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Research Article

Liquid chromatography tandem mass spectrometry method for simultaneous determination of losartan and its active metabolite in human plasma

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ABSTRACT

A highly selective, sensitive and reproducible liquid chromatography tandem mass spectrometry (LC-MS/MS) method for simultaneous determination of losartan and its active metabolite (EXP3174) in human plasma, using candesartan as an internal standard (IS), was described. Chromatographic separation was carried out in the Luna HST 2.5µm C18 (50x3 mm.). The mobile phases were 0.05% formic acid and acetonitrile at a ratio of 3.3:6.7 (v/v). The mass spectrometry was operated in positive electrospray ionization and multiple reactions monitoring (MRM) mode. Liquid-liquid extraction (LLE) was employed with the mixture of ethylacetate and hexane at a ratio of 9:1 (v/v). The method was developed and fully validated according to the USFDA guidance. The limit of detection (LOD) of losartan and EXP3174 were 0.10 and 0.20 ng/mL, respectively and the lower limit of quantification (LLOQ) of both losartan and EXP3174 were 0.5 ng/mL. This method demonstrated good linearity (r^2>0.999) ranged from 0.5-2,500 ng/mL for both losartan and EXP3174. Accuracy and precision of the method were acceptable with high recovery of extraction. Neither anticoagulant nor matrix affected the analysis. This method was successfully applied to determine the concentrations of losartan and EXP3174 in human plasma in a bioequivalence study.

1. INTRODUCTION

Losartan potassium is a highly selective angiotensin II type 1 (AT1) receptor antagonist indicated for treatment of hypertension[1-3]. It is chemically described as 2-butyl-4-chloro-l-[p-(o-1H-tetrazol-5-ylphenyl) benzyl] imidazole-5-methanol monopotassium salt. The empirical formula is $C_{22}H_{22}ClKN_6O$. Losartan is mainly metabolized by the cytochrome P450 system. Oxidation of the C_5-hydroxymethyl on the imidazole ring to the 5-carboxylic acid produces an active metabolite of losartan called losartan carboxylic acid or EXP3174[4].

Although several LC-MS/MS methods for detection of losartan and its active metabolite have been reported, there are still some analytical limitations such as large volume of plasma samples needed, large solvent consumption and narrow linearity range (1-500 ng/mL)[5-7].

We developed a sensitive and wider linearity range (0.5-2,500 ng/mL) LC-MS/MS method in a positive ionization mode for simultaneous determination of losartan and EXP3174 in human plasma. Only 0.1 mL per sample was required for an analysis. This method was fully validated according to the Guidance for Industry, Bioanalytical Methods Validation, U.S. Department of Health and Human Service, Food and Drug Administration, Center for Drug Evaluation and Research (USFDA CDER, 2001, BP)[8] and subsequently used in a bioequivalence study of healthy Thai volunteers.

2. MATERIALS AND METHODS

2.1. Instrumentation

The high performance liquid chromatography with mass spectrometry system (LC-MS/MS) was performed on separated module of Acquity Ultra Performance LC™, (Waters, Co., Ltd. USA) equipped with Quattro Micro mass spectrometer, (Micromass Technologies, UK). The data management system was performed by Masslynx 4.1 SCN627, (Micromass Technologies, UK).

Chromatographic separation was carried on the Luna HST C_{18} column (3 mm x 50 mm, 2.5 µm) with Analytical Guard Cartridge System from Phenomenex Ltd., USA at 30±5°C. An isocratic elution of mobile phase was performed on a mixture of 67% acetonitrile and 33% water containing 0.05% formic acid at a flow rate of 0.250 mL/min. The sample injection volume was 5 µL with a 4-minute total run time. The mass spectrometer was operated in the positive electrospray ionization (ESI) mode. Quantification was performed in multiple reaction monitoring (MRM) mode. The optimized mass spectrometric parameters were set as follows: source temperature at 120°C, desolvation temperature at 350°C, cone gas flow rate at 30 L/Hr and desolvation gas flow rate at 650 L/Hr. The parameters set to the best abundant and specific daughter ions included capillary voltage of 1 kV, cone voltage of 30 V for losartan, 32 V for EXP3174 and 28 V for IS. The collision energies for the detection of losartan, EXP3174 and IS were set to 37 eV, 24 eV and 12 eV, respectively.

2.2. Chemicals and reagents

The reference standard of losartan potassium was obtained from The United States Pharmacopeial Convention, Inc., USA. Losartan carboxylic acid and an internal standard (IS), Candesartan, were obtained from TLC PharmaChem Inc., Canada. Their structures were presented in Figure 1. HPLC grade of acetonitrile was purchased from Fisher Scientific UK, the United Kingdom and Merck, Darmstadt, Germany. HPLC grade of methanol was purchased from Fisher Scientific UK, the United Kingdom. Analytical reagent grade of formic acid, ethylacetate and hexane were purchased from Scharlau, Barcelona, Spain. Type I water was produced by a Milli-Q water purification system, Millipore Corporation, USA. Drug-free human plasma was provided through the courtesy of the Department of Transfusion Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.

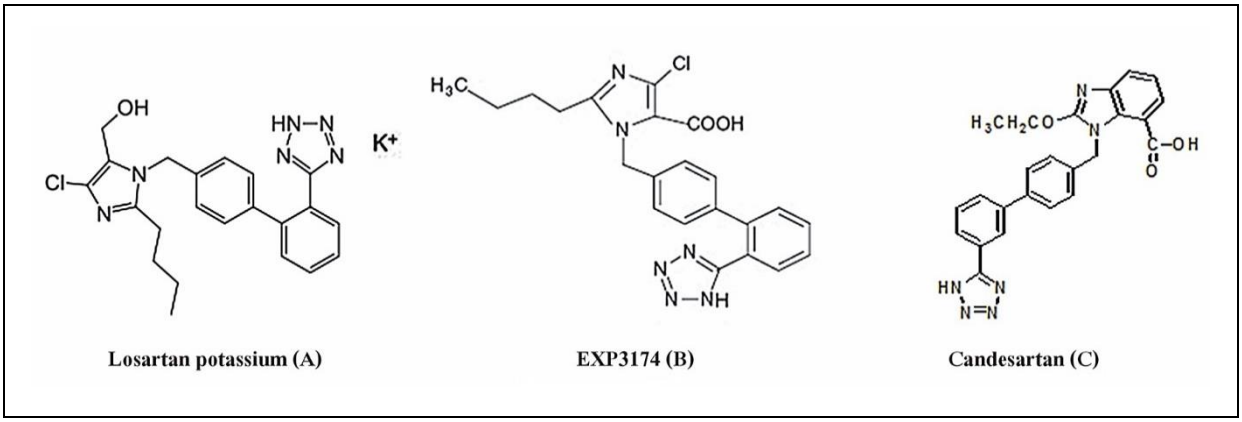

Figure 1. Chemical structure of losartan potassium (A), EXP3174 (B) and Candesartan, IS (C).

2.3. Preparation of stock and working solutions

The stock solutions of losartan and EXP3174 were prepared in methanol at a concentration of 700 µg/mL (calculated for the free base) and 800 µg/mL, respectively. The working solutions were prepared by dilution of stock solution with 50% methanol to acquire different concentrations of working solution which ranged from 10-50,000 ng/mL. The stock solution of candesartan, an internal standard, was dissolved in methanol containing 0.5% ammonium hydroxide. The working solutions were obtained by diluting the candesartan stock solution to 100 ng/mL with 50% methanol. All stock and working solutions were stored in a -70±10°C freezer.

2.4. Preparation of calibration curves and quality control samples

The calibration standard and quality control sample were prepared by spiked losartan and EXP3174 working solution into pooled human blank plasma to get the desired concentration of 0.5, 1, 10, 100, 700, 1,300, 1,950 and 2,500 ng/mL. The concentration of QC samples at LLOQ, LQC, MQC and HQC were 0.5, 1.5, 1,200 and 1,900 ng/mL, respectively and were prepared in a similar manner for both losartan and EXP3174.

2.5. Sample preparation

The sample extraction was achieved by liquid-liquid extraction technique. The 5 µL of IS working solution and 30 µL of 1 M formic acid solution were added to 100 µL of plasma. Then, one milliliter of ethylacetate and hexane mixture at 9:1 (v/v) was added, vortex-mixed for 10 minutes, and centrifuged for 15 minutes at 4ºC and 10,000 rpm. 800 µL of organic layer was transferred to a conical polypropylene tube and evaporated under nitrogen stream. The residue was reconstituted by adding 100 µL of acetonitrile and 0.05% formic acid at 1:1 (v/v) and transferred to a vial. A 5 µL aliquot of each sample was injected into the LC-MS/MS system for the analysis.

2.6. Method validation

This developed method was fully validated according to the Guidance for Industry, Bioanalytical Methods Validation, U.S. Department of Health and Human Service, Food and Drug Administration, Center for Drug Evaluation and Research (USFDA CDER, 2001, BP).

2.6.1. Specificity and selectivity

The specificity and selectivity were evaluated by screening a minimum of six individual sources of human blank plasma. The six sources of human blank plasma showing no interference at the retention time of analyte and IS were selected and pooled, which used for full method validation.

2.6.2. Linearity

Calibration curve consisted of blank sample (plasma without IS), zero sample (plasma with IS) and eight points standard calibration curve range from 0.5 to 2,500 ng/mL. Three calibration curves, accepted in accuracy and precision, were used to establish linearity. The lowest standard on the calibration curve was the lower limit of quantification (LLOQ), which the analyte signal to noise ratio (S/N) was at least five times more than extracted human blank plasma.

2.6.3. Accuracy and precision

Intra-day accuracy and precision were assessed by analyzing six replicates of the quality control samples at LLOQ, LQC, MQC and HQC in a single batch run. The inter-day accuracy and precision were assessed by analyzing 18 replicates of the quality control samples at each concentration through three accuracy and precision batches runs on two consecutive method validation days.

2.6.4. Extraction recovery and process efficiency

The recovery of losartan and EXP3174 in plasma was assessed by analyzing six replicates of the quality control samples at LQC, MQC and HQC. The relative recovery was determined by comparing the peak areas of plasma spiked standards before extraction (pre-extraction) to the peak areas of plasma spiked standards post extraction (post-extraction). The process efficiency was determined by comparing the peak areas of plasma spiked standards before extraction (pre-extraction) to the peak areas of neat standard solution (un-extraction).

2.6.5. Matrix effect

The matrix effects of losartan and EXP3174 in six individual sources of plasma, each source were assessed by analyzing three replicates of the quality control samples at LQC, MQC and HQC. The matrix factors were

determined by comparing the peak areas of plasma spiked standards post extraction (post-extraction) to the peak areas of neat standard solution (un-extraction). The IS normalized matrix factors were calculated by dividing the matrix factor of the analyte by the matrix factor of the internal standard.

2.6.6. Stability

The stability of losartan and EXP3174 in plasma was assessed by analyzing quality control samples at LQC, MQC and HQC under a variety of storage and processing conditions. Three aliquots of each concentration quality control samples were taken to evaluate the bench top or short term stability, freeze and thaw stability, post-preparative stability and long term stability. Bench top stability was assessed after exposure of the plasma samples to room temperature (25±2ºC) for 4 h, freeze and thaw stability was evaluated after undergoing three cycles of freeze at -70±10°C and thaw at 25±2ºC, post-preparative stability determined by storing the reconstituted sample under auto-sampler condition 10±5°C for 72 h and dry sample condition at -70±10°C for 5 days before being analyzed. Long term stability was analyzed after storage quality control samples at -70±10°C for 60 days.

2.6.7. Anticoagulant effect

Anticoagulant effect was determined by analyzing sets of six replicates of the quality control samples at LQC, MQC and HQC with two different anticoagulants, lithium heparin which used in clinical samples and citrate phosphate dextrose which used for method validation.

2.6.8. Dilution integrity

Dilution integrity test was performed by preparing samples at a concentration approximately 1.7 times of 85% the upper limit of quantification (ULOQ) concentration and frozen for a period of at least 24 h. On the day of evaluation, the dilution integrity standard was diluted by a factor of two (1:2), five (1:5) and twenty (1:20) times with human blank plasma (six determinations per dilution factor).

2.6.9. Robustness

Robustness will be evaluated when changes occurred, different columns lots (same type) and different solvents lots (reconstitution solvents and mobile phase), on the same instrument. Intra-day robustness was assessed by analyzing six replicates of the quality control samples at LLOQ, LQC, MQC and HQC in a single batch run. The inter-day robustness was assessed by analyzing 12 replicates of the quality control samples at each concentration through two robustness batches runs on two consecutive method validation days.

3. RESULTS AND DISCUSSION

3.1. Method development: LC-MS/MS

The concentrations of losartan and EXP3174 in human plasma, using candesartan as an internal standard were analyzed by combined reversed phase liquid chromatography and tandem mass spectrometry (LC-MS/MS).

The multiple reaction monitoring (MRM) transitions in both positive and negative ion mode were compared. Positive ion mode gave more intense response. The mass spectra revealed parent and daughter scan at m/ z of 422.99>207.02 and 422.99>180.03 for losartan (Figure 2A), 436.96>234.96 and 436.96>206.99 for EXP3174 (Figure 2B) and 441.02>262.94 for an IS (Figure 2C), respectively. The LC-MS/MS in MRM mode under the optimized conditions provided a highly sensitive and selective method for simultaneous determination of losartan and EXP3174.

3.2. Method development: sample preparation

Losartan and EXP3174 are weak acids. The pK_a values of the acidic nitrogen in the tetrazole ring are 5.6 and 5.4 for losartan and EXP3174, respectively. The pK_a value for the carboxy group in EXP3174 is 4.2, all of them exist as nonionic forms in solution at a low pH (≤3.0).[5] In this study, the liquid-liquid extraction method was acidified to less than pH 3.0 with 1 M formic acid. Liquid-liquid extraction was used to clean up the sample before injecting into the LC-MS/MS. This helped minimize the suppression or enhancement of ionization in LC-MS/MS. One milliliter of ethylacetate and hexane mixture at 9:1 (v/v), in formic acid was adjusted to reach an optimal condition that produces the highest recovery and the best peak shape for chromatogram of losartan, EXP3174 and candesartan. This method provided less solvent consumption compared to the earlier liquid-liquid extraction method which used 2 mL of methyl-*t*-butyl ether (MTBE).[7]

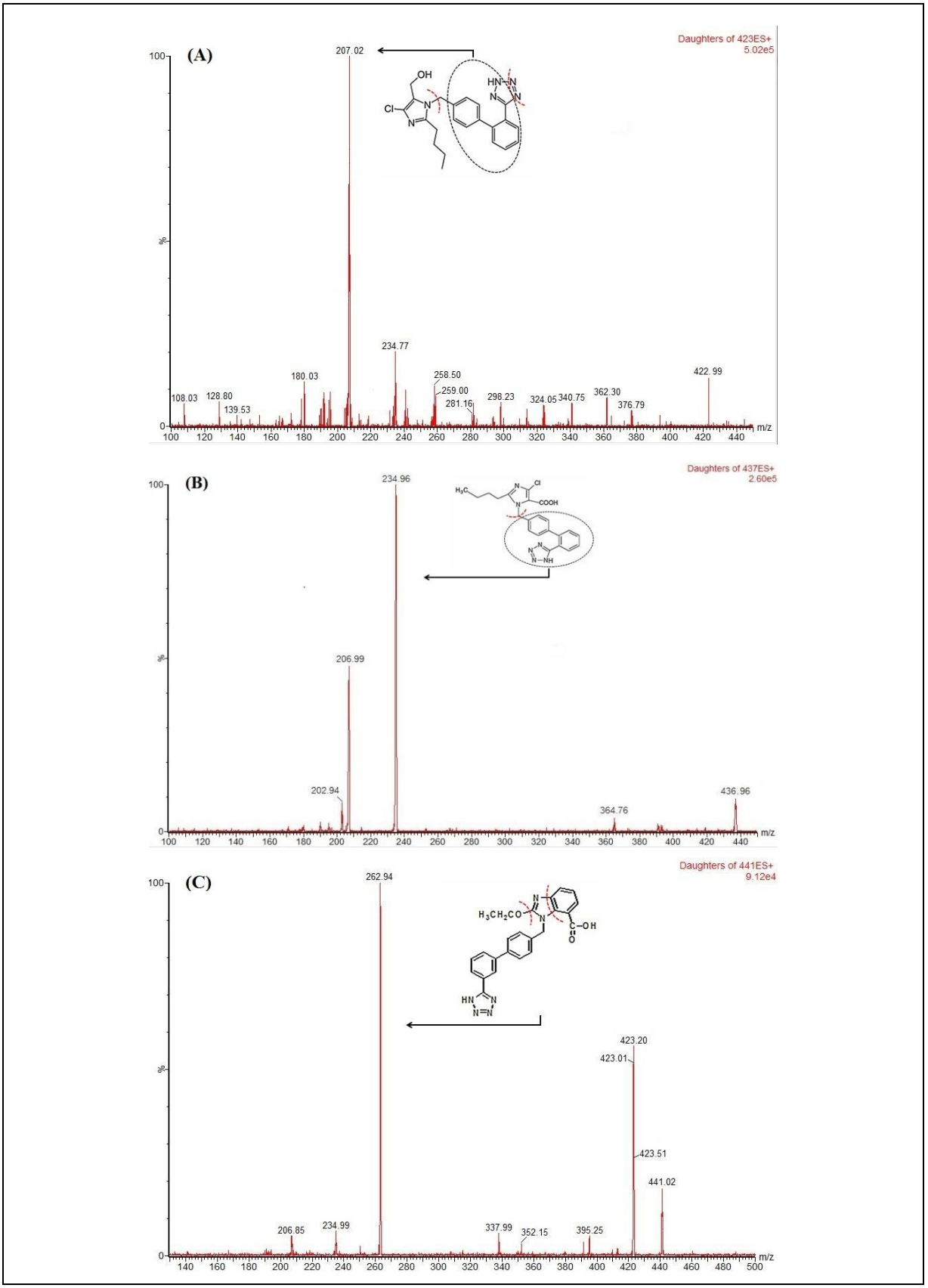

Figure 2. Product ion MS/MS spectra of losartan (A), EXP3174 (B) and candesartan, IS (C).

3.3. Assay performance and validation

3.3.1. Specificity and selectivity

The specificity and selectivity of the method were assessed in human blank plasma. The chromatogram showed no interference at the retention time of losartan, EXP3174 and IS (Figure 3A) which were 1.45, 1.71 and 1.47 minutes, respectively (Figure 3B).

Figure 3C shows the chromatogram of the calibration standard sample at 1300 ng/mL and Figure 3D illustrates the clinical plasma sample at 2.5 h after oral administration of a single dose of 100 mg losartan potassium. The developed method proved its selectivity and specificity for losartan, EXP3174 and IS.

3.3.2. Linearity and sensitivity

Limit of detection (LOD) for losartan and EXP3174 was 0.1 and 0.2 ng/mL, respectively. The lower limit of quantification (LLOQ) was 0.5 ng/mL for both losartan and EXP3174. The percentage accuracy and the coefficient of variation of LLOQ were 95.78% and 3.90% for losartan, 99.20% and 3.81% for EXP3174, respectively. The calibration curve was best fitted by a least squares linear regression model y = mx+b, weighing by 1/x, where y is the peak area ratio of analyte and its internal standard, m is the slope of the calibration curve, x is the analyte concentrations and b is the y-axis intercept of the calibration curve. An eight-point standard calibration curve was linear within the

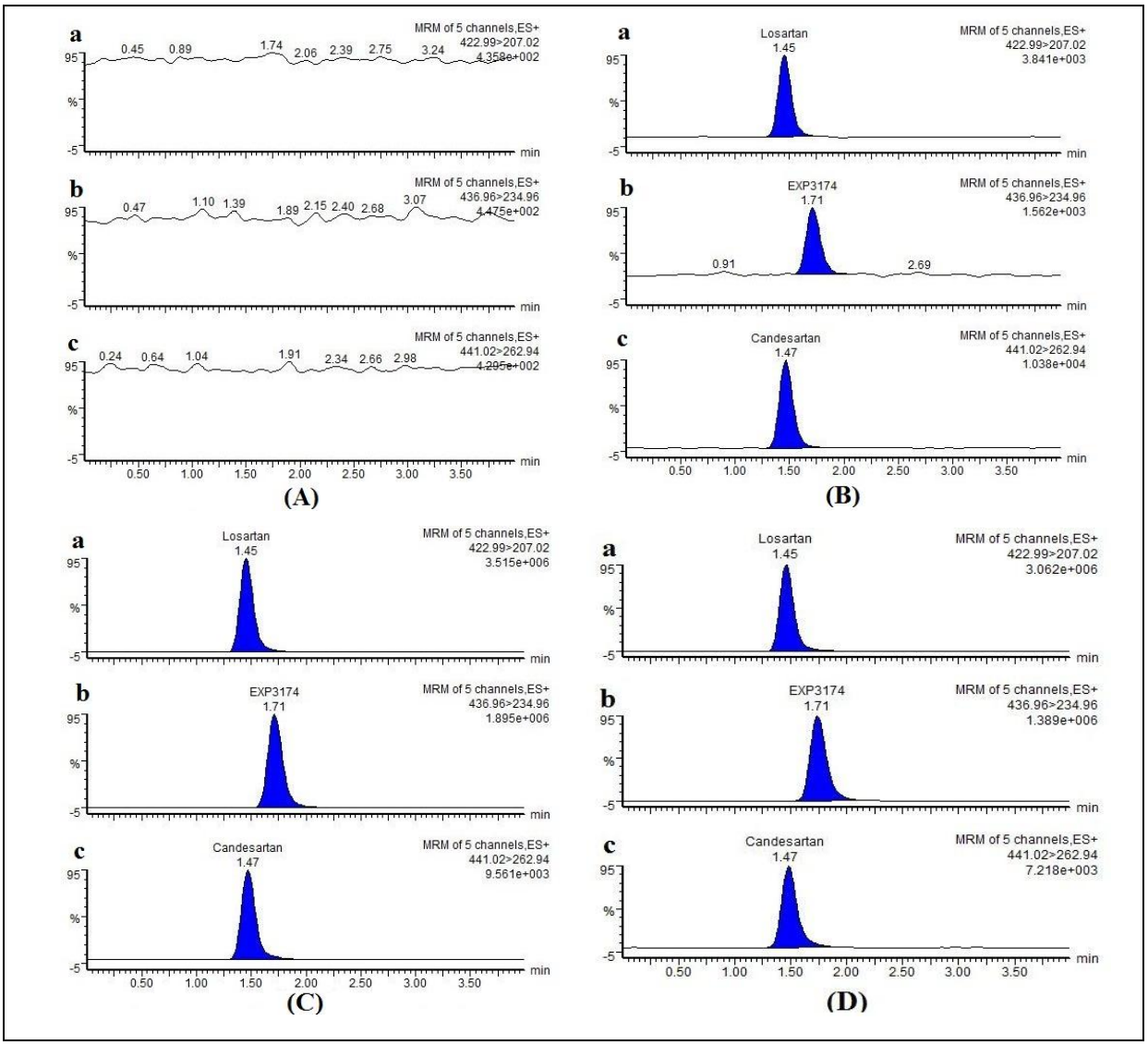

Figure 3. The chromatograms of extracted (A) blank plasma, (B) LLOQ sample and (C) calibration standard sample at 1300 ng/mL, (D) clinical plasma sample at 2.5 h after an oral administration of 100 mg losartan potassium. (a) Losartan, (b) EXP3174 and (c) candesartan (IS).

concentration ranged from 0.5-2,500 ng/mL for both analytes. This method provided wider linearity range of losartan and EXP3174 in human plasma compared to earlier reported methods which linearity was in the range of 1-1,000 ng/mL for both losartan and EXP3174.[5-7] The determination coefficient of $r^2 > 0.995$ was achieved for all of the calibration curves as shown in Figure 4A and 4B. The results of the three sets of calibration curve were summarized in Table 1.

3.3.3. Accuracy and precision

The intra- and inter-day accuracy and precision for the quality control samples are shown in Table 2. The accuracy and precision results were within the acceptance criteria according to USFDA guideline, ±20% of the nominal value for LLOQ and ±15% of the nominal value for others. These indicated that the developed method had good precision, accuracy and reproducibility for an analysis of losartan and EXP3174 in human plasma.

3.3.4. Extraction recovery and process efficiency

The exaction recovery of losartan at LQC, MQC and HQC was 74.79%, 87.99%, 79.84% and 70.44%, 88.67%, 80.74% for EXP3174, respectively. The process efficiency of losartan at LQC, MQC and HQC was 89.79%, 88.45%, 97.82% and 86.16%, 88.75%, 95.92% for EXP3174, respectively. For IS, the exaction recovery and process efficiency were 81.86% and 88.78%, respectively. These results indicated that this developed method was highly efficient and reproducible.

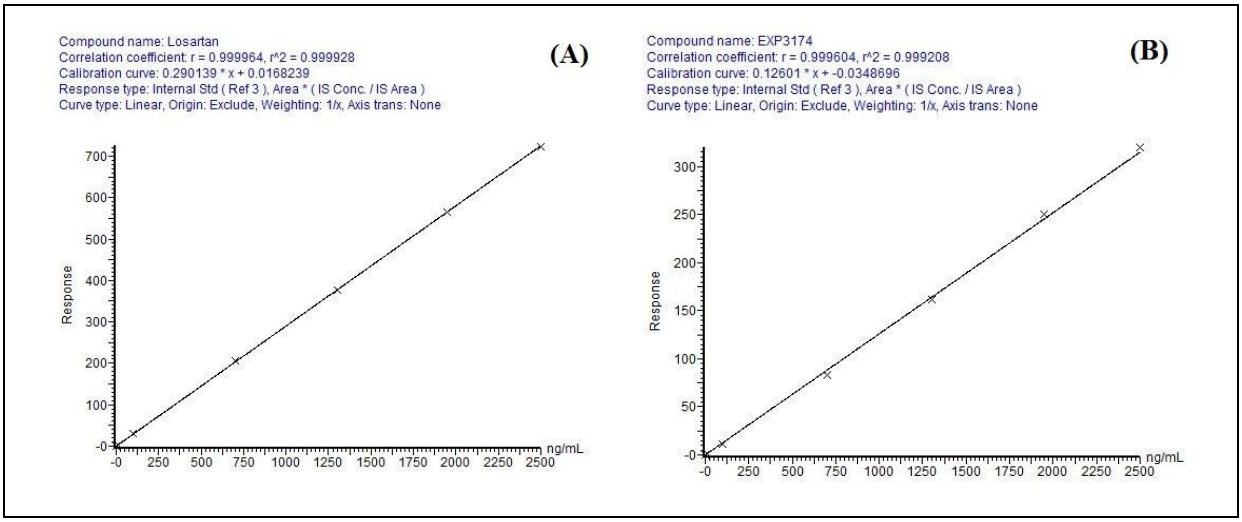

Figure 4. The calibration curve of losartan (A) and EXP3174 (B).

Table 1 The accuracy and precision of calibration curve for losartan and EXP3174 in human plasma

Nominal Concentration (ng/mL)	Losartan (n=3)			EXP3174 (n=3)		
	Mean±SD (ng/mL)	Accuracy (%)	CV (%)	Mean±SD (ng/mL)	Accuracy (%)	CV (%)
0.50	0.4787±0.0621	95.74	12.97	0.5862±0.0031	117.24	0.53
1.00	0.9866±0.0151	98.66	1.53	1.0152±0.0544	101.52	5.36
10.00	9.9644±0.8632	99.64	8.66	9.2847±0.5847	92.85	6.30
100.00	106.5219±1.4026	106.52	1.32	89.8965±2.3214	89.90	2.58
700.00	699.8295±21.2577	99.98	3.04	636.8026±21.8512	90.97	3.43
1300.00	1296.5066±6.8025	99.73	0.52	1259.4830±34.8214	96.88	2.76
1950.00	1935.6903±17.1863	99.27	0.89	1991.4613±6.8110	102.13	0.34
2500.00	2511.5219±44.1280	100.46	1.76	2569.3643±56.1224	102.77	2.18

n = number of replicate

Table 2 The intra and inter day accuracy and precision of losartan and EXP3174 in human plasma

Losartan							
QC samples	**Batch**	**Intra-day (n=6)**			**Inter-day (n=18)**		
		Mean±SD (ng/mL)	**Accuracy (%)**	**Precision (%)**	**Mean±SD (ng/mL)**	**Accuracy (%)**	**Precision (%)**
LLOQ (0.5 ng/mL)	**1**	0.4982±0.0212	99.64	4.26	0.4769±0.0427	95.38	8.95
	2	0.4697±0.0677	93.94	14.41			
	3	0.4628±0.0175	92.56	3.78			
LQC (1.5 ng/mL)	**1**	1.5443±0.0432	102.95	2.80	1.4207±0.1031	94.71	7.26
	2	1.3504±0.0670	90.03	4.96			
	3	1.3674±0.0464	91.16	3.39			
MQC (1200 ng/mL)	**1**	1318.2624±23.2439	109.86	1.76	1297.1212±30.5464	108.09	2.35
	2	1309.9081±19.2292	109.16	1.47			
	3	1263.1932±12.1884	105.27	0.96			
HQC (1900 ng/mL)	**1**	2085.1183±20.6250	109.74	0.99	1992.0293±73.2549	104.84	3.68
	2	1971.3266±18.6908	103.75	0.95			
	3	1919.6430±16.4276	101.03	0.86			
EXP3174							
QC samples	**Batch**	**Intra-day (n=6)**			**Inter-day (n=18)**		
		Mean±SD (ng/mL)	**Accuracy (%)**	**Precision (%)**	**Mean±SD (ng/mL)**	**Accuracy (%)**	**Precision (%)**
LLOQ (0.5 ng/mL)	**1**	0.5421±0.0265	108.42	4.89	0.5104±0.0482	102.08	9.44
	2	0.4549±0.0242	90.98	5.32			
	3	0.5343±0.0320	106.86	5.99			
LQC (1.5 ng/mL)	**1**	1.3614±0.0389	90.76	2.86	1.4068±0.0676	93.79	4.81
	2	1.3781±0.0565	91.87	4.10			
	3	1.4809±0.0276	98.73	1.86			
MQC (1200 ng/mL)	**1**	1193.4269±19.6433	99.45	1.65	1225.0194±43.9237	102.08	3.59
	2	1278.0817±28.8836	106.51	2.26			
	3	1203.5495±14.4872	100.30	1.20			
HQC (1900 ng/mL)	**1**	1925.3205±23.9735	101.33	1.25	1976.7851±47.5048	104.04	2.40
	2	2032.3064±7.1309	106.96	0.35			
	3	1972.7284±12.2476	103.83	0.62			

n = number of replicate

3.3.5. Matrix effect

Matrix effect expressed by matrix factor (MF) and IS normalized matrix factor. The IS normalized matrix factor for losartan and EXP3174 ranged from 0.95 – 1.08 and 0.89 – 1.08 at the three quality control concentration levels, with %CV ranged from 2.86% – 4.00% and 2.86% – 5.94%, respectively. The matrix factor for IS and its %CV were 1.04 and 2.88%. The matrix factor and IS normalized matrix factor between 0.85-1.15 indicates that other interfering substances in the plasma sample cannot significantly suppress or enhance ionization.

3.3.6. Stability

Stability studies were conducted and covered all of the conditions in the study, shown in Table 3. Losartan and EXP3174 were stable at 25±2°C for 4 h, three cycles of freeze and thaw, at -70±10°C for 60 days. The extracted samples were also stable in the auto-sampler at 10±5°C for 72 h and in the freezer at -70±10°C for 5 days. In addition, re-injection reproducibility was evaluated to determine whether an analytical batch run could be reanalyzed in the case of instrument failure for 24 h. These indicated that losartan and EXP3174 were stable in human plasma under storage or handling conditions.

3.3.7. Anticoagulant effect

The percentage accuracy of two different types of anticoagulant, lithium heparin and citrate phosphate dextrose were 95.43-99.85 and 100.02-103.57 for losartan and 97.90-105.41 and 91.67-103.90 for EXP3174, respectively. These results were within the acceptable ranges, suggested no anticoagulant effect when two different anticoagulants were used.

Table 3 The stability of losartan and EXP3174 in human plasma.

Losartan				
Conditions	**QC samples (n=3)**	**Mean±SD (ng/mL)**	**Accuracy (%)**	**Variation (%)**
Short term stability at 25±2ºC for 4 h	LQC (1.5 ng/mL)	1.5516±0.0259	103.44	6.65
	MQC (1200 ng/mL)	1221.3843±33.1923	101.78	1.76
	HQC (1900 ng/mL)	1834.4657±30.5443	96.55	2.33
Freeze and thaw stability at -70±10 °C for 3 cycles	LQC (1.5 ng/mL)	1.3953±0.0453	93.02	4.10
	MQC (1200 ng/mL)	1237.6764±11.9595	103.14	0.45
	HQC (1900 ng/mL)	1880.6622±31.5088	98.98	0.13
Long term stability at -70±10 °C for 60 days	LQC (1.5 ng/mL)	1.5887±0.0088	105.91	9.20
	MQC (1200 ng/mL)	1257.3447±24.1499	104.78	1.13
	HQC (1900 ng/mL)	1904.7785±9.7508	100.25	1.42
Post-preparative stability at 10 ± 5°C for 72 h	LQC (1.5 ng/mL)	1.5383±0.0156	102.55	5.73
	MQC (1200 ng/mL)	1210.3062±18.5233	100.86	2.65
	HQC (1900 ng/mL)	1898.4623±33.6247	99.92	1.08
Post-preparative stability at -70±10 °C for 5 days	LQC (1.5 ng/mL)	1.5726±0.1104	104.84	8.09
	MQC (1200 ng/mL)	1252.3164±28.3782	104.36	0.73
	HQC (1900 ng/mL)	1821.6226±31.6173	95.87	3.01
Re-injection reproducibility for 24 h	LQC (1.5 ng/mL)	1.6454±0.0339	109.69	3.59
	MQC (1200 ng/mL)	1273.7509±14.0632	106.15	0.76
	HQC (1900 ng/mL)	1926.6326±4.2584	101.40	2.61
EXP3174				
Conditions	**QC samples (n=3)**	**Mean±SD (ng/mL)**	**Accuracy (%)**	**Variation (%)**
Short term stability at 25±2ºC for 4 h	LQC (1.5 ng/mL)	1.4938±0.0792	99.59	3.43
	MQC (1200 ng/mL)	1175.5413±33.9391	97.96	1.80
	HQC (1900 ng/mL)	1904.9847±27.5826	100.26	1.61
Freeze and thaw stability at -70±10 °C for 3 cycles	LQC (1.5 ng/mL)	1.5618±0.1113	104.12	0.96
	MQC (1200 ng/mL)	1218.1806±6.3654	101.52	5.49
	HQC (1900 ng/mL)	1966.6532±38.6524	103.51	4.90
Long term stability at -70±10 °C for 60 days	LQC (1.5 ng/mL)	1.4379±0.0200	95.86	7.05
	MQC (1200 ng/mL)	1184.1393±25.2186	98.68	2.54
	HQC (1900 ng/mL)	1962.3618±12.8129	103.28	4.67
Post-preparative stability at 10 ± 5°C for 72 h	LQC (1.5 ng/mL)	1.4941±0.1185	99.61	3.41
	MQC (1200 ng/mL)	1163.9817±16.6335	97.00	0.79
	HQC (1900 ng/mL)	1967.4526±33.2738	103.55	4.94
Post-preparative stability at -70±10 °C for 5 days	LQC (1.5 ng/mL)	1.5705±0.1334	104.70	1.53
	MQC (1200 ng/mL)	1216.1035±43.2223	101.34	5.31
	HQC (1900 ng/mL)	1963.9689±27.4097	103.37	4.76
Re-injection reproducibility for 24 h	LQC (1.5 ng/mL)	1.6249±0.0099	108.33	9.22
	MQC (1200 ng/mL)	1301.7167±39.4763	108.48	2.27
	HQC (1900 ng/mL)	2085.5122±27.1650	109.76	1.38

n = number of replicate

3.3.8. Dilution integrity

Percentage accuracy of two-, five- and twenty-time diluted samples were 106.89, 113.62, 113.8 for losartan and 113.11, 106.48, 94.86 for EXP3174, respectively. The results indicated that clinical samples could be diluted if the concentration exceeded the upper limit of qualification.

3.3.9. Robustness

Robustness will be evaluated when changes occurred, such as different columns lots or solvents lots (extraction solvents, reconstitution solvents and mobile phase), on the same instrument. The coefficient of variation of intra- and inter-day precision were 1.14-5.86% and 1.55-8.57% for losartan, 1.37-4.68% and 1.51-8.79% for EXP3174, respectively. The intra- and inter-day accuracy were 90.36-105.12% and 96.48-102.96% for losartan, 92.35-110.02% and 95.28-102.44% for EXP3174, respectively. The results suggested the robustness of the method.

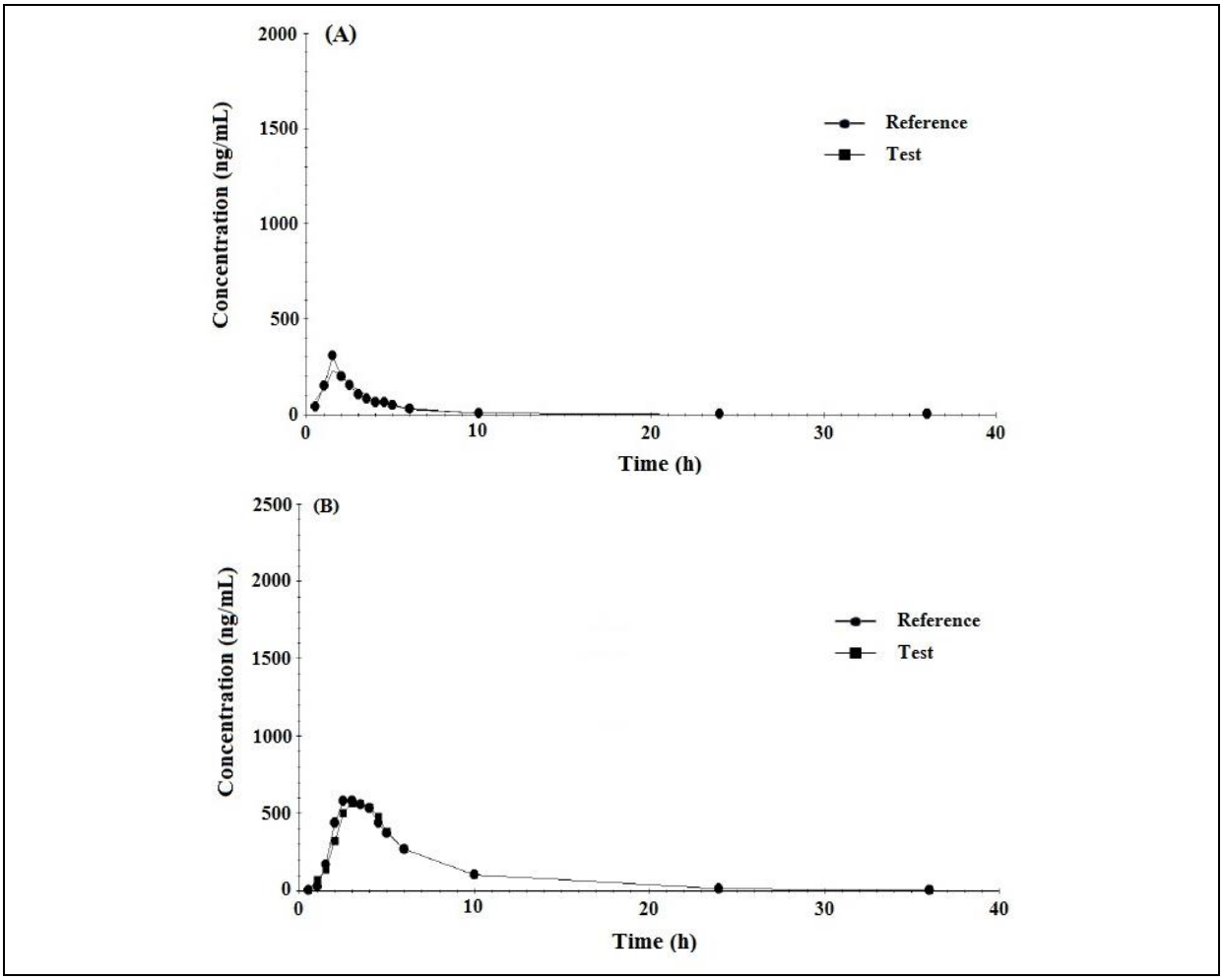

Figure 5. Mean plasma concentration-time profiles of (A) Losartan and (B) EXP3174 after oral administration of single dose of 100 mg losartan potassium tablet in 60 healthy volunteers.

3.3.10. Application

The validated method was applied to quantify losartan and EXP3174 concentrations in human plasma samples in a bioequivalence study of single oral 100 mg-losartan potassium tablets in healthy Thai volunteers (Figure 5). The Institutional Review Board of Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand approved the study protocol.

4. CONCLUSIONS

We developed a simple, sensitive and selective LC-MS/MS method for simultaneous determination of losartan and its metabolite in human plasma. The method was fully validated according to the USFDA guidance. Compared to previously reported method, it consumed smaller volume of solvent and offered wider linearity range (0.5–2,500 ng/mL) for both losartan and EXP3174. A 4-minute run time per sample made it possible to analyze more than 300 samples per day.

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Conflict of interest

None to declare

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Ethical approval

The Institutional Review Board of Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand approved the study protocol. (COA no.Si048/2010)

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