Determination of isofraxidin and astilbin by HPLC in rat plasma and its application after orally administration the extract of *Sarcandra glabra*

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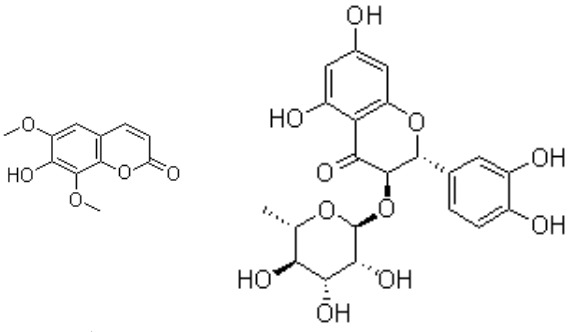
Abstract: *Sarcandra glaber* is a common traditional Chinese medicine used to treat psoriasis and other infectious diseases, isofraxidin and astilbin are the main components of it. In order to study the pharmacokinetics of *Sarcandra glabra*, an HPLC method for simultaneous determination of isofraxidin and astilbin in rat plasma was established. Plasma samples were prepared using solid phase extraction method. C_{18} column with a guard was used, mobile phase was consisted of A (methanol) and B (0.1% aqueous acetic acid) with gradient elution as follows: 0 ~ 4min, A: 35%, B: 65%; 4 ~ 10min, A: 35% ~ 45%, B: 65% ~ 55%; 10 ~ 20min, A: 45%, B: 55%. The flow rate was 1.2 mL/min from 0 to 4 min, 1.0 mL/min from 4 to 20 min. The detection wavelength was 300 nm. A linear correlation between drug amount and peak area was established for isofraxidin in the range of 20-320 ng and for astilbin in the range of 19-304 ng. The recovery was over 68% for both compounds, the accuracy was within 8%, and the inter-day and intra-day precisions were all less than 8%. The pharmacokinetics of isofraxidin and astilbin was studied after orally administration the extract of *Sarcandra glabra.*

Keywords: Isofraxidin; astilbin; HPLC, pharmacokinetic; *Sarcandra glabra.*

INTRODUCTION

Sarcandra glaber (thumb) Nakai, an ever green shrub growing in south China and Japan belonging to the family *Chloranthaceae*, is a common traditional Chinese medicine. It has antibiosis, anti-inflammation, non-specific immuno-enhancement and anticancer effects (Li *et al.,* 2007, He RR *et al.,* 2009, and Yuan *et al.,* 2008), therefore it is usually used to treat cancer and infectious diseases, such as pneumonia, psoriasis, appendicitis and gastroenteritis (Wang J *et al.,* 2007, Lin *et al.,* 2006).

Isofraxidin and astilbin (fig. 1) are the main components of *Sarcandra glaber*. Astilbin has anti-bacteria, anti-inflammation, immuno-suppressing effects, and it could inhibit coenzyme A reductase (Fei *et al.,* 2005, Yi *et al.,* 2008). Isofraxidin has anti-stress, anti-fatigue and immuno-regulating effects. All these are related to the effect of *Sarcandra glaber*. Since drug concentration *in vivo* could reflect the effectiveness and toxicity of drugs (Zhang, 2011), therefore, investigating the pharmacokinetics of isofraxidin and astilbin can provide more understanding about the effect of *Sarcandra glaber*, and this will be useful for guiding its clinical application. Thus, establishing a reliable *in vivo* analytical method is absolutely essential.



A B

Although there were some reports on the analysis of astilbin and isofraxidin in plasma/serum (Sun *et al.,* 2007; Guo *et al.,* 2007), these methods could only determine one component. Compared to determining each component one by one, simultaneous determination of two or more components is more economical and time-saving (Gao *et al.,* 2012). Here, we reported an HPLC method for the simultaneous determination of astilbin and isofraxidin for the first time, and thereafter, the pharmacokinetics of these two components were studied after orally administration the extract of *Sarcandra glaber.*

MATERIALS AND METHODS

Chemicals and reagents

Astilbin was purchased from Guang Dong Hanfang medical Company (purity >98%) (Guangzhou, China), isofraxidin was from Chinese national institution of biological and pharmaceutical products (purity >98%) (Bei jing, China). HPLC grade methanol was purchased from Siyou Biological Company of Handan (Hebei

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province, China). All other reagents were of analytical grade.

Chromatographic conditions

Agilent 1200 system with G1314A UV detector was used for analysis, and the detection wavelength was 300 nm, column oven temperature 25°C, and a online solvent degasser system was used. A Gemini C_{18} (150×4.6 mm,5 μm) column with security guard C_{18} (4.6×2.0 mm, 5 μm) (Phenomenex, USA) was used, the mobile phase was composed of methanol (A) and 0.1% acetic acid (B) in gradient mode (0 ~ 4 min, A: 35%, B: 65%; 4~ 10 min, A: 35% ~ 45%, B: 65% ~ 55%;10 ~ 20 min, A: 45%, B: 55%). The flow rate was 1.2 mL/min during 0~ 4 min, and 1.0 mL/min during 4 ~ 20 min.

Extraction of Sarcandra glaber

320 g of *Sarcandra glaber* was coarsely smashed and socked in 3.2 L of 70% ethanol for 0.5 h, and then heated to boil, keeping boiling for 0.5 h, the extract was filtrated. Gruffs were extracted again, the filtrates were combined and condensed to 100 mL. Then the extract was stored at -20°C until use.

Preparation of standard and quality control solutions

Individual stock solutions of astilbin and isofraxidin (1 mg/mL) were prepared in methanol and stored at 4°C. Standard solutions containing 1, 2, 4, 8, 12, 16 μg/mL of each component were prepared by diluting the stock solutions using methanol. The same procedure was used for preparing standard solution (2.0 μg/mL) and quality control (QC) solutions, which contained 2.0, 6.0 and 16 μg/mL of each component. Calibration standards and QC samples were prepared by adding 50 μL of standard or QC solution to 200 μL of blank rat plasma.

Plasma sample collection

Five male Wister rats (weight 350 ± 20g) were purchased from Guangdong medical laboratory animal centre (Guangzhou, China). Prior to experiment animals were kept under fast condition overnight. All animal studies were reviewed and approved by the animal and ethics review committee of Guangzhou University of Chinese Medicine and Euthanasia and disposal of carcass was in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Rats were anaesthetized and orally administrated with the water extract of *Sarcandra glaber* (the dosage was 1.5 mL/100g). After intra-gastric administration of the drug, at predetermined time points (5min, 15min, 0.5, 1, 1.5, 2, 4, 6, 8 h), 0.5 mL of blood was collected via retro-orbital plexus puncture. After centrifugation at 13000 rpm for10 min, the supernatant was transferred to a 2 mL polypropylene Eppendorf vial and stored at -80°C until analysis.

Sample preparation

Solid-phase extraction method was used for sample preparation. After activated and equilibrated using methanol (1 mL) and demineralized water (1 mL) respectively, Waters Oasis HLB C_{18} cartridge (1 mL, 30 mg) was used for the extraction. 200 μL of plasma and equal amount of water was swirled for 30 s and then loaded onto the cartridge, thereafter, it was eluted with 1 mL of water, sucked dry and eluted with 0.5mL of

Table 1: Recovery of astilbin and isofraxidin from plasma (n=5)

Compound	Spiked concentration (ng)	Observed concentration (ng)	Recovery (%)	RSD (%)
isofraxidin	40	28.2	70.4	1.13
	120	84.8	70.7	0.51
	240	172.6	71.9	0.01
astilbin	38	26.1	68.7	3.33
	114	93.6	82.1	0.79
	228	177.8	78.0	0.75

Table 2: Intra-day and inter-day precision and accuracy of astilbin and isofraxidin in spiked plasma samples (n=5)

Compound	Spiked concentration (ng)	Observed concentration (ng)	Accuracy (%)	RSD (%)	Precision (RSD %)	
					Intra-day (n=5)	Inter-day (n=15)
Atilbin	38	39.0	102.6	0.45	1.39	7.23
	114	118.0	103.5	1.03	4.07	4.89
	228	218.2	95.7	0.98	4.49	5.14
Isofraxidin	40	45.0	112.6	0.87	3.82	4.17
	120	113.8	94.8	0.35	0.96	5.12
	240	216.0	90.0	6.78	6.77	7.12

methanol. The methanol elution was collected, and then mixture of methanol and 0.1% glacial acetic acid (1:1) 0.5 mL was added, the mixture was swirled for 10 s, filtered with 0.22 μm milipore member, and the filtrate was used for analysis.

Method verification

Calibration curve

Mix blank rat plasma with the appropriate standard solutions, and then these samples were extracted by solid-extraction method as previously described in "sample preparation" section. Calibration curves were constructed by plotting the peak area against the content of different concentrated samples.

Accuracy and precision

Replicate analysis (n=6) of QC samples at three concentration levels on three different validation days were used for determining the accuracy and precision. The accuracy was expressed by (mean observed concentration)/ (spiked concentration) ×100%, and the precision by the relative standard deviation (RSD %).

The limit of quantitation and detectability

The detectability was determined as S/N ratio about 7:1, and the limit of quantiation was determined as the lowest point in the calibration curve.

Recovery

The extraction recovery of both compounds at three QC levels was appraised by analysing the samples as described above and comparing the peak areas with those obtained from direct injection of the compounds dissolved in the supernatant of the processed blank plasma.

Selectivity

The selectivity of the proposed methodology was evaluated by assaying six blank rat plasma samples for potential interfere at the retention time of astilbin and isofraxidin.

RESULTS

Selectivity

Representative chromatograms of blank plasma, plasma spiked with astilbin and isofraxidin, and rat plasma after orally administration of *Sarcandra glaber* are shown in fig. 2. The retention time of isofraxidin and astilbin was 9.83 and 13.64 min respectively. The blank sample showed no peaks at the corresponding retention time of astilbin and isofraxidin. Evidently, it had no interference on the elution of astilbin and isofraxidin. Thus, the chromatographic condition could be used for the determination of astilbin and isofraxidin. Since the polarity of astilbin and isofraxidin is quite different, in iso-gradient mode a longer time is needed for elution. Therefore, the gradient method was used in this study.

Sample preparation

Sample extraction step is a key element for accuracy and sensitivity of the analytical method, especially for biological samples. Currently the most widely used sample extraction methods are liquid-liquid extraction (LLE), protein precipitation (PPT) and solid-phase extraction (SPE). In this paper, we compared these three methods. LLE method gave a poor recovery compared to SPE and PPT, and compared to SPE, the baseline noise of the PPT method was too large and declined the sensitivity. Therefore, the SPE method was selected.

Calibration curve and recovery

The calibration curves were Y=4.437X-1.254 (r^2= 0.999) and Y=4.3803X-0.5902 (r^2= 0.999) in the content range of 20 ng to 320 ng for astilbin and isofraxidin respectively. The detectability was 9.5 ng for isofraxidin and 10 ng for astilbin. The detection limit was set as the lowest concentration in the calibration curve (astilbin 19 ng, isofraxidin 20 ng). The extraction recovery results of both compounds at three QC levels are listed in table 1. The recovery was higher than 68.7% for astilbin and 70.4% for isofraxidin.

Accuracy and precision

The accuracy and precision results are shown in table 2. The accuracy at three levels were among 95.7~103.5% and 90.0~112.6% for astilbin and isofraxidin respectively. The RSD of intra-day was less than 4.49% and 6.77%, and the RSD of inter-day precision was less than 7.23% and 7.12% for astilbin and isofraxidin respectively. The values assessed were considered acceptable.

Stability

Stability of the two compounds in rat plasma was investigated by analyzing three replicates of QC samples, and the conditions listed below were used: short-term stability at 20°C for 8h and at 4°C for 16h, after three freeze/thaw cycles (-80°C to 20°C), the results are listed in table 3. The results showed that all samples were stable during the specified storage conditions and analytical process.

Application of the method

This method was used to determine the plasma content of astilbin and isofraxidin after orally administration of the water extract of *Sarcandra glaber*. The typical serum chromatogram is shown in fig. 2. At all the points, only the peak of isofraxidin was detected. Isofraxidin was absorbed and eliminated quickly, the concentration- time profile is shown in fig. 3. The T_{max} was 30min, and was eliminated completely within 4 h.

DISCUSSION

Determination method

Generally, in the development of analytical method, the injection volume was constant since many types of

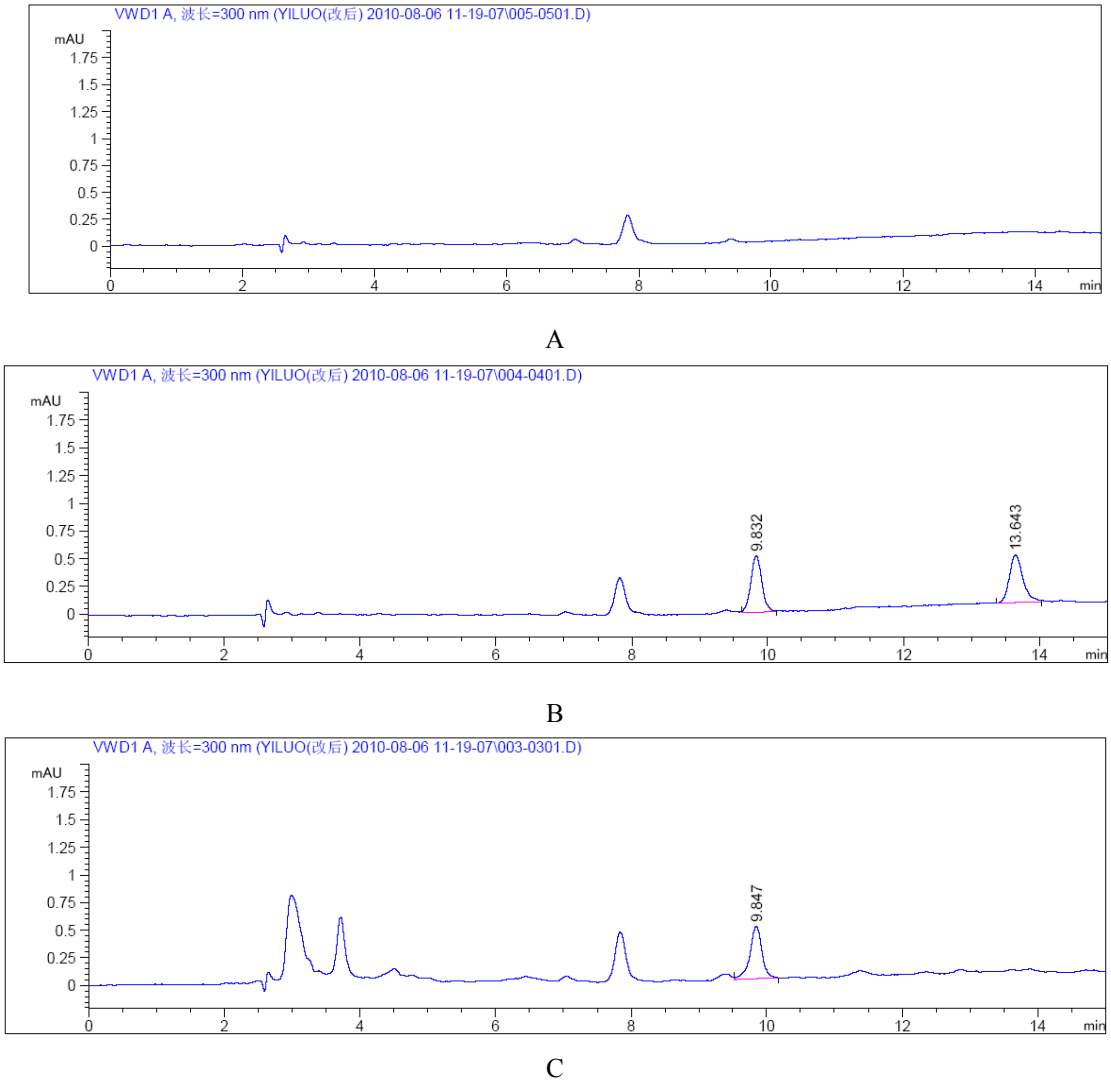

Fig. 2: Representative HPLC-UV chromatograms (λ=300 nm) of blank plasma (a), blank plasma spiked with QC samples (b) and rat plasma after orally administration of the extraction of *Sarcandra glaber* (t=0.5h).

equipment provide a fixed quantity ring. In this paper, a changeable injection volume could be achieved in the range of 10 µL to 1000 µL. Therefore, for the samples with low drug concentration, the content of the drugs in the sample could be determined by increasing injection volume, therefore in this paper, the calibration curve was constructed by peak areas against drug content, and the concentration of drugs in the plasma was a calibrated result.

Pharmacokinetics study

From fig. 2 it is shown that astilbin had not detected. In order to determine astilbin, the injection volume was increased to 800 µL. However, there was no trace of astilbin. Study by Wang et al showed that astilbin had poor permeation across Caco-2 cell line and it is the substrate of P-gp (Wang X.D. *et al.,* 2009), and in *in vivo* condition astilbin as a flavonoid may be metabolized by kinds of enzymes like other flavonoid (Liang *et al.,* 2009), all these might lead to a poor bioavailability. Besides, there are many components in *Sarcandra glaber,* and the other constituents in the extract may affect the absorption and metabolism of astilbin, the concrete reason needs further study.

From fig. 3, it is found that isofraxidin absorbed and eliminated quickly. Similar results has been reported by Sun *et al.,* who studied the pharmacokinetics of isofraxidin in rats after orally administration of isofraxidin (Sun *et al.,* 2007), implying that the pharmacokinetic behavior of isofraxidin was not affected by other components in the extract. Absorption into the body is the capital terms for drug action, and the results of this paper suggested that isofraxidin may be one of the active components of *Sarcandra glaber.*

Table 3: Stability of isofraxidin and astilbin under various conditions

Analyst	Conditions	Added amount (ng)	Measured amount (ng)	RSD (%)
Isofraxidin	20°C 8h	40	45.46	1.11
		120	115.98	0.97
		240	220.72	0.90
	4°C 16h	40	46.81	1.17
		120	117.76	0.98
		240	220.13	0.92
	Freeze thaw	40	46.21	1.13
		120	118.99	0.99
		240	223.06.	0.95
Astilbin	20°C 8h	38	37.46	0.98
		114	117.76	1.03
		228	219.61	0.96
	4°C 16h	38	40.32	1.06
		114	116.58	1.02
		228	217.60	0.95
	Freeze thaw	38	38.51	0.92
		114	118.00	0.89
		228	219.96	0.91

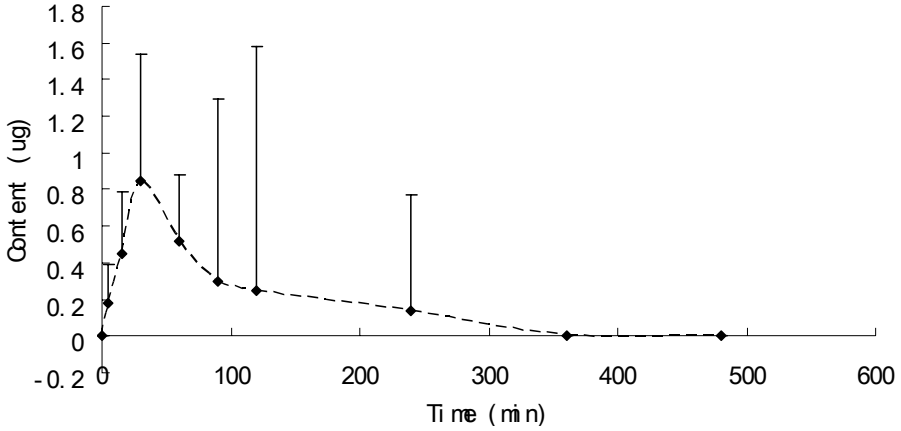

Fig. 3: The concentration-time profile of isofraxidin in rats after orally administration the extract of *Sarcandra glaber.*

CONCLUSION

In this paper, an HPLC method for simultaneous determination of astilbin and isofraxdin *in vivo* was established for the first time, and the pharmacokinetic behavior of isofraxidin after orally administration of the extract of *Sarcandra glabe* was studied. The established method was accurate, precise, simple and easy to perform. The pharmacokinetic results showed that isofraxidin might be the main components of *Sarcandra glabe,* and it could be used as the quality control index of the herb.

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