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Short communication

Determination of telmisartan in human plasma by liquid chromatography–tandem mass spectrometry

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Abstract

A rapid, selective and sensitive method for the determination of the angiotensin II receptor antagonist, telmisartan, in human plasma has been developed. Telmisartan and the internal standard, diphenhydramine, were extracted from plasma using diethyl ether–dichloromethane (60:40, v/v), and separated on a Zorbax extend C_{18} column using methanol–10 mM ammonium acetate (85:15, v/v) adjusted to pH 4.5 after mixing with formic acid as mobile phase. Detection was carried out by multiple reaction monitoring on a Q-trap™ LC–MS/MS system with an ESI interface. The assay was linear over the range 0.5–600.0 ng/ml with a limit of quantitation of 0.5 ng/ml and a limit of detection of 0.05 ng/ml. Intra- and inter-day precision were <6.7% and <8.1%, respectively, and the accuracy was in the range 88.9–111.0%. The assay was applied to a pharmacokinetic study of telmisartan given as a single oral dose (80 mg) to healthy volunteers.

Keywords: Telmisartan; LC–MS/MS

1. Introduction

Telmisartan (4-((2-*n*-propyl-4-methyl-6-(1-methylbenzimidazol-2-yl)-benzimidazol-1-yl)methyl)-biphenyl-2-carboxylic acid) is an angiotensin II receptor antagonist widely used in the treatment of hypertension. It undergoes minimal biotransformation in the liver to form the inactive telmisartan 1-*o*-acylglucuronide as its principal metabolite [1]. The long half-life and selectivity of telmisartan for angiotensin II receptors allows once daily dosing with minimal side effects [2,3].

For pharmacokinetic study, various analysis methods including radiolabeled drug [4] and HPLC with fluorimetric detection [5] have been developed for the determination of telmisartan in biosamples. However, these methods suffer from disadvantages such as low sensitivity, extensive sample preparation, larger biosamples and long analytical run time.

Liquid chromatography with tandem mass spectrometry (LC–MS/MS) is widely employed for bioassay of drugs [6,7], but has not been applied to the determination of telmisartan. In this paper, we describe an LC–MS/MS method for the determination of telmisartan in human plasma and its application to a clinical pharmacokinetic study in healthy volunteers given a telmisartan 80 mg tablet.

2. Experimental

2.1. Materials and reagents

Telmisartan (99.0%) and diphenhydramine (99.9%) (Fig. 1) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, P.R. China). Methanol was HPLC grade and purchased from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals were analytical grade and used without further purification. Distilled, deminer-

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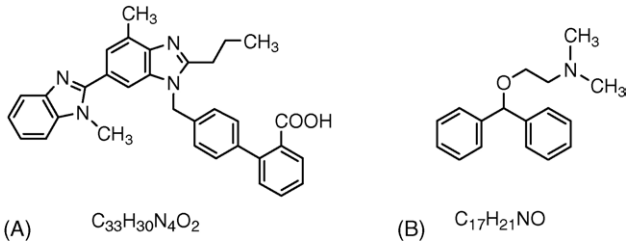

Fig. 1. Structures of telmisartan (A) and diphenhydramine (B) (internal standard).

alized water was produced by a Milli-Q Reagent Water System (Millipore, MA, USA).

2.2. Preparation of standard solutions

Stock solutions of telmisartan and diphenhydramine (both 0.5 mg/ml) were prepared in methanol and stored at 4 °C. Standard solutions of telmisartan (0.5, 1.0, 3.0, 10.0, 30.0, 100.0, 300.0 and 600.0 ng/ml) were prepared by dilution of the stock solution with methanol:water (50:50, v/v). An internal standard working solution (10 ng/ml diphenhydramine) was similarly prepared. Low, medium and high quality control (QC) solutions (1.0, 30.0, 500.0 ng/ml) were also prepared. The limit of detection (LOD) was investigated using standards with concentrations of 0.10, 0.05 and 0.01 ng/ml.

2.3. Instrumentation and conditions

The LC–MS system consisted of an Agilent 1100 series HPLC (Agilent Technologies, Palo Alto, CA, USA) coupled to an Applied Biosystems Sciex Q-trap™ mass spectrometer (Applied Biosystems Sciex, Ontario, Canada). Isocratic chromatography was carried out on a 150 mm × 4.6 mm, 5 μm Zorbax extend C_{18} column maintained at 35 °C using a mobile phase of methanol–10 mM ammonium acetate (85:15, v/v) adjusted to pH 4.5 after mixing with formic acid. The flow rate was 1.0 ml/min and the column effluent was split so that approximately 0.5 ml/min entered the detector, which was equipped with an ion-spray source and operated in the positive ion mode. Optimum ion source parameters were as follows: curtain gas = 20 p.s.i.; ion spray voltage = 1500 V; temperature = 500 °C; ion source gas 1 = 60 p.s.i.; ion source gas 2 = 50 p.s.i. The collision gas was set to medium mode and the interface heater to on mode. Hydrophilic impurities were diverted to waste for 60 s after an injection using a 10-way switching valve. Data acquisition was carried out by Analysis 1.4 software on a *DELL* computer.

2.4. Sample preparation

Liquid–liquid extraction of mixtures of thawed plasma (50 μl) and internal standard working solution (50 μl) was carried out by shaking with 3 ml diethyl ether–dichloromethane (60:40, v/v) for 10 min. After centrifugation at 2000 × *g* for 10 min, the organic phase was transferred to another tube and evaporated to dryness at 40 °C under a gentle stream of nitrogen. Residues were reconstituted in 200 μl aliquots of mobile phase and 20 μl injected into the LC–MS system.

2.5. Assay validation

Calibration standards and QC samples ($n = 6$) were analyzed on three separate days. Linearity of calibration curves based on peak areas was assessed by weighted ($1/x^2$) least-squares analysis. Intra- and inter-day precision was calculated as coefficient of variation (CV) and accuracy as relative error. The limit of quantitation (LOQ) was determined as the concentration below which the inter-day CV exceeded 20%. The LOD was determined as the concentration with signal-to-noise ratio of 3. The recoveries of telmisartan and the internal standard were evaluated by comparing peak areas of extracted QC samples and internal standard with those of reference solutions reconstituted in blank plasma extracts, respectively. Matrix effects were evaluated by comparing peak areas of QC solutions and internal standard solutions reconstituted in blank plasma extracts with that of the same solutions injected directly into the LC–MS system.

Stability in plasma was assessed in the autosampler at room temperature for 8 h and on storage at −20 °C for 90 days. The effect of three freeze-thaw cycles was also investigated.

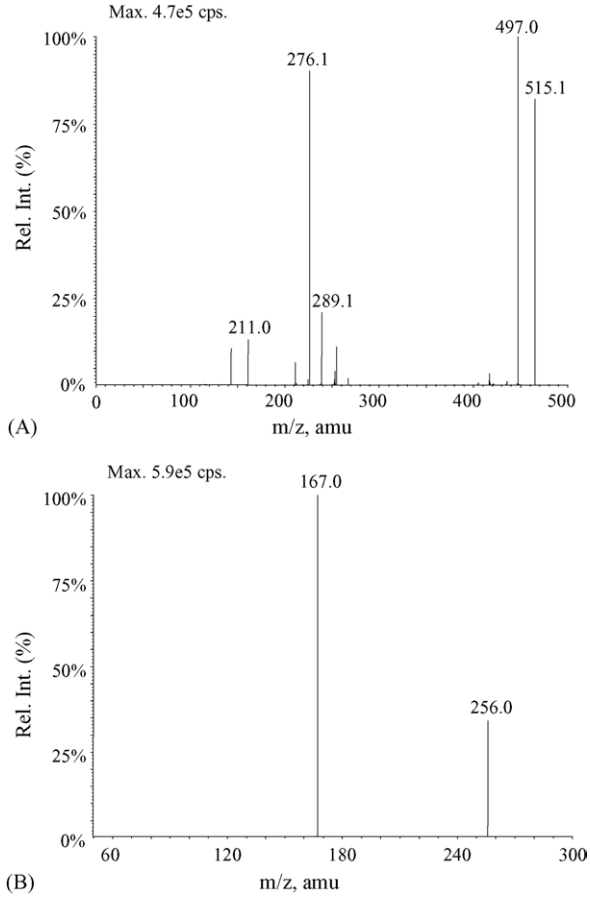

Fig. 2. Full-scan product ion spectra of $[M+H]^+$ for (A) telmisartan and (B) diphenhydramine.

2.6. Pharmacokinetic study

Telmisartan was determined in plasma of healthy volunteers ($n = 22$) after administration of an 80 mg tablet (Micardis). Blood samples (1 ml) were collected before the dose and at 0.5, 1.0, 1.5, 2.0, 3.0, 5.0, 8.0, 12.0, 24.0, 48.0, 72.0 and 96.0 h post-dose. Plasma was obtained by centrifugation of whole blood at $2000 \times g$ for 5 min and stored at $-20\,^{\circ}C$ prior to analysis.

3. Results and discussion

3.1. Mass spectrometry

Positive electrospray ionization mass spectra of telmisartan and diphenhydramine are shown in Fig. 2. Multiple reaction monitoring (MRM) was performed at unit resolution using the mass transition ion-pairs m/z 515.1 → 276.1 for telmisartan (declustering potential (DP) 90 eV; collision energy (CE) 21 eV) and m/z 256.0 → 167.0 for diphenhydramine (DP 20 eV; CE 15 eV).

3.2. Chromatography

Various combinations of acetonitrile, methanol, acetic acid and formic acid were investigated to optimize the mobile phase for sensitivity, speed and peak shape. The inclusion of 10 mM ammonium acetate instead of pure water reduced matrix effects without decreasing response. Peak shape was improved by using formic acid to adjust the mobile phase pH to 4.5. Further improvement in peak shape with reduced cycle time was achieved by splitting the column effluent and increasing the flow rate. Of a number of C_{18} columns (Nova-Pak, Nucleosil and Hypersil) evaluated, Zorbax extend gave the best chromatography. With a total flow rate of 1.0 ml/min (splitting 0.5 ml/min to the ion source), the cycle time was 2.6 min allowing a sample throughput of 180–220 samples per day.

3.3. Assay validation

Typical chromatograms are shown in Fig. 3. The assay was linear over the concentration range 0.5–600.0 ng/ml ($r > 0.996$)

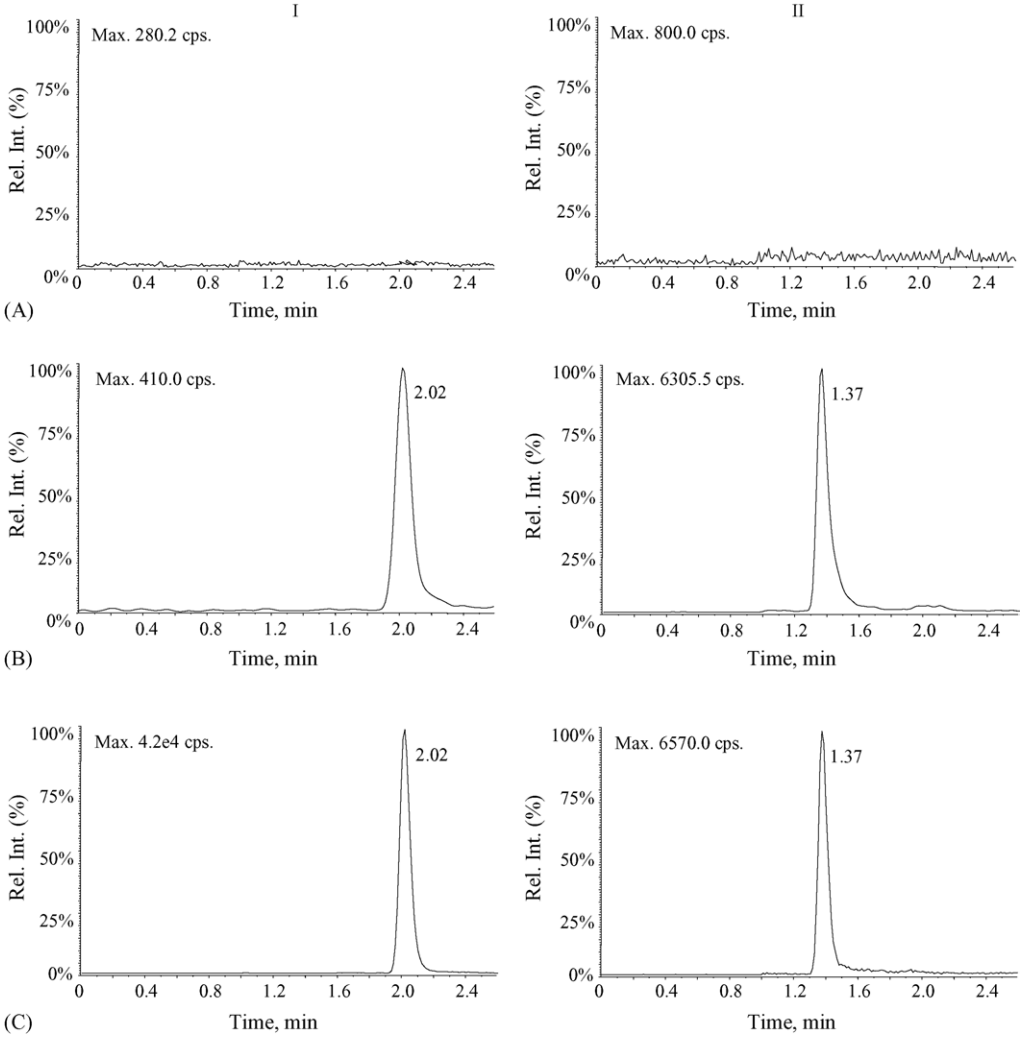

Fig. 3. Representative single reaction monitoring chromatograms of (A) blank plasma, (B) plasma spiked with telmisartan and diphenhydramine at the limit of quantitation (0.5 ng/ml) and (C) a plasma sample 1.5 h after an oral administration of a telmisartan 80 mg tablet to healthy volunteers. Peak I, telmisartan; Peak II, diphenhydramine.

Table 1
Precision and accuracy for the determination of telmisartan in human plasma (data are based on assay of six replicates on three different days)

Nominal concentration (ng/ml)	Calculated concentration (ng/ml)	Intra-day CV (%)	Inter-day day CV (%)	Relative error (%)
1.00	1.03	4.5	8.1	2.9
30.0	31.1	6.7	3.6	3.6
500.0	499.2	5.4	4.5	−0.2

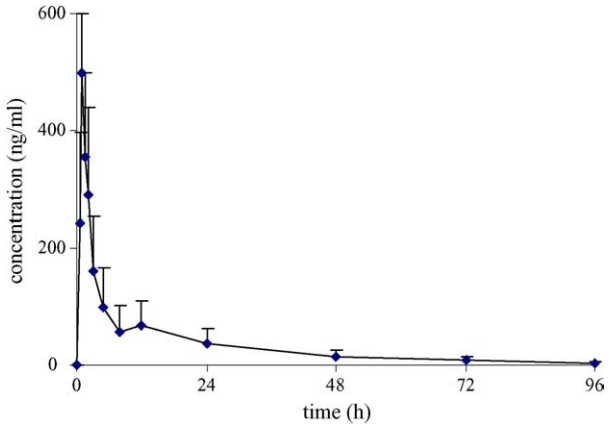

Fig. 4. Plasma concentration–time profile for telmisartan after administration of a telmisartan 80 mg tablet. Data are mean ± S.D. for 22 healthy volunteers.

with an LOD of 0.05 ng/ml. Intra- and inter-day precision were 4.5–6.7% and 3.6–8.1%, respectively and accuracy was 88.9–111.0% (Table 1).

The recoveries of telmisartan at 1.0, 30.0 and 500.0 ng/ml were 79.4%, 77.6% and 78.4%, respectively. The recovery of the internal standard was 75.0%. In relation to matrix effects, the relative errors based on mean peak areas were −6.5%, −7.2% and −6.3% at 1.0, 30.0 and 500.0 ng/ml, respectively. The relative error for diphenhydramine was −7.3%. The results indicate that no co-eluting endogenous substances significantly influenced the ionization of telmisartan and the internal standard. Telmisartan was stable under all the conditions evaluated with mean recoveries of 94.3–105.6% of the nominal concentrations.

3.4. Pharmacokinetic study

The plasma concentration–time profile for telmisartan after a single oral dose of 80 mg is shown in Fig. 4. The mean maximum concentration (C_{max}) was 507 ± 100 ng/ml occurring at 1.0 ± 0.2 h. The mean plasma elimination half-life ($t_{1/2}$) was 20.4 ± 7.7 h and the mean area under the plasma concentration–time curve (AUC) was 3200 ± 1650 ng h/ml.

4. Conclusion

A highly selective, sensitive and rapid method for the determination of telmisartan in human plasma is reported using high-performance liquid chromatography with detection by tandem mass spectrometry. Acceptable precision and accuracy is obtained within the standard curve range of 0.5–600.0 ng/ml. The LOD of the method is 0.05 ng/ml. The method requires only 50 μl of plasma and allows high sample throughput due to a simple sample preparation procedure and short run time.

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