

# Qualitative and quantitative determination of 15 main active constituents in *Fructus Sophorae pill* by liquid chromatography tandem mass spectrometry

Xu-ran Zhi<sup>1,2</sup>, Zhi-yong Zhang<sup>1</sup>, Pei-pei Jia<sup>1</sup>, Xiao-xu Zhang<sup>1</sup>, Lin Yuan<sup>1</sup>, Ning Sheng<sup>1</sup>, Lan-tong Zhang<sup>1</sup>

<sup>1</sup>Department of Pharmaceutical Analysis, School of Pharmacy, Hebei Medical University, 361 East Zhongshan Road, Shijiazhuang, Hebei 050017, <sup>2</sup>Department of Pharmacy, Hebei General Hospital, Shijiazhuang, Hebei 050051, PR China

Submitted: 26-01-2014

Revised: 27-03-2014

Published: 21-01-2015

## ABSTRACT

**Background:** *Fructus Sophorae pill*, one of the traditional Chinese medicine, was widely used for hemorrhoids, hypertension and odontalgia. This paper describes a sensitive and specific assay for the determination of the 15 active constituents (sophoricoside, genistin, genistein, rutin, quercetin, kaempferol, baicalein, baicalin, naringin, naringenin, hesperidin, neohesperidin, wogonin and cimifugin, prim-O-glucosylcimifugin) in *Fructus Sophorae pill*. **Materials and Methods:** Chromatographic separation was performed on a C<sub>18</sub> column with acidified aqueous methanol gradients at a flow rate of 0.8 mL/min. The identification and quantification of the analytes were achieved by use of a hybrid quadrupole linear ion trap mass spectrometer. Multiple-reaction monitoring scanning was applied to quantification with switching electrospray ion source polarity between positive and negative modes. **Results:** The proposed method was used to analyze 40 batches of samples with good linearity (r, 0.9990-0.9999), intraday precisions (RSD, 0.14-2.55%), interday precisions (RSD, 0.51-2.81%), stability (RSD, 0.31-2.65%), and recovery (RSD, 1.29-2.95%) of the 15 compounds. In addition, the hierarchical cluster analysis, including a method called furthest neighbor and nearest neighbor, was employed to classify samples according to characteristics of the 15 constituents. **Conclusion:** The results indicated that the analytical method was rapid, reliable, simple and suitable for the quality evaluation of *Fructus Sophorae pill*.

**Key words:** Dosage form, *Fructus Sophorae pill*, Hierarchical cluster analysis, HPLC-ESI-MS/MS, Qualitative and quantitative determination

## INTRODUCTION

Traditional Chinese medicine (TCM) is receiving more and more attention in the world due to its accurately clinical practice. *Fructus Sophorae pill* is an ancient TCM widely used all over the world, which is prepared from *Fructus Sophorae* (processing with stir-frying), charred *Radix Sanguisorbae*, *Radix Scutellariae Baicalensis*, *Fructus Aurantii Submaturus* (processing with stir-frying), *Radix Angelicae Sinensis*, and *Radix Saposhnikovia*.<sup>[1]</sup> Modern pharmacological and clinical studies have shown that some components in *Fructus Sophorae pill* possessed hemostatic properties, anticancer, anti-obesity, anti-hypertension

and it is widely used for hemorrhoids, hypertension and odontalgia.<sup>[2,3]</sup> In Chinese medicines, some active constituents were usually regarded as index component for quality control of these Chinese prescriptions.<sup>[4]</sup> Up to now, baicalin was regarded as the index compound for the quality control of *Fructus Sophorae pill* which had been recorded in Chinese Pharmacopoeia.<sup>[1]</sup> The determination of major active components in *Fructus Sophorae pill* had been reported in several published papers, such as sophoricoside, genistein, quercetin, naringin and wogonin, using analytical methods including high performance liquid chromatography (HPLC),<sup>[5-7]</sup> high performance capillary electrophoresis (HPCE)<sup>[8,9]</sup> and thin layer chromatography (TLC) scanning.<sup>[10]</sup> However, these methods suffered from long run time, low resolution and sensitivity and few analytes. Quality control is one of the most important problems for the development and application of TCM, and manufacturers

### Address for correspondence:

Dr. Lantong Zhang, Department of Pharmaceutical Analysis, School of Pharmacy, Hebei Medical University, 361 East Zhongshan Road, Shijiazhuang 050017, PR China. E-mail: zhanglantong@263.net

### Access this article online

#### Website:

www.phcog.com

#### DOI:

10.4103/0973-1296.149739

#### Quick Response Code:



have played a particular consideration on its safety and efficacy.<sup>[11,12]</sup> The effect of a TCM always results from the synergy of multiple components, but the conventional quality control methods cannot represent the real quality of herb medicine. Because the methods are the simply quantitative analysis of a few or one component. So quantitative analysis of multiple components is becoming more and more indispensable for quality evaluation of TCM. Meanwhile, an increasing number of researchers have begun to use this technology in TCM, especially for Chinese prescriptions.<sup>[13-15]</sup> In order to further apply TCM and enhance the clinical safety, an reliable and accurate method based on the multiple constituents is needed to develop for quality evaluation of *Fructus Sophorae pill*. HPLC-MS/MS is a selective and sensitive detection method that can detect the trace amounts of constituents. It cannot only identify all the peaks of the analytes by comparison of precursor and production ions with standards, retention times but also provide adequate structural information.

Nowadays, we first validated and developed a accurate and simple HPLC-MS/MS method for simultaneous analysis of 15 main active constituents in *Fructus Sophorae pill*, including sophoricoside, genistein, genistin, quercetin, rutin and kaempferol in *Fructus Sophorae*,<sup>[16,17]</sup> baicalein, baicalin, wogonin in *Radix Scutellariae Baicalensis*,<sup>[18,19]</sup> naringin, naringenin, hesperidin, neohesperidin in *Fructus Aurantii Submaturus*,<sup>[20,21]</sup> and cimifugin, prim-O-glucosylcimifugin in *Radix Saposnikoviae*.<sup>[22]</sup> Their structures are listed in Figure 1. With the development of this method, due to the high selectivity of the multiple-reaction monitoring (MRM) mode, optimization of chromatographic separation and the sample pre-processing are greatly simplified. In addition, the application of the parent and product ions monitoring are expected to identification of known molecules and increase the specificity of detection.<sup>[23]</sup> In order to reduce the analysis time, an electrospray ionization source was operated in positive and negative mode at the same time. To the best of our knowledge, the new method provides the best specificity and sensitivity for determination of 15 active constituents in *Fructus Sophorae pill*. In addition, *Fructus Sophorae pill* involved two dosage forms, including honeyed pills and water-honeyed pills, which were acquired from different pharmaceutical companies in China. It is necessary to comprehensively control the quality of *Fructus Sophorae pill* with an effective statistical method. Thus, 40 batches of *Fructus Sophorae pill* were first detected by the developed method combined with hierarchical clustering analysis (HCA) to illustrate the difference among various samples and find unqualified manufacture corporation, and ensure its safety and efficacy.

## MATERIALS AND METHODS

### Chemicals and materials

HPLC-grade methanol was provided by J. T. Baker (Philipsburg, USA). Purified water was purchased from Wahaha (Hangzhou Wahaha Group Co., Ltd.). Analytical-grade methanol, used for sample preparation, was obtained from Tianjin Chemical (Tianjin, China). HPLC-grade formic acid was provided by Dikma Technology Corporation (Lake Forest, USA).

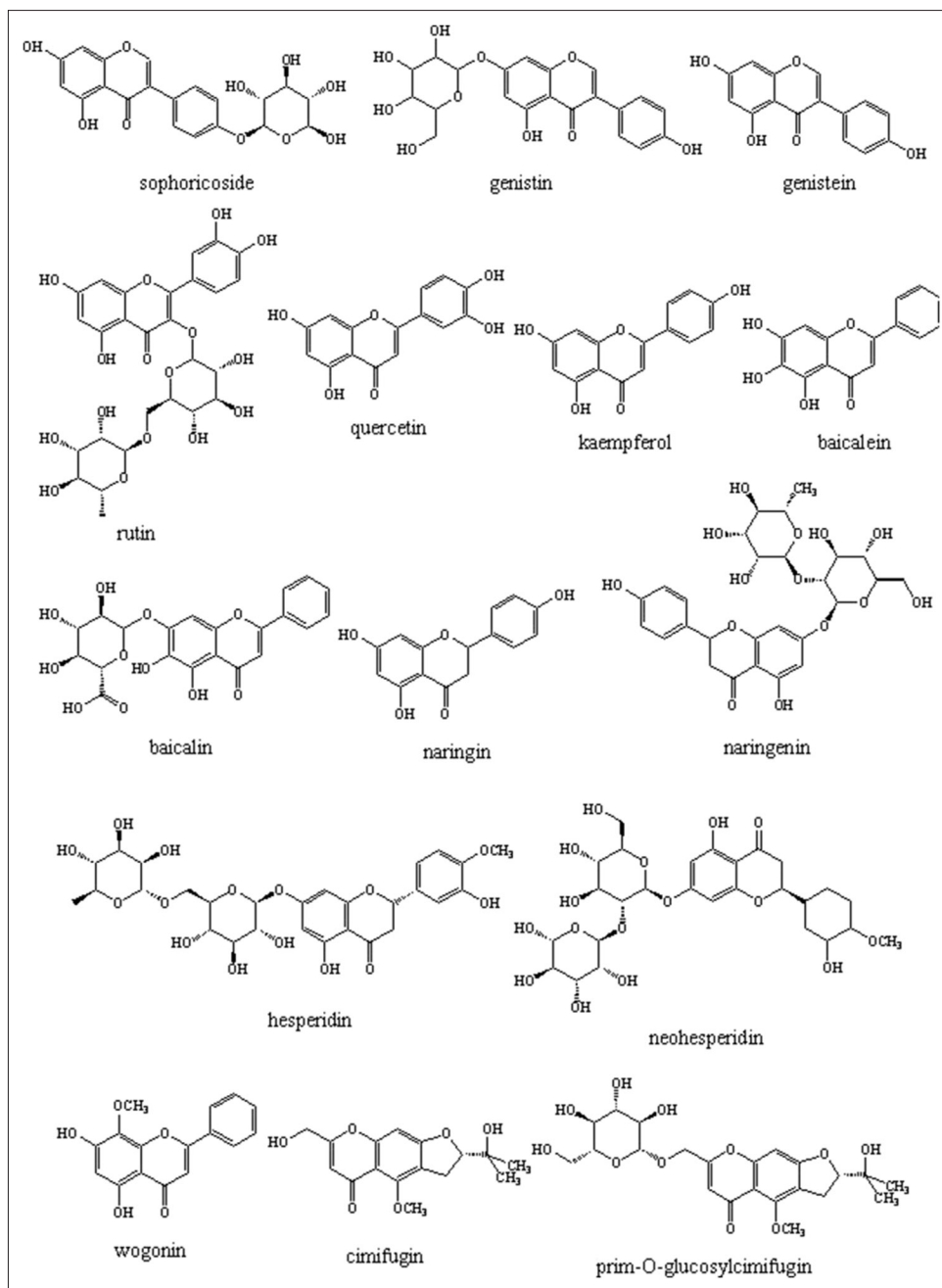
Sophoricoside (11061521), genistein (11012521), genistin (11080316) and kaempferol (11042524) were provided by Shanghai Tauto Biotech Co., Ltd., China. Quercetin (100081-200406), rutin (100080-200707), and baicalin (715-200010) were purchased from National Institute for the Control of Pharmaceutical and Biological Products. The standards including baicalein (FY12970712), wogonin (12930717), naringin (FY17910711), naringenin (FY17900719), hesperidin (FY11290618), neohesperidin (FY11300518), cimifugin (FY16230608), prim-O-glucosylcimifugin (FY16220622) were provided by Nantong Feitu Biological Technology Co., Ltd. The purities of the above components were more than 98% according to HPLC analysis.

The 40 batches of *Fructus Sophorae pill* were obtained from different manufacturers in China: Taifu Pharmaceutical Co., Ltd. (samples 1-10), Taiji Pharmaceutical Co., Ltd. (samples 11-18), Wanghe Pharmaceutical Co., Ltd. (samples 19-20), Huakang Pharmaceutical Co., Ltd. (samples 21), Daren Tang Pharmaceutical Co., Ltd. (samples 22-27), Puji Tang Pharmaceutical Co., Ltd. (samples 28-32), Sanjiu Pharmaceutical Co., Ltd. (samples 33-35), Shuangren Pharmaceutical Co., Ltd. (samples 36-38), Pingguang Pharmaceutical Co., Ltd. (samples 39-40). These samples referred to two dosage forms, including 21 batches of water-honeyed pills (marked as samples 1-21) and 19 batches of honeyed pills (marked as samples 22-40).

### Standard solutions and sample preparation

The appropriate amount of standards were accurately weighed and dissolved in methanol as 15 components of stock solutions, respectively. In order to make the calibration curve, stock solutions were diluted to provide a series of standard solutions with gradient concentration. We also prepared the mixture of the standards stock solution. All the solutions were kept in a refrigerator at 4°C for analysis.

All the samples were cut into pieces and milled into the homogeneous size. 0.5 g pulverized powder was accurately



**Figure 1:** The chemical structures of the 15 compounds in *Fructus Sophorae pill*

weighed and ultrasonically extracted (30 min) with 30 mL 75% methanol in a conical flask. And the extracted solution cooled at room temperature, and then was adjusted to the original weight by adding 75% methanol. Before HPLC injection, the supernatant was filtered by a 0.45  $\mu\text{m}$  microporous membrane.

#### Instrumentation and analytical conditions

For quantitative analysis of the 15 components, all experiments were executed in positive and negative mode at the same time by using an Agilent Series 1200 liquid chromatograph (Agilent, USA) equipped with a binary

pump, an autosampler and a vacuum degasser, connected to a 3200 QTRAP™ system (Applied Biosystems, Foster City, CA, USA) from Applied Biosystems/MDS Sciex, TurboIonspray interface and a hybrid triple quadrupole linear ion trap mass spectrometer equipped with TurboV sources.

The chromatographic separation was carried out at 30°C on a Diamonsil C<sub>18</sub> column (150 × 4.6 mm, 5  $\mu\text{m}$ ). The mobile phase consisted of A (methanol) and B (0.3% aqueous formic acid). The elution program was optimized as follows: With the range of 0-8 min, 25%-45% A; 8-18 min,

45%-65% A; 18-27 min, 65%-95% A; 27-30 min, and 95%-95% A. Before inject each sample, this was followed by the equilibration period of 6 min. The flow rate of mobile phase was kept at 0.8 mL/min. And the injection volume was 10  $\mu$ L.

The operating conditions for the ESI interface were as follows: The turbo spray temperature was 650°C; the ion spray voltage was set to 5500-4500 V, respectively; the curtain gas was set at 25 arbitrary units; nebuliser gas (gas 1) and heater gas (gas 2) were kept at 60 and 65 arbitrary units, respectively. Entrance potential (EP) and Collision cell exit potential (CXP) were set at 10.0/-10.0 V and 5.0/-5.0 V, respectively. The full-scan mass covered the range from  $m/z$  100 to 1000. Declustering potential (DP), collision energy (CE) and the precursor-to-product ion pairs, for each component are listed in Table 1. Other parameters were also optimized for maximum abundance of the ion of interest by the automatic tuning procedure of the instrument. All results were synchronized and controlled by Analyst software (Versions 1.5.2, Applied Biosystems/MDS Sciex).

#### Method validation

We utilized an external standard method for the quantification. The standard solution was applied to generate a series of concentrations by diluting with methanol. The calibration curves consisted of six different concentrations for each reference analyte in triplicate. The linearity of each calibration curves was constructed by plotting the peak areas of the reference analytes. LOQ and LOD under the chromatographic conditions used were separately determined at S/N of 10 and 3, respectively. For each target component, the stock solutions mentioned above were diluted with methanol to a series of appropriate concentrations. And an

aliquot of the diluted solutions was injected into HPLC for determination to analyse the LOQ and LOD.

Determining the precision of the method utilized measurement of intra- and inter-day variability. Measurement of intra-day precision was performed in six replications prepared from the *Fructus Sophorae pill* sample 22 on the same day, while the inter-day variability was performed over three consecutive days. The relative standard deviation (RSD) was taken as a measurement of precision. To evaluate sample stability, the sample solution was tested at room temperature and analyzed in triplicate every 8 h within 48 h.

Accuracy of the method was evaluated by spiking the 15 compounds with three different concentration levels (low, middle and high) to the known amounts of *Fructus Sophorae pill*. Then the resultant samples were analyzed and extracted with the method. Average recoveries were evaluated via the following equation: Recovery (%) = (amount found – original amount)/amount spiked  $\times$  100%, and RSD (%) = (SD/mean)  $\times$  100%.

Matrix effects are an inherent aspect of electrospray ionization because matrix components can analyte ionization efficiency (competing ions) and affect the properties of the droplets (e.g. viscosity changes).<sup>[24]</sup> In this study, sample 22 was operated as described in “sample preparation”, then 15 mL of the extract was spiked with a one-fold mixed standard solution of the 15 compounds at three concentration levels (low, middle and high). And another 15 mL of the extract was diluted one-fold with 75% methanol. Each level prepared triplicate samples.

**Table 1: HPLC-ESI-MS<sup>n</sup> data of the 15 components from *Fructus Sophorae pill***

Compounds	MW	t <sub>R</sub> (min)	Ionization mode	MS <sub>1</sub> (m/z)	MS <sub>2</sub> (m/z)	DP (V)	CE (eV)
Sophoricoside	432.27	16.87	ESI-	431.2	268.0	-86	-45
Genistin	432.27	14.28	ESI-	431.1	268.2	-85	-44
Genistein	270.24	22.81	ESI-	268.7	133.0	-65	-44
Rutin	610.51	16.63	ESI-	609.3	301.0	-71	-50
Quercetin	302.33	21.75	ESI-	301.0	150.9	-55	-32
Kaempferol	286.23	24.20	ESI-	284.6	92.9	-72	-53
Baicalin	270.24	24.23	ESI-	269.3	65.1	-54	-62
Baicalin	446.37	19.52	ESI-	445.4	268.7	-70	-34
Naringin	580.53	15.05	ESI-	579.3	150.9	-81	-54
Naringenin	272.25	21.56	ESI-	271.2	150.9	-63	-25
Hesperidin	610.56	15.14	ESI-	609.4	301.2	-56	-37
Neohesperidin	610.56	15.68	ESI-	609.5	301.1	-80	-43
Wogonin	284.26	26.48	ESI+	285.2	270.1	55	34
Cimifugin	306.31	15.45	ESI+	307.1	259.2	71	41
Prim-O-glucosylcimifugin	468.45	12.62	ESI+	469.4	307.2	70	43

HPLC: High performance liquid chromatography; ESI: Electrospray ionization

Matrix effect was determined via the following equation:

$$\text{Matrix effect (\%)} = (A - B)/C \times 100\%$$

A: The peak area of the analyte in the spiked sample matrix.  
B: The peak area of the analyte in the unspiked sample matrix.  
C: The peak area of the standard solutions in 75% methanol at the same concentrations.

### Chemometric data analysis

In this study, HCA, including a method called furthest neighbor and nearest neighbor, was employed to classify samples according to characteristics of the 15 constituents. And HCA of 40 samples were performed by using SPSS software (SPSS Statistics 19, SPSS Inc., USA).

## RESULTS AND DISCUSSION

### Optimization of the extraction conditions

A variety of times, solvents and extraction methods were determined to acquire the best extraction efficiency. Ultrasonic bath extraction has advantages to refluxing extraction, such as less solvent, rapidness and convenience. And it is a commonly used extraction method for quantitative determination of TCM. Therefore, extraction method chosen ultrasonic bath extraction. To assess the optimal duration of extraction and solvent ratio, a comparative study on different water-methanol mixtures containing 25%, 50%, 75% and 100% (v/v) methanol was performed with ultrasonic bath extraction. The results indicated that 75% methanol showed the highest extraction efficiency and most suitable. To screen optimal extraction time, the samples were extracted with 75% methanol for 15, 30, 45 and 60 min. The results showed that 15 analytes were completely extracted within 30 min. Finally, extraction with 75% methanol in an ultrasonic bath for 30 min was adopted.

### Optimization of the chromatographic and mass spectrometric conditions

The precursor ions and product ions of the 15 reference analytes were clearly obtained in MS1 (Q1) and MS2 (product ion) mode by infusing individual standard solutions at a flow rate of 10  $\mu\text{L}/\text{min}$ . The conditions of mass spectral were achieved in both positive and negative mode in a single run, and the ionization of the wogonin, cimifugin and prim-O-glucosylcimifugin responded much better in positive ion mode than negative ion mode, whereas the others was more efficient in negative ion mode. For this reason, the mass spectral conditions were optimized in positive- and negative-ion modes at the same time. To get the richest relative abundance of

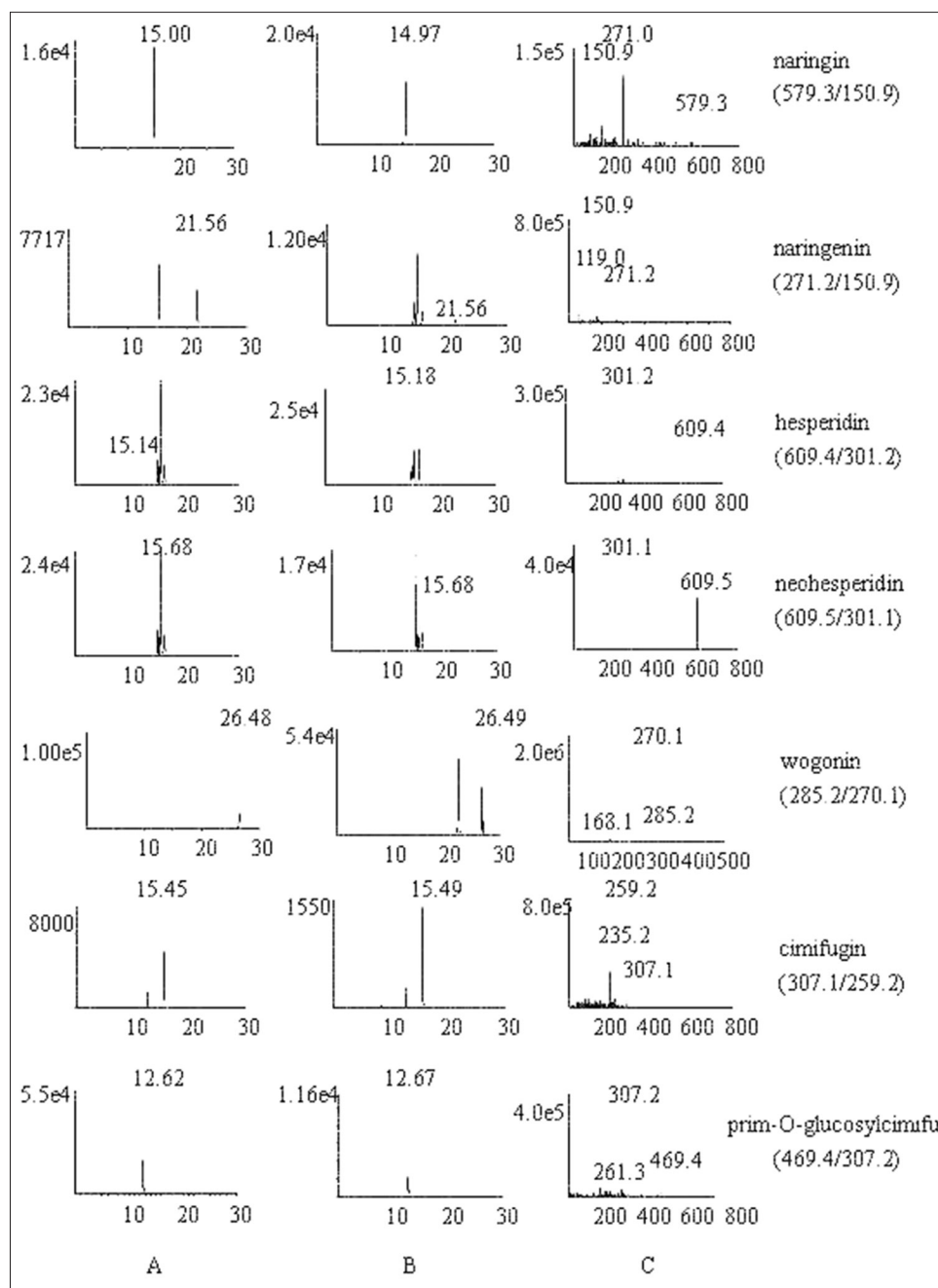
precursor and product ions and obtain the maximum sensitivity of  $[\text{M}-\text{H}]^-$ ,  $[\text{M} + \text{H}]^+$ , the parameters of CE and DP were optimized. The highest peak intensity was the base for selecting the ions used for quantitative analysis.

On the analytes, neohesperidin and hesperidin are isomers with identical product ion of 301.0.<sup>[25]</sup> Genistein and sophoricoside are structural isomers, then genistein is the hydrolysis product of them.<sup>[26]</sup> Therefore, the optimization of the LC conditions was the important key to achieving the better resolution of adjacent peaks. Different mobile phase compositions were optimized in view of obtaining shorter analysis time, higher peak responses and good peak shape of target analytes in chromatograms. acetonitrile-water and methanol-water were compared, but no obvious differences were observed. Because of the high-price and high-toxicity of acetonitrile, methanol-water was selected. The effect of different buffers was tested, including ammonium acetate (0.5, 1, and 2 mmol/L), acetic acid (0.1 ‰, 0.3‰, 0.5 ‰, and 1‰), and formic acid (0.1‰, 0.3‰, 0.5‰, and 1‰). The results indicated that formic acid could eliminate the peak tailing of the target analytes and more enhance the resolution by comparing with ammonium acetate and acetic acid. And the mobile phase with 0.3‰ formic acid could improve the ionization efficiency. In addition, other chromatographic conditions were also evaluated, including flow rates (0.8 and 1.0 mL/min), column temperatures (25°C and 30°C) and analytical columns (Sapphire C<sub>18</sub> column and Diamonsil C<sub>18</sub> column). Eventually, the optimal condition of the Diamonsil C<sub>18</sub> column (150 × 4.6 mm, 5  $\mu\text{m}$ ) with the flow rate of 0.8 mL/min at the column temperature of 30°C was beneficial for improving the ionization of analytes detected mode and could reproduce retention time and guarantee sharp peak shape. The typical extract ions chromatograms (XIC) of MRM chromatograms of standards and sample are shown in Figure 2.

### Analytical method validation

The linear regression results indicate good linear correlation. The correlation coefficients (r) for all the analytes in the concentration range were >0.9990. LOQ and LOD expressed by 10- and 3-fold of the ratio of the S/N were also obtained, respectively. Detailed information regarding linear ranges, calibration curves, LOQ and LOD is listed in Table 2.

The overall interday and intraday precisions for the targeted analytes were less than 2.81% and 2.55%, respectively. The average recovery was in the range of



**Figure 2:** Representative extraction chromatograms (XIC) of multiple-reaction monitoring (MRM) chromatograms of sophoricoside, genistin, genistein, rutin, quercetin, kaempferol, baicalein, baicalin, wogonin, naringin, naringenin, hesperidin, neohesperidin, cimifugin and prim-O-glucosylcimifugin (A) standards; (B) samples; (C) monitored MRM transitions of 15 standards

97.25%-102.8% with RSD ranging from 1.29% to 2.95% characterizing good reliability and accuracy of the method. The detailed data are given in Table 3. When the solution was kept at room temperature, all investigated components were stable within 48 h (RSD < 2.65%). The matrix effect results indicated that the method is no significant matrix effect. These results showed that the established assay was a useful and reliable method for evaluation of the quality of *Fructus Sophorae pill*.

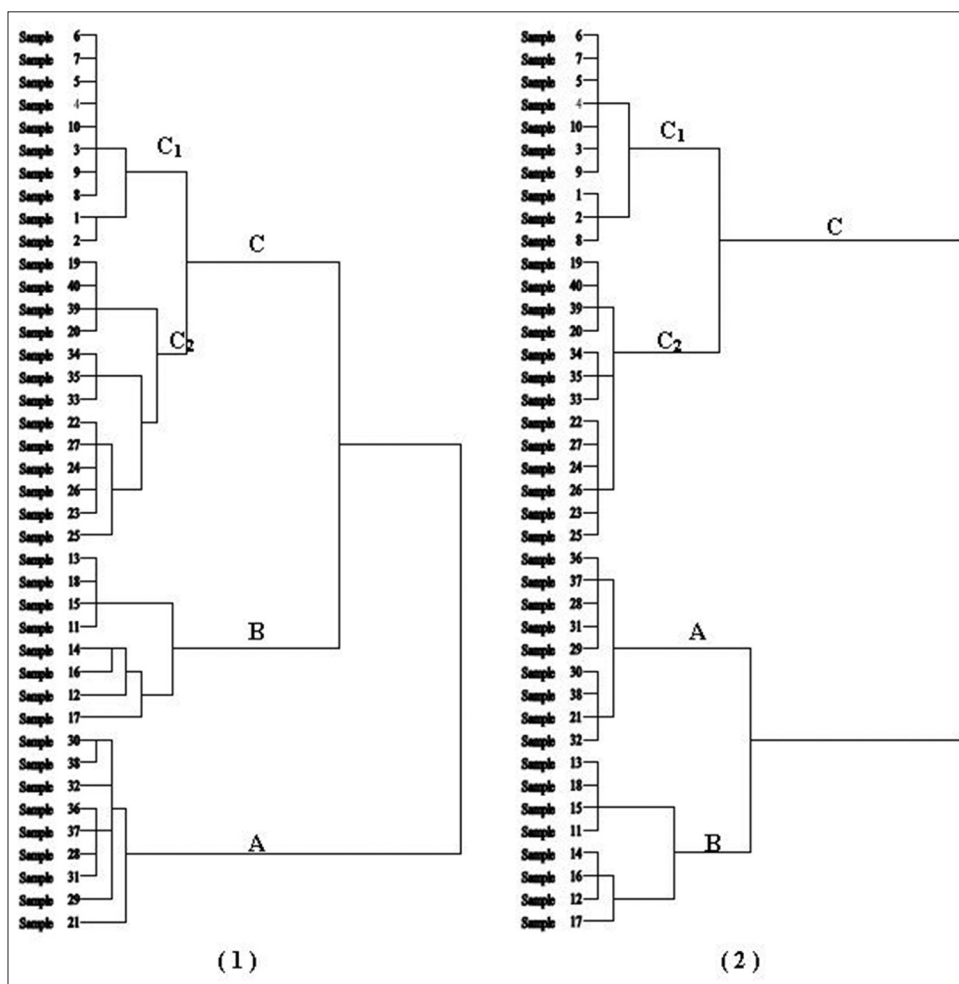
### Quantitative analysis of samples

The developed analytical method was subsequently applied for the determination of 15 investigated analytes in 40 batches of *Fructus Sophorae pill*. The analysis time was reduced to 30 min in a single run by switching the ion source polarity between positive and negative modes. In addition, MRM scanning mode provided good sensitivity because it accordingly enhanced the response of analytes and significantly reduced the noise levels.

**Table 2: Calibration curves, linear ranges, LOD and LOQ of 15 markers**

Compound	Regression equation	r	Linear range (µg/mL)	LOD (ng/mL)	LOQ (ng/mL)
Sophoricoside	$Y=2.89e^4 X-8.23e^3$	0.9995	1.226-38.70	4.085	12.26
Genistin	$Y=7.15e^4 X-7.87e^3$	0.9990	0.2185-6.900	0.6243	1.824
Genistein	$Y=3.14e^5 X-7.41e^3$	0.9994	0.0546-1.725	0.2185	0.6070
Rutin	$Y=4.53e^4 X-4.19e^3$	0.9994	0.2850-9.000	0.8906	1.900
Quercetin	$Y=4.34e^5 X-1.09e^3$	0.9998	0.0051-0.4100	0.2540	0.6350
Kaempferol	$Y=8.73e^4 X-4.69e^2$	0.9999	0.0100-1.920	0.0775	0.1664
Baicalein	$Y=9.77e^4 X-3.17e^3$	0.9994	0.3100-9.789	1.240	3.875
Baicalin	$Y=1.71e^4 X-2.33e^4$	0.9992	2.137-67.47	5.340	10.68
Naringin	$Y=3.44e^4 X+1.06e^3$	0.9993	0.0560-10.80	0.1867	0.6222
Naringenin	$Y=4.94e^5 X-1.04e^3$	0.9998	0.0054-0.1720	0.2700	0.7714
Hesperidin	$Y=2.64e^4 X+6.27e^3$	0.9992	0.1607-30.90	0.7652	2.009
Neohesperidin	$Y=2.93e^4 X+4.18e^3$	0.9997	0.5225-16.50	3.483	10.45
Wogonin	$Y=4.87e^5 X+9.72e^4$	0.9991	0.1977-6.244	0.9885	2.197
Cimifugin	$Y=1.17e^5 X+2.16e^3$	0.9994	0.0098-0.3108	0.9842	3.281
Prim-O-glucosylcimifugin	$Y=2.74e^5 X+5.82e^3$	0.9992	0.0486-1.536	1.216	4.053

LOD: Limit of detection (S/N=3); LOQ: Limit of quantification (S/N=10)



**Figure 3:** Dendrograms of hierarchical clustering for the 40 tested samples of *Fructus Sophorae pill* and its confused plants using nearest neighbor (1) and furthest neighbor (2) methods

Therefore, some minor components in *Fructus Sophorae pill* could also be accurately checked out. The calibration curves were used for the quantitative determination

of 15 compounds. The detailed contents are listed in Table 4. It is found that the total contents of samples in the range of 17.68-42.50 mg/g. It demonstrated

**Table 3: Intra- and inter-assay and accuracy of the 15 active components**

Compound	Precision (n=6)		Accuracy (n=3)					Stability 48h, n=3 RSD%
	Intraday RSD%	Interday RSD%	Original (µg)	Spiked (µg)	Found (µg)	Recovery (%)	RSD%	
Sophoricoside	1.86	1.74	4701.98	3785.09	8453.76	99.12	1.75	0.62
				4890.06	9496.19	98.04	2.24	
				5736.41	10507.80	101.2	1.69	
Genistin	1.52	1.89	45.50	37.35	82.68	99.56	2.34	1.75
				49.14	93.99	98.69	2.53	
				57.78	103.25	99.96	1.39	
Genistein	0.84	0.96	20.17	15.94	35.70	97.44	1.46	0.82
				20.10	40.17	99.52	2.84	
				24.09	44.46	100.8	2.08	
Rutin	0.43	0.53	250.93	206.52	455.08	98.85	1.93	1.05
				256.20	503.01	98.39	1.81	
				316.18	574.51	102.3	1.69	
Quercetin	2.55	2.48	4.60	3.87	8.49	100.5	1.71	1.20
				4.81	9.36	99.05	2.07	
				5.68	10.18	98.24	1.48	
Kaempferol	2.19	2.50	8.44	7.19	15.54	98.62	2.11	2.65
				8.57	16.92	99.01	1.89	
				10.23	18.76	100.8	2.21	
Baicalein	1.67	1.95	105.76	85.35	190.14	98.87	1.59	0.58
				111.68	215.98	98.69	1.41	
				127.12	232.34	99.57	2.74	
Baicalin	0.14	0.64	1416.58	1147.43	2580.75	101.5	2.01	1.41
				1432.16	2828.68	98.60	2.62	
				1706.97	3116.89	99.61	1.63	
Naringin	0.31	0.51	170.57	137.82	312.19	102.8	1.85	0.31
				173.98	341.30	98.13	1.92	
				206.22	372.75	98.04	2.95	
Naringenin	2.02	2.28	2.19	1.78	3.95	99.18	1.77	1.75
				2.23	4.39	98.54	2.05	
				2.63	4.74	97.25	1.97	
Hesperidin	1.15	1.29	658.79	540.21	1191.60	98.63	1.48	0.68
				662.75	1312.13	98.58	2.16	
				793.19	1469.19	102.8	2.54	
Neohesperidin	2.17	2.81	287.84	233.44	518.97	99.01	1.86	2.34
				294.17	586.69	101.6	1.93	
				349.44	634.31	99.15	1.29	
Wogonin	0.76	0.85	63.08	50.46	112.78	98.49	1.96	0.85
				65.10	127.47	98.91	2.18	
				76.45	137.62	97.50	2.64	
Cimifugin	1.87	1.59	4.89	4.01	8.93	100.8	1.88	1.18
				5.09	9.90	98.53	2.42	
				5.89	10.70	98.71	1.76	
Prim-O-glucosylcimifugin	1.32	1.47	21.67	17.87	39.37	99.05	1.84	1.12
				21.69	43.54	100.8	2.75	
				26.22	47.59	98.88	1.87	

RSD: Relative standard deviation

that *Fructus Sophorae pill* samples were obviously different. Samples 17, 32, and 21 had the highest total contents (42.50, 41.97 and 41.33 mg/g), while samples 3, 9, and 5 had the lowest total contents (17.68, 18.16 and 18.38 mg/g). By dealing with the data, the mean value of the total contents of 40 batches samples was 29.62 mg/g. Among them, sophoricoside was the

highest investigated components, and the mean content was 16.08 mg/g; secondly, baicalin was 7.01 mg/g and follow by hesperidin at 2.48 mg/g. On the other hand, the contents of naringenin, quercetin, and cimifugin were relatively low. Neohesperidin was not detected in samples 1-18, 33-35 and 39-40. Meanwhile, multiple active components, including micro and macro



Table 4: Contents of the 15 active components in *Fructus Sophorae pill*

Sample no.	Content of each compound in 40 samples (µg/g)															Total	
	Sophoricoside	Genistin	Genistein	Rutin	Quercetin	Kaempferol	Baicalin	Baicalein	Baicalin	Naringin	Naringenin	Hesperidin	Neohesperidin	Wogonin	Cimifugin		Prim- o- glucosylcimifugin
1	1.46×10 <sup>4</sup>	208.50	146.59	799.33	15.37	59.25	556.88	2.83×10 <sup>3</sup>	47.23	8.38	3.60×10 <sup>3</sup>	-	-	393.54	23.64	77.10	2.33×10 <sup>4</sup>
2	1.44×10 <sup>4</sup>	230.08	168.35	856.02	19.32	61.56	503.48	2.84×10 <sup>3</sup>	48.67	9.58	3.44×10 <sup>3</sup>	-	-	288.26	21.64	70.03	2.29×10 <sup>4</sup>
3	1.02×10 <sup>4</sup>	204.41	107.47	677.50	12.29	54.00	466.77	3.06×10 <sup>3</sup>	40.67	6.97	2.38×10 <sup>3</sup>	-	-	315.57	28.34	69.06	1.77×10 <sup>4</sup>
4	1.16×10 <sup>4</sup>	188.63	130.47	741.32	14.23	57.14	490.15	3.04×10 <sup>3</sup>	45.48	8.11	2.94×10 <sup>3</sup>	-	-	349.42	17.89	57.03	1.97×10 <sup>4</sup>
5	1.09×10 <sup>4</sup>	136.80	118.21	696.19	12.33	55.24	503.29	3.03×10 <sup>3</sup>	38.48	6.53	2.54×10 <sup>3</sup>	-	-	258.41	15.14	50.73	1.84×10 <sup>4</sup>
6	1.11×10 <sup>4</sup>	185.67	125.14	731.57	12.42	53.01	515.01	2.91×10 <sup>3</sup>	43.63	7.92	3.01×10 <sup>3</sup>	-	-	381.87	15.92	66.20	1.92×10 <sup>4</sup>
7	1.10×10 <sup>4</sup>	232.95	144.66	870.57	18.84	58.45	520.88	2.81×10 <sup>3</sup>	41.30	7.33	2.82×10 <sup>3</sup>	-	-	318.38	18.52	68.92	1.87×10 <sup>4</sup>
8	1.27×10 <sup>4</sup>	162.44	111.46	664.60	12.74	51.96	481.02	2.94×10 <sup>3</sup>	47.13	8.59	3.17×10 <sup>3</sup>	-	-	361.88	19.89	73.73	2.12×10 <sup>4</sup>
9	1.03×10 <sup>4</sup>	193.68	138.09	721.46	13.80	57.14	513.84	3.05×10 <sup>3</sup>	39.97	7.62	2.82×10 <sup>3</sup>	-	-	340.72	18.12	65.99	1.82×10 <sup>4</sup>
10	1.18×10 <sup>4</sup>	224.93	118.58	1.26×10 <sup>3</sup>	14.75	117.31	574.21	2.85×10 <sup>3</sup>	44.13	7.97	2.99×10 <sup>3</sup>	-	-	319.55	19.35	70.38	1.97×10 <sup>4</sup>
11	1.51×10 <sup>4</sup>	291.92	128.30	1.64×10 <sup>3</sup>	17.98	122.75	806.74	1.12×10 <sup>4</sup>	71.14	12.63	5.51×10 <sup>3</sup>	-	-	497.11	51.53	108.44	3.31×10 <sup>4</sup>
12	1.88×10 <sup>4</sup>	254.75	108.60	1.41×10 <sup>3</sup>	11.49	104.60	565.23	1.02×10 <sup>4</sup>	52.32	10.94	4.27×10 <sup>3</sup>	-	-	471.79	48.41	121.78	3.92×10 <sup>4</sup>
13	1.43×10 <sup>4</sup>	235.45	111.89	1.24×10 <sup>3</sup>	14.94	99.34	689.97	1.08×10 <sup>4</sup>	63.17	12.69	4.49×10 <sup>3</sup>	-	-	349.11	36.41	92.27	3.17×10 <sup>4</sup>
14	1.75×10 <sup>4</sup>	258.77	118.67	1.29×10 <sup>3</sup>	12.81	118.42	777.62	1.09×10 <sup>4</sup>	64.34	12.28	4.92×10 <sup>3</sup>	-	-	454.16	37.34	98.57	3.59×10 <sup>4</sup>
15	1.40×10 <sup>4</sup>	275.66	127.27	1.39×10 <sup>3</sup>	16.53	127.63	789.22	1.17×10 <sup>4</sup>	73.19	13.20	5.71×10 <sup>3</sup>	-	-	391.60	41.42	99.20	3.31×10 <sup>4</sup>
16	1.71×10 <sup>4</sup>	227.40	134.59	1.82×10 <sup>3</sup>	19.44	130.27	800.90	1.21×10 <sup>4</sup>	75.36	14.60	6.06×10 <sup>3</sup>	-	-	460.06	45.34	104.45	3.79×10 <sup>4</sup>
17	2.06×10 <sup>4</sup>	238.66	113.54	1.18×10 <sup>3</sup>	14.11	111.18	666.61	1.01×10 <sup>4</sup>	55.32	11.89	4.90×10 <sup>3</sup>	-	-	377.44	56.14	116.41	4.25×10 <sup>4</sup>
18	1.41×10 <sup>4</sup>	185.34	179.36	283.88	21.73	151.14	903.93	4.91×10 <sup>3</sup>	81.17	11.52	111.56	300.89	300.89	402.22	35.28	95.42	3.21×10 <sup>4</sup>
19	1.83×10 <sup>4</sup>	164.76	244.56	310.65	18.39	118.95	835.97	4.49×10 <sup>3</sup>	80.55	13.17	96.09	308.73	308.73	1.16×10 <sup>3</sup>	117.75	113.39	2.68×10 <sup>4</sup>
20	1.73×10 <sup>4</sup>	214.97	100.20	1.22×10 <sup>3</sup>	13.71	57.09	329.44	1.27×10 <sup>4</sup>	2.82×10 <sup>3</sup>	15.74	619.31	174.23	174.23	1.01×10 <sup>3</sup>	104.68	108.88	2.52×10 <sup>4</sup>
21	1.96×10 <sup>4</sup>	171.36	75.98	945.14	7.32	31.80	398.34	5.34×10 <sup>3</sup>	642.46	8.23	2.48×10 <sup>3</sup>	3.31×10 <sup>3</sup>	3.31×10 <sup>3</sup>	174.23	59.43	83.73	4.13×10 <sup>4</sup>
22	1.77×10 <sup>4</sup>	132.82	68.99	876.52	6.64	28.21	411.65	4.24×10 <sup>3</sup>	548.73	6.60	2.99×10 <sup>3</sup>	1.08×10 <sup>3</sup>	1.08×10 <sup>3</sup>	237.59	18.42	81.63	2.92×10 <sup>4</sup>
23	1.68×10 <sup>4</sup>	151.73	64.28	832.34	6.05	28.04	375.98	4.29×10 <sup>3</sup>	742.77	5.86	2.23×10 <sup>3</sup>	986.15	986.15	209.77	13.23	66.53	2.74×10 <sup>4</sup>
24	1.69×10 <sup>4</sup>	138.94	58.82	761.71	5.56	22.61	367.79	4.08×10 <sup>3</sup>	487.12	5.46	1.91×10 <sup>3</sup>	878.47	878.47	195.71	17.70	61.69	2.68×10 <sup>4</sup>
25	1.59×10 <sup>4</sup>	197.31	74.11	818.46	7.37	30.33	429.80	4.99×10 <sup>3</sup>	621.59	7.12	2.08×10 <sup>3</sup>	857.05	857.05	160.50	11.50	57.10	2.48×10 <sup>4</sup>
26	1.71×10 <sup>4</sup>	190.12	85.22	924.41	8.37	33.54	440.33	5.26×10 <sup>3</sup>	639.85	8.67	2.89×10 <sup>3</sup>	1.03×10 <sup>3</sup>	1.03×10 <sup>3</sup>	198.06	15.01	74.01	2.77×10 <sup>4</sup>
27	1.77×10 <sup>4</sup>	183.82	92.15	947.29	5.44	12.03	505.67	1.03×10 <sup>4</sup>	2.56×10 <sup>3</sup>	7.36	575.34	3.86×10 <sup>3</sup>	3.86×10 <sup>3</sup>	214.84	18.70	68.53	2.96×10 <sup>4</sup>
28	1.86×10 <sup>4</sup>	182.40	73.21	1.05×10 <sup>3</sup>	3.15	11.60	469.07	9.90×10 <sup>3</sup>	1.78×10 <sup>3</sup>	6.68	394.29	2.96×10 <sup>3</sup>	2.96×10 <sup>3</sup>	166.93	15.22	62.85	3.80×10 <sup>4</sup>
29	1.77×10 <sup>4</sup>	222.20	98.79	941.46	6.01	17.92	534.72	1.11×10 <sup>4</sup>	2.92×10 <sup>3</sup>	8.53	690.55	3.51×10 <sup>3</sup>	3.51×10 <sup>3</sup>	241.37	22.42	72.83	4.01×10 <sup>4</sup>
30	2.00×10 <sup>4</sup>	168.35	81.38	969.76	3.78	14.28	494.19	1.05×10 <sup>4</sup>	2.79×10 <sup>3</sup>	7.06	497.98	3.17×10 <sup>3</sup>	3.17×10 <sup>3</sup>	161.20	15.80	63.66	3.73×10 <sup>4</sup>
31	1.83×10 <sup>4</sup>	200.35	98.26	938.99	5.21	17.60	538.72	1.12×10 <sup>4</sup>	3.14×10 <sup>3</sup>	8.37	665.15	3.66×10 <sup>3</sup>	3.66×10 <sup>3</sup>	208.16	19.27	71.20	4.20×10 <sup>4</sup>
32	2.12×10 <sup>4</sup>	139.47	75.54	1.08×10 <sup>3</sup>	6.84	35.88	472.99	6.72×10 <sup>3</sup>	49.98	9.90	2.81×10 <sup>3</sup>	-	-	269.94	10.93	30.98	2.84×10 <sup>4</sup>
33	1.67×10 <sup>4</sup>																

Table 4 Contd...

Table 4: Continue...

Sample no.	Content of each compound in 40 samples (µg/g)																
	Sophoricoside	Genistin	Genistein	Rutin	Quercetin	Kaempferol	Baicalin	Baicalein	Baicalin	Naringin	Naringenin	Hesperidin	Neohesperidin	Wogonin	Cimifugin	Prim-o-glucosylcimifugin	Total
34	1.57×10 <sup>4</sup>	138.25	56.63	971.50	5.35	29.37	412.08	5.59×10 <sup>3</sup>	42.96	8.19	2.77×10 <sup>3</sup>	-	-	259.21	7.42	38.89	2.60×10 <sup>4</sup>
35	1.64×10 <sup>4</sup>	156.42	73.17	967.68	6.35	31.35	457.55	5.95×10 <sup>3</sup>	44.47	8.66	2.97×10 <sup>3</sup>	-	-	255.65	9.15	29.20	2.73×10 <sup>4</sup>
36	1.75×10 <sup>4</sup>	209.71	147.41	1.14×10 <sup>3</sup>	3.30	25.11	217.52	1.05×10 <sup>4</sup>	3.04×10 <sup>3</sup>	7.89	255.89	2.93×10 <sup>3</sup>	2.93×10 <sup>3</sup>	131.91	6.31	142.23	3.63×10 <sup>4</sup>
37	1.79×10 <sup>4</sup>	215.34	145.95	1.23×10 <sup>3</sup>	3.78	27.55	271.06	1.08×10 <sup>4</sup>	3.29×10 <sup>3</sup>	8.02	290.78	2.80×10 <sup>3</sup>	2.80×10 <sup>3</sup>	120.11	7.93	139.72	3.72×10 <sup>4</sup>
38	1.95×10 <sup>4</sup>	232.23	150.71	1.20×10 <sup>3</sup>	3.89	30.05	274.00	1.12×10 <sup>4</sup>	3.37×10 <sup>3</sup>	8.62	310.41	3.09×10 <sup>3</sup>	3.09×10 <sup>3</sup>	136.63	9.70	144.54	3.96×10 <sup>4</sup>
39	1.86×10 <sup>4</sup>	254.41	256.95	337.29	32.96	181.52	1.07×10 <sup>3</sup>	4.62×10 <sup>3</sup>	33.36	6.39	663.01	-	-	1.26×10 <sup>3</sup>	40.61	225.31	2.76×10 <sup>4</sup>
40	1.78×10 <sup>4</sup>	252.01	244.23	285.68	30.20	195.89	964.38	4.82×10 <sup>3</sup>	34.69	6.41	678.16	-	-	1.04×10 <sup>3</sup>	36.08	239.90	2.66×10 <sup>4</sup>

-Not available for quantification

components, are frequently considered to be responsible for the therapeutic effects, and therefore the analysis of multiple components is more reasonable for quality evaluation of *Fructus Sophorae pill*. These results indicated that the distinctions of the contents of 15 investigated analytes were apparent in the samples from different companies or even different batches from the same company. Thus, it was a efficient and reliable method as a simultaneous analysis of multiple constituents in *Fructus Sophorae pill*.

HCA is a statistical method for finding relatively homogeneous clusters of cases based on measured characteristics and has been widely utilized to quality control, origin discrimination and species authentication of TCM.<sup>[27,28]</sup> This method begins with each observation being regarded as a separate cluster, and then combines them until all observations group to one cluster. The hierarchical clustering process can be represented as a dendrogram or tree, where each step in the clustering process is illustrated by a connection of the tree.<sup>[29]</sup> In this study, HCA with two methods, furthest neighbor and nearest neighbor were applied to classify the *Fructus Sophorae pill* samples from different companies. The clustering results illustrated are shown in Figure 3. It was found that the two clustering methods can obtain nearly identical data, that is, all the measured samples were falled into three clusters: Sample 21 (Huakang), sample 28-32 (Puji Tang) and sample 36-38 (Shuangren) were in cluster A, sample 11-18 (Taiji) were in cluster B and the other samples were in cluster C, which was further divided into two subgroups (C<sub>1</sub> and C<sub>2</sub>). Sample 1-10 (Taifu) were in subgroup C<sub>1</sub> and the others were in subgroup C<sub>2</sub>, formed by the sample 1-10 (Taifu), sample 19-20 (Wanghe), sample 22-27 (Daren Tang), sample 33-35 (Sanjiu) and sample 39-40 (Pingguang). These samples could be obviously classified to a identical group, no matter what chemometric method is applied. More importantly, this division and distinction clearly demonstrated that chemical profiles from the same company were nearly identical, whereas chemical profiles from different companies produced significant difference. Furthermore, these samples involved two different dosage forms, including water-honeyed pills (samples 1-21) and honeyed pills (samples 22-40). It is demonstrated in Figure 3 that there is no significant variations in terms of content between water-honeyed pills and honeyed pills.

## CONCLUSIONS

A selective, sensitive and rapid HPLC- MS/MS method performing in both positive and negative modes at the

same time has been firstly validated and developed for the separation and quantitative analysis of 15 components of *Fructus Sophorae pill*. The developed method was successfully applied for determination of 40 batches of *Fructus Sophorae pill* from different companies. The results demonstrated that the contents of 15 investigated analytes were distinct from different companies or even different batches from the same company. But chemical profiles were nearly identical in the same company by HCA. The HPLC-MS/MS method offered a good alternative for routine analysis of TCM and could be applied as a reliable quality-control method for *Fructus Sophorae pill*.

## ACKNOWLEDGEMENTS

The work received financial support from the Natural Science Foundation of Hebei Province- CSPC Pharmaceutical Joint Research Fund, China (H2012206079).

## REFERENCES

- Pharmacopoeia Commission of PRC, editors. Pharmacopoeia of the People's Republic of China. Vol. 1. Beijing: China Medical Science Press; 2010. p. 1182-3.
- Kite GC, Veitch NC, Boalch ME, Lewis GP, Leon CJ, Simmonds MS. Flavonol tetraglycosides from fruits of *Styphnolobium japonicum* (Leguminosae) and the authentication of *Fructus Sophorae* and *Flos Sophorae*. *Phytochemistry* 2009;70:785-94.
- Wang HL, Duan FY, Ding JH, Li CE. Research progress of Huaijiao Pills. *Prog Mod Biomed* 2007;7:793-5.
- Yang R, Zeng HJ, Wang QW, Guo C, Li JJ, Qu LB. Simultaneous determination of eight active components in Chinese medicine 'JiangYaBiFeng' tablet by HPLC coupled with diode array detection. *J Pharm Biomed Anal* 2011;55:552-6.
- Wei LX, Wang MD, Zhang Y, Wang G, Xue XQ, Zhao QH. Simultaneous determination of four effective components in Huaijiao Pills by RP-HPLC. *Chin Pharm J* 2011;46:549-52.
- Meng J, Wang JL. Simultaneous determination of sophoricoside, wogonin and naringin in Huaijiao Pills by HPLC. *J Chin Med Mater* 2011;34:638-9.
- Bian QQ, Yang PZ. Determination of genistein and quercetin in Huaijiao Pills by RP-HPLC. *Chin Tradit Pat Med* 2005;27:766-9.
- Gao YS, Dang CG, Li H. Determination of four effective components in Huaijiao Pills by HPCE. *Chin J Pharm* 2007;38:290-2.
- Sun L, Zheng BJ, Suo RZ. HPCE analysis of geulstein in Huaijiao Pills. *Chin J Pharm Anal* 2004;24:361-2.
- Pan JW, Zhang TZ, Zheng LJ. Quantitative determination of genistin and total genistein in the fruit of *Fructus Sophorae* and Huaijiao Pills by TLC scanning method. *Chin J Anal Lab* 2004;23:25-7.
- Li SP, Zhao J, Yang B. Strategies for quality control of Chinese medicines. *J Pharm Biomed Anal* 2011;55:802-9.
- Liang XM, Jin Y, Wang YP, Jin GW, Fu Q, Xiao YS. Qualitative and quantitative analysis in quality control of traditional Chinese medicines. *J Chromatogr A* 2009;1216:2033-44.
- Jiang Y, David B, Tu PF, Barbin Y. Recent analytical approaches in quality control of traditional Chinese medicines. *Anal Chim Acta* 2010;657:9-18.
- Sanchez-Avila N, Priego-Capote F, Ruiz-Jimenez J, Luque de Castro MD. Fast and selective determination of triterpenic compounds in olive leaves by liquid chromatography-tandem mass spectrometry with multiple reaction monitoring after microwave-assisted extraction. *Talanta* 2009;78:40-8.
- Yin LH, Lu BN, Qi Y, Xu LN, Han X, Xu YW, et al. Simultaneous determination of 11 active components in two well-known traditional Chinese medicines by HPLC coupled with diode array detection for quality control. *J Pharm Biomed Anal* 2009;49:1101-8.
- Chang L, Ren YP, Cao L, Sun YG, Sun Q, Zhang LT. Simultaneous determination and pharmacokinetic study of six flavonoids from *Fructus Sophorae* extract in rat plasma by LC-MS/MS. *J Chromatogr B* 2012;904:59-64.
- Wei MH, Wang BJ. The study in the development of the traditional Chinese medicine *Fructus Sophorae*. *Asia Pac Tradit Med* 2010;6:115-9.
- Kim YH, Jeong DW, Paek IB, Ji HY, Kim YC, Lee HS. Liquid chromatography with tandem mass spectrometry for the simultaneous determination of baicalein, baicalin, oroxylin A and wogonin in rat plasma. *J Chromatogr B* 2006;844:261-7.
- Liu WD, Yan LG, Fang Y, Liu MS. Utility of UPLC-ESI-TOF/MS for rapid analysis of the constituents in *Scutellariae Radix*. *Inf Tradit Chin Med* 2012;29:20-4.
- Jia Q, Bai Y, Ma Y, Pang W, Su WW. Chemical constituents of *Fructus Aurantii* and *Fructus Aurantii Immaturus* by HPLC-ESI-MS. *China Tradit Herb Drugs* 2005;36:169-72.
- Li XL, Xiao HB, Liang XM, Shi DZ, Liu JG. LC-MS/MS determination of naringin, hesperidin and neohesperidin in rat serum after orally administrating the decoction of *Bulpleurum falcatum* L. and *Fructus aurantii*. *J Pharm Biomed Anal* 2004;34:159-66.
- Kim MK, Yang DH, Jung M, Jung EH, Eom HY, Han SB. Simultaneous determination of chromones and coumarins in *Radix Saposhnikovia* by high performance liquid chromatography with diode array and tandem mass detectors. *J Chromatogr A* 2011;1218:6319-30.
- Sun DX, Dong LX, Guo PP, Shi XW, Gao J, Wang CY, et al. Simultaneous detection of flavonoids and phenolic acids in *Herba Lysimachiae* and *Herba Desmodii Styracifolii* using liquid chromatography tandem mass spectrometry. *Food Chem* 2013;138:139-47.
- Hyeyoung K, Steven JL, Lucia GA. Variability of matrix effects in liquid and gas chromatography-mass spectrometry analysis of pesticide residues after QuEChERS sample preparation of different food crops. *J Chromatogr A* 2012;1270:235-45.
- Tong L, Zhou DD, Gao J, Zhu YH, Sun H, Bi KS. Simultaneous determination of naringin, hesperidin, neohesperidin, naringenin and hesperetin of *Fructus aurantii* extract in rat plasma by liquid chromatography tandem mass spectrometry. *J Pharm Biomed Anal* 2012;58:58-64.
- Chen CY, Zhou JE, Yan GF, Zhang J, Du N, Lu Y. Metabolism of sophoricoside in homogenized liver of rats. *J Shanghai Jiaotong Univ Med Sci* 2005;25:232-4.
- Kong WJ, Wang JB, Zang QC, Xing XY, Zhao YL, Liu W. Fingerprint-efficacy study of artificial *Calculus bovis* in quality control of Chinese materia medica. *Food Chem* 2011;127:1342-7.
- Qiao S, Shi R, Liu M, Zhang C, Yang W, Wang Q. Simultaneous quantification of flavonoids and phenolic acids in *Herba Scutellariae barbatae* and its confused plants by high performance liquid chromatography-tandem mass spectrometry.

Food Chem 2011;129:1297-304.

29. Zhao Y, Zhou X, Chen HG, Gong XJ, Cai ZW, Zhou CY. Determination of dehydroevodiamine in *Evodia rutaecarpa* (Juss.) Benth by high performance liquid chromatography and classification of the samples by using hierarchical clustering analysis. *Fitoterapia* 2009;80:415-20.

**Cite this article as:** Zhi Xr, Zhang Zy, Jia Pp, Zhang Xx, Yuan L, Sheng N, *et al.* Qualitative and quantitative determination of 15 main active constituents in *Fructus Sophorae* pill by liquid chromatography tandem mass spectrometry. *Phcog Mag* 2015;11:196-207.

**Source of Support:** Nil, **Conflict of Interest:** None declared.