Quality control of *Semen Ziziphi Spinosae* standard decoction based on determination of multi-components using TOF-MS/MS and UPLC-PDA technology

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1. Introduction

*Semen Ziziphi Spinosae* (SZS), the dried seeds of *Ziziphus jujuba* Mill. var. *spinosa* (Bunge) Hu ex H. F. Chou derived from Rhamnaceae species [1], was firstly documented and classified as high-grade goods in *Shen Nong Materia Medica*, a medicinal work of E. Han Dynasty in ancient China. It has been used to treat insomnia, anxiety, neurasthenic, hyperhidrosis, forgettery, dreaminess and other symptoms for thousands of years in China as the most frequently used herb in Chinese medicines in ancient China. It has been used to treat insomnia, anxiety, neurasthenic, hyperhidrosis, forgettery, dreaminess and other symptoms for thousands of years in China as the most frequently used herb in Chinese medicines, in which active constituents had not been clearly revealed. Therefore, it is urgent to establish an improved and comprehensive method to reveal bioactive components and further quality assessment of SZS preparations with water extraction. Herbal standard decoction of SZS standard decoction based on the quality of SZS preparations, which are mostly focused on analysis of certain type of chemicals based on HPLC, UV or TLC, etc [8–11]. It is generally accepted that most of traditional Chinese medicines generate medical effect by the synergic action of a variety of chemical components. Current quantitative methods in the literature may not comprehensively evaluate the quality of SZS preparations and these conventional analytical methods may result in longer running time and less sensitivity [12]. Therefore, it is urgent to establish an improved and comprehensive method to reveal bioactive components and further quality assessment of SZS preparations with water extraction. Herbal standard decoction of single herbal pieces in water decoction is the standard reference to measure if the quality of herbal preparations complies with the raw herbal pieces and confirms the unification of clinical administration and dosage [13]. Therefore, it is necessary to establish a method to unify the efficiency of herbal preparations. A sensitive, fast and comprehensive method for the quality assessment of *Semen Ziziphi Spinosae* (SZS) standard decoction with characterization of its chemical components was developed and validated. UPLC-Q/TOF-MS/MS system was used to identify thirty-six chemical components of SZS standard decoction which included nucleosides, phenolic acids, alkaloids, and flavonoids. Furthermore, a UPLC-PDA method was validated to simultaneously determine adenosine, protocatechuic acid, magnoflorine, catechin, protocatechin, vicenin II, spinosin, kaempferol-3-rutinoside, and 6‴-feruloylspinosin which represent four species of characteristic compounds. The qualitative method had been validated according to Chinese Pharmacopoeia (2015 edition) in terms of linearity, repeatability, recovery and stability for all analytes, with the results showing good precision, accuracy and stability. In conclusion, the method using UPLC combined with MS and PDA provided a novel way for the standardization and identification of SZS standard decoction, and also offered a basis for qualitative analysis and quality assessment of the preparations for SZS standard decoction.

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comprehensive quality control (QC) method to standardize operation of manufacturers of SZS standard decoction and unify the therapeutic benefit of different SZS preparations.

SZS standard decoction was prepared according to “Management standard of traditional Chinese medicine (TCM) decoction in medical institutions” [14]. In this study, a rapid and reliable chemical profiling and multicomponent quantitative analysis method based on mass spectroscopy and chromatography was developed. The UPLC-MS is fairly fast and accurately in identifying seven types of water-soluble active components in SZS standard decoction. Simultaneously, nine active components subject to four species, namely, nucleosides (adenosine), phenolic acids (protocatechuic acid), alkaloids (magnoflorine), and flavonoids (catechin, protocatechin, vicenin II, spinosin, kaempferol-3-rutinoside, and 6‴-feruloylspinosin) were quantitatively determined by the UPLC-PDA method. And chemical structures of nine reference compounds are displayed in Fig. 1. In a word, the method integrating chromatography and mass spectroscopy analysis would offer comprehensive and reliable information for the identification and QC of SZS standard decoction and preparations.

2. Experimental

2.1. Materials and reagents

Spinosin was from Liaoning Institute for Drug Control (Shenyang, China). Adenosine, protocatechuic acid, catechin, protocatechin, magnoflorine, vicenin II, kaempferol-3-rutinoside and 6‴-feruloylspinosin were bought from Chengdu Chroma Bio-
technology Co. Ltd (Chengdu, China). The purities of all reference substances were tested with HPLC method (≥ 98%).

SZS was provided from GuoDa Pharmacy (Shenyang, China). HPLC-grade acetonitrile was purchased from Fisher Scientific (Fisher, Scientific, USA). Phosphoric acid (HPLC grade) and formic acid (HPLC grade) were obtained from Yuwang Chemical Reagent (Shandong, China). Purified water was purchased from Wahaha Group Co. Ltd. (Hangzhou, China).

2.2. Mass spectrometry system for identification

The samples were analyzed by an Agilent 1260 UPLC system consisting of an on-line degasser, a quaternary pump, an auto-sampler, and a column temperature controller. The chromatographic column was Waters Cortecs® T3 column (2.1 mm × 100 mm, 2.7 μm). The mobile phase contained 0.1% formic acid in water (A) and acetonitrile (B) with a flow rate of 0.4 mL/min using a gradient program as follows: 0–2% B from 0 to 5 min, 2–5% B from 5 to 10 min, 5–10% B from 10 to 15 min, 10–13% B from 15 to 20 min, 13–18% B from 20 to 24 min, 18–24% B from 24 to 32 min and 24–90% B from 32 to 40 min. And the injection volume of the sample and mixed standard solution was 3 μL.

The Agilent 1260 UPLC system was connected to a Triple TOF™ 5600 (AB SCIEX, Foster City, CA) with an ESI interface. Mass range was set at m/z 50–2000. The optimum parameters of MS/MS detector were set as follows: ion spray voltage was 4500 V for positive ion mode and −4500 V for negative ion mode; ion source temperature was 550 °C; ion source gas 1 was 50 psi; ion source gas 2 was 50 psi; curtain gas was 30 psi; collision energy was 10 V in MS mode and 45 V in MS² mode; and declustering potential was all set at 80 V. Peak View® Software V. 2.2 was used for data collection and processing.

2.3. UPLC-PDA system for quantification

Waters H-class UPLC (Waters Corporation, America) have been carried for chromatography system equipped with a quaternary solvent delivery system, an on-line degasser, an auto-sampler, a column temperature controller and a photodiode array detector. All experimental data was collected and managed with Empower 2 software.

The chromatographic separation was achieved with a Waters Cortecs® T3 column (2.1 mm × 100 mm, 2.7 μm) with the temperature of 30 °C. Detection wavelength for determination was set at 210 nm. The mobile phase was 0.03% phosphoric acid in water (A) and acetonitrile (B) with the flow rate of 0.4 mL/min. The gradient elution program was as followed: 0–1% B in 0–3.5 min, 1–2% B in 3.5–6.5 min, 2–5% B in 6.5–10 min, 5–10% B in 10–17 min, 17–22% B in 10–13 min, 22–26% B in 13–18 min, 26–29% B in 18–20 min, 29–32% B in 20–24 min and 24–90% B in 32–35 min. The sample injection volume was 1 μL.

2.4. Preparation of SZS standard decoction

The SZS pieces was extracted with 8 times amount of water. After soaked for 30 min, and refluxed for 30 min, the mixture was immediately filtered through a 120-mesh sieve. Then the filtrate was extracted with 6 times amount of water, with refluxing time for 20 min and immediate filtered again. The twice combined extraction decoction was concentrated to a 0.2 g/mL by reducing pressure.

2.5. Preparation of the mixed standard solution and sample solution

Stock solutions of adenosine, protocatechuic acid, catechin, protocatechin, magnoflorine, vicenin II, spinosin, kaempferol-3-rutinoside and 6′-feruloylspinosin were separately prepared in water. The stock solution of each analyte was further diluted with water to obtain the working solutions so as to prepare the calibration solutions. All solutions were kept at −4 °C and then stand to room temperature before use.

Before injected into the instrument, the SZS standard decoction solution was centrifuged at 12,000 rpm for 5 min, and the liquid supernatant was filtered through 0.22 μm membranes.

3. Results and discussion

3.1. Identification of the main chemical components

The basic peak chromatograms (BPC) of SZS standard decoction in positive and negative ion modes are displayed in Fig. 2. Multiple data processing methods like mass defect filtering, product ion filtering and neutral loss filtering with full scan mode could improve the accuracy and efficiency of analytical process. In Fig. 3, TOF/MS and TOF/MS² spectroscopy data of Vicenin II (a characteristic compound in SZS) are displayed in the negative ion mode, which show the cracking situation of C-glycosylflavonones compounds. The ion peaks at m/z 473.1112 [M–H–120]– and m/z 353.0674 [M–H–120–120]– were formed due to the breakage of one or two molecules of glucose. Similarly, fragment behavior of m/z [M–H–90]– 503.1190 and [M–H–90–120]– 383.0769 resulted from the different fracture disposition. Mass spectral information was concluded and confirmed by the literatures. Thirty-six compounds were found out and tentatively identified, covering 15 flavonoids, 6 amino acids, 5 alkaloids, 5 nucleosides, 2 phenolic acids, 2 triterpenoid saponins, and 1 lactone [15–29], among which eleven compounds were unambiguously identified by comparing retention time and fragments information with reference standards. The mass error for all identified compounds was within ± 5 ppm. And the identified components and their MS data are listed in Table 2.

3.2. The selection of index components in determination

With the phytochemical studies going further, a variety of compounds have been isolated from SZS, QC items proposed in current literature focus on saponins, flavones or triterpenes, but it is hard to speculate that certain types of components contribute to the total pharmacological actions. Thus, the conventionally used single or few markers approach has gradually been substituted and multi-components determination method has stood out for the quality assessment of TCM and their preparations [30]. To date, saponins and flavones are popularly acknowledged to be bioactive for sedative and hypnotic effects [31,32]. Alkaloids is also proved to shorten sleep onset and prolong sleep time of mice induced by pentobarbital[33]. In addition, adenosine and protocatechuic acid exhibit antimicrobial, anti-inflammatory and immunity enhance activities [34,35]. In summary, five main types of ingredients all may be beneficial to the total pharmacological effect of SZS and its preparations. In this study, adenosine, protocatechuic acid, catechin, protocatechin, magnoflorine, vicenin II, kaempferol-3-rutinoside and 6′-feruloylspinosin were chosen as index components, which were composed of nucleosides, phenolic acids, alkaloids, and flavonoids that all have UV absorption and can be determined by UPLC-PDA. Saponins were furthermore analyzed by HPLC-ELSD in the other study because of weak UV absorption. The established method was suitable for routine quantitative analysis and quality assessment of SZS standard decoction.

3.3. Optimization of chromatographic conditions

Chromatographic column, mobile phase composition and detection wavelength were emphatically optimized to obtain a better resolution and an appropriate retention time of the investigated components.
Two hydrophilic analytical columns with different particle sizes and manufacturers, namely, Agilent Poroshell 120 SB-Aq (1.8 mm × 100 mm, 2.7 μm) and Waters Cortecs T3 (2.1 mm × 100 mm, 2.7 μm), were compared for separation of water-soluble compounds in SZS standard decoction. Then Waters Cortecs T3 was proved to make the better separation and response at the same UPLC system.

Different compositions of mobile phase and gradient elution were investigated to obtain good resolution and sharp peaks for quantitative analysis. Most of the compounds are slightly acidic; thus the addition of acid (phosphoric acid, formic acid and glacial acetic acid) in mobile phase could help to enhance the resolution and eliminate the peak tailing of the analytes. The result indicated that acetonitrile and water with 0.03% phosphoric acid could achieve ideal peak shapes and resolution, in which acidic pH could guarantee the molecularity of most acidic compounds for analysis and was found to have little influence on the peak shape of magnoflorine.

The maximum adsorption wavelengths of nine compounds were scanned in UV spectra (190–400 nm) with three dimension chromatograms of PDA. All target components showed an appropriate absorption at 210 nm simultaneously with smooth baseline and less interference. Allowing of the simplicity and operability for the method, the wavelength of 210 nm was chosen as the detection wavelength for multi-component determination.

Additionally, some other chromatographic variables were also investigated, such as column temperatures (25, 30, 35 °C), and flow rates (0.35, 0.4, 0.5 mL/min). In result, column temperature had little influence on the separation, though the variation of flow rate may lead to time-consuming or poor resolution. As displayed in Table 3, the optimized values: column temperature of 30 °C and flow rate of 0.4 mL/min could achieve the best peak parameters and resolution with shorter time.

### 3.4. Method validation for UPLC-PDA system

#### 3.4.1. Specificity

The specificity was examined by comparing the chromatograms of blank solvent, standard solution and sample solutions. The retention time of target peak in the chromatogram of the
Table 2
Identification of chemical constituents in SZS standard decoction by UPLC-Q/TOF-MS/MS.

| No. | Rt (min) | Formula | Measured mass (m/z) | Error (ppm) | Identification | Ref. |
|-----|----------|---------|---------------------|-------------|----------------|-----|
| 1   | 1.342    | C₆H₉O₇  | 191.0204[M-H]⁻ | 0.8         | Citric acid    | [15]|
| 2   | 1.766    | C₁₂H₁₆N₄O₂ | 233.1131[M-H]⁻ | -4.8        | Glutamic acid dipeptide | [16-18]|
| 3   | 1.926    | C₈H₁₄N₂O₂ | 132.1015[M-H]⁻ | -1.2        | Aminocaproic acid | [16-18]|
| 4   | 2.024    | C₁₂H₁₆N₄O₂ | 243.0623[M-H]⁻ | 0.2         | Uridine monophosphate | [19]|
| 5   | 2.297    | C₁₀H₁₈N₂O₆P | 328.0448[M-H]⁻ | -0.8        | Cyclic adenosine monophosphate | [19]|
| 6   | 2.885    | C₁₂H₁₆N₄O₂ | 344.0401[M-H]⁻ | -0.2        | Cyclic guanosine monophosphate | [19]|
| 7   | 4.124    | C₁₄H₂₂O₇ | 164.0724[M-H]⁻ | 3.9         | Phenylalanine | [16-18]|
| 8   | 4.644    | C₁₂H₁₄N₂O₂ | 266.0897[M-H]⁻ | -0.5        | Adenosine¹ | [19]|
| 9   | 4.825    | C₁₂H₁₄N₂O₅ | 282.0846[M-H]⁻ | 0.8         | Guanosine hydrate | [19]|
| 10  | 4.993    | C₆H₁₀O₄ | 153.0197[M-H]⁻ | 2.4         | Protocatechuic acid² | [20]|
| 11  | 5.818    | C₁₂H₁₈N₂O₂ | 252.1468[M-H]⁻ | 0.8         | Cyl[Arginine-proline] | [16-18]|
| 12  | 8.465    | C₁₈H₂₄O₆ | 137.0250[M-H]⁻ | 4.2         | Hydroxybenzoic acid | [16-18]|
| 13  | 9.143    | C₁₂H₁₄N₂O₂ | 203.0831[M-H]⁻ | -1.5        | Tryptophan | [16-18]|
| 14  | 13.382   | C₁₂H₁₄O₄ | 289.0723[M-H]⁻ | 1.9         | Epicatechin³ | [21]|
| 15  | 15.612   | C₁₂H₁₄N₂O₄ | 448.7965[M+H]⁺ | 447.893 | 6-Glu-Coicaraine | [22]|
| 16  | 16.882   | C₁₂H₁₄N₂O₄ | 314.1752[M+H]⁺ | -0.3        | Norsynephrine | [23]|
| 17  | 18.668   | C₁₂H₁₄N₂O₄ | 286.1440[M+H]⁺ | 318.1356 | Coicaraine | [22, 24, 25]|
| 18  | 19.258   | C₁₂H₁₄O₇ | 289.0724[M-H]⁻ | 1.9         | Cathaëine⁴ | [21]|
| 19  | 20.060   | C₁₂H₁₄O₇ | 342.1702[M-H]⁻ | 314.1627 | Magnoflorine⁴ | [26]|
| 20  | 21.409   | C₁₂H₁₄O₇ | 593.1561[M-H]⁻ | -1.4        | Vicnin I² | [24-26]|
| 21  | 24.900   | C₁₂H₁₄O₇ | 593.1654[M-H]⁻ | -0.8        | Isovitexin-2′-O-β-D-glucopyranoside | [25-27]|
| 22  | 24.950   | C₁₂H₁₄O₇ | 609.1808[M-H]⁺ | -1         | Isopinosine | [26]|
| 23  | 25.299   | C₁₂H₁₄O₇ | 609.1808[M-H]⁺ | -1         | Spinosisina⁴ | [22, 25, 26]|
| 24  | 25.765   | C₁₂H₁₄O₇ | 595.1656[M-H]⁺ | -2.5        | Vitexin-4′-O-glucoside | [26]|
| 25  | 27.859   | C₁₂H₁₄O₇ | 595.1656[M-H]⁺ | -2.5        | Kaempferol-3-rutinoside⁴ | [25, 26]|
| 26  | 28.22    | C₁₂H₁₄O₇ | 771.1242[M-H]⁺ | -0.9        | Nerviliordin J | [25]|
| 27  | 28.366   | C₁₂H₁₄O₇ | 729.2028[M-H]⁺ | 0.3         | 6-P-hydroxybenzoylsinosin | [22, 25]|
| 28  | 29.630   | C₁₂H₁₄O₇ | 755.2176[M-H]⁺ | -0.8        | 6-P-coumarylsinosin | [22, 25]|
| 29  | 29.670   | C₁₂H₁₄O₇ | 875.2183[M-H]⁺ | -0.5        | 7-O-(6′-O-Feruloylglucosyl)-isoyctiside or 4-O-(6′-O-Feruloylglucosyl)-swertisin | [25]|
| 30  | 29.696   | C₁₂H₁₄O₇ | 815.2387[M+H]⁺ | -0.7        | 6′-Sinapoylsinosin | [27]|
| 31  | 30.256   | C₁₂H₁₄O₇ | 875.2283[M+H]⁺ | -0.5        | 6′-Feruloylsinosin⁴ | [25, 27]|
| 32  | 32.211   | C₁₂H₁₄O₇ | 1205.3622[M-H]⁻ | 0.3         | Jujuboside A⁵ | [25, 27, 28]|
| 33  | 34.511   | C₁₂H₁₄O₇ | 1043.5431[M-H]⁻ | 0.5         | Jujuboside B⁶ | [25, 27, 28]|
| 34  | 35.117   | C₁₂H₁₄O₇ | 632.3808[M+H]⁺ | 0.2         | Amphine D | [22, 28]|
| 35  | 35.461   | C₁₂H₁₄O₇ | 455.3521[M+H]⁺ | -0.2        | Ebelin lactone | [29]|
| 36  | 35.650   | C₁₂H₁₄O₇ | 447.1280[M+H]⁺ | -1.3        | Swertisin | [25-27]|

¹ Identified with reference standard.
sample solution was in conformity with that in standard solution, and the representative chromatogram is shown in Fig. 4.

3.4.2. Calibration curves and linear ranges
The linearity range of calibration curves was based on the concentration of the sample solution. Standard stock solutions were prepared and diluted into six concentration levels for constructing the external standard calibration lines by plotting the peak area against the concentration of each compound. As shown in Table 4, excellent linearity over the test ranges was achieved ($r \geq 0.9996$).

3.4.3. Precision and stability
The inter-day precision was verified by analyzing the samples in three replicates at three different concentrations (low, medium, high). The intra-day variability was investigated with the same solution in three replicates on three successive days, respectively. The relative standard deviation (RSD) calculated as measurement were all within 2.0%. And stability of sample solution was also analyzed by comparing the peak areas of the analytes in triplicate from the same sample solution after storing at 0, 2, 4, 8, 12, and 24 h. The RSD values were not more than 1.8%. We could conclude that the method has good precision and repeatability, and the analytes were relatively stable in sample solution at room temperature for 2 days.

3.4.4. Accuracy
Accuracy was further verified with a recovery test performed by adding three concentration levels (low, medium and high) of the mixed standard references into a certain amount of sample solution. The fortified solutions were determined with the developed method and evaluated by calculating the ratio of the detection and addition amount. The results of accuracy test were between 85.4% and 104.7% with RSDs less than 3.0%, which is recorded in detail at Table 5.

3.4.5. Ruggedness
In order to introduce the analytical method into different laboratories, some important factors were slightly adjusted to investigate the robustness, including flow rate ($\pm 0.1$ mL/min), injection volume (0.5, 1, 2 μL), wavelength (203, 210, 220 nm), column temperature ($\pm 5$ °C), and acid concentration ($\pm 0.01\%$ phosphoric acid). The affection was assessed by the RSDs of total content of nine marker compounds. At different conditions, the RSDs were within 5.0%.

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### Table 3
Results of different chromatographic conditions for SZS standard decoction (ie. spinosin).

| No. | Factors                     | Level                        | Rt (min) | N³ |
|-----|-----------------------------|------------------------------|----------|----|
| 1   | Chromatography column       | Waters Cortecs® T3 (2.1 mm × 100 mm, 2.7 μm) | 19.45    | 22,911 |
|     |                             | Agilent Poroshell 120 SB-Aq (1.8 mm × 100 mm, 2.7 μm) | 21.67    | 20,920 |
| 2   | Flow rate                   | 0.35 mL/min                  | 21.22    | 23,109 |
|     |                             | 0.4 mL/min                   | 19.60    | 23,320 |
|     |                             | 0.5 mL/min                   | 17.95    | 22,990 |
| 3   | Column temp.                | 25 °C                        | 20.13    | 22,391 |
|     |                             | 30 °C                        | 19.77    | 22,402 |
|     |                             | 35 °C                        | 19.15    | 19,920 |

¹ Retention time.
² Number of theoretical plates.

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Fig. 4. UPLC chromatograms of reference substance (A) and sample of SZS standard decoction (B): 1-Adenosine, 2-Protocatechuic acid, 3-Catechin, 4-Protocatechin, 5-Magnoflorine, 6-Vicenin II, 7-Spinosin, 8-Kaempferol-3-rutinoside, 9-6‴-Feruloylspinisin.
3.5. Sample analysis

The established analytical method was successfully utilized for the simultaneous determination of adenosine, protocatechuic acid, catechin, protocatechin, magnoflorine, vicenin II, spinosin, kaempferol-3-rutinoside and 6‴-feruloylspinosin in 10 samples of SZS standard decoction, and the contents result is demonstrated in Table 6. The total content arrangement of nine compounds varied from 656.59 to 1002.09 μg/g in 10 batches of samples. Although Hebei province is traditionally regarded as a genuine producing area of SZS, the highest content of 1002.09 μg/g was found in the batch from the city of Jining, Shandong province. However, the content of three

### Table 4
Regression equations, correlation coefficients and linear ranges of nine components.

| Analytes          | Linearity equation          | r     | Linear range (μg/mL) |
|-------------------|-----------------------------|-------|----------------------|
| Adenosine         | $Y = 1.004 \times 10^4 X + 5.081 \times 10^2$ | 0.9998 | 1.816–36.31          |
| Protocatechuic acid | $Y = 1.401 \times 10^4 X + 1.511 \times 10^3$ | 0.9998 | 1.122–22.44          |
| Catechin          | $Y = 2.323 \times 10^3 X + 3.582 \times 10^4$ | 0.9998 | 1.793–35.87          |
| Protocatechin     | $Y = 2.495 \times 10^4 X + 1.647 \times 10^4$ | 0.9998 | 1.236–24.72          |
| Magnoflorine      | $Y = 9.970 \times 10^3 X + 1.511 \times 10^4$ | 0.9998 | 10.63–212.6          |
| Vicenin II        | $Y = 1.321 \times 10^4 X - 2.9831$ | 0.9996 | 16.29–325.8          |
| Spinosin          | $Y = 7.446 \times 10^3 X + 4.285 \times 10^4$ | 0.9996 | 1.233–24.67          |
| Kaempferol-3-rutinoside | $Y = 6.408 \times 10^4 X + 3.778 \times 10^3$ | 0.9999 | 0.394–7.380          |
| 6‴-Feruloylspinosin | $Y = 7.607 \times 10^4 X + 5.009 \times 10^4$ | 0.9998 | 4.043–80.86          |

### Table 5
The results of recovery tests analyzed for SZS standard decoction (n = 9).

| Analytes          | Original (μg) | Spiked (μg) | Found (μg) | Recovery (%) | RSD (%) |
|-------------------|---------------|-------------|------------|--------------|---------|
| Adenosine         | 25.51         | 13.07       | 38.36      | 98.3         | 1.1     |
|                   | 26.14         | 51.36       | 30.57      | 98.9         | 1.5     |
|                   | 39.21         | 64.40       | 39.61      | 99.2         | 1.5     |
|                   | 22.32         | 6.470       | 38.81      | 98.8         | 1.8     |
| Protocatechuic acid | 4.57         | 4.464       | 8.61       | 90.6         | 2.6     |
|                   | 6.696         | 10.77       | 92.6       | 1.8          |         |
|                   | 8.967         | 26.75       | 97.7       | 1.2          |         |
| Catechin          | 17.99         | 17.93       | 35.91      | 99.9         | 0.8     |
|                   | 26.90         | 44.02       | 96.8       | 0.7          |         |
|                   | 6.180         | 18.16       | 98.7       | 1.1          |         |
| Protocatechin     | 12.06         | 12.36       | 24.06      | 97.1         | 1.6     |
|                   | 18.54         | 29.74       | 95.3       | 2.0          |         |
|                   | 96.76         | 288.8       | 104.7      | 1.3          |         |
| Magnoflorine      | 187.5         | 191.6       | 377.8      | 99.3         | 0.4     |
|                   | 290.3         | 478.5       | 100.3      | 0.9          |         |
|                   | 3.073         | 9.071       | 93.1       | 2.1          |         |
| Vicenin II        | 6.20          | 6.146       | 12.27      | 98.7         | 1.1     |
|                   | 9.220         | 15.16       | 97.1       | 1.9          |         |
|                   | 6.180         | 18.16       | 98.7       | 1.1          |         |
| 6‴-Feruloylspinosin | 52.12        | 53.27       | 105.5      | 99.9         | 1.2     |
|                   | 87.33         | 140.1       | 100.8      | 0.7          |         |

### Table 6
Quantitative analytical results of 9 compounds in 10 batches of SZS standard decoction (1-Adenosine, 2-Protocatechuic acid, 3-Catechin, 4-Protocatechin, 5-Magnoflorine, 6-Vicenin II, 7-Spinosin, 8-Kaempferol-3-O-rutinoside, 9-6‴-Feruloylspinosin).

| No. | Origin     | Content (μg/g) | Total contents (μg/g) |
|-----|------------|---------------|-----------------------|
| 1   | Hebei Xingtai | 47.33, 6.72  | 47.33, 6.72, 37.22, 30.01, 273.2, 7.10, 168.51, 26.11, 70.95, 667.15 |
| 2   | Hebei Zanhuang | 47.04, 6.38  | 47.04, 6.38, 30.09, 23.24, 293.1, 6.57, 372.55, 30.02, 68.91, 677.90 |
| 3   | Hebei Shijiazhuang | 46.93, 7.04 | 46.93, 7.04, 28.11, 20.09, 262.9, 7.72, 186.53, 27.22, 69.55, 656.59 |
| 4   | Hebei Anguo | 54.22, 5.26  | 54.22, 5.26, 48.55, 27.84, 386.4, 10.15, 256.42, 34.36, 102.82, 926.02 |
| 5   | Liaoqing Chaoyang | 40.63, 7.21 | 40.63, 7.21, 42.74, 24.85, 342.4, 9.06, 216.54, 30.49, 85.74, 799.66 |
| 6   | Liaoqing Laiyang | 41.63, 7.14 | 41.63, 7.14, 42.97, 24.92, 344.2, 9.10, 214.46, 31.26, 86.11, 801.79 |
| 7   | Shandong Linyi | 52.26, 8.80  | 52.26, 8.80, 52.28, 30.02, 376.7, 9.65, 259.42, 35.21, 98.62, 922.96 |
| 8   | Shandong Jining | 46.68, 7.13  | 46.68, 7.13, 46.63, 26.91, 369.9, 9.49, 236.71, 32.33, 93.30, 869.08 |
| 9   | Shandong Laiwu | 65.80, 9.34  | 65.80, 9.34, 51.47, 32.21, 405.1, 11.52, 264.29, 41.08, 100.70, 981.51 |
| 10  | Shandong Jining | 65.96, 9.69  | 65.96, 9.69, 52.49, 31.78, 406.8, 11.41, 270.43, 44.02, 109.51, 1002.09 |
samples (Xingtai, Zhanhua and Shijiazhuang) from Hebei province were with the lower content, not more than 700 μg/g. Many reasons may explain the content variation of analyzed samples, such as the occasionality of detected samples, geographical environment of raw herbal materials, frying process and storage conditions.

4. Conclusions
In this study, an improved and comprehensive method for the QC of SZS standard decoction based on the analysis of multi-components was developed using UPLC coupled with MS and PDA methods. UPLC-MS was a powerful driving force for identification of various constituents in SZS standard decoction. Moreover, quantitative analysis was performed with UPLC-PDA, which allows the simultaneous analysis of the multi-components of SZS standard decoction based on the analysis of multi-components of SZS standard decoction. Based on the above, the method for analysis of SZS standard decoction defined in this study was extensively used for the quality control of SZS standard decoction or other dosage forms.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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