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LC/MS fingerprinting of *Shenmai injection*: A novel approach to quality control of herbal medicines

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Abstract

Chromatographic fingerprinting has been recommended as a potential and reliable strategy for the quality control of herbal medicines. Although varieties of chromatographic techniques, particularly HPLC, have been widely employed, hyphenated chromatographic approach has not been sufficiently exploited in chromatographic fingerprinting. In this work, LC/MS fingerprinting of *Shenmai injection* was developed. Thirty ginsenosides as well as seven ophioponins were selected to construct the LC/MS fingerprint using selective ion monitoring (SIM) mode, while previous HPLC fingerprint [H.J. Zhang, Y.J. Wu, Y.Y. Cheng, J. Pharm. Biomed. Anal. 31 (2003) 175–183] only represents the ginsenosides. Subsequently, the proposed LC/MS fingerprints were applied to identifying the product manufacturers. All the samples were accurately classified based on their LC/MS fingerprints in conjunction with principal components analysis (PCA). This study would be potentially helpful to improve the quality control ability of fingerprinting-based strategy for complex herbal medicines.

Keywords: Chromatographic fingerprinting; LC/MS; *Shenmai injection*; Quality control of herbal medicines; *Radix Ginseng Rubra*; *Radix Ophiopogonis*

1. Introduction

Due to the significant expansion of the use of the alternative medicine including traditional herbal medicines, it is very critical to develop a high standard of quality control in assessing the multi-components mixtures to guarantee their identity, consistency and authenticity [2–5]. Chromatographic fingerprinting, a meaningful quality control method of herbal medicines, has gained rising attentions in recent years [6–9].

After chromatographic fingerprints (chromatograms representing the chemical characteristics of herbal medicines) are acquired, the identity, consistency and authenticity of samples can be determined by comparison of their fingerprints using chemometrics methods. Up to now, varieties of chromatographic techniques including TLC [10], GC [11], HPLC [12–16] and HSCCC [17] have already been employed to develop fingerprints. It is well known that hyphenated chromatographic approaches such as GC/MS, LC/MS and CE/MS could show greatly improved performances in terms of correction of retention time shift, selectivity, chromatographic separation abilities and measurement precision [18]. However, it has not been exploited, and fewer works are reported in chemical fingerprinting [19].

In this study, *Shenmai injection*, derived from traditional Chinese medicine *Shen Mai San* [20] and made from *Radix Ginseng Rubra* and *Radix Ophiopogonis* [21], was investigated as a typical example to develop LC/MS fingerprinting for evaluating the quality of herbal medicine. *Shenmai injection* has been used to treat coronary atherosclerotic cardiopathy and viral myocarditis, and it is also capable of raising tumor patient's immunity. Its main effective components are ginsenosides and ophioponins [1,22]. Currently, the compendial quality control method of *Shenmai injection* is determination of the total content of ginsenosides, which is set to the least 0.8 mg ginsenosides in 1 mL of injection. Apparently, this standard is not sufficient for quality control. In our early researches [1,14,23], we have reported an HPLC fingerprinting approach for evaluating the quality of *Shenmai injection*s such as determining the lot-to-lot consistency and distinguishing the manufacturers. However, the proposed HPLC fingerprint of *Shenmai injection* was very similar with that of Radix Ginseng. Almost all the peaks of the fingerprint were attributed to ginsenosides, which indicated

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the constituents of *Radix Ophiopogonis* were not sufficiently characterized in HPLC fingerprint-based quality control strategy. The present study aims to develop LC/MS fingerprinting method in which ginsenosides as well as ophioponins were chemically represented. The proposed LC/MS fingerprint was applied to identify the different manufacturers of *Shenmai injection*s. This fingerprinting approach is equally applicable to other herbal medicines.

2. Experimental

2.1. Reagents and materials

Acetonitrile and methanol were of HPLC grade from Tedia (Fairfield, USA). Acetic acid and phosphoric acid (A.R. grade) for analysis were purchased from Hangzhou Reagent Company (Hangzhou, PR China). Water was purified by a Milli-Q academic water purification system (Milford, MA).

Standards of ginsenoside Rg_1, Rg_2, Rb_1, Rb_2, Rb_3, Re, Rc and Rh1 were purchased from College of Pharmacy, Jilin University (Changchun, PR China). *Shenmai injection*s were purchased from three different manufacturers in China, respectively. *Radix Ginseng Rubra* and *Radix Ophiopogonis* were supplied by Qingchunbao Pharmaceutical Co., Ltd. (Hangzhou, PR China).

2.2. Sample preparation

A 5 mL of *Shenmai injection* was added on a RP-C_{18} column (500 mg, SUPELCO Park Bellefonte, USA) after a filtration through 0.45 μm nylon film, and successively eluted with 2 mL of water and 5 mL of methanol. The methanol eluate was collected for analysis. The sample injection was 20 μL.

2.3. HPLC/MS analysis

An Agilent 1100 series MSD, operating in the selective ion monitoring (SIM) mode, hyphenated with the Agilent 1100 HPLC system (Agilent Company, Germany) was used for HPLC/MS analysis. The column used was a Hypersil C18 column (4.6 mm × 250 mm, 5 μm, Hanbang Science & Technology, PR China) coupled with Agilent C18 pre-column (4 mm × 10 mm, 5 μm).

The mobile phase was solvent A (HAc:CH_3CN = 0.02:100) and solvent B (HAc:H_2O = 0.02:100) with gradient elution at 40 °C as follows: 24–32% A at 0–10 min, 32% A for 5 min, 32–36% A at 15–25 min, 36% A for 20 min, 36–52% A at 45–60 min, 52–60% A at 60–70 min, 60% A for 10 min, 60–90% A at 80–90 min, 90–95% A at 90–95 min. The flow rate was 0.5 mL/min. The effluent was monitored at 202 nm. MS conditions were as follows: negative ion mode, fragmentor voltage 250 V, drying gas N_2 flow rate 12 L/min, the drying gas temperature 350 °C and the capillary voltage 3500 V. The characteristic ions used for the determination by SIM were listed in Table 3.

In order to identify the peaks of the fingerprints, Esquire LC-00075 series (Bruker, Switzerland) ion-trap mass spectrometer with electrospray ionization (ESI) source was used in HPLC/MS/MS method. The HPLC conditions for HPLC/MS/MS analyses were the same as those used for HPLC/MS analysis. ESI-MS conditions of HPLC/MS analysis were as follows: negative ion mode, drying gas N_2 flow rate 8 L/min, temperature 320 °C, pressure of Nebulizer 12 psi, octapole voltage 2.35 V, ion-trap voltage 32.2 V and scan range 400–1400 u.

For analyzing glycosides from *Radix Ophiopogonis*, the mobile phase consisted of (A) 0.02% aqueous acetic acid and (B) acetonitrile using a linear gradient as follows: 76–68% A at 0–10 min, 68–48% A at 10–30 min, 48–28% A at 30–40 min and 28–10% A at 40–45 min. The flow rate was 0.3 mL/min. ESI-MS/MS conditions were as follows: negative ion mode, separation width 0.9, fragment amplification 1.5–3.0 and scan range 100–1100 u.

3. Results and discussion

3.1. MS/MS analysis of ophioponins in Shenmai injection

As mentioned above, the major compositions of *Shenmai injection*, 39 ginsenosides derived from ginseng, have been illustrated in our previous study [20]. But compounds from *Radix Ophiopogonis* in the preparation have not been clarified. In present work, HPLC/MS/MS was employed, and seven ophioponins were identified based on MS data and published literature data. The total ion chromatogram of extract of *Radix Ophiopogonis* was shown in Fig. 1.

From MS/MS spectra, information on the sequence of sugars and on the aglycone could be provided. Then some compounds were tentatively identified by comparing their molecular weight and MS/MS data with the reference data from literature [24–29]. For instance, peak 3 exhibits $[M-H]^-$ ions at *m*/*z* 869. The fragment ions of *m*/*z* 737 and 591 were detected as shown in Fig. 2. It indicated the successive loss of one molecule of arabinose and rhamnose, respectively, and proved to be ophiogenin 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinofuranosyl-(1 → 4)-β-D-glucopyranoside]. The MS/MS data and the identification of each compound were listed in Table 1 and Fig. 3.

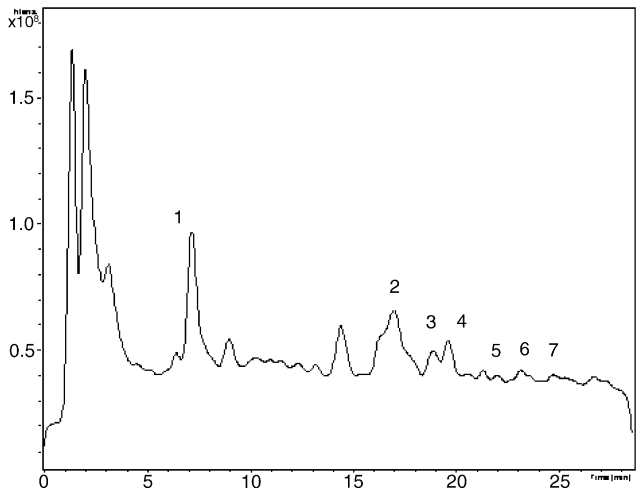

Fig. 1. MS-TIC chromatogram of extracts of *Radix Ophiopogonis*.

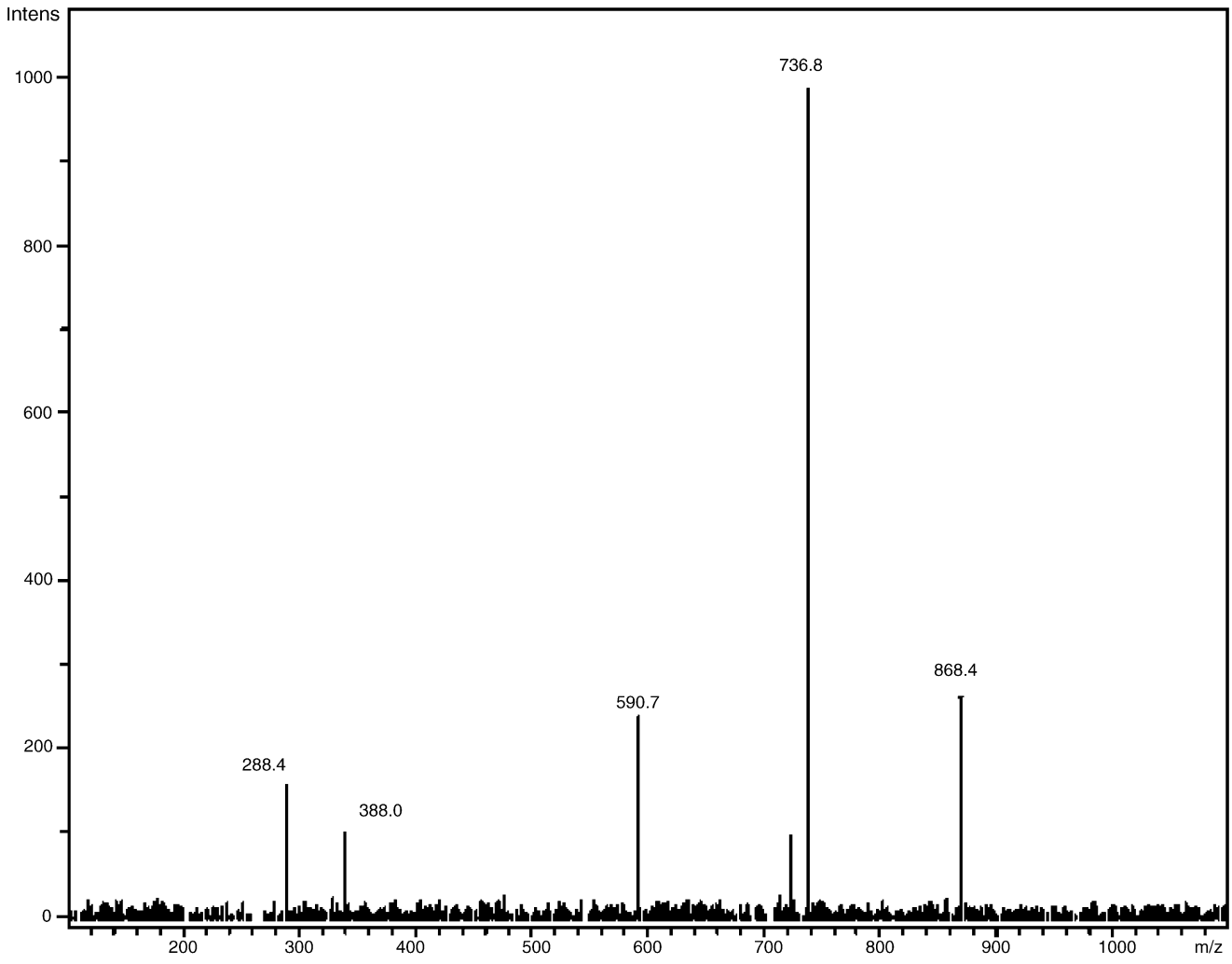

Fig. 2. The MS/MS spectra of peak 3 of extract of *Radix Ophiopogonis*.

3.2. LC/MS fingerprint of Shenmai injection

3.2.1. Optimization of LC/MS conditions

The fragmentor voltage of the mass spectrum can affect the formation and intensity of the molecular ions. In order to get better performance for LC/MS analysis, the fragmentor voltage was optimized. Due to ginsenosides are major constituents of *Shenmai injection*, nine major ginsenosides were selected as representative constituents to perform this task. The results were summarized in Table 2. As can be seen in Table 2, 250 V is adequate for LC/MS analysis of *Shenmai injection*. Moreover, the online MS-TIC chromatogram of *Shenmai*

Table 1
HPLC/MS^n data and identification of ophioponins

Peak	Ret. time	$[M-H]^-$	Identity	Fragment ion *m/z*
1	6.5	447	2-Bornanol, (1*S*,2*R*)-form, *O*-[α-L-arabinofuranosyl-(1 → 6)-β-D-glucopyranoside]	315$[M-H-Ara]^-$
2	16.8	753	Ophiogenin 3-*O*-[α-L-rhamno-pyranosyl-(1 → 2)-β-D-glucopyranoside]	607$[M-rha]^-$
3	18.2	869	Spirost-5-ene-3,17-diol, (3β,17αOH,25*R*)-form, 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinofuranosyl-(1→4)-β-D-glucopyranoside]	737$[M-Ara]^-$, 591$[M-Ara-rha]^-$
4	18.8	737	Spirost-5-ene-3,17-diol, (3β,17αOH,25R)-form, 3-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside]	591$[M-rha]^-$
5	23	341	2′-Hydroxyophiopogonone A	205$[M-136]^-$, 177$[M-136-28]^-$
6	23.6	327	Ophiopogonanone A	205$[M-122]^-$, 177$[M-122-28]^-$
7	25.0	325	Methylophiopogonone B	211$[M-114]^-$, 183$[M-114-28]^-$

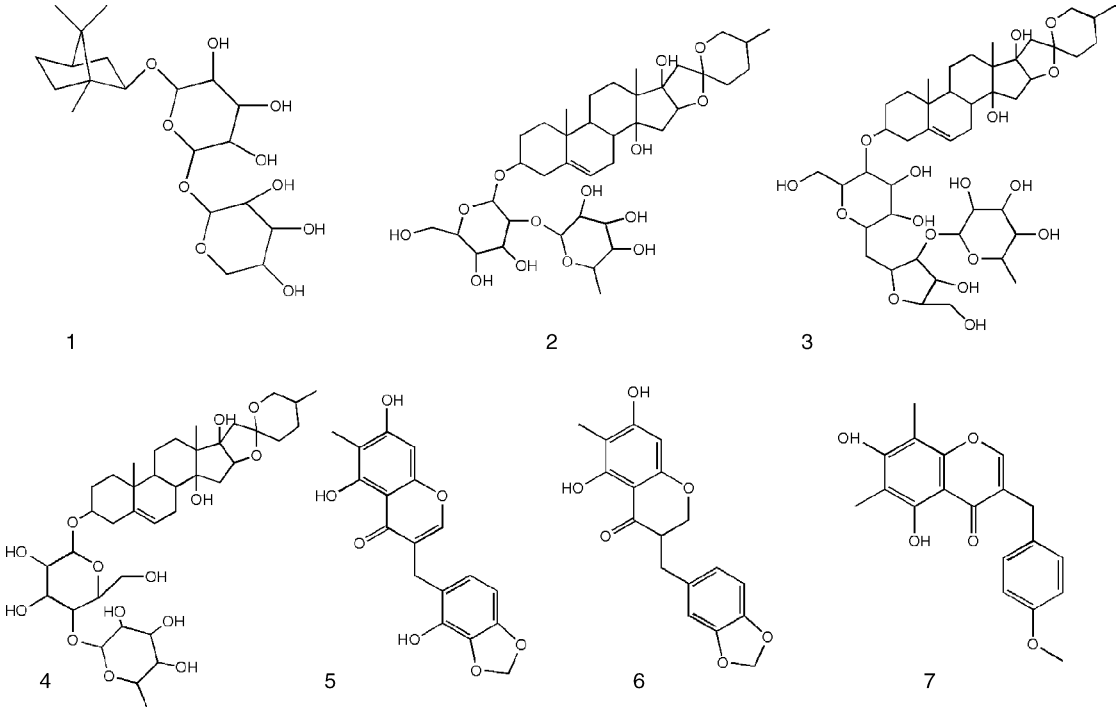

Fig. 3. Structures of identified compounds in extracts of *Radix Ophiopogonis*.

injection under different fragmentor voltage (see Fig. 4) also showed that intensity on 250 V is higher than that of 50 and 150 V.

3.2.2. Identification of LC/MS fingerprint of Shenmai injection

So far a conclusion can be generalized that the major constituents in *Shenmai injection* are ginsenosides coming from *Radix Ginseng Rubra* and several compounds derived from *Radix Ophiopogonis*. Therefore, ions of those compounds can be monitored to represent chemical characteristics of *Shenmai injection* by using LC/MS. Here, 30 ion peaks from ginseng and 7 ion peaks from *Radix Ophiopogonis* were selected to construct the LC/MS fingerprint using SIM mode. A representative MS-TIC fingerprint was shown in Fig. 5. During MS scan analysis, each ion within the mass range is sequentially analyzed, while in a SIM analysis only selected ions are analyzed. A greater sensitivity and individual profiles of the selected ions are thus obtained. The details of the selected ions of LC/MS fingerprint were presented in Table 3.

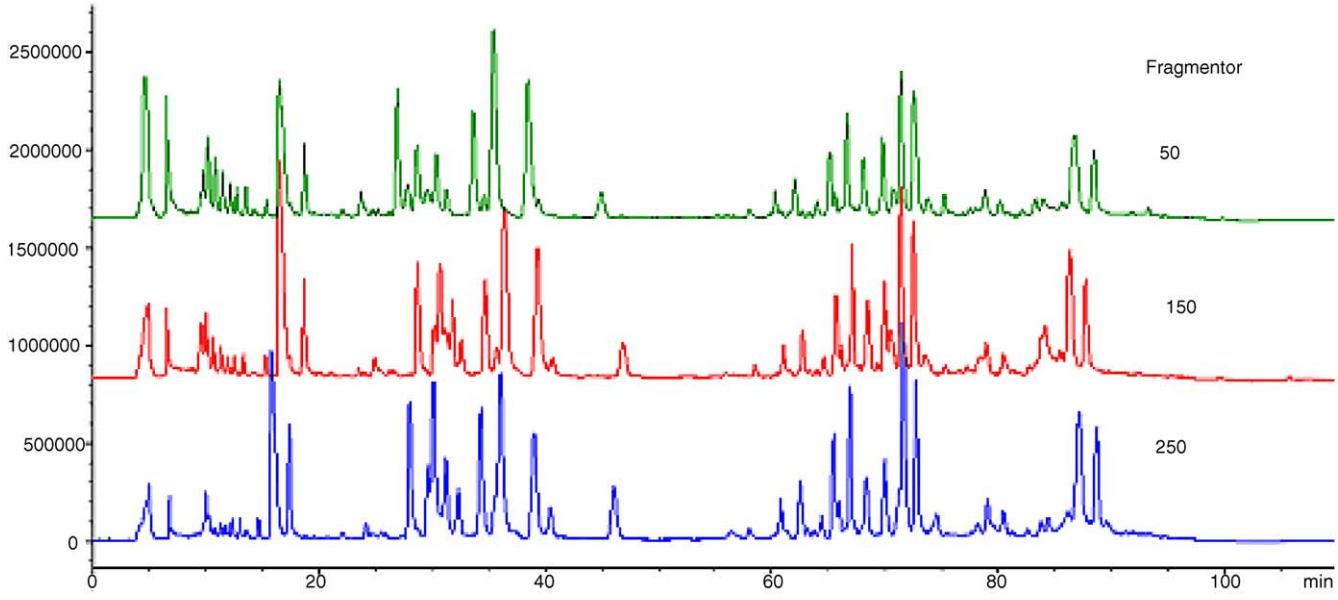

Fig. 4. MS-TIC chromatograms of *Shenmai injection* under different fragmentor voltage.

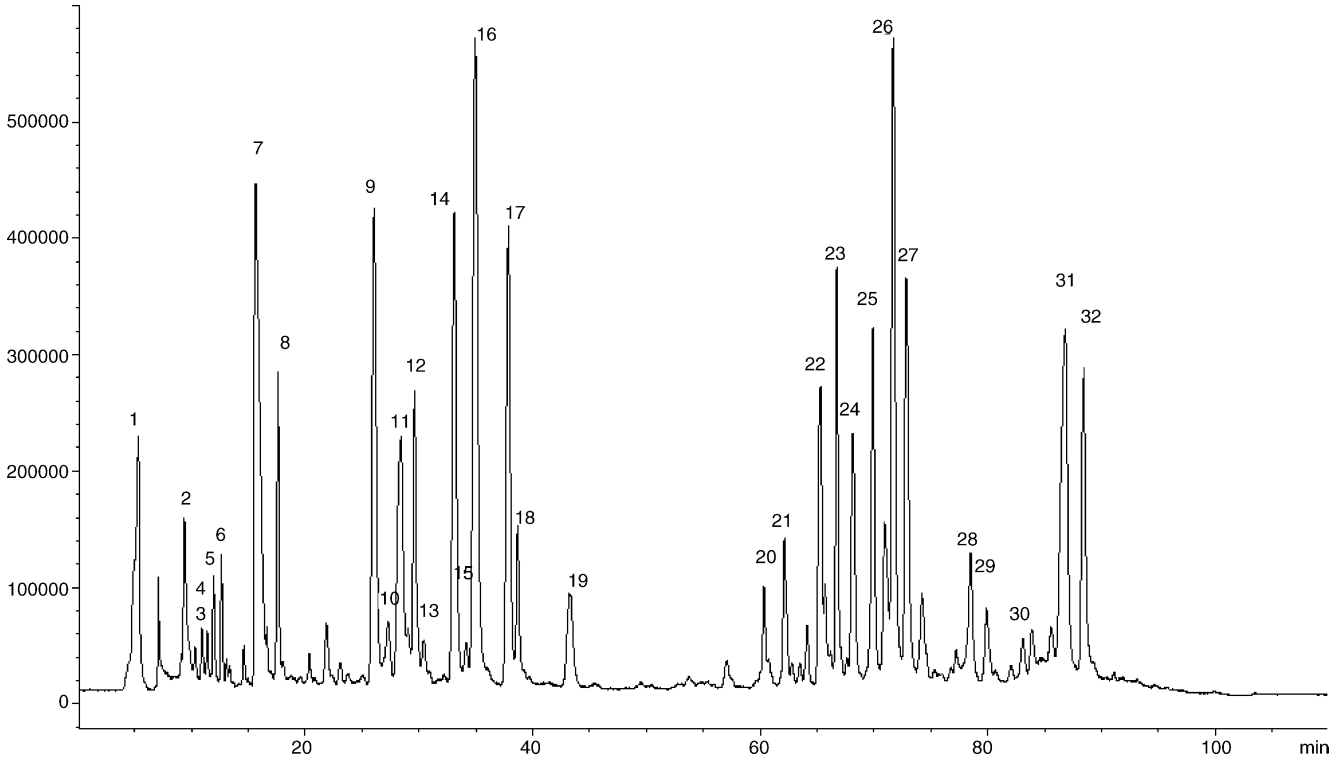

Fig. 5. A representative LC/MS fingerprint of *Shenmai injection*.

3.3. Distinguish manufacturers using LC/MS fingerprints

Distinguishing among same-product manufacturers is an important concern for both health authorities and the public, which also is one of major challenges associated with implementing chromatographic fingerprinting. In the following study, LC/MS fingerprints of 21 samples, 6 samples from manufacture A, 4 samples from manufacture B and all others from manufacture C, were investigated as a method for their quality control.

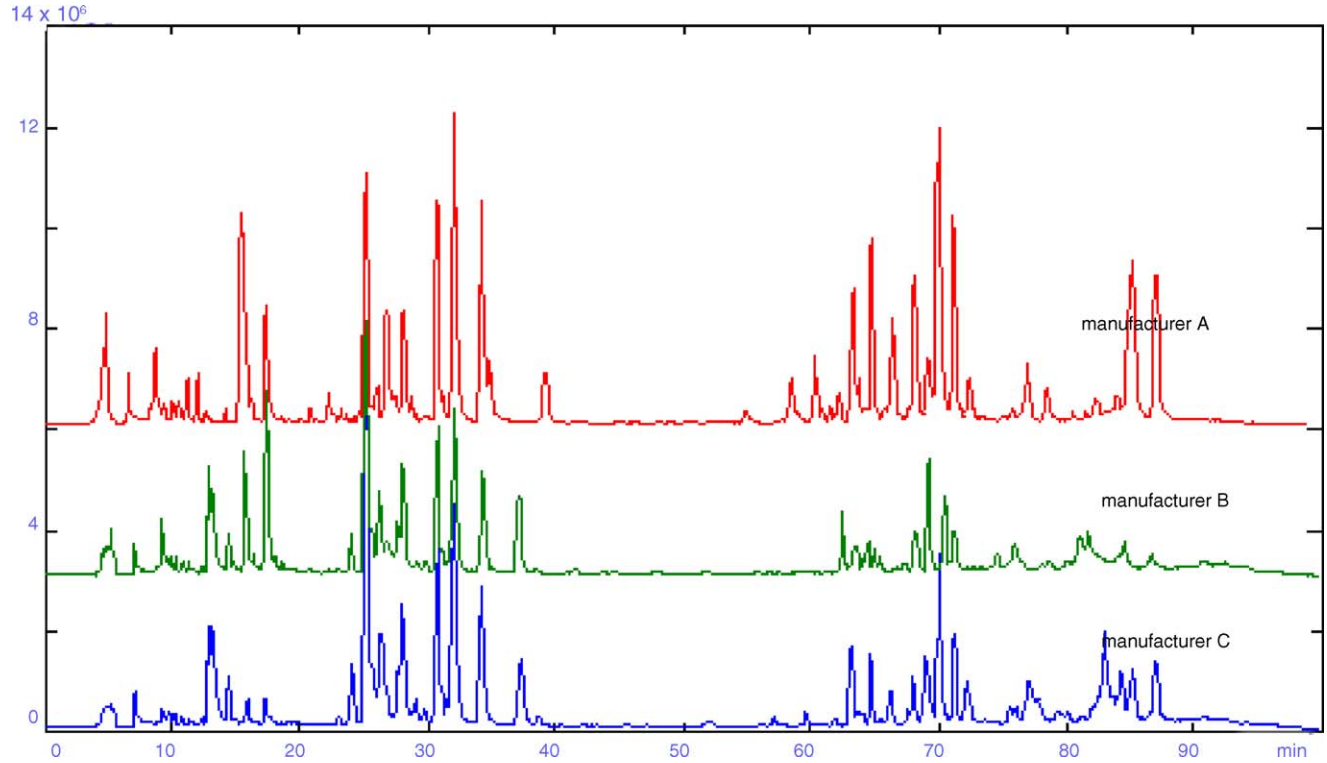

Fig. 6. Representative LC/MS fingerprints of different manufacturers.

Table 2
Intensity of respective ions of nine ginsenosides under different fragmentor voltage

Fragmentor	50	100	150	200	250	300	350
Rg_1				▲	※	♦	
Rg_2				▲	※	♦	
Rb_1					♦	▲	※
Rb_2					♦	▲	※
Rb_3					♦	▲	※
Re					♦	▲	※
Rc					♦	▲	※
Rh_1			▲	※	♦		

(※) The highest intensity of ions; (♦) the second highest intensity of ions; (▲) the third highest intensity of ions.

LC/MS fingerprints were mathematically represented by a 37-dimensional vector $\mathbf{x}_i = [x_{i1}, x_{i2}, \ldots, x_{i37}]^t$, where x_{ij} was the absolute area of the peak j in the fingerprint and the superscript t indicates the transpose of matrix. The representative LC/MS fingerprints of the *Shenmai injection*s from each of three manufacturers were shown in Fig. 6. As revealed by Fig. 6, the samples from three manufacturers had similar chemical patterns. It is difficult to distinguish manufactures by direct visual inspection. In this regard, a well-known chemometrics approach, principal components analysis (PCA) [30], was further employed as a potential tool to distinguish among same-product manufacturers based on their LC/MS fingerprints. The score plots derived from the first two PCs are shown in Fig. 7, where

Table 3
Identification of constituents on LC/MS fingerprint

Peak	Ret. Time	Compounds	$[M-H]^-$	$[M+AcO]^-$	Resource
1	5.26	–	233	–	☆
2	9.33	–	825	–	◊
3	10.86	–	715	–	◊
4	11.33	–	715	–	◊
5	11.91	–	301	–	☆
6	12.62	Re	945	–	◊
	12.62	Rg_1	799	–	◊
7	15.60	–	301	–	☆
8	17.55	2-Bornanol; (1*S*,2*R*)-form, *O*-[α-L-arabinofuranosyl-(1 → 6)-β-D-glucopyranoside]	447	–	☆
9	26.01	Rf	799	–	◊
10	27.24	Rb_1	1107	–	◊
11	28.36	Ro	955	–	◊
12	29.56	Notoginseng R_2	769	–	◊
13	30.35	Rc	1077	–	◊
14	33.07	Rg_2	783	–	◊
15	34.11	Rb_2	1077	–	◊
	34.11	Rb_3	1077	–	◊
16	34.87	Rh_1	–	697	◊
	34.87	Rg_2 iso	783	–	◊
17	37.80	Rh_1 iso	–	697	◊
18	38.63	–	793	–	◊
19	43.25	Rd	945	–	◊
20	60.27	–	781	–	◊
21	62.02	–	781	–	◊
22	65.24	Rg_6 iso	765	–	◊
	65.24	Ophiogenin 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinofuranosyl-(1 → 4)-β-D-glucopyranoside]	869	–	☆
23	66.66	Rg_6 iso	765	–	◊
24	68.07	Rk_3/Rh_4	655	–	◊
25	69.83	Rk_3/Rh_4	655	–	◊
26	71.64	20(R)Rg_3	783	–	◊
27	72.77	20(R)Rg_3	783	–	◊
28	78.46	Rs_3 iso	825	–	◊
29	79.83	Rs_3 iso	825	–	◊
30	83.02	2′-Hydroxyophiopogonone A	341	–	☆
31	86.65	Rk_1/Rg_5	765	–	◊
32	86.65	Ophiopogonanone A	327	–	☆
	88.37	Rk_1/Rg_5	765		◊

☆, derived from *Radix Ophiopogonis*; ◊, derived from *Red Ginseng*.

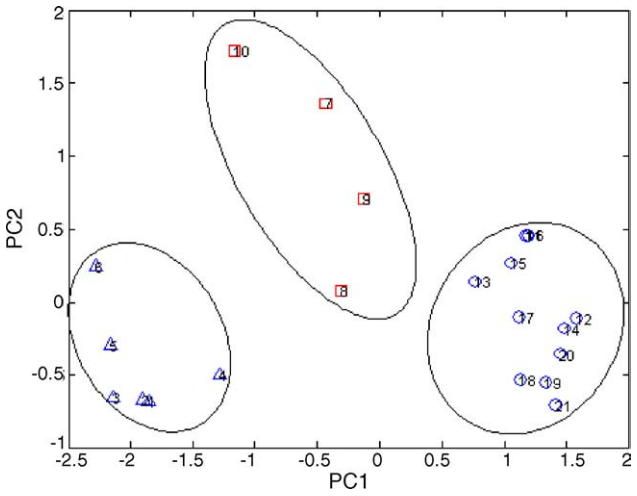

Fig. 7. Distribution of samples on the plan of the first two principal components: (△) manufacturer A; (□) manufacturer B; (◯) manufacturer C.

each sample is represented as a marker. It is noticeable that the samples were clearly clustered in three domains (i.e. manufacturer A, B and C). Therefore, the presented LC/MS fingerprints conjugated with chemometrics approach offers a powerful way for distinguishing among same-product manufacturers of herbal medicines.

4. Conclusions

To date, little attention has been paid to develop hyphenated chromatographic approach in chromatographic fingerprinting for quality evaluation of complex herbal medicines. In this study, we reported our preliminary results of developing an LC/MS fingerprinting of *Shenmai injection* for quality control. Thirty ginsenosides as well as seven ophioponins were chemically represented in the proposed LC/MS fingerprint. Based on the LC/MS fingerprints in conjunction with principal components analysis, manufacturers of samples were accurately identified, which indicate that the presented LC/MS fingerprint approach coupled with chemometrics method offers a powerful tool for the quality control of herbal medicines. Clearly, this study would be potentially helpful to improve the quality control ability of fingerprinting-based strategy for complex herbal medicines.

Acknowledgments

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