

Acknowledgment

This study was financially supported by the National High Technology Research and Development Program of China (Project 2004AA2Z3765) and Sankyo Co. Ltd. (Japan).

References

[1] K. Koga, S. Yamagishi, M. Takeuchi, Y. Inagaki, S. Amano, T. Okamoto, T. Saga, Z. Makita, M. Yoshizuka, Mol. Med. 8 (2002) 591.
[2] L.R. Schwocho, H.N. Masonson, J. Clin. Pharmacol. 41 (2001) 515.
[3] N. Kobayashi, I. Fujimori, M. Watanabe, T. Ikeda, Anal. Biochem. 287 (2000) 272.
[4] H. Nakamura, T. Inoue, N. Arakawa, Y. Shimizu, Y. Yoshigae, I. Fujimori, E. Shimakawa, T. Toyoshi, T. Yokoyama, Eur. J. Pharmacol. 512 (2005) 239.
[5] P. Laeis, K. Puchler, W. Kirch, J. Hypertens. 19 (2001) S21.
[6] US Department of Health and Human Services Food and Drug Administration, Guidance for Industry: Bioanalytical Method validation, US Department of Health and Human Services: Rockville, MD, 2001. Website: http://www.fda.gov/cder/guidance/index.htm.
[7] State food and drug administration, The Guidance of bioavailability and bioequivalence study technique for chemistry drug in human (No. [H]GCL2-1) 2005. Website: http://www.sda.gov.cn/gsz05106/08.pdf.
[8] P.J. Taylor, Clin. Chem. 38 (2005) 328.