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High-performance liquid chromatography–electrospray ionization mass spectrometry determination of sodium ferulate in human plasma

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Abstract

A selective and sensitive high-performance liquid chromatography-electrospray ionization mass spectrometry method has been developed for the determination of sodium ferulate in human plasma. The sample preparation was a liquid–liquid extraction and chromatographic separation was achieved with an Agilent ZORBAX SB-C_{18} (3.5 μm, 100 mm × 3.0 mm) column, using a mobile phase of methanol–0.05% acetic acid 40:60 (v/v). Standard curves were linear ($r^2 = 0.9982$) over the concentration range of 0.007–4.63 nM/ml and had acceptable accuracy and precision. The within- and between-batch precisions were within 12% relative standard deviation. The lower limit of quantification (LLOQ) was 0.007 nM/ml. The validated HPLC–ESI-MS method has been used successfully to study sodium ferulate pharmacokinetics, bioavailability and bioequivalence in 20 healthy volunteers.

Keywords: Sodium ferulate; HPLC/ESI-MS; Human plasma; Pharmacokinetics

1. Introduction

Sodium ferulate, Fig. 1(A), the sodium salt of ferulic acid (4-hydroxy-3-methoxycinnamic acid), is one of the essential components of the sodium ferulate and aspirin capsule that contains 50 mg sodium ferulate, 20 mg aspirin, 25 mg cinnarizine and 10 mg Vitamin B_1. The preparation is used for preventing and treating cerebrovascular disease in China. Pharmacological studies showed that sodium ferulate had been found to inhibit platelet aggregation and metabolism of arachidonic acid, increase coronary blood flow, relax or stimulate smooth muscle, possess anti-arrhythmic affects, anti-oxidative, immunostimulating, anti-inflammatory, effects, etc. [1–4].

The therapeutic daily dose of sodium ferulate 50 mg given orally. The $t_{1/2}$ is very short, which results in lower plasma concentrations in the elimination phase, and hence a sensitive analytical method is needed for its determination in plasma. Several methods for the quantification of ferulate in plasma have been reported. Xu et al. [5] developed a HPLC method with UV detector to assay ferulate in dog plasma with LLOQ of 20 ng/ml and LOD of 5.14 ng/ml. Xia et al. [6] determined the plasma concentration of ferulate in Xinshu oral liquid by HPLC-UV and the LLOQ of ferulic acid was 0.25 μg/ml. The HPLC analysis time of these two methods was long (about 9 min), and the sensitivity was insufficient. Wittemer and Veit [7] established a HPLC–coulometric-array detection method to determination of six metabolites derived from artichoke leaf extract in human plasma and the LLOQ of ferulic acid was 2.2 ng/ml. The sample preparation and extraction procedure of these methods were time consuming. Cremin et al. [8] reported a HPLC–ESI-MS method for the analysis of hydroxycinnamates such as caffeic acid, ferulic acid and chlorogenic acid in human plasma and urine, while the sample preparation was complex, the retention time of ferulic acid was as long (12.5 min) and the recovery was only 47.5.

The purpose of this paper was to explore the high selectivity and sensitivity of a single quadrupole MS system with an electrospray interface for the development and validation of a robust reversed-phase LC–MS method for the quantification of ferulate in human plasma. It was essential to establish an assay with an

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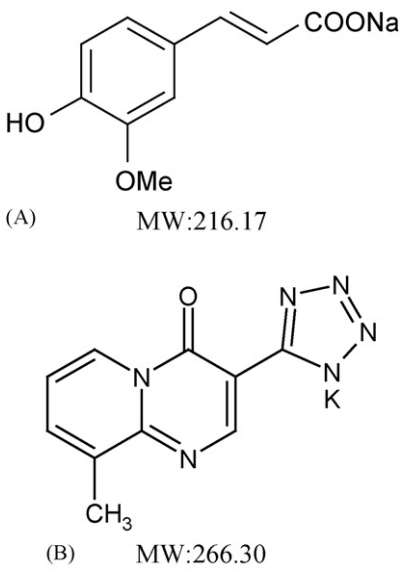

Fig. 1. Chemical structures of sodium ferulate (A) and pemirolast potassium (B).

LLOQ in the low pM/ml range. At the same time, this method was efficient in analyzing large numbers of plasma obtained for pharmacokinetic, bioavailability or bioequivalence studies after therapeutic doses of sodium ferulate.

2. Experimental

2.1. Chemicals and reagents

Sodium ferulate reference standard (98.5%, purity) was supplied by Dezhou Deyao Company (Dezhou, PR China); sodium ferulate and aspirin test capsules were obtained from Zhongsheng Haitian Company (Zhengzhou, PR China); sodium ferulate and aspirin reference capsules were purchased from Zhuhai Limin Company (Zhuhai, PR China); pemirolast potassium reference standard (internal standard, 99.7%, purity) was supplied by Nantong Feima Company (Nantong, PR China). HPLC grade methanol was purchased from VWR International Company (Darmstadt, Germany). Other chemicals were of analytical grade and used as received. Water was purified by redistillation before use.

2.2. Instrumentation and operating conditions

The HPLC system consisted of a Shimadzu LC-10AD pump, a Shimadzu DGU-14AM degasser, a Shimadzu SIL-HTc autosampler and a Shimadzu CTO-10Avp column oven (Shimadzu, Kyoto, Japan). The column was an Agilent ZORBAX SB-C_{18} (3.5 μm, 100 mm × 3.0 mm) and was operated at 30 °C. The mobile phase consisted of methanol: 0.05%acetic acid 40:60 (v/v) and was set at a flow rate of 0.4 ml/min.

Mass spectrometric detection was performed using a Shimadzu LCMS-2010A quadrupole mass spectrometer with an electrospray ionization (ESI) interface. All measurements were carried out under the negative ESI mode, the ($[M-Na]^-$, *m/z* 193.10) for sodium ferulate and ($[M-K]^-$, *m/z* 227.10) for pemirolast potassium were selected as detecting ions, respectively. The MS operating conditions were optimized as follows: nebulizer gas rate 1.5 l/min, CDL temperature 280 °C, block temperature 230 °C, probe voltage: −4.5 kV. The quantification was performed via peak area ratio (peak area of analyte/peak area of IS). Data acquisition and processing were accomplished using Shimadzu LCMS solution Software for LCMS-2010A system.

2.3. Preparation of stock solutions

Primary stock solutions of sodium ferulate for the preparation of standards and quality controls (QC) were prepared from separate weightings.

The primary stock solutions were prepared in methanol at a concentration of 4.6 μM/ml and stored at 4 °C protected from light until used in order to prevent sodium ferulate from decomposing [9].

The stock solution of pemirolast potassium (IS) was prepared in methanol at a concentration of 3.8 μM/ml and was stored at 4 °C.

Working solutions of sodium ferulate were prepared daily in methanol by appropriate dilution at 0.7, 2.3, 4.6, 23.1, 46.3, 92.5, 231.3, 370.1 and 462.6 nM/ml.

The stock solution of pemirolast potassium was further diluted with methanol to prepare the working internal standard solution containing 187.8 nM/ml of pemirolast potassium.

2.4. Sample preparation

A 1 ml aliquot of the collected plasma sample from a human volunteer was pipetted into a 10 ml centrifuge tube. The working internal standard solution (20 μl × 187.8 nM/ml) and 5 ml acetic acid ethyl ester were added and the samples were vortexed for 3 min. After centrifugation at 1600 × *g* for 10 min, the organic phase was transferred to another 10 ml centrifuge tube and evaporated to dryness under a gentle stream of nitrogen gas in water bath at 40 °C. The residue was redissolved in 100 μl mobile phase. Aliquots of 10 μl were injected into the LC–MS system.

To prepare the standard calibration samples and QC samples, 20 μl internal standard solution was added to 1 ml blank human plasma. The following procedures were the same as described above. Calibration standards were prepared to achieve the final standard concentrations of 0.007, 0.023, 0.046, 0.23, 0.46, 0.93, 2.31 and 4.63 nM/ml for ferulate.

All manipulations of plasma and stock solutions were performed under non-actinic light.

2.5. Method validation

The method validation assays were carried out according to the FDA document [10].

Analyses of blank samples of the healthy persons' blank plasma were obtained from six sources. Each blank sample was tested for the visible interference. In order to evaluate the matrix effect on the ionization of analytes, 10 μl four different

concentration levels of ferulate working solutions (0.7, 23.1, 92.5, 370.1 nM/ml) were added to the dried extracts of 1 ml blank sample, respectively, and subsequently dried. The residues were dissolved in 100 μl mobile phase. The same concentration levels of ferulate were dried directly and dissolved with the same volume of the mobile phase. The matrix effect on internal standard was evaluated using the same method.

Standard curves were prepared by determining the best-fit of peak area ratios (peak area of analyte/peak area of IS) versus concentration, and fitted to the equation $R = bC + a$ by weighted least-squares regression with a weighting factor of $1/C^2$.

Standard curves of eight concentrations of ferulate ranging 0.007–4.63 nM/ml were extracted and assayed. The lower limit of detection (LLOD) and the lower limit of quantification (LLOQ) were determined as the concentrations with a signal-to-noise ratio of 3 and 10, respectively.

Precision and accuracy were assessed by determining QC plasma samples at four concentration levels on the three different validation days. The concentration of each sample was determined using standard curve prepared and analyzed on the same day.

The extraction recovery of ferulate was determined by comparing the ferulate/IS peak area ratios (R_1) obtained from extracted plasma samples with those (R_2) from standard solutions at the same concentration. This procedure was repeated for the four different concentrations of 0.007, 0.23, 0.93, 3.70 nM/ml ($n = 5$).

The short-term temperature stability of ferulate was assessed by keeping QC plasma samples at room temperature for 6 h. The long-term stability was evaluated by keeping QC plasma samples at low temperature (−20 °C) for 5 days. The post-preparative stability was determined by placing QC samples under the autosampler conditions (4 °C) for 24 h. The freeze and thaw stability was tested by analyzing QC plasma samples undergoing three freeze (−20 °C)-thaw (room temperature) cycles on consecutive days. The ferulate working solutions were also assessed for stability at room temperature for 6 h.

3. Clinical study design

This was an open randomized, balanced, two-period crossover study in 20 Chinese healthy men. Each volunteer received in random order, single oral dose of sodium ferulate and aspirin test capsules or reference capsules in cycle. Blood samples (5 ml) for assay of plasma concentrations of ferulate were collected at the time of 0, 0.17, 0.25, 0.33, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 6.0 h after oral administration. They were put into lithium heparin tubes and were immediately centrifuged at 1600 × *g* for 10 min. The plasma obtained was frozen at −20 °C in coded polypropylene tubes until analysis.

4. Results and discussion

4.1. Selection of IS

It is necessary to use an internal standard to get high accuracy when HPLC is equipped with MS as the detector. Pemirolast potassium was adopted because of its similarity of retention time, ionization and extraction efficiency as well as less endogenous interference in plasma at *m*/*z* 227.10. The structures of sodium ferulate and pemirolast potassium are shown in Fig. 1.

4.2. Sample preparation

Liquid–liquid extraction was necessary and important because this technique could not only purify but also concentrate the sample. Diethyl ether, acetic acid ethyl ester and *n*-hexane-isopropanol (95:5, v/v) were all attempted and acetic acid ethyl ester was finally adopted because of its high extraction efficiency, and the extracted endogenous compounds did not interfere. 0.1 ml of 1.0 M hydrochloric acid was added to the plasma in order to enhance the extraction efficiency.

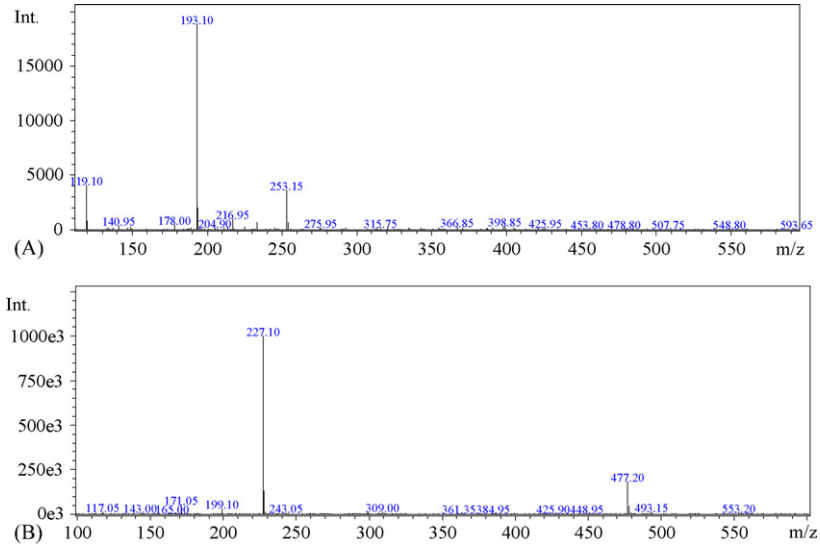

Fig. 2. Negative ion electrospray mass spectra of sodium ferulate (A) and pemirolast potassium (B).

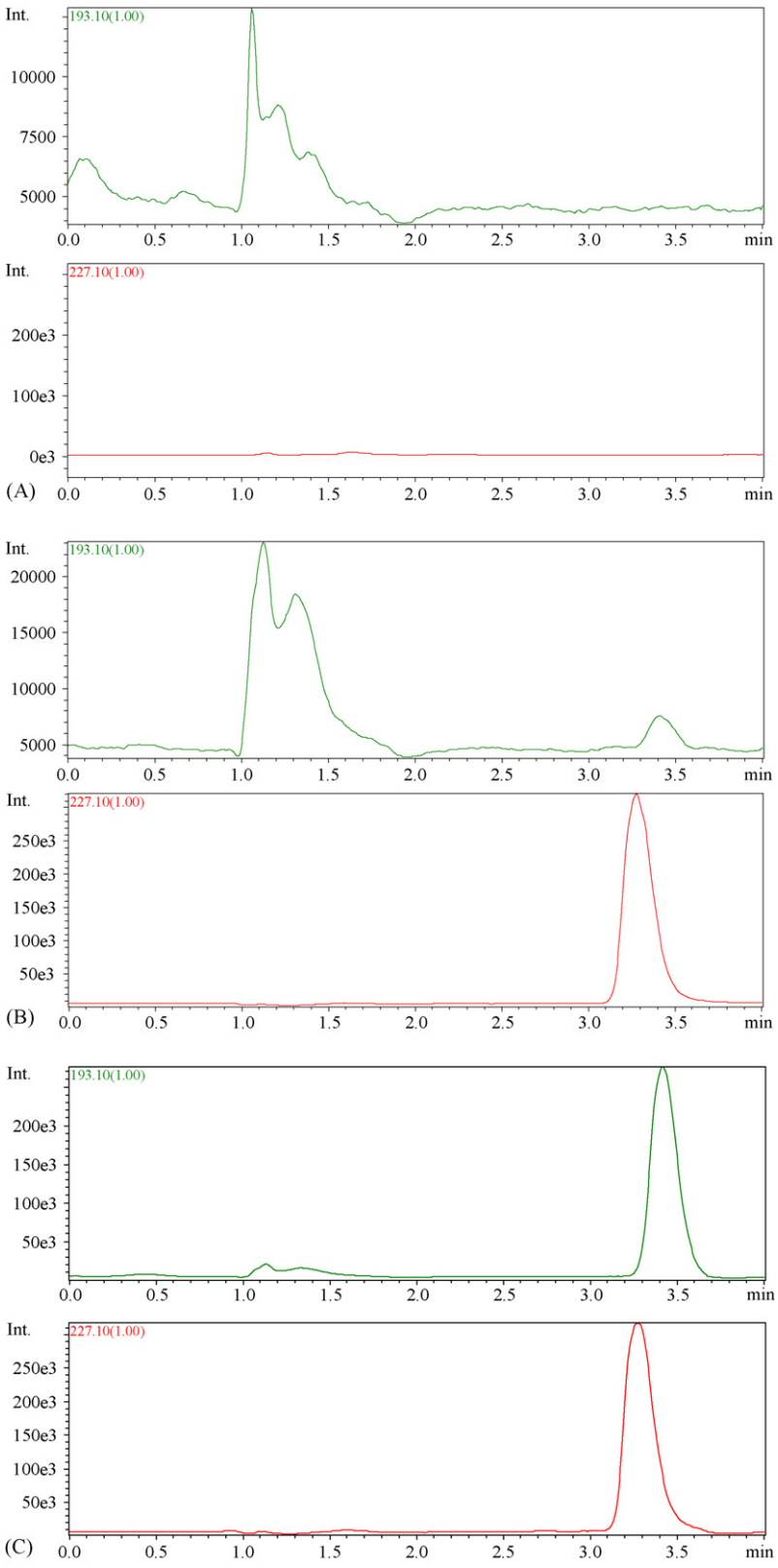

Fig. 3. The SIM (—) chromatograms of (A) blank plasma sample, (B) LLOQ (concentration of ferulate = 0.007 nM/ml, concentration of IS = 3.76 nM/ml) and (C) supplemented plasma sample (concentration of ferulate = 2.31 nM/ml, concentration of IS = 3.76 nM/ml). The retention times of ferulate and IS were 3.4 min and 3.3 min, respectively.

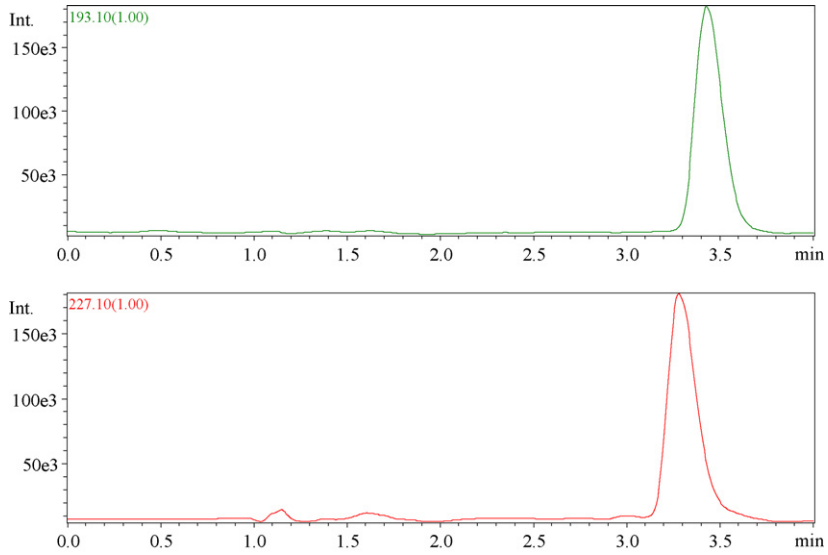

Fig. 4. The SIM (—) chromatograms of plasma sample obtained at 0.5 h from a subject who received a single oral dose (50 mg). The retention times of ferulate (2.72 nM/ml) and the IS (3.76 nM/ml) were 3.4 min and 3.3 min, respectively.

4.3. Separation and selectivity

Negative eletrospray ionization mass spectra of sodium ferulate and IS are shown in Fig. 2, respectively. According to the mass spectra, *m/z* 193.10 ($[M-\mathrm{Na}]^-$) of sodium ferulate and *m/z* 227.10 ($[M-\mathrm{K}]^-$) of pemirolast potassium were selected for monitoring.

The SIM (—) chromatograms of supplemented plasma are depicted in Fig. 3C. As shown, the retention times of sodium ferulate and the IS were 3.4 and 3.3 min, respectively.

Short retention times were obtained by using an elution system of methanol: 0.05% acetic acid (40:60, v/v) as the mobile phase. The total HPLC/MS analysis time was 4 min per sample. A representative chromatogram of a plasma sample obtained at 0.5 h from a subject who received a single oral dose (50 mg) is shown in Fig. 4.

4.4. Method validation

Fig. 3A shows a HPLC chromatogram for a blank plasma sample. No significant interference from endogenous substance with analyte or IS were detected. The matrix effect on the ionization of analyte or IS was evaluated by comparing the peak area of analytes redissolved in extracted blank samples (the final solution of blank plasma after being extracted and redissolved) with that redissolved in the eluent at the same concentration level. All ratios were between 85% and 115%, which meant no matrix effect for sodium ferulate and pemirolast potassium in this method.

Table 2
Recoveries of ferulate from plasma ($n=5$)

Added (nM/ml)	Recovery (mean ± S.D., %)	R.S.D. (%)
0.007	91.9 ± 10.2	11.1
0.23	92.0 ± 6.7	7.3
0.93	90.7 ± 3.6	3.9
3.70	91.6 ± 2.9	3.2

The linear regression of the peak ratios versus concentrations was fitted over the concentration range of 0.007–4.63 nM/ml in human plasma. The mean equation (curve coefficients ± S.D.) of the standard curve was $R=0.3559\ (\pm 0.0061)C-0.0000055\ (\pm 0.0003411)$ ($r^2=0.9982$), where R corresponds to the peak area ratio of ferulate to the IS and C refers to the concentration of ferulate added to plasma over a concentration range of 0.007–4.63 nM/ml.

The lower limit of quantification for ferulate proved to be 0.007 nM/ml (LLOQ) and the lower limit of detection (LLOD) was 0.003 nM/ml. Fig. 3B shows the chromatogram of an extracted sample that contained 0.007 nM/ml (LLOQ) of ferulate.

Table 1
The within- and between-batch precision, accuracy of the method for determination of ferulate (within-batch: $n=5$; between-batch: $n=5$ series per day × 3 days)

Added conc. (nM/ml)	Within-batch			Between-batch		
	Detected conc. (mean ± S.D., nM/ml)	Accuracy (%)	R.S.D. (%)	Detected conc. (mean ± S.D., nM/ml)	Accuracy (%)	R.S.D. (%)
0.007	0.0074 ± 0.0008	105.50	11.2	0.0074 ± 0.0007	106.33	9.8
0.23	0.23 ± 0.02	101.73	7.7	0.23 ± 0.02	99.78	7.4
0.93	0.90 ± 0.04	97.84	4.2	0.90 ± 0.04	98.05	4.0
3.70	3.64 ± 0.11	98.39	3.1	3.70 ± 0.13	100.03	3.6

Table 3
Data showing stability of ferulate in human plasma at different QC levels ($n = 5$)

	Accuracy (mean ± S.D., %)			
	0.007 (nM/ml)	0.23 (nM/ml)	0.93 (nM/ml)	3.70 (nM/ml)
Short-term stability (6 h, room temperature)	99.6 ± 9.5	98.3 ± 7.2	98.8 ± 3.4	105.2 ± 3.1
Freeze and thaw stability (3 cycles, −20 °C, room temperature)	100.6 ± 9.8	96.4 ± 8.1	104.1 ± 3.1	101.8 ± 3.2
Long-term stability (5 days, −20 °C)	98.4 ± 10.2	98.5 ± 7.0	103.8 ± 3.6	99.7 ± 3.3
Post-preparative stability (24 h, 4 °C)	106.9 ± 10.8	107.3 ± 3.9	106.2 ± 5.0	98.6 ± 3.2

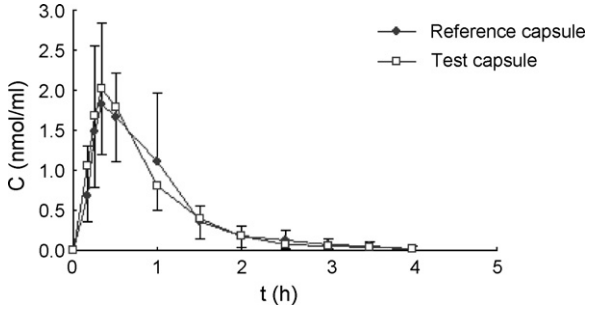

Fig. 5. Mean drug plasma concentration–time curve of ferulate in 20 volunteers after oral administration of reference or test sodium ferulate and aspirin capsule. Each point represents a mean ± S.D. ($n = 20$).

Table 4
Pharmacokinetic parameters of 20 healthy male volunteers after oral administration of ferulate in single dose study

Parameters	Test capsules	Reference capsules
T_{max} (h)	0.4 ± 0.2	0.5 ± 0.3
$T_{1/2}$(Kel) (h)	0.70 ± 0.26	0.69 ± 0.25
$MRT_{0\to\infty}$ (h)	0.85 ± 0.19	0.96 ± 0.29
C_{max} (nM ml^{-1})	2.45 ± 0.61	2.47 ± 0.75
$AUC_{0\to4}$ (nM h ml^{-1})	1.89 ± 0.39	1.94 ± 0.58
$AUC_{0\to\infty}$ (nM ml^{-1})	1.90 ± 0.39	1.97 ± 0.60

Data for within-batch and between-batch precision and accuracy of the method for ferulate are presented in Table 1. The accuracy deviation values are within 20% for 0.007 nM/ml and 15% for 0.23, 0.93 and 3.70 nM/ml of the actual values. The precision determined at each concentration level does not exceed 12% of the relative standard deviation (R.S.D.). The results, calculated using one-way ANOVA, indicated that the values were within the acceptable range ($\alpha = 0.05$) and the method was accurate and precise.

The mean extraction recovery of ferulate, determined at four concentrations (0.007, 0.23, 0.93, 3.70 nM/ml), were 91.9, 92.0, 90.7, 91.6% ($n = 5$), respectively. The extraction recovery determined for ferulate was shown to be consistent, precise and reproducible. Data are shown below in Table 2.

Table 3 summarizes the freeze and thaw stability, short-term stability, long-term stability and post-preparative stability data of ferulate, which showed the stability of the samples.

The stability of working solutions were stable at room temperature for 6 h.

4.5. Results of pharmacokinetic study

The method was applied to analyze plasma samples obtained from 20 healthy volunteers, which received a single dose of sodium ferulate and aspirin capsule in the bioequivalence study. The procedure developed was sensitive enough to assure the quantitative analysis of ferulate in plasma with acceptable accuracy over a period of 6 h after a single oral administration. The mean plasma concentration–time profiles of 20 volunteers are represented in Fig. 5. Pharmacokinetic parameters of the test capsules and the reference capsules are listed in Table 4. The test capsule was found to be bioequivalent to the reference one.

5. Conclusion

The described LC/ESI-MS method for determination of sodium ferulate in human plasma was proved to be rapid, selective and sensitive. The analysis time was short, liquid–liquid extraction procedure simple. These make the method suitable for the analysis of a large number of samples resulting from the pharmacokinetic, bioavailability or bioequivalence study of sodium ferulate in humans.

References

[1] N. Nenadis, H.Y. Zhang, M.Z. Tsimidou, J. Agric. Food Chem. 51 (2004) 1874–1879.
[2] H. Kikuzaki, M. Hisamoto, K. Hirose, K. Akiyama, H. Taniguchi, J. Agric. Food Chem. 50 (2002) 2161–2168.
[3] S.Y. Ou, K.C. Kwok, J. Sci. Food Agric. 84 (2004) 1261–1269.
[4] Y.J. Fu, M. He, Acta Pharm. Sin. 39 (2004) 325–327.
[5] S. Xu, J.H. Hu, F.Q. Li, Chin. Tradit. Pat. Med. 27 (2005) 1062–1065.
[6] D.Y. Xia, T. Guo, W.H. Pan, Y. Sui, D.S. Dang, Zhongcaoyao 35 (2004) 36–38.
[7] S.M. Wittemer, M. Veit, J. Chromatogr. B 793 (2003) 367–375.
[8] P. Cremin, S. Kasin-Karakas, A.L. Waterhouse, J. Agric. Food Chem. 49 (2001) 1747–1750.
[9] Y.H. Kang, X.Y. Huang, G.L. Tan, Progr. Pharm. Sci. 28 (2004) 80–84.
[10] Guidance for Industry, Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), May 2001.