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Quantitative determination of rhein in human plasma by liquid chromatography–negative electrospray ionization tandem mass/mass spectrometry and the application in a pharmacokinetic study

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ABSTRACT

To investigate the pharmacokinetics of rhein in human, a liquid chromatography–electrospray ionization tandem mass/mass spectrometry (HPLC–ESI-MS/MS) method for the determination of rhein in human plasma is established in this study. Indomethacin is used as the internal standard (I.S.). The plasma samples are analyzed after protein precipitation with methanol, and the LC separation is performed on an Agilent Eclipse XDB-C18 column with a mobile phase of acetonitrile–0.2% formic acid water (70:30, v/v). The electrospray-ion source is performed in the negative mode. The multi-reaction monitoring mode is selected and the ion selected channels are set at *m/z*: 283.1 → 238.9 for rhein ($[M-H]^- \rightarrow [M-CO_2-H]^-$) and *m/z*: 356.2 → 312.0 for indomethacin ($[M-H]^- \rightarrow [M-CO_2-H]^-$), respectively. Calibration curve is linear over the range of 1.0–8000.0 ng/ml. The chromatographic separation is achieved within 12 min. The lower limits of quantification (LLOQ) is 1.0 ng/ml. The intra- and inter-run precisions are less than 4.65% and 8.28%, respectively. The method is successfully applied to determine the plasma concentrations of rhein in Chinese volunteers.

1. Introduction

Rhein(1.8-dihydroxy-3-carboxy-anthraquinone), a lipophilic anthraquinone derived from a traditional Chinese herbal medicine *Rheum palmatum* L., is traditionally used to treat many different diseases in China. Rhein has many types of bioactivities in restoring the first-phase insulin secretion and protecting the islets function [1], shifting the Th1/Th2 responses by inhibiting T-box expressed in T-cells (T-bet) expression and enhancing GATA-binding protein-3 (GATA-3) expression [2], inhibiting the viability of MCF-7 and MDA-MB-435s breast cancer cells [3], affecting angpt2 and tie2 related to angiogenesis [4], inhibiting the hypertrophy of renal proximal tubular epithelial cells [5], and so on. Therefore, rhein can be used in the treatments of anti-inflammatory, anti-viral reagents and anti-tumor, etc. The pharmacokinetics of rhein in rats have been studied in previous studies [6–11].

In published studies, Several LC–MS [12–15], TLC [16] and HPLC [17–19] methods have been used for the determination of rhein in rats plasma, and only HPLC method has been used for the quantification of rhein in human plasma [20,21], in which the most sensitive assay is the LC–MS/MS method with an LLOQ of 10 ng/ml [12]. As searched through Medline, Science Direct and Google using key words such as pharmacokinetics, rhein, human and determination, the method of HPLC–ESI-MS/MS for the determination of rhein in human plasma is not available. So, to evaluate the pharmacokinetics of rhein in human, we needed to develop a simple and sensitive method for the determination of rhein in human plasma. This study developed a simple and sensitive LC–ESI-MS/MS method with an LLOQ as low as 1.0 ng/ml for the quantification of rhein in human plasma with one-step precipitation. The method is successfully applied to study the pharmacokinetics of rhein in healthy Chinese volunteers.

2. Experimental

2.1. Chemicals and reagents

The standard substances of rhein (Fig. 1A, 99.7%, purity) and indomethacin (Fig. 1B, 99.8%, purity) to were both obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The test formulation was rhein capsule, which (the quality of preparation C/W GMP defined by Food and Drug Administration of China) was provided by the Chemical and Pharmaceutical Preparation Lab of Nanjing Drum Tower Hospital (Nanjing, China). Methanol and acetonitrile were both of HPLC grade (Merck, Darmstadt, Germany). The deionized water

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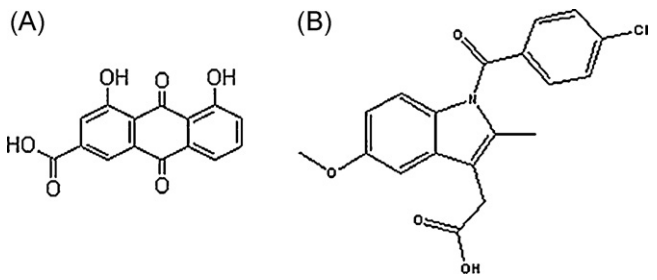

Fig. 1. Chemical structures of rhein (A) and indomethacin (B).

was purified with Milli-Q water system (Millipore Corporation, Bedford, Massachusetts, France) and used throughout the study.

2.2. Instrumentation and conditions

Analysis of samples and standards was performed using an LC–MS–MS system (Agilent Technologies, Inc., USA). The HPLC–ESI-MS/MS equipment consisted of a Quat pump, a Hip-ALS SL$^+$ auto-sampler, and an Agilent 6460A Triple Quadrupole Tandem Mass/Mass Spectrometry (Agilent Technologies, Inc., USA) equipped with an ESI ion source. Masshunter software was used for data acquisition and analysis. LC separation was performed on an Agilent Eclipse XDB-C18 column (4.6 mm × 150 mm, 5 μm, Agilent Technologies, Inc., USA) with a mobile phase of acetonitrile–0.2% formic acid water (70:30, v/v) at a flow rate of 0.3 ml/min. The column temperature was maintained at 35 °C. HPLC–ESI-MS/MS was carried out using nitrogen to assist nebulization. Tuning the ion spray, operated in the negative ionization mode, for rhein resulted in a 4000 V spray voltage and a 350 °C capillary temperature with the nitrogen sheath at a flow rate of 11 l/min. Nebulizer was set at 55 psi. The tube lens offsets were both selected at 135 V and the Delta EMV(−) was set at −200 V. The multi-reaction monitoring (MRM) mode was used and the collision energy was selected at 10 V and 8 V for rhein and indomethacin, respectively. The target ions were set at m/z: 283.1 → 238.9 for rhein and 356.2 → 312.0 for indomethacin (I.S.), respectively.

2.3. Preparation of stock and working solutions

The stock solutions of rhein (1.0 mg/ml) and internal standard (0.5 mg/ml) were prepared in methanol and stored at −20 °C. Standard solutions of rhein with concentrations of 100.0, 10.0, 1.0, and 0.1 μg/ml, were prepared by serial dilution of rhein stock solution with methanol in separate 25 ml volumetric flasks. A solution containing 0.5 μg/ml internal standard was also obtained by further dilution of I.S. stock solution with methanol.

2.4. Sample preparation

All frozen standards and samples were allowed to thaw at room temperature and homogenized by vortex. A 0.2-ml aliquot plasma sample was transferred to a 1.5 ml centrifuge tube together with 20 μl of I.S. (0.5 μg/ml). The sample mixture was mixed with 0.60 ml of methanol and vortex mixed for approximate 5 min, then allowed to stand for 5 min to deproteinize and the precipitate was removed by centrifugation at 14,000 rpm for 5 min. The supernatant was pipetted into an injected vial and a 10 μl aliquot was injected into the HPLC–ESI-MS/MS system for analysis.

2.5. Calibration standards and quality controls

Calibration standards of rhein were prepared by spiking appropriate amounts of the standard solutions in 0.20 ml blank plasma obtained from healthy volunteers.

Standard curves were prepared in the range of 1.0–8000.0 ng/ml for rhein at concentrations of 1.0, 5.0, 20.0, 100.0, 500.0, 2000.0, and 8000.0 ng/ml. The calibration curve was prepared and assayed along with quality control (QC) samples and each run of unknown plasma samples.

The QC samples were prepared at concentration levels of 2.0, 100.0, and 4000.0 ng/ml for rhein using the same method of preparing the calibration standards and stored at −70 °C. The amounts of QC samples were over 5% of test samples. QC samples were analyzed with processed test samples at intervals in each run. The results of the QC samples provided the basis of accepting or rejecting the run.

3. Assay validation

3.1. Specificity

The specificity of the assay was checked by comparing the chromatograms of six different batches of blank human plasma with the corresponding spiked plasma. Each blank plasma sample was tested using the protein precipitation procedure and HPLC–ESI-MS/MS conditions to ensure no interference of rhein and I.S. from plasma.

3.2. Linearity of calibration curve and LLOQ

To evaluate the linearity, calibration standards of seven rhein concentration levels at 1.0, 5.0, 20.0, 100.0, 500.0, 2000.0, and 8000.0 ng/ml were prepared and assayed on different 5 days. The calibration curve was constructed by plotting the peak-area ratios of rhein to the I.S. Versus the concentrations of rhein, using weighted least squares linear regression. The accuracy of every concentration was calculated and the calibration curve would be accepted on the basis of the accuracy for 1.0 ng/ml within ±20% and for other concentrations within ±15%. The plasma sample containing 1 ng/ml of rhein (LLOQ) was prepared and assayed for five replicates, and its precision (within 15%) and accuracy (within ±20%) were defined [22].

3.3. Precision and accuracy

Validation samples of three concentrations (2.0, 100.0 and 4000.0 ng/ml) for rhein were prepared and analyzed on three separate runs to evaluate the accuracy (five replicates on each run), intra-day and inter-day precision of the analytical method. Assay precision was calculated using the relative standard deviation (RSD, %). Accuracy is defined as the relative deviation in the calculated value (E) of a standard from that of its true value (T) expressed as a percentage (RE%). It was calculated by using the formula:

$$\mathrm{RE\%} = \frac{E - T}{T} \times 100.$$

3.4. Extraction recovery

The extraction recoveries of rhein were evaluated by analyzing five replicates at concentrations of 2.0, 100.0 and 4000.0 ng/ml for rhein. The recovery was calculated by comparison of the peak areas extracted from plasma samples with those of injected standards.

3.5. Stability

The stability of rhein in plasma was studied under a variety of storage and handling conditions using the QC samples (2.0, 100.0 and 4000.0 ng/ml) (five replicates for each level). The short-term temperature stability was assessed by analyzing QC samples those were kept at room temperature (25 °C) for 4 h. The processed QC samples were kept at 4 °C (as sample injector set) for 24 h to valuate the stability of storage in sample injector. Freeze–thaw stability (−70 °C in plasma) was checked through three cycles. The QC samples were stored at 25 °C for 0.5 h and thawed unassisted at room temperature (25 °C). When completely thawed, the samples were refrozen for 24 h under the same conditions and thawed unassisted at room temperature. The freeze–thaw cycles were repeated three times, and then analyzed on the third cycle. The long-term stability was performed at −70 °C in plasma for 8 weeks. The stock solutions of rhein and I.S. were stored at −70 °C for 8 weeks and the stability of stock solutions was also evaluated.

3.6. Matrix effect (ME)

The matrix effect (ME) was examined by comparing the peak areas of rhein and I.S. between two different sets of samples. The samples were prepared by serial dilution of standard solution with blank plasma and mobile phase, respectively and the obtained peak areas were defined as *A* and *B*. ME was calculated by using the formula: ME (%) = $A/B \times 100$. The matrix effect of the assay was evaluated at three concentration levels of 2.0, 100.0 and 4000.0 ng/ml for rhein and 0.5 μg/ml for I.S. Five samples at each level were analyzed. The blank plasma samples used in this study were five different batches of human blank plasma. If the ME values were within the range of 85–115%, an endogenous matrix effect was recommend negative.

3.7. Clinical study design and pharmacokinetic analysis [23]

The clinical pharmacokinetic study was approved by the Ethic Committee of Nanjing Drum Tower Hospital the Affiliated Hospital of Nanjing University Medical School. The volunteers were recruited at Nanjing Medical University and wrote informed consent to participate in the study according to the principles of the Declaration of Helsinki [24]. 36 healthy adult male Chinese volunteers were eligible based on the following criteria: age 18–30 years and body mass index between 20 and 24 kg/m^2; no smoking status and no history or evidence of a renal, gastrointestinal, hepatic, or hematologic, or any acute or chronic disease, or any allergy to any drugs; no history of using any kind of drugs within 30 days. All volunteers were evenly assigned into 3 groups by a simple randomization method and oral administrated a single dose of rhein capsule (50, 100 and 200 mg) with 200 ml of water after fasting for 12 h, respectively. 3.0-ml blood was drawn before drug administration (for baseline measurements) and at 0.33, 0.67, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, 16.0, 24.0, 36.0, 48.0 and 72.0 h after dosing. The blood samples were drawn into a vacuum tube with heparin sodium as an anticoagulant and immediately centrifuged at 1000 × *g* for 10 min. The separated plasma was transferred into another clean, dry tube and stored at −70 °C until quantitative analysis for the determination of rhein in plasma was performed.

The plasma concentrations of these blood samples were determined using the LC–MS/MS method, and the main pharmacokinetic parameters were calculated. The maximum plasma concentrations (C_{max}) and the time to those (t_{max}) were noted directly. The elimination half-life ($t_{1/2}$) was calculated using the formula $t_{1/2} = 0.693/k_e$. The area under the plasma concentration–time curve from the start of administration to the time of the last determined concentration (AUC_{0-72}) was calculated using the linear trapezoidal rule. The area under the plasma concentration–time curve to time infinity ($AUC_{0-\infty}$) was calculated as follows: $AUC_{0-\infty} = AUC_{0-72} + C_{72}/k_e$, in which the C_{72} was the plasma concentration of rhein at 72 h post-dose. Analysis of variance (ANOVA) was performed to test for the different dose on pharmacokinetic variable.

4. Results and discussion

4.1. Condition of chromatography

In order to achieve the excellent specificity under ESI(−) condition, the percentage of organic phase in the mobile phase was set high enough (70%) so as to avoiding the hydrophilic response-suppressing endogenous interferent which broken away from column before rhein. Compared with methanol, acetonitrile was selected as the organic phase due to the better selectivity. The test results showed that better peak shapes could be achieved by adding a certain amount of formic acid into the aqueous portion. The concentration of formic acid in deionized water was also investigated. The test results showed that exorbitant amount of formic acid would decreased the MS sensitivity of rhein and the ratio of 2‰ was suitable. This result was on account of the negative mode in selected ion monitoring being selected for the detection of rhein. According to this, a mobile phase of 2‰ formic acid water–acetonitrile (30:70, v/v) was selected in the method. In this study, norfloxacin, ibuprofen and indomethacin were investigated as I.S., respectively. Comparing with norfloxacin and ibuprofen, indomethacin had more similar retention to rhein and satisfactory resolution from rhein and other peaks [25]. The different column temperatures of 25, 35 and 45 °C were tested and the results showed that rhein and I.S. could be separated preferably from the interference of the endogenous substance at the column temperature of 35 °C. The analysis time of each injection was also set as long as 12 min so as to avoiding the response-suppressing endogenous interference and matrix effect. Different volumes of methanol (400, 600, 800 μl) were added respectively into 200 μl of plasma sample to deproteinize. As results showed, the preferable purity and concentration were achieved by adding 3 times volume of methanol into plasma sample.

4.2. Conditions for MS/MS

Because rhein had only an aglycone without glycosyl in its structure, meanwhile, its benzene ring was combined with –OH and –COOH, it was a medium-polarity compound. Usually, the electrospray ionization (ESI) was used for medium-polarity to high-polarity substance, so the ESI was adopted for the assay of rhein for its medium-polarity property. On account of its structure, rhein had considerable acidity and $[M-H]^-$ was more suitable to be achieved. As results showed (Fig. 2), in negative ionization and full scan mode, *m*/*z*: 283.1 $[M-H]^-$ was selected as mother ion for the detection of rhein. In product ion mode, collision energy was optimized, and when the value was 10 eV, *m*/*z*: 238.9$[M-CO_2-H]^-$ was the most abundant as the daughter ion for the detection of rhein. In order to gain enough response at LLOQ, the voltage of electron multiplier tube was set at −200 V(EMV(−)). Under these LC–MS/MS conditions, all MS parameters were fixed and enough sensibility and specificity were achieved, furthermore, the value of S/N (LLOQ) was satisfied with the detection (>10). Under LC–MS/MS conditions, *m*/*z*: 356.2$[M-H]^-$ and 312.0$[M-CO_2-H]^-$ was selected as mother ion and product ion for the detection of indomethacin (I.S.), respectively (Fig. 3). In optimized experiments, although the more response was achieved with increase of the parameters being set for collision energy, indomethacin would break into pieces completely as no conspicuous fragment

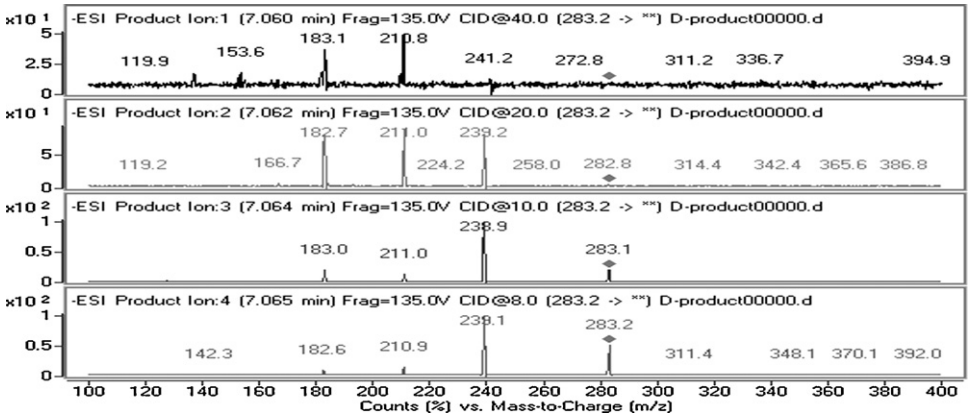

Fig. 2. ESI(−) tandem mass spectra of rhein at collision energies (CE) of 40 V, 20 V, 10 V and 8 V.

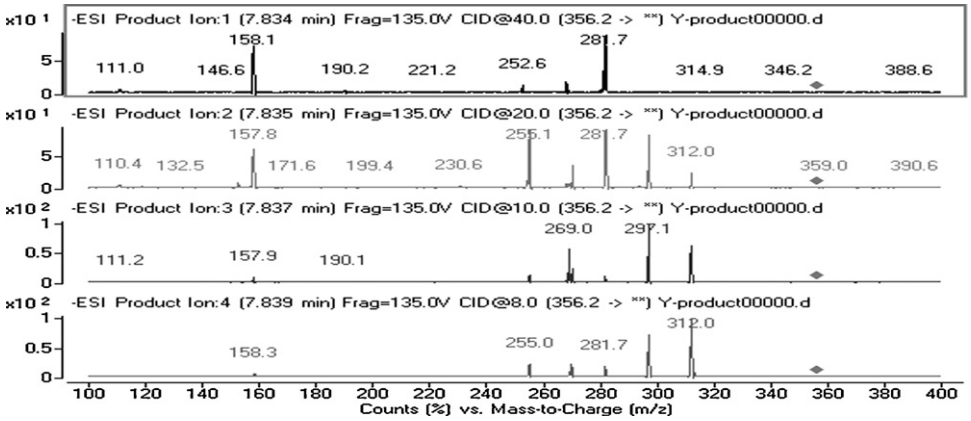

Fig. 3. ESI(−) tandem mass spectra of indomethacin at collision energies (CE) of 40 V, 20 V, 10 V and 8 V.

ions had enough abundant to be satisfied with detection when the parameters were above a certain extent. So, 8 eV was suitable, relatively. Under this MS conditions, although the response of indomethacin was not so high comparing with ibuprofen and norfloxacin, and the concentration of indomethacin in plasma was set at a considerable level (0.5 μg/ml), indomethacin was finally selected as I.S. for its similar retention and satisfactory resolution.

4.3. Method validation

4.3.1. Selectivity

Selectivity was assessed by comparing the chromatograms of six different batches of blank human plasma with the corresponding spiked plasma. Fig. 4 showed the typical chromatograms of a blank (a), a LLOQ for rhein in plasma and I.S. (b), a spiked plasma sample with rhein (500.0 ng/ml) and I.S. (c), and plasma sample from one healthy volunteer at 3.0 h after a single dose of rhein capsule administration (d). There was no significant interference from endogenous substances observed at the retention times of rhein and indomethacin.

Table 1
Matrix effect evaluation of rhein and I.S. in human plasma ($n = 5$).

Samples	Nominal concentration (ng/ml)	Matrix effect (%)
Rhein	2.0	98.3
	100.0	100.7
	4000.0	102.6
I.S.	500.0	97.8

Note: *n*, number of replicates.

4.3.2. Matrix effect

The matrix effect of the method was evaluated at three rhein concentration levels of 2.0, 100.0 and 4000.0 ng/ml and the I.S. concentration level of 0.5 μg/ml. Five samples at each level were analyzed. The blank plasma samples used in this study were from five different batches of human blank plasma. As shown in Table 1, the results obtained were well within the acceptable limit, which indicated that there was no matrix effect observed in this study.

4.3.3. Linearity of calibration curves and LLOQ

Five calibration analyses were performed on five consecutive days and the correlation coefficient >0.99 confirmed that the

Table 2
Precision and accuracy of the assay for determination of rhein in plasma ($n = 3$ runs, 5 replicates per run).

Spiked concentration (ng/ml)	Mean found concentration (ng/ml)	Inter-run RSD (%)	Intra-run RSD (%)
2	2.05 ± 0.17	8.28	4.65
100	98.26 ± 6.84	6.32	3.12
4000	3754.13 ± 232.76	5.46	3.26

Note: RSD, relative standard deviation.

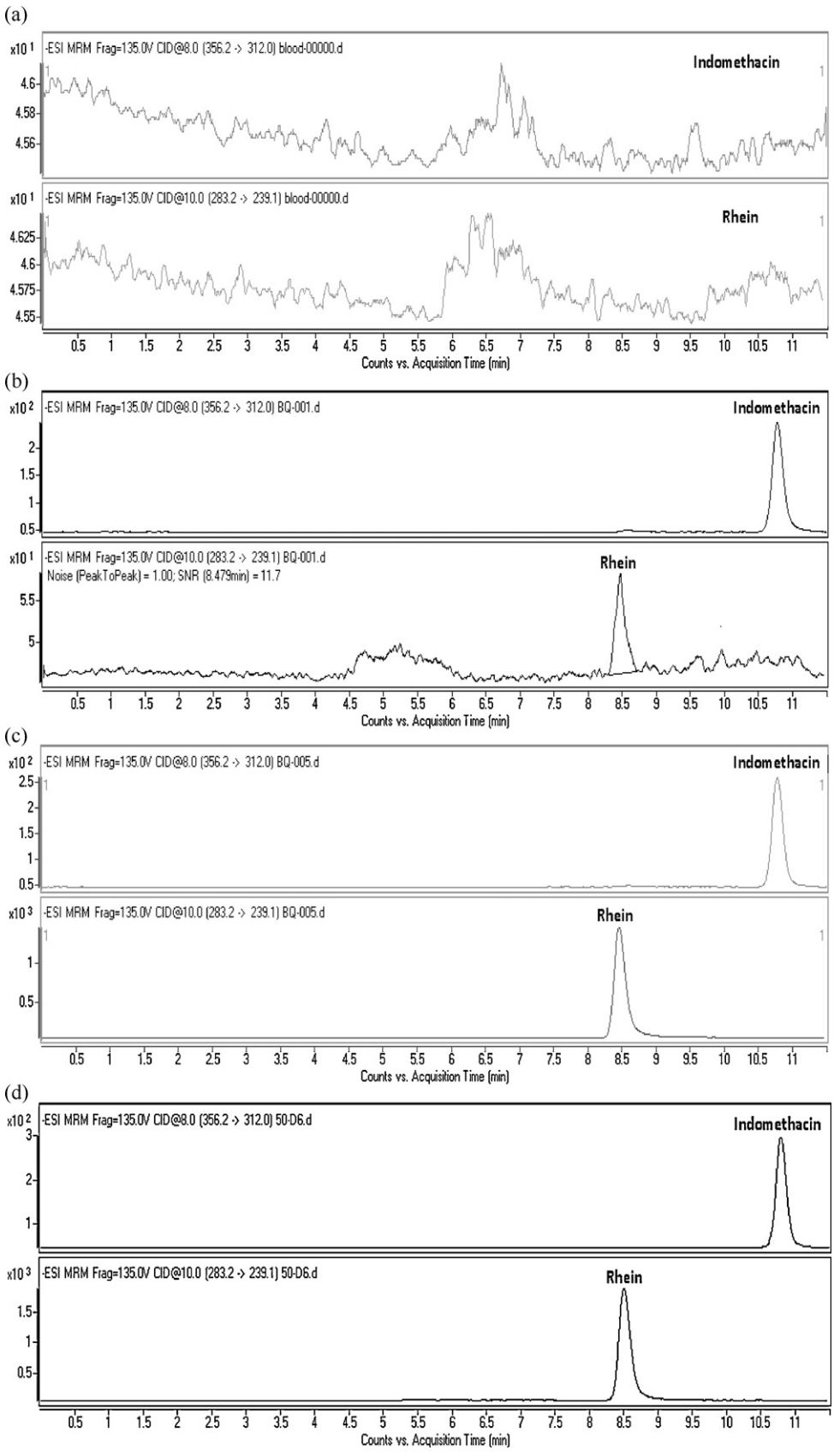

Fig. 4. The typical chromatograms of a blank (a), a LLOQ for rhein in plasma and I.S. (b), a spiked plasma sample with rhein (500.0 ng/ml) and I.S. (c), and plasma samples from healthy volunteers (d).

Table 3
The mean extraction recoveries data of rhein in plasma ($n = 5$).

Spiked concentration (ng/ml)	Extraction recovery (%, mean ± SD)	RSD (%)
2	94.25 ± 7.62	8.08
100	92.17 ± 5.44	5.90
4000	91.68 ± 5.08	5.54

Note: *n*, number of replicate; SD, standard deviation; RSD: relative standard deviation.

calibration curves were linear over the concentration ranges of 1.0–8000.0 ng/ml for the rhein. The mean calibration curve had a slope of 20.7844 ± 0.8054, an intercept of 4.3269 ± 0.1505 (weighting factor was $1/C^2$). The LLOQ for rhein in plasma was 1.0 ng/ml.

4.3.4. Precision and accuracy

Rhein plasma samples at three concentration levels of 2.0, 100.0, and 4000.0 ng/ml were analyzed for accuracy and precision. The data obtained for rhein are shown in Table 2. The precision was calculated by using one-way ANOVA. For the three concentration levels of rhein, the intra-day precision was less than 4.65%, the inter-day precision was less than 8.28% and the accuracy was within ±12.45%. The data obtained for rhein were within the acceptable limits to meet the guidelines for bio-analytical methods [22].

4.3.5. Extraction recovery

The mean extraction recoveries were measured at three different concentration levels for rhein (2.0, 100.0, and 4000.0 ng/ml) by comparing the peak areas of rhein prepared in plasma with those obtained from direct injection of standards dissolved in the processed blank plasma. The data of recovery obtained are shown in Table 3.

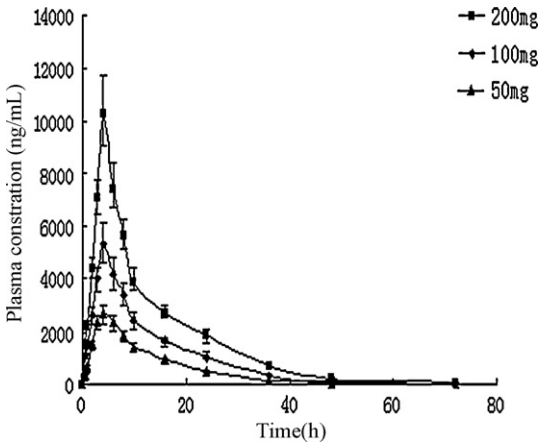

Fig. 5. The mean plasma concentration–time curve of rhein after a single dose of 50, 100 and 200 mg oral administration in Chinese healthy volunteers.

4.3.6. Stability

The results of stability experiments showed that no significant degradation occurred at room temperature (25 °C) for 4 h, sample injector temperature (4 °C) for 24 h, −70 °C for 8 weeks and the three freeze–thaw cycles for rhein plasma samples. The determination concentration of rhein in plasma for various kinds of stability experiments was shown in Table 4.

The standard solutions of rhein and indomethacin at 500 ng/ml were prepared by spiking the stock solutions with methanol, respectively. By comparing the peak areas of rhein and I.S. in stock solutions prepared 8 weeks before with which prepared immediately. The mean ratios of peak areas were 98.5% and 94.6% for rhein and indomethacin, respectively, and these results indicated that the

Table 4
Stability data of rhein in human plasma ($n = 5$).

Stability	Mean (±SD) spiked concentration (ng/ml)		
	2.0	100.0	4000.0
Baseline	2.05 ± 0.17	98.26 ± 6.84	3754.13 ± 232.76
Stability (24 h at 4 °C)	2.03 ± 0.14	100.15 ± 4.65	3926.75 ± 196.32
Short-term stability (4 h at 25 °C)	2.02 ± 0.13	101.44 ± 5.31	3864.15 ± 206.88
Long-term stability (8 weeks at −70 °C)	1.98 ± 0.21	97.26 ± 7.53	3709.95 ± 158.83
Freeze–thaw stability (3 freeze-and-thaw cycles)	1.99 ± 0.18	99.36 ± 8.24	3795.32 ± 214.79

Note: *n*, number of replicate; SD, standard deviation.

Table 5
Pharmacokinetic parameters for rhein after a single oral dose of 50, 100 or 200 mg in Chinese healthy subjects. All data are means (SD).

Variable dose (mg)	No. of subjects	$t_{1/2}$ (h)	C_{max} (ng/ml)	T_{max} (h)	MRT (h)	AUC_{0-t} (ng h/ml)	$AUC_{0-\infty}$ (ng h/ml)
50	20	7.92 ± 0.85	2705.75 ± 168.75	4.18 ± 0.36	8.25 ± 0.92	21226.94 ± 985.46	22652.48 ± 1215.21
100	20	7.34 ± 0.65	5347.37 ± 256.42	4.00 ± 0.25	7.91 ± 0.64	47413.35 ± 2956.27	51365.73 ± 3429.15
200	20	8.34 ± 1.21	10225.13 ± 652.18	3.75 ± 0.19	8.06 ± 0.77	100143.42 ± 4892.44	110585.63 ± 5913.22

MRT, mean residence time.

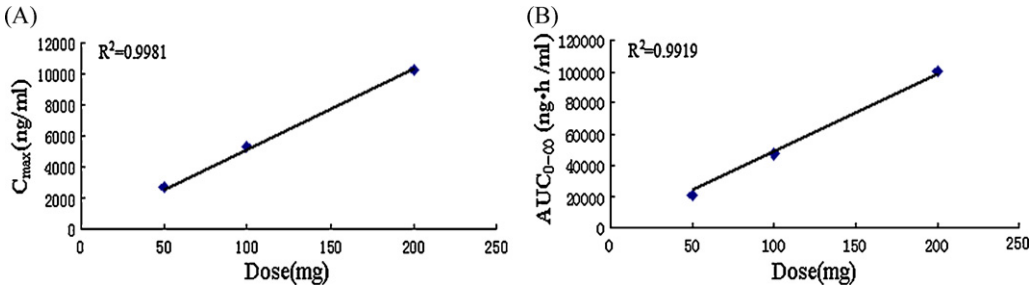

Fig. 6. Linear pharmacokinetics of (A) C_{max} and (B) $AUC_{0-\infty}$ of rhein after a single dose of 50, 100, or 200 mg in Chinese healthy subjects.

stock solutions of rhein and I.S. stored at −70 °C for 8 weeks were both stabile.

4.4. Pharmacokinetic studies

The developed method was applied to the determination of rhein in human plasma and the pharmacokinetic study. The mean plasma concentration–time curve of rhein after a single dose of 50, 100 and 200 mg is shown in Fig. 5. The main pharmacokinetic parameters of rhein were calculated and summarized in Table 5. Other than C_{max} and $AUC_{0-\infty}$, ANOVA analyses showed that no significant differences appeared in pharmacokinetic parameters with different doses. In the range of 50–200 mg, C_{max} and $AUC_{0-\infty}$ were proportional to the dose (Fig. 6.) and T_{max} and MRT and $t_{1/2}$ did not change following dose escalation. Therefore, linear pharmacokinetics were found for rhein in Chinese healthy subjects after s single oral-dose administration in the range of 50–200 mg.

5. Conclusion

The assay achieved good sensitivity and specificity for the determination of rhein in human plasma. No significant interferences caused by endogenous compounds were observed. This simple and sensitive assay is suitable for pharmacokinetic studies of rhein in human subjects.

References

[1] H. Du, J. Shao, P. Gu, B. Lu, J. Wang, Z. Liu, Effect of rhein treatment on first-phase insulin secretory function in db/db mice, China J. Chin. Mater. Med. 35 (2010) 2764–2767.

[2] X.Y. Sheng, M. Wang, M. Lu, B.L. Xi, H.G. Sheng, Y.Q. Zang, Rhein ameliorates fatty liver disease through negative energy balance, hepatic lipogenic regulation, and immunomodulation in diet-induced obese mice, Am. J. Physiol. – Endocrinol. Metab. 300 (2011) 886–893.

[3] V.E. Fernand, J.N. Losso, R.E. Truax, E.E. Villar, D.K. Bwambok, S.O. Fakayode, M. Lowry, I.M. Warner, Rhein inhibits angiogenesis and the viability of hormone-dependent and independent cancer cells under normoxic or hypoxic conditions in vitro, Chem. Biol. Interact. 192 (2011) 220–232.

[4] Z.H. He, R. Zhou, M.F. He, C.B. Lau, G.G. Yue, W. Ge, P.P. But, Anti-angiogenic effect and mechanism of rhein from *Rhizoma Rhei*, Phytomedicine 18 (2011) 470–478.

[5] D.Q. Yu, Y. Gao, X.H. Liu, Effects of rhein on the hypertrophy of renal proximal tubular epithelial cells induced by high glucose and angiotensin II in rats, J. Chin. Med. Mater. 33 (2010) 570–574.

[6] W.F. Tang, X. Huang, Q. Yu, F. Qin, M.H. Wan, Y.G. Wang, M.Z. Liang, Determination and pharmacokinetic comparison of rhein in rats after oral dosed with Da-Cheng-Qi decoction and Xiao-Cheng-Qi decoction, Biomed. Chromatogr. 21 (2007) 1186–1190.

[7] Y. Takizawa, T. Morota, S. Takeda, M. Aburada, Pharmacokinetics of rhein from Onpi-to, an Oriental herbal medicine, in rats, Biol. Pharm. Bull. 26 (2003) 613–617.

[8] C.S. Shia, Y.C. Hou, S.H. Juang, S.Y. Tsai, P.H. Hsieh, L.C. HO, P.D.L. Chao, Metabolism and pharmacokinetics of san-Huang-Xie-Xin-Tang, a polyphenol-rich Chinese medicine formula, in rats and ex-vivo antioxidant activity, Evidence-based Complem. Altern. Med. (2011), doi:10.1093/ecam/nep124, Article ID721293, 9pp.

[9] X.H. Jiang, D. Zhang, S.J. Chen, Studies on determination and pharmacokinetics of rhubarb anthraquinones in rabbit plasma by high performance liquid chromatography, Chin. J. Chromatogr. 21 (2003) 251–254.

[10] Y. Peng, J.G. Sun, G.J. Wang, Pharmacokinetic study of rhein and its carboxyl-esterification derivatives in rats, Chin. J. Nat. Med. 7 (2009) 228–233.

[11] Y.Y. Ren, H.L. Gong, W.F. Tang, M.F. Wan, Y. Huang, Effects of ranitidine on pharmacokinetics of rhein from Dachengqi Decoction in rats after oral administration, J. Chin. Integr. Med. 7 (2009) 868–872.

[12] B. Zan, R. Shi, T. Wang, J. Wu, N. Cheng, Simultaneous quantification of multiple active components from Xiexin decoction in rat plasma by LC–ESI-MS/MS: application in pharmacokinetics, Biomed. Chromatogr. 25 (2011) 816–826.

[13] F. Xu, Y. Liu, H. Dong, R. Song, Z. Zhang, Pharmacokinetic comparison in rats of six bioactive compounds between Da-Cheng-Qi decoction and its parent herbal medicines, Nat. Prod. Commun. 5 (2010) 795–800.

[14] C. Zhu, Z. Zheng, Z. Chen, X. Liang, Determination of rhein in plasma of rat by HPLC/MS, J. Chin. Med. Mater. 25 (2002) 646–649.

[15] H.T. Li, H. Sun, X.J. Wang, W.J. Sun, G.Z. Jiao, D.X. Zhou, L. Zhou, H.X. Cao, G.M. Zhang, Simultaneous determination by UPLC–ESI-MS of scoparone, capillarysisin, rhein, and emodin in rat urine after oral administration of Yin Chen Hao Tang preparation, J. Sep. Sci. 31 (2008) 659–666.

[16] J.H. Lee, J.M. Kim, Pharmacokinetic analysis of rhein in *Rheum undulatum* L, J. Ethnopharmacol. 84 (2003) 5–9.

[17] H. Gong, W. Tang, H. Wang, Q. Xia, X. Huang, Effects of food and gender on the pharmacokinetics of rhein and emodin in rats after oral dosing with Da-Cheng-Qi decoction, Phytother. Res. 25 (2011) 74–80.

[18] H.F. Zhang, Y.P. Shi, Temperature-assisted ionic liquid dispersive liquid–liquid micro-extraction combined with high performance liquid chromatography for the determination of anthraquinones in Radix et Rhizoma Rhei samples, Talanta 82 (2010) 1010–1016.

[19] Y. Li, J.P. Gao, X. Xu, L.X. Dai, Simultaneous determination of baicalin, rhein and berberine in rat plasma by column-switching high-performance liquid chromatography, J. Chromatogr. B 838 (2006) 50–55.

[20] A. Ojha, R. Rathod, H. Padh, H. Padh, Simultaneous HPLC–UV determination of rhein and aceclofenac in human plasma, J. Chromatogr. B 877 (2009) 1145–1148.

[21] W. Zhu, X.M. Wang, L. Zhang, X.Y. Li, B.X. Wang, Pharmacokinetic of rhein in healthy male volunteers following oral and retention enema administration of rhubarb extract: a single dose study, Am. J. Chin. Med. 33 (2005) 839–850.

[22] Guidance for Industry, Bio-Analytical Method Validation, no. 2, Department of Health and Human Services, U.S. Food and Drug Administration, 2001, pp. 929–935.

[23] S.C. Chow, J.P. Liu (Eds.), Statistical Design and Analysis in Pharmaceutical Science: Validation, Process Controls, and Stability, Marcel Decker, Inc., New York, NY, USA, 1995, ISBN 0-8247-9336-6.

[24] Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects, World Medical Association (WMA), 2006, http://www.wma.net/e/policy/b3.htm (accessed on 16.08.06).

[25] L.R. Snyder, J.J. Kirkland, J.L. Glajch, Practical HPLC Method Development, John Wiley & Sons, Inc., New York, 1997, p. 659.