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Development and validation of liquid chromatography–mass spectrometry method for the determination of telmisartan in human plasma

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Abstract

A sensitive liquid chromatographic–electrospray ionization mass spectrometric method was developed and validated for fast determination of telmisartan in human plasma. Plasma of 0.1 mL was deprotienated with methanol, centrifugation, evaporation to dryness and dissolving in mobile phase, samples were separated using a Hypersil-Keystone C18 reversed-phase column (150 mm × 2.1 mm i.d., 5 μm), together with a mobile phase containing of acetonitrile–10 mM ammonium acetate (42:58, v/v), 0.2% acetic acid and was isocratically eluted at a flow rate of 0.2 mL/min. Telmisartan and its internal standard, valsartan, were measured by electrospray ion source in positive selective ion monitoring mode. The method demonstrated linearity from 1 to 2000 ng/mL ($r = 0.9988$). The limit of quantification for telmisartan in plasma was 1 ng/mL with good accuracy and precision. The mean sample extract recovery of the method were higher than 82 and 78% for telmisartan and internal standard (IS), respectively. The within-run and between-run precision ranged from 3.4 to 8.9% and 5.9 to 11.2% (relative standard deviation, R.S.D.), respectively.

Keywords: Telmisartan; LC–MS; Electrospray ionization

1. Introduction

Telmisartan, 4-((2-*n*-propyl-4-methyl-6-(1-methylbenzimidazol-2-yl)-benzimidazol-1-yl)methyl)-biphenyl-2-carboxylic acid (Fig. 1), is a new highly selective, non-peptide angiotensin II type 1 (AT1)-receptor antagonist angiotensin that lowers blood pressure through blockade of the renin–angiotensin–aldosterone system (RAAS) [1] and widely used in treatment of hypertension. It can selectively block the angiotensin type I (AT1) receptor, which responsible for vasoconstriction and for salt and water retention. As an angionist of angiotensin type I receptor, unlikely losartan and eprosartan, which can be rejected by receptor due to high concentration of angiotensin II, telmisartan shows the non-competitive inhibition. In in vitro studies, it has been shown that telmisartan is eightfold more potent than losartan [2]. Animal models have demonstrated that telmisartan, when administered by i.v. or p.o., provides dose-dependent and persistent inhibition of the angiotensin II-induced pressor response[3–5]. The therapy with this drug offers a good quality of life for hypertensive patients due to the absence of side effects and its once daily administration. Studies in healthy volunteers and patients with mild-to-moderate hypertension have consistently found that telmisartan has a long terminal elimination half-life of ∼24 h [6–17]. Telmisartan has become one of the most important advances in the treatment of hypertension.

A few analytical methods for the determination of telmisartan have reported. Among these analytical techniques, capillary zone electrophoresis (CZE) method [18–20] and

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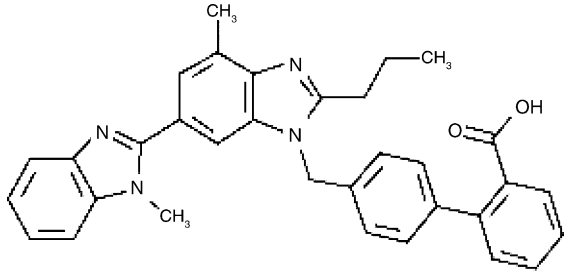
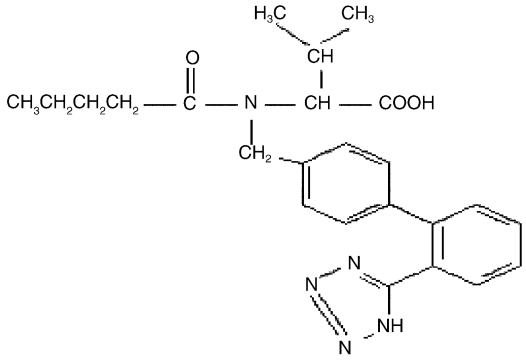


Telmisartan



Valsartan

Fig. 1. Chemical structures of telmisartan and valsartan (IS).

micellar electrokinetic chromatographic method [21] have been described. High-performance liquid chromatographic (HPLC) methods coupled with UV [22–25] and fluorescence [26] detection have also been reported. Recently, the polarography methods were also published [27,28]. However, these methods are not ideal ones to meet with the clinical drug monitoring and pharmacokinetics study because of the interference in plasma samples and low sensitivity of methods. In the present study, a method developed by using liquid chromatographic–mass spectrometry (LC–MS) for the determination of telmisartan in human plasma solved these problems. In the method, 0.1 mL of plasma sample was undergone deprotienating, centrifuging, drying and dissolving with mobile phase. The interference materials to the influence of the measurement were removed. The availability of the method was also validated in the experiment, and the conditions of the separation and ionization were optimized, making the method more suitable for the telmisartan clinical drug monitoring and pharmacokinetics study.

2. Experimental

2.1. Reagents and chemicals

Telmisartan and valsartan (internal standard, IS) were purchased from China Supervise Institute of Drug and Biological Preparation (Beijing, China). All solvents were HPLC grade and were obtained from TEDIA Company Inc. (Fairfield, OH, USA). Other reagents were of analytical grade, and all water used was Milli-Q grade.

2.2. Equipment

The HPLC system consisted of a Shimadzu LC-10ADvp pump, an SCL-10ADvp system controller, a CTO-10Avp column oven, an FCV-10ALvp low pressure gradient unit, a DGU-14A degasser and an SPD-M10Avp diode array detector (Shimadzu, Kyoto, Japan). The samples were dried on Savant drier (USA). The mass spectrometer was an LCMS-2010 single quadrupole equipped with electrospray ionisation interface (Shimadzu, Kyoto, Japan). The data was collected and processed using LCMSsolution software. The Speed Vacplus Model vacuum drier (Savant, USA) was used in the preparation of samples.

2.3. Chromatographic conditions

Chromatographic separations were performed using a Thermo Hypersil-Keystone Hypurity C18 (150 mm × 2.1 mm, 5 μm) analytical column. The oven temperature was set at 40 °C. The mobile phase consists of acetonitrile–10 mM ammonium acetate (42:58, v/v) containing 0.2% acetic acid and was isocratically eluted at a flow rate of 0.2 mL/min.

2.4. Mass spectrometer conditions

An LCMS-2010 quadrupole mass spectrometer was interfaced with electrospray ionization (ESI) probe. The temperatures were maintained at 250, 250, 200 °C for the probe, CDL and block, respectively. The voltages were set at 4.5 kV, −30 V, 25 V, 150 V and 1.5 kV for the probe, CDL, Q-array 1, 2, 3 bias, Q-array RF and detector, respectively. The flow rate of nebulizer gas was 4.5 L/min. The ions of selection monitoring were decided by positive scanning from m/z 100–600. For the quantification of telmisartan, the protonated molecule ions of valsartan at $m/z = 436$ (IS) and telmisartan at $m/z = 515$ were monitored. Tuning of mass spectrometer was performed with the help of autotuning function of LCMSsolution software (Version 2.02) using tuning standard solution (polypropylene glycol). Optimization and calibration of mass spectrometer were achieved with autotuning.

2.5. Analytical procedure

2.5.1. Preparation of stock solutions, calibration standard and quality control samples

A stock solution of telmisartan at concentration of 200 μg/mL was prepared in methanol. The internal standard (valsartan) was also prepared as a stock solution (1 mg/mL) in methanol and was further diluted with methanol to give a concentration of 100 ng/mL and used for all analyses. A serial calibration curve samples at concentration of 1, 5, 10, 50, 100, 500, 1000 and 2000 ng/mL of telmisartan were freshly

prepared by diluting standard solutions with blank plasma. The quality control (QC) samples were prepared by diluting standard solutions with blank plasma to form concentrations of 1, 200 and 2000 ng/mL of telmisartan. Fifty microliters of internal standard (100 ng/mL) was added to 0.1 mL of calibration curve samples and quality control samples. The further processing both calibration curve samples and quality control samples were the same as described in the following section (Section 2.5.2) for collection and preparation of the samples. All standard stock solutions were prepared once a month and stored at −20 °C.

2.5.2. Collection and preparation of the samples

Twenty-four healthy volunteers (11 female, 13 male) received the investigation. The average age of volunteers was 24 years old within the range of 20–29. The mean of body weights was 59.3 kg (45–76 kg) and the mean of body heights was 168.5 cm (156–182 cm). Following written informed consent, volunteers took an 80 mg tablet of telmisartan with 200 mL of water, and were not allowed drinking, smoking and had low fat food at 2 h after taking drug. Blood samples were collected in heparinized tubes pre-dose (0 h) and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72 and 96 h post-dose. Plasma was immediately separated by centrifugation at 4000 rpm and stored −20 °C until analysis.

A plasma sample (0.1 mL) was placed in a 1.5 mL Eppendorf tube. After the addition of 50 μL of a 100 ng/mL solution of internal standard, the tube was briefly vortexed and 0.2 mL of methanol was added into the tube. After vortexing for 30 s, the tube was centrifuged at 14,000 rpm for 10 min at 5 °C and the supernatant was transferred to another clear Eppendorf tube. The extract was evaporated to dryness in a Speed Vacplus Model vacuum drier. The residue was reconstituted with 100 μL of mobile phase, vortexed for 30 s and centrifuged at 14,000 rpm for 5 min and 5 μL of supernatant was injected onto the analytical column.

2.6. Assessment of matrix effect and method selectivity

For matrix effect, three concentration solutions of standard (1, 200, 2000 ng/mL) and internal standard (50 ng/mL) were dried and reconstituted in mobile phase (neat standard). Fif-

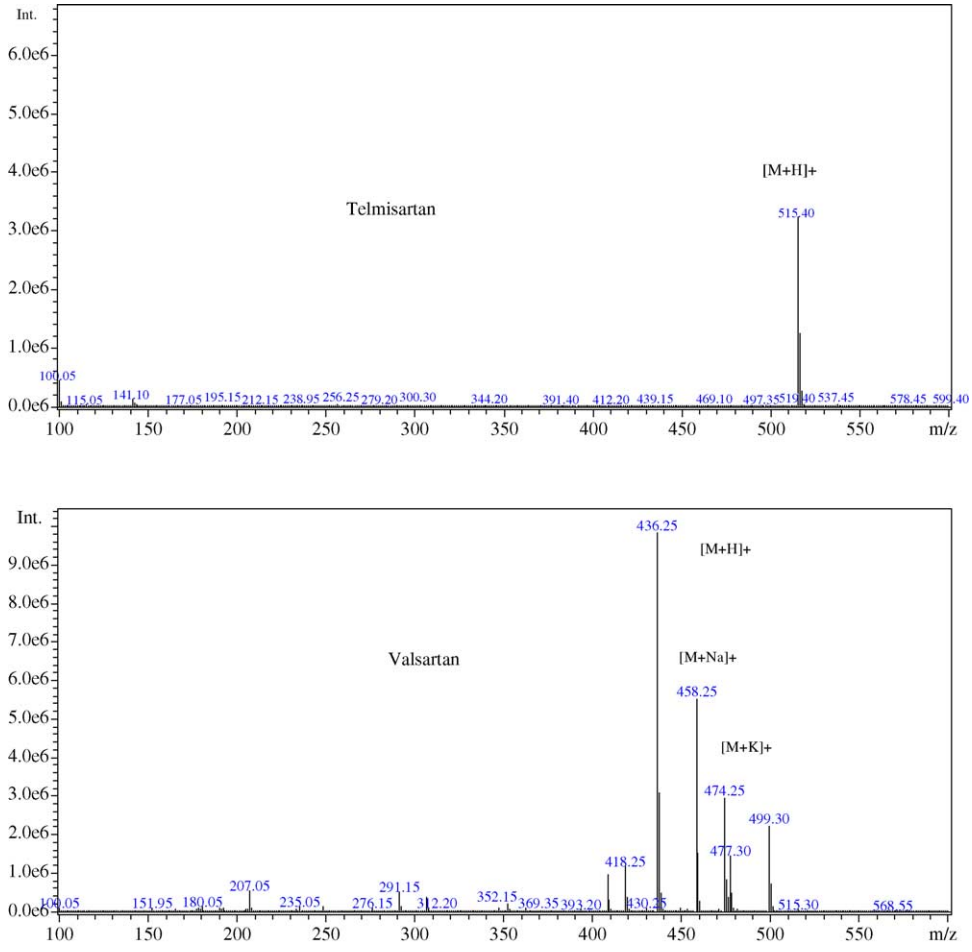


Fig. 2. ESI–MS positive ion scanning spectra of telmisartan and valsartan (IS). Chromatographic conditions: column, Thermo Hypersil-Keystone Hypurity C18 (150 mm × 2.1 mm, 5 μm); oven temperature, 40 °C; mobile phase, acetonitrile–10 mM ammonium acetate (42:58, v/v) containing 0.2% acetic acid; flow rate, 0.2 mL/min. Mass spectrometer: electrospray ionization (ESI) source; positive scan *m/z*, 100–600; temperature—probe, 250 °C; CDL, 250 °C; block, 200 °C; voltage—capillary, 4.5 kV; CDL, −30 V; Q-array 1, 2, 3 bias, 25 V; Q-array RF, 150 V; detector, 1.5 kV. Nebulizer gas flow rate: 4.5 L/min.

Table 1
Peak areas of neat standard of telmisartan and valsartan and the same standard spiked into plasma before and after extraction ($n = 5$)

Norminal concentration (ng/mL)	Telmisartan					Valsartan				
	Peak area[a] (A)	Peak area[b] (A')	Peak area[c] (A'')	Matrix effect[d] (A'/A, ×100)	Recovery[e] (A''/A', ×100)	Peak area[a] (B)	Peak area[b] (B')	Peak area[c] (B'')	Matrix effect[d] (B'/B, ×100)	Recovery[e] (B''/B', ×100)
1	24367 (3.5)[f]	23794 (4.1)	21352 (3.8)	97.6	89.7	1283748 (2.4)	1293716 (3.6)	1027364 (4.6)	100.8	79.4
200	5017326 (2.9)	5026736 (3.4)	4439836 (4.0)	100.2	88.3	1302739 (3.1)	1282957 (3.1)	996452 (3.4)	98.5	77.7
2000	49163548 (2.6)	50154374 (3.0)	43284738 (4.5)	102.0	86.3	1260866 (2.7)	1253821 (2.7)	982631 (4.1)	99.4	77.9
Mean				99.9	88.1				99.7	78.3
CV%				2.2	1.9				1.2	1.2

[a] Neat standard and IS.
[b] Standard and IS spiked after extraction.
[c] Standard and IS spiked before extraction.
[d] Matrix effect (%), expressed as the ratio of the mean areas of standard and IS spiked into plasma post-extraction to the peak areas of the neat standard and IS multiplied by 100. A value >100% indicates ionization enhancement and a value <100% indicates ionization suppression.
[e] Recovery (%), expressed as the ratio of the mean peak areas of standard or IS spiked before extraction to the mean peak areas of standard or IS spiked post-extraction multiplied by 100.
[f] Numbers in parentheses are coefficient of variation (CV%).

teen plasma samples (0.1 mL) of five different sources (five per each source) were placed into 1.5 mL Eppendorf tubes. The samples were processed as in "Section 2.5.2" and the residues were reconstituted in 100 μL of mobile phase containing 1, 200, 2000 ng/mL of standard and 50 ng/mL of internal standard, respectively. Other 15 plasma samples from five different sources spiked with standard (1, 200, 2000 ng/mL, respectively) and internal standard (50 ng/mL) before extraction were deproteined. The residues were dissolved in 100 μL of mobile phase. For assessment of selectivity, six lots of blank plasma from different sources were prepared as in "Section 2.5.2" and analyzed.

3. Results and discussion

3.1. Selection of HPLC and MS conditions

Many methods for the determination of telmisartan have been reported [18–28], but, to our best knowledge, the LC–MS method for the determination of telmisartan in biological samples has been seldom reported. In the present study, to select an appropriate ionization mode in LC–MS analysis, telmisartan and valsartan (IS) were scanned with ESI and APCI positive and negative ion modes using injection standard solutions. In different ionization modes, the base peak intensity of positive ion was higher than those of negative ion, and the efficiency of ionization in ESI was higher than APCI. Fig. 2 shows the positive ion mass spectra of telmisartan and valsartan (IS) by ESI scanning from m/z 100 to 600. Only protonated ion m/z 515 $[M+H]^+$ was observed in telmisartan spectra and m/z 436 $[M+H]^+$, 458 $[M+Na]^+$, 474 $[M+K]^+$ were occurred in valsartan scanning spectra. This is the reason why there is a ketonic group in the molecular of valsartan, and Na^+, K^+ ions were easily added. Therefore, the ESI positive ion mode was selected in this study.

The separation and ionization of telmisartan and valsartan were affected by the components of mobile phase. The mobile phase pH affected not only the retention time, but also the ionization efficiency of telmisartan and valsartan. The retention time increased with the increment of mobile phase pH, especially valsartan. The acidity of mobile phase benefited to the ionization of telmisartan and valsartan. Thus, the sensitivity of telmisartan was improved by increasing acidity of mobile phase because of raising the rate of ionization.

3.2. Validation of the method

3.2.1. Matrix effect

The matrix effect and the possibility for ionization suppression or enhancement for telmisartan and valsartan in different plasma samples (lots, subjects) were examined. The coefficients of variation (CVs, %) of the mean peak areas of telmisartan and valsartan at any given concentration in five different plasma lots were small (<5%), strongly indicat-

Table 2
Intermediate precision, accuracy and linear regression parameters of telmisartan determination in human plasma

Added concentration (ng/mL)	Mean measured concentration, $n = 5$ (ng/mL)	Precision (R.S.D., %)	Mean relative error[a] (%)
1.0	1.05	11.8	5.0
10.0	9.75	6.3	2.5
50.0	46.89	4.7	6.2
100.0	95.75	5.4	4.3
500.0	509.36	3.8	1.9
1000.0	992.18	5.2	0.8
2000.0	1966.07	4.5	1.7
Calibration curve			
Slope	0.0297		
Intercept	0.05113		
Correlation coefficient	0.9988		

[a] Mean relative error = |mean measured concentration − added concentration| × 100/added concentration.

ing little or no difference in ionization efficiency and consistent recovery of standard and internal standard from different plasma lots. In addition, by comparing peak areas of standard and internal standard for samples spiked after extraction from plasma with the analogous peak areas obtained by injecting neat standard and IS directly, the extent of the absolute matrix effect was estimated (Table 1). The values >100% indicate ionization enhancement in plasma versus neat standards; whereas, values <100% indicate ionization suppression. The data presented in Table 1 indicated that the mean "absolute" matrix effect for telmisartan and valsartan (99.9 and 99.7%, respectively) was negligible.

3.2.2. Selectivity

Six lots of plasma extracts from different sources were analyzed. Interference peaks at the retention time of telmisartan and valsartan were not observed in any of the plasma lots. In addition, telmisartan and valsartan were separately injected and selective ions were monitored. Fig. 3(A) showed one of representative chromatogram of six lots of blank plasma extracts. Fig. 3(B) showed the selective ion chromatogram of the plasma sample at the lowest limit of detection (0.1 ng/mL). No cross-talk was observed.

3.2.3. Sensitivity and linearity

The limit of quantitation (LOQ) is 1 ng/mL using 0.1 mL plasma. A good signal-to-noise ratio (10:1) was observed at the LOQ indicating that the corresponding valve could be reached. The calibration curve of telmisartan was linear over the range from 1 to 2000 ng/mL with the correlation coefficient of 0.9988. The results demonstrate acceptable accuracy, reproducibility and good fit to the non-weighted regression lines. The calibration curve had the regression equation of $y = 0.0297x + 0.05113$, where y was the peak area ratio of telmisartan to internal standard, x was the concentration of telmisartan. Representative calibration curve parameters for the method from intra-day standard curve replicates are showed in Table 2. Intra-assay precision and accuracy were

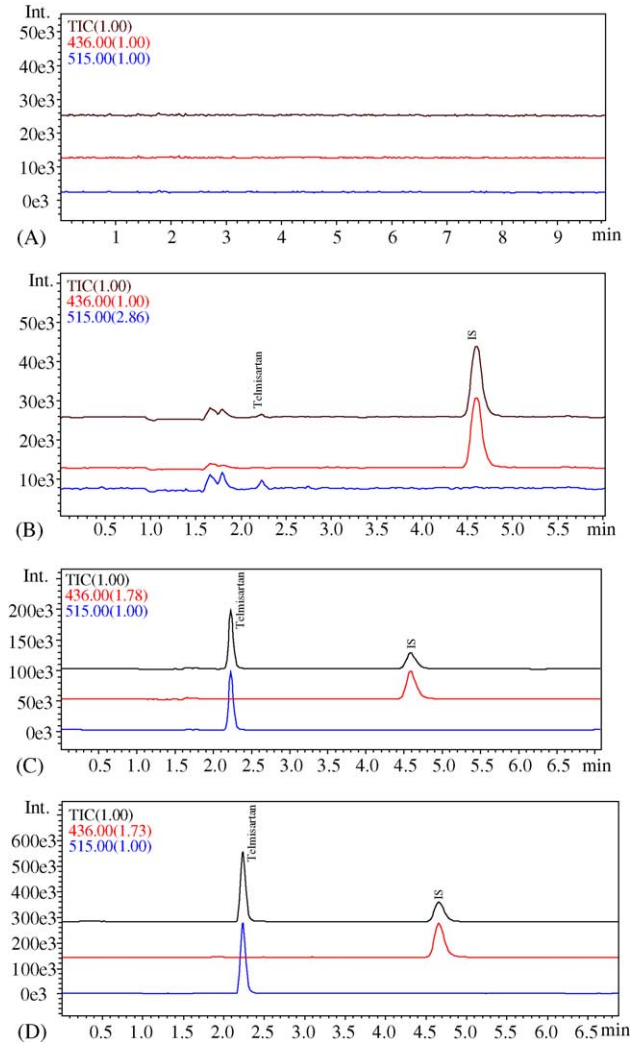


Fig. 3. Selective and total ion chromatograms of telmisartan and valsartan (IS). Positive ion monitored at *m/z* 436 (valsartan, IS), 515 (telmisartan), (A) blank plasma, (B) blank plasma spiked with 0.1 ng/mL (the lowest limit of detection) of telmisartan and IS, (C) blank plasma spiked with standard (50 ng/mL) and IS and (D) human plasma sample after administration of telmisartan and spiked with IS.

Table 3
Reproducibility and accuracy for telmisartan of quality control sample in human plasma ($n = 5$)

Nominal concentration (ng/mL)	Mean found concentration (ng/mL)	Precision (R.S.D., %)	Mean relative error[a] (%)
Within-run			
1	1.08	8.9	8.0
100	98.3	4.7	1.7
2000	1976.4	4.3	1.2
Between-run			
1	1.10	11.2	10.0
100	95.4	5.9	4.6
2000	1964.8	7.2	1.8

[a] Mean relative error = |mean measured concentration − added concentration| × 100/nominal concentration.

Table 4
Stability data for telmisartan in human plasma

Conditions	Recovery[a] (%)
Storage in plasma at room temperature (RT) for 24 h	95
Storage in plasma at −20 °C for 2 months	94
Storage in processed plasma extract at RT for 24 h	97
Freeze–thawed four times	98

[a] Recovery is expressed as the response of telmisartan stored in plasma following stability conditions compared to the response of telmisartan freshly spiked in plasma.

very satisfactory for all the concentrations tested. Relative standard deviation (R.S.D.) values were less than 11.8% at all concentrations.

3.2.4. Reproducibility and accuracy

The within-run and between-run reproducibility of the method for plasma is summarized in Table 3 by analysis of replicates ($n = 5$) of QC samples containing known concentrations of 1.0, 100 and 2000 ng/mL of telmisartan. The precision of the method was described as R.S.D. among each assay. The within-run R.S.Ds were always below 8.9% and the between-run R.S.Ds below 11.2%. The accuracy of the method was evaluated by analysis of the quality control samples spiked with standard solutions and expressed as a percentage error of measured concentrations versus nominal concentrations. Precision and accuracy were calculated at each concentration.

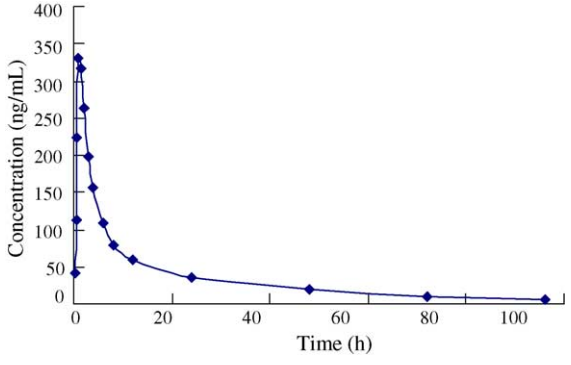


Fig. 4. Plasma concentration–time profile of telmisartan from 18 healthy volunteers following a single oral dose of 80 mg.

3.2.5. Extraction recovery

The recoveries of telmisartan and IS were calculated by comparing the areas obtained from spiked blank plasma ($n = 5$) with those obtained from injecting directly standard solutions with similar concentrations in methanol. The mean extraction recoveries were higher than 82 and 78% for telmisartan and IS, respectively.

3.2.6. Stability

Telmisartan is shown to be stable under conditions of storage and processing. It is stable extract at room temperature for at least 24 h. The analyte is also stable in human plasma when stored at −20 °C for at least 2 months and at room temperature for at least 24 h. It is stable under the influence of freeze–thaw cycles. Table 4 shows the stability data of telmisartan subsequent to various storage conditions and freeze–thaw cycles.

3.3. Pharmacokinetics study

The method was successfully used to perform the determination of plasma concentrations of telmisartan after oral administration of 80 mg dose to 18 healthy volunteers. Fig. 4 shows mean plasma concentration–time profile of

Table 5
Mean (±S.D.) pharmacokinetic parameters for telmisartan in healthy volunteers ($n = 18$)

T_{max} (h)	1.2 ± 0.3
$T_{1/2}$ (h)	26.4 ± 2.5
C_{max} (ng/mL)	387.0 ± 56.0
$AUC_{0\rightarrow 96}$ (h ng/mL)	3400.7 ± 637.3
CL/F (L/h)	22.9 ± 4.1

telmisartan. The pharmacokinetic parameters were shown in Table 5.

4. Conclusions

A fast, sensitive, specific LC–ESI–MS method for determination of telmisartan in human plasma was developed and validated. Significantly lower limit of quantification (1.0 ng/mL) was achieved in plasma, compared with the previously published methods. The steps of sample preparation were uncomplicated using 0.1 mL plasma sample. The method has been successfully applied to the pharmacokinetics study and produced satisfactory results demonstrate that the method is accurate, reliable and sensitive.

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