Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 44 (2007) 737–742
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Simultaneous determination of six major stilbenes and flavonoids in *Smilax china* by high performance liquid chromatography

Bo Shao, Hong-zhu Guo *, Ya-jun Cui, Ai-hua Liu, Hai-lan Yu, Hui Guo, Man Xu, De-an Guo *

The State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, No. 38 Xueyuan Road, Beijing 100083, PR China

Received 5 November 2006; received in revised form 4 March 2007; accepted 9 March 2007
Available online 14 March 2007

Abstract

A simple, sensitive and specific HPLC method was developed for simultaneous determination of the six major active constituents in *Smilax china*, namely taxifolin-3-*O*-glycoside (**1**), piceid (**2**), oxyresveratrol (**3**), engeletin (**4**), resveratrol (**5**) and scirpusin A (**6**), respectively. The samples were separated on an Aglient Zorbax XDB-C_{18} column with gradient elution of acetonitrile and 0.02% phosphoric acid (v/v) at a flow rate of 1.0 ml/min and detected at 300 nm. The six target compounds were completely separated within 35 min. All calibration curves showed good linearity ($r^2 > 0.999$) within test ranges. The reproducibility was evaluated by intra- and inter-day assays and R.S.D. values were less than 3.7%. The recoveries were between 93.7 and 103.0%. The method was successfully applied to the analysis of six constituents in 15 commercial samples of *S. china*. The results indicated that the developed HPLC assay was readily utilized as a quality control method for *S. china*.

Keywords: *Smilax china*; Stilbenes; Flavonoids; High performance liquid chromatography

1. Introduction

The tuber of *Smilax china* (Liliaceae) is a traditional Chinese medicine, named as Ba Qia (or Jin Gang Teng) in Chinese. It is indigenous to Hubei, Zhejiang, Jiangsu and Guangxi Provinces of China [1].

In traditional Chinese medicine, the main actions of *S. china* are diuresis, detoxifcation and dissipating stasis [1]. Modern pharmacological studies were mainly focused on its anti-inflammatory, anti-tumor and anti-bacterial activities [2–11].

Stilbenes, flavonoids and steroidal saponins are the main constituents of *S. china*. According to our research and the literatures, about 10 stilbenes and flavonoids [12,13] have been identified in *S. china* and they possess various biological activities, such as anti-oxidant [14–19], anti-inflammatory [20–23], anti-tumor [15,24] and antivirus effects [25]. Hence, they could be considered as the 'marker compounds' for the chemical evaluation or standardization of *S. china*. The development of quality control methods for determination of the major stilbenes and flavonoids in *S. china* was an essential issue for the effective clinical use of this medicinal herb. But until now, few studies on the quantitative determination of chemical constituents in *S. china* have been reported. The authentication of commercial samples of *S. china* was generally carried out using classical procedures performed by thin layer chromatography (TLC) [1]. It was reported that the content of total flavonoids was determined by spectrophotometric analysis [26] and a single constituent - kaempferol was quantified by HPLC method [27]. Besides, there has been no quantitative analysis for stilbenes in *S. china* until now. In this study, a HPLC method was developed for simultaneous determination of six major stilbenes and flavonoids, namely taxifolin-3-*O*-glycoside (**1**), piceid (**2**), oxyresveratrol (**3**), engeletin (**4**), resveratrol (**5**) and scirpusin A (**6**), respectively. Their structures were shown in Fig. 1. The developed method was simple, sensitive and specific, and could be used for the comprehensive evaluation of the quality of *S. china*.

* Corresponding authors. Tel.: +86 10 82801516; fax: +86 10 82802700.
E-mail address: gda@bjmu.edu.cn (D.-a. Guo).

0731-7085/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2007.03.008

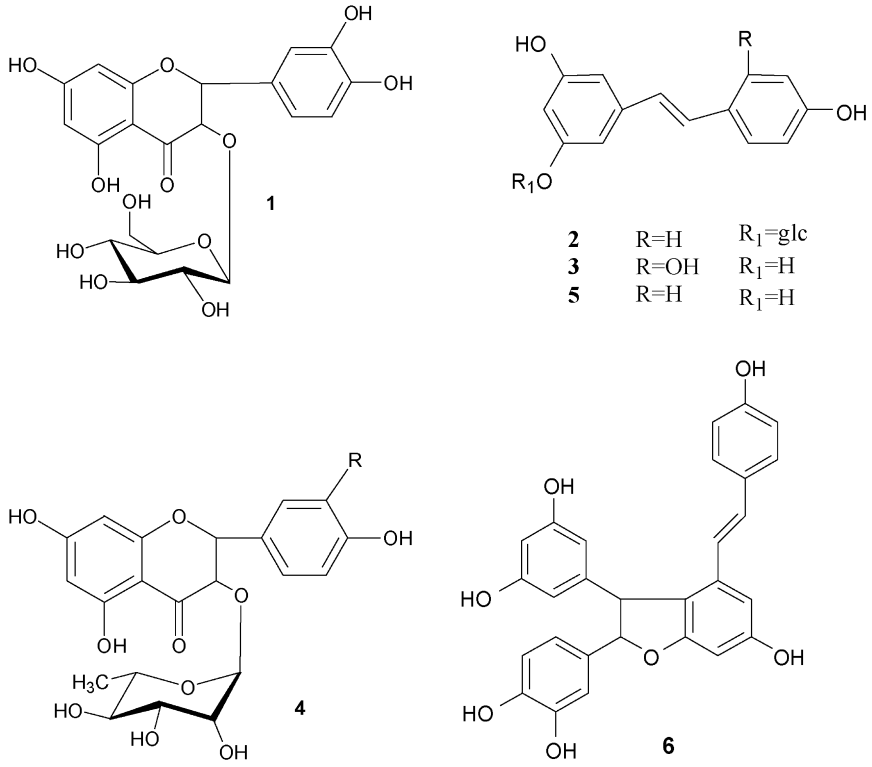

Fig. 1. Structures of compounds **1–6** identified from *S. china*. (**1**) Taxifolin-3-*O*-glycoside; (**2**) piceid; (**3**) oxyresveratrol; (**4**) engeletin; (**5**) resveratrol; (**6**) scirpusin A.

2. Experimental

2.1. Chemicals and materials

Acetonitrile was of HPLC grade (J.T. Baker, Phillipsburg, NJ, USA). Deionized water was purified by Milli-Q system (Millipore, Bedford, MA, USA). Phosphoric acid was of analytical grade from Beijing Reagent Company (Beijing, PR China). Fifteen commercial herbal samples of *S. china* were obtained locally from drug stores or markets in different provinces of China and authenticated by De-an Guo, Professor of Pharmacognosy, Peking University. Voucher specimens (NO. BMU020817A-BMU020831A) were deposited at Herbarium of School of Pharmaceutical Sciences, Peking University.

The standard compounds **1–6** were isolated by the author from the tuber of *S. china*. The powdered tuber (9 kg) was extracted with 95% EtOH and 50% EtOH at room temperature. It was concentrated *in vacuo* and then extracted with petroleum (60–90 °C fraction), EtOAc and *n*-BuOH. The EtOAc fraction (100 g) was subjected to repeated chromatography on silica gel column and eluted with petroleum ether–acetone or $CHCl_3$–MeOH gradient solvent system. Further purification was performed by using semi-preparative HPLC with MeOH–H_2O solvent system to yield compounds **1** (18 mg), **2** (15 mg), **3** (80 mg), **4** (20 mg), **5** (300 mg) and compound **6** (30 mg) which was isolated from this genus for the first time. Compounds **1–5** were identified by direct comparison of their ^{1}H NMR, ^{13}C NMR and MS (LC-MS) spectral data with those reported in the literatures [28,29,12]. Their purities were all proved to be above 98% by HPLC analysis. Structures of the six compounds are shown in Fig. 1.

2.2. Apparatus and chromatographic conditions

An Agilent 1100 liquid chromatography system (Agilent Technologies Deutschland, Waldbronn, Germany), equipped with a quaternary solvent delivery system, an autosampler and UV detector, was used. An Agilent Zorbax XDB-C_{18} column (250 mm × 4.6 mm, 5 μm) connected with a Zorbax XDB-C_{18} guard column (20 mm × 4 mm, 5 μm) at temperature of 30 °C was applied for all analyses. UV absorption was measured at 300 nm. The eluents were A (acetonitrile) and B (0.02% aqueous phosphoric acid, v/v). The gradient program was used as follows: initial 0–6 min, linear change from A–B (13:87, v/v) to A–B (15:85, v/v); and then held for 3 min; 9–17 min, linear change from A–B (15:85, v/v) to A–B (19:81, v/v); 17–28 min, linear change from A–B (17:83, v/v) to A–B (28:72, v/v) and then held for 9 min. Re-equilibration duration was 15 min between individual runs. The flow rate was 1.0 ml/min and aliquots of 10 μl were injected.

2.3. Sample preparation

The dried powders of *S. china* samples (0.2 g, 40 mesh) were accurately weighed and extracted by refluxing with 10 ml methanol for 2 h. Then the resultant mixture was adjusted to the original weight and the supernatant were filtered through

0.45 μm membrane prior to HPLC injection. All samples were prepared in triplicate.

2.4. Calibration curves

To prepare standard solutions, reference compounds **1**–**6** were accurately weighted and dissolved in methanol, then diluted to the concentration ranges of **1** (1.52–45.45 μg/ml), **2** (0.50–29.85 μg/ml), **3** (1.02–60.90 μg/ml), **4** (1.52–91.35 μg/ml), **5** (2.00–120.00 μg/ml) and **6** (0.81–32.48 μg/ml) for the construction of calibration curves. The calibration curve for each compound was performed with six different concentrations in triplicate using the same HPLC method as described above.

3. Results and discussion

3.1. ^{1}H, ^{13}C NMR and MS data of compound 6

Scirpusin A (**6**): Brown amorphous powder; For ^{1}H NMR (500 MHz, DMSO-d_6), δ7.15 (2H, d, J = 8.5 Hz, H-2, 6), 6.68 (2H, d, J = 9.0 Hz, H-3, 5), 6.84 (1H, d, J = 16.0 Hz, H-7), 6.59 (1H, d, J = 16.0 Hz, H-8), 6.25 (1H, d, J = 2.0 Hz, H-12), 6.60 (1H, d, J = 2.0 Hz, H-14), 6.06 (3H, s, H-2′, 4′, 6′), 4.37 (1H, d, J = 5.0 Hz, H-7′), 5.27 (1H, d, J = 5.0 Hz, H-8′), 6.70 (1H, d, J = 2.0 Hz, H-10′), 6.70 (1H, d, J = 8.0 Hz, H-13′), 6.58 (1H, dd, J = 8.5, 2.0 Hz, H-14′); For ^{13}C NMR (125 MHz, DMSO-d_6), δ127.9 (C-1), 127.8 (C-2), 115.5 (C-3), 157.4 (C-4), 115.5 (C-5), 127.8 (C-6), 129.0 (C-7), 122.0 (C-8), 134.8 (C-9), 118.5 (C-10), 160.8 (C-11), 95.9 (C-12), 158.5 (C-13), 103.1 (C-14), 146.3 (C-1′), 105.4 (C-2′), 158.7 (C-3′), 101.0 (C-4′), 158.7 (C-5′), 105.4 (C-6′), 55.3 (C-7′), 92.5 (C-8′), 132.6 (C-9′), 112.7 (C-10′), 145.3 (C-11′), 145.3 (C-12′), 115.4 (C-13′), 116.8 (C-14′); ESI–MS m/z: $[M-H]^-$ 469.3.

3.2. Extraction procedure

In order to obtain the quantitative extraction, variables involved in the procedure such as extraction method, extraction solvent and extraction time were optimized. Refluxing was compared with ultrasonic extraction. It was found that refluxing extraction was more effective with little interference. Hence the refluxing extraction was chosen as the preferred method. The 20% methanol, 50% methanol, 70% methanol and methanol were performed as extraction solvents to analyze the effect of the solvent on extraction efficiency. The results showed that pure methanol was the most suitable extraction solvent, which allowed extraction of all the constituents in high yields. The influence of the extraction time on the efficiency of extraction was also investigated. Powdered samples were extracted with methanol for 30, 60, 90 and 120 min, respectively. The results suggested that the highest amount of constituents were obtained with the extraction time of 120 min. After extraction, the residue was further extracted with methanol for additional 60 min, and almost no components were detected by HPLC. Therefore, later samples were extracted for 120 min.

3.3. Optimization of separation conditions

The chromatographic conditions were optimized to obtain chromatograms with better resolution of adjacent peaks within shorter time. Different types of chromatographic columns were tested. Agilent Zorbax SB-C_{18} column, Zorbax Extend-C_{18} column and Zorbax XDB-C_{18} column are suitable to different kinds of chemical constituents and in different pH ranges. The *S. china* extract showed different retention behaviors on these columns. Zorbax XDB-C_{18} column was proved to be better than others.

Different mobile phase compositions were also optimized. Various mixtures of water and methanol were used as mobile phase but separation was not satisfactory. However, when methanol was replaced by acetonitrile, the situation was greatly improved and satisfactory resolution was obtained. Addition of acid in mobile phase was found to enhance the resolution and eliminate the peak tailing of the target compounds. So the type and concentration of acids (0.3% formic acid, 1% acetic acid and 0.02% phosphoric acid) were examined. As a result, acetonitrile and water containing 0.02% phosphoric acid was chosen as the eluting solvent system that gave desired separation. And gradient elution was applied.

It was also suggested that separation was better when column temperature was kept at 30 °C than 20, 25, 35 and 40 °C.

According to the absorption maxima of six standards on the UV spectra with three-dimensional chromatograms of HPLC-DAD detection, the monitoring wavelength was set at 300 nm.

3.4. Method validation

3.4.1. Linearity

Linear regression analysis for each compound was performed by plotting the peak area versus concentration by the external standard method. The calculated results were given in Table 1.

Table 1
Linear relation between peak area and concentration (n = 6)

Compound	Regression equation	r^2	Linear range (μg/ml)	LOD (ng/ml)	LOQ (ng/ml)
1	$y = 11.969x - 1.7081$	0.9997	1.52–30.30	3.75	15.00
2	$y = 45.602x - 1.9385$	0.9998	0.50–9.95	1.30	4.00
3	$y = 27.224x - 0.8752$	0.9999	1.02–60.90	2.20	6.80
4	$y = 13.665x - 0.1611$	0.9995	1.52–91.35	1.88	7.50
5	$y = 47.618x - 1.1952$	0.9998	2.00–120.00	1.00	2.50
6	$y = 19.109x - 1.4282$	0.9997	0.81–48.72	2.50	10.00

In the regression equation $y = ax + b$, x refers to the concentration of the compound (μg/ml), y the peak area, and r^2 is the correlation coefficient of the equation. LOD: limit of detection; LOQ: limit of quantification.

Table 2
Intra- and inter-day repeatability for the major constituents in *S. china*

Compound	Intra-day ($n=6$)						Inter-day ($n=3$)	
	Day 1		Day 2		Day 3		Mean ± S.D.	R.S.D. (%)
	Mean ± S.D.[a]	R.S.D. (%)	Mean ± S.D.	R.S.D. (%)	Mean ± S.D.	R.S.D. (%)		
1	0.11 ± 0.00	1.1	0.11 ± 0.00	1.4	0.12 ± 0.00	2.9	0.11 ± 0.00	2.7
2	0.05 ± 0.00	1.8	0.05 ± 0.00	2.0	0.05 ± 0.00	3.7	0.05 ± 0.00	3.1
3	0.35 ± 0.01	1.7	0.36 ± 0.01	1.5	0.36 ± 0.01	2.8	0.35 ± 0.01	2.4
4	0.19 ± 0.00	2.0	0.19 ± 0.01	3.2	0.20 ± 0.01	2.7	0.19 ± 0.01	3.2
5	0.56 ± 0.01	1.4	0.55 ± 0.01	1.5	0.54 ± 0.02	3.4	0.55 ± 0.01	2.6
6	0.32 ± 0.01	1.6	0.31 ± 0.01	2.0	0.31 ± 0.00	0.9	0.31 ± 0.01	2.0
Total	1.57 ± 0.02	1.2	1.58 ± 0.02	1.1	1.57 ± 0.02	1.5	1.57 ± 0.02	1.3

[a] Data were mg constituents per gram drug.

All the compounds showed good linearity ($r^2 > 0.999$) in a relatively wide concentration range.

3.4.2. Limits of detection and quantification

The limit of detection (LOD) and quantification (LOQ) under the chromatographic conditions were determined by measuring the magnitude of analytical background by injecting blank samples and calculating the signal-to-noise ratio for each compound by injection series of solutions until the S/N ratio 3 for LOD and 10 for LOQ, then six replicate injections of the solution gave the R.S.D. less than 3%. LOD and LOQ were reported in Table 1 for each compound.

3.4.3. Repeatability

Measurement of intra- and inter-day variability was utilized to determine the repeatability of the method, which was evaluated using multiple preparations of a sample. The intra-day repeatability was examined on six replicate samples (sample no. 1) that were extracted and analyzed within 1 day, and inter-day repeatability was determined for three independent days. The quantity of each ingredient presented in this herbal sample was determined from corresponding calibration curve. The relative standard deviation (R.S.D.) was taken as a measure of method repeatability. The results were shown in Table 2, indicating that the intra- and inter-day R.S.D. values of the six compounds were all less than 3.7%, which showed good reproducibility of the developed method.

3.4.4. Recovery

In the recovery test, it involved the spiking known quantities of the mixed standard solution to known amounts of powdered *S. china* samples (sample no. 1) prior to the extraction. The fortified samples were then extracted and analyzed with the HPLC method described above. The added standard solutions were prepared in three different concentration levels (high, medium and low) in the concentration range of calibration curve and triplicate fortified samples were prepared at each level. For comparison, an unspoken sample was concurrently prepared and analyzed. The accuracy was calculated with the value of detected versus added amounts. The recovery of the method was in the range of 93.7–103.9%, with R.S.D. less than 4.0%. Considering the results, the method was deemed to be accurate (Table 3).

3.4.5. Stability

Stability was tested with standard solution and sample solution that were stored at room temperature and analyzed every 12 h within 3 days, and the analytes were found to be rather stable within 72 h (R.S.D. < 3.0%).

3.5. Application

The developed analytical method was then applied to simultaneously determine the six constituents in 15 commercial samples of *S. china*. All six compounds were detected in herbal samples. Peaks in the obtained chromatograms were identified by comparing the retention times and UV spectra with those of the standards. Representative chromatograms are shown in Fig. 2. Retention parameters for **1**–**6** were 15.90, 18.02, 23.21, 25.10,

Table 3
Recoveries of the six major constituents in *S. china* ($n=3$)

Compound	Added (μg/ml)	Detected (μg/ml)[a]	Recovery (%)[b]	R.S.D. (%)[c]
1	0.98	0.91 ± 0.04	93.7	0.3
	1.95	1.90 ± 0.10	97.6	0.7
	3.90	3.81 ± 0.11	97.5	0.7
2	0.23	0.23 ± 0.01	101.7	0.7
	0.45	0.45 ± 0.02	100.1	0.9
	0.90	0.88 ± 0.02	97.6	0.7
3	1.43	1.38 ± 0.33	96.3	1.9
	2.86	2.74 ± 0.18	95.6	1.0
	5.74	5.96 ± 0.85	103.9	4.0
4	1.82	1.79 ± 0.19	98.0	1.5
	3.65	3.69 ± 0.06	101.2	0.4
	7.30	7.25 ± 0.26	99.4	1.4
5	2.56	2.64 ± 0.16	103.0	0.7
	5.13	5.23 ± 0.55	102.0	2.1
	10.25	9.82 ± 0.36	95.8	1.2
6	1.11	1.03 ± 0.18	94.6	1.9
	2.22	2.19 ± 0.09	98.6	0.9
	4.44	4.41 ± 0.16	99.4	1.2

[a] Calculated by subtracting the total amount after spiking from the amount in the herb before spiking. Data were means of three experiments.
[b] Calculated as detected amount/added amount × 100%. Data were means of three experiments.
[c] R.S.D. (%) = (S.D./mean) × 100.

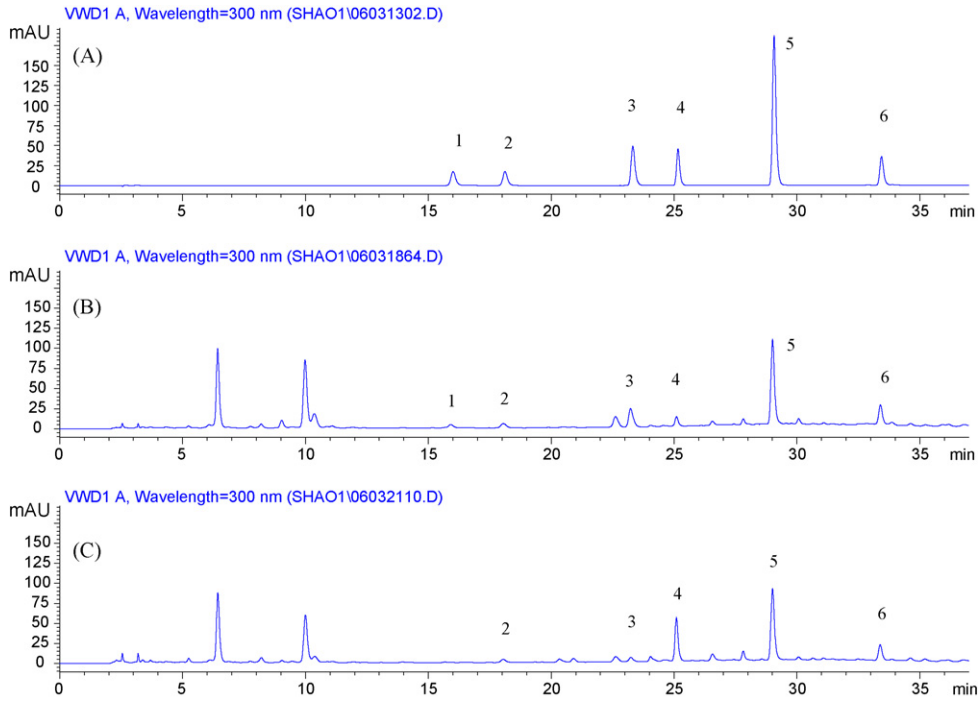

Fig. 2. HPLC chromatograms of standard mixture (A), sample No. 1 (B), sample No. 12 (C). (**1**) Taxifolin-3-*O*-glycoside; (**2**) piceid; (**3**) oxyresveratrol; (**4**) engeletin; (**5**) resveratrol; (**6**) scirpusin A.

28.99 and 33.39 min, respectively. The contents of the six compounds in the samples were quantified and the results were shown in Table 4 with the mean values of three replicate injections.

From the results, it was easy to note that there is a significant variability in the contents of stilbenes and flavonoids among 15 samples. For example, resveratrol (**5**) was the most dominant constituent in many samples. The contents varied from 0.01 to 1.47 mg/g. Obvious variation could also be found in other constituents. The total amounts of six constituents varied from 0.22 to 5.74 mg/g in 15 samples. In fact, a number of reasons may contribute to the differences in the level of active ingredients among various samples, such as genetic variation, plant origin, drying process and storage conditions, etc. These all suggested that each procedure involved should be standardized in the future. Then the quality of *S. china* could be assured.

Variations of these "marker compounds" may influence the potencies of *S. china*. However, the relationship among the quantities of these two types of compounds, their pharmacologically activities and potencies of *S. china* needs to be clarified. Further

Table 4
Contents of six major constituents in different *S. china* samples

No.	Origin[a]	Content (mg/g)						
		(**1**)	(**2**)	(**3**)	(**4**)	(**5**)	(**6**)	Total
1	Tongcheng City, Hubei Prov.	0.11	0.05	0.35	0.19	0.56	0.32	1.57
2	Chongyang City, Hubei Prov.	0.07	0.06	0.79	1.15	0.82	0.32	3.21
3	Yueyang City, Hubei Prov.	0.09	0.06	0.08	0.19	0.55	0.44	1.41
4	Chibi City, Hubei Prov.	0.54	0.06	0.15	0.03[b]	0.74	0.42	1.93
5	Wuhan City, Hubei Prov.	1.29[b]	0.07	2.24[b]	0.36	1.47	0.31	5.74
6	Beijing	0.03	0.02	0.58	0.72	0.51	0.12	1.98
7	Shenyang City1, Liaoning Prov.	nd	0.02	nd	0.94	0.01[b]	0.07	1.05
8	Shenyang City2, Liaoning Prov.	nd	0.02	0.44	0.32	0.55	0.27	1.61
9	Heze, Shandong Prov.	nd	0.01[b]	0.05	0.05	0.13	0.09	0.33
10	Haiyang City, Shandong Prov.	nd	0.03	0.47	0.04	0.32	0.26	1.11
11	Jinan City, Shandong Prov.	nd	0.05	0.02[b]	0.06	0.27	0.14	0.54
12	Zhengzhou City, Henan Prov.	nd	0.03	0.07	0.84	0.48	0.27	1.69
13	Jixi City, Anhui Prov.	nd	0.02	0.02[b]	0.02[b]	0.06	0.10	0.22
14	Shanghai	nd	0.01[b]	0.31	0.09	0.13	0.09	0.63
15	Chengdu, Sichuan Prov.	0.24	0.04	0.21	1.60	0.01[b]	0.21	2.30

nd: not detected. (**1**) Taxifolin-3-*O*-glycoside; (**2**) piceid; (**3**) oxyresveratrol; (**4**) engeletin; (**5**) resveratrol; (**6**) scirpusin A.

[a] All samples were collected in China.

[b] Out of test range.

studies on the pharmacological activities of these two types of compounds in *S. china* extracts are currently in progress in our laboratory.

In contrast to the previous reported methods in analysis of *S. china* [1,26,27], i.e. utilizing TLC, spectrophotometric techniques and HPLC assay, this newly developed HPLC method provided much higher specificity, precision and accuracy. By quantification of six major constituents, the quality of *S. china* could be effectively evaluated.

4. Conclusion

This is the first report on the simultaneous determination of six major stilbenes and flavonoids in *S. china*, which was proved to be simple, rapid and accurate. This HPLC assay can be readily utilized as a suitable quality control method for the determination of the major constituents in *S. china*.

Acknowledgements

We thank Program for Changjiang Scholars and Innovative Team in University (985-2-063-112) and the Ministry of Science and Technology of China (2006BAI08B03-03 and 2002BA906A29) for financial support of this work.

References

[1] National Commission of Chinese Pharmacopoeia, Pharmacopoeia of Peoples Republic of China, Chemical Industry Press, Beijing, 2005, pp. 216.
[2] State Administration of Traditional Chinese Medicine of People's Republic of China. *Zhong-hua-ben-cao*; Shanghai Science and Technology Publisher, Shanghai, China, 1999, pp. 157–160.
[3] X.S. Shu, Z.H. Gao, X.L. Yang, J. Ethnopharmacol. 103 (2006) 327–332.
[4] W.Y. Li, L.H. Zhou, Shi Zhen, J. Tradit. Chin. Med. Res. 7 (1996) 272–273.
[5] D.S. Chen, Y.N. Lu, J. Wang, Chin. J. Hosp. Pharm. (Chin. Hosp. Pharm. J.) 20 (2000) 544–545.
[6] Y.N. Lu, D.S. Chen, J.G. Deng, L.Q. Tian, J. Chin. Med. Mater. 26 (2003) 344–346.
[7] Y.N. Lu, D.S. Chen, X.Z. Xiong, L.Q. Tian, J.G. Deng, Chin. J. Hosp. Pharm. 24 (2004) 517–519.
[8] J.L. Ruan, J. Zou, Y.L. Cai, Herald Med. 24 (2005) 670–672.
[9] X.S. Shu, Z.H. Gao, X.L. Yang, J. Chin. Mater. Med. 31 (2006) 239–243.
[10] C.Y. Wen, P. Feng, X.H. Ding, X.J. Wang, Beijing J. Tradit. Chin. Med. 4 (2001) 44–45.
[11] X.J. Wang, P. Feng, C.Y. Wen, Chin. J. Pathophysiol. 12 (1996) 614–615.
[12] F. Feng, W.Y. Liu, Y.S. Chen, J.H. Liu, S.X. Zhao, J. China Pharm. Univ. 34 (2003) 119–121.
[13] H.L. Ruan, Y.H. Zhang, W. Zhao, Y.F. Tan, Z.L. Sun, J.Z. Wu, Nat. Prod. Res. Dev. 14 (2002) 35–36, 41.
[14] K. Roemer, M. Mahyar, Drugs Today 38 (2002) 571–580.
[15] M. Jang, L. Cai, G. Udeani, Science 275 (1997) 218–220.
[16] P. Lorenz, S. Roychowdhury, Nitric Oxide 9 (2003) 63–76.
[17] L.X. Zhu, Z.Y. Jin, Pharmacol. Clin. Chin. Mater. Med. 21 (2005) 16–18.
[18] G. Schmeda-Hirschmann, Phytother. Res. 10 (1996) 683–685.
[19] A. Saleem, H. Kivelae, J. Biosci. 58 (2003) 351–354.
[20] K. Subbaramaiah, A.J. Dannenberg, Adv. Exp. Med. Biol. 492 (2001) 147–157.
[21] J. Xu, X. Li, P. Zhang, Z.L. Li, Y. Wang, Arch. Pharm. Res. 28 (2005) 395–399.
[22] N.H. Shin, S.Y. Tyu, H. Lee, Planta Medica 64 (1998) 283–284.
[23] K.O. Chung, J. Pharm. Pharmacol. 55 (2003) 1695–1700.
[24] K.P. Bhat, J.M. Pezzuto, Ann. N. Y. Acad. Sci. 957 (2002) 210–229.
[25] G. Yang, J. Zhou, Y. Li, C. Hu, Planta Medica 71 (2005) 569–571.
[26] S.W. Liu, Y.X. Xu, Resour. Dev. Market 21 (2005) 481–482, 486.
[27] S.X. Liu, Chin. Tradit. Herbal Drugs 35 (2004) 284–285.
[28] B. Baderschneider, P. Winterhalter, J. Agric. Food Chem. 49 (2001) 2788–2798.
[29] Y. Hua, J.Y. Zhou, W. Ni, C.X. Chen, Nat. Prod. Res. Dev. 13 (2001) 16–18.