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# Identification of multiple constituents in the traditional Chinese medicine formula Sheng-Mai San and rat plasma after oral administration by HPLC–DAD–MS/MS

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ABSTRACT

Sheng-Mai San (SMS), a traditional Chinese medicine formula, has been used for the treatment of cardiovascular disease in Asia over long period of time. While its effectiveness has been confirmed by clinical use, its active chemical constituents remain unclear. In this paper, an HPLC–DAD–MS/MS method is described for the efficient and rapid identification of the chemical constituents in SMS extract. MS/MS fragmentation behavior of authentic compounds was proposed for aiding the structural identification of the components. A total of 53 compounds were identified or tentatively characterized by comparing their retention times, UV and MS spectra with those of authentic compounds or literature data. HPLC/UV and MS techniques were employed to screen for the potential bioactive components in rat plasma after oral administration of SMS. Twenty-five compounds including 14 prototype components and 11 metabolites were detected in dosed rat plasma compared with blank rat plasma. This identification and structural elucidation of the chemical constituents in the medicine formula and rat plasma may provide important experimental data for further pharmacological and clinical research.

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## 1. Introduction

Traditional Chinese medicine (TCM) has attracted much attention due to their effectiveness against many diseases with proved safety over long period of clinical use. It is widely accepted that complex synergistic effects among vast number of chemical constituents existing in TCM are responsible for the therapeutic effects of TCM [1,2]. However, the detection and structural characterization of each chemical constituents contained in herbal prescriptions are often challenging and become the major obstacle for the further pharmacological investigation. The development of sensitive and reliable analytical methods for these herbal prescriptions is therefore prerequisite for the identification of their pharmacological active constituents. Direct coupling of high-performance liquid chromatography with tandem mass spectrometry (HPLC–MS/MS) has been proven to be an efficient tool for the rapid on-line analysis of the known compounds and elucidation of unknown compounds in complex matrix and therefore become a valuable analytical technique in TCM research [3,4].

Sheng-Mai San (SMS) is a traditional Chinese formula, consisting of three herbs, *Radix ginseng* (*Red ginseng*), *Radix ophiopogonis* and *Fructus schisandrae*, has long been used for the treatment of loss of essence-energy and excessive body fluids. Recent studies have shown its effectiveness in the treatment of coronary atherosclerotic cardiopathy and viral myocarditis [5,6]. Some chemical components, such as ginsenosides, lignans, homoisoflavonoids and steroidal glycosides, have been suggested to be responsible for the pharmacological activities of SMS [7–10]. None of these individual chemical components, however, can recapture all the pharmacological activities of SMS. Therefore the active chemical constituents of SMS and its pharmacological mechanism underlying still largely remain unknown.

Currently the study of the chemical components in SMS has been mainly based on identifying chemical constituents in individual herb extracts, while there were only sparse data for comprehensive investigation on the constituents of SMS [5]. Furthermore, biological studies with SMS mainly focused on pharmacokinetics of one or few bioactive components. This approach offers limited information towards identifying active chemical constituents of SMS since the pharmacokinetic properties of each chemical constituent have not been taken into account. Since only the compounds absorbed into the blood have the chance to show pharmacological bioactivities, the chemical constituents profiling of SMS in plasma will not only greatly reduce the number of chemicals involved for further investigation, but also help to reveal the potential active chemical constituents [11]. In order to investigate the effectiveness of SMS,

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comprehensive investigation on the profile constituents of the formula is necessary. Therefore, in this study, an HPLC–DAD–MS/MS method was firstly developed to systematic qualitative investigation of the chemical constituents of SMS extract and to screen for multiple absorbed components in rat plasma after oral administration of SMS extract.

## 2. Experimental

### 2.1. Chemicals and materials

Standards of ginsenoside Rg1, Rb1, Rb2, Rc, Rd, Rg3, Rg2, Rh1 and Schizandrol A, Schizandrol B, Schizandrin A, Schizandrin B, Schizandrin C and Schisantherin A were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP) (Beijing, China). The ten homoisoflavonoids standards, including desmethylisophiopogonone B, 5,7,4′-trihydroxy-3′,5′-methoxy-6,8-dimethylhomoisoflavanone, 5,7,3′-trihydroxy-4′-methoxy-6,8-dimethylhomoisoflavanone, ophiopogonanone E, 5,7,2′-trihydroxy-3′,4′-methylenedioxy-8-methylhomoisoflavanone, ophiopogonanone A, 5,7-dihydroxy-4′-methoxy-6-methylhomoisoflavanone, methylophiopogonone A, methylophiopogonanone A, methylophiopogonanone B and the eight steroidal saponin standards, including ruscogenin-1-O-α-L-rhamnopyranosyl-(1 → 2)-4-O-sulfate-α-L-arabinopyranoside-3-O-β-D-glucopyranoside, ruscogenin-1-O-α-L-rhamnopyranosyl-(1 → 2)-4-O-sulfate-β-D-fulopyranoside-3-0-β-D-glucopyanoside, ruscogenin-1-O-α-L-rhamnopyranosyl-(1 → 2)-4-O-sulfate-α-L-arabinopyranoside, ophiogenin-3-O-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside, prazarifenin A 3-O-α-L-rhamnopyranosyl-(1–2)-β-D-glucopyranoside, ophiopogonin D, ophiopogonin D′ and ophiopogonin B were isolated and purified from *Radix ophiopogonis* in the authors' laboratory and their structures were elucidated by their spectral data (MS, $^{1}H$ and $^{13}C$ NMR), and their purities were above 98% as determined by HPLC.

HPLC-grade acetonitrile was purchased from Tedia (Fairfield, OH, USA). AR-grade acetic acid and methanol were obtained from Jiangsu Hanbon (Jiangsu, China). Water for HPLC analysis was purified by a Milli-Q academic water purification system (Milford, MA, USA).

*Radix ginseng* (*Red ginseng*), *Radix ophiopogonis* and *Fructus schisandrae* were purchased from Fengyuan Tongling crude drug company (Anhui, China) and were identified by Professor Bo-Yang Yu. The voucher specimens were deposited in our laboratory.

### 2.2. Animals

Fifteen male Sprague–Dawley (SD) rats (220 ± 20 g body weight) were obtained from the Laboratory Animal Center of Nantong University and were randomly separated into a control group (5 rats) and a dosed group (10 rats). The experimental animals were housed under the above conditions for 3 days acclimatization and fasted for 12 h with free access to water, prior to the experiments. All procedures were in accordance with the National Institute of Health's guidelines regarding the principles of animal care (2004).

### 2.3. Sample preparation

#### 2.3.1. Preparation of plant samples for analysis

The powdered sample of *Radix ginseng* (20 g) was immersed in 200 ml distilled water and decocted by boiling for 1 h. The operation was repeated twice with 160 and 120 ml water, respectively. The extracts were combined and concentrated to approximate 100 ml. An aliquot of 1.5 ml of the extract was added into a 10 ml polypropylene test tube and then mixed with equal volume of water. The solution was extracted four times with 6–6 ml of water-saturated n-butanol. Extraction was performed by vortexing for 30 s and centrifugation for 10 min at 8000 rpm. The n-butanol fractions were transferred into a glass tube and dried at 50 °C under a stream of nitrogen. The residue was dissolved in 1 ml methanol. After centrifugation at 12,000 rpm for 10 min, an aliquot of 20 μl solution was injected into the HPLC/MS/MS system.

The preparation of *Radix ophiopogonis* and *Fructus schisandrae* was identical with that of *Radix ginseng*. SMS sample was prepared by combining *Radix ginseng* (20 g), *Radix ophiopogonis* (60 g) and *Fructus schisandrae* (30 g). Then the mixture was extracted three times with 1100 ml, 880 ml and 660 ml water for 1 h, respectively. The extracts were combined and concentrated to approximate 100 ml, and this concentration solution was used for oral administration. The sample for chemical analysis was prepared with the same procedure as that for *Red ginseng*. All sample solutions were stored at −20 °C and used at room temperature.

#### 2.3.2. Preparation of blood plasma samples for analysis

The SMS extract was orally administered to rats at a dose of 1.35 ml/100 g body weight three times a day, and the control rats were orally administered with physiological saline in the same way. The rats were anesthetized by inhalation of diethyl ether 60 min after last administration and the blood samples were collected in heparinized tubes and centrifuged to separate plasma at 3000 rpm for 10 min.

All plasma samples from one group of rats were combined into one sample to eliminate the individual variability. The plasma samples were pretreated by solid phase extraction (SPE) before LC/MS analysis. Supelclean™ LC-18 SPE columns (3 ml/500 mg volume, Supelco, USA) were first preconditioned with 3 ml of methanol, and then equilibrated with 3 ml of deionized water. The plasma samples (1.5 ml) were loaded onto the preconditioned SPE columns directly. After the sample had been absorbed, the cartridge was washed with 6 ml water and then eluted with 3 ml methanol, which was evaporated under a stream of nitrogen at 40 °C. The residue was dissolved in 100 μl methanol for HPLC/MS/MS analysis. After centrifugation at 12,000 rpm for 10 min and an aliquot of 20 μl was injected into the HPLC/MS/MS system.

### 2.4. HPLC–DAD–MS/MS analysis

All analyses were carried out using a Waters Alliance HPLC system (Waters, Milford, MA, USA), equipped with a PDA 2996 photo diode array detector (Waters, Milford, MA, USA), a Micromass Quattro micro™ API benchtop triple quadrupole mass spectrometer (Waters MS Technologies, Manchester, UK), a Z-spray electrospray ionization (ESI) source operated in both negative and positive mode, controlled by MassLynx™ software (version 4.0, Waters, Milford, MA, USA) for data acquisition and processing.

Sample solutions were separated on an Alltima C18 column (4.6 mm × 250 mm, i.d. 5 μm). A linear gradient elution of acetonitrile–acetic acid (100:0.02, v/v) (A) and water–acetic acid (100:0.02, v/v) (B) was used for the separation of samples. The gradient programmer was as follows: 0–30 min, 8–18% A; 30–80 min linear increase to 50% A; 80–115 min linear increase to 100% A. The solvent flow rate was 1 ml/min and 20 μl of sample solution was injected in each run. Column temperature was maintained at 35 °C. The effluent was introduced into a PDA detector (scanning range 190–400 nm, resolution 1.2 nm) and subsequently into an electrospray source (desolvation temperature 400 °C, capillary voltage 3.0 kV, cone voltage 30 V). The split ratio of HPLC flow between PAD detector and MS detector was 2:1. Helium was used

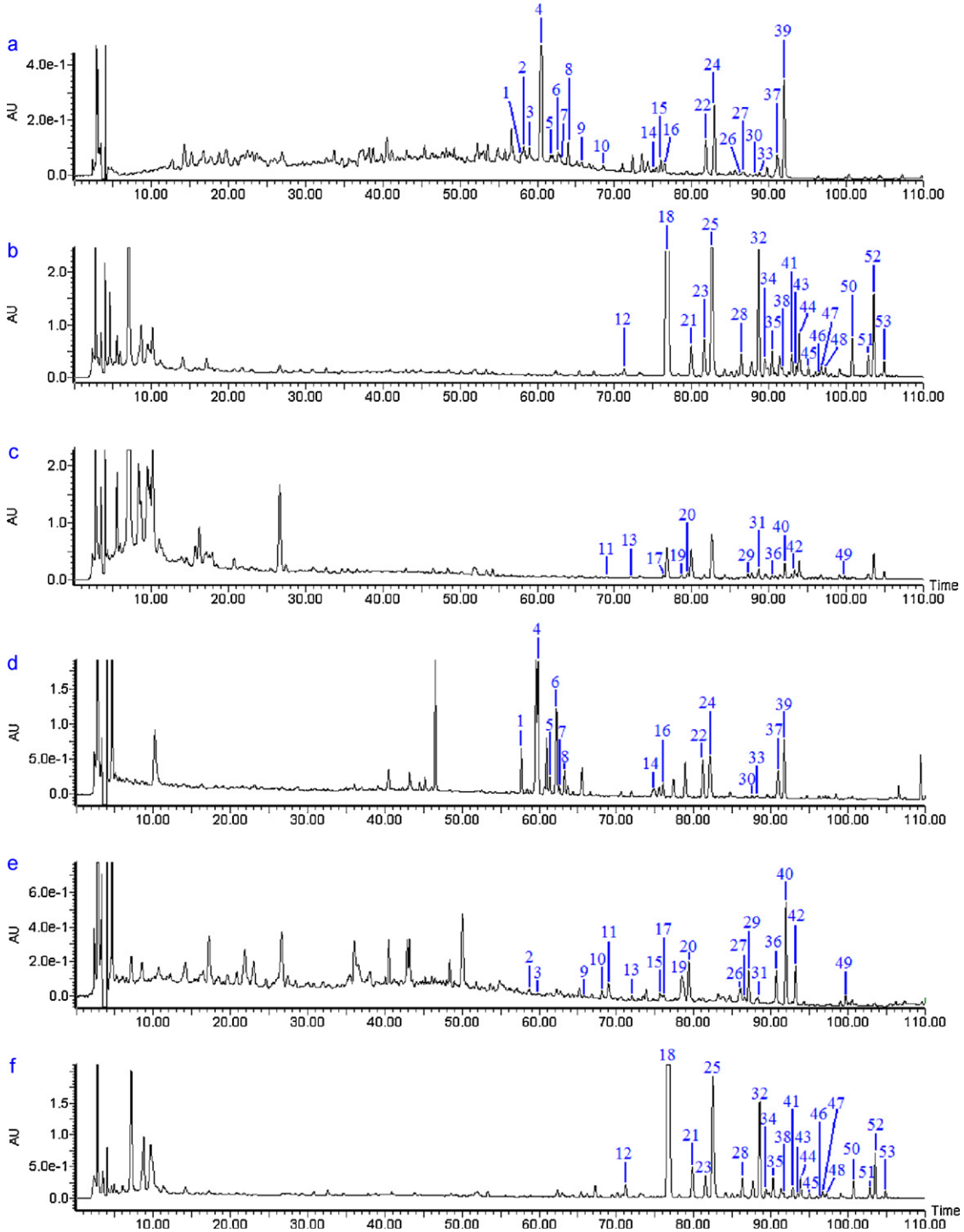



**Fig. 1.** HPLC–DAD chromatograms of extracts of SMS at 203 nm (a), 254 nm (b), 296 nm (c), *Radix ginseng* at 203 nm (d), *Radix ophiopogonis* at 203 nm (e), and *Fructus schisandrae* at 254 nm (f).

as collision gas (collision energy 30 V) and nitrogen as desolvation gas (500 l/h).

## 3. Results and discussion

### 3.1. HPLC analysis of SMS and single herb extracts

In order to obtain chromatograms with good separation and strong total ion current (TIC), acetonitrile–acetic acid (100:0.02)/water–acetic acid (100:0.02, v/v) were found to be the optimal mobile phase in both HPLC and MS analyses. Compared with methanol, acetonitrile remarkably improved separation of the major constituents in SMS. In addition, the addition of acetic acid has substantial effect on selectivity and efficiency of some compounds.

Ginsenosides, ligans, steroidal glycosides and homoisoflavonoids were the main constituents of SMS extract. According to the study on the characteristic UV profile of these main constituents, ginsenosides and steroidal glycosides were detected at 203 nm, while lignans and homoisoflavonoids were performed

at 254 nm and 296 nm, respectively. Fifty-three compounds from SMS were detected under the current HPLC condition and were characterized by comparing their retention times and UV spectra in the chromatogram of SMS with those of authentic compounds and single herb extracts. The representative HPLC–DAD chromatograms of the extract of SMS and single crude drugs are presented in Fig. 1.

### 3.2. HPLC–MS/MS analysis of SMS extract

#### 3.2.1. Tandem mass spectrometry of authentic compounds

In order to obtain MS fragmentation patterns of constituents from SMS extract, 32 authentic compounds were studied by HPLC–DAD–MS/MS in both negative and positive ion modes. The authentic compounds could be classified into four groups according to their chemical structures and their dominant fragmentation pathways were studied. In the full scan mass spectra, most of the authentic compounds exhibited $[M-H]^-$ and/or $[M+H]^+$ ions of sufficient abundance that could be subjected to MS/MS analysis. The proposed fragmentation patterns were helpful for the structural identification of constituents with similar fragmentation patterns in SMS extract and the bioactive constituents in rat plasma after oral administration of SMS.

#### 3.2.2. HPLC–MS/MS analysis of the SMS extract

The screening, identification and further characterizing of components in SMS extract as well as in *Radix ginseng*, *Radix ophiopogonis* and *Fructus schisandrae* were performed by HPLC–DAD–MS/MS in both the positive and negative ion modes.

Twenty-seven peaks in the HPLC–DAD and HPLC–MS/MS (TIC) chromatograms were unequivocally identified by comparisons of their retention times, MS data and UV spectra with those of authentic compounds. The other 26 peaks were identified tentatively by comparing their UV spectrum, molecular weight and structural information from MS/MS spectra with published literature data. The retention time ($t_R$), λ-max, MS data and the most characteristic fragments of the reference compounds and identified peaks are listed in Table 1. Their chemical structures are shown in Table 2. The UV chromatograms at 254 nm, 296 nm, 203 nm and MS TIC chromatograms of SMS extract were presented in Fig. 2.

*3.2.2.1. Identification of ginsenosides in SMS extract.* The fragmentation of 8 ginsenosides standards was studied and the major fragmentation mechanisms of ginsenosides were concluded. In the negative MS experiments, the common fragmentation behavior was the successive or simultaneous losses of sugar units substituted at O–C (20), O–C (3) or O–C (6). The aglycone ions at *m/z* 475 or 459 could be observed occasionally. The positive ion mode could give more fragment ions except for the losses of the sugar moieties. The characteristic ions at *m/z* 443, 425 and 407 were observed for the protopanaxadiol type, while fragment ions for the protopanaxatriol type were at *m/z* 441, 423 and 405 [12,13]. Compared the retention time and MS spectra of SMS extract with those of authentic compounds, peaks 1, 5, 6, 7, 8, 22 and 24 were unambiguously identified.

Based on fragmentation patterns, the chemical structures of peaks 4, 14, 17, 30, 33, 37 and 39 in the complex mixtures were identified, by comparison of their retention behavior and MS/MS spectra with the literature data [14–16]. Among them, peaks 14, 17, 37 and 39 showed the same $[M-H]^-$ ions at *m/z* 765. However, in the positive-ion MS/MS spectra, peaks 14 and 17 afforded ions at *m/z* 603,441,423,405, while peaks 37 and 39 showed ions at *m/z* 587, 443, 425, 407. After comparison with the literature data, peaks 14 and 17 were identified as a pair of the geometric isomers ginsenoside F4 or Rg6. Peaks 37 and 39 were identified as another pair of geometric isomers ginsenoside Rg5 or Rk1 [15].

*3.2.2.2. Identification of steroidal saponins in SMS extract.* The eight steroidal saponins were summarized as four types of aglycone skeletons namely ruscogenin type, diosgenin type, ophiogenin type and prazerigenin A type. The fragmentation patterns of the authentic compounds were summarized, and positive ion mode was found to be more suitable for the identification of steroidal saponins than negative mode. In the negative ion mode, a series of fragment ions were produced by the successive or simultaneous losses of sugar units from $[M-H]^-$ ions. In the positive ions mode, the MS/MS spectra of the $[M+H]^+$ ions also displayed ions arising from the eliminated sugar moieties substituted at O–C (1) or O–C (3), followed by the characteristic fission of aglycone to form the diagnostic ions $[Aglycone+H]^+$, $[Aglycone+H-H_2O]^+$, $[Aglycone+H-144]^+$, and $[Aglycone+H-H_2O-144]^+$. Compared the retention time and MS/MS spectra of SMS with those of authentic compounds, peaks 3, 9, 10, 15, 26 and 27 were unambiguously identified.

Ophiopogonin D (peak 26, $t_R$ = 86.33 min) was used to characterize the fragmentation pathways in the HPLC/MS/MS experiments. It showed $[M-H]^-$ at *m/z* 853, $[M+CH_3COO]^-$ at *m/z* 913 and afforded ions at *m/z* 721 owing to the loss of a xylose residue. In the positive ion mode, it displayed $[M+H]^+$ at *m/z* 855 and afforded ions at *m/z* 723, 577, and 431, originating from the successive or simultaneous loss of xylose, fucopyranose and rhamnose residues, respectively. The diagnostic ions at *m/z* 413, 287, 269 and 251 resulted from sequential loss of 18 Da, 144 Da, 18 Da and 18 Da, respectively. The elimination of 144 Da was produced by the cleavage of E-ring [17,18]. Similar fragmentation pathways were observed in the spectra of other steroidal saponins. It should be noted that compound 10 (ophiogenin type) and compound 15 (prazerigenin A type) displayed some differences in the fragmentation patterns from ophiopogonin D, the MS/MS spectra of $[M+H]^+$ ions generally yielded a series of fragment ions originating from the successive losses of $H_2O$ (18 Da) before losing the sugar units.

*3.2.2.3. Identification of homoisoflavonoids in SMS extract.* Homoisoflavonoids are a type of special flavonoids with their B-rings and C-rings connected by an additional $CH_2$ group. According to the MS/MS analysis of 10 homoisoflavonoid standard compounds, their diagnostic ions were $[M-H-B\text{-}ring-CH_2+H]^-$ at *m/z* 207, 193 or 223 and $[M-H-B\text{-}ring]^-$ at *m/z* 218 or 204 from the loss of the benzyl part of the B-ring. Some common fragmentations occurred by losses of $CH_3$ (15 Da), $H_2O$ (18 Da), $CO_2$ (44 Da), $CH_2O_2$ (46 Da) as well as by an RDA-like cleavage [19].

For example, peak 29 (ophiopogonanone A, $t_R$ = 87.27 min) exhibited the $[M-H]^-$ ion at 327 and afforded ions at *m/z* 309, 283 and 281, originating from the successive loss of $H_2O$ (18 Da), $CO_2$ (44 Da) and $CH_2O_2$ (46 Da), respectively. This suggested the presence of a hydroxyl group and a methylenedioxy group. In addition, the presence of ions at *m/z* 193 were ascribed to the cleavage of C3–C9 bond [9].

Peaks 11, 16, 19, 36, 40 and 42 were unambiguously identified by comparison with the authentic compounds. Peaks 2, 13, 31 and 49 were deduced by comparing with the literature data, they were tentatively identified as 5, 7, 2′, 4′-tetrahydroxy-8-methoxy-6-methylhomoisoflavanone [19], 5,7,4′-trihydroxy-6-methylhomoisoflavanone [20], 5,7-dihydroxy-4′-methoxy-6-methylhomoisoflavanone [19] and 6-formyl-isoophiopogonanone A [9], respectively.

*3.2.2.4. Identification of lignans in SMS extract.* Peaks 18, 25, 44, 50, 52 and 53 were unambiguously identified by comparing with the authentic compounds. Based on similar fragmentation

**Table 1**
Characterization of compounds in extract of SMS by HPLC–DAD–MS/MS.

| Peak no. | Retention time (min) | λ-max (nm) | Compound | Plant material | Negative ions (*m*/*z*) | Positive ions (*m*/*z*) |
|---|---|---|---|---|---|---|
| 1[a] | 57.70 | – | Ginsenoside Rf | RG | $[M-H]^-$: 799, $[M-H-Glc]^-$: 637, $[M-H-2Glc]^-$: 475 | $[M+Na]^+$: 823, $[M+H]^+$: 801, $[M+H-Glc-2H_2O]^+$: 603, $[M+H-Glc-3H_2O]^+$: 585, $[Aglycone+H-3H_2O]^+$: 441, $[Aglycone+H-4H_2O]^+$: 423, $[Aglycone+H-5H_2O]^+$: 405 |
| 2 | 58.90 | 296 | 5,7,2′,4′-tetrahydroxy-8-methoxy-6-methyl homoisoflavanone | RO | $[M-H]^-$: 345, $[M-H-CH_3]^-$: 330, $[M-H-B\text{-}ring-CH_2+H]^-$: 223 | $[M+Na]^+$: 369, $[M+H]^+$: 347 |
| 3[a] | 59.39 | – | Ruscogenin1-O-α-L-rhamnopyranosyl-(1 → 2)-4-O-sulfate-α-L-arabinopyranoside-3-O-β-D-glucopyranoside | RO | $[M-H]^-$: 949, $[M-H-Rha]^-$: 803, $[M-H-Glc]^-$: 787 | $[M+H]^+$: 951, $[M+H-Rha]^+$: 805, $[M+H-SO_3-Rha]^+$: 725, $[Aglycone+H]^+$: 431, $[Aglycone+H-H_2O]^+$: 413, $[Aglycone+H-2H_2O]^+$: 395 $[Aglycone+H-144]^+$: 287, $[Aglycone+H-144-H_2O]^+$: 269 |
| 4 | 60.55 | – | Ginsenoside Ro | RG | $[M-H]^-$: 955, $[M-H-Glc]^-$: 793 | $[M+Na]^+$: 978, $[M+H-Glc]^+$: 795, $[M+H-Glc-H_2O]^+$: 777, $[Aglycone+H-H_2O]^+$: 439 |
| 5[a] | 62.02 | – | 20(*S*)-ginsenoside Rg2 | RG | $[M-H]^-$: 783, $[M+CH_3COO]^-$: 843, $[M-H-Rha]^-$: 637, $[M-H-Rha-Glc]^-$: 475 | $[M+H]^+$: 785, $[M+H-Rha]^+$: 639, $[M+H-Rha-H_2O]^+$: 621, $[M+H-Rha-2H_2O]^+$: 603, $[Aglycone+H-3H_2O]^+$: 441, $[Aglycone+H-4H_2O]^+$: 423, $[Aglycone+H-5H_2O]^+$: 405 |
| 6[a] | 62.68 | – | 20(*R*)-ginsenoside Rg2 | RG | $[M-H]^-$: 783, $[M-H-Rha]^-$: 637 | $[M+H]^+$: 785, $[M+H-Rha]^+$: 639, $[Aglycone+H-3H_2O]^+$: 441, $[Aglycone+H-4H_2O]^+$: 423, $[Aglycone+H-5H_2O]^+$: 405 |
| 7[a] | 62.96 | – | 20(*S*)-ginsenoside Rh1 | RG | $[M-H]^-$: 637, $[M+CH_3COO]^-$: 697, $[M-H-Glc]^-$: 475 | $[M+H]^+$: 639, $[M+H-H_2O]^+$: 621, $[M+H-2H_2O]^+$: 603, $[M+H-3H_2O]^+$: 585, $[M+H-4H_2O]^+$: 567, $[Aglycone+H-3H_2O]^+$: 441, $[Aglycone+H-4H_2O]^+$: 423, $[Aglycone+H-5H_2O]^+$: 405 |

Table 1 (*Continued*)

| Peak no. | Retention time (min) | λ-max (nm) | Compound | Plant material | Negative ions (*m*/*z*) | Positive ions (*m*/*z*) |
|---|---|---|---|---|---|---|
| 8[a] | 64.00 | – | 20(*R*)-ginsenoside Rh1 | RG | $[M-H]^-$: 637, $[M+CH_3COO]^-$: 697, $[M-H-Glc]^-$: 475 | $[M+H]^+$: 639, $[M+H-H_2O]^+$: 621, $[M+H-2H_2O]^+$: 603, $[M+H-3H_2O]^+$: 585, $[M+H-4H_2O]^+$: 567, $[Aglycone+H-3H_2O]^+$: 441, $[Aglycone+H-4H_2O]^+$: 423, $[Aglycone+H-5H_2O]^+$: 405 |
| 9[a] | 65.72 | – | Ruscogenin 1-O-α-L-rhamnopyranosyl-(1 → 2)-4-O-sulfate-α-L-arabinopyranoside | RO | $[M-H]^-$: 787, $[M-H-H_2O]^-$: 769, $[M-H-SO_3-2H_2O]^-$: 671 | $[M+H]^+$: 789, $[M+H-SO_3]^+$: 709, $[M+H-Rha]^+$: 643, $[M+H-SO_3-Rha]^+$: 563, $[Aglycone+H]^+$: 431, $[Aglycone+H-H_2O]^+$: 413, $[Aglycone+H-144]^+$: 287, $[Aglycone+H-144-H_2O]^+$: 269, $[Aglycone+H-144-2H_2O]^+$: 251 |
| 10[a] | 68.52 | – | Ophiogenin 3-O-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside | RO | $[M-H]^-$: 753, $[M+CH_3COO]^-$: 813, $[M-H-Rha]^-$: 607 | $[M+Na]^+$: 777, $[M+H-H_2O]^+$: 737, $[M+H-2H_2O]^+$: 719, $[M+H-3H_2O]^+$: 701, $[M+H-H_2O-Rha]^+$: 591, $[M+H-2H_2O-Rha]^+$: 573, $[M+H-3H_2O-Rha]^+$: 555, $[Aglycone+H-H_2O]^+$: 429, $[Aglycone+H-2H_2O]^+$: 411, $[Aglycone+H-3H_2O]^+$: 393, $[Aglycone+H-144-2H_2O]^+$: 267 |
| 11[a] | 69.33 | 262 | Desmethylisophiopogonone B | RO | $[M-H]^-$: 297, $[M-H-B\text{-}ring-CH_2+H]^-$: 191 | $[M+H]^+$: 299, $[M+Na]^+$: 321 |
| 12 | 71.25 | 217, 254 | Gomisin S | SC | – | $[M+H]^+$: 433, $[M+H-H_2O]^+$: 415, $[M+H-H_2O-CH_3O]^+$: 384 |
| 13 | 72.13 | 295 | 5,7,4′-trihydroxy-6-methyl homoisoflavanone | RO | $[M-H]^-$: 299, $[M-H-B\text{-}ring-CH_2+H]^-$: 193 | $[M+H]^+$: 301, $[M+Na]^+$: 323, $[M+Na]^+$: 789 |
| 14 | 75.00 | – | Ginsenoside Rg6/ginsenoside F4 | RG | $[M-H]^-$: 765, $[M-H-Rha]^-$: 619 | $[M+H]^+$: 767, $[M+H-Rha]^+$: 621, $[M+H-Rha-H_2O]^+$: 603, $[M+H-Rha-2H_2O]^+$: 585, $[M+H-Rha-3H_2O]^+$: 567, $[Aglycone+H-3H_2O]^+$: 441, $[Aglycone+H-4H_2O]^+$: 423, $[Aglycone+H-5H_2O]^+$: 405 |

Table 1 (*Continued*)

| Peak no. | Retention time (min) | λ-max (nm) | Compound | Plant material | Negative ions (*m*/*z*) | Positive ions (*m*/*z*) |
|---|---|---|---|---|---|---|
| 15[a] | 76.00 | – | Prazarifenin A 3-O-α-L-rhamnopyranosyl-(1–2)-β-D-glucopyranoside | RO | $[M-H]^-$: 737, $[M+CH_3COO]^-$: 797, $[M-H-Rha]^-$: 591 | $[M+Na]^+$: 761, $[M+H]^+$: 739, $[M+H-H_2O]^+$: 721, $[M+H-2H_2O]^+$: 703, $[Aglycone+H]^+$: 431, $[Aglycone+H-H_2O]^+$: 413, $[Aglycone+H-2H_2O]^+$: 395, $[Aglycone+H-3H_2O]^+$: 377, $[Aglycone+H-144-H_2O]^+$: 269, $[Aglycone+H-144-2H_2O]^+$: 251 |
| 16[a] | 76.27 | 296 | 5,7,4′-trihydroxy-3′,5′-methoxy-6,8-dimethyl homoisoflavanone | RO | $[M-H]^-$: 373, $[M-H-CH_3O]^-$: 342, $[M-H-2CH_3O]^-$: 311, $[M-H-B\text{-}ring-CH_2+H]^-$: 207 | $[M+H]^+$: 375, $[M+Na]^+$: 397 |
| 17 | 76.47 | – | Ginsenoside Rg6/ginsenoside F4 | RG | $[M-H]^-$: 765, $[M-H-Rha]^-$: 619, $[M-H-Rha-H_2O]^-$: 601 | $[M+H]^+$: 767, $[M+H-H_2O]^+$: 749, $[M+H-2H_2O]^+$: 731, $[M+H-Rha-H_2O]^+$: 603, $[M+H-Rha-2H_2O]^+$: 585, $[M+H-Rha-3H_2O]^+$: 567, $[Aglycone+H-3H_2O]^+$: 441, $[Aglycone+H-4H_2O]^+$: 423, $[Aglycone+H-5H_2O]^+$: 405 |
| 18[a] | 76.77 | 216,252,280 | Schizandrol A | SC | – | $[M+H]^+$: 433, $[M+H-H_2O]^+$: 415, $[M+H-H_2O-CH_3O]^+$: 384 |
| 19[a] | 78.80 | 296 | 5,7,3′-trihydroxy-4′-methoxy-6,8-dimethyl homoisoflavanone | RO | $[M-H]^-$: 343, $[M-H-CO_2]^-$: 299, $[M-H-B\text{-}ring-CH_2+H]^-$: 207 | $[M+H]^+$: 345, $[M+Na]^+$: 367 |
| 20[a] | 79.47 | 296 | Ophiopogonanone E | RO | $[M-H]^-$: 359, $[M-H-CH_3]^-$: 344, $[M-H-B\text{-}ring-CH_2+H]^-$: 223, $[M-H-B\text{-}ring-CH_2+H-CH_3]^-$: 208 | $[M+H]^+$: 361, $[M+Na]^+$: 383 |
| 21 | 79.93 | 214,254,294 | Gomisin D | SC | – | $[M+H]^+$: 531, $[M+H-CH_2O_2]^+$: 485, $[M+H-C_6H_{10}O_3]^+$: 401, $[M+H-C_6H_{10}O_3-H_2O]$: 383 |

Table 1 (*Continued*)

| Peak no. | Retention time (min) | λ-max (nm) | Compound | Plant material | Negative ions (*m*/*z*) | Positive ions (*m*/*z*) |
|---|---|---|---|---|---|---|
| 22[a] | 81.32 | – | 20(*S*)-ginsenoside Rg3 | RG | $[M-H]^-$: 783, $[M-H-Glc]^-$: 621 | $[M+Na]^+$: 807, $[M+H]^+$: 785, $[M+H-H_2O]^+$: 767, $[M+H-2H_2O]^+$: 749, $[M+H-3H_2O]^+$: 731, $[M+H-Glc-H_2O]^+$: 605, $[M+H-Glc-2H_2O]^+$: 587, $[M+H-Glc-3H_2O]^+$: 569, $[Aglycone+H-2H_2O]^+$: 443, $[Aglycone+H-3H_2O]^+$: 425, $[Aglycone+H-4H_2O]^+$: 407 |
| 23 | 81.63 | 220,258,280 | Gomisin J | SC | – | $[M+H]^+$: 389, $[M+H-CH_3O]^+$: 358, $[M+H-CH_3O-CH_3]^+$: 343 |
| 24[a] | 82.49 | – | 20(*R*)-ginsenoside Rg3 | RG | $[M-H]^-$: 783, $[M-H-Glc]^-$: 621 | $[M+Na]^+$: 807, $[M+H]^+$: 785, $[M+H-H_2O]^+$: 767, $[M+H-2H_2O]^+$: 749, $[M+H-3H_2O]^+$: 731, $[M+H-Glc-H_2O]^+$: 605, $[M+H-Glc-2H_2O]^+$: 587, $[M+H-Glc-3H_2O]^+$: 569, $[Aglycone+H-2H_2O]^+$: 443, $[Aglycone+H-3H_2O]^+$: 425, $[Aglycone+H-4H_2O]^+$: 407 |
| 25[a] | 82.60 | 220,255,284 | Schizandrol B | SC | – | $[M+Na]^+$: 439, $[M+H]^+$: 417, $[M+H-H_2O]^+$: 399 |
| 26[a] | 86.12 | – | Ophiopogonin D | RO | $[M-H]^-$: 853, $[M+CH_3COO]^-$: 913, $[M-H-Xyl]^-$: 721, $[Aglycone-H]^-$: 429 | $[M+Na]^+$: 877, $[M+H]^+$: 855, $[M+H-Xyl]^+$: 723, $[M+H-Xyl-Rha]^+$: 577, $[M+H-Xyl-Rha-H_2O]^+$: 559, $[Aglycone+H]^+$: 431 $[Aglycone+H-H_2O]^+$: 413, $[Aglycone+H-2H_2O]^+$: 395, $[Aglycone+H-3H_2O]^+$: 377, $[Aglycone+H-144]^+$: 287, $[Aglycone+H-144-H_2O]^+$: 269 |
| 27[a] | 86.31 | – | Ophiopogonin D′ | RO | $[M-H]^-$: 853, $[M+CH_3COO]^-$: 913, $[M-H-Xyl]^-$: 721 | $[M+Na]^+$: 877, $[M+H]^+$: 855, $[M+H-Xyl]^+$: 723, $[M+H-Xyl-H_2O]^+$: 705, $[M+H-Xyl-Rha]^+$: 577, $[M+H-Xyl-Rha-H_2O]^+$: 559, $[M+H-Xyl-Rha-2H_2O]^+$: 541, $[M+H-Xyl-Rha-3H2O]^+$: 523, $[Aglycone+H]^+$: 415, $[Aglycone+H-H_2O]^+$: 397, $[Aglycone+H-144]^+$: 271, $[Aglycone+H-144-H_2O]^+$: 253 |

Table 1 (*Continued*)

| Peak no. | Retention time (min) | λ-max (nm) | Compound | Plant material | Negative ions (*m*/*z*) | Positive ions (*m*/*z*) |
|---|---|---|---|---|---|---|
| 28 | 86.42 | 214,248,278 | Trigloylgomisin H | SC | – | $[M+Na]^+$: 523, $[M+H]^+$: 501, $[M+H-H_2O]^+$: 483, $[M+H-C_5H_{10}]^+$: 431, $[M+H-C_5H_8O_2]^+$: 401 |
| 29[a] | 87.27 | 294 | Ophiopogonanone A | RO | $[M-H]^-$: 327, [M−H−B-ring+H]$^-$: 205, [M−H−B-ring−$CH_2$]$^-$: 192 | $[M+H]^+$: 329, $[M+Na]^+$: 351 |
| 30 | 88.37 | – | Ginsenoside Rs3/isomer | RG | $[M-H]^-$: 825, [M−H−AC]$^-$: 783 | $[M+Na]^+$: 849, $[M+H-H_2O]^+$: 809, $[M+H-2H_2O]^+$: 791, [Aglycone+H−$2H_2O$]$^+$: 443, [Aglycone+H−$3H_2O$]$^+$: 425, [Aglycone+H−$4H_2O$]$^+$: 407 |
| 31 | 88.49 | 293 | 5,7-dihydroxy-4′-methoxy-6-methyl homoisoflavanone | RO | $[M-H]^-$: 313, [M−H−$CO_2$]$^-$: 297, [M−H−B-ring−$CH_2$+H]$^-$: 191 | $[M+H]^+$: 315, $[M+Na]^+$: 337 |
| 32 | 88.63 | 214,250,285 | Angeloylgomisin H | SC | – | $[M+Na]^+$: 523, $[M+H]^+$: 501, $[M+H-H_2O]^+$: 483, $[M+H-C_5H_{10}]^+$: 431, $[M+H-C_5H_8O_2]^+$: 401 |
| 33 | 88.75 | – | Ginsenoside Rs3/isomer | RG | $[M-H]^-$: 825, [M−H−AC]$^-$: 783 | $[M+Na]^+$: 849, [M+Na−$H_2O$]$^+$: 831, $[M+H-H_2O]^+$: 809, $[M+H-2H_2O]^+$: 791, [Aglycone+H−$2H_2O$]$^+$: 443, [Aglycone+H−$3H_2O$]$^+$: 425, [Aglycone+H−$4H_2O$]$^+$: 407 |
| 34 | 89.48 | 212,254,280 | Benzoylgomisin H | SC | – | $[M+H]^+$: 523, $[M+H-H_2O]^+$: 505, $[M+H-2H_2O]^+$: 487, $[M+H-C_7H_6O_2]^+$: 401 |
| 35 | 90.40 | 218,255,280 | Benzoylgomisin Q | SC | – | $[M+H]^+$: 553, $[M+H-C_7H_6O_2]^+$: 431, $[M+H-C_7H_6O_3]^+$: 415, $[M+H-C_7H_6O_2-C_2H_4O]^+$: 387 |
| 36[a] | 90.87 | 264 | Methylophiopogonone A | RO | $[M-H]^-$: 339, [M−H−CO]$^-$: 311, [M−H−B-ring−$CH_2$]$^-$: 204 | $[M+H]^+$: 341, $[M+Na]^+$: 363 |
| 37 | 91.38 | – | Ginsenoside Rk1/ginsenoside Rg5 | RG | $[M-H]^-$: 765 | $[M+H]^+$: 767, $[M+H-H_2O]^+$: 749, $[M+H-2H_2O]^+$: 731, [M+H−$H_2O$−Glc]$^+$: 587, [Aglycone+H−$2H_2O$]$^+$: 443, [Aglycone+H−$3H_2O$]$^+$: 425, [Aglycone+H−$4H_2O$]$^+$: 407 |
| 38 | 91.78 | 220 | Tigloylgomisin P | SC | – | $[M+NH_4]^+$: 532, [M+H−CO−$H_2O$]$^+$: 469, $[M+H-C_5H_8O_2]^+$: 415, [M+H−$C_5H_8O_2$−$CH_3O$]$^+$: 384, [M+H−$C_5H_8O_2$−$C_2H_4O$]$^+$: 371 |

Table 1 (*Continued*)

| Peak no. | Retention time (min) | λ-max (nm) | Compound | Plant material | Negative ions (*m*/*z*) | Positive ions (*m*/*z*) |
|---|---|---|---|---|---|---|
| 39 | 91.95 | – | Ginsenoside Rk1/ginsenoside Rg5 | RG | $[M-H]^-$: 765 | $[M+H-H_2O]^+$: 749, $[M+H-2H_2O]^+$: 731, $[M+H-H_2O-Glc]^+$: 587, $[M+H-2H_2O-Glc]^+$: 569, $[M+H-3H_2O-Glc]^+$: 551, $[Aglycone+H-2H_2O]^+$: 443, $[Aglycone+H-3H_2O]^+$: 425, $[Aglycone+H-4H_2O]^+$: 407 |
| 40[a] | 92.05 | 296 | Methylophiopogonanone A | RO | $[M-H]^-$: 341, $[M-H-CH_3]^-$: 325, $[M-H-B\text{-}ring-CH_2]^-$: 206, $[M-H-B\text{-}ring-CH_2-CO]^-$: 178 | $[M+H]^+$: 343, $[M+Na]^+$: 365 |
| 41 | 92.97 | 224,256,284 | Gomisin G | SC | – | $[M+H]^+$: 537, $[M+H-C_7H_6O_2]^+$: 415, $[M+H-C_7H_6O_2-C_2H_4O]^+$: 371, $[M+H-C_7H_6O_2-C_2H_4O-CO]^+$: 343 |
| 42[a] | 93.32 | 296 | Methylophiopogonanone B | RO | $[M-H]^-$: 327, $[M-H-CH_3]^-$: 312, $[M-H-B\text{-}ring-CH_2]^-$: 206, $[M-H-B\text{-}ring-CH_2+H-CO]^-$: 178 | $[M+H]^+$: 329, $[M+Na]^+$: 351 |
| 43 | 93.58 | 218,252,290 | SchisantherinC/ schisantherin B | SC | – | $[M+Na]^+$: 537, $[M+H]^+$: 515, $[M+H-C_5H_8O_2]^+$: 415, $[M+H-C_5H_8O_2-C_2H_4O]^+$: 371 |
| 44[a] | 93.92 | 222,254,285 | Schisantherin A | SC | – | $[M+NH4]^+$: 554 $[M+H-C_7H_6O_2]^+$: 415, $[M+H-C_7H_6O_2-C_2H_4O]^+$: 371, $[M+H-C_7H_6O_2-C_2H_4O-CH_3O]^+$: 340 |
| 45 | 95.10 | 219, 250 | Schisanhenol | SC | – | $[M+H]^+$: 403, $[M+H-H_2O]^+$: 385, $[M+H-H_2O-CH_3]^+$: 370, $[M+H-H_2O-CH_3-CH_3O]^+$: 339 |
| 46 | 96.45 | 218,254,284 | Gomisin B | SC | – | $[M+Na]^+$: 537, $[M+H]^+$: 515, $[M+H-C_5H_8O_2]^+$: 415 |
| 47 | 96.77 | 217 | Gomisin E | SC | – | $[M+Na]^+$: 537, $[M+H]^+$: 515, $[M+H-C_6H_{10}O_3]^+$: 385 |
| 48 | 97.30 | 222,255,280 | Gomisin F | SC | – | $[M+Na]^+$: 537, $[M+H]^+$: 515, $[M+H-C_5H_8O_2]^+$: 415, $[M+H-C_5H_8O_2-CO]^+$: 387 |
| 49 | 99.78 | 274 | 6-formyl-isoophiopogonanone A | RO | $[M-H]^-$: 355, $[M-H-CO]^-$: 327, $[M-H-CO-B\text{-}ring-CH_2+H]^-$: 193 | $[M+H]^+$: 357, $[M+Na]^+$: 379 |
| 50[a] | 100.78 | 216,248,280 | Schizandrin A | SC | – | $[M+H]^+$: 417, $[M+H-CH_3O]^+$: 386, $[M+H-C_5H_{10}]^+$: 347 |
| 51 | 102.87 | 216,254,280 | Gomisin N | SC | – | $[M+H]^+$: 401, $[M+H-CH_3O]^+$: 371, $[M+H-C_5H_{10}]^+$: 331, $[M+H-C_5H_{10}-CH_3O]^+$: 300 |

Table 1 (*Continued*)

| Peak no. | Retention time (min) | λ-max (nm) | Compound | Plant material | Negative ions (*m*/*z*) | Positive ions (*m*/*z*) |
|---|---|---|---|---|---|---|
| 52[a] | 103.57 | 216,254,280 | Schizandrin B | SC | – | $[M+H]^+$: 401, $[M+H-CH_3O]^+$: 371, $[M+H-C_5H_{10}]^+$: 331 |
| 53[a] | 104.92 | 220,258,284 | Schizandrin C | SC | – | $[M+H]^+$: 385, $[M+H-CH_3O]^+$: 354 |

RG, *Red ginseng*; RO, *Radix ophiopogonis*; SC, Schisandra chinensis; Glc, glucose; Xyl, xylose; Fuc, fucose; Rha, rhamnose; AC, 6-O-acetyl.
[a] Compared with authentic compounds.

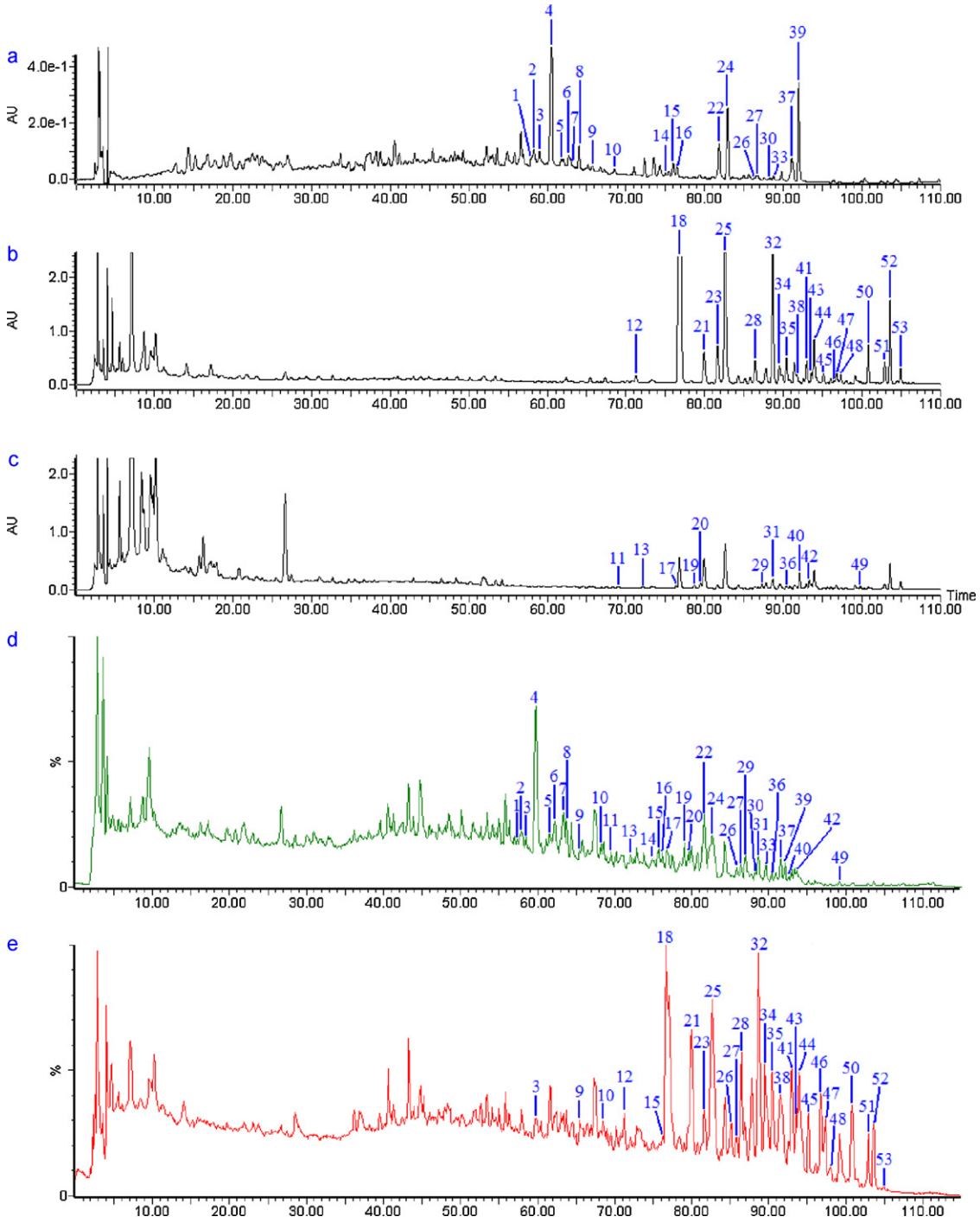



**Fig. 2.** HPLC–DAD–MS/MS analysis of extract of SMS. (a) HPLC–DAD chromatogram at 203 nm; (b) HPLC–DAD chromatogram at 254 nm; (c) HPLC–DAD chromatogram at 296 nm; (d) Negative ion mode MS spectra; and (e) Positive ion mode MS spectra.

**Table 2**
Chemical structures of compounds identified in SMS extract and rat plasma sample after oral administration of SMS extract.

Ginsenosides

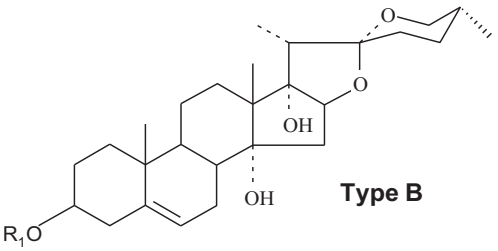
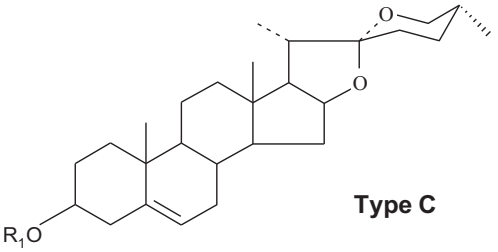
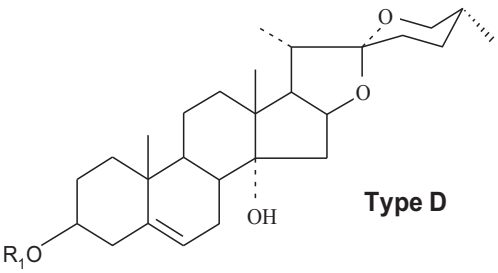
| Peak no. | Type | Ginsenoside | $R_1$ | $R_2$ | $R_3$ | MW |
|---|---|---|---|---|---|---|
| | | | | | | |
| 1 | A | Rf | –H | –O–Glc$^2$–Glc | H | 800 |
| 4 | D | Ro | – | – | – | 956 |
| 5/6 | A | 20(S)-Rg2/20(R)-Rg2 | –H | –O–Glc$^2$–Rha | H | 784 |
| | | | | | | |
| 7/8 | A | 20(S)-Rh1/20(R)-Rh1 | –H | –O–Glc | H | 638 |
| 14/17 | B | F4 | –H | –O–Glc$^2$–Rha | – | 766 |
| 14/17 | C | Rg6 | –H | –O–Glc$^2$–Rha | – | 766 |
| | | | | | | |
| 22/24 | A | 20(S)-Rg3/20(R)-Rg3 | –Glc$^2$–Glc | –H | –H | 784 |
| 30/33 | A | Rs3/iso | –Glc$^2$–Glc$^6$–AC | –H | –H | 826 |
| | | | | | | |
| 37/39 | B | Rg5 | –Glc$^2$–Glc | –H | – | 766 |
| 37/39 | C | Rk1 | –Glc$^2$–Glc | –H | – | 766 |
| M11 | A | Protopanaxadiol | –H | –H | –H | 460 |

Steroidal saponins

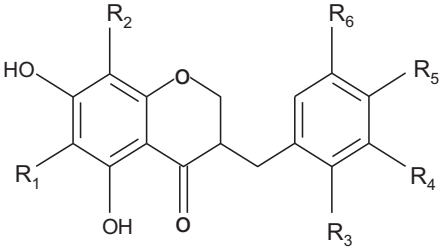
| Peak no. | Type | Compound | $R_1$ | $R_2$ | MW |
|---|---|---|---|---|---|
| 3 | A | Ruscogenin1-O-α-L-rhamno pyranosyl-(1 → 2)-4-O-sulfate-α-L-arabinopyranoside-3-O-β-D-glucopyranoside | -Rha-4-O-sulfate –Ara | -Glc | 950 |

**Table 2**
(*Continued*)

Steroidal saponins

| Peak no. | Type | Compound | $R_1$ | $R_2$ | MW |
|---|---|---|---|---|---|
| 9 | A | Ruscogenin1-O-α-L-rhamnopyranosyl-(1 → 2)-4-O-sulfate-α-L-arabinopyranoside | -α-L-Rha-(1 → 2)-4-O-sulfate-α-L-Ara | H | 788 |
| | | | | |  Type B |
| 10 | B | Ophiogenin3-O-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside | -α-L-Rha-(1 → 2)-β-D-Glc | – | 754 |
| | | | | |  Type C |
| 15 | D | PrazarifeninA 3-O-α-L-rhamnopyranosyl-(1–2)-β-D-glucopyranoside | -α-L-Rha-(1–2)-β-D-Glc | – | 738 |
| 26 | A | Ophiopogonin D | -α-L-Rha-(1 → 2)-β-D-Xyl (1 → 3)-β-D-Fuc | – | 854 |
| | | | | |  Type D |
| 27 | C | Ophiopogonin D′ | -α-L-Rha-(1 → 2)-β-D-Xyl(1 → 3)-β-D-Glc | – | 854 |

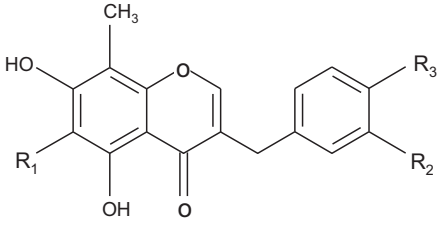
Homoisoflavonoids



| Peak no. | Compound | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ | MW |
|---|---|---|---|---|---|---|---|---|
| 2 | 5,7,2′,4′-Tetrahydroxy-8-methoxy-6-methyl homoisoflavanone | $CH_3$ | $OCH_3$ | OH | H | OH | H | 346 |
| 13 | 5,7,4′-Trihydroxy-6-methyl homoisoflavanone | $CH_3$ | H | H | H | OH | H | 300 |
| 16 | 5,7,4′-Trihydroxy-3′,5′-methoxy-6,8-dimethyl homoisoflavanone | $CH_3$ | $CH_3$ | OH | $OCH_3$ | OH | $OCH_3$ | 374 |
| 19 | 5,7,3′-Trihydroxy-4′-methoxy-6,8-dimethyl homoisoflavanone | $CH_3$ | $CH_3$ | H | OH | $OCH_3$ | H | 344 |
| 20 | Ophiopogonanone E | $CH_3$ | $OCH_3$ | OH | H | $OCH_3$ | H | 360 |
| 29 | Ophiopogonanone A | $CH_3$ | H | H | –O–$CH_2$–O– | | H | 328 |
| 31 | 5,7-Dihydroxy-4′-methoxy-6-methyl homoisoflavanone | $CH_3$ | H | H | H | $OCH_3$ | H | 314 |
| 40 | Methylophiopogonanone A | $CH_3$ | $CH_3$ | H | –O–$CH_2$–O– | | H | 342 |
| 42 | Methylophiopogonanone B | $CH_3$ | $CH_3$ | H | H | $OCH_3$ | H | 328 |

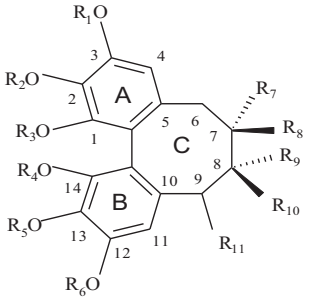
Table 2 ( *Continued* ).

| Peak no. | Compound | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ | MW |
|---|---|---|---|---|---|---|---|---|
| 49 | 6-Formyl-isoophiopogonanone A | CHO | $CH_3$ | H | –O–$CH_2$–O– | | H | 356 |

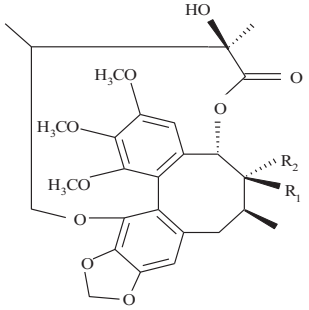



| Peak no. | Compound | $R_1$ | $R_2$ | $R_3$ | – | – | – | MW |
|---|---|---|---|---|---|---|---|---|
| 11 | Desmethylisoophiopogonone B | H | H | OH | – | – | – | 298 |
| 36 | Methylophiopogonone A | $CH_3$ | –O–$CH_2$–O– | – | – | – | – | 340 |

Lignans




| Peak no. | Compound | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ | $R_7$ | $R_8$ | $R_9$ | $R_{10}$ | $R_{11}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 12 | Gomisin S | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | OH | H | H | $CH_3$ | H |
| 18 | Schizandrol A | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | H | H | $CH_3$ | OH | H |
| 23 | Gomisin J | H | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | H | $CH_3$ | H | $CH_3$ | H | H |
| 25 | Schizandrol B | –$CH_2$– | | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | H | $CH_3$ | OH | H |
| 28 | Trigloylgomisin H | $CH_3$ | $CH_3$ | Tigloyl | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | H | $CH_3$ | OH | H |
| 32 | Angeloylgomisin H | $CH_3$ | $CH_3$ | Angeloyl | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | H | $CH_3$ | OH | H |
| 34 | Benzoylgomisin H | $CH_3$ | $CH_3$ | Benzoyl | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | H | $CH_3$ | OH | H |
| 35 | Benzoylgomisin Q | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | H | $CH_3$ | $CH_3$ | OH | Benzoyl |
| 38 | Tigloylgomisin P | –$CH_2$– | | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | H | $CH_3$ | OH | $CH_3$ | –OTig |
| 41 | Gomisin G | –$CH_2$– | | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | OH | $CH_3$ | H | –OBz |
| 43 | Schisantherin C | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | –$CH_2$– | | $CH_3$ | OH | $CH_3$ | H | –OTig |
| | Schisantherin B | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | –$CH_2$– | | $CH_3$ | OH | $CH_3$ | H | –OAng |
| 44 | Schisantherin A | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | –$CH_2$– | | $CH_3$ | OH | $CH_3$ | H | –Obz |
| 45 | Schisanhenol | H | $CH_3$ | H | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | H | $CH_3$ | $CH_3$ | H |
| 46 | Gomisin B | –$CH_2$– | | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | H | OH | $CH_3$ | –OAng |
| 48 | Gomisin F | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | –$CH_2$– | | $CH_3$– | H | OH | $CH_3$ | –OAng |
| 50 | Schizandrin A | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | H | $CH_3$ | H | H |
| 51 | Gomisin N | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | –$CH_2$– | | $CH_3$ | H | $CH_3$ | H | H |
| 52 | Schizandrin B | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | –$CH_2$– | | $CH_3$ | H | $CH_3$ | H | H |
| 53 | Schizandrin C | –$CH_2$– | | $CH_3$ | $CH_3$ | –$CH_2$– | | $CH_3$ | H | $CH_3$ | H | H |
| M6 | 7,8-Dihydnoxy-3-demethyl-schizandrin | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | H | $CH_3$ | H | OH | $CH_3$ | OH | H |
| M7 | 7,8-Dihydnoxy-2-demethyl-schizandrin | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | H | H | OH | $CH_3$ | OH | H |
| M9 | 7,8-Dihydnoxy-schizandrin | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | H | OH | $CH_3$ | OH | H |




| Peak no. | Compound | $R_1$ | $R_2$ | – | – | – | – | – | – | – |
|---|---|---|---|---|---|---|---|---|---|---|
| 21 | Gomisin D | OH | $CH_3$ | – | – | – | – | – | – | – |
| 47 | Gomisin E | $CH_3$ | H | – | – | – | – | – | – | – |

**Table 3**
MS and MS/MS data (*m*/*z*) of the absorbed compounds and metabolites in rat plasma after oral administration of SMS.

| Peak no. | Retention time (min) | MS (*m*/*z*) | MS/MS (*m*/*z*) | Identification |
|---|---|---|---|---|
| 7[a] | 63.20 | $[M-H]^-$: 637, $[M+CH_3COO]^-$: 697, $[M+H]^+$: 639 | $[M+H-H_2O]^+$: 621, $[M+H-2H_2O]^+$: 603, $[Aglycone+H-3H_2O]^+$: 441, $[Aglycone+H-4H_2O]^+$: 423, $[Aglycone+H-5H_2O]^+$: 405 | Ginsenoside Rh1 |
| 18[a] | 76.98 | $[M+H]^+$: 433, $[M+Na]^+$: 455 | $[M+H-H_2O]^+$: 415, $[M+H-H_2O-CH_3O]^+$: 384, $[M+H-C_3H_5O_3]^+$: 360 | Schizandrol A |
| 21 | 79.70 | $[M+H]^+$: 531, $[M+Na]^+$: 553 | $[M+H-CH_2O_2]^+$: 485, $[M+H-C_6H_{10}O_3]^+$: 401, $[M+H-C_6H_{10}O_3-H_2O]$: 383 | Gomisin D |
| 22[a] | 81.67 | $[M-H]^-$: 783, $[M+H]^+$: 785, $[M+Na]^+$: 807 | $[M-H-Glc]^-$: 621, $[Aglycone-H]^-$: 475, $[Aglycone+H-2H_2O]^+$: 443, $[Aglycone+H-3H_2O]^+$: 425, $[Aglycone+H--4H_2O]^+$: 407 | Ginsenoside Rg3 |
| 25[a] | 82.62 | $[M+H]^+$: 417, $[M+Na]^+$: 439 | $[M+H-H_2O]^+$: 399 | Schizandrol B |
| 28 | 86.39 | $[M+H]^+$: 501, $[M+Na]^+$: 523 | $[M+H-H_2O]^+$: 483, $[M+H-C_5H_{10}]^+$: 431, $[M+H- C_5H_8O_2]^+$: 401 | Trigloylgomisin H |
| 30 | 88.46 | $[M-H]^-$: 825, $[M+H]^+$: 827 | $[M+H-H_2O]^+$: 809, $[M+H-AC]^+$: 785, $[Aglycone+H-2H_2O]^+$: 443, $[Aglycone+H-3H_2O]^+$: 425, $[Aglycone+H-4H_2O]^+$: 407 | Ginsenoside Rs3/isomer |
| 32 | 88.72 | $[M+Na]^+$: 523, $[M+H]^+$: 501 | $[M+Na-H_2O]^+$: 505, $[M+H-H_2O]^+$: 483, $[M+H-C_5H_{10}]^+$: 431, $[M+H-C_5H_8O_2]^+$: 401 | Angeloylgomisin H |
| 34 | 89.5 | $[M+H]^+$: 523, | $[M+H-H_2O]^+$: 505, $[M+H-2H_2O]^+$: 487, $[M+H-C_7H_6O_2]^+$: 401 | Benzoylgomisin H |
| 35 | 90.45 | $[M+H]^+$: 553 | $[M+H-C_7H_6O_2]^+$: 431, $[M+H-C_7H_6O_3]^+$: 415, $[M+H-C_7H_6O_2-C_2H_4O]^+$: 387 | Benzoylgomisin Q |
| 44[a] | 93.95 | $[M+H]^+$: 537 | $[M+H-C_7H_6O_2]^+$: 415, $[M+H-C_7H_6O_2-C_2H_4O]^+$: 371, $[M+H-C_7H_6O_2-C_2H_4O-CH_3O]^+$: 340 | Schisantherin A |
| 45 | 95.1 | $[M+H]^+$: 403, | $[M+H-H_2O]^+$: 385, $[M+H-H_2O-CH_3]^+$: 370, $[M+H-H_2O-CH_3-CH_3O]^+$: 339 | Schisanhenol |
| 50[a] | 100.70 | $[M+H]^+$: 417, $[M+Na]^+$: 439, | $[M+H-CH_3O]^+$: 386, $[M+H-C_5H_{10}]^+$: 347 | Schizandrin A |
| 52[a] | 103.57 | $[M+H]^+$: 401, | $[M+H-CH_3O]^+$: 371, $[M+H-C_5H_{10}]^+$: 331 | Schizandrin B |
| M1 | 41.03 | $[M+H]^+$: 434, | $[M+H-H_2O]^+$: 416, $[M+H-H_2O-CH_3O]^+$: 385 | Isomers of 7,8-dihydroxy- |
| M3 | 50.35 | $[M+H]^+$: 434, $[M+Na]^+$: 456 | $[M+H-H_2O]^+$: 416, $[M+H-H_2O-CH_3O]^+$: 385 | |
| M4 | 51.03 | $[M+H]^+$: 434, $[M+Na]^+$: 456 | $[M+H-H_2O]^+$: 416, $[M+H-H_2O-CH_3O]^+$: 385 | |

Table 3 (*Continued*).

| Peak no. | Retention time (min) | MS (*m*/*z*) | MS/MS (*m*/*z*) | Identification |
|---|---|---|---|---|
| M5 | 53.37 | $[M+H]^+$: 434, $[M+Na]^+$: 456 | $[M+H-H_2O]^+$: 416, $[M+H-H_2O-CH_3O]^+$: 385 | |
| M8 | 56.15 | $[M+H]^+$: 434, $[M+Na]^+$: 456 | $[M+H-H_2O]^+$: 416, $[M+H-H_2O-CH_3O]^+$: 385 | |
| M6 | 54.08 | $[M+H]^+$: 434, $[M+Na]^+$: 456 | $[M+H-H_2O]^+$: 416, $[M+H-H_2O-CH_3O]^+$: 385 | 7,8-Dihydroxy-3-demethyl-schizandrin |
| M7 | 55.37 | $[M+H]^+$: 434, $[M+Na]^+$: 456 | $[M+H-H_2O]^+$: 416, $[M+H-H_2O-CH_3O]^+$: 385 | 7,8-Dihydroxy-2-demethyl-schizandrin |
| M9 | 64.17 | $[M+H]^+$: 449, $[M+Na]^+$: 471 | $[M+H-H_2O]^+$: 431, $[M+H-H_2O-CH_3O]^+$: 400, $[M+H-H_2O-CH_3O-CO]^+$: 372 | 7,8-Dihydroxy-schizandrin |
| M2 | 41.90 | $[M+H]^+$: 418, $[M+Na]^+$: 440 | $[M+H-H_2O]^+$: 400, $[M+H-H_2O-CH_3O]^+$: 369 | Demethylated metabolites of schizandrol A |
| M10 | 65.38 | $[M+H]^+$: 418, $[M+Na]^+$: 440 | $[M+H-H_2O]^+$: 400, $[M+H-H_2O-CH_3O]^+$: 369 | |
| M11 | 93.44 | $[M+H]^+$: 461 | $[M+H-H_2O]^+$: 443, $[M+H-2H_2O]^+$: 425, $[M+H-3H_2O]^+$:407 | Protopanaxadiol |

Glc, glucose; AC, 6-O-acetyl.

[a] Compared with authentic compounds.

patterns, the chemical structures of peaks 12, 21, 23, 28, 32, 34, 35, 38, 39, 41, 43, 45, 46, 47, 48 and 51 were identified and some isomers were distinguished tentatively [16,21–23,25]. The characteristic fragmentations resulted from the successive or simultaneous losses of $H_2O$ (18 Da), $CH_3O$ (31 Da), $CH_2O_2$ (46 Da), $C_5H_{10}$ (70 Da), $C_5H_8O_2$ (100 Da), $C_7H_6O_2$ (122 Da) or $C_6H_{10}O_3$ (130 Da). The presence of the $[M+H-C_7H_6O_2]^+$ ion suggested that there was a benzoyloxy group at the eight-membered diene ring in its structure, while the occurrence of the $[M+H-C_5H_8O_2]^+$ ion suggested the presence of an angeloyloxy or tigloyloxy group in their structures.

Peak 21 exhibited $[M+H]^+$ ion at *m*/*z* 531 and yielded ions at *m*/*z* 485, 401 and 383, originating from the losses of $CH_2O_2$ (46 Da), $C_6H_{10}O_3$ (130 Da) and $H_2O$ (18 Da), respectively. Hence, it was plausibly characterized as gomisin D. Peak 47 gave a $[M+H]^+$ ion at *m*/*z* 515, with the occurrence of the $[M+H-C_6H_{10}O_3]^+$ at *m*/*z* 385, suggesting that it possessed a similar structure to gomisin D. Therefore, peak 47 was tentatively identified as gomisin E [23].

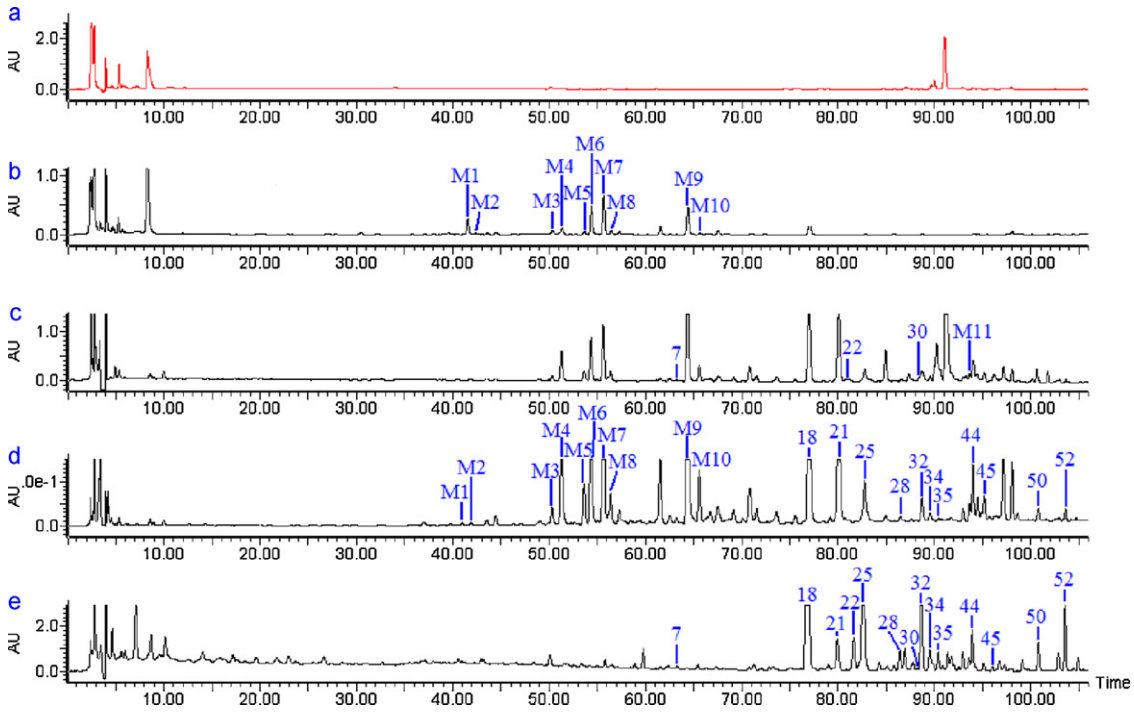



**Fig. 3.** HPLC–DAD chromatograms of rat plasma samples and SMS extract. (a) Blank rat plasma; (b) rat plasma sample after oral administration of schizandrol A; (c) rat plasma sample after oral administration of SMS extract at 203 nm; (d) rat plasma sample after oral administration of SMS extract at 254 nm; and (e) SMS extract.

Peaks 28 and 32 displayed the same $[M+H]^+$ ions at $m/z$ 501. They both afforded product ions at $m/z$ 483, 431 and 401 in the MS/MS spectrum, corresponding to successive losses of $H_2O$, $C_5H_{10}$ and $C_5H_8O_2$. After comparison with the literature data [23], they were identified as trigloylgomisin H and angeloylgomisin H, respectively. Similar fragmentation patterns were found in the spectra of peaks 34 and 38. According to the literature data, they were tentatively identified as benzoylgomisin H and tigloylgomisin P, respectively [23,25].

### 3.3. HPLC–MS/MS analysis of plasma sample after oral administration of SMS extract

To clarify the active constituents responsible for the pharmacological action, it is necessary to know the chemical constituent profile *in vivo* [26]. Therefore, the rat plasma after oral administration of SMS extract was analyzed by the same HPLC–DAD–MS/MS method used for SMS extract.

Various sample preparation methods were tested to select an efficient clean up of the plasma sample for obtaining better recovery of the target compounds. The methods included protein precipitation with acetonitrile, methanol, liquid–liquid extraction with water-saturated n-butanol and solid-phase extraction with various sorbents. Finally, solid-phase extraction with Supelclean™ LC-18 SPE columns was chosen because it ensured the simultaneous extraction of all target compounds and less interference from the co-eluted endogenous matrixes.

In order to screen and identify the structures of absorbed components of SMS extract and their metabolites, the rat plasma samples were analyzed by the described HPLC/MS/MS method. HPLC chromatograms of SMS extract, rat plasma samples collected after administered SMS, schizandrol A and blank plasma sample are shown in Fig. 3. The retention time ($t_R$), λ-max, MS data and the fragments of the metabolites are listed in Table 3. Their chemical structures are shown in Table 2.

There were 14 peaks displayed in the profiles of dosed plasma and SMS extract, whereas there were no equivalent peaks in the profile of the blank plasma. Thus, these compounds were defined as prototype components. According to the retention time and mass spectra with those of authentic compounds, compounds 7, 18, 22, 25, 44, 50 and 52, were designated as ginsenoside Rh1, schizandrol A, ginsenoside Rg3, schizandrol B, schisantherin A, schizandrin A and schizandrin B, respectively. Compounds 21, 28, 30, 32, 34, 35 and 45 were tentatively characterized as gomisin D, trigloylgomisin H, ginsenoside Rs3, angeloylgomisin H, benzoylgomisin H, benzoylgomisin Q and schisanhenol by comparing MS/MS spectra of sample with those of literature data, respectively [16,23,25].

The other 11 peaks marked as M1–M11, which only appeared in dosed plasma, were assumed to be exogenous metabolites derived from SMS (see Fig. 3). The UV spectrum of peaks M7 and M9 were found to be very similar to that of schizandrol A, indicating that they might come from a similar structure. In order to further confirm their origin and identify the structure of the metabolites, reference compound of schizandrol A was orally administered to rats. The results indicated that M1–M10 were also found in rat plasma after administration of schizandrol A, and their retention time and fragmentation behavior were identical with those in rat plasma after administration of SMS (see Fig. 3). As a result, peaks M1–M10 were identified as metabolites of schizandrol A.

The molecular weight of M9 was 448 Da, which was 16 Da more than that of schizandrol A. Therefore, M9 was supposed to be a hydroxy metabolite of schizandrol A. Compared with literature data [27], M9 was identified as 7,8-dihydroxy-schizandrin, one of the main metabolites of schizandrol A. M6 and M7 showed the same $[M+H]^+$ ions at $m/z$ 434 and $[M+Na]^+$ ions at $m/z$ 456, and they also displayed the identical fragment ions at $m/z$ 416 and 385, respectively. According to the literature data, they were identified as 7,8-dihydroxy-3-demethyl-schizandrin and 7,8-dihydroxy-2-demethyl-schizandrin, respectively [27]. In addition, M1, M3–M5 and M8 exhibited the same $[M+H]^+$ ion and possessed similar fragment ions in MS/MS spectra as those of M6 and M7, but the retention times of them were different. They were tentatively identified as the isomers of 7,8-dihydroxy-2-demethyl-schizandrin and 7,8-dihydroxy-3-demethyl-schizandrin based on the literature data [28]. M2 and M10 showed $[M+H]^+$ ion at $m/z$ 418 and afforded similar fragment ions at $m/z$ 400 and 369, respectively. By referring to the literature data, they were tentatively identified as demethylated metabolites of schizandrol A [28]. M11 showed $[M+H]^+$ ion at $m/z$ 461 and displayed product ions at $m/z$ 443, 425 and 407 in the MS/MS spectrum. According to the literature data [29], it was identified as protopanaxadiol.

Homoisoflavonoids and steroidal glycosides are major groups of constituents in SMS. However, no prototype components and related metabolites of them were detected in the drug-containing plasma, possibly attributed to the fact that the content of homoisoflavonoids and steroidal glycosides in SMS extract was very low and they were poorly absorbed into blood and quickly excreted through urine and feces.

## 4. Conclusion

In this study, an HPLC–DAD–MS/MS method was established for identifying multiple components in the extract of SMS. As a result, 53 compounds including 14 ginsenosides, 6 steroidal saponins, 12 homoisoflavonoids and 21 lignans in the complex system were successfully separated and identified by comparing retention times, UV and MS spectra with those of authentic compounds and literature data. To screen the potentially bioactive components in SMS, rat plasma samples were similarly investigated after the administration of SMS. The analysis of the fragmentation pathways of the compounds in SMS extract greatly facilitated the identification of metabolites from SMS, as most of these metabolites retained the structural features of the parent compounds. 25 compounds including 14 prototype components and 11 metabolites were identified. Furthermore, the origin of the metabolites was confirmed by comparing their mass spectra and chromatographic behavior with those in rat plasma sample obtained after administration of authentic compounds. The quantitative determination of the identified constituents was not among the aims of the study. This identification and structural elucidation of the constituents in SMS and rat plasma provided essential data for further active chemical constituents identification and pharmacological research of SMS. It would be also helpful to better understand the pharmacodynamic profile of SMS, which will facilitate its clinical usage and quality control during production.

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