Journal of Pharmaceutical and Biomedical Analysis 49 (2009) 862–867

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Simultaneous quantification of losartan and active metabolite in human plasma by liquid chromatography–tandem mass spectrometry using irbesartan as internal standard

Budi Prasaja[a,*], Lucy Sasongko[b], Yahdiana Harahap[c], Hardiyanti[a], Windy Lusthom[a], Matthew Grigg[d]

[a] PT Clinisindo Laboratories, Jakarta, Indonesia
[b] School of Pharmacy, Bandung Institute of Technology, Indonesia
[c] Department of Pharmacy, Faculty of Mathematics and Natural Science, University of Indonesia
[d] Applied Biosystems, Australia, 52 Rocco Drive, Scoresby, 3079, Australia

ARTICLE INFO

Article history:
Received 27 November 2008
Received in revised form 8 January 2009
Accepted 8 January 2009
Available online 19 January 2009

Keywords:
Losartan
Losartan acid
LC–MS/MS
Deproteination
Simple extraction

ABSTRACT

A simple and sensitive liquid chromatography–tandem mass spectrometry (LC–MS/MS) method employing electronspray ionization was developed and validated for quantification of losartan and its carboxylic acid metabolite in human plasma using irbesartan as internal standard (IS). Following a simple pretreatment procedure, the analytes were separated using a gradient mobile phase on reverse phase C_{18} column. Selected reaction monitoring was specific for losartan, losartan acid and irbesartan. The method validation demonstrated the specificity, lower limit of quantification, accuracy and precision of measurements. The assay exhibited a linear dynamic range of 2.0–400 ng/mL for losartan and 1.85–370 ng/mL for losartan acid. A run time of 3.5 min for each sample made it possible to analyze more than 200 samples per day. The validated method has been successfully used to analyze human plasma samples for application in bioavailability/bioequivalence studies.

1. introduction

Losartan, 2-butyl-4-chloro-1-[[2′-(1H-tetrazol-5-yl)[1,1'biphenyl]-4-yl]methyl]-1H-imidazole-5-methanol, $C_{22}H_{23}ClN_6O$, is the first orally available angiotensin II-receptor used as an antihypertensive agent. Losartan and its active metabolite block the vasoconstrictor and aldosterone secreting effects of angiotensin II by type AT_1 receptor blockage. Following oral administration, losartan is rapidly absorbed, reaching maximum concentrations 1–2 h post administration. The active metabolite, losartan acid, is 10–40 times more potent by weight than losartan [1–3].

Numerous analytical methods for determination of losartan in human plasma based on ultraviolet or fluorescence detection are reported for extraction and determination of losartan in biological matrices [4–7]. These methods however mostly need extracting steps that are sophisticated and time consuming with some interfering materials that could affect the overall determination of losartan.

2. Experimental

2.1. Chemical and reagents

Losartan potassium was manufactured by U.S. Pharmacopeia (Rockville, MD, USA). Losartan acid was synthesized at Synfine Research, Inc (Richmond Hill, Ontario, Canada) and Irbesartan was manufactured by Zhuhai Sanxin Fine Chemical Co., Ltd (Zhuhai City, Guangdong, China). Methanol was from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA). Formic acid and acetonitrile were from Merck (Darmstadt, Germany). Distilled water was from a Purelab UHQ PS (High Wycombe, Bucks, UK) was used. Drug free control human plasma was obtained from Indonesia Red Cross (Jakarta, Indonesia).

2.2. Sample preparation

A plasma sample (250 μL) was transferred to a 1.5 mL polypropylene microtest tube, the 20 μL of internal standard (IS) working solution (10 μg/mL irbesartan in methanol) were added. The samples were briefly mixed. Then, 250 μL acetonitrile was added, vortex-mixed for 10 s and centrifuged for 10 min at 3000 rpm. A 15 μL aliquot was injected for LC–MS/MS analysis. This preparation was chosen as a simple and rapid sample preparation.

* Corresponding author. Tel.: +62 21 7388 9918; fax: +62 21 7388 5453.
E-mail address: budi.prasaja@clinisindo.com (B. Prasaja).

0731-7085/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2009.01.007

Table 1
Gradient elution timetable and mass spectrometer parameters.

Time (min)	%A	%B
0.01	30	70
1.50	90	10
2.50	90	10
3.00	30	70
3.50	30	70

Parameter	Value
Source temperature (°C)	600
Dwell time (ms)	150
GS1 (psi)	50
GS2 (psi)	60
Curtain gas (psi)	40
CAD gas (psi)	5
Ion spray voltage (V)	4500
Entrance potential (V)	5
Declustering potential/DP (V)	55 (Losartan) 50 (Losartan acid)
Collision cell entrance potential/CEP (V)	28 (Losartan) 20 (Losartan acid)
Collision energy (V)	46 (Losartan) 88 (Losartan acid)
Collision cell exit potenstial (V)	2 (Losartan) 2 (Losartan acid)
Mode of analysis	Negative
Ion transition for	
Losartan (*m/z*)	420.73/126.70
Losartan acid (*m/z*)	434.67/156.90
Irbesartan (*m/z*)	426.87/192.00

A = acetonitrile containing 0.1% (v/v) formic acid; *B* = water containing 0.1% (v/v) formic acid.

2.3. LC–MS/MS instrument and conditions

The HPLC consisted of a Shimadzu Prominence system (Kyoto, Japan) equipped with two LC-20AD pump units, a DGU-20A5 degasser, a SIL-20A autosampler, a CTO-20AC column oven and a CBM-20A system controller. The chromatographic separation was carried out on a Phenomenex® Synergy 4 μ POLAR-RP 80A, 50 × 2.0 mm i.d. (Torrance, CA, USA) at 40 °C using a Phenomenex® guard column AQ C18, 4 × 2.0 mm i.d. (Torrance, CA, USA). A gradient elution was carried out at a flow rate 0.7 mL/min for a 3.5 min run time. Mass spectrometric detection was performed using an API 3200 triple quadrupole instrument (Concord, Ontario, Canada) using MRM. A turbo-ion spray source using electrospray ionization in negative mode was used. The parameters of the mass spectrometer and gradient elution are summarized in Table 1. Data processing was performed using Analyst™ software (version 1.4.1).

2.4. Calibration standards and quality control samples

A stock solution of losartan was prepared in water at 500 μg/mL (calculated for the free base) and a losartan intermediate solution (100 μg/mL) was prepared by diluting the losartan stock solution with water. The intermediate losartan solution was diluted as appropriate with water to give Quality Control (QC) solutions at 120 ng/mL, 3000 ng/mL and 6000 ng/mL. In a similar manner, calibration standards were created at the following concentration 8000 ng/mL, 4000 ng/mL, 2000 ng/mL, 1000 ng/mL, 500 ng/mL, 200 ng/mL, 100 ng/mL, 40 ng/mL.

A stock solution of losartan acid was prepared in methanol at 370 μg/mL and a losartan acid intermediate solution (92.5 stock μg/mL) was prepared by diluting the losartan acid stock solution with methanol. The losartan acid intermediate solution was diluted as appropriate with water:methanol (1:1) to give Quality Control (QC) solutions at 111 ng/mL, 2775 ng/mL and 5550 ng/mL. In a similar manner, calibration standards were created at the following concentration 7400 ng/mL, 3700 ng/mL, 1850 ng/mL, 925 ng/mL, 462 ng/mL, 185 ng/mL, 92.5 ng/mL, 37 ng/mL.

All solutions were stored in refrigerator (4 °C) when not in use. Calibration and QC samples were prepared by spiking 50 μL of losartan intermediate solution and 50 μL of losartan acid intermediate solution into 900 μL of blank plasma at corresponding concentrations. The QC samples were stored frozen (−20 °C) until used and calibration samples were prepared fresh for every batch

2.5. Method validation

Plasma samples were quantified using the ratio of the peak area of losartan and losartan acid to that of IS as the assay parameter. For the calibration standards, peak area ratios were plotted against analyte plasma concentrations. A linear regression was used with a $1/x^2$ weighting factor applied. The acceptance criterion for calibration curve was a correlation coefficient (r) of 0.99 or better.

Limit of quantification (LOQ) was defined as the lowest concentration at which the precision expressed by relative standard deviation is lower than 20% and inaccuracy (bias) expressed by relative difference of the measured and true value is also lower than 20%. The method specificity was evaluated by screening six lots of blank plasma.

Table 2
Precision and accuracy of the method for determining losartan concentrations in plasma samples.

Concentration added (ng/mL)	Intra-batch			Inter-batch		
	Concentration measured (Mean value ± SD; ng/mL)	RSD (%)	Bias (%)	Concentration measured (Mean value ± SD; ng/mL)	RSD (%)	Bias (%)
6.00	6.11 ± 0.47	7.77	1.77	6.11 ± 0.38	6.29	1.75
150.06	152.43 ± 7.69	5.05	1.58	151.60 ± 3.25	2.15	1.03
300.12	314.67 ± 18.17	5.77	4.85	310.34 ± 13.24	4.27	3.41

Table 3
Precision and accuracy of the method for determining losartan acid concentrations in plasma samples.

Concentration added (ng/mL)	Intra-batch			Inter-batch		
	Concentration measured (Mean value ± SD; ng/mL)	RSD (%)	Bias (%)	Concentration measured (Mean value ± SD; ng/mL)	RSD (%)	Bias (%)
5.54	5.29 ± 0.39	7.35	−4.40	5.23 ± 0.29	5.46	−5.49
138.45	136.09 ± 4.83	3.55	−1.71	135.55 ± 6.17	4.55	−2.09
276.90	282.97 ± 10.36	3.66	2.19	283.02 ± 15.24	5.39	2.21

The intra-batch precision and accuracy were determined by analyzing five sets of QC samples in a batch. The inter-batch precision and accuracy were determined by analyzing ten sets of QC samples on five different batches. The acceptance criteria for intra- and inter-batch precision was 15% or better, and the inaccuracy was 15% or better.

The absolute recovery of the losartan, losartan acid and IS were determined by comparing the peak areas of extracted plasma standards to the peak areas of post extraction plasma blanks spiked at corresponding concentration.

The stability of the analytes and IS in plasma were assessed by analyzing QC samples at two concentrations (low and high), respectively, in duplicate ($n = 2$), under different temperature and timing conditions. The results were compared with those for freshly prepared QC samples, and the percentage concentration deviation was calculated.

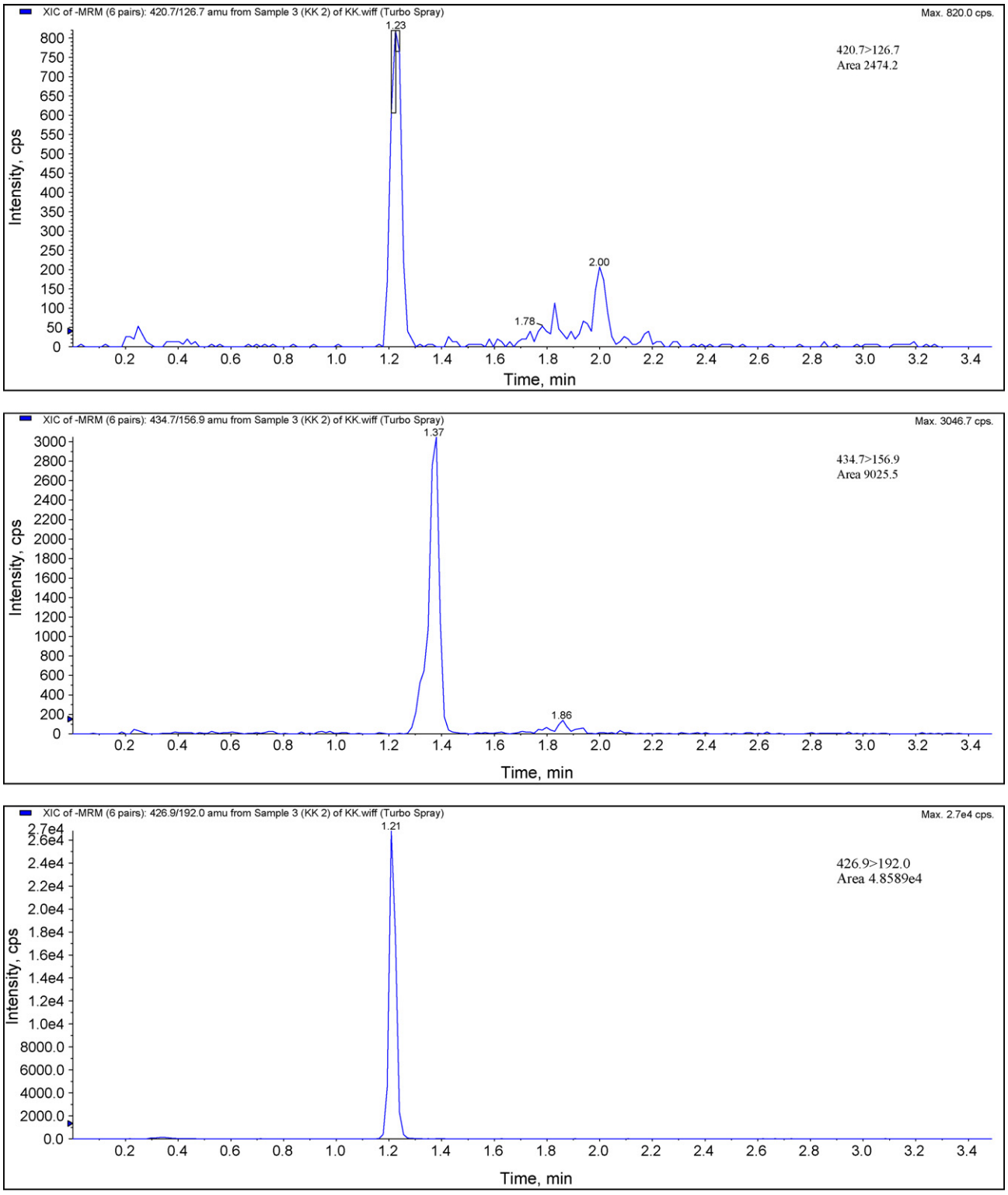

Fig. 1. Chromatograms of blank human plasma spiked with losartan and losartan acid at LLOQ and IS.

2.6. PK Study

The present method has been applied in a randomized crossover bioequivalence study in 24 healthy volunteers following single oral administration of 50 mg losartan from either PT Novell Pharmaceutical Laboratories, Indonesia or Merck Sharp & Dohme Ltd, UK (Cozaar®) pharmaceutical companies. The drugs were administered under fasting conditions and blood sampling were carried out at 0 h, 0.5 h, 1 h, 1.5 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 12 h, 16 h, and 24 h after drug administration. Following standing for 30 min and centrifuge (3000 rpm, 10 min), the plasma was removed and stored at −20 °C until analysis.

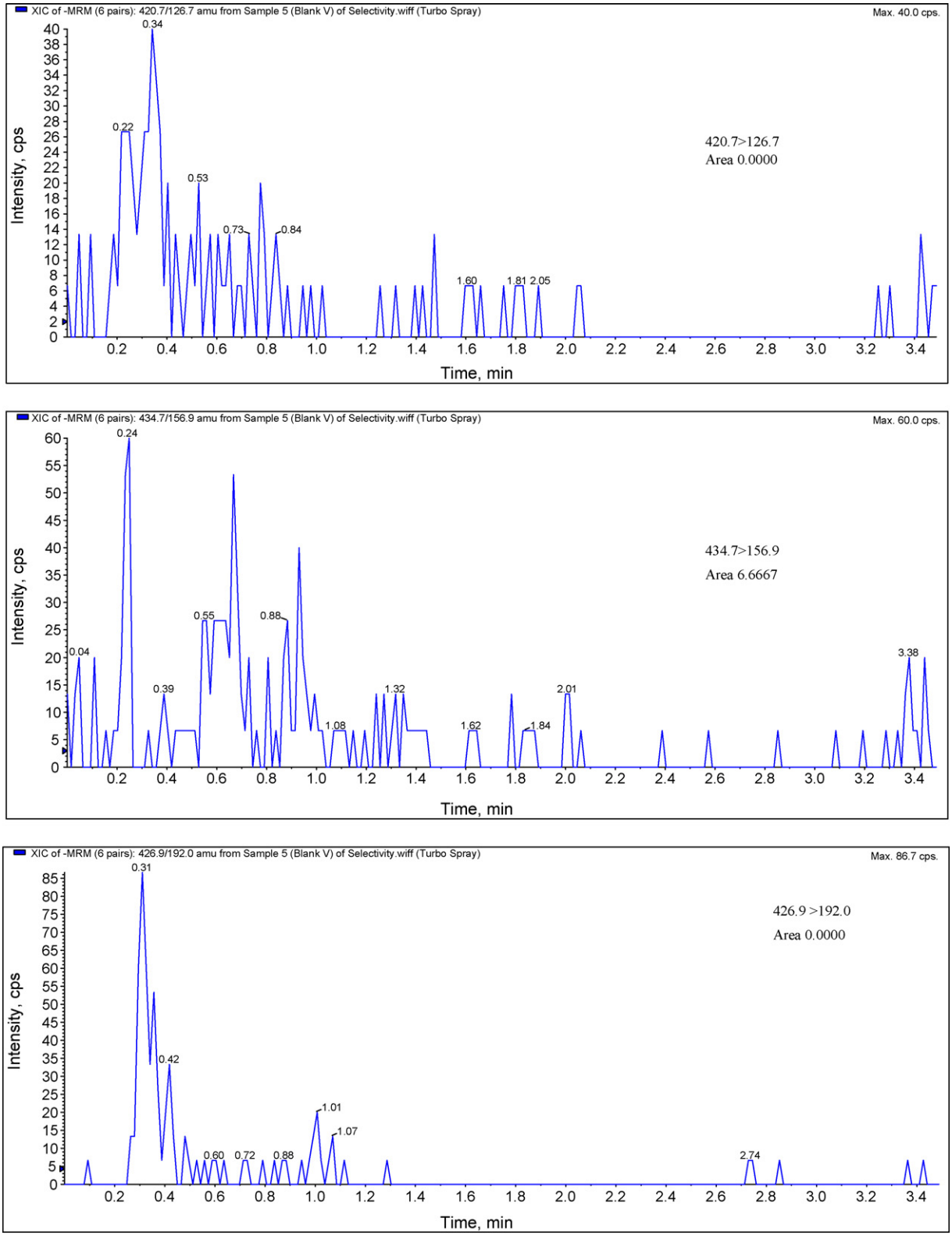

Fig. 2. Chromatograms of blank plasma only.

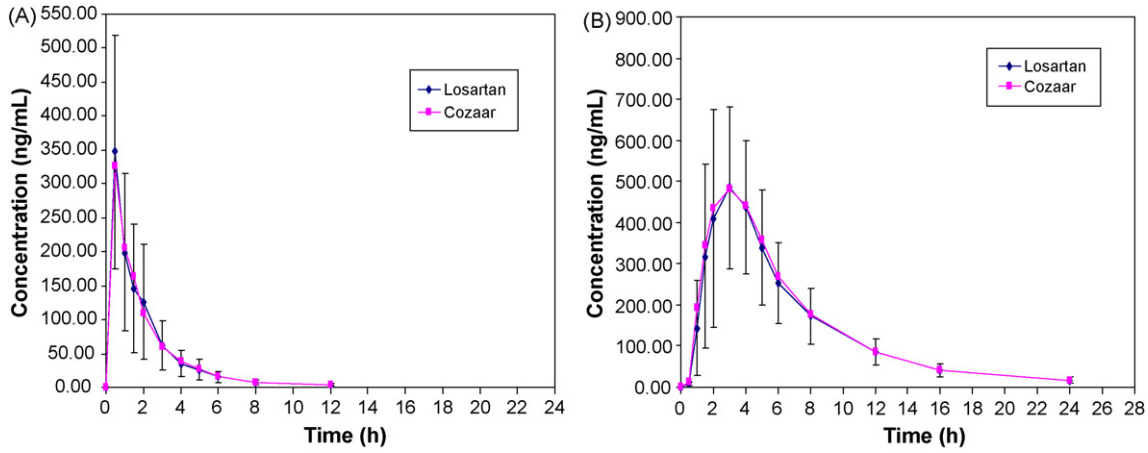

Fig. 3. Mean plasma concentration of Losartan (A) and Losartan acid (B) after oral administration of single dose of two brands to 24 healthy human volunteers.

3. Result and discussion

3.1. LC/MS/MS conditions

The concentrations of losartan and its active metabolite losartan acid, and irbesartan as internal standard were analyzed by combined reversed phase liquid chromatography and tandem mass spectrometry (LC–MS–MS). The multiple reaction monitoring (MRM), monitoring transitions in both positive and negative mode were compared and a more intense response observed in negative ion mode.

Product ion spectra were observed for all compounds leading to MRM monitoring of 420.7 > 126.7 for losartan, 434.7 > 156.9 for losartan acid and 426.9 > 192.0 for irbesartan. The conditions were chosen following optimization of the fragmentation conditions. This method provided limits of quantitation for analysis of losartan and its active metabolite in human plasma that were much lower compared to earlier LC-UV methods employed which ranged between 5 ng/mL and 10 ng/mL [4,8]. This method has been successfully validated over the range 2–400 ng/mL for losartan, and 1.85–370 ng/mL for losartan acid.

3.2. Calibration curve

The eight-point calibration curve was linear over the concentration range 2.0–400 ng/mL for losartan and 1.85–370 ng/mL for losartan acid. The best linear fit and least-squares residual for the calibration curve were achieved with a $1/x^2$ weighing factor. The mean correlation coefficient (r) of the weighted calibration curve generated during the validation was more than 0.999 and 0.9979 for losartan and losartan acid.

3.3. Specificity and limit of quantification

Human blank plasma samples from six different subjects were extracted and no endogenous peak that interfered with the quantitation of losartan, losartan acid and IS. The limits of quantification were established at 1.99 ng/mL for losartan and 1.85 ng/mL for losartan acid. Representative chromatograms of blank human plasma spiked with losartan and losartan acid at LLOQ and IS and chromatograms of blank plasma only are shown in Figs. 1 and 2.

3.4. System suitability of the method

The performance of the system was shown to be optimal by analyses of standard solution (100 ng/mL) in five replicate at the beginning of each sample sequence. The criterion was 2% relative standard deviation of analyte peak area. The samples for checking the suitability of the systems were well within the acceptable %RSD.

3.5. Precision and accuracy

The intra-batch precision and accuracy of the method are illustrated in Tables 2 and 3. Five sets of quality control samples (low, medium and high concentration) were analyzed with calibration samples in one batch. The intra-batch precision range from 5.05% to 7.77% and from 3.55% to 7.35% and the accuracy from 1.58% to 4.85% and from −4.40% to 2.19% for losartan and losartan acid

The inter-batch precision and accuracy were evaluated by processing a set of calibration and quality control samples (three levels analyzed twice) in five separate batches. The samples were prepared in advance and stored at −20 °C. The inter-batch precision range from 2.15% to 6.29% and from 4.55% to 5.46% and the accuracy from 1.03% to 3.41% and from −5.49% to 2.21% for losartan and losartan acid was observed.

3.6. Recovery

Recoveries of the analytes and IS were good and reproducible. The recoveries of losartan and losartan acid were 79.03%, 81.90%, 90.24% and 81.45%, 82.85%, 85.65% at low, medium and high QC samples. The recovery of IS was 87.35%

3.7. Stability

Stability of stock solutions were tested and established at room temperature for 0 and 6 h, and under refrigeration (4 °C) for 36 days. Stability of the analytes and IS in human plasma were tested for short term, freeze-thaw and long term stability. All the stability studies were conducted at two concentration levels (low and high) with three determinations for each.

For short term stability, spiked plasma was left to stand at the ambient temperature for 6 h before they were further prepared. No significant decrease of the analyte concentration was observed. The stability data of the analytes in plasma over three freeze-thaw cycles indicate that the analytes were stable in human plasma for three freeze-thaw cycles, when stored at −20 °C and thaw to room temperature. For long term stability, two sets of samples (low and high) were stored in the freezer at −20 °C for 50 days. The samples were then analyzed using freshly prepared calibration samples. The samples were stable for a period of 50 days at −20 °C.

3.8. PK study

Plasma samples were periodically collected up to 24 h after oral administration of 50 mg dose to 24 healthy volunteers. The mean plasma concentration–time curve of losartan and losartan acid is shown in Fig. 3. The C_{max} of the test and reference formulation for losartan were 331.2 ng/mL and 335.0 ng/mL. The C_{max} of the test and reference formulation for losartan acid were 512.1 ng/mL and 535.9 ng/mL, respectively. The plasma level of losartan reached a maximum 0.5–3 h after administration and 1.5–5 h for losartan acid. This method has been applied successfully to analyze 2160 plasma samples from a bioequivalence study and no carry-over was observed during analyze.

4. Conclusion

A simple and sensitive LC/MS/MS method for the quantification of losartan and active metabolite in human plasma was developed and validated. The simplicity of the assay, with simple pretreatment procedure using acetonitrile, and sample turnover of 3.5 min per sample, make it an attractive procedure for the high-throughput bioanalysis of losartan.

References

[1] M. Burnier, Circulation 103 (2001) 904–912.
[2] D.W. Sifton, Physicians' Desk Reference Medical Economics. 55th ed. (2001) 1668–1670.
[3] D.A. Sica, T.W. Gehr, S. Ghosh, Clin. Pharmacokinet. 44 (2005) 797–814.
[4] A. Zarghi, S.M. Foroutan, A. Shafaati, A. Khoddam, Arzneim-Forsch/Drug. Res. 25 (2005) 569–572.
[5] H. Jalalizadeh, E. Souri, H. Farsam, M. Ansari, Iranian J. Pharm. Ther. 2 (2003) 18–21.
[6] J.J.I. Tamimi, I.I. Salem, S. Mahmood Alam, Q. Zaman, R. Dham, Biopharm. Drug Dispos. 26 (2005) 205–210.
[7] R. Zaidenstein, S. Soback, M. Gips, B. Avni, V. Dishi, Y. Weissgarten, A. Golik, E. Scapa, Ther. Drug Monit. 23 (2001) 369–373.
[8] D. Farthing, D. Sica, I. Fakhry, A. Pedro, T.W. Gehr, J. Chromatogr. B Biomed. Sci. Appl. 704 (1997) 374–378.