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Development and validation of liquid chromatography–tandem mass spectrometric method for simultaneous determination of fosinopril and its active metabolite fosinoprilat in human plasma

Cui Shuangjin [a], Feng Fang [a,*], Ma Ming [a], Liu Han [a], Chen Yun [b]

[a] *Department of Pharmaceutical Analysis, China Pharmaceutical University, Nanjing 210009, China*
[b] *Institute of Dermatology, Chinese Academy of Medical Sciences and Peking Union Medical College, Nanjing 210042, China*

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Abstract

A highly sensitive and selective liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was developed and validated for simultaneous determination of the prodrug fosinopril and its major active metabolite fosinoprilat for pharmacokinetic studies in healthy subjects. In order to get the lower limit of quantification (LLOQ), especially for analysis of fosinopril, key points of the method have been investigated including chromatographic conditions and selection of LC–MS/MS conditions. The analytes were extracted from plasma samples by liquid–liquid extraction, separated on a reversed-phase C_8 column using gradient procedure, and detected by tandem mass spectrometry with a triple quadrupole ionization interface. The analytes and internal standard zaleplon were detected using positive electrospray ionization (ESI) in the SRM mode. The LLOQ of the method down to 0.1 ng mL^{-1} for fosinopril and 1.0 ng mL^{-1} for fosinoprilat were identifiable and reproducible. The standard calibration curves for both fosinopril and fosinoprilat were linear over the ranges of 0.1–15.0 and 1.0–700 ng mL^{-1} in human plasma, respectively. The within- and between-batch precisions (relative standard deviation (RSD)%) and the accuracy were acceptable. The validated method was successfully applied to reveal the pharmacokinetic properties of fosinopril and fosinoprilat after oral administration.

Keywords: Fosinopril; Fosinoprilat; Liquid chromatography–tandem mass spectrometry; Human plasma; Pharmacokinetics

1. Introduction

Fosinopril sodium, an angiotensin-converting enzyme (ACE) inhibitor, is a phosphinic acid ester prodrug, which is in vivo rapidly hydrolyzed to the pharmacologically active diacid, fosinoprilat [1,2]. Fosinoprilat, which has been shown to be effective in the treatment of hypertention, is cleared by both hepatic and renal routes [3,4].

Because of lower oral dosage (usually 10–20 mg) and rapid metabolism to active metabolite [5], the concentration of the parent drug fosinopril in human plasma is very low. Till now, to the best of our knowledge, few data are available regarding pharmacokinetics of fosinopril in humans because of the lack of highly sensitive analytical methods. As it was important to study the distribute behaviors of fosinopril in vivo and to indicate the possible action mechanism of parent drug, an assay capable of quantifying fosinopril at concentration down to level of pg mL^{-1} is essential.

Several methods have been reported to determine fosinoprilat in biological fluids [6–9,11], while all of them have their own disadvantages to analysis of batches of biological samples. Gas chromatography with nitrogen–phosphorus detection (NPD) needed time-consuming derivatization [6]. Liquid chromatography (LC) using microemusion as eluent took long chromatographic run time [7]. Radioimmunoassay (RIA) lacked specificity [8,9]. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) method referred complex extraction procedures [10]. And all the methods above could not analyze fosinopril and fosinoprilat simultaneously for pharmacokinetic study, although HPLC-UV and CE methods [11–15] have been used for the determination of fosinopril and fosinoprilat in pharmaceutical formulations.

The purpose of this work was to develop a simple, sensitive high-performance liquid chromatography/positive ion electro-

* Corresponding author.
E-mail address: fengfang1@126.com (F. Feng).

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spray tandem mass spectrometry method for the simultaneous quantification of fosinopril and fosinoprilat in human plasma to reveal the characters of pharmacokinetics of both compounds, especially those of fosinopril.

2. Experimental

2.1. Chemicals and reagents

Fosinopril (98.0%), fosinoprilat (98.0%) and zaleplon (internal standard, 99.0%) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol (HPLC-grade) was purchased from Merck Company (America). Ammonium acetate (analytical grade) and hydrochloric acid were from Nanjing Chemical Co. (Nanjing China). Distilled water, doubly distilled in our laboratory, was used throughout the study. Dichlormethane, diethyl ether and other chemicals used were all of analytical grade and from Nanjing Chemical Co. (Nanjing China).

2.2. Instrumentation

A Thermo Electron TSQ Quantum ultra tandem triple quadrupole mass spectrometer equipped with electrospray ionization (ESI) source (San Jose, CA, USA), an Electron surveyor LC pump and autosampler were used in tandem for gradient elution. The data processing was carried out using Xcalibur 1.1 software (Thermo-Electron). Peak integration and calibration were carried out using LC Quan software (Thermo-Electron).

2.3. Chromatographic conditions

Chromatography was performed on a LiChrospher-C_8 column (250 mm × 4.6 mm I.D. 5 μm, Hanbon Ltd., Jiangsu, China). The column was maintained at 25 °C.

A gradient elution procedure was used by on-line mixing eluent A and eluent B with liquid flow-rate 1.0 mL min^{-1} and post-column split ratio 1:1. Methanol was used as eluent A and 10 mmol L^{-1} ammonium acetate aqueous was as eluent B. The gradient procedure was as follows: from 0 to 2.0 min, 40% B; from 2.01 to 10.0 min, 15% B; from 10.01 to 12.0 min, 40% B.

2.4. Mass spectrometric conditions

The mass spectrometer was operated in the positive ion detection mode. Quantification was performed using selected reaction monitoring (SRM) of the transitions of *m/z* 436.01 → *m/z* 390.05 for both fosinopril and fosinoprilat (see Section 3), and the transitions of *m/z* 305.98 → *m/z* 263.97 for zaleplon (as internal standard, I.S.) with a scan time of 0.5 s per transition.

Nitrogen was used as the sheath gas, ion sweep gas, and auxiliary gas, with the optimum values set at 35, 0, and 5 psi, respectively. The spray voltage was 3500 V. Temperature of the heated capillary was set to 350 °C. Argon was used as collision gas at a pressure of approximately 0.5 m Torr and the optimized collision energy was −20 eV. The mass spectrometer was operated at unit mass resolution (peak width at half-height set at 0.7) for both Q1 and Q3.

2.5. Preparation of standard and quality control samples

Stock solutions of fosinopril and fosinoprilat were prepared by dissolving the accurately weighed reference compounds in methanol to give a final concentration of 50 μg mL^{-1} for both. Solution of I.S. was prepared in methanol at the concentration 50 μg mL^{-1} and diluted to 500 ng mL^{-1} with methanol.

Blank human plasma (drug free) was obtained from Nanjing Blood Donor Service (Jiangsu, China). Calibration curves were prepared by spiking appropriate standard solutions of the parent drug and its active metabolite, respectively, to 1.0 mL of blank plasma. Concentrations in plasma samples were 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 15.0 ng mL^{-1} for fosinopril and 1.0, 5.0, 10.0, 50.0, 150, 400, 700 ng mL^{-1} for fosinoprilat. Quality control (QC) samples were separately prepared in blank plasma samples (1.0 mL) at the concentration of 0.5, 5.0, 15.0 ng mL^{-1} for fosinopril and of 5.0, 400, 700 ng mL^{-1} for fosinoprilat, respectively. The spiked plasma samples (standards and quality controls) were extracted on each analytical batch along with the unknown samples and samples thus made were stored at –20 °C until analysis.

2.6. Sample preparation

Plasma was obtained by centrifugation of blood treated with the anticoagulant sodium heparin. To a 1.0 mL aliquot of plasma sample, 20 μL of internal standard (500 ng mL^{-1} zaleplon in methanol), 200 μL of hydrochloric acid (1.0 mol L^{-1}) were added. The samples were briefly mixed, and 5 mL of mixed solvent ether–dichloromethane (3:1, v/v) were added. The mixture was vortex-extracted for 3 min. After centrifugation at 4000 × *g* for 10 min, the upper organic layer was removed and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The dry residues were reconstituted with 100 μL of solution consisted of eluents A and B (8:2) and 20 μL aliquot was injected into the LC–MS/MS system for analysis.

2.7. Method validation

The method validation assays were carried out according to the currently accepted U.S. Food and Drug Administration (FDA) guidance for Industry (Bioanalytical Method Validation), May 2001 [16].

The specificity of the method was tested by analyzing blank plasma samples from six healthy humans. Each blank sample was tested for interference using the proposed extraction procedure and chromatographic/mass spectroscopic conditions compared with an aqueous solution of the analytes at a concentration near the lower limit of quantification (LLOQ).

The matrix effect experiments were carried out by extracting blank plasma from six different sources, reconstituting the final extract in mobile phase containing a known amount of the analytes, analyzing the reconstituted extracts and then comparing the peak areas of the analytes with those of standard solutions

consisted of eluents A and B (8:2). If the ratio is <85% or >115%, an exogenous matrix effect is implied.

Plasma samples were quantified using the peak area ratios of fosinopril and fosinoprilat to that of the I.S. and standard curves in the form of $y = A + Bx$, where y represents the plasma concentration of analytes and x represents the ratios analytes peak area to that of I.S. To evaluate linearity, plasma calibration curves were prepared and were analyzed on 5 separate days.

The accuracy and precision were also determined by replicate analyses ($n = 5$) of QC samples at three concentration levels in three separated days. The accuracy was expressed by (mean observed concentration)/(spiked concentration) × 100% and the precision by relative standard deviation (RSD%). The concentration of each sample was determined using the calibration curve and analyzed on the same day.

The extraction recoveries of fosinopril and fosinoprilat at three QC levels were evaluated by comparing peak areas of analytes obtained from plasma samples with those obtained from the standard solutions at the same concentration.

Freeze and thaw stability: QC plasma samples at three concentration levels were stored at the storage temperature for (−20 °C) for 24 h and thawed unassisted at room temperature. When completed thawed, the samples were refrozen for 24 h under the same conditions. The samples were analyzed after five freeze (−20 °C)–thaw (room temperature) cycles.

Short-term temperature stability: QC plasma samples at three concentration levels were kept at room temperature for a period that exceeded the routine preparation time of the samples (about 6 h).

Long-term stability: QC plasma samples at three concentration levels kept at low temperature (−20 °C) were studied for a period of 5 days.

Postpreparative stability: The autosampler stability was conducted by re-analyzing extracted QC samples kept under autosampler conditions (4 °C) for 24 h.

Stock solution stability: The stability of fosinopril and fosinoprilat and the I.S. working solutions were evaluated at room temperature for 2 weeks.

Standard curves in each analytical run were used to calculate the concentrations of fosinopril and fosinoprilat in the unknown samples in the run. They were prepared along with the unknown samples in the same batch and analyzed in middle of the run. The QC samples in duplicates at three concentrations (0.5, 5.0, 15.0 ng mL^{-1} for fosinopril and 5.0, 400, 700 ng mL^{-1} for fosinoprilat) were prepared and were analyzed along with processed test samples at intervals in each batch.

2.8. Application of the assay

To demonstrate the reliability of this method for the study of pharmacokinetics of fosinopril and fosinoprilat, it was used to determine concentrations of both in plasma samples 0–36 h after administration of 20 mg fosinopril sodium to 20 healthy Chinese volunteers. The pharmacokinetic study approved by the Ethics Committee. All volunteers gave written informed consent to participate in the study according to the principles of the Declaration of Helsinki. Blood samples were drawn in heparinized tubes at 0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10, 12, 15, 24 and 36 h after oral administration. The obtained plasma samples were immediately separated by centrifugation at 2000 × g for 10 min and stored frozen at −20 °C until analysis.

3. Results and discussion

3.1. Selection of LC–MS/MS conditions

Fosinopril and fosinoprilat contain a phosphinate group, a carboxy group and amide group chemically. Theoretically, they could be determined in both positive ESI mode and negative ESI mode. At the initial period of study, the possibility of using positive mode and negative mode under electrospray ionization source was investigated. In the end, the positive mode was chosen since the sensitivity for the parent drug fosinopril and its active metabolite was adequate for the clinical pharmacokinetic studies under a low dosage of 20 mg.

During the study, it was found that MS parameters had a crucial impact on the responses of both fosinopril and fosinoprilat. At the beginning, all the MS parameters were optimized automatically by instrument itself under flow injection mode to get high signal intensities of fosinopril and fosinoprilat. Fig. 1A and B shows the product-ion spectra of the $[M+H]^+$ ions of fosinopril (A), fosinoprilat (B) thus obtained. But very small signal for both analytes was observed when HPLC was coupled with MS/MS, even if different eluting conditions were used. A standard solution (1.0 μg mL^{-1}) of fosinopril and fosinoprilat were then infused into the HPLC/MS/MS system to optimize the MS parameters one by one manually. An important phenomenon emerged while collision energy was changed. The protonated molecules $[M+H]^+$ (m/z 436.01) for the active metabolite fosinoprilat was broken to pieces remarkably when the collision pressure changed from 0 to 0.7 m Torr. The main fragment ion at m/z 390.05 showed high MS signal intensity. While for fosinopril, when the collision pressure raised, signal detected by the m/z 436.01 → m/z 390.05 SRM channel became obvious (Fig. 2A, peak II) and the area of chromatographic peak was about 40 times large than that detected by the m/z 564.70 → m/z 436.29 SRM channel (Fig. 2A, peak I). Signal detected by the m/z 564.70 → m/z 390.05 SRM channel (Fig. 2B, peak III) was much smaller than that by the m/z 436.01 → m/z 390.05 SRM channel (Fig. 2B, peak IV). In the end, the m/z 436.01 → m/z 390.05 SRM channel was chosen for quantification of fosinopril to get high sensitivity.

Several compounds, such as enalaprilat, telmisartan and zaleplon, were investigated to be used as an I.S. It was found that the retention time of enalaprilat is not suitable. Telmisartan, although also an acid, had unstable recovery under the selected conditions. Zaleplon, which had stable MS response, reproducible recovery and a suitable retention time, was selected in the end. In the positive ESI mode, selected reaction monitoring (SRM) of the transition of m/z 305.98 → m/z 263.97 was chosen for zaleplon (Fig. 1C).

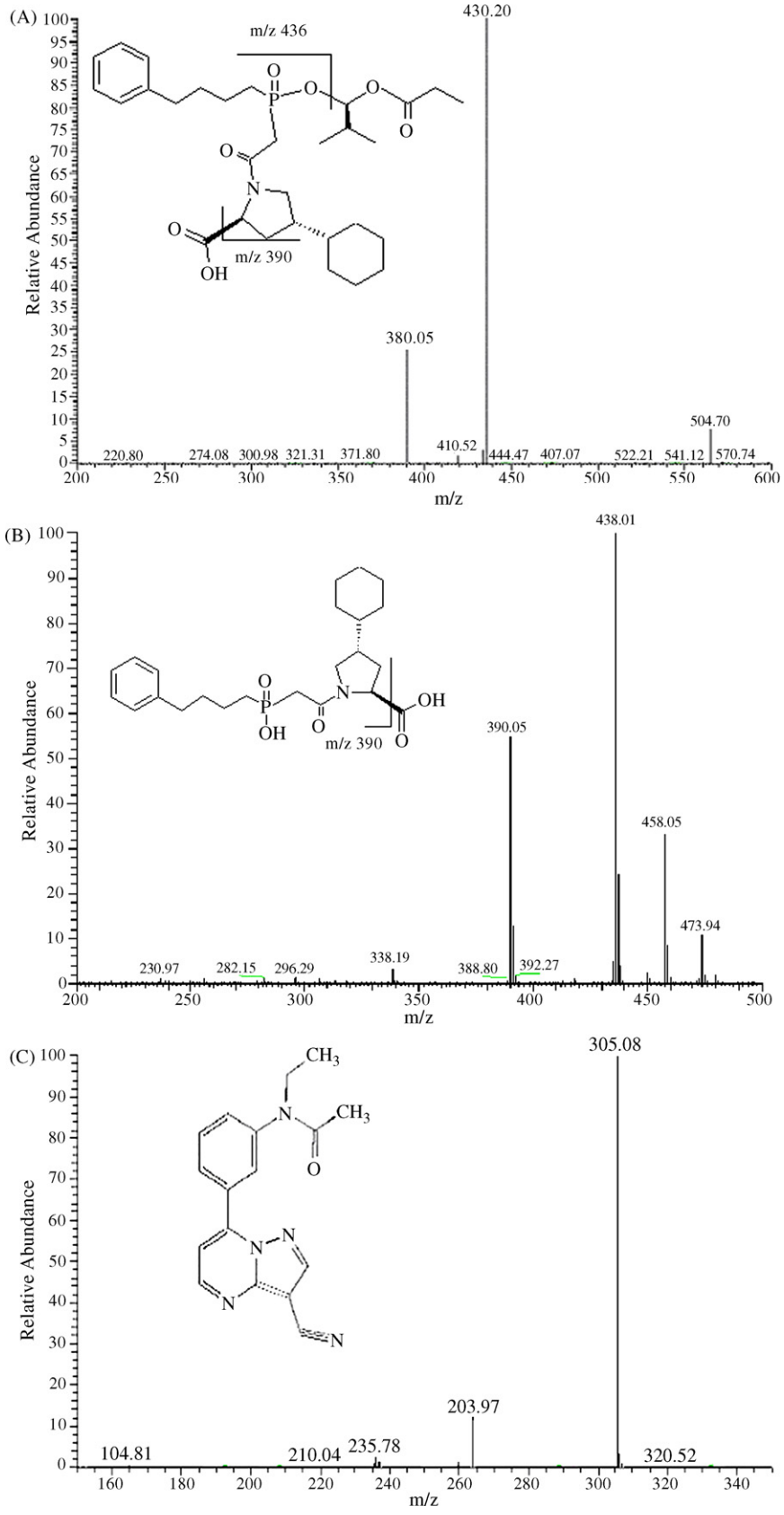

Fig. 1. Product-ion spectra of the $[M+H]^+$ ions of fosinopril (A), fosinoprilat (B) and zaleplon (C).

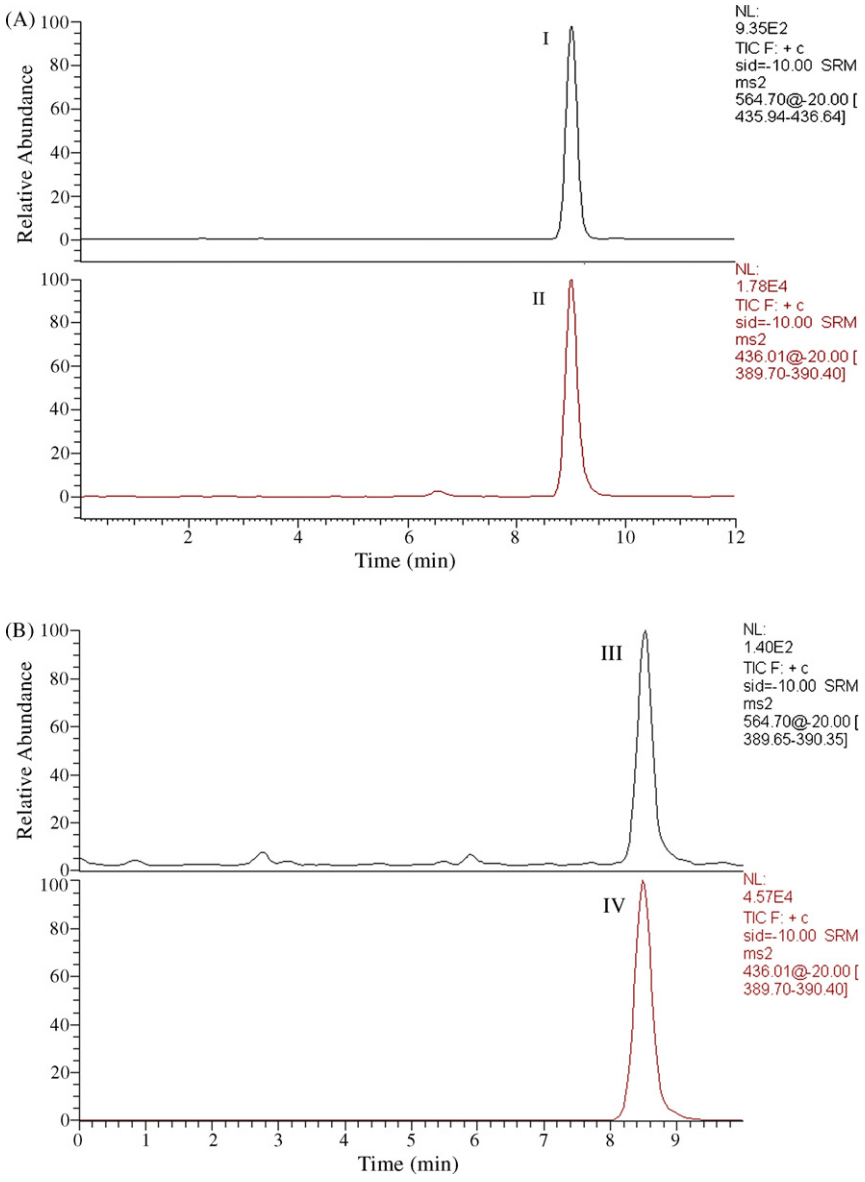

Fig. 2. Selected reaction monitoring chromatograms of fosinopril with different SRM channels. (A) Selected reaction monitoring chromatograms of fosinopril (50 ng mL^{-1}) with two SRM channels: I: *m/z* 564.70 → 436.29; II: *m/z* 436.01 → 390.05. (B) Selected reaction monitoring chromatograms of fosinopril (100 ng mL^{-1}) with two SRM channels: III: *m/z* 564.70 → 390.05; IV: *m/z* 436.01 → 390.05.

3.2. Optimization of chromatographic conditions

High sensitivity was very important in this study, especially to the parent drug fosinopril. It was found that although the mass spectra revealed higher signals for fosinopril and fosinoprilat, the LLOQ of fosinopril was not adequate when HPLC was coupled with MS system. Considering that the possibility of phosphorylated compounds be adsorbed and even trapped in the stainless steel surfaces in LC–ESI-MS hardware [17], we attempted methanol–0.1% ammonia aqueous according to the published literature [18]. No improvement was got and retention time for the analytes on the analytical column was small. Several other mobile phases were investigated later including addition of 10 mmol L^{-1} ammonium acetate or 0.1% formic acid to organic and aqueous phases. It was found that the presence of a low amount of ammonium acetate in the HPLC eluent could not only improve the sensitivity by promoting the ionization of the analytes and optimize peak shape, but also release fosinoprilat adsorbed. And methanol has the merit to produce higher sensitivity and lower background noise on ESI interface than acetonitrile.

There are carboxy group and phosphinic acid group in the structure of fosinoprilat, while fosinopril is the phosphinic acid ester prodrug. The difference between the polarity of both analytes made it necessary to use gradient procedure, or else a run time would be long when isocratic procedure be used. To achieve quick separation on baseline and eliminate the matrix effect, the gradient procedure was designed carefully. It was found that if the percentage of eluent B were not suitable, either double-peak shape for fosinoprilat, or peaks overlap for fosinopril and fosinoprilat would appear.

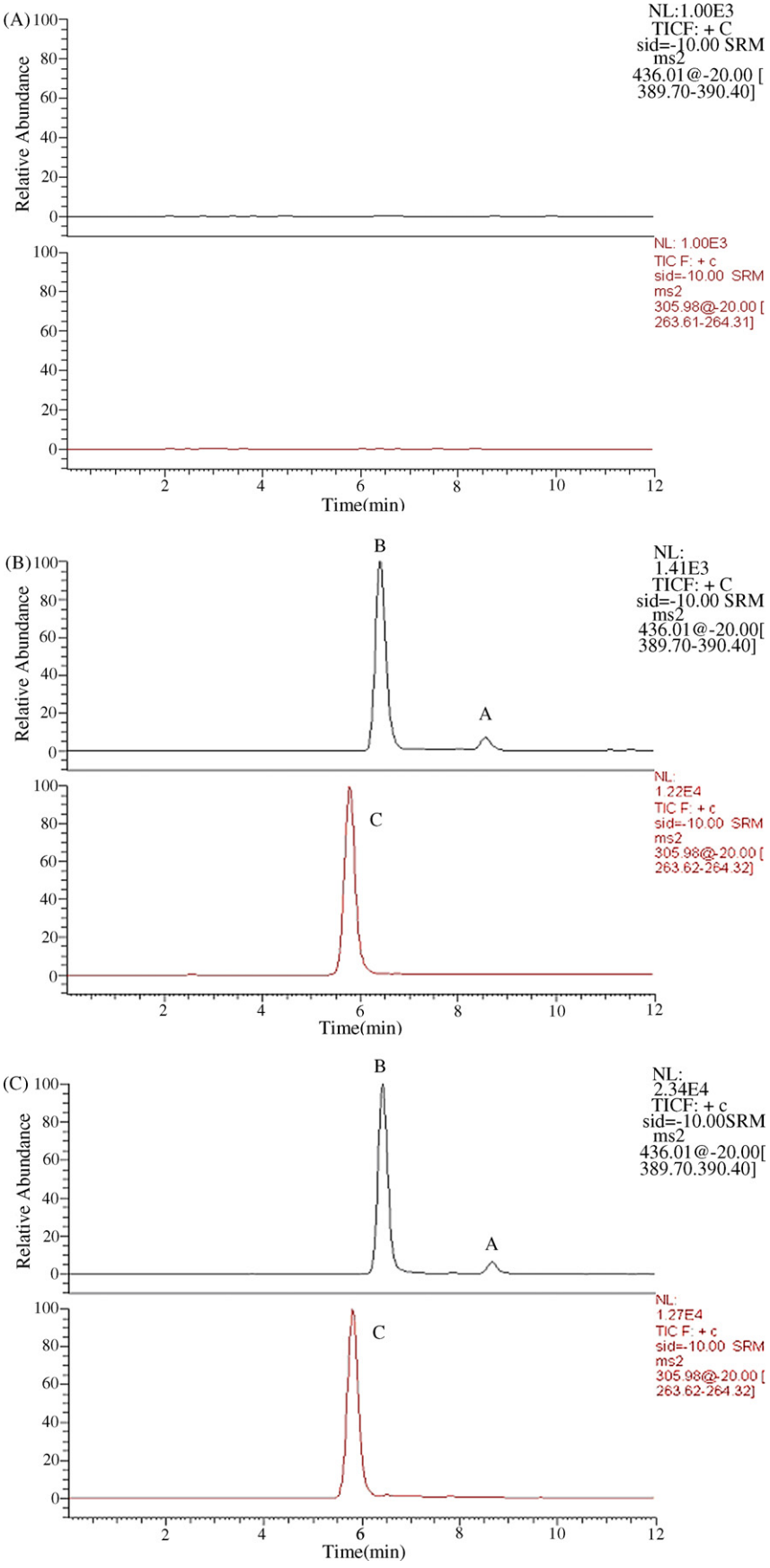

Fig. 3. Representative SRM chromatograms of fosinopril (A), fosinoprilat (B) and I.S. (C) in human plasma. (A) Blank plasma sample. (B) Plasma sample spiked with A (0.1 ng mL^{-1}), B (1.0 ng mL^{-1}) and C (20.0 ng mL^{-1}). (C) volunteer plasma sample 4.0 h after oral dose of 20 mg fosinopril sodium.

Fig. 3 shows the typical chromatograms of a blank plasma sample, a blank plasma sample spiked with fosinopril (0.1 ng mL^{-1}) and fosinoprilat (1.0 ng mL^{-1}) and zaleplon (20 ng mL^{-1}), and a plasma sample from a healthy volunteer 4.0 h after an oral administration. There is no interference from endogenous substances to the analytes and I.S. Typical retention times for fosinopril, fosinoprilat and zaleplon were 8.7, 6.4 and 5.8 min, respectively.

No matrix effect for fosinopril (RSD = 3.6%) and fosinoprilat (RSD = 3.0%) was observed for the six different plasma pools indicating that no undetected co-eluting compounds that could influence the ionization of the analytes.

3.3. Sample preparation

Jemal and Mulvana [10] used solid-phase cartridge to extract fosinopril and its metabolite from plasma samples. In the present experiment, a liquid–liquid extraction method was evaluated. Since fosinopril and fosinoprilat (diacid metabolite) are both acidic compounds, acidification of plasma samples could not only get higher ratio of extraction for fosinopril and fosinoprilat, but also reduce the rate of hydrolysis of fosinopril to fosinoprilat. Three different acids including hydrochloric acid, phosphoric acid and formic acid (1 mol L^{-1}) with different volume 100, 200 and 300 μL were tested then. The better and more reproducible recovery was obtained with 200 μL of hydrochloric acid (1 mol L^{-1}).

Different extraction solvents were also investigated. They were dichloromethane, diethyl ether, ethyl acetate, hexamethylene, ether–dichloromethane (1:1, v/v), ether–dichloromethane (2:1, v/v), diethyl ether–dichloromethane (3:1, v/v), and diethyl ether–dichloromethane (4:1, v/v). Finally, ether–dichloromethane (3:1, v/v) was selected to get a recovery of extraction of about 80%.

3.4. Linearity, precision, accuracy and lower limits of quantification

Visual inspection of the plotted duplicate calibration curves and correlation coefficients >0.999 confirmed that the calibration curves of fosinopril and fosinoprilat were linear over the concentration range of 0.1–15 ng mL^{-1}, 1.0–700 ng mL^{-1}, respectively. The relative calibration graphs are given, respectively, by the equations $y = (0.500 \pm 0.282) + (11.953 \pm 0.611)x$ for fosinoprilat and $y = (0.0164 \pm 0.0164) + (8.6741 \pm 0.641)x$ for fosinopril. Where y represents the plasma concentration of analytes and x represents the ratios analytes peak area to that of I.S. Results of the calibration curves for fosinopril and fosinoprilat LC–MS/MS determination are given in Table 1.

The lower limits of quantification (LLOQ) offered by the present LC–MS/MS was 0.1 ng mL^{-1} for fosinopril and 1.0 ng mL^{-1} for fosinoprilat, which was more sensitive than the reported methods [6–10].

The method showed good precision and accuracy. Table 2 summarizes the within- and between-batch precisions and accuracies for fosinopril and fosinoprilat from QC samples, respectively. In this assay, the accuracy deviation values are within 15% of the actual values. The precision determined at each concentration level does not exceed 10% of the relative standard deviation.

3.5. Extraction recovery and storage stability

The recoveries observed (value ± SD standard deviation, $n = 5$) were 54.6 ± 6.1, 55.1 ± 3.1 and 61.5 ± 3.0% (0.5,

Table 1
Results of calibration curves for fosinopril and fosinoprilat LC–MS/MS determination

Fosinopril				Fosinoprilat			
Added C. (ng mL^{-1})	Mean	RSD (%)	Accuracy (%)	Added C. (ng mL^{-1})	Mean	RSD (%)	Accuracy (%)
0.1	0.11	8.55	105.1	1.0	1.09	4.20	111.5
0.2	0.20	7.26	97.5	5.0	4.43	3.37	90.8
0.5	0.45	4.41	90.8	10.0	9.69	3.24	99.2
1.0	1.04	5.59	103.9	50.0	49.41	7.19	101.2
2.0	2.04	1.34	102.0	150.0	147.7	3.54	100.9
5.0	4.96	2.33	99.2	400.0	388.4	3.77	99.6
15.0	15.00	0.19	100.0	700.0	683.4	1.04	100.0

$n = 5$.

Table 2
The within-and between-batch precision and accuracy of the method for determination of fosinopril and fosinoprilat in human plasma ($n = 3$ days, five replicates per day)

Compounds	Added C. (ng mL^{-1})	Found C. (ng mL^{-1})	Within-batch RSD (%)	Between-batches RSD (%)	Mean accuracy (%)
Fosinopril	0.2	0.21 ± 0.01	3.61	6.88	104.3
	5.0	5.13 ± 0.09	1.47	2.60	102.6
	15.0	16.1 ± 0.43	2.64	0.66	107.4
Fosinoprilat	5.0	5.09 ± 0.15	2.31	5.20	101.8
	400	431.5 ± 9.36	2.00	2.61	107.9
	700	771.0 ± 9.36	1.13	1.16	110.1

Table 3
The stability of fosinopril and fosinoprilat in human plasma at different levels ($n = 5$)

	Accuracy (mean ± SD%)					
	Fosinopril			Fosinoprilat		
	0.2 (ng mL^{-1})	5.0 (ng mL^{-1})	15.0 (ng mL^{-1})	5.0 (ng mL^{-1})	400 (ng mL^{-1})	700 (ng mL^{-1})
Freeze and thaw stability	94.0 ± 1.0	96.7 ± 2.7	110.0 ± 2.9	109.8 ± 1.3	114.2 ± 0.5	112.2 ± 0.7
Short-term stability	86.6 ± 1.5	94.8 ± 3.2	106.8 ± 1.9	106.3 ± 1.1	110.1 ± 3.7	114.1 ± 0.2
Long-term stability	85.8 ± 8.7	100.5 ± 7.8	114.7 ± 4.8	113.6 ± 0.2	112.7 ± 0.2	112.9 ± 1.2
Postpreparative stability	95.8 ± 4.3	101.2 ± 2.1	104.9 ± 2.8	103.9 ± 3.3	107.6 ± 2.1	113.0 ± 3.1

5.0, 15.0 ng mL^{-1}, respectively) for fosinopril, 91.4 ± 1.8, 81.8 ± 1.2 and 95.1 ± 1.7% (5.0, 400, 700 ng mL^{-1}, respectively) for fosinoprilat. Fosinopril gave by the lower recovery, but since, reproducibility was evaluated positively, the relative standard deviation of different concentrations were all below 11.2%, this result was considered acceptable, especially taking into account the adequate LLOQ.

Table 3 summarized the freeze and thaw stability, short-term stability, long-term stability and postpreparative stability data of fosinopril and fosinoprilat. All the results showed good stability during these tests and there were no stability-related problems during the routine analysis of samples for pharmacokinetic and bioavailability studies. The stability of the working solutions was tested at room temperature. The stock solutions of fosinopril and fosinoprilat were stable for 2 weeks. The solution of I.S. was proved stable for 2 months.

3.6. Application

The method was applied to determine the plasma concentration of fosinopril and fosinoprilat after an oral administration of fosinopril sodium (20 mg) in 20 healthy Chinese volunteers (between 18 and 25 years old). Mean plasma concentration–time profiles of fosinopril and fosinoprilat are presented in Fig. 4. The main pharmacokinetic parameters of fosinopril and fosinoprilat in 20 volunteers were calculated. After oral administration of 20 mg fosinopril, the mean C_{max}-values for fosinopril sodium and fosinoprilat were 4.61 ± 2.34 and 409.43 ± 136.28 ng mL^{-1}, respectively. Corresponding mean

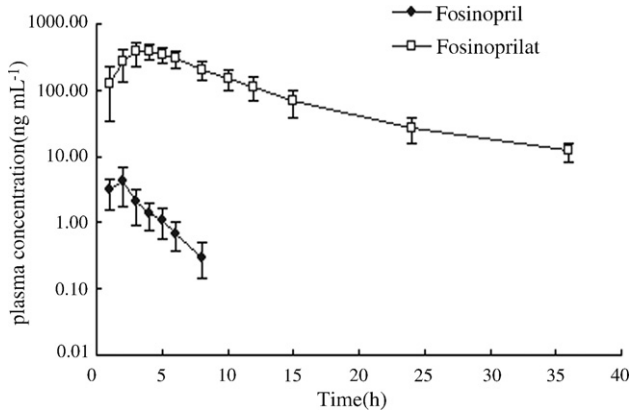

Fig. 4. Mean plasma concentration–time profiles of fosinopril and fosinoprilat after an oral administration of 20 mg fosinopril sodium in 20 healthy volunteers. ($n = 20$, $x \pm SD$).

T_{max}-values were 1.21 ± 0.42 h for fosinopril and 3.74 ± 0.87 h for fosinoprilat, respectively. The mean plasma elimination half-life of fosinopril was 2.72 ± 1.75 h and for fosinoprilat was 7.25 ± 0.81 h.

Because of the lack of sensitive determining method, it has never been reported to study pharmacokinetics of fosinopril in human being [6–10,18–20]. The present method makes it possible to determine the concentration changes of fosinopril as time changes and therefore could reveal the characters of fosinopril. According to the LLOQ, fosinopril could be determined 8 h after dosage

4. Conclusions

An LC–MS/MS method was developed and validated for simultaneous determination of fosinopril and fosinoprilat whose concentration are at different order of magnitude in human plasma. Using the method, the clinical pharmacokinetic characters of fosinopril were first revealed. The method proved to be superior with respect to sensitivity and selectivity for both fosinopril and its active metabolism fosinoprilat, compared with those analytical methods reported previously. The method was successfully applied for the clinical research of fosinopril and fosinoprilat in 20 volunteers after an oral dose of 20 mg fosinopril sodium.

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