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Short communication

Analysis of 'SHENMAI' injection by HPLC/MS/MS

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Abstract

An HPLC/MS/MS method was developed for the analysis of 'SHENMAI' injection, composed of red ginseng and ophiopogon. The constituents of 'SHENMAI' were found to be similar with those of ginseng and 39 ginsenosides were detected. By the studies of MS and MS/MS spectra and the comparison with literature data, most of these ginsenosides were identified. Based on this study, suggestions were put forward to improve the quality control system of 'SHENMAI' injection.
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Keywords: SHENMAI injection; Red ginseng; Ginsenosides; Quality control; HPLC/MS

1. Introduction

'SHENMAI', made from red ginseng and ophiopogon, is one of the most widely used herbal medicines in Traditional Chinese Medicine. It's used to treat cardiomyopathy, miocardial infarction and hypertension. Although the main constituents in ginseng and ophiopogon were found to be ginsenosides and ophioponins, respectively [1], so far, the active compounds were not exactly clear yet. Thus, it led to the difficulty in the quality control of 'SHENMAI' injection. The present quality control system is established on the HPLC fingerprint technology, by which a quality assessment model was built on the data analysis of HPLC chromatograms of different batches of 'SHENMAI' injections. However, due to the unknown of constituents of 'SHENMAI' and the mechanism of the activity, this model could not accurately reflect the pharmaceutical activity of 'SHENMAI'.

The present study aims to develop a HPLC/MS/MS method to analyze 'SHENMAI' injection and its composition plants, i.e. ginseng and ophiopogon, to characterize the constituents of the 'SHENMAI' and its difference with ginseng and ophiopogon. This study would be potentially helpful to optimize the fingerprint model.

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Type A

Ginsenoside	R_1	R_2	MW
Ra_1	Glc^2-Glc	Glc^6-$Arap^4$-Xyl	1210
Ra_2	Glc^2-Glc	Glc^6-$Araf^2$-Xyl	1210
Ra_3	Glc^2-Glc	Glc^6-Glc^3-Xyl	1240
Rb_1	Glc^2-Glc	Glc^6-Glc	1108
Rb_2	Glc^2-Glc	Glc^2-Arap	1078
Rb_3	Glc^2-Glc	Glc^2-Xyl	1078
Rc	Glc^2-Glc	Glc^6-Araf	1078
Rd	Glc^2-Glc	Glc	946
Rg_3	Glc^2-Glc	H	784
Rh_2	Glc	H	622
Rs_1	Glc^2-Glc^6-Ac	Glc^6-Arap	1120
Rs_2	Glc^2-Glc^6-Ac	Glc^6-Araf	1120
Rs_3	Glc^2-Glc^6-Ac	H	826
Malonyl-Rb_1	Glc^2-Glc^6-Ma	Glc^6-Glc	1194
Malonyl-Rb_2	Glc^2-Glc^6-Ma	Glc^6-Arap	1164
Malonyl-Rc	Glc^2-Glc^6-Ma	Glc^6-Araf	1164
Malonyl-Rd	Glc^2-Glc^6-Ma	Glc	1032
F_2	Glc	Glc	784

Type B

Ginsenoside	R_1	R_2	R_3	MW
Re	H	Glc	O-Glc^2-Rha	946
Rf	H	H	O-Glc^2-Glc	800
20-gluco-Rf	H	Glc	O-Glc^2-Glc	962
Rg_1	H	Glc	O-Glc	800
Rg_2	H	H	O-Glc^2-Rha	784
Rh_1	H	H	O-Glc	638
Notoginsenoside R_2	H	H	O-Glc^2-Xyl	770
F_1	H	Glc	OH	638

Type C

Ginsenoside	R_1	R_2	MW
Rg_5	Glc-Glc-	H	766
Rh_3	Glc-	H	604
Rh_4	H	Glc-O-	620
Rs_4	Ac-Glc-Glc-	H	808
Rs_6	H	Ac-Glc-O-	662
F_4	H	Rha-Glc-O-	766

Type D

Ginsenoside	R_1	R_2	MW
Rg_6	H	Rha-Glc-O-	766
Rk_1	Glc-Glc	H	766
Rk_2	Glc-	H	604
Rk_3	H	Glc-O-	620
Rs_5	Ac-Glc-Glc-	H	808
Rs_7	H	Ac-Glc-O-	662

Ro

	R_1	R_2	MW
Ro	Glc^2-Glc	Glc	956

Glc=β-D-glucose Rha=α-L-rhamnose
Arap=α-L-arabinose(pyranose)
Araf=α-L-arabinose(furanose)
Xyl=β-D-xylose
Ac=6′-O-acetyl

Fig. 1. Structures of ginsenosides.

Table 1
Solvent gradient program of HPLC analysis

Time (min)	A (%)	B (%)
0	70	30
10	60	40
15	60	40
25	54	46
65	35	65
75	25	75

2. Experimental

2.1. Samples preparation

'SHENMAI' injection, together with the extracts of red ginseng and ophiopogon, were supplied by a Chinese pharmaceutical company. All the samples were filtered through a 0.45 μm film before HPLC analysis.

The plant materials, Panax ginseng C.A. Mey and Ophiopogon japonicus (Thunb)Ker-Gawl, were identified by pharmacognostic specialist, Professor Luan Lianjun. The voucher specimens of red ginseng and ophiopogon were deposited in our laboratory.

Ginsenosides Rb_1, Rb_2, Rb_3, Rc, Rd, Re, Rg_1, Rg_2, 20(s)-Rg_3 and Rh_1 were kindly presented by Professor Xu Jingda, college of pharmacy, Jilin University, PR China.

The structures of ginsenosides were listed in Fig. 1.

2.2. Solvents and reagents

Acetonitrile was HPLC grade from Tedia (Fairfield, OH); acetic acid for analysis from Hangzhou Reagent Company (Hangzhou, PR China); Water was purified by a Milli-Q academic water purification system (Milford, MA).

2.3. Instrumentation and conditions

2.3.1. HPLC-UV analysis

Agilent 1100 series HPLC system (Waldbronn, Germany) equipped with quaternary pump, vacuum degasser, autosampler, diode-array detector, column heater–cooler and ChemStation system was used in HPLC-UV analysis. The chromatographic separation was performed on a Hypersil C_{18} column (5 μm, 4.6×250 mm^2) from Hanbang Science & Technology (Jiangsu, PR China). A linear gradient elution of A ($CH_3COOH:H_2O$ = 0.01:100) and B ($CH_3COOH:H_2O:CH_3CN$ = 0.01:20:80) was used. The gradient is presented in Table 1. The solvent flow rate was 0.5 ml/min and column temperature was set at 40 °C. The UV detection wavelength was set at 202 nm.

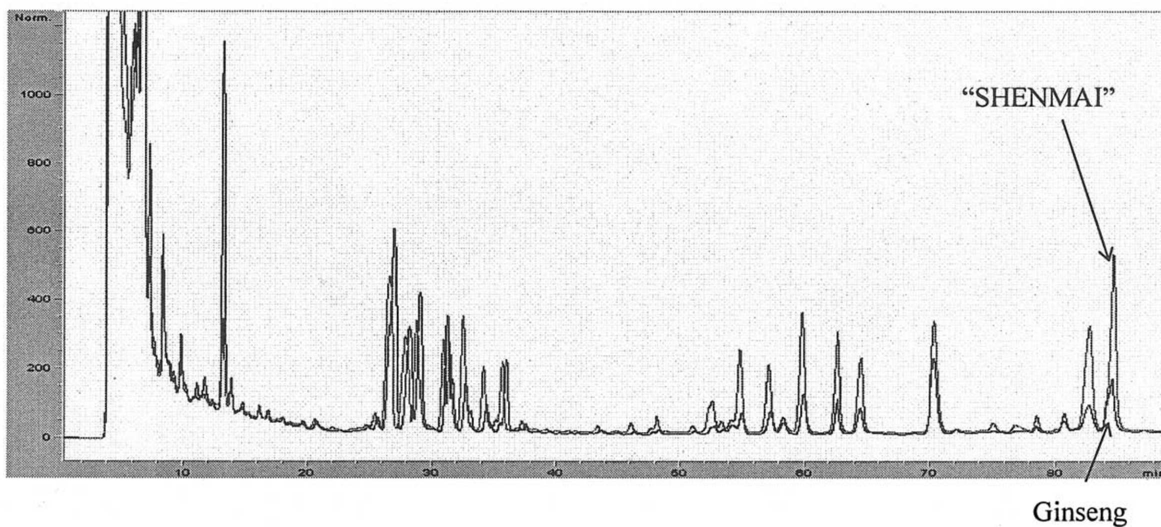
2.3.2. HPLC-ESI-MS analyses

The HPLC conditions for HPLC-MS analyses were the same as those used for HPLC-UV analysis. Esquire LC-00075 series (Bruker, Switzerland) ion-trap mass spectrometer with electrospray ionization was used in HPLC-MS method. ESI-MS conditions of HPLC-MS analysis were as follows: negative ion mode, drying gas N_2, 8 l/min, temperature 320 °C, pressure of Nebulizer 12 psi, octapole voltage 2.35 V, ion-trap voltage 32.2 V, scan range 400–1400 u. ESI-MS/MS conditions were as follows: negative ion mode, separation width 0.9, fragment amplification 1.5, scan range 200–1300 u.

3. Results and discussions

3.1. HPLC analysis of 'SHENMAI' injection and extracts of ginseng and ophiopogon

The typical HPLC-UV chromatograms of SHENMAI injection, ginseng and ophiopogon extract were presented in Fig. 2. The constituents in 'SHENMAI' injection were well separated on reversed-phase column with the linear gradient elution. No obvious peak was detected in ophiopogon extract under different elution gradients with the wavelength scanned from 190 to 390 nm. Furthermore, no ion peak attributed to ophioponins was found in the MS analysis of ophiopogon extract via direct injection. Comparing the chromatograms of 'SHENMAI' and ginseng extract, it's found that their composition were very similar with each other. However, the quantities of each constituent between 'SHENMAI' and ginseng



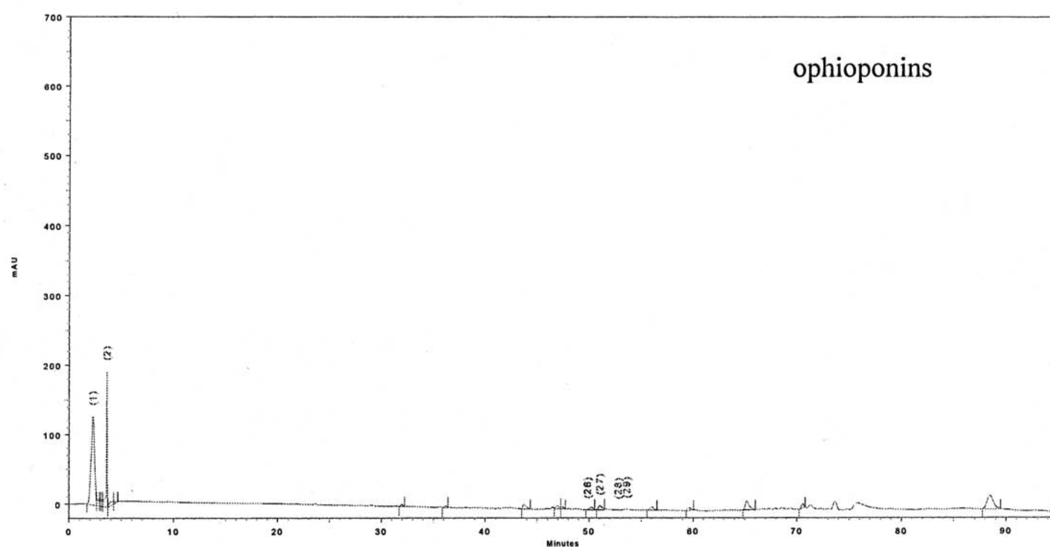


Fig. 2. Chromatograms of 'SHENMAI' injection, ginseng and ophiopogon extract.

showed a big difference, especially the less polarity constituents.

It indicated that the quality of 'SHENMAI' injection relied on ginseng much more than ophiopogon. Moreover, some changes could have taken place in the course of procedure, which resulted in the difference of quantities between the constituents in 'SHENMAI' and ginseng.

3.2. HPLC-UV-ESI-MS^n analysis of 'SHENMAI' injection

In HPLC-UV chromatogram, 29 peaks, which were all attributed to ginsenosides, were detected. Each peak had its corresponding peak in HPLC-MS chromatogram except peak 27 (Fig. 3). According to the MS spectra of corresponding

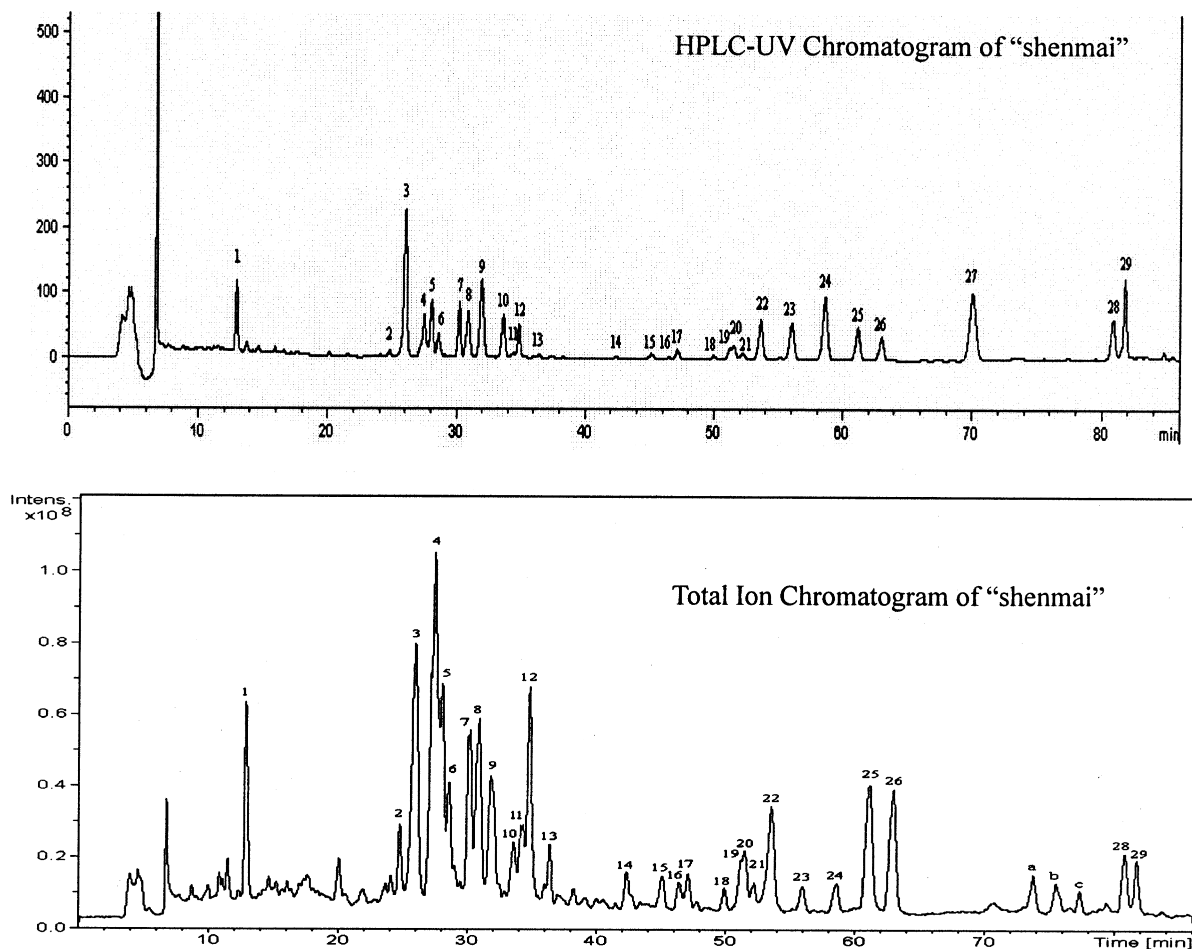


Fig. 3. HPLC-UV Chromatograph and MS-TIC Chromatograph of 'SHENMAI'.

peak in HPLC-UV chromatogram, it could be considered that most constituents in SHENMAI injection were well separated and detected.

In MS spectra, most ginsenosides, excluding malonyl-ginsenosides, exhibited their quasi-molecular ions $[M-H]^-$, adduct ions $[M+Cl]^-$ and $[M+AcO]^-$, some of them showed $[M+M-H]^-$. Since malonyl-ginsenosides are thermally unstable compounds, they would decompose in capillary at 320 °C and lose one molecular CO_2 to produce quasi-molecular ion $[M-CO_2-H]^-$ and adduct ion $[M-CO_2+Cl]^-$, instead of $[M-H]^-$(Table 2). Chlorine-contained solvents were not employed in HPLC-MS analyses, presence of adduct ions $[M+Cl]^-$ and $[M-CO_2+Cl]^-$ were ascribed to contamination of ion resource.

Compared the retention time and MS spectra of SHENMAI injection sample with those of reference compounds, peak 1, 3, 5, 7–9, 12, 26 were attributed to ginsenoside Re, Rg_1, Rb_1, Rc, Rb_2, Rb_3, Rg_2, Rh_1, Rd and 20(s)-Rg_3, respectively (Table 2). Re and Rg_1, Rb_3 and Rg_2, have same HPLC retention time. They were recognized according to their MS spectral characteristics. Other peaks were identified by careful studies of their MS and MS–MS spectra and by comparison with the literature data ([2–4]). For example, peak 22 and 28 had same m/z value of quasi-molecular

Table 2
HPLC-UV-ESI-MS identification

Peak	Retention time	Identification	$[M-H]^-$	$[M+Cl]^-$	$[M+AcO]^-$	$[M-H-CO_2]^-$	$[M-CO_2+Cl]^-$	$[M+M-H]^-$
1	12.911	Re	945	981	–	–	–	–
1	12.911	Rg_1	799	835	859	–	–	–
2	24.75	Ra_1/Ra_2	1209	1245	–	–	–	–
3	25.994	Rf	799	835	–	–	–	–
3	25.994	Rb_1	1107	1143	–	–	–	–
4	27.440	Ro	955	–	–	–	–	–
5	28.044	Rc	1077	1113	–	–	–	–
6	28.563	Notoginsenoside R_2	769	805	–	–	–	–
7	30.187	Rb_2	1077	1113	–	–	–	–
8	30.875	Rb_3	1077	1113	–	–	–	–
8	30.875	Rg_2	783	819	–	–	–	–
9	31.917	Rh_1	637	673	697	–	–	1275
9	31.917	Rg_2 iso.	783	819	–	–	–	–
9	31.917	Malonyl-Rb_1/iso.	–	–	–	1149	–	–
10	33.596	Rh_1 iso.	637	673	697	–	–	1275
11	34.34	Malonyl-Rb_2/Rb_3/Rc	–	–	–	1119	1155	–
12	34.815	Rd	945	981	–	–	–	–
13	36.385	Rb_2/Rb_3/Rc iso.	1077	–	–	–	–	–
13	36.385	Malonyl-Rb_2/Rb_3/Rc	–	–	–	1119	1155	–
14	42.37	Malonyl-Rd/iso.	–	–	–	987	1023	–
15	45.11	Not reported	781	817	–	–	–	–
16	46.45	Rg_2 iso.	783	819	–	–	–	–
16	46.45	Malonyl-Rd/iso.	–	–	–	987	1023	–
17	47.105	Not reported	781	817	–	–	–	–
18	49.96	Not reported	751	787	–	–	–	–
19	51.2	Rg_6 iso.	765	801	–	–	–	–
20	51.486	Rg_6 iso.	765	801	–	–	–	–
21	52.180	Not reported	751	787	–	–	–	–
22	53.580	F4/Rg_6	765	–	–	–	–	–
23	55.993	Rk_3/Rh_4	619	655	679	–	–	1239
24	58.582	Rk_3/Rh_4	619	655	679	–	–	1239
25	61.125	20(R)Rg_3	783	819	–	–	–	–
26	62.961	20(S)Rg_3	783	819	–	–	–	–
27	69.997	Not detected	–	–	–	–	–	–
28	80.833	Rk_1/Rg_5	765	801	–	–	–	–
29	81.741	Rk_1/Rg_5	765	801	–	–	–	–
A	73.8	Rs_3 iso.	825	861	–	–	–	–
B	75.52	Rs_3 iso.	825	861	–	–	–	–
C	77.35	Ro iso.	955					

ion in the MS spectra. But they could be distinguished using MS–MS spectra. The MS–MS fragment ion (Table 3, Fig. 4) of peak 22 indicated the successive loss of rhamnose and glucose, proved itself to be F4 or Rg_6, while the MS–MS fragment ion (Table 3, Fig. 5) of peak 28 showed the loss of two glucoses, proved to be Rk_1 or Rg5.

Several isomers were also detected. Some of them were epimers, including 20(R/S) Rg_3 as peak 25/26, 20(R/S) Rg_2 as peak 8/9 and 20(R/S) Rh_1 as peak 9/10. Some others were geometric isomers, especially those less polarity ginsenosides, for example Rk_1 and Rg_5 (peak 28, 29), which were different just in the position of the double bond.

Table 3
HPLC-UV-ESI-MS^n identification

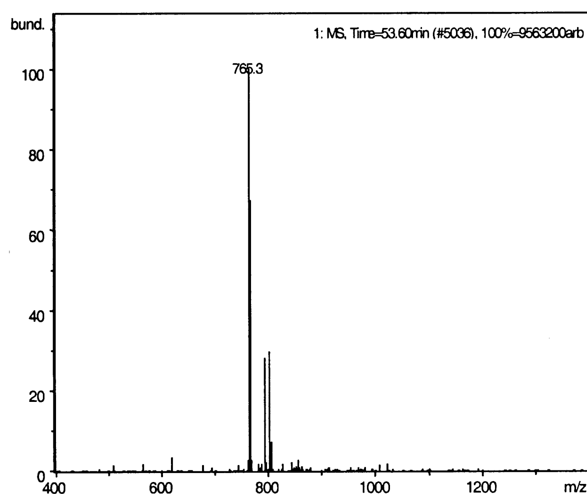
Peak	Identification	Fragment ion m/z
3	Rf	637[M-H-Glc]$^-$; 475[M-H-2Glc]$^-$
4	Ro	793[M-H-Glc]$^-$; 631[M-H-2Glc]$^-$; 613[M-H-2Glc-H_2O]$^-$; 587[M-H-2Glc-CO_2]$^-$; 569[M-H-2Glc-CO_2-H_2O]$^-$
6	Notoginsenoside R_2	637[M-H-xyl]$^-$; 475[M-H-xyl-Glc]$^-$
19	Rg_6 iso.	619[M-H-Rham]$^-$; 601[M-H-Rham-H_2O]$^-$;
20	Rg_6 iso.	619[M-H-Rham]$^-$; 601[M-H-Rham-H_2O]$^-$;
22	F4/ Rg_6	619[M-H-Rham]$^-$; 601[M-H-Rham-H_2O]$^-$; 457[M-H -Rham-Glc]$^-$
25	20(R)Rg_3	621[M-H-Glc]$^-$; 459[M-H-2Glc]$^-$
26	20(S)Rg_3	621[M-H-Glc]$^-$; 459[M-H-2Glc]$^-$
28	Rk_1/Rg_5	603[M-H-Glc]$^-$; 441[M-H-2Glc]$^-$
29	Rk_1/Rg_5	603[M-H-Glc]$^-$
A	Rs_3 iso.	783[M-Ac]$^-$; 621[M-Ac-Glc]$^-$; 459[M-Ac-2Glc]$^-$

MS/MS spectra of peaks, such as 2, 11, 13, 16, 23, 24, and so on, were not detected, probably because of their quantities were so low that the signals were covered by the noise. These peaks were identified by comparing their molecular weight with the literature data. The corresponding data of peak 15,17,18,21 were not found in the literature, they could be supposed to be new ginsenosides which probably were the dehydrated derivatives of notoginsenoside R_2 and ginsenoside Rf. The study of these compounds was in process.

4. Conclusions

The quality control has always been the necklace of the development of plant drugs. Although a few strategies were put forward and some progresses were achieved, for example, the fingerprint strategy [5], in which the similarity comparison was used to control the quality, but it's far from solving this problem.

In this study, 'SHENMAI' injection was analyzed by HPLC/MS/MS method and the main constituents were identified. It showed that ginseng would play a much more important role than ophiopogon to affect the quality of 'SHENMAI' injection. Moreover, the big difference between the quantities of the less polarity ginsenosides of ginseng and 'SHENMAI' indicated that these compounds would probably take effects on the bioactivity of 'SHENMAI'. So, some suggestions could be put forward to improve the quality control. Firstly, the quality of ginseng is the key to guarantee the quality of 'SHENMAI'. Sec-



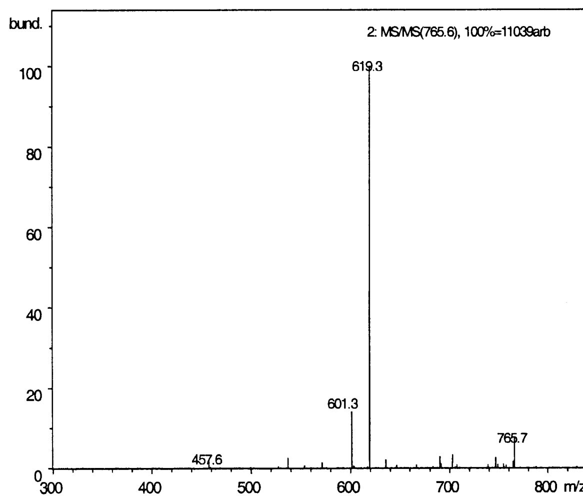


Fig. 4. MS and MS^n spectra of peak 22.

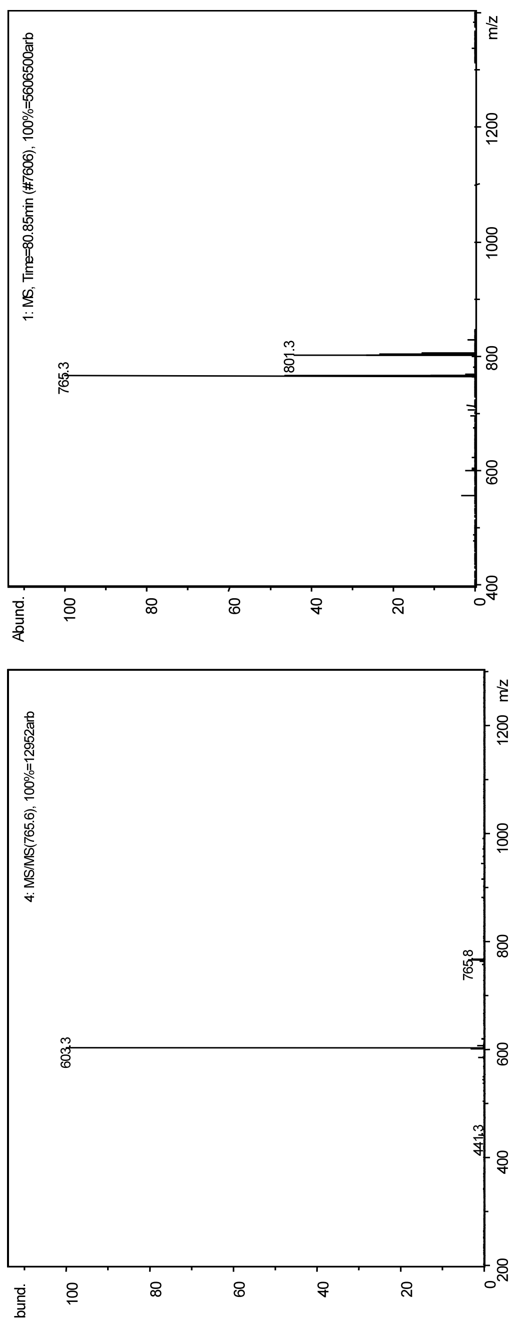


Fig. 5. MS and MS^n spectra of peak 28.

ondly, the less polarity ginsenosides could be some of the main active compounds in 'SHENMAI' injection. This suggestion was already confirmed by report [6]. Based on this study and correlative reports of the ginsenosides' bio-activities, further pharmacology tests of the ginsenosides in 'SHENMAI' injection could be designed and the indexes for fingerprint could be optimized to improve the quality control efficiency.

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References

[1] Xu Rensheng, Natural Product Chemistry, Science Press, Beijing, 1988.
[2] K. Samukawa, H. Yamashita, H. Matsuda, M. Kubo, Chem. Pharm. Bull. 43 (1995) 137.
[3] N. Fuzzati, B. Gabetta, K. Jayakar, R. Pace, F. Peterlongo, J. Chromatogr. A 854 (1999) 69.
[4] S.W. Kwon, S.B. Han, I.H. Park, J.M. Kim, M.K. Park, J.H. Park, J. Chromatogr. A 921 (2001) 335.
[5] A. Hasler, O. Sticher, B. Meier, J. Chromatogr. 605 (1992) 41.
[6] I.H. Park, L.Z. Piao, S.W. Kwon, Y.J. Lee, S.Y. Cho, M.K. Park, J.H. Park, Chem. Pharm. Bull. 50 (2002) 538.