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Original Article

Determination of candesartan or olmesartan in hypertensive patient plasma using UPLC-MS/MS

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ABSTRACT

Candesartan and olmesartan are angiotensin II receptor blockers (ARBs) used for the treatment of hypertension and heart failure. Quantitation methods for candesartan and olmesartan were developed using ultra-high performance liquid chromatography-tandem mass spectrometry following protein precipitation. Candesartan was separated using 5 mM ammonium formate (A) and 100% acetonitrile (B) and olmesartan was separated using 2 mM ammonium formate with 0.1% formic acid (A) and 100% acetonitrile (B). Separation was performed using an isocratic method with a Thermo hypersil GOLD C18 column. Electrospray ionization was used for analyte ionization and detection of candesartan, olmesartan, and the internal standards by multiple reaction monitoring. Developed method showed excellent linearity ($r > 0.99$) in the concentration range of 2–500 ng/mL for candesartan and 5–2,500 ng/mL for olmesartan. Accuracies were 86.70–108.8% for candesartan and 87.87–112.6% for olmesartan. These methods were able to successfully measure plasma candesartan or olmesartan concentrations in hypertensive patients. This study can be used for pharmacokinetic studies of candesartan or olmesartan in humans.

Keywords: Candesartan; Olmesartan; Angiotensin II Receptor Blockers; Liquid Chromatography; Mass Spectrometry

INTRODUCTION

Angiotensin II receptor blockers (ARBs) are effective and well-tolerated antihypertensive agents [1]. Among the various ARBs, candesartan cilexetil and olmesartan medoxomil are deesterified during absorption in the form of prodrugs to form the active forms, candesartan and olmesartan, respectively [2]. Candesartan binds more strongly to the AT1 receptor and dissociates more slowly compared with other ARBs [3]. In clinical studies of patients with hypertension, administration of 40 mg of telmisartan, 8 mg of candesartan cilexetil, or 80 mg of valsartan for 3 months resulted in a similar decrease in systolic blood pressure (SBP) and diastolic blood pressure (DBP) in all groups [4].

Olmesartan exhibits a strong blood pressure lowering effect with rapid onset, long-term action, and good tolerability [3]. In clinical studies, the average SBP and DBP at 24 hours of

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drug administration were approximately 3–5 mmHg lower in patients receiving olmesartan compared with patients receiving losartan, valsartan, or irbesartan [5]. These results indicate that olmesartan has a higher antihypertensive effect compared with other ARBs.

Candesartan and olmesartan do not exhibit a significant risk of drug-food interactions, however, they potentially interact with several other drugs [6]. For example, co-administration with non-steroidal anti-inflammatory drugs, such as celecoxib, ibuprofen, diclofenac, and naproxen, and candesartan, may lead to increased blood pressure and swelling in heart failure patients [6]. Also, both olmesartan medoxomil and candesartan cilexetil are substrates for carboxylesterase 2 (CES2). Many phenolic compounds contained in vegetables and fruits, and medications, such as aspirin and simvastatin, exhibit an inhibitory potential for CES2 [7]. Since candesartan and olmesartan show a dose-response relationship in the therapeutic dose range (candesartan cilexetil: 2–32 mg; olmesartan medoxomil: 2.5–40 mg) [8], medication compliance may be evaluated by determining candesartan or olmesartan plasma levels in patients whose blood pressure does not decrease following drug administration. To analyze candesartan and olmesartan in these clinical cases, a method is needed to detect the target drug in complex matrices rapidly and with high sensitivity.

In previous studies, several liquid chromatography-mass spectrometry (LC-MS) methods have been reported to measure candesartan or olmesartan in human plasma samples. The main disadvantages of conventional analytical methods are that they require complex extraction procedures or long running times. The method proposed for quantifying candesartan in human plasma [9] and the method for quantifying olmesartan use a sample preparation technique involving solid phase extraction (SPE) [10]. Sample preparation using SPE requires a separate cartridge. A method for quantifying candesartan developed by Bonthu et al. [11] and a method for quantifying olmesartan by Kumar et al. [12] use a sample preparation technique that requires liquid-liquid extraction (LLE). Sample preparation techniques using LLE required evaporation, drying, and reconstitution steps with liquid nitrogen.

In our study, we simplified the sample preparation steps compared with SPE and LLE using protein precipitation. The advantage is that there is no need for cartridges for analyte separation or evaporation, or drying and reconstitution steps with liquid nitrogen during sample preparation.

The main purpose of this study was to develop a rapid and simple analysis method using ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) to determine the concentration of candesartan or olmesartan in plasma obtained from outpatients.

METHODS

Reagents

Candesartan (purity 99%) and olmesartan (purity 98%) were purchased from Aladdin (Shanghai, China), acetonitrile and deionized water from Merck (Darmstadt, Germany), and ammonium formate and formic acid from Sigma-Aldrich (St. Louis, MO, USA). The internal standard (IS), candesartan-d_4 (purity 98.9%), was obtained from Toronto Research Chemicals (Toronto, ON, Canada). All the mobile phases were used LC grade and the other reagents were used extra-pure grade.

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Chromatography conditions

The determination of plasma candesartan or olmesartan concentrations were done using the Acquity™ UPLC (Waters, Milford, MA, USA) with a Xevo TQ-MS triple quadrupole tandem mass spectrometer (Waters). Sample separation was performed using a Thermo Hypersil Gold C18 column (150 × 2.1 mm, 1.9 μm). The temperatures of column oven and autosampler temperatures were maintained to 40°C and 10°C, respectively, during analysis. The mobile phase for measuring candesartan included (a) 5 mM ammonium formate and (b) acetonitrile. The mobile phase for measuring olmesartan consisted of (a) 2 mM ammonium formate with 0.1% formic acid and (b) acetonitrile. An isocratic method (A:B = 10:90) with a flow rate of 0.4 mL/min was used to quantitate both drugs. Mass detection was performed using the electrospray ionization positive mode with multiple reaction monitoring. Mass transitions were set to m/z 441.16 → 263.21 for candesartan, m/z 447.30 → 207.20 for olmesartan, and m/z 445.20 → 267.20 for the candesartan-d_4 (IS). The data were analyzed using Masslynx version 4.1 software (Waters).

Stock and working solutions

Each stock solution (1 mg/mL) was prepared by dissolving candesartan or olmesartan certified standards in 100% methanol. The same solvent was used to prepare working solutions for candesartan and olmesartan. The concentrations for the working solutions were as follows: candesartan; 20, 50, 100, 250, 500, 1,000, 2,000, 5,000 ng/mL, and olmesartan; 50, 100, 500, 1,000, 2,500, 5,000, 15,000, 25,000 ng/mL. As an IS, candesartan-d_4 was used for both candesartan and olmesartan and a standard solution of 100 μg/mL was prepared in 100% methanol. The IS used in the analysis was diluted with methanol to 50 ng/mL for the candesartan assay and 600 ng/mL for the olmesartan assay.

Sample preparation

Candesartan or olmesartan were extracted using protein precipitation. The 100 μL sample of plasma was transferred to a 1.7 mL microcentrifuge tube. Then, 50 μL of IS (50 ng/mL for candesartan and 600 ng/mL for olmesartan) were added. Next, 500 μL of 100% acetonitrile was added and vortexed (candesartan: 10 minutes; olmesartan: 5 minutes). Supernatants were centrifuged at 13,000 rpm for 5 minutes and the supernatants were transferred to a new vial for analysis.

Method validation

Developed quantitative method was validated based on the "Bioanalytical Method Validation Guidance for Industry" published by US Food and Drug Administration and "Guideline on Bioanalytical Method Validation" published by Korea Ministry of Food and Drug Safety [13,14]. The linearity, sensitivity, accuracy, precision, carry-over, matrix effect, stability, and dilution integrity of the bioanalytical method were evaluated.

Linearity and sensitivity

The linearity for candesartan and olmesartan in whole analytical batches was evaluated over the range of 2–500 ng/mL and 5–2,500 ng/mL, respectively. The calibration curves consisted of at least 8 concentrations including the double blank sample (without adding analytes and IS), blank (with only IS), and lower limit of quantification (LLOQ), respectively. They consisted of a set of standard samples to generate calibration curves. The accuracy of the calibration curve samples should be within 20% of the theoretical value at the LLOQ and within 15% of the theoretical value for low quality controls (LQC, concentration about 3 times the lowest limit), medium quality controls (MQC, the middle level of the calibration curve), and high quality controls (HQC, 80% of the maximum limit of the calibration curve).

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Accuracy and precision

The accuracy and precision of the assay for candesartan and olmesartan was evaluated using LLOQ, LQC, MQC, HQC samples. Accuracy and precision were assessed by repeating measurements 5 times within each batch (intra-batch), and assessing 3 independent batches (inter-batch). Accuracy and precision should be within ± 20% for LLOQ and within ± 15% for LQC, MQC, and HQC.

Carry-over and matrix effect

The carry-over of candesartan and olmesartan was evaluated by injecting the upper limit of quantification (ULOQ) and then injecting a double blank sample. When the double blank sample was injected, the peak areas of the analyte and IS in the double blank sample should be less than 20% and 5% of the LLOQ, respectively. The matrix effect was evaluated by analyzing the quality control sample. The quality control samples at LQC and HQC are measured with 6 different biological samples the coefficient of variation (CV) should be within 15%.

Stability and dilution integrity

The stabilities of candesartan and olmesartan were evaluated under a variety of conditions including reinjection, processed sample stability, short-term, short-term stock, and freeze-thaw stability (FTS). LQC and HQC that satisfied each storage condition should be analyzed at least 3 times. In this case, the average value for each concentration should be within 15% of the theoretical value. Short-term stability samples were all evaluated for 10 hours at room temperature and FTS was evaluated after 3 freeze-thaw cycles at −80°C. The reinjection stability was evaluated by comparing the determined concentrations of the LQC and HQC samples analyzed in the previous batch with newly prepared samples, and the pre-treatment sample stability was analyzed after overnight storage at 10°C in the autosampler. To evaluate the dilution integrity, the test samples were analyzed after using dilution quality control sample (DiQC), at a concentration 2-fold greater than ULOQ, and diluted 10- and 5-fold.

Method application

Determination of plasma candesartan or olmesartan concentrations in human plasma

A clinical study was conducted at the Dongguk University Hospital Clinical Trial Center (Ilsan, Korea) in 29 subjects who agreed to participate voluntarily. The study was approved by the Dongguk University Hospital Institutional Review Board (IRB No. 2017-11-009). To quantify the candesartan and olmesartan concentration at steady-state, a total of 34 and 24 plasma samples were collected in 17 and 12 cardiovascular patients. The patients had already continued daily treatment of candesartan or olmesartan at least 1 month prior to the enrollment. V1 was the first visit after enrollment and V2 was 2 months after first visit. All patients were administered their study drugs according the clinically recommended regimens which were 8 or 16 mg daily for candesartan cilexetil or 20 or 40 mg daily for olmesartan medoxomil, respectively. Sample preparation and analysis of the collected plasma samples were performed in the same manner as the validation set.

RESULTS

Methods development

The mass spectra and chemical structures of candesartan and olmesartan are shown in **Figure 1**. During method development, the chromatographic conditions were improved to achieve

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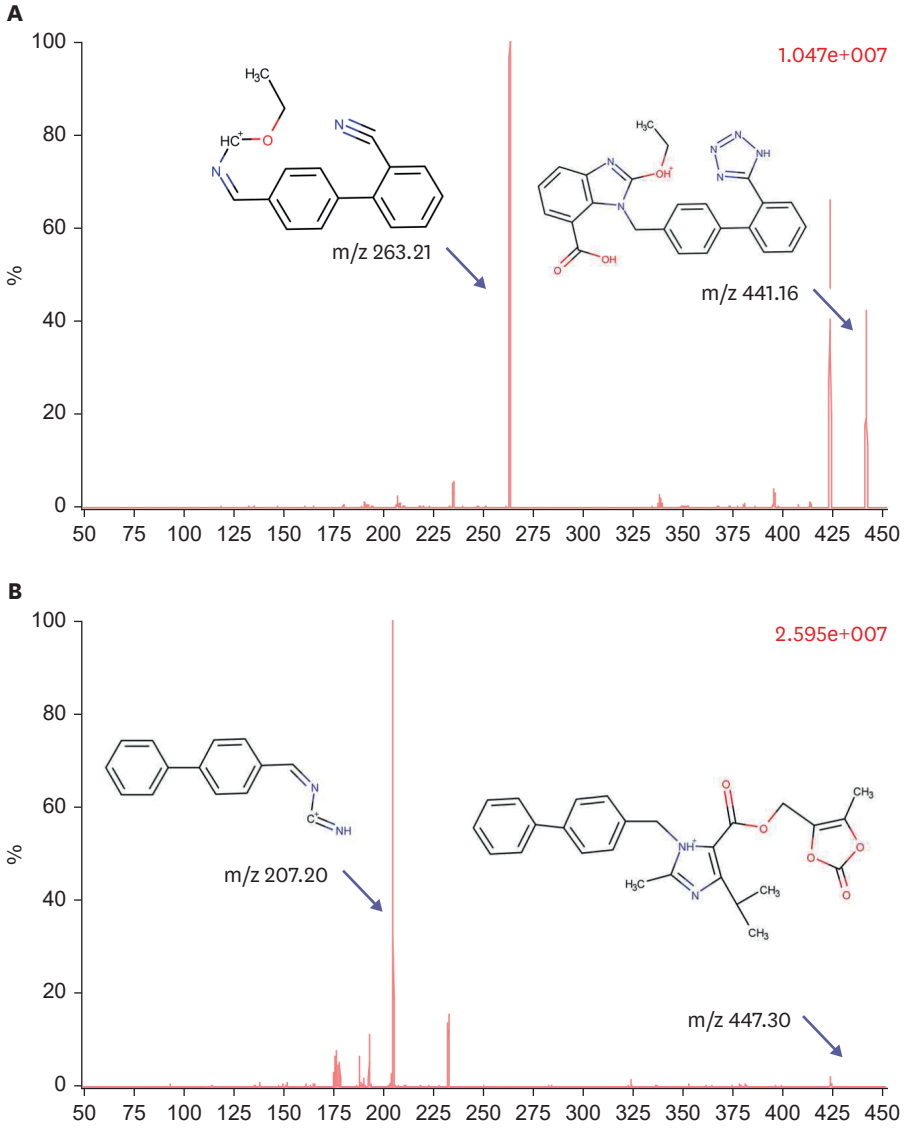

Figure 1. Mass spectrum and chemical structure of (A) candesartan and (B) olmesartan in electrospray ionization positive mode.

good separation of candesartan or olmesartan. The final analytical methods were established as follows: Separation was carried out with an isocratic method (A:B = 10:90) using 5 mM ammonium formate (a) and 100% acetonitrile (b) for candesartan, and 2 mM ammonium formate with 0.1% formic acid (a) and 100% acetonitrile (b) for olmesartan. Chromatography was performed using a Thermo Hypersil GOLD C18 column (150 × 2.1 mm, 1.9 μm). The separation was evaluated using acetonitrile, formic acid, methanol, ammonium acetate, and ammonium formate, however, ammonium formate and acetonitrile showed the good results for the quantitation of candesartan, and formic acid and acetonitrile yielded the best results for the quantitation of olmesartan. The ULOQ chromatograms for candesartan and olmesartan before and after these improvements are shown in **Figure 2**. A gradient method (initial: 40% B; 0–1.0 minutes: 85% B; 1.0–1.4 minutes: 85% B; 1.4–1.5 minutes: 40% B; 1.5–5.0 minutes: 40% B) was used for candesartan and olmesartan. Candesartan was eluted at 1.69 minutes with a sensitivity of 9.15×10^5 (counts per second [cps]) (**Figure 2A**). An isocratic method was used for

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candesartan, which was eluted at 0.84 minutes with a sensitivity of 3.67×10^6 cps (**Figure 2B**). For candesartan, the run time was reduced by approximately 0.9 minutes and the sensitivity was improved from 9.15×10^5 to 3.67×10^6 cps. A above gradient method was used for olmesartan, which was eluted at 1.12 minutes with a sensitivity of 6.27×10^6 cps (**Figure 2C**). An isocratic method was used for olmesartan, which was eluted at 0.88 minutes with a sensitivity of 2.38×10^7 cps (**Figure 2D**). For olmesartan, peak shouldering was observed in the front portion with broad peaks over the entire chromatogram, however, the peak shape was improved from previous conditions. The retention time was reduced by approximately 0.4 minutes and the sensitivity improved from 6.27×10^6 to 2.38×10^7 cps. The results showed a good peak shape and sensitivity, and the analytes were well separated. Finally, the isocratic method was used for candesartan and olmesartan analysis.

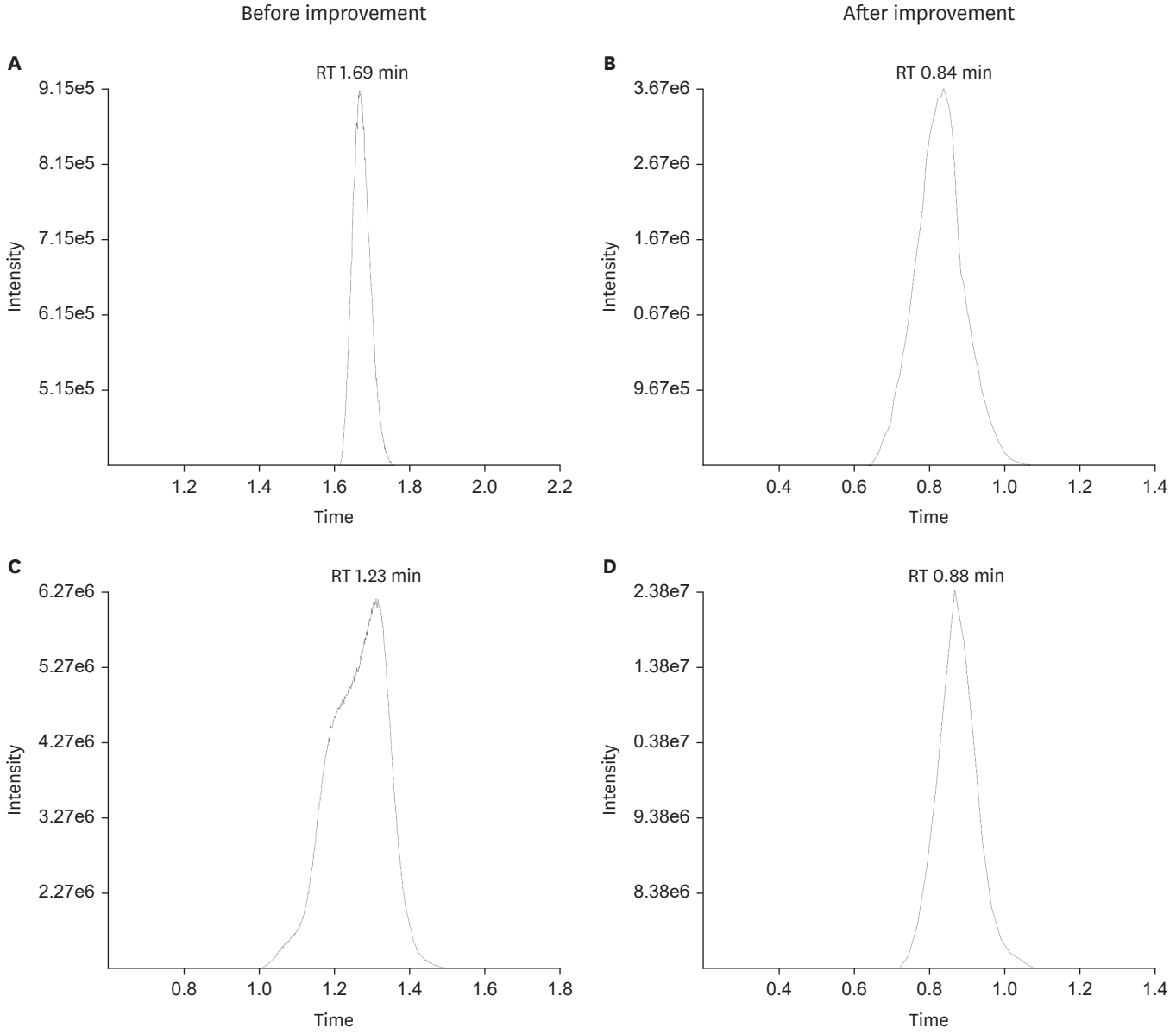

Figure 2. Representative chromatogram using a gradient method: (A) ULOQ for candesartan, (C) ULOQ for olmesartan, and using an isocratic method: (B) ULOQ for candesartan, (D) ULOQ for olmesartan.
ULOQ, upper limit of quantification; RT, retention time.

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Method validation

Linearity and sensitivity

Calibration curves were established with a weighting factor of 1/x range from 2–500 ng/mL ($r \geq 0.999$ and $r^2 \geq 0.998$) for candesartan and 5–2,500 ng/mL ($r \geq 0.999$ and $r^2 \geq 0.996$) for olmesartan. Both the candesartan and olmesartan curves showed excellent linearity. The signal-to-noise ratio for the LLOQ of candesartan (2 ng/mL) and olmesartan (5 ng/mL) was 130.94 and 839.59, respectively, which was greater than 10. Double blank samples and chromatograms of LLOQ for candesartan and olmesartan are shown in **Figure 3**.

Accuracy and precision

The results of accuracy and precision for the 4 concentrations (LLOQ, LQC, MQC, and HQC) of candesartan and olmesartan are shown in **Table 1**. The candesartan inter- and intra-batch accuracies were 94.97–107.1% and 86.70–108.8% (LLOQ; 86.70–100.6%), respectively, and intra- and inter-day precision was within 10.0%. For olmesartan, the inter- and intra-batch

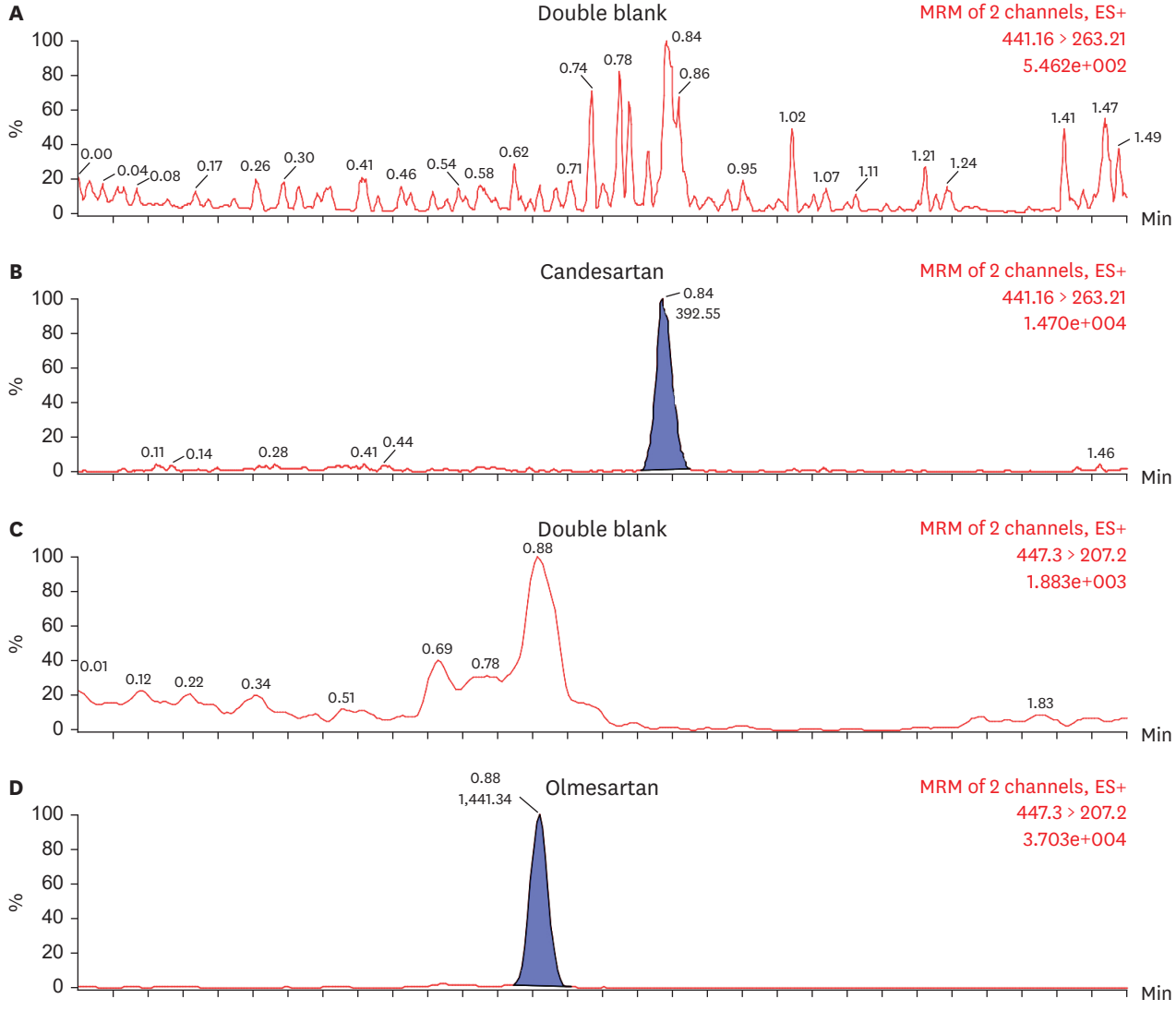

Figure 3. Representative chromatogram of (A) double blank human plasma for candesartan, (B) LLOQ for candesartan, (C) double blank human plasma for olmesartan and (D) LLOQ for olmesartan.
LLOQ, lower limit of quantification.

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Table 1. Accuracy and precision of the validation of the quality control samples

Concentration	Candesartan				Olmesartan			
	LLOQ (2 ng/mL)	Low (6 ng/mL)	Medium (45 ng/mL)	High (400 ng/mL)	LLOQ (5 ng/mL)	Low (15 ng/mL)	Medium (150 ng/mL)	High (2,000 ng/mL)
Accuracy (%)								
Batch 1	100.6	108.7	104.0	103.8	112.6	95.36	97.62	101.6
Batch 2	86.70	108.8	101.9	102.5	91.28	87.87	94.44	99.27
Batch 3	97.60	103.8	88.33	95.17	92.96	90.04	94.99	90.90
Inter-batch	94.97	107.1	98.07	100.5	98.95	91.09	95.69	97.26
Precision (CV, %)								
Batch 1	8.80	4.74	5.32	2.54	2.66	3.44	2.67	1.35
Batch 2	5.73	2.44	5.77	2.29	3.63	4.22	4.47	2.60
Batch 3	0.28	3.85	2.59	0.64	0.12	5.90	5.17	2.25
Inter-batch	10.0	8.64	4.35	4.35	10.60	4.18	5.27	5.27

CV, coefficient of variation; LLOQ, lower limit of quantification.

accuracies were 91.09–98.95% and 87.87–112.6% (LLOQ; 91.28–112.6%), respectively, and the intra- and inter-day precision was within 10.6%. For all analysis batches, candesartan and olmesartan met the accuracy and precision criteria within ± 20% for LLOQ and within ± 15% for LQC, MQC, and HQC.

Carry-over and matrix effect

Five double blank samples were injected after each ULOQ sample was injected to identify any carry-over effect of candesartan and olmesartan. The results showed that both candesartan and olmesartan exhibited no interference after a double blank sample injection. After injecting LQC and HQC samples prepared from 6 different individual plasma samples, the CV in LQC and HQC were 7.2% and 9.6%, respectively, for candesartan, and 7.1% and 4.2%, respectively, for olmesartan. There was no significant matrix effect in human plasma with LQC and HQC samples and the matrix effect results for each of candesartan and olmesartan are shown in **Table 2**.

Stability and dilution integrity

The stability of each working and stock solution of candesartan and olmesartan were evaluated under various conditions. The results demonstrated that our method exhibited acceptable stability (**Table 3**). The treated samples were stable for 24 hours in an auto sampler. The working and stock solutions of candesartan and olmesartan were stable for 11 hours at laboratory temperature. Each of 6 LQC and HQC samples for both drugs were satisfied the acceptance criteria, within 15% of the nominal concentration change after freeze-thaw cycles. The difference after reinjection was lower than 15% compared with the nominal concentration of test and reference samples. These results indicate that candesartan and olmesartan are stable under the experiment conditions.

Table 2. Matrix effect results of candesartan or olmesartan at 2 concentrations (n = 3)

Number	Candesartan		Olmesartan	
	Low (6 ng/mL)	High (400 ng/mL)	Low (15 ng/mL)	High (2,000 ng/mL)
Subject 1	10.94	661.18	92.54	13,450.27
Subject 2	11.75	750.83	110.95	12,962.61
Subject 3	13.12	770.04	93.94	11,918.19
Subject 4	13.22	728.14	100.65	12,642.72
Subject 5	12.90	879.41	94.65	12,737.59
Subject 6	12.37	806.35	94.81	12,285.84
CV, %	7.23	9.62	7.11	4.20

Values are presented as peak area ratio.
CV, coefficient of variation.

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Table 3. Stability data of candesartan or olmesartan under various conditions at 2 concentrations (n = 3)

Concentration	Candesartan		Olmesartan	
	Low (6 ng/mL)	High (400 ng/mL)	Low (15 ng/mL)	High (2,000 ng/mL)
Stability	% change		% change	
Reinjection	−14.5	−4.55	5.52	6.36
Autosampler for 24 hours	−1.51	−3.16	1.46	−2.22
Three freeze-thaw cycles	−3.63	−5.90	−1.52	−3.13
Plasma at room temperature for 11 hours	8.70	9.94	12.4	−2.06
Stock at room temperature for 11 hours	−4.05	1.66	−5.25	−9.40

To evaluate the dilution integrity of candesartan and olmesartan, each DiQC sample was diluted 10- and 5-fold. The results for candesartan and olmesartan satisfied the accuracy (85–115%) and precision (CV ≤ 15%) parameters, and reproducibility was observed when the sample diluted 10-fold. The results of the dilution integrity analysis for candesartan and olmesartan are shown in **Table 4**.

Method application

Determination of plasma candesartan or olmesartan concentration in human plasma

The method was applied to the determination of candesartan or olmesartan concentration in plasma obtained from hypertension patients. The candesartan (2–500 ng/mL) or olmesartan (5–2,500 ng/mL) concentration range was successfully determined and showed adequate reproducibility. Chromatograms for candesartan or olmesartan in plasma of hypertension patients are shown in **Figure 4**. The mean ± standard deviation (SD) plasma candesartan concentration for 17 patients were 77.8 ± 80.6 ng/mL, and mean ± SD plasma olmesartan concentration for 12 patients were 425 ± 335 ng/mL.

DISCUSSION

An analytical method to determine plasma candesartan or olmesartan concentration using the UPLC-MS/MS system has been successfully developed and validated. All of the assay parameters satisfied the acceptance criteria for linearity, accuracy, sensitivity, matrix effect, carry-over, stability, and reproducibility. Using the UPLC-MS/MS system, a small injection volume (3 μL) was required for quantitation and the assay run time was relatively short (candesartan: 1.5 minutes, olmesartan: 2 minutes). In this study, developed method showed an excellent linearity (r > 0.99) over a concentration range from 2–500 ng/mL for candesartan

Table 4. Dilution integrity of candesartan or olmesartan in human blank plasma (n = 5)

Number	Candesartan						Olmesartan					
	10-fold dilution			5-fold dilution			10-fold dilution			5-fold dilution		
	Measured concentration (ng/mL)	Accuracy (%)	Precision (CV, %)	Measured concentration (ng/mL)	Accuracy (%)	Precision (CV, %)	Measured concentration (ng/mL)	Accuracy (%)	Precision (CV, %)	Measured concentration (ng/mL)	Accuracy (%)	Precision (CV, %)
1	84.63	84.63		174.82	87.41		481.60	96.32		963.88	96.39	
2	90.73	90.73		173.39	86.69		479.45	95.89		967.96	96.80	
3	85.46	85.46	3.60	162.42	81.21	2.82	483.62	96.72	2.38	979.82	97.98	2.51
4	90.49	90.49		170.61	85.30		484.31	96.86		980.83	98.08	
5	91.23	91.23		170.23	85.11		507.88	101.6		1,025.7	102.6	
Mean	88.51	88.51	-	170.29	85.14	-	487.37	97.47	-	983.64	98.36	-
SD	3.19	-	-	4.80	-	-	11.62	-	-	24.64	-	-

Nominal concentration: 2-fold concentration of upper limit of quantification.
CV, coefficient of variation; SD, standard deviation.

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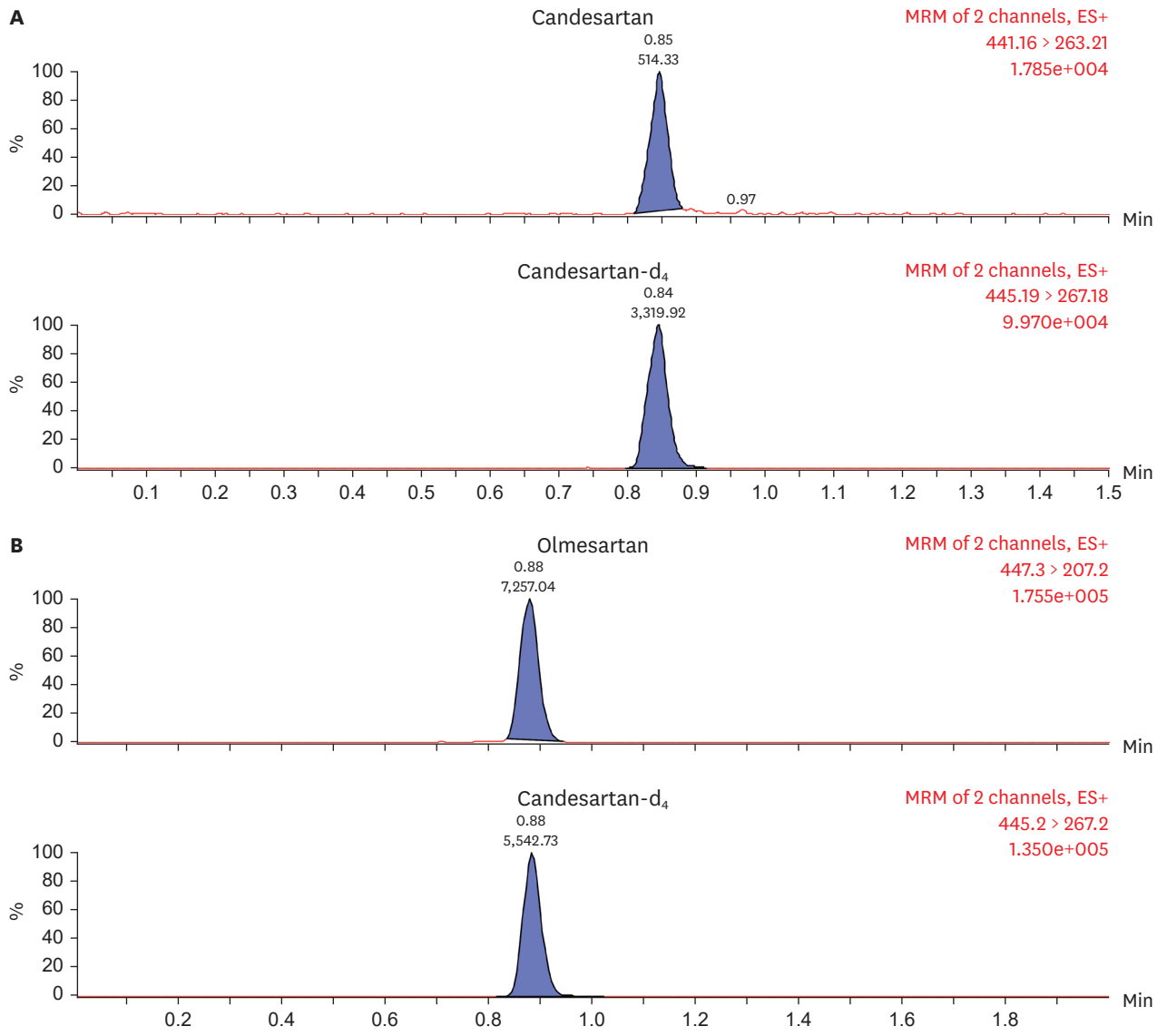

Figure 4. Representative chromatogram of (A) candesartan and ISs in plasma after oral administration of 8 mg candesartan, (B) olmesartan and ISs in plasma after oral administration of 20 mg olmesartan.
IS, internal standard.

and 5–2,500 ng/mL for olmesartan. Under various stability conditions, the % change in values for candesartan and olmesartan at 2 concentrations (LQC and HQC) were within 15%. Stability in the plasma was obtained during storage and analysis conditions.

The analysis of candesartan or olmesartan in our study exhibited sufficient accuracy and precision as in previous studies. Comparative data from previous studies on the analysis of candesartan or olmesartan are shown in **Table 5**. In the case of candesartan in previous studies, the accuracy and precision were 89.2–111% and 0.9–16.9% [15-18], whereas in our study, the accuracy and precision were 86.70–108.8% and 0.28–8.8%. In the case of olmesartan in previous studies, the accuracy and precision were 87.7–109.5% and 1.0–10.4% [12,19-21], whereas in our study, the accuracy and precision were 87.87–112.6% and 0.12–5.9%. In previous studies using LLE, organic solvents such as diethyl

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Table 5. Comparison of analysis methods from previous studies and this study

Variable	Sample	Preparation	Equipment condition	Analysis time (minute)	LLOQ (ng/mL)	Accuracy (%)	Precision (CV, %)	Reference
Candesartan	Plasma	PP	LC-MS	1.5	2.0	86.7–108.8	0.28–8.80	Present study
	Plasma/Urine	SPE	LC-MS	4.5	1.0	89.2–111.0	0.90–16.9	[15]
	Plasma	LLE	LC-MS	2.0	1.0	95.0–107.3	0–9.0	[16]
	Plasma	LLE	LC-MS	2.5	1.0	95.4–102.2	1.90–4.10	[17]
	Plasma	PP	LC-MS	2.5	2.0	95.5–104.3	1.30–8.00	[18]
Olmesartan	Plasma	PP	LC-MS	2.0	5.0	87.9–112.6	0.12–5.90	Present study
	Plasma	LLE	LC-MS	3.0	5.0	93.9–100.0	3.10–10.4	[12]
	Plasma	LLE	LC-MS	4.2	5.0	87.7–109.5	1.40–6.40	[19]
	Plasma	PP	LC-MS	10.0	0.2	100.1–103.2	2.40–7.40	[20]
	Plasma	LLE	LC-MS	6.0	10.0	88.9–97.8	1.00–1.90	[21]

CV, coefficient of variation; LLOQ, lower limit of quantification; SPE, solid phase extraction; LLE, liquid-liquid extraction; PP, protein precipitation; LC-MS, liquid chromatography-mass spectrometry.

ether or dichloromethane were used and nitrogen evaporation was also required. The protein precipitation method and UPLC-MS/MS system used in the present study yielded similar levels of accuracy and precision as that of previous studies without the additional concentration and nitrogen evaporation steps.

Our assay has the advantage of shortening the analysis runtime and enabling the analysis to be performed with high throughput (**Table 5**). In previous studies, the average run time was 2.88 (2–4.5) minutes for candesartan and 5.8 (3–10) minutes for olmesartan, and approximately 21 samples were analyzed per hour for candesartan and 10 samples for olmesartan. In the present study, the run time of candesartan was 1.5 minutes and that of olmesartan was 2 minutes, and approximately 40 samples for candesartan and 30 samples for olmesartan were analyzed per hour. Therefore, the advantage of our assay is the ability to process more samples per hour with an analysis that shows similar accuracy and precision.

Plasma concentrations of candesartan or olmesartan in 29 hypertension patients were successfully measured using our newly developed method. The mean ± SD plasma candesartan concentration for 17 patients were 77.8 ± 80.6 ng/mL. The mean ± SD plasma olmesartan concentration for 12 patients were 425 ± 335 ng/mL.

The cause of this large deviation among patient' samples is unclear, however, there are several possibilities. It is difficult to clearly understand the deviations in plasma concentration because dosing timing and diet are generally not controlled in clinical studies. In addition, dose compliance is also a major factor that can affect plasma candesartan or olmesartan concentrations. Irregular administration or ingestion of wine, tea, or some natural products containing phenolic compounds and flavonoids could be interfere conversion to an active form.

In conclusion, a validated rapid and simple UPLC-MS/MS method was provide to quantitate candesartan or olmesartan in plasma from hypertension patients. This method has been successfully applied to measure candesartan or olmesartan in the plasma of hypertension patients and may be applied to the analysis of the concentration of candesartan, olmesartan, and various analytes.

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