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Short communication

Liquid chromatographic–tandem mass spectrometric method for the simultaneous quantitation of telmisartan and hydrochlorothiazide in human plasma

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ABSTRACT

A rapid and sensitive method using liquid chromatography–tandem mass spectrometry (LC–MS/MS) has been developed for the simultaneous determination of telmisartan and hydrochlorothiazide in human plasma. Sample preparation involved liquid–liquid extraction with diethyl ether–dichloromethane (60:40, v/v). The analytes and internal standard, probenecid, were separated on a Venusil XBP-C_8 column using gradient elution with acetonitrile–10 mM ammonium acetate–formic acid at a flow rate of 1.2 mL/min. Detection was by electrospray negative ionization mass spectrometry using multiple reaction monitoring of the transitions at m/z 513.0 → 469.4 for telmisartan, m/z 295.9 → 268.9 for hydrochlorothiazide and m/z 283.9 → 239.9 for probenecid. For both analytes, the method was linear in the range 1.00–600 ng/mL with intra- and inter-day precision (as relative standard deviation) ≤10.6% and accuracy (as relative error) ≤4.2%. The assay was successfully applied to a pharmacokinetic study in 9 healthy volunteers given a single oral dose of a combination tablet containing telmisartan 80 mg and hydrochlorothiazide 12.5 mg.

1. Introduction

Telmisartan (TEL) (Fig. 1), an angiotensin II receptor blocker, is widely used in the treatment of hypertension and heart failure [1,2]. Hydrochlorothiazide (HCT) (Fig. 1), a thiazide diuretic, is also used to treat mild to moderate hypertension, usually in combination with other antihypertensive agents with different mechanisms of action [3]. This is not only because blood pressure control is often inadequate using monotherapy but also because combination therapy can simplify dosing regimens, improve compliance, decrease side effects and reduce cost. TEL and HCT is such a combination which, although more effective than monotherapy with either drug [4], has not been investigated with respect to the pharmacokinetics of the two drugs in combination.

Analytical methods for TEL suitable for use in human pharmacokinetic studies have been reported based on high-performance liquid chromatography (HPLC) with detection by mass spectrometry (LC–MS) [5] and tandem mass spectrometry (LC–MS/MS) [6]. Both methods use electrospray ionization (ESI) in the positive ion mode and provide a limit of quantitation of 0.5–1.0 ng/mL using 50–100 μL plasma. The LC–MS/MS assay developed in our laboratory [6] is particularly advantageous in respect of high sample throughput since it has a very short cycle time of only 2.6 min. An LC–MS/MS assay for HCT equally suitable for use in human pharmacokinetic studies has also been developed but in this case it uses ESI in the negative ion mode [7]. It is noteworthy that although assay of both TEL and HCT has been performed by HPLC as part of a multiple-dose pharmacokinetic study involving concurrent administration, the two assays were carried out independently [8].

In this paper, we describe a simple and sensitive LC–MS/MS method for the simultaneous quantitation of TEL and HCT in human plasma using probenecid as internal standard (I.S.) (Fig. 1). The assay has been successfully applied to a pharmacokinetic study of a combination tablet containing TEL 80 mg and HCT 12.5 mg.

2. Experimental

2.1. Chemicals and reagents

TEL (purity 99.7%) was provided by Boehringer Ingelheim Pharma GmbH & Co. (Ingelheim, Germany); HCT (purity 99.4%)

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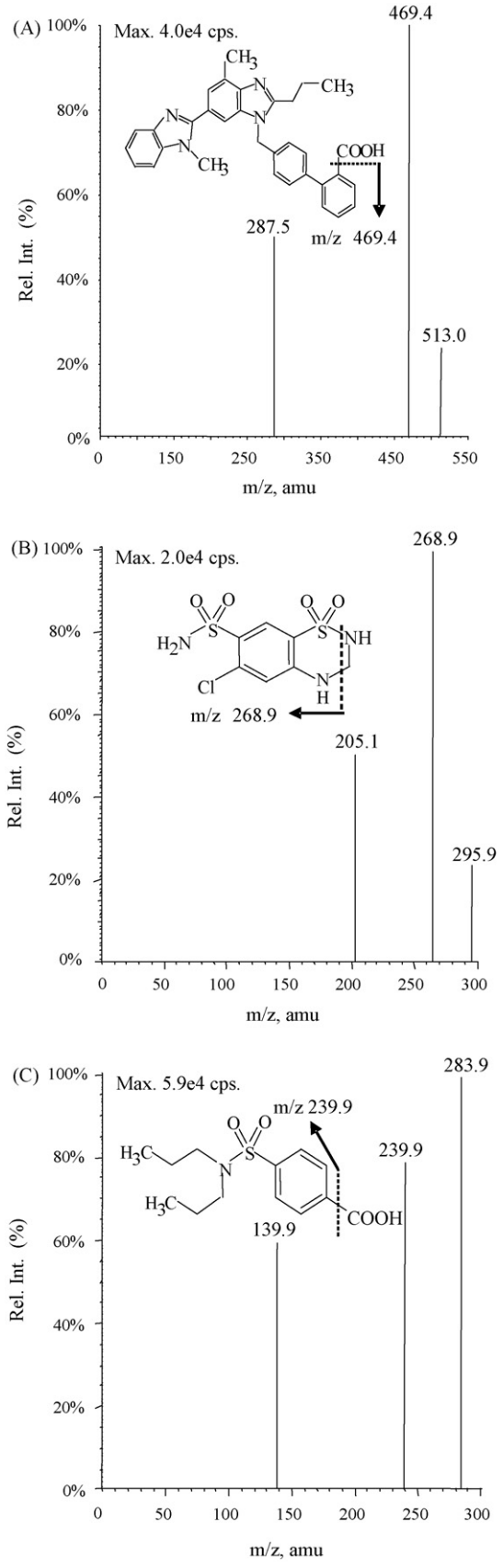

Fig. 1. Full-scan product ion mass spectra of $[M-H]^-$ ions of (A) TEL, (B) HCT and (C) probenecid.

and probenecid (purity >99.5%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile was HPLC grade purchased from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals were of analytical grade. Distilled water, prepared from demineralized water, was used throughout the study.

2.2. Instrumentation

LC–MS/MS analysis was performed using an Agilent 1100 Series HPLC system (Agilent Technologies, Palo Alto, CA, USA) coupled to an Applied Biosystems Sciex API 4000 mass spectrometer (Applied Biosystems Sciex, Ontario, Canada). Applied Biosystems/MDS Sciex Analyst Software was used for data acquisition and processing.

2.3. LC–MS/MS

Chromatography was performed on a Venusil XBP-C_8 column (4.6 mm × 50 mm i.d., 5 μm; Agela Technologies Inc., Delaware, USA) maintained at 25 °C. Gradient elution using 10 mM ammonium acetate containing 0.1% formic acid as solvent A and acetonitrile as solvent B was as follows: 0–0.2 min, 45% B; 0.2–0.8 min, 45–80% B; 0.8–1.3 min, 80–90% B; 1.3–2.0 min, 90% B; 2.0–2.5 min, 90–45% B; 2.5–3.5 min, 45% B. The flow rate was 1.2 mL/min and an approximately 1:1 (v/v) split of the column eluant was employed.

The mass spectrometer was equipped with an ESI source operating in the negative ion mode. Unit resolution was applied to both Q1 and Q3. TEL, HCT and probenecid were monitored by multiple reaction monitoring (MRM) of the transitions m/z 513.0 → 469.4, m/z 295.9 → 268.9 and m/z 283.9 → 239.9, respectively. The dwell time for each MRM transition was set at 200 ms. MS parameters, optimized by infusing solutions into the mass spectrometer using a syringe pump, were as follows: Nebulizer gas (GS1), heater gas (GS2), curtain gas and collision gas (all N_2) 45, 45, 10 and 5 psi, respectively; ion spray voltage −4000 V; source temperature 500 °C; declustering potentials (DP) and collision energies (CE), −90 V and −28 eV for TEL, −80 V and −25 eV for HCT and −60 V and −22 eV for probenecid, respectively.

2.4. Preparation of calibration standards and quality control (QC) samples

Solutions (1 mg/mL) of TEL and HCT were prepared separately in methanol. They were mixed and diluted with methanol:water (50:50, v/v) to yield a stock solution containing 50 μg/mL of each analyte. This solution was then used to spike blank plasma to give a series of calibration standards containing 1.00, 3.00, 10.0, 30.0, 100, 300 and 600 ng/mL TEL and HCT. Low, medium and high concentration QC samples containing 3.00, 30.0 and 300 ng/mL of each analyte were prepared independently using the same procedure. A 1 mg/mL stock solution of probenecid in methanol was diluted with methanol:water (50:50, v/v) to give a 500 ng/mL I.S. working solution. In each analytical run, calibration standards, QC samples and unknowns were treated together.

2.5. Sample preparation

Plasma samples stored at −80 °C were allowed to thaw at room temperature and then subjected to liquid–liquid extraction (LLE). To 100 μL plasma in a glass-stoppered 10 mL tube, 100 μL phosphate buffer (pH 7.4), 100 μL I.S. working solution and 3 mL diethyl ether–dichloromethane (60:40, v/v) were added. The mixture was vortex-mixed for 30 s, centrifuged at 2000 × g for 5 min, and the organic layer transferred to another clean glass tube and evaporated

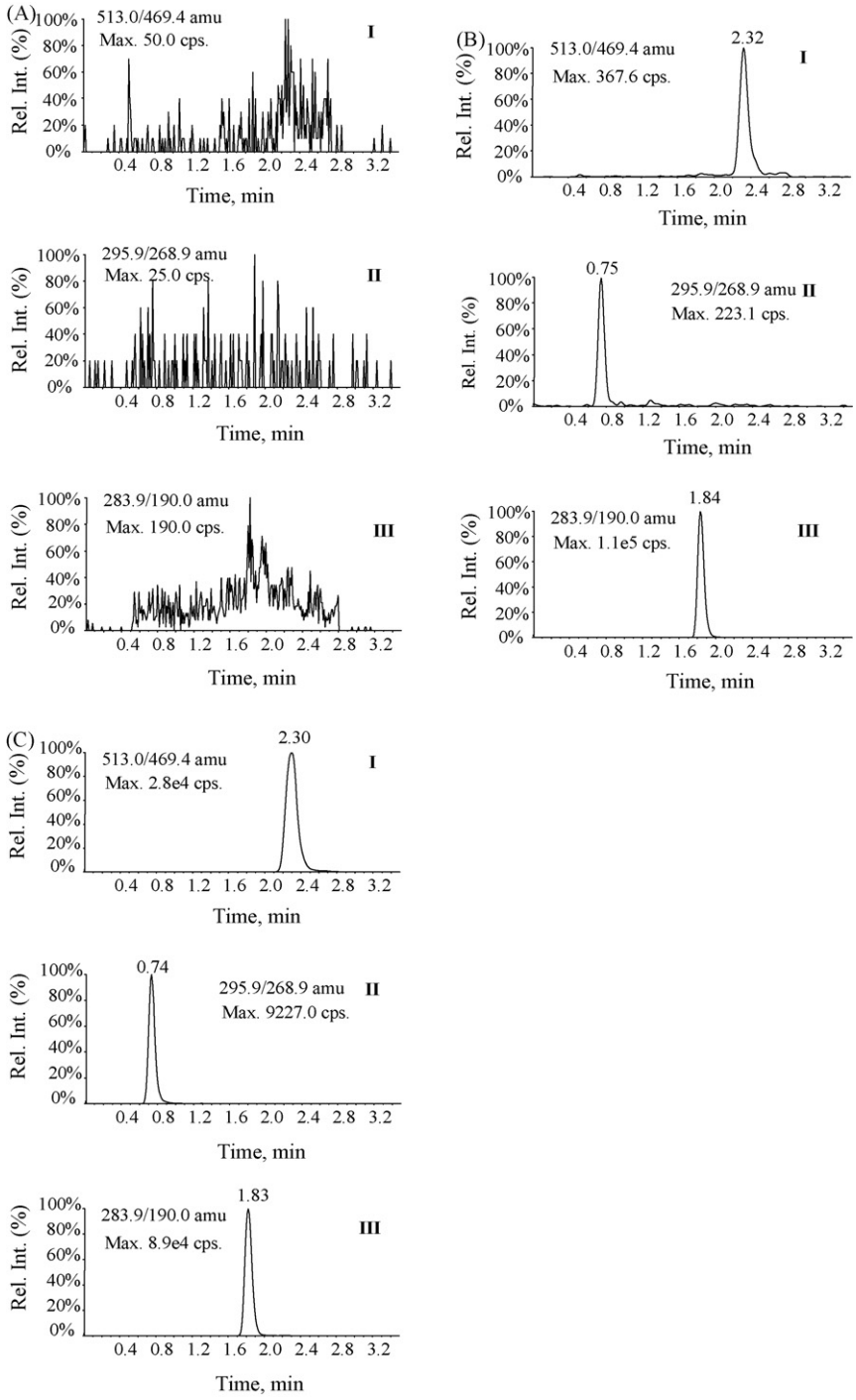

Fig. 2. Typical MRM chromatograms of (I) TEL, (II) HCT and (III) probenecid in human plasma: (A) blank plasma; (B) blank plasma spiked with 1.0 ng/mL TEL and 1.0 ng/mL HCT; and (C) a plasma sample from a human volunteer 1.5 h after oral administration of a combination tablet containing TEL 80 mg and HCT 12.5 mg.

to dryness at 40 °C with nitrogen. The residue was reconstituted in 200 μL mobile phase and 10 μL injected into the LC–MS/MS system.

2.6. Assay validation

The method was validated for specificity, linearity, precision, accuracy, recovery, matrix effects and stability according to FDA guidelines [9]. Blank human plasma samples from six different individuals were used to evaluate specificity of the method. Standard curves based on peak area ratio of analyte to I.S. were prepared in triplicate on three separate occasions. Linearity was assessed by least-squares regression with a weighting index of $1/x^2$. Intra- and inter-day precision as relative standard deviation (R.S.D.) and accuracy as relative error (R.E.) were determined on the basis of six replicates of QC samples included in each run. The lower limit of quantitation (LLOQ) was defined as the concentration with accuracy of ±15% and precision <15%. Recovery was determined in quadruplicate by comparing peak areas of QC samples to those of post-extraction blank plasma samples spiked with equivalent amounts of each analyte. Matrix effects were evaluated by compar-

Table 1
Accuracy and precision for the simultaneous determination of TEL and HCT in human plasma (data are based on assay of six replicates on three different days)

Analyte	Added conc. (ng/mL)	Found conc. (ng/mL) (mean ± S.D.)	Intra-day R.S.D. (%)	Inter-day R.S.D. (%)	R.E. (%)
TEL	3.00	3.12 ± 0.18	5.5	7.2	4.1
	30.0	30.1 ± 1.4	3.5	9.1	0.48
	300	310 ± 18	4.7	10.6	3.4
HCT	3.00	3.03 ± 0.16	5.6	1.8	1.0
	30.0	31.3 ± 1.1	3.5	2.9	4.2
	300	296 ± 9	3.1	1.9	−1.4

ing peak areas of post-extraction blank plasma samples spiked with both analytes with peak areas of post-extraction water spiked at the same concentrations. Stability tests based on analysis of QC samples in triplicate were as follows: Three freeze-thaw cycles; storage at room temperature for 6 h; storage at −80 °C for 60 days.

2.7. Pharmacokinetic study

A clinical trial was performed in 9 healthy volunteers receiving a single oral dose of a combination tablet containing TEL 80 mg and HCT 12.5 mg. The clinical protocol was approved by the Ethics Committee of Peking University First Hospital, China. All volunteers read the protocol and gave written informed consent before entering the study. Blood samples (3 mL) were collected into heparinized tubes before and at 0.25, 0.50, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12, 24, 36, 48, 72 and 96 h after administration. Plasma was separated by centrifugation at 2000 × *g* for 10 min and stored frozen at −80 °C until analysis.

3. Results and discussion

3.1. Mass spectrometry

Full-scan product ion spectra of TEL, HCT and I.S. are shown in Fig. 1. Deprotonated molecular ions for TEL, HCT and I.S. were at *m*/*z* 513.0, 295.9 and 283.9, respectively. Using the most intense product ion of each compound led to choice of the following transitions for MRM acquisition: TEL *m*/*z* 513.0 → 469.4; HCT *m*/*z* 295.9 → 268.9; and I.S. *m*/*z* 283.9 → 239.9. Transitions for TEL and probenecid arise from loss of carbon dioxide from their carboxylic acid groups whereas the transition for HCT arises from the loss of HCN.

Table 2
Stability data for TEL and HCT based on triplicate assay of QC samples

Conditions	Analyte	Added conc. (ng/mL)	Found conc. (ng/mL) (mean ± S.D.)
Three freeze-thaw cycles	TEL	3.00	3.17 ± 0.04
		30.0	31.4 ± 1.8
		300	307 ± 3
	HCT	3.00	2.96 ± 0.09
		30.0	31.0 ± 0.9
		300	304 ± 22
Stability at room temperature for 6 h	TEL	3.00	3.29 ± 0.06
		30.0	28.3 ± 0.6
		300	293 ± 3
	HCT	3.00	3.13 ± 0.10
		30.0	28.9 ± 0.6
		300	278 ± 1
Storage at −80 °C for 60 days	TEL	3.00	3.12 ± 0.09
		30.0	28.9 ± 0.7
		300	308 ± 24
	HCT	3.00	2.90 ± 0.11
		30.0	31.5 ± 2.2
		300	300 ± 13

3.2. Chromatography

Chromatography using different analytical columns and mobile phase compositions was evaluated to optimize specificity and selectivity. On C_{18} columns (Nucleosil, Hypersil, Zorbax and Venusil), TEL was subject to peak tailing using mobile phases containing acetonitrile, methanol, ammonium acetate and formic acid. However, using a Venusil XBP-C_8 column and a mobile phase composed of acetonitrile and 10 mM ammonium acetate, all analytes gave good peak shape and satisfactory retention time. In addition, inclusion of formic acid (0.1%) in the mobile phase was found to reduce matrix effects without decreasing response. Because of the different polarities of the three compounds, a gradient elution was used which shortened the retention times of TEL and I.S. without significantly affecting that of HCT. Under these conditions, retention times for TEL, HCT and I.S. were typically 2.32, 0.75 and 1.84 min, respectively and the run time was 3.5 min. Probenecid was a suitable I.S. since it ionized well in the negative ion mode, gave a similar retention time to that of the analytes and did not cause ion suppression.

3.3. Assay validation

As shown in Fig. 2A, the assay was free of interference from endogenous peaks arising from plasma. Chromatograms at the LLOQ of TEL and HCT are shown in Fig. 2B.

The assay was found to be linear for both TEL and HCT in the range 1.00–600 ng/mL with typical regression lines of $y = 0.00408x + 0.00059$ ($r = 0.9974$) and $y = 0.00204x + 0.00006$ ($r = 0.9978$), respectively. For both analytes, the LLOQ was 1.00 ng/mL.

As shown in Table 1, intra- and inter-day precisions were, respectively, <5.5% and <10.6% for TEL and <5.6% and <2.9% for HCT. Accuracy ranged from 0.48 to 4.1% for TEL and −1.4 to 4.2% for HCT.

At low, medium and high QC concentrations, the recoveries were, respectively, 79.4 ± 3.7%, 84.2 ± 3.1% and 83.1 ± 1.4% for TEL and 73.7 ± 2.6%, 72.3 ± 2.5% and 72.6 ± 1.6% for HCT. The mean recovery of the I.S. was 52.3 ± 3.1%.

Matrix effects were absent as shown by the fact that concentrations of analytes as a percentage of nominal concentrations for low, medium and high QC samples were, respectively, 102 ± 3.7%, 108 ± 4.9% and 106 ± 2.3% for TEL and 98.0 ± 1.6%, 104 ± 1.2% and 98.3 ± 0.3% for HCT. The percent nominal concentration of I.S. was 98.2 ± 6.6%.

Table 3
Pharmacokinetic parameters for TEL and HCT after oral administration of a combination tablet (data are mean ± S.D., $n = 9$)

Parameter	TEL	HCT
T_{max} (h)	1.22 ± 0.44	1.50 ± 0.25
C_{max} (ng/mL)	344 ± 157	82.4 ± 26.0
AUC_{0-t} (ng h/mL)	2456 ± 958	510 ± 122
$t_{1/2}$ (h)	26.1 ± 4.8	7.75 ± 0.95

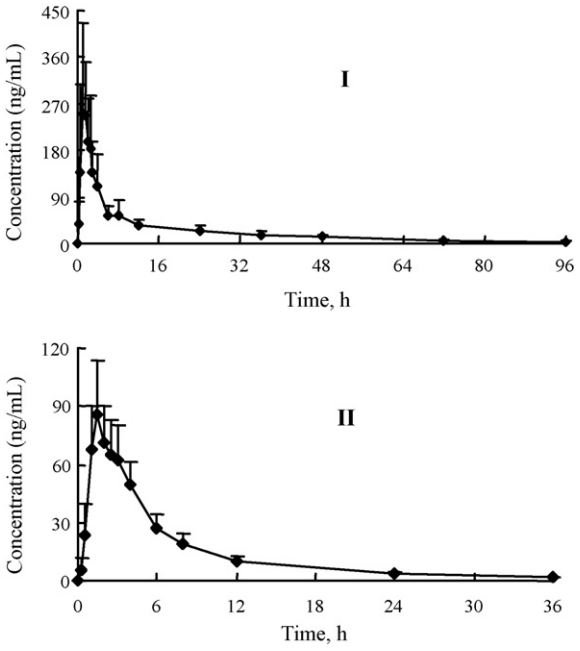

Fig. 3. Mean plasma concentration–time profiles for (I) TEL and (II) HCT after oral administration of a combination tablet containing TEL 80 mg and HCT 12.5 mg (data are mean ± S.D., $n = 9$).

Results of stability tests are summarized in Table 2. There was no significant degradation of either analyte on storage at −80 °C for 60 days, after three freeze-thaw cycles and on storage at room temperature for 6 h.

3.4. Pharmacokinetic study

Plasma concentration–time profiles and corresponding pharmacokinetic parameters for TEL and HCT after a single oral dose of a combination tablet containing TEL 80 mg and HCT 12.5 mg are shown in Fig. 3 and Table 3, respectively. Li et al. [6] reported pharmacokinetic parameters for an oral 80 mg dose of TEL alone of C_{max} 507 ± 100 ng/mL, $t_{1/2}$ 20.4 ± 7.7 h and AUC_{0-t} 3200 ± 1650 ng h/mL. Corresponding parameters for an oral 12 mg dose of HCT alone reported by Liu et al. [7] were C_{max} 68.8 ± 26.3 ng/mL, $t_{1/2}$ 8.98 ± 2.41 h and AUC_{0-t} 421 ± 155 ng h/mL. These data appear to indicate that, in combination, TEL and HCT show mutual inhibition of absorption. However, in a study involving multiple doses of TEL 160 mg plus HCT 25 mg, Yong et al. [8] reported pharmacokinetic parameters that were not significantly different from the corresponding parameters for each drug administered alone at the same dose. Therefore, further research is required to substantiate the foregoing putative pharmacokinetic drug interaction.

4. Conclusions

A rapid and sensitive LC–MS/MS method has been developed for the simultaneous quantitation of TEL and HCT in human plasma. The assay involves relatively simple sample preparation, has a short run time and gives good accuracy and precision. The assay has been successfully applied to a pharmacokinetic study involving a single oral dose of a combination tablet containing TEL 80 mg and HCT 12.5 mg in healthy volunteers.

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