Comparison of pharmacokinetic behavior of two iridoid glycosides in rat plasma after oral administration of crude *Cornus officinals* and its jiuzhipin by high performance liquid chromatography triple quadrupole mass spectrometry combined with multiple reactions monitoring mode

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ABSTRACT

Objective: The present study examined the pharmacokinetic profiles of two iridoid glycosides named morroniside and loganin in rat plasma after oral administration of crude and processed *Cornus officinals.* **Materials and Methods:** A rapid, selective and specific high-performance liquid chromatography/electrospray ionization tandem mass spectrometry with multiple reactions monitoring mode was developed to simultaneously investigate the pharmacokinetic profiles of morroniside and loganin in rat plasma after oral administration of crude *C. officinals* and its jiuzhipin. **Results:** The morroniside and loganin in crude and processed *C. officinals* could be simultaneously determined within 7.4 min. Linear calibration curves were obtained over the concentration ranges of 45.45-4800 ng/mL for all the analytes. The intra-and inter-day precisions relative standard deviation was lesser than 2.84% and 4.12%, respectively. **Conclusion:** The pharmacokinetic parameters of two iridoid glucosides were also compared systematically between crude and processed *C. officinals*. This paper provides the theoretical proofs for further explaining the processing mechanism of Traditional Chinese Medicines.

Key words: High performance liquid chromatography-triple quadrupole mass spectrometry, morroniside and loganin, multiple reactions monitoring mode, pharmacokinetics, rat plasma

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INTRODUCTION

Cornus Officinals, a famous Chinese herbal medicine, is derived from the dry ripe sarcocarp of *C. officinalis* Sieb. et Zucc (Cornaceae) and recorded in Chinese Pharmacopoeia, which is a classic resource on Traditional Chinese Medicine (TCM).[1] Owing to its biological and pharmacological activities such as anti-inflammation, anti-virus and anti-oxidation, *C. officinals* has increasingly drawn much attentions as one of the most popular and cherished herbal medicine in the clinic in the world and can be used for medicine, hygienic food and cosmetic.[2,3]

The crude TCM and its processed product-jiuzhipin (JZP) are often used clinically. Appropriate pharmaceutical processing may reduce toxicity or side-effects, potentiate the beneficial effects, change the pharmacological properties, preserve active constituents, facilitate administration, improve flavor or eliminate unpleasant taste and increase purity of Chinese medicine.[4,5] In China, the processing methods for crude TCM have been practiced since the Tang dynasty and are documented in Chinese pharmacopoeia.[6] Previous studies showed that the crude *C. officinals* is better in astringing yin for arresting sweating, which has been used intensively for the treatment of spontaneous sweating, night sweating, spermatorrhea and enuresis.[7,8] The typical example is *C. officinals* powder

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for treating kidney deficiency and frequent micturition incontinence. *C. officinals* after stewed with yellow rice wine is stronger in nourishing kidney, astringing semen and reducing urination, which has been used diffusely for curing dizziness, coldness and pain in waist, frequent micturition, enuresis, impotence and prospermia. Simultaneously, *C. officinals* warmly dredges up by dint of wine and its acidity is reduced.[9,10]

A previous report revealed that the 39 compounds in crude and JZP and 10 compounds in rat plasma after oral administration of *C. officinals* were detected.[11] It provides a helpful chemical proof for further pharmacology and active mechanism research. However, biological studies with *C. officinals* have mainly been focused on the pharmacokinetics of one or a few bioactive components.[12] This approach offers limited information toward studying the pharmacology of *C. officinals* since the pharmacokinetic properties of each chemical constituent have not been taken into account. Until now, most investigations have been based on the pharmacodynamics or pharmacology of crude *C. officinals* and very little attention has been devoted to the pharmacokinetic study of multiple components of processed *C. officinals in vivo*.

Iridoid glycosides are the major active components widely distributed in crude *C. officinals* and its processed products. Exploring dynamic of iridoid glycosides in dialysate from *C. officinals* and its processed products may help to explain why crude and processed *C. officinals* have traditionally been used for treating different clinical symptoms and to expatiate upon the processing mechanism.[13] To the best of our knowledge, several high-performance liquid chromatography (HPLC)/ultraviolet methods have been applied to the quantification of single morroniside or loganin from crude *C. officinals*, but not from its processed products.[14] However, these methods cannot be used to study the pharmacokinetics of multiple absorbed components, due to the fact that the plasma sample used for testing at each time point cannot exceed 10% of the total plasma volume of an animal if multiple time points need to be measured. Thus, it is necessary to develop a more comprehensive and global assay to fully evaluate the pharmacokinetics of iridoid glycosides from crude and processed *C. officinals*.

Therefore, we hypothesized that two iridoid glucosides named morroniside and loganin in JZP may exhibit pharmacokinetic properties presenting with increased C_{max} and delayed $T_{1/2}$ following oral administration, which appear to be beneficial for the pharmacological activities. In the present study, a rapid, selective and specific HPLC- electrospray ionization tandem mass spectrometry (ESI-MS/MS) with multiple reactions monitoring (MRM) mode was firstly developed to simultaneously investigate the pharmacokinetic profiles of morroniside and loganin *in vivo* and to screen potentially bioactive components in rat plasma after oral administration of crude *C. officinals* and its JZP. The pharmacokinetic behaviors of two iridoid glucosides were also compared systematically between the crude and processed *C. officinals*. This paper provides the theoretical proofs for further explaining the processing mechanism of TCMs.

EXPERIMENTAL

Materials and reagents

The crude and processed forms of *C. officinals* and its JZP were collected from Henan suppliers. Loganin was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Morroniside was obtained from Shanghai Shangyi Biotechnology Co. Ltd., (Shanghai, China). The purity of each standard compound was greater than 98% by HPLC analysis. The structures of these two compounds are shown in Figure 1. HPLC-grade acetonitrile and methanol was obtained from Merck (Darmstadt, Germany) and Fisher Scientific Corporation (Loughborough, UK), respectively. Deionized water was purified using the Milli-Q system (Millipore, Bedford, MA, USA) and HPLC grade formic acid was purchased from Honeywell Company (Morristown, New Jersey, USA). All other chemicals were of analytical grade and commercially available.

Instrument and analytical conditions

Chromatographic experiments were performed on an Agilent 1200 series HPLC system (Santa Clara, USA) with the mobile phase consisting of (A) methanol acetic acid (0.1%, v/v) and (B) aqueous acetic acid (0.1%, v/v) using a gradient elution of 10-90% B at 0-10 min. An Agilent Zorbax Extend C_{18} (100 mm × 3.0 mm, 3.5 μm) was employed with a flow rate of 0.6 mL/min. The column temperature was set at 40°C and the injection volume was 5 μL.

Determination was performed using an Agilent Technologies 6410 Triple Quad liquid chromatography/MS equipped with

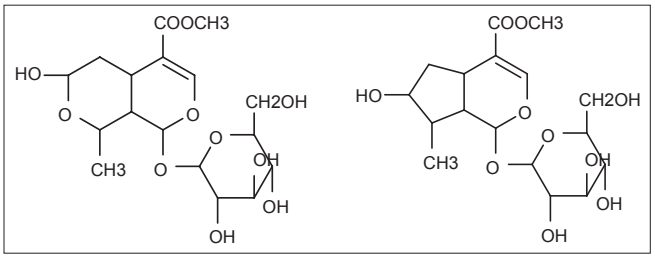

Figure 1: The chemical structures of two iridoid glucosides in *Fructus corni*

electrospray ionization (ESI). The compounds were ionized in the positive and negative ion polarity mode. The ionization source conditions were as follows: Spray voltage of 4000 V (+), 3600 V (−), source temperature of 100°C and desolvation temperature of 350°C. Nitrogen was used as nebulizer gas and pressure was set at 40 p.s.i at a flow rate of 10 L/min. The pressure of high purity nitrogen was 0.15 MPa for collision-induced dissociation (CID). Quantification was operated at MRM modes. The delta potential of the electron multiplier (EMV) was set to 400 V for data acquisition. The summary of MS/MS detection parameters is shown in Table 1. Data acquisition and processing were performed by Agilent Mass Hunter Workstation.

Preparation of sample solutions

The 100 g powder of *C. officinals* and its JZP samples were transferred to the round-bottom flasks with ten-fold volumes of distilled water for about 2 h, respectively. A few grains of pumice were added and then boiled on a water-bath under a reflux condenser for about 2 h. After cooling to the room temperature, the sample solutions were filtered and the filtrates were collected. The solid residues were treated again as described above (for 1 h). The collected filtrates were mixed and evaporated to a final volume of 50 mL under reduced pressure at a temperature not exceeding 60°C. The extracted solutions were filtered through 5 layer gauzes and concentrated to approximately 2 g crude drug per milliliter (this concentration of the solution was used for oral administration) and finally the solutions were lyophilized.

Animals and blood sampling

Sprague-Dawley rats (8 weeks old, 200 ± 20 g) were obtained from the Laboratory Animal Center of Zhejiang Chinese Medical University (Zhejiang, China). The experimental protocol was approved by the University Ethics Committee for the use of experimental animals and conformed to the Guide for Care and Use of Laboratory Animals. Rats were housed in groups of two or three with 12 h light/12 h dark cycle at a temperature of 22°C ± 1°C, relative humidity of 60% ±10%, for 1 week. Immediately before the day of administration, the rats were fasted for 12 h, but were allowed water *ad libitum*. Then, aqueous solutions of crude and processed *C. officinals* extracts were administered to the rats at a dose of 1.0 mL/100 g body weight, respectively. The rats were anesthetized by inhalation of diethyl ether after administration. At 5, 10, 15, 30, 45, 60, 90, 120, 180, 240 and 360 min following oral administration, the blood samples were collected from the caudal vein into heparinized tubes and centrifuged at 10,000 rpm for 15 min to separate plasma and stored at −80°C until analysis.

A simple liquid-liquid extraction method was applied to extract two iridoid glucosides from rat plasma. Briefly, to 200 μL of the plasma sample, 15 μL of methanol was added (volume of the corresponding working solution for calibration curve and quality control [QC] samples). Then, the mixture was vortexed for 1 min and extracted with 2 mL ethyl acetate by vortex-mixing for 5 min. The upper layer was transferred to a clean tube after centrifugation at 12,000 rpm for 10 min. The organic phase was evaporated to dryness under a gentle stream of nitrogen gas. The obtained residue was reconstituted in 50 μL methanol and centrifuged at 12,000 rpm for another 5 min. The above operations were carried out at room temperature. And then, an aliquot of 5 μL supernatant was injected into HPLC-MS system for analysis.

Pharmacokinetic analysis

The data of plasma concentration versus time was subjected to a non-compartmental pharmacokinetic analysis using Kinetica 5.0 (Thermo Electron Corp., Philadelphia, PA) to obtain an estimate of various pharmacokinetic parameters. Maximum plasma concentration (C_{max}) and time of maximum concentration (T_{max}) were obtained directly from the plasma concentration-time plots. The elimination rate constants (k) were determined by linear regression on the logarithmic transformation of the last four data points of the curve. The elimination half-life ($T_{1/2}$) was calculated by the following equation: $T_{1/2} = 0.693/k$. The area under the plasma concentration versus time curve up to the last time (t) (AUC_{0-t}) was determined using the trapezoidal rule. The AUC0−∞ values were calculated by adding the value of $Ct \times k^{-1}$ to AUC_{0-t}.

RESULTS AND DISCUSSION

Method validation

Selectivity

The specificity of the method was presented by comparing MRM chromatograms of morroniside and loganin from

Table 1: MS/MS detection parameters for morroniside and loganin in *Fructus corni*

Compound	Precursorion (m/z)	Production	Dwell time (ms)	Fragmentation (V)	Collision energy (eV)
Morroniside	451	243	750	100	12
		179			15
Loganin	435	389	175	100	10
		227			10

MS: Mass spectrometry

crude and processed *C. officinals* for a blank rat plasma sample, a spiked plasma sample and a plasma sample from a rat 3 h after oral administration of crude and processed *C. officinals* extracts. The morroniside and loganin were eluted at 6.0 min and 7 min approximately. As shown in Figure 2, no interfering peaks were found at the retention time of morroniside and loganin. Two bioactive compounds were confirmed by the mass spectral analyses [Figure 3].

Linearity of the calibration curve and lower limit of quantification (LLOQ)

The standard curves of the peak area (Y) to the concentration (C) were constructed using $1/x^2$ weighted linear least-squares regression model. The standard curves, correlation coefficients and linear ranges of morroniside and loganin in plasma were y = 0.6433 × –24.602 (45.45-2272.72 ng/mL) and y = 1.3161 × –51.830 (96.00-4800 ng/mL). All the marker substances showed good linearity ($r^2 \geq 0.9994$). LLOQ of the two analytes were 45.45-96.00 ng/mL.

Accuracy and precision

Accuracy, intra- and inter-day precisions were evaluated from the results of QC samples. Six replicates of QC samples at three concentration levels were determined on three different days. The mean values and relative standard deviation (RSD) for QC samples were calculated over

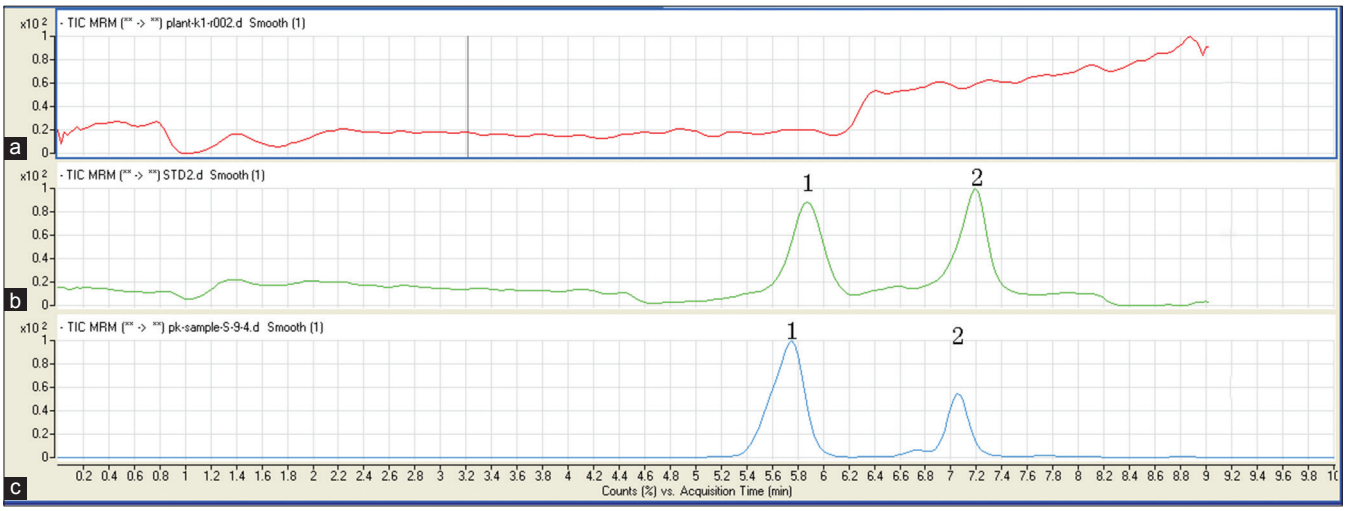

Figure 2: Liquid chromatography/mass spectrometry chromatographic profiles of *Fructus corni* samples. (a) Blank plasma; (b) blank plasma spiked with morroniside and logain; (c) plasma samples after oral administration *Fructus Corni* extracts; (1) morroniside; (2) logain

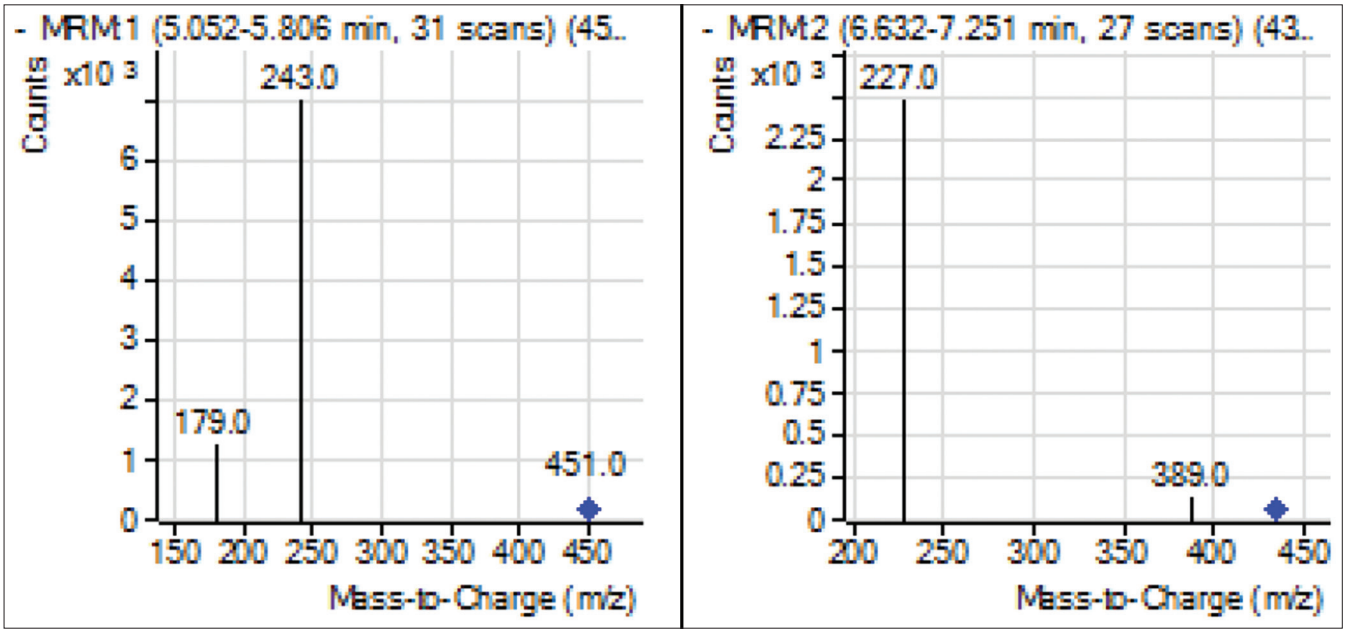

Figure 3: Mass spectra of two bioactive compounds. Each compound was collected from high-performance liquid chromatography and analyzed by mass spectra

three validation days. The intra- and inter-day precisions were expressed by RSD. Table 2 summarizes the intra- and inter-day precisions and accuracies of morroniside and loganin at three concentration levels (low, middle and high). The intra- and inter-day precisions (RSD) of these analytes were all lesser than 2.84% and 4.12%. These results were within the acceptance criteria and indicated that the method was accurate, reliable and reproducible.

Recovery

The extraction recovery of analytes at three QC levels was conducted by calculating at each standard concentration as the ratio of the peak area for extracted blank plasma spiked before extraction relative to peak area of the equivalent blank plasma samples spiked after the extraction.

The recoveries of morroniside and loganin from rat plasma are shown in Table 3. The mean recoveries of morroniside and loganin were more than 79% at three concentration levels (low, medium and high). This result indicated that the efficiency was acceptable and the method was accurate.

Stability

QC samples of morroniside and loganin at three concentrations were used for stability experiments. The stabilities of morroniside and loganin were tested under different conditions. It was indicated that this new method for the simultaneous determination of morroniside and loganin in rat plasma offered satisfactory stability, with accuracy in the range between 7.48% and 14.82%.

Application to pharmacokinetic study

This HPLC-MS/MS method was developed and used successfully in the pharmacokinetic studies in rats. The mean plasma concentration-time curves of morroniside and loganin in rats after oral administration of crude *C. officinals* and its JZP extracts are shown in Figures 4 and 5. Their pharmacokinetic parameters are listed in Table 4. In general, morroniside and loganin could be absorbed and eliminated rapidly in rats, for it was detected in plasma at 5 min. As shown in Figures 4 and 5, the plasma levels of morroniside and loganin after administration of JZP extracts were much higher than those after oral administration of crude extracts. The $T_{1/2}$ of morroniside and loganin after used with JZP extracts were longer than that after used with crude extracts. The AUC_{0-t} and C_{max} of morroniside and loganin after administration of JZP were significantly higher than those after administration of crude product. The information described above might be helpful for further studies on the pharmacokinetics of *C. officinals* and beneficial for application of this TCM in clinical therapy.

CONCLUSION

A novel HPLC-ESI-MS/MS with MRM mode was firstly developed and validated for comparison of pharmacokinetics of two iridoid glucosides in rat plasma. The analytical procedure was then successfully applied to a pharmacokinetic study of the analytes after oral administration of crude *C. officinals* and its jiuzhipin and it could help to investigate the processing mechanism of *C. officinals*. To the best of our knowledge, this paper is the first study reporting the comparison of pharmacokinetics

Table 3: Recoveries of morroniside and loganin at three different spiked level in rat plasma samples

Added (ng/mL)	Found (ng/mL)	Recovery (%)	RSD (%) (*n*=6)
Morroniside			
45.45	38.41	84.51	1.48
227.27	186.08	81.88	1.77
2272.72	1811.47	79.71	0.82
Loganin			
96	81.05	84.43	1.03
480	396.94	82.70	1.28
4800	3912.89	81.52	1.34

RSD: Relative standard deviation

Table 2: The intra- and inter-day accuracy and precisions of morroniside and loganin in rat plasma at low, medium, and high concentration levels

Compounds spiked conc. (ng/mL)	Intra-day			Inter-day		
	Measured concentration (ng/mL)	Accuracy (%)	Precision (%)	Measured conc. (ng/mL)	Accuracy (%)	Precision (%)
Morroniside						
45.45	47.84±0.80	5.26	1.75	44.54±0.53	−2.01	1.17
227.27	217.56±3.22	−4.27	1.42	215.88±2.39	−5.01	1.05
2272.72	2428.61±26.80	6.86	1.18	2423.79±23.30	6.65	1.03
Loganin						
96	100.21±2.54	4.38	2.64	87.28±2.33	−9.09	2.42
480	497.79±7.96	3.71	1.66	504.45±19.76	5.09	4.12
4800	4609.00±136.51	−3.98	2.84	5148.05±66.93	7.25	1.39

Table 4: Mean pharmacokinetic parameters of morroniside and loganin in rat plasma after oral administration of crude processed *Cornus officinals* and JZP extracts

Parameters	Crude		Processed	
	Morroniside	Loganin	Morroniside	Loganin
k (1/min)	0.00668±0.00069	0.00816±0.00041	0.00389±0.0015	0.005187±0.0017
$t_{1/2}$ (min)	103.79±10.83	85±4.31	178.2±67.48	133.64±34.42
C_{max} (mg/L)	375.53±103.91	435.14±68.62	928.77±245.68	1251.11±266.65
AUC_{0-t}	39491.99±4209.05	33076.06±3455.78	92824.42±8775.57	135331.61±36275.78
MRT	190.54±23.35	176.46±32.01	250.76±51.38	195.77±37.82

JZP: Jiuzhipin; AUC: Area under the plasma concentration versus time curve up to the last time; MRT: Mean residence time

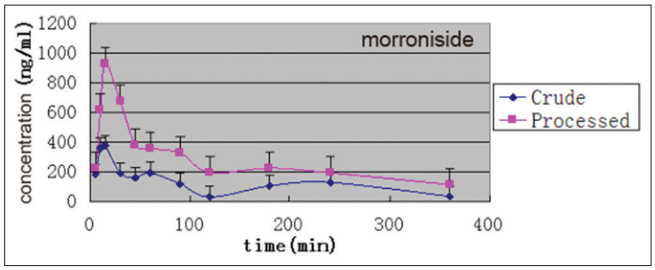

Figure 4: Mean plasma concentration-time curves of morroniside after oral administration of crude *Fructus corni* extracts to rats (n = 6)

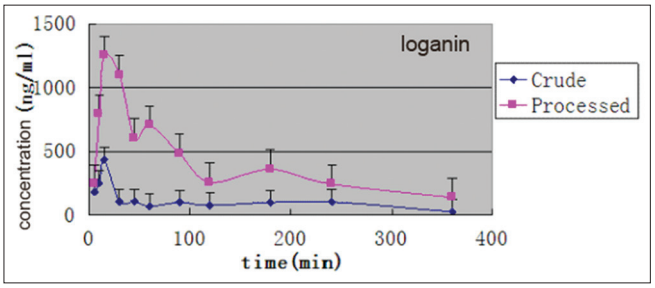

Figure 5: Mean plasma concentration-time curves of logain after oral administration of processed *Fructus corni* extracts to rats (n = 6)

of bioactive compounds after oral administration of Chinese herbal medicine extracts to rats. The described novel method has high sensitivity and specificity and would offer a good alternative for simultaneous analysis of the process of absorbing multi-components into the body after administration of Chinese herbal medicine.

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