Journal of Pharmaceutical Analysis 2011;1(4):284–290

Contents lists available at ScienceDirect

Journal of Pharmaceutical Analysis

www.elsevier.com/locate/jpa
www.sciencedirect.com

ORIGINAL ARTICLE

Simultaneous quantification of five major active components in capsules of the traditional Chinese medicine 'Shu-Jin-Zhi-Tong' by high performance liquid chromatography

Xing-Xin Yang, Xiao-Xia Zhang, Rui-Miao Chang, Yan-Wei Wang, Xiao-Ni Li*

College of Pharmacy, Shanxi Medical University, 56 Xinjian South Road, Taiyuan 030001, China

Received 29 June 2011; accepted 19 August 2011
Available online 22 October 2011

KEYWORDS

High performance liquid chromatography;
Quality control;
'Shu-Jin-Zhi-Tong' capsules;
Traditional Chinese medicine

Abstract A simple and reliable high performance liquid chromatography (HPLC) method has been developed for the simultaneous quantification of five major bioactive components in 'Shu-Jin-Zhi-Tong' capsules (SJZTC), for the purposes of quality control of this commonly prescribed traditional Chinese medicine. Under the optimum conditions, excellent separation was achieved, and the assay was fully validated in terms of linearity, precision, repeatability, stability and accuracy. The validated method was applied successfully to the determination of the five compounds in SJZTC samples from different production batches. The HPLC method can be used as a valid analytical method to evaluate the intrinsic quality of SJZTC.

1. Introduction

Traditional Chinese medicine (TCM) has a long history dating back several thousands of years, and is widely used for treating various chronic diseases [1] (for example, rheumatism and arthritis), in East Asian countries such as China, Japan and Korea [2]. The increasing popularity of TCMs can be attributed to their effectiveness and relatively low toxicity. With the growing use of herbal products, quality control is required to guarantee their safety and efficacy. Hence, the development of efficient methods to evaluate and control the quality of TCMs is important.

In the past decades, a large number of analytical strategies have been designed to evaluate the quality of medicinal herbs or herbal preparations. These include quantification of a single compound or multiple components, as well as fingerprint analysis. Single marker compound quantification is simple and convenient, but it does not afford sufficient quantitative information for the other active constituents in complex TCMs [3]. Fingerprint analysis can control the quality consistency and stability of herbal products, but it cannot provide accurate quantification of analytes in TCMs [4]. Despite the

*Corresponding author. Tel./fax: +86 351 4690114.
E-mail address: ninili1235@163.com (X.-N. Li).

Peer review under responsibility of Xi'an Jiaotong University.
doi:10.1016/j.jpha.2011.08.002

Production and hosting by Elsevier

requirement of many reference standards, multi-component determination remains widely used to evaluate and control the quality of TCMs because of the advantage of simultaneous quantification of many markers from different herbal products for control of total quality [5,6]. In the process, techniques such as HPLC [7], gas chromatography (GC) [8], high performance capillary electrophoresis (HPCE) [9], gas chromatography–mass spectrometry (GC–MS) [10] and liquid chromatography–mass spectrometry (LC–MS) [11] are often used. However, HPLC is simple, reliable and inexpensive, and has been widely used for quantitative analysis of herbal medicines.

The herbal preparation 'Shu-Jin-Zhi-Tong' capsules (SJZTC) has been widely used in China. It contains seven medicinal materials: Rhizoma Corydalis, Rhizoma Corydalis Decumbentis, Rhizoma Cibotii, Radix Scutellariae, Cortex Cinnamomi, Herba Lycopodii and Flos Carthami. The remedy is particularly valuable for the treatment of conditions including rheumatism, arthritis and scapulohumeral periarthritis. Various chemical and pharmacological studies have shown that tetrahydropalmatine [12,13], protopine [14,15], protocatechuic aldehyde [16], baicalin [17] and cinnamaldehyde [18] from Rhizoma Corydalis or Rhizoma Corydalis Decumbentis, Rhizoma Corydalis Decumbentis or Rhizoma Corydalis, Rhizoma Cibotii, Radix Scutellariae and Cortex Cinnamomi, respectively, possess significant pharmacological actions and are the major active components in SJZTC. Thus, the development of simple and effective methods for the simultaneous quantification of these active compounds would be of significant value for the quality control of SJZTC, but to date, none has been reported.

We present here a convenient, sensitive and reliable HPLC method, which allows the simultaneous quantification of the five major active components, protocatechuic aldehyde, protopine, tetrahydropalmatine, baicalin and cinnamaldehyde (chemical structures shown in Fig. 1), of SJZTC. The developed method was successfully applied to the quantitative analysis of the five major compounds in the samples of SJZTC from different production batches for quality evaluation and control. This is the first report of the simultaneous quantification of the five components of SJZTC.

2. Experimental

2.1. Chemicals and reagents

HPLC grade acetonitrile and methanol were purchased from Fisher Scientific (Fairlawn, NJ, USA). Deionized water was purified by a Milli-Q Water Purification System (Millipore, Billerica, MA, USA). All other reagents used were of analytical reagent grade or higher. Reference standards of protocatechuic aldehyde, protopine, tetrahydropalmatine, baicalin and cinnamaldehyde were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), in purities greater than 98%. The products of SJZTC were kindly provided by the Department of Pharmaceutical Analysis, College of Pharmacy, Shanxi Medical University (Taiyuan, China).

2.2. Preparation of standard solutions

Standard stock solutions of the five reference standards (protocatechuic aldehyde (173 μg/mL), protopine (138 μg/mL), tetrahydropalmatine (1060 μg/mL), baicalin (479 μg/mL) and cinnamaldehyde (14 μg/mL)) were prepared by dissolving the respective working standard substance in methanol. They were then diluted with methanol to the concentrations required. All the solutions were stored at 4 °C until use.

2.3. Preparation of sample solutions

Sample preparation was performed with an ultrasonic cleaning bath. In brief, the powder from SJZTC (about 0.2 g) accurately weighed was transferred into a 50-mL conical flask with stopper, and 30 mL of 60% aqueous methanol solution was added. Ultrasonication (250 W, 44 KHz) was performed at room temperature for 40 min, and then the same solvent was added to compensate for the lost weight during the extraction. The resultant mixture was centrifuged at 4000 rpm for 10 min, and then the supernatant was collected and filtered through a 0.45 μm syringe filter before injection into the HPLC system for analysis. The contents of the selected compounds in SJZTC were obtained from the corresponding calibration curves.

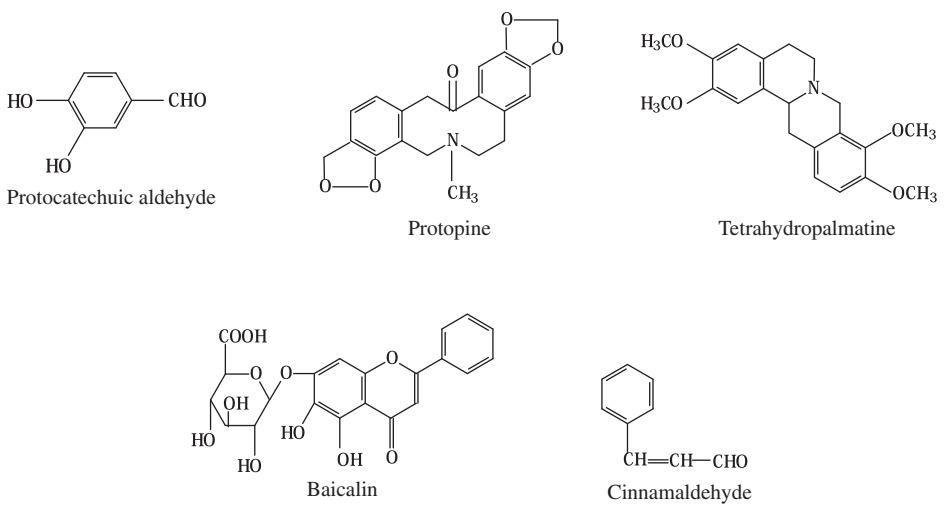

Figure 1 The chemical structures of the tested compounds.

2.4. Apparatus and chromatographic conditions

All analyses were performed on a Shimadzu (Kyoto, Japan) HPLC system, consisting of two model LC-10AD$_{VP}$ pumps and a model SIL-10AF auto-sampler, equipped with a model SPD-10A$_{VP}$ UV–vis detector. Data integration was performed using Shimadzu Class-$_{VP}$ software. Separation was achieved using a Waters Symmetry C_{18} column (250 mm × 4.6 mm ID, 5 μm) and the column temperature was maintained at 30 °C. The mobile phase comprising acetonitrile (A) and 0.1% phosphoric acid (B) was used to elute the target components with a gradient program (0–10 min, 5%A; 10–11 min, 5%A→37%A; 11–23 min, 37%A; 23–24 min, 37%A→63%A; 24–30 min, 63%A→69%A). The sample injection volume, flow rate and detection wavelength were set at 5 μL, 1.0 mL/min and 280 nm, respectively.

2.5. Analytical method validation

2.5.1. Linearity, limits of detection and quantification

The stock solutions containing the five analytes were prepared and diluted with methanol to the appropriate concentration for construction of calibration curves. At least six concentrations of the five compounds were analyzed under optimum HPLC conditions in triplicate, and then the calibration curves were constructed by plotting the peak areas versus the concentration of each analyte. The regression equations were calculated in the form of $y=ax+b$, where y and x correspond to the peak area and compound concentration, respectively.

The lowest concentration of working solution was diluted with methanol to a series of appropriate concentrations, and aliquots of diluted solutions were injected into the HPLC system for analysis. The limits of detection (LOD) and quantification (LOQ) for each compound under the optimal chromatographic conditions were determined at a signal-to-noise (S/N) ratio of 3 and 10, respectively.

2.5.2. Precision, repeatability and stability

The measurement of intra- and inter-day variability was utilized to evaluate the precision of the developed method. The intra-day precision was investigated for the mixed standards using six replicates within one day, and inter-day precision was determined in duplicates for consecutive three days. Variations were expressed by relative standard deviations (RSD).

The repeatability of the established method was examined at three levels (1.6 g, 2.0 g and 2.4 g) of the SJZTC sample (sample number 20090310), which were extracted and analyzed as mentioned above in triplicates. The repeatability is presented as RSD ($n=3$).

Stability of the sample solution was also assessed at room temperature. The same sample solution was analyzed in triplicate every 4 h within 24 h. RSD was taken as a measure of stability.

2.5.3. Accuracy

A recovery test was used to evaluate the accuracy of the developed method. Three quantities (low, medium and high) of the authentic standards were added to the known real sample (SJZTC, sample number 20090310). Resultants were then extracted and analyzed as described above. The quantity of each analyte was subsequently realized from the corresponding calibration curve. The recovery of each compound was calculated by the formula: recovery (%)=(amount found−original amount)/amount added × 100%, and RSD (%)=(S.D./mean) × 100%.

3. Results and discussion

3.1. Optimization of the sample preparation method

Refluxing and ultrasonication are often used to extract the compounds of interest from the matrix. One of the important disadvantages of refluxing is the loss of target compounds due to ionization, hydrolysis and oxidation during extraction [19]. In addition, refluxing is time-consuming and suffers from low efficiency of the extraction and consumption of solvent [20]. These shortcomings have led to the consideration of ultrasonic extraction, which has been widely used in TCMs analysis [21].

During the extraction process, the choice of extraction solvent, sample–solvent ratio and extraction time are critical for high extraction efficiency. Anhydrous and aqueous methanol or ethanol solutions are often used as the extraction solvent. In our study, different concentrations (10%, 20%, 40%, 60%, 80% and 100%) of methanol and ethanol solutions were used to extract the target components from SJZTC (sample number 20090310). The results exhibited in Fig. 2A and B indicated that the extraction values of all targets gradually increased with an increase in the concentrations of ethanol or methanol when the concentration of methanol was <60% or the concentration of ethanol was <80%. Extraction with either 60% or 80% aqueous methanol resulted in no differences in the efficiency. Anhydrous methanol and ethanol did not benefit efficient extraction. The extraction values with 60% aqueous methanol were higher than those with 80% aqueous ethanol. Thus, 60% aqueous methanol was proven to be the best solvent choice, allowing efficient extraction of all target analytes in high yield. A suitable sample–solvent ratio was also investigated and four ratios (1:50, 1:100, 1:150 and 1:200, w/v) were tested. The sample–solvent ratio controlled at 1:150 was found to be optimal (Fig. 2C). In addition, different extraction times (20, 30, 40 and 50 min) were also optimized with 40 min being sufficient for the extraction (Fig. 2D).

The optimized extraction conditions were therefore as follows: samples were extracted by ultrasonication using 60% aqueous methanol as the extraction solvent (1:150, w/v) for 40 min. A second extraction of the residue was carried out to ensure the efficiency of the extraction protocol. No analyte peaks were detected in the second extract, indicating complete extraction of the five components under these extraction conditions.

3.2. Optimization of chromatographic conditions

The selection of HPLC conditions was guided by the requirement for obtaining chromatograms with better resolution of adjacent peaks within a short time especially when large amounts of samples were analyzed. The complex compositions of herbal products often make it difficult to obtain satisfactory separation, and it is generally necessary to optimize various chromatographic parameters such as the mobile phase, column temperature and flow rate. In the present study, different mobile phases consisting of acetonitrile–water and methanol–water in

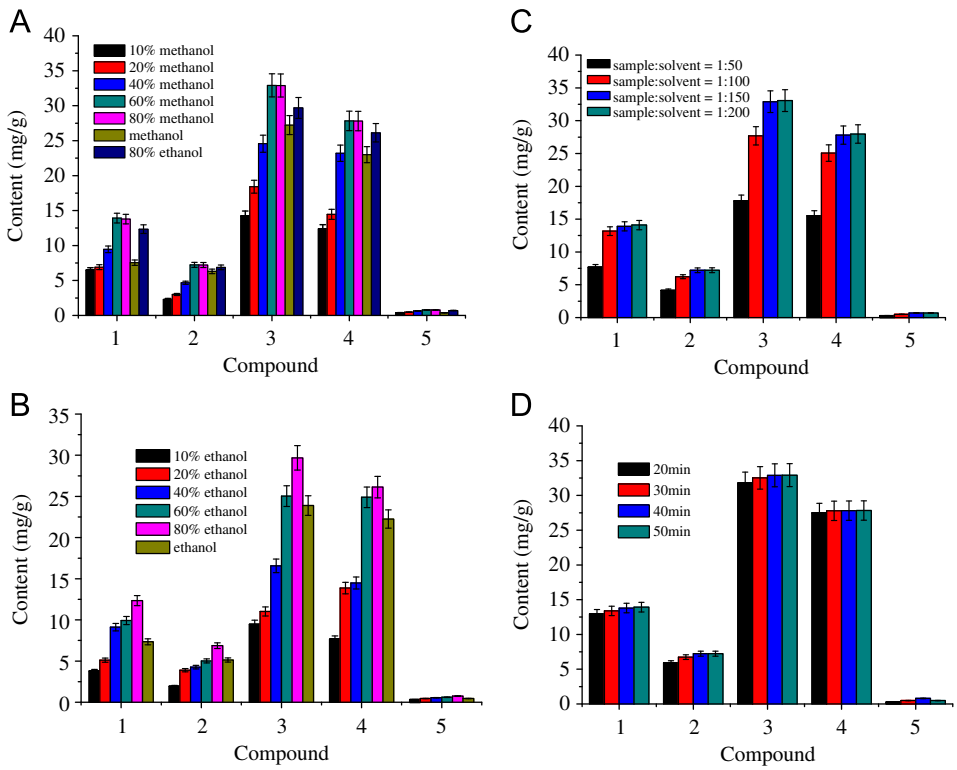

Figure 2 The optimization of suitable extraction conditions for the five compounds in SJZTC including protocatechuic aldehyde (**1**), protopine (**2**), tetrahydropalmatine (**3**), baicalin (**4**) and cinnamaldehyde (**5**). (A) The influence of different concentrations of methanol on the extraction; (B) the influence of different concentrations of ethanol on the extraction; (C) the influence of the sample–solvent ratio with 60% methanol as the extraction solvent; (D) the extraction time to the extraction efficiency of the analytes with 60% methanol as the extraction solvent (1:150, w/v).

various proportions were compared under different gradient elution modes but failed to give satisfactory separation of the five analytes. Phosphate buffer, formic acid, acetic acid and phosphoric acid additives were therefore investigated, and phosphoric acid was found to improve separation. The effects of column temperature and flow rate were also investigated and the results showed that the separation was optimal at 30 °C and 1.0 mL/min, respectively. Finally, a gradient elution program was chosen so as to ensure that each run was completed within 30 min. In addition, 280 nm was chosen as the wavelength for detection of the analytes of interest in accordance with related literature reports [22–25]. After many tests, excellent separations of the five compounds in SJZTC were achieved in a single run detected at 280 nm and the representative chromatograms are shown in Fig. 3, in which chromatograms A and B correspond to mixed standards and SJZTC sample (sample number 20090310), and the peaks 1, 2, 3, 4 and 5 represent protocatechuic aldehyde, protopine, tetrahydropalmatine, baicalin and cinnamaldehyde, respectively.

3.3. *Validation of the method*

3.3.1. *Linearity, LOD and LOQ*

The parameters from the linear calibration curve with the R^2, linear range and regression equation, LOD and LOQ of the five compounds are listed in Table 1. As a result, each coefficient of determination (R^2) was >0.9987, as determined by least square analysis, suggesting good linearity between the peak areas (y) and the compound concentrations (x) over a wide concentration range. The LOD and LOQ of each compound were in the ranges 0.04–0.15 and 0.12–0.31 μg/mL, respectively, which indicated high sensitivity under these HPLC conditions.

3.3.2. *Precision, repeatability, stability and accuracy*

As shown in Table 2, the precision, repeatability, stability and accuracy of all analytes are listed. The intra- and inter-day precision of the five active components were in the ranges 0.51–1.64% and 1.48–2.12%, respectively, indicating that the method described was precise enough for the quantitative evaluation of the analytes in SJZTC. Repeatability with RSD < 4% demonstrated that the developed analytical method was reproducible for all components examined. The sample solutions were stable within 24 h with RSD < 5%. Average recoveries of the investigated targets ranged from 97.31% to 102.35% and RSD values were all < 3% ($n=9$), which demonstrated that the developed HPLC method was sufficiently reliable and accurate for the measurement of the compounds analyzed.

3.4. *Sample analysis*

The developed HPLC method was successfully applied to the simultaneous determination of the five major active components (protocatechuic aldehyde, protopine, tetrahydropalmatine, baicalin

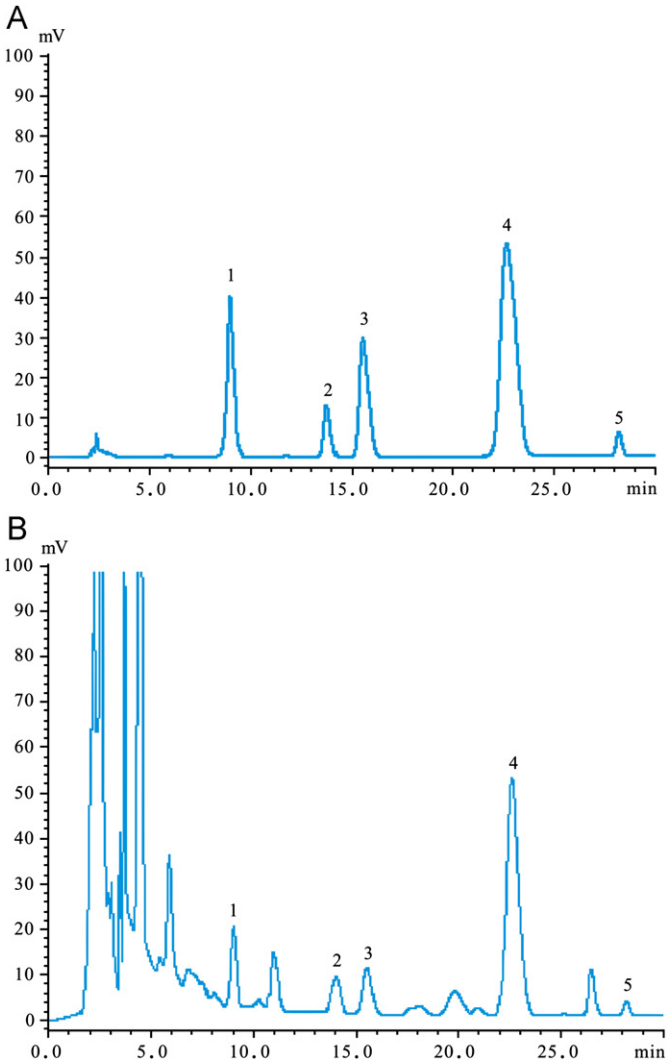

Figure 3 Representative chromatograms for simultaneous quantification of the five active compounds in SJZTC. (A) Mixed standards; (B) SJZTC sample (sample number 20090310). Peak 1=protocatechuic aldehyde, 2=protopine, 3=tetrahydropalmatine, 4=baicalin and 5=cinnamaldehyde.

Table 1 Regression data, LODs and LOQs of the five compounds.

Compound	Regression equation ($y=ax+b$)[a]	R^2	Linear range (μg/mL)	LOD[b] (μg/mL)	LOQ[c] (μg/mL)
Protocatechuic aldehyde	$y=33.824x+40.153$	0.9994	17.3–173.0	0.09	0.20
Protopine	$y=27.993x+63.726$	0.9993	13.8–138.0	0.15	0.31
Tetrahydropalmatine	$y=29.407x-142.59$	0.9996	106.0–1060.0	0.06	0.17
Baicalin	$y=38.914x-115.07$	0.9997	47.9–479.0	0.04	0.12
Cinnamaldehyde	$y=51.258x+4.291$	0.9987	0.28–14.00	0.13	0.24

[a] $y=ax+b$, y refers to the peak area, and x refers to the concentration of the reference compound (μg/mL).
[b] LOD refers to the limits of detection, $S/N=3$.
[c] LOQ refers to the limits of quantity, $S/N=10$.

and cinnamaldehyde) in 20 samples of SJZTC collected from different production batches. The measured concentrations (mg/g) of the five compounds in the samples are summarized in Table 3. Of these, tetrahydropalmatine was found to be the most abundant bioactive component (31.92–46.26 mg/g) of SJZTC. The concentrations of protocatechuic aldehyde, protopine and baicalin were in the ranges 8.48–17.05, 5.73–11.64 and 16.33–27.89 mg/g, respectively. In addition, the concentration of cinnamaldehyde was not

Table 2 Precision, repeatability, stability and recovery of the five compounds.

Compound	Precision (RSD, %, $n=6$)		Repeatability (RSD, %, $n=3$)			Stability (RSD, %, $n=6$)	Recovery (%, $n=9$)	
	Intra-day	Inter-day	LL[a]	ML[b]	HL[c]		Mean	RSD
Protocatechuic aldehyde	0.72	1.80	2.46	0.73	1.03	3.15	99.27	1.39
Protopine	0.96	2.04	3.57	2.28	1.22	1.47	102.35	2.58
Tetrahydropalmatine	1.28	1.56	0.87	1.32	0.65	1.28	101.10	1.74
Baicalin	0.51	1.48	1.35	1.16	1.10	0.93	99.98	0.89
Cinnamaldehyde	1.64	2.12	3.89	3.51	1.73	4.41	97.31	2.72

[a]LL (low lever) refers to 1.6 g of sample 20090310.
[b]ML (middle lever) refers to 2.0 g of sample 20090310.
[c]HL (high lever) refers to 2.4 g of sample 20090310.

Table 3 Quantification of the 5 active components in the various batches of SJZTC products.

No. of batches	Contents (mg/g)[a]				
	1[b]	2	3	4	5
20080318	10.25±0.18	6.48±0.07	34.26±0.67	27.87±0.51	0.08±0.00
20080507	9.51±0.11	10.19±0.19	42.41±0.78	16.33±0.34	0.07±0.00
20080611	13.68±0.23	8.92±0.11	42.80±0.73	27.59±0.50	0.10±0.00
20080709	13.46±0.19	10.37±0.18	32.29±0.57	27.56±0.57	ND[c]
20080803	10.74±0.20	8.78±0.10	42.28±0.75	27.40±0.48	0.07±0.00
20080901	8.48±0.10	10.38±0.15	42.04±0.73	27.38±0.69	0.22±0.00
20081010	9.30±0.11	11.64±0.20	42.29±0.65	16.82±0.32	ND
20081102	13.87±0.24	10.37±0.14	45.30±0.71	21.95±0.50	0.27±0.00
20081203	13.91±0.26	10.34±0.12	42.57±0.79	27.79±0.64	0.09±0.00
20090109	13.78±0.21	9.98±0.10	31.92±0.61	27.84±0.61	0.19±0.00
20090310	13.90±0.16	7.21±0.08	32.89±0.58	27.81±0.59	0.35±0.00
20090402	13.95±0.21	10.41±0.12	42.30±0.82	27.79±0.52	ND
20090604	16.41±0.31	10.40±0.17	42.26±0.70	23.18±0.61	ND
20090715	10.58±0.17	10.33±0.13	42.61±0.74	27.89±0.75	0.40±0.00
20090907	8.86±0.09	10.37±0.15	42.44±0.64	18.37±0.35	0.83±0.01
20091103	14.19±0.28	5.73±0.05	42.83±0.73	19.25±0.39	0.21±0.00
20091202	13.43±0.17	8.41±0.07	42.09±0.52	27.83±0.73	0.57±0.01
20100108	13.66±0.20	10.54±0.13	39.27±0.67	21.07±0.56	0.48±0.01
20100205	13.47±0.22	10.59±0.18	46.26±0.83	19.30±0.42	0.75±0.01
20100310	17.05±0.38	9.86±0.12	40.14±0.78	27.88±0.71	0.92±0.01

[a]Data of the contents are expressed as mean±S.D. ($n=3$).
[b]1=protocatechuic aldehyde, 2=protopine, 3=tetrahydropalmatine, 4=baicalin and 5=cinnamaldehyde.
[c]Not detected.

more than 1 mg/g in all capsules and was not detected in some samples of SJZTC. The phenomenon described above and the data shown in Table 3 indicated that the concentrations of the investigated compounds were not consistent in different production batches of SJZTC, despite being prepared by the same protocol and via the same manufacturing process. This variation could be due to subtle changes in manufacturing processes and the different origins of the raw materials, which might influence the quality and stability of this product. Hence, the simultaneous quantification of the five major active components in SJZTC would be a valuable tool to improve quality control of this preparation. The method described here allows the evaluation of SJZTC quality via the detection of the five main active components, and the average contents of protocatechuic aldehyde, protopine, tetrahydropalmatine, baicalin and cinnamaldehyde were >13, 9, 39, 22 and 0.3 mg/g, respectively. This HPLC method may therefore be employed as a useful tool to evaluate the quality of this popular herbal remedy.

4. Conclusion

TCMs are becoming increasingly popular worldwide. Efficient protocols to evaluate and control the quality of herbal products are urgently needed. This is the first report of the simultaneous quantification of the five main active components in SJZTC which was proved to be simple, rapid, sensitive and accurate. The assay method was then successfully applied in the quantitative determination of the major biologically active constituents from 20 batches of SJZTC. The proposed method could be used to improve the quality control of SJZTC preparations ensuring the efficacy and quality of the

product. This method may also be useful in controlling the quality of other related pharmaceutical preparations containing Rhizoma Corydalis, Rhizoma Corydalis Decumbentis, Rhizoma Cibotii, Radix Scutellariae and Cortex Cinnamomi.

Acknowledgment

This research was funded by a grant from the Scientific and Technological Innovation Project Foundation of Shanxi China (No. 20090321099).

References

[1] K. Chen, Progress in traditional Chinese medicine, Trends Pharmacol. Sci. 16 (6) (1995) 182–187.
[2] D. Normile, Asian medicine: the new face of traditional Chinese medicine, Science 299 (5604) (2003) 188–190.
[3] Y.L. Zhang, X.X. Yang, X.N. Li, Quantitative analysis of astragaloside IV in traditional Chinese medicine 'Huang-Qi-Si-Wu' Capsules by HPLC/UV, J. Chin. Pharm. Sci. 19 (3) (2010) 223–228.
[4] C.F. Qiao, Q.B. Han, J.Z. Song, et al., Chemical fingerprint and quantitative analysis of fructus psoraleae by high-performance liquid chromatography, J. Sep. Sci. 30 (6) (2007) 813–818.
[5] C. Feng, Y.L. Cai, J.L. Ruan, Simultaneous determination of 10 active components in traditional Chinese medicine "YIGONG" capsule by RP-HPLC-DAD, J. Pharm. Biomed. Anal. 47 (2) (2008) 442–447.
[6] L.H. Yin, B.N. Lu, Y. Qi, et al., Simultaneous determination of 11 active components in two well-knowntraditional Chinese medicines by HPLC coupled with diode arraydetection for quality control, J. Pharm. Biomed. Anal. 49 (4) (2009) 1101–1108.
[7] L. Xu, X. Han, Y. Qi, et al., Multiple compounds determination and fingerprint analysis of Lidanpaishi tablet and keli by high-performance liquid chromatography, Anal. Chim. Acta 633 (1) (2009) 136–148.
[8] Q. Ye, C.H. Deng, Determination of camphor and borneol in Flos Chrysanthemi Indici by UAE and GC-FID, J. Chromatogr. Sci. 47 (4) (2009) 287–290.
[9] Q.C. Chu, T. Wu, L. Fu, et al., Simultaneous determination of active ingredients in *Erigeron breviscapus* (Vant.) Hand-Mazz. by capillary electrophoresis with electrochemical detection, J. Pharm. Biomed. Anal. 37 (3) (2005) 535–541.
[10] C.H. Deng, J. Ji, N. Li, et al., Fast determination of curcumol, curdione and germacrone in three species of *Curcuma rhizomes* by microwave-assisted extraction followed by headspace solid-phase microextraction and gas chromatography–mass spectrometry, J. Chromatogr. A 1117 (2) (2006) 115–120.
[11] Y.F. Du, P.W. Liu, Z.F. Yuan, et al., Simultaneous qualitative and quantitative analysis of 28 components in *Isodon rubescens* by HPLC–ESI–MS/MS, J. Sep. Sci. 33 (4–5) (2010) 545–557.
[12] Y.C. Oh, J.G. Choi, Y.S. Lee, et al., Tetrahydropalmatine inhibits pro-inflammatory mediators in lipopolysaccharide-stimulated THP-1 cells, J. Med. Food 13 (5) (2010) 1125–1132.
[13] C. Wang, S.W. Wang, G.R. Fan, et al., Screening of antinociceptive components in *Corydalis yanhusuo* W.T. Wang by comprehensive two-dimensional liquid chromatography/tandem mass spectrometry, Anal. Bioanal. Chem. 396 (5) (2010) 1731–1740.
[14] S.A. Saeed, A.H. Gilani, R.U. Majoo, et al., Anti-thrombotic and anti-inflammatory activities of protopine, Pharmacol. Res. 36 (1) (1997) 1–7.
[15] L. Ustünes, G.M. Laekeman, B. Gözler, et al., In vitro study of the anticholinergic and antihistaminic activities of protopine and some derivatives, J. Nat. Prod. 51 (5) (1988) 1021–1022.
[16] S.Q. Han, Chemiluminescence determination of pharmacologically active compounds by capillary electrophoresis, Luminescence 20 (6) (2005) 405–410.
[17] C.R. Li, L.M. Zhou, G. Lin, et al., Contents of major bioactive flavones in proprietary traditional Chinese medicine products and reference herb of radix Scutellariae, J. Pharm. Biomed. Anal. 50 (3) (2009) 298–306.
[18] Z.D. He, C.F. Qiao, Q.B. Han, et al., Authentication and quantitative analysis on the chemical profile of cassia bark (cortex cinnamomi) by high-pressure liquid chromatography, J. Agric. Food Chem. 53 (7) (2005) 2424–2428.
[19] G. Paganga, N. Miller, C.A. Rice-Evans, The polyphenolic content of fruit and vegetables and their antioxidant activities. What does a serving constitute, Free Radical Res. 30 (2) (1999) 153–162.
[20] X. Yan, M. Suzuki, M. Ohnishi-Kameyama, et al., Extraction and identification of antioxidants in the roots of yacon (*Smallanthus sonchifolius*), J. Agric. Food Chem. 47 (11) (1999) 4711–4713.
[21] G. Rocco, C. Toledo, I. Ahumada, et al., Determination of polychlorinated biphenyls in biosolids using continuous ultrasound-assisted pressurized solvent extraction and gas chromatography-mass spectrometry, J. Chromatogr. A 1193 (1–2) (2008) 32–36.
[22] X.Z. Li, P. Lei, S. Liu, Determination of 3 kinds of components in huanglian jiedu decoction by HPLC under different UV wavelengths, China J. Chin. Mat. Med. 31 (20) (2006) 1686–1688 (in Chinese).
[23] F.H. Xu, Y.H. Luo, W.K. Chen, et al., HPLC determination of three effective ingredients in *Corydalis decumbens* (Thunb.) Pers. injection, Chin. J. Pharm. Anal. 28 (9) (2008) 1494–1496 (in Chinese).
[24] X.R. Zhan, X.Y. Shi, W. Gai, et al., HPLC simultaneous determination of danshensu, protocatechuic aldehyde, hesperidin and salvianolic acid B in Rukuaixiao tablets, Chin. J. Pharm. Anal. 28 (5) (2008) 689–692 (in Chinese).
[25] R. Dai, K. Li, Q. Li, et al., Determination of mangiferin, jateorrhizine, palmatine, berberine, cinnamic acid, and cinnamaldehyde in the traditional Chinese medicinal preparation Zi-Shen pill by high-performance liquid chromatography, J. Chromatogr. Sci. 42 (4) (2004) 207–210.