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HPLC determination of telmisartan in human plasma and its application to a pharmacokinetic study

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A sensitive, simple, and accurate HPLC method was developed for the assay of telmisartan in human plasma. Using naproxen as internal standard, the assay involved liquid-liquid extraction of the compound from acidified plasma into organic solvent and reversed-phase chromatography with fluorescence detection. The assay was shown to be linear from 0.5 to 1000 ng/mL. In 24 healthy volunteers, the plasma concentrations of the drug were determined after a single oral dose of 160 mg.

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 (ARA II) widely used in the treatment of hypertension (Sharpe et al. 2001). HPLC methods with fluorescence detection have been reported for the determination of telmisartan in biological fluids and the clean-up step was based on the automated column-switching technique (Stangier et al. 2000a, 2000b, 2000e), solid phase extraction (Torrealday et al. 2003) and liquid-liquid extraction (Stangier et al. 2000d; Yong et al. 2000). However, the full details of methods using liquid-liquid extraction were not presented. The present paper describes a simple and reproducible HPLC method using liquid-liquid extraction to detect the plasma concentrations in 24 male healthy volunteers. Its pharmacokinetic applicability is also demonstrated.

2. Investigations, results and discussion

A typical chromatogram of a plasma sample is shown in Fig. 1. No endogenous interfering peaks were found at the retention time of telmisartan and the internal standard (I.S.). The retention time of naproxen (I.S.) and telmisartan were 4.0 and 5.4 min, respectively, and the run time was 7 min.

The calibration curves were linear in the concentration range 0.5 $\sim$ 1000 ng/mL ($r = 0.9991$). A typical calibration curve obtained from 7 points was $y = 0.0044x - 0.0009$, where y represents the peak area ratio of telmisartan to I.S., x represents the concentration of telmisartan, and the linear regression weighted by 1/x (the reciprocal of the nominal telmisartan concentration).

The results of intra-day and inter-day accuracy and precision evaluation for QC samples are shown in Table 1 and Table 2. For telmisartan, both intra-day and inter-day precision CV % were all less than 10%, while those of accuracy PE % were less than 15%. The limit of quantitation defined as the concentration with acceptable accuracy and precision (below 20%) was 0.5 ng/mL, which was more sensitive than the results reported in the literature of liquid-liquid extraction (Stangier et al. 2000c; Yong et al. 2000).

The method reported here using one-step liquid-liquid extraction of telmisartan in plasma after acidic treatment was satisfactory. Ethyl acetate has been demonstrated to be appropriate for extracting both telmisartan and internal standard. Based on direct comparison of peak areas from ex-

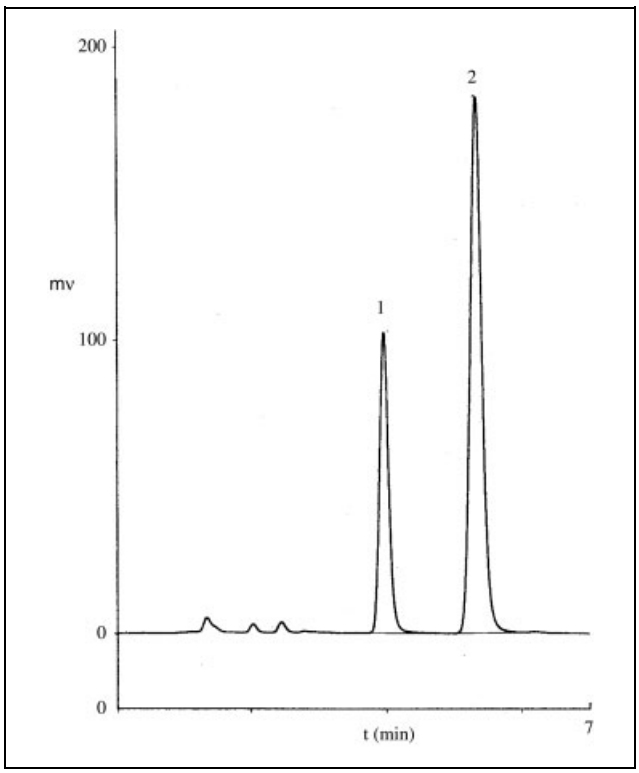

Fig. 1: Typical chromatogram obtained from a volunteer 3 h after dosing: (1) I.S.; (2) 529.45 ng/mL of telmisartan

Table 1: Intra-assay accuracy and precision for telmisartan in human plasma

Added conc. (ng/mL)	Calculated conc. (ng/mL)	PE %	CV %
0.5	0.52	3.79	5.10
1.0	1.02	2.23	4.08
300	289.71	−3.43	4.77
800	853.27	6.66	3.71

Table 2: Inter-assay accuracy and precision for telmisartan in human plasma

Added conc. (ng/mL)	Calculated conc. (ng/mL)	PE %	CV %
0.5	0.53	5.89	1.16
1.0	1.03	3.49	2.58
300	288.25	−3.92	1.41
800	831.71	3.96	3.04

tracts versus nonextracted samples, the mean absolute recoveries of telmisartan were $82 \pm 5\%$, $79 \pm 5\%$ and $88 \pm 4\%$ at 1.0, 300, 800 ng/mL concentration, respectively ($n = 6$). The mean absolute recovery of I.S. was found to be $93 \pm 3\%$ ($n = 10$).

Stock solutions of telmisartan in methanol were stable for at least 2 months at 4 °C. The analytes reconstituted in the mobile phase were also stable at ambient conditions (no control of temperature in the autosampler) for at least 24 h, thus allowing us to automate the procedure. No significant degradation in plasma samples (spiked or clinical) during 2 months at −20 °C was detected. Furthermore, telmisartan was stable for at least three freeze-thaw cycles. The results of accuracy and precision for the ability to dilute samples were 4% and 5%, respectively ($n = 6$).

The method was successfully used to perform the determination of plasma concentrations of telmisartan. Fig. 2 shows the mean blood telmisartan concentration versus time profiles from 24 male volunteers after oral administration of a 160-mg dose of telmisartan. The pharmacokinetic parameters, derived by non-compartmental analysis using the WinNonlin Program (Version 4.1, Pharsight Corporation, Mountain View, CA, USA), are summarized in Table 3. High interindividual variability was demonstrated in this study, which was consistent with previous pharmacokinetic evaluations (Smith et al. 2000; Stangier et al. 2000c).

Table 3: Pharmacokinetic parameters for telmisartan after a single oral administration of 160 mg telmisartan in 24 male volunteers

Pharmacokinetic parameter	Mean ± SD	Range
C_{max} (ng/mL)	2816 ± 1684	719.9–5798
AUC (ng h/mL)	12103 ± 13971	1938–71022
T_{max} (h)	0.93 ± 0.36	0.5–2.0

In conclusion, naproxen was the most appropriate internal standard because of its good recovery and separation under the chromatographic method developed. Liquid-liquid extraction further provides a simple and practical way to process plasma samples containing telmisartan with quantitative recovery. The simplicity of extraction, high selectivity, the short run time, and the good reproducibility make this procedure ideal for the detection of telmisartan in a large series of samples generated by clinical studies.

3. Experimental

3.1. Chemicals and reagents

Telmisartan was obtained from Baoguang Pharmaceutical Co. (Sichuan, P.R. China). Naproxen, used as I.S. was kindly provided by Shanghai Institute for Drug Control (Shanghai, P.R. China). Acetonitrile and methanol were HPLC grade from Burdick & Jackson Co. (Muskegon, MA, USA). Water filtered through Millipore was used during the entire HPLC procedure. All other chemicals and solvents used were of analytical grade.

3.2. Standard solutions

A stock solution of telmisartan was prepared at a concentration of 500 µg/mL in methanol and diluted to 100, 10 and 1 µg/mL in methanol for the working solutions. The stock solution of naproxen contained 1 mg/mL in methanol and was diluted to 100 µg/mL in methanol for the working solutions. Stock solutions and working solutions were maintained at 4 °C. Telmisartan standard samples (0.5, 2, 10, 100, 400, 700 and 1000 ng/mL) were prepared by spiking human plasma with appropriate volumes of the working solutions prepared as mentioned above. Quality control (QC) sam-

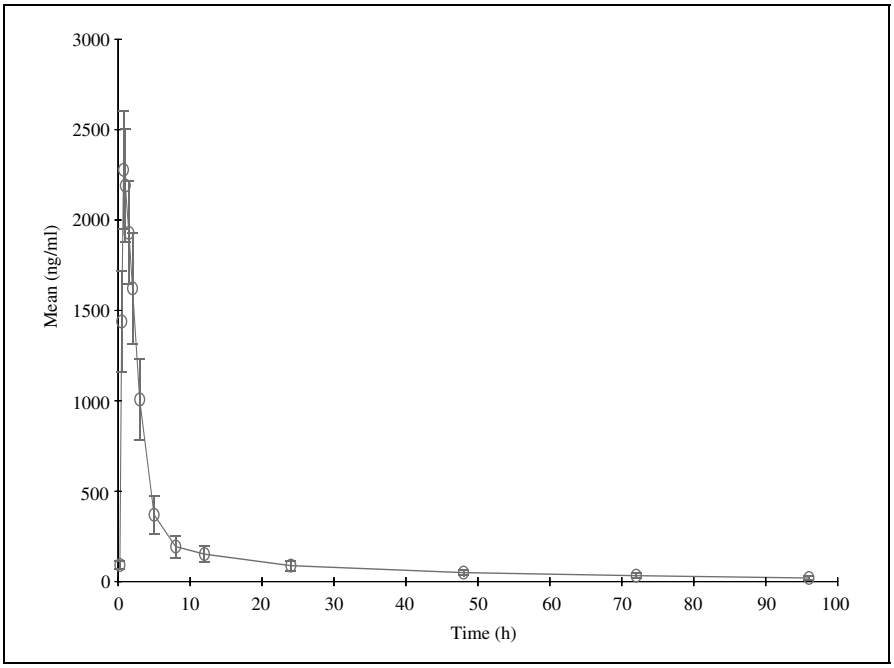

Fig. 2:
Plasma concentration-time profiles (Mean ± SE) of telmisartan in 24 healthy male volunteers following oral administration of 160 mg of telmisartan

ples (0.5, 1.0, 300 and 800 ng/mL) were independently prepared in the same manner. All standards and QC samples were stored at −20 °C until analysis.

3.3. Extraction procedure

0.5 mL of standard, QC, or an unknown human plasma sample was combined with 25 μL of working solution of I.S. and made acidic with 25 μL of 1 mol/L hydrochloric acid. The solutions were violently vortexed with 4 mL of ethyl acetate for 5 min. They were centrifuged at 4000 g for 10 min and the organic phase was transferred to a clean tube and evaporated to dryness at 50 °C with the aid of a gentle stream of N_2. 200 μL of mobile phase was added to the residue. After vortexing for 2 min and centrifugating for 5 min, the supernatant was transferred to an autosampler vial. A 20 μL volume was injected into the HPLC system for quantitation.

3.4. Chromatographic conditions

The HPLC system (Shimadzu, Kyoto, Japan) consisted of a LC-10AD pump, SIL-10A autoinjector and RF-10AXL fluorescence detector. Data were collected and analysed by Class LC-10 software (version 1.63, Shimadzu, Japan). Separation of analytes was performed using a Kromasil C_8 (AKZONOBEL, Bohus, Sweden) column (150 × 4.6 mm, 5 μm) preceded by a 0.5 μm precolumn filter (Waters, MA, USA).
The HPLC system was equilibrated with the mobile phase consisting of acetonitrile − 0.02 mol/L KH_2PO_4 (50 : 50, v/v), at a flow rate of 1.2 mL/min. The injection volume was 20 mL and the chromatographic peaks were detected at an excitation wavelength of 305 nm and an emission wavelength of 365 nm. The column temperature was maintained at 25 °C.

3.5. Assay validation

The intra-day and inter-day variability of telmisartan were assayed (six replicates) at 0.5, 1.0, 300 and 800 ng/mL on the same day and on five sequential days, respectively. Precision was characterized by the coefficients of variation (CV, %) whereas accuracy was expressed as a percentage error (PE, %) of nominal versus measured concentration.
The ability to dilute samples originally above the upper limit of the standard curve was estimated by the samples of telmisartan at a concentration of 3000 ng/mL which were diluted to 600 ng/mL by blank plasma.

3.6. Pharmacokinetics

The HPLC method developed was used to investigate the plasma profile of telmisartan after a single 160 mg oral dose (Micardis®, Boehringer Ingelheim, Germany). A clinical study on 24 male healthy volunteers was conducted under fasting conditions. Mean age ± SD was 21.0 ± 1.49 (range 19–25) and mean body weight (kg) was 70.0 ± 6.76 (range 58–89). Following written informed consent, volunteers received a 160 mg oral dose of telmisartan tablets with 250 mL water. Blood samples were collected in heparinized tubes, pre-dose and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 5, 8, 12, 24, 48, 72 and 96 hour post-dose. Plasma was immediately separated by centrifugation at 3000 g for 10 min, and stored in polypropylene tubes at −20 °C until analysis. The study protocol was approved by the Ethnic Committee of Huashan hospital.

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