



ORIGINAL ARTICLE

Quality analysis of commercial samples of *Ziziphi spinosae* semen (*suanzaoren*) by means of chromatographic fingerprinting assisted by principal component analysis



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Abstract Due to the scarcity of resources of *Ziziphi spinosae* semen (ZSS), many inferior goods and even adulterants are generally found in medicine markets. To strengthen the quality control, HPLC fingerprint common pattern established in this paper showed three main bioactive compounds in one chromatogram simultaneously. Principal component analysis based on DAD signals could discriminate adulterants and inferiorities. Principal component analysis indicated that all samples could be mainly regrouped into two main clusters according to the first principal component (PC1, redefined as Vicenin II) and the second principal component (PC2, redefined as zizyphusine). PC1 and PC2 could explain 91.42% of the variance. Content of zizyphusine fluctuated more greatly than that of spinosin, and this result was also confirmed by the HPTLC result. Samples with low content of jujubosides and two common adulterants could not be used equivalently with authenticated ones in clinic, while one reference standard extract could substitute the crude drug in pharmaceutical production. Giving special consideration to the well-known bioactive saponins but with low response by end absorption, a fast and cheap HPTLC method

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for quality control of ZSS was developed and the result obtained was commensurate well with that of HPLC analysis. Samples having similar fingerprints to HPTLC common pattern targeting at saponins could be regarded as authenticated ones. This work provided a faster and cheaper way for quality control of ZSS and laid foundation for establishing a more effective quality control method for ZSS.

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1. Introduction

Ziziphi spinosae semen (ZSS, *suanzaoren* in Chinese) is the dried and ripe seeds of *Ziziphus jujuba* Mill. var. *spinosa* (Bunge) Hu ex H. F. Chou. As a popular herb used long in China, ZSS has the functions of supplementing the liver, quieting the heart, arresting the sweat and promoting the production of the body fluid from views of Traditional Chinese Medicine. It is the most frequently used herb in Chinese materia medica preparations to treat anxiety and insomnia for its main sedative and hypnotic effects [1]. Increasing literature has reported its other pharmacological actions, including protecting the cardiovascular system [2], regulating immune function [3], and antioxidation [4]. This herb mainly comes from wild resources and the demand for it exceeds supply largely. Wider application of the herb in the field of health care in recent years has worsened the bad situation of resources [5]. Medicine markets are glutted with adulterants and inferior goods.

With phytochemical studies going deeper, myriads of compounds have been isolated from this herb, among which saponins, flavones and alkaloids dominate. To date, saponins and flavones are popularly acknowledged to be bioactive. For example, spinosins exert potential effect on pentobarbital-induced sleep [6]. Flavones and saponins can prolong sleeping time of mice, but saponins have more effective sedative and hypnotic functions compared with flavones [7]. Two saponins can prolong the suprathreshold barbiturate-induced sleeping time [8], and Cao's team suggested that jujubosides may be a good resource of lead compound for novel hypnotics [9]. Besides, alkaloid fraction is also proved to shorten sleep onset and prolong sleep time induced by pentobarbital [10], and total alkaloids can significantly decrease the locomotor activity of mice [11]. To sum up, three major types of ingredients may all contribute to the total pharmacological actions of this herb. Quality control items stipulated in Chinese Pharmacopeia 2010 edition focus on spinosin and jujuboside A, but it is hard to imagine that these two compounds contribute to pharmacological actions while other compounds with similar structures do not. Thus, the historically used one-size-fits-all approach, single or few markers mode, has gradually faded away and chromatographic fingerprint has come into the spotlight of the quality control of Chinese medicinal materials (CMM) and their preparations [12]. Combination of fingerprint techniques with chemometrics makes it more powerful for CMM's quality control [13]. To date, many papers introducing fingerprint or quantitation methods targeting several markers of ZSS have been published [14–17], but the reasons for markers selection have not been rationally explained, and more importantly, high cost compared with the price of the drug for quality analysis is another big problem. In consideration of the uneven quality level of ZSS at present, this study aimed at finding out rational detection indexes for quality control of ZSS and establishing a cheap and fast quality assessment method to discriminate the unqualified samples. The new method established could be used for rapid quality assessment for multiple samples. Not only should the adulterants be distinguished, but also the inferior samples should be treated differently.

2. Experimental

2.1. Chemicals and apparatus

Spinosin, jujuboside A and B (Purchased from Shanghai Winherb Medical S&T Development Co., Ltd.); LOT: jujuboside A-100415, jujuboside B-100320, and spinosin-100327; acetonitrile for high performance liquid chromatography (HPLC) analysis was from chromatographic grade from Merck (Darmstadt, Germany); all water used were purified by a Milli-Q system (Millipore, MA, USA); and other chemicals and reagents were all of analytical grade.

High performance thin layer chromatography (HPTLC) equipment consisted of an automatic TLC sampler 4, TLC development twin-through chamber, TLC scanner 3 with WinCats software and Digistore TLC documentation device (all from CAMAG, Muttenz, Switzerland). HPLC was performed on an Agilent 1200 series HPLC system with an autosampler and a diode array detector (DAD) (Agilent, Santa Clara, CA, USA). MS data were obtained on a 3200 QTRAP LC-MS/MS system by AB Sciex with an electrospray ionization source (AB Sciex, Framingham, MA, USA).

2.2. Samples

Twenty-seven batches samples were collected, and S#01 was from Beijing Institute for Drug Control; S#02 from Xingtai in Hebei Province; S#3 from Taiwan; S#04&05 from Hong Kong; S#06 from Xi'an in Shaanxi Province; S#07 from Chaozhou in Guangdong Province; S#08&09 from Chengdu Huaantang and Tai Chi drugstore in Sichuan Province; S#10 from Hongxiang Pharmaceutical Co., Ltd. in Yunnan Province; S#11&12 from Tongrentang and Dechengda drugstore in Beijing; S#13 from Shengxing drugstore in Guizhou Province; S#14 from Bozhou in Anhui Province; S#15 from Zhuhai Institute for Drug Control; S#16-23 from Jisheng, Jiabaohua, Minshengtang, and Jinxingtang drugstores and Red Cross Outpatient Clinic in Zhuhai, and S#16&19 had been parched; S#24&25, one sample and its standard extract were supplied by Youli Biotech Co., Ltd. in Guangdong Province; in addition, two common adulterants, S#26-*Ziziphi mauritiana* semen and S#27-*Hoveniae acerbae* semen, were also collected. All samples were identified by Professor Peishan Xie from the State Key Laboratory for Quality Research in Chinese Medicine, Macau University of Science and Technology.

2.3. Preparation of samples and standard solutions

Dried powder (80 mesh sieve passed) of samples was introduced into a flask and was refluxed with 20 mL petroleum ether (40–60 °C) at 90 °C for 1 h. After cooling at room temperature (25 °C), extracted solution was filtered through a filter paper, and the residue was washed with 5 mL fresh petroleum ether. The filtrate

was discarded, and after the residuary solvent completely evaporated, the sample was refluxed with 20 mL methanol for 1 h sequentially. Extracted solution was filtered and the residue was washed with 5 mL fresh methanol, and the filtrate was merged with the eluent. Combined solution was evaporated to dryness under reduced pressure. The residue was dissolved with methanol and transferred to a 1 mL volumetric flask, and the solution was diluted to the mark. For preparation of solution of the standard extract, 1.25 g fine powder was accurately weighed and extracted with 200 mL methanol by ultrasonication for 30 min. Then, extracted solution was filtered through filter paper, and the residue was washed with 5 mL fresh methanol. The filtrate was merged with the eluent and evaporated to dryness. The residue was dissolved with methanol and transferred into a 10 mL volumetric flask, and the solution was diluted to the mark. In addition, standard solutions containing jujuboside A and jujuboside B 1.0 mg/mL respectively in methanol were prepared. All well

mixed solutions were passed through microporous membranes with I.D. 0.45 μm , and subsequent solutions were collected as test solutions.

2.4. Experimental conditions

2.4.1. HPTLC conditions

HPTLC plate: silica gel 60 pre-coated plate (Merck, Germany, LOT: HX805149); developing solvent: dichloromethane–butyl acetate–methanol–water (10:20:15:4); sample application: 2 μL ; development: dried the sample loaded plate with the presence of phosphorus pentoxide under reduced pressure for more than 4 h before developing, developing distance was 6 cm, then evaporated to dryness with a steam of cold air; observation: sprayed 5% aluminum chloride in ethanol for flavone detection and 5% vanillin in concentrated sulfuric acid solution for saponin detec-

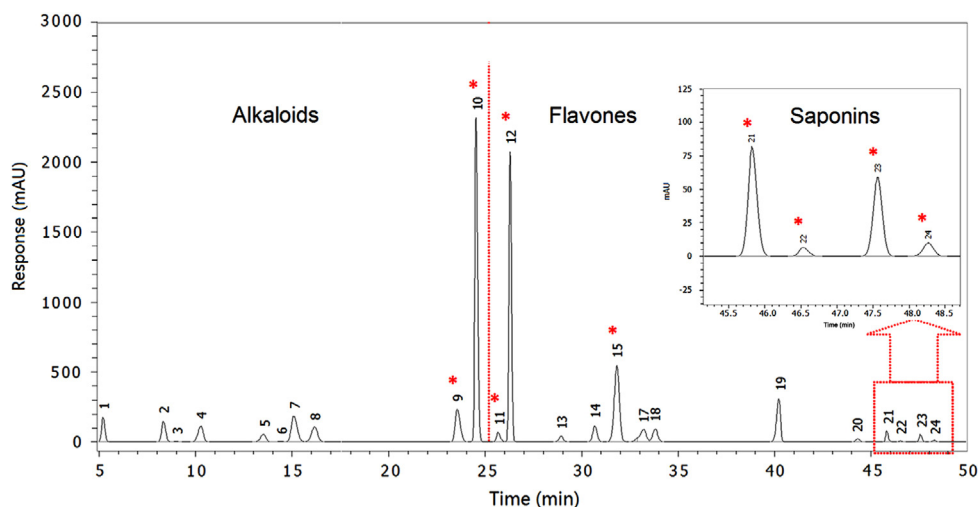


Fig. 1 HPLC common pattern of ZSS at 203 nm based on 24 batches of samples, bioactive components of three major types represented. Elution order was alkaloids, flavones, and saponins successively. Saponins peaks seemed relatively low for the low response to diode array detector.

Table 1 Structural information of major peaks.

Peak no.	t_R (min)	Compound	λ_{max} (nm)	MS fragments
2	8.28	TBD	258	427.4 [M–H] [–] ; 381.2, 281.3, 255.2, 145.1
4	10.24	TBD	260, 292	315.3 [M–H] [–] ; 297.3, 279.2, 171.1
5	13.49	TBD	248	205.8 [M]/[M+H] ⁺ ; 188.7, 130.0
7	14.99	TBD	218, 278	449.0 [M]/[M+H] ⁺ ; 287.1, 269.9, 237.8
8	16.16	TBD	250	443.5 [M–H] [–] ; 397.5, 297.2, 281.3
9	23.35	Coclaurine [18]	224, 282	286.9 [M+H] ⁺ ; 269.8, 175.9
10	24.44	Zizyphusine [18]	216, 268, 302	342.8 [M] ⁺ ; 297.9, 282.9, 265.9, 222.8
11	25.63	Vicenin II [19]	270, 337	593.2 [M–H] [–] ; 503.3, 473.0, 413.2, 383.0, 353.2
12	26.24	Spinosin ^a	270, 338	607.5 [M–H] [–] ; 427.2, 367.1, 337.2, 307.3
15	31.60	6 ^{'''} -feruloylspinosin or 6 ^{'''} -feruloylisospinosin [19]	274, 330	783.5 [M–H] [–] ; 607.3, 427.3, 367.3, 337.1, 307.2
19	40.24	TBD [19]	274, 330	1118.8 [M–H] [–] ; 762.1, 663.3, 394.8, 352.3, 293.0
21	45.85	Jujuboside A ^a	E.A.	1205.8 [M–H] [–] ; 1073.9, 927.5, 765.8, 749.9, 603.8
22	46.54	Jujuboside A ₁ or jujuboside D [19]	E.A.	1205.8 [M–H] [–] ; 1073.9, 927.5, 749.8, 603.2
23	47.60	Jujuboside B ^a	E.A.	1043.8 [M–H] [–] ; 911.8, 894.0, 766.0, 749.7, 603.7
24	48.32	Jujuboside B1 [19]	E.A.	1043.9 [M–H] [–] ; 912.0, 894.0, 765.9, 749.7, 603.6

TBD: to be determined.

^aCompared with reference substance; E.A.: end absorption.

tion, heated the plate at 90 °C until bands were clearly visible, after 10 min cooling, inspected under both UV 365 nm and visible light.

2.4.2. HPLC and MS conditions

HPLC Column: ZORBAX SB C₁₈ (4.6 mm × 250 mm, 5 μm; Agilent); column temperature: 35 °C; mobile phase: A-acetonitrile, B-0.1% formic acid in water; gradient: 0–20 min, 5–12% A; 20.01–35 min, 20–22%; 35.01–50 min, 25–55%; flow rate: 1 mL/min; injection volume: 10 μL; detection wavelength: 204 nm; ionization source: ESI; ionization voltage: 4200 V; nebulizing GS1: 50; nebulizing GS2: 50; declustering potential and collision energy are mass dependent. Positive mode was used for alkaloids analysis and negative mode for flavones and saponins.

2.5. Data processing

All HPTLC images and HPLC chromatograms were imported into ‘Chromap’ software developed by Chromap Institute of Herbal Medicine Research (Zhuhai, China) for statistical analysis. All analyses were based on the value of peak area of HPLC fingerprint and digital scanning profile of HPTLC fingerprint.

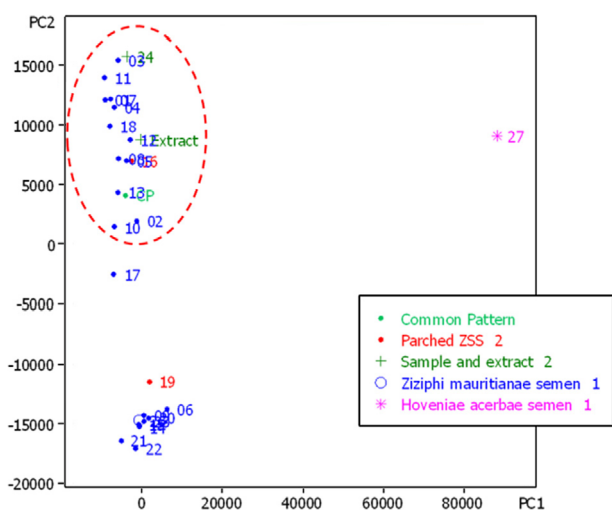


Fig. 2 PCA plot of HPLC fingerprint of ZSS. One of the adulterant S#27 could be easily isolated, and other samples were divided into two groups according to the scores of PC2. PC1 and PC2 could explain 91.42% of the variance. Centralization was used to pretreat the original data.

2.6. Method validation

Analysis was repeated six times to test the precision on S#11; six replicate samples of S#18 were prepared to determine the repeatability; S#24 was analyzed after being prepared at time of 0, 4, 8, 12 and 24 h for stability test. All chromatograms obtained were processed by ‘Chromap’ and similarities were all over 0.99. It indicated good precision, repeatability and sample stability of the method. All items met the requirements for fingerprint analysis.

3. Results and discussion

3.1. HPLC fingerprint analysis

HPLC fingerprint common pattern was constructed based on mean value of the peak area from 24 batches of samples (one extract and two adulterants excluded). The whole chromatogram was regularly divided into three sections according to UV absorption and MS data of each peak. The first section comprised several alkaloids peaks with retention time of less than 25 min. The second one consisted of flavones peaks with retention time between 25 and 45 min, and the third was made up of four saponins peaks with retention time from 45 to 50 min (Fig. 1).

Peaks #10, #21 and #23 were unambiguously designated as spinosin, jujuboside A and jujuboside B, respectively, by comparison with reference substances. Other asterisk marked peaks were tentatively identified by HPLC–MS/MS technique based on their characteristics of mass fragmentation. Information of major peaks is shown in Table 1.

Principal component analysis (PCA) was employed to group all samples. As a result, all collected samples except sample #27 were classified into two main groups (Fig. 2).

After dimension reduction, the first and second principal components (PC1 and PC2) could explain 91.42% of the variance. PC1 was redefined as vicenin II (loading: 0.85, highest in PC1). One of the adulterants, sample #27 could be easily isolated by score of PC1. In fact, two adulterants could be distinguished from genuine samples just by their appearances (Fig. 3). *Ziziphi mauritanae* semen was lighter in color. *Hoveniae acerbae* semen (sample #26) was irregular and smaller in size but darker in color. This law was not universally applicable, since some samples collected from pharmacies had been pulverized or even parched. It is very hard to differentiate them. Thus, further analysis was necessary to qualify them.

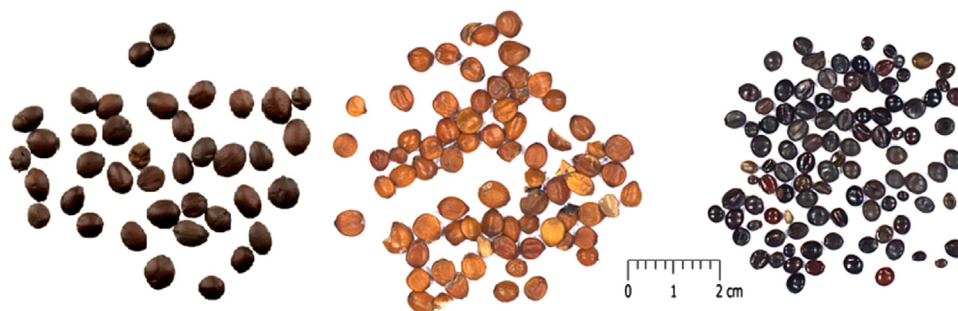


Fig. 3 Appearance comparison of *suanzaoren* with its adulterants. Left: *Ziziphi spinosae* semen, middle: *Ziziphi mauritanae* semen, right: *Hoveniae acerbae* semen.

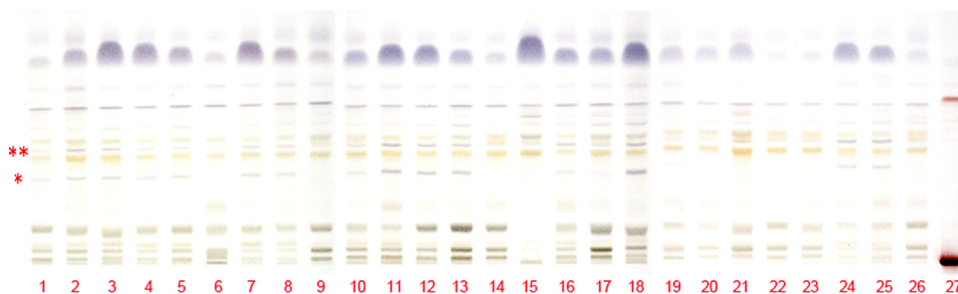


Fig. 4 HPTLC image targeting saponins of ZSS. * shows band of jujuboside A, and ** shows band of jujuboside B. Content of jujubosides varied greatly from batch to batch, and many samples along with two adulterants contained trace or no jujuboside A.

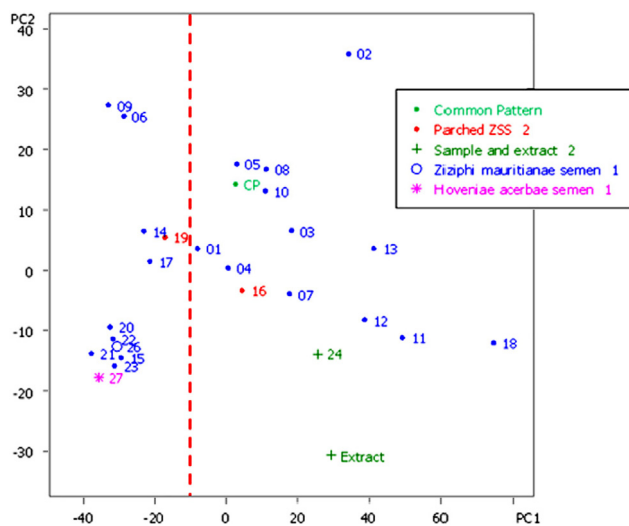


Fig. 5 PCA plot of HPTLC fingerprint of ZSS. All samples were divided into two groups by the red dashed line according to the scores of PC1, and PC1 can be redefined mainly as jujuboside A.

Other samples clustered into two groups according to the score of PC2, and PC2 was redefined mainly as peak 10, zizyphusine (loadings: -0.78 , highest in PC2). Samples of group 1 (16 batches, shown in the red dotted ellipse) were richer in zizyphusine than those of group 2. One parched sample S#16 belonged to group 1 and another S#19 to group 2, and parching process can hardly affect the bioactive ingredients of ZSS by Zhou's research [20]. Constituents of the extract (sample #25) were commensurate well with those of its original sample #24, and it is an irresistible trend for extract reference substance instead of crude drug to be used due to the inconsistency of the inner quality.

3.2. HPTLC fingerprint analysis

Considering that saponins have very low response with absorption at 203 nm and do not bear the due weight in statistical analysis based on DAD signal, a convenient and cheap HPTLC method targeting only the saponins was developed. Bluish gray bands of saponins were observed by derivatization with vanillin in sulfuric acid. Obviously, samples differed mainly in content of jujuboside A and jujuboside B (Fig. 4), and these two major bioactive

ingredients could hardly be found in many samples especially for the two adulterants.

HPTLC image was imported into Chromap software allowing for profile scanning, and peaks with R_f value between 0.2 and 0.7 were integrated. Fingerprints produced were used for another PCA analysis. The result is shown in Fig. 5. Jujuboside A and B got high loadings in PC1, 0.84 and 0.43, respectively. According to PC1, samples were classified into two parts. The right part showed samples which were poor in jujuboside A and B, and these samples were inferior with regard to the important roles that saponins play in the pharmacological actions. This result perfectly matched that of the PCA analysis of HPLC fingerprint, and it suggested that this simple HPTLC method could be effectively used for quality control of ZSS to discriminate adulterants and inferiorities.

Two markers, spinosin and jujuboside A, are introduced to qualify ZSS in Chinese pharmacopeia 2010 edition. From the results of our study, whether spinosin should be selected is controversial. PCA analysis of HPLC fingerprint did not show great impact of flavones on the quality, and it was confirmed by HPTLC analysis that contents of two major flavones including spinosin were relatively stable for all genuine goods, even for one of the adulterants, sample #26-Ziziphi mauritiana semen (Fig. 6).

4. Conclusion

In this work, a representative HPLC fingerprint common pattern was established based on 24 batches of samples collected from places all over China. Bioactive components of three major types were expressed simultaneously in the fingerprint chromatogram, and major peaks were designated by comparison with reference substances or reference data from literatures. This informative HPLC chromatogram provided a wider view of the material basis at a time for *Zizyphus spinosae* semen.

Principal component analysis, as a powerful and promising tool, helped to hit the components which affected the quality of ZSS most through dimension reduction. In view of the low response of saponins to diode array detector, another fast and cheap HPTLC detection method targeting mainly at the well acknowledged bioactive saponins was developed to confirm the HPLC result. As expected, analysis based on scanning profile of HPTLC image gave a consistent result: samples low in zizyphusine were poor in jujuboside A and B correspondently. It was also testified that contents of two major flavones including spinosin in ZSS samples were relatively stable, even for one of the adulterants collected. Thus, it may be questionable to choose spinosin as a marker to

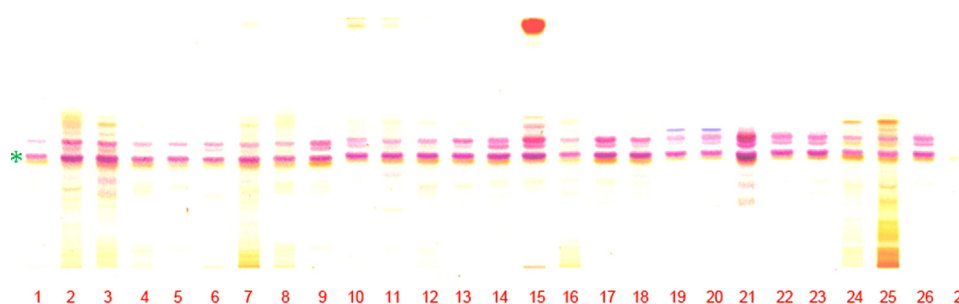


Fig. 6 Inverse image of the HPTLC chromatogram of ZSS under UV 366 nm. * shows band of spinosin; content of spinosin was relatively stable, even for one of the adulterants-S#26. Thus, selection of spinosin as marker for quality assessment was questionable.

assess the quality of ZSS in Chinese pharmacopeia. HPLC fingerprint provided an overview of three major components, and HPTLC method was fast, effective and cheap to discriminate adulterants and inferiorities from the genuine goods. The method established in this work was more rational for quality control of ZSS to date, but more work should be done to clarify the further relationship between the ingredients and the pharmacological actions.

References

- [1] K.F. Chung, C.K. Lee, Over-the-counter sleeping pills: a survey of use in Hong Kong and a review of their constituents, *Hosp. Psychiat.* 24 (2002) 430–435.
- [2] X.J. Chen, C.L. Yu, J.F. Liu, Protective effects of total saponins of semen *Ziziphi spinosae* on cultured rat myocardial cells, *Acta Pharmacol. Sin.* 11 (1990) 153–155 (in Chinese).
- [3] J. Xie, L. Guo, G. Pang, et al., Modulation effect of Semen *Ziziphi spinosae* extracts on IL-1-(beta), IL-4, IL-6, IL-10, TNF-(alpha) and IFN-(gamma) in mouse serum, *Nat. Prod. Res.* 25 (2011) 464–467.
- [4] Z. Peng, H. Zhang, S. Cheng, et al., Protective effect of semen *Ziziphi spinosae* on superoxide dismutase reduction in mice with endotoxin fever, *China J. Chin. Mater. Med.* 20 (1995) 369–370 (in Chinese).
- [5] C. Ye, Brief introduction of identification method for *Ziziphi spinosae* semen and its adulterants, *Prac. J. Med. Pharm.* 23 (2006) 1506 (in Chinese).
- [6] L.E. Wang, X.Y. Cui, S.Y. Cui, et al., Potentiating effect of spinosin, a C-glycoside flavonoid of Semen *Ziziphi spinosae*, on pentobarbital-induced sleep may be related to postsynaptic 5-HT(1A) receptors, *Phytomed. Int. J. Phytother. Pytopharmacol.* 17 (2010) 404–409.
- [7] J.G. Jiang, X.J. Huang, J. Chen, et al., Comparison of the sedative and hypnotic effects of flavonoids, saponins, and polysaccharides extracted from Semen *Ziziphus jujube*, *Nat. Prod. Res.* 21 (2007) 310–320.
- [8] J.G. Jiang, X.J. Huang, J. Chen, Separation and purification of saponins from Semen *Ziziphus jujube* and their sedative and hypnotic effects, *J. Pharm. Pharmacol.* 59 (2007) 1175–1180.
- [9] J.X. Cao, Q.Y. Zhang, S.Y. Cui, et al., Hypnotic effect of jujubosides from Semen *Ziziphi spinosae*, *J. Ethnopharmacol.* 130 (2010) 163–166.
- [10] Y. Ma, H. Han, S.Y. Nam, et al., Cyclopeptide alkaloid fraction from *Ziziphi spinosae* Semen enhances pentobarbital-induced sleeping behaviors, *J. Ethnopharmacol.* 117 (2008) 318–324.
- [11] J.W. Fu, W. Qiao, C.H. Chen, Study on sedative and hyponotic effect of total alkaloid from Semen *Ziziphi spinosae*, *J. Tianjin Med. Univ.* 11 (2005) 52–54 (in Chinese).
- [12] P.S. Xie, S.B. Chen, Y.Z. Liang, et al., Chromatographic fingerprint analysis—a rational approach for quality assessment of traditional Chinese herbal medicine, *J. Chromatogr. A* 1112 (2006) 171–180.
- [13] Y.Z. Liang, P.S. Xie, K. Chan, Quality control of herbal medicines, *J. Chromatogr. B* 812 (2004) 53–70.
- [14] M. Zhang, Y. Zhang, J. Xie, Simultaneous determination of jujuboside A, B and betulinic acid in semen *Ziziphi spinosae* by high performance liquid chromatography–evaporative light scattering detection, *J. Pharmaceut. Biomed. Anal.* 48 (2008) 1467–1470.
- [15] K.H. Shin, H.Y. Kim, W.S. Woo, Determination of flavonoids in seeds of *Zizyphus vulgaris* var. *spinosus* by high performance liquid chromatography, *Planta Med.* 44 (1982) 94–96.
- [16] C. Niu, J. Zhang, Quantitative analysis and chromatographic fingerprinting of the semen *Zizyphi spinosae* by ultra-high-performance liquid chromatography coupled with diode-array detector, *J. Sep. Sci.* 34 (2011) 2989–2996.
- [17] J.J. Liu, B. Chen, S.Z. Yao, Simultaneous analysis and identification of main bioactive constituents in extract of *Zizyphus jujuba* var. *sapinosa* (*Zizyphi spinosae* semen) by high-performance liquid chromatography–photodiode array detection–electrospray mass spectrometry, *Talanta* 71 (2007) 668–675.
- [18] B. Han, M. Park, Y. Han, Aporphine and tetrahydrobenzylisoquinoline alkaloids from the seeds of *Zizyphus vulgaris* var. *spinosus*, *Arch. Pharm. Res.* 12 (1989) 263–268.
- [19] W.J. Wang, J.G. Luo, L.Y. Kong, HPLC-ESI-MS(n) analysis of chemical constituents in Semen *Ziziphi spinosae*, *China J. Chin. Mater. Med.* 34 (2009) 2768–2773 (in Chinese).
- [20] X. Zhou, H.S. Yu, J. Zhang, et al., Comparative studies on the HPLC fingerprints of the Semen *Ziziphi spinosae* (SZS) and the parched Semen *Ziziphi spinosae*(pSZS), *Chin. J. Drug Appl. Monit.* 4 (2007) 26–28 (in Chinese).