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UPLC/Q-TOF-MS Analysis for Identification of Hydrophilic Phenolics and Lipophilic Diterpenoids from Radix Salviae Miltiorrhizae

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Summary. A rapid method has been used for simultaneous identification of both hydrophilic and lipophilic compounds from Radix Salviae Miltiorrhizae (RSM, the root of *Salvia miltiorrhiza* B_{GE}.) by ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC/Q-TOF-MS). A total of 58 compounds extracted by methanol were detected and tentatively identified within 20 min, including hydrophilic phenolics, lipophilic diterpenoids, a verbascose, and several organic acids. These compounds were separated on an Acquity UPLC BEH C18 column and identified based on tandem mass spectrometry (MS/MS) fragmentation patterns under the positive and negative ion modes, respectively. Among them, micranthin B and 9-oxo-10E,12Z-octadecadienoic acid were reported in RSM for the first time. Their fragmentation patterns in electrospray ionization (ESI)–MS/MS spectra were first investigated by matching their accurate molecular masses. This contribution presented one of the first reports on the analysis of hydrophilic phenolics and lipophilic diterpenoids from Radix Salviae Miltiorrhizae using UPLC/Q-TOF-MS. The results demonstrated that UPLC/Q-TOF-MS method could be applied to rapidly and expediently describe and provide comprehensive chemical information for simultaneous analysis of two different polar components in RSM.

Key Words: UPLC/Q-TOF-MS, phenolics, diterpenoids, fragmentation patterns, Radix Salviae Miltiorrhizae

Introduction

Radix Salviae Miltiorrhizae (RSM), the dried root of *Salvia miltiorrhiza* B_{GE}, commonly called "Danshen", has been widely used as a popular herb in Traditional Chinese Medicine for the treatment of coronary heart disease, cerebrovascular disease, hepatitis, chronic renal failure, dysmenorrhea, insomnia, and inflammatory disease [1–6]. Previous phytochemical studies on RSM indicated that hydrophilic depsides derivatives and lipophilic diterpenoids were the major constituents of this herb [7, 8]. Danshensu, protocatechualdehyde, salvianolic acid B, tanshinone I, tanshinone IIA, and cryptotanshinone *(Fig. 1)* are six major active compounds in Danshen. Pharmacol-

ogical studies established that these compounds possessed anti-ischemic, antioxidant, and antitumor activities [9, 10].

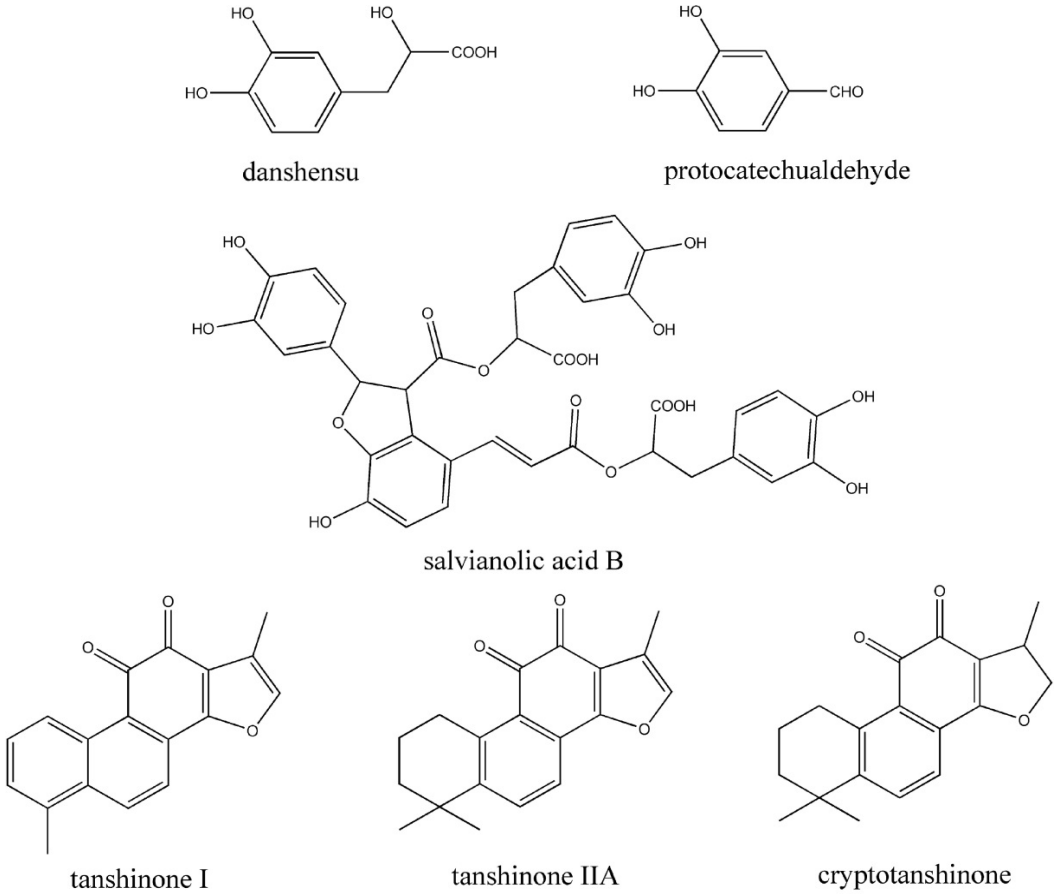

Fig. 1. The chemical structures of six major constituents in Radix Salviae Miltiorrhizae

Some analytical techniques were utilized for separation and identification of constituents in RSM, such as high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), and liquid chromatography hyphenated with tandem mass spectrometry (LC–MS/MS) [11–13]. However, HPTLC had a disadvantage of low resolution, which resulted in inaccuracy. A single HPLC or UPLC method only dealt with the separation of constituents in RSM, which raised the possibility of incomplete analysis due to the absence of fragmentation patterns. Furthermore, low sensitivity and time-consuming procedure emerged in the separation section of LC–MS/MS analysis. The aim of this research was to develop a rapid, conven-

ient, and reliable analytical method to simultaneously detect water-soluble and lipid-soluble compounds in RSM. UPLC has several advantages over other chromatographic techniques, which include short analysis time, low-solvent consumption, high sensitivity, and high resolution when hyphenated with a Q-TOF MS detector [14]. Compounds in a mixture could be effi-

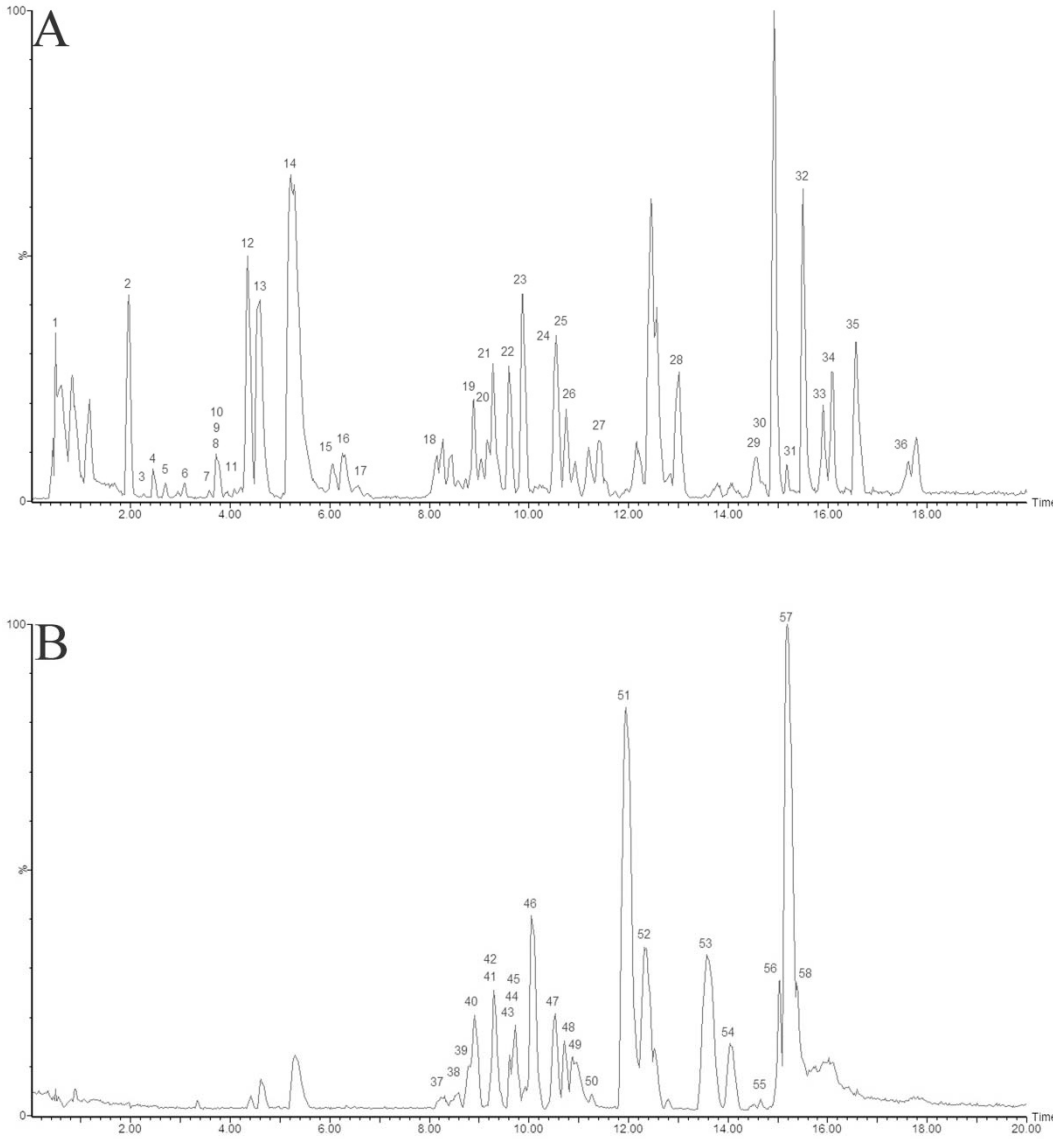

Fig. 2. Total ion current profile of Radix Salviae Miltiorrhizae (A is negative ion mode, B is positive mode)

ciently separated and characterized by UPLC and MS, respectively [15, 16]. All these advantages suggest that UPLC/Q-TOF-MS is a powerful technique for the rapid isolation and simultaneous identification of components found in RSM.

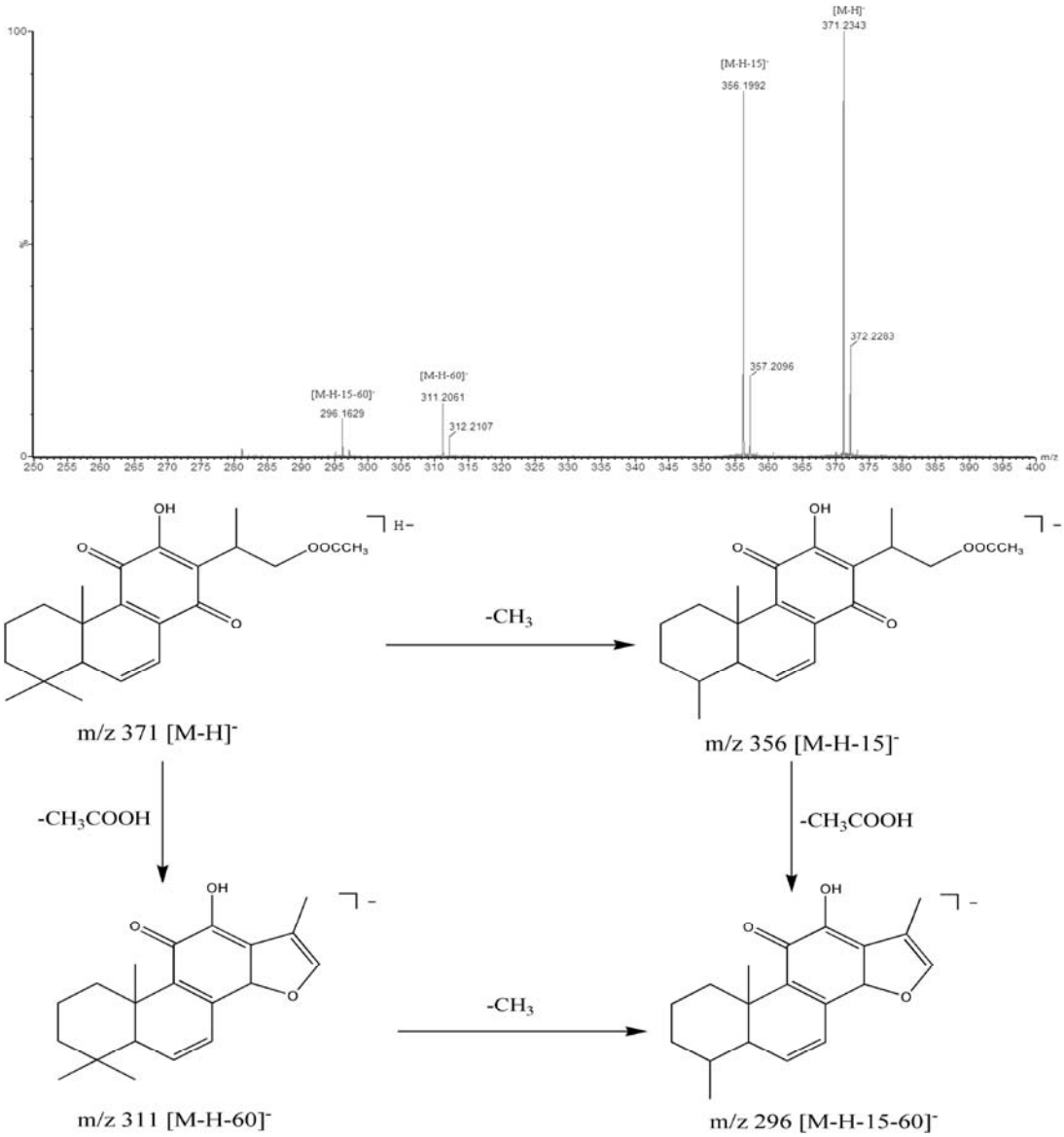

Fig. 3. Fragmentation pathways of micranthin B

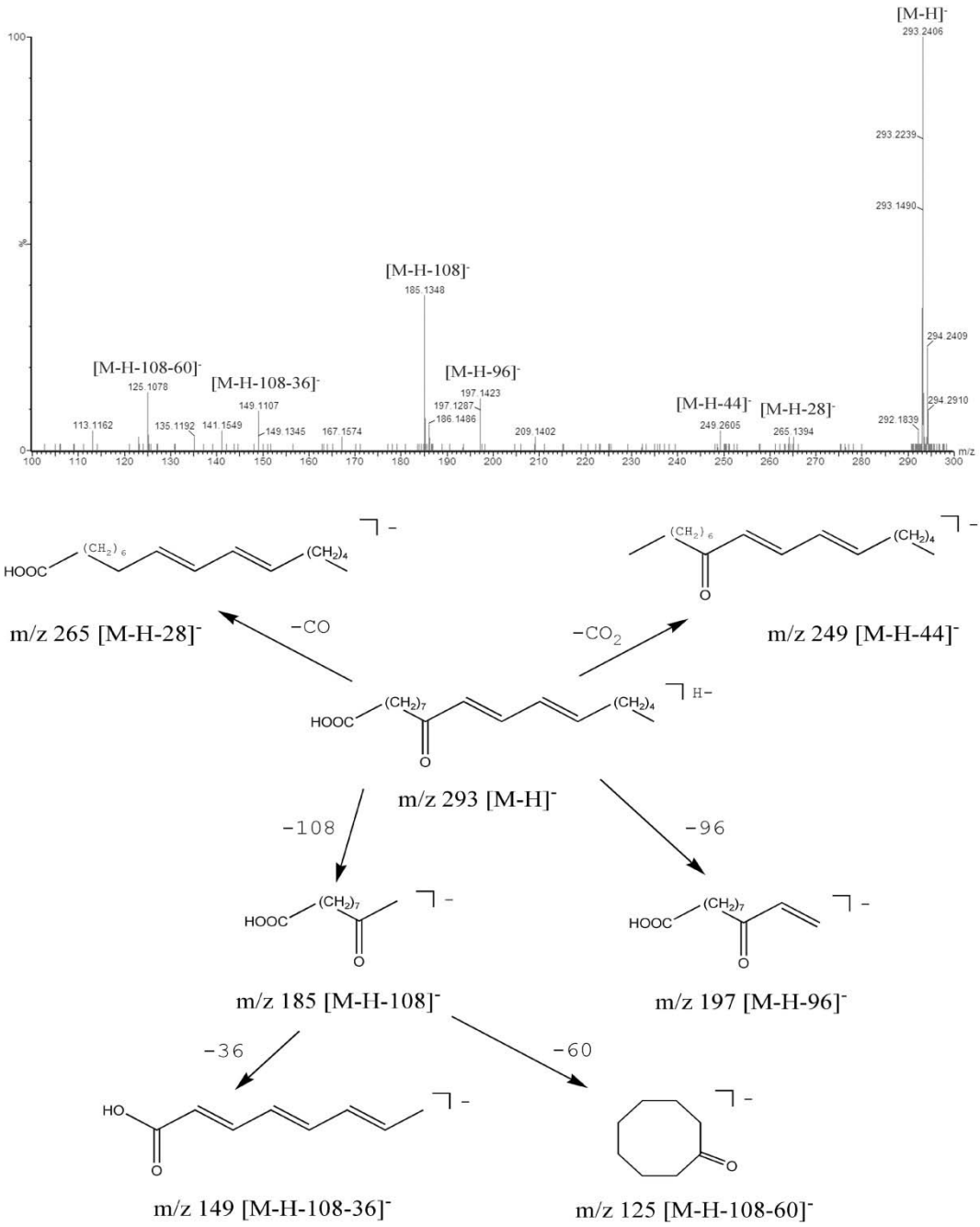

Fig. 4. Fragmentation pathways of 9-oxo-10E,12Z-octadecadienoic acid

In this study, an UPLC/Q-TOF-MS method has been developed for the identification of components in RSM utilizing an Acquity UPLC BEH C18 column and a Q-TOF-MS detector. Fifty-eight compounds including hydrophilic phenolics, lipophilic diterpenoids, a verbascose, and several organic acids were detected and tentatively identified by comparing their accurate masses and fragment information based on correlative references. Micranthin B and 9-oxo-10E,12Z-octadecadienoic acid were firstly reported in RSM. The major fragmentation behaviors of these two compounds obtained by UPLC–MS were firstly researched in this study *(Figs 3* and *4)*. This study demonstrated that UPLC/Q-TOF-MS could provide a rapid and convenient method for simultaneous isolation and identification of hydrophilic and lipophilic compounds, which is suitable and significant for multicomponent analysis and further quality investigation in RSM.

Experimental

Herbal Materials

RSM was purchased from Zhixin Group Co., Ltd. (Guangzhou City, Guangdong Province, China) in March, 2013. The plant was identified by Prof. Jizhu Liu (Pharmacognostics Department, Guangdong Pharmaceutical University). The voucher specimen (No. 121201) was deposited in the Central Laboratory, Guangdong Pharmaceutical University.

Solvents and Reagents

Methanol and acetonitrile used for UPLC analysis were obtained from Merck Company (Darmstadt, Germany). Formic acid of HPLC grade for UPLC analysis was purchased from CNW Technologies GmbH Germany. Water used for UPLC analysis was purchased from Watson's Food and Beverage (Guangzhou City, Guangdong Province, China). Leucine–enkephalin (Sigma, USA) was used as the lock mass.

Sample Preparation

Approximately 10.0 g of dried RSM were extracted ultrasonically in 50 mL of methanol for 1 h. Then the sample solution was centrifuged for 10 min at about 13,000 rpm. After centrifugation, the supernatant was transferred into an UPLC sample vial as the test solution for UPLC analysis.

UPLC Analysis

UPLC analysis was performed on a Waters Acquity UPLC system (Waters, Milford, USA) equipped with a binary high-pressure pump, automatic sample injector, and column manager. Separation was carried out on an Acquity UPLC BEH C18 column (2.1 mm × 50 mm, 1.7 μm, Waters, USA) with an Acquity UPLC BEH C18 VanGuard Pre-Column (2.1 mm × 5 mm, 1.7 μm, Waters, USA) at a flow rate of 0.3 mL min^{-1} and a temperature of 25 °C. Solvent A (0.1% aqueous formic acid) and solvent B (acetonitrile) were used as mobile phase. A linear gradient elution was run under the conditions as follows: 0 min, 2% B; 0.5 min, 3% B; 2 min, 20% B; 7 min, 25% B; 7.5 min, 50% B; 14 min, 52% B; 14.5 min, 85% B; 19 min, 86% B; and 20 min, 100% B. The injection volume was 1 μL.

MS Conditions

MS analysis was performed on a Micromass Q-Tof micro (Waters, USA) equipped with an electrospray ionization (ESI) source, direct injection device, and automatic calibration technique of lock spray. MS data were collected and processed by Masslynx software under the positive and negative ion modes, respectively. The ESI source conditions were applied as follows: full scan data acquisition was performed from *m/z* 100 to 1000; the capillary voltage is 3000 V; cone voltage, 30 V; source temperature, 100 °C; and desolvation temperature, 350°C. Nitrogen and argon were used as the cone and collision gases, respectively. The cone and desolvation gas flows rates were 50 L h^{-1} and 500 L h^{-1}, respectively. Leucine–enkephalin was used as the lock mass ($[M+H]^+$ *m/z* 556.2771, $[M-H]^-$ *m/z* 554.2615).

Results and Discussion

Optimization of UPLC Condition

The optimization of elution system was aimed at increasing the resolution and decrease the peak tailing of water-soluble phenolics and lipid-soluble diterpenoids. The mobile phase constitutions such as methanol–0.1% formic acid, acetonitrile–0.4% formic acid, and acetonitrile–0.1% formic acid were investigated. The results showed that the mobile phase of acetonitrile–0.1% formic acid could not only improve the resolution of adjacent peaks but also depress the tailing of the peaks in the total ion current (TIC) profile *(Fig. 2)*. The isocratic elution could not meet the request owing to the complexity of the components in Radix Salviae Miltiorrhizae. Therefore, a linear gradient

elution was selected for the simultaneous separation of water-soluble and lipid-soluble compounds in Danshen.

Selections of Ion Mode and Collision Energy

Negative and positive ion modes were necessarily selected to optimize the mass spectra in order to ensure accuracy and reproducibility. The experiments illustrated that water-soluble constituents (danshensu, etc.) in RSM displayed strong sensitivity in negative ion mode due to the structure feature with hydroxyl or carboxyl, which could make them more vulnerable to acidification, while lipid-soluble compounds (tanshinone I, etc.) showed low sensibility for negative ion mode. This enabled us to draw a conclusion that it is suitable and reasonable to choose a negative ion mode for the water-soluble components and a positive ion mode for the lipid-soluble components. The selection of collision energy aimed at obtaining the best condition of ESI–MS/MS ionization for water-soluble and lipid-soluble compounds from RSM. Different collision energies including 15 eV, 20 eV, 25 eV, and 30 eV were investigated. The results suggested that the collision energy with 20 eV could provide the best fragmentation behaviors of ionization for multiple ingredients in Danshen.

Fragmentation Patterns of Micranthin B and 9-Oxo-10E,12Z-Octadecadienoic Acid

The results of MS data of compounds in Danshen are given in *Table I*, which include elemental compositions, measured and calculated masses, mass errors, and fragment ions. Fifty-eight compounds in RSM were measured within 5 ppm error by comparison of their calculated values, indicating mass accuracy and high resolution.

Compounds micranthin B and 9-oxo-10E,12Z-octadecadienoic acid were first found in RSM; hence, it was essential and significant to investigate their fragmentation pathways in ESI–MS/MS spectra. Although micranthin B has characteristic structure of phenanthraquinone, it is especially vulnerable to appear on the negative ion mode due to its –OH and –$OOCCH_3$ groups, which could make it easily have a loss of H and quickly show strong sensitivity for negative ion mode. The deprotonated molecular ion $[M-H]^-$ of micranthin B at *m/z* 371.1847 may generate a product ion at *m/z* 311 after the loss of an acetic acid (60 Da) molecule. A product ion at *m/z* 296 was formed after the disconnection of a methyl (15 Da) derived from

Table I. Identification of components in Radix Salviae Miltiorrhizae using UPLC/Q-TOF-MS

Peak (No.)	RT (min)	Ion mode	Measured mass *(m/z)*	Calculated mass *(m/z)*	Error (ppm)	MS/MS *(m/z)*	Formula	Identification
1	0.48	$[M-H]^-$	827.2645	827.2669	−2.9	827, 665, 545, 503, 443, 383, 341, 179, 164	$C_{30}H_{52}O_{26}$	Verbascose
2	1.94	$[M-H]^-$	197.0444	197.0450	−3.0	395, 197, 179, 151, 135, 123, 109, 107	$C_9H_{10}O_5$	Danshensu
3	2.13	$[M-H]^-$	153.0190	153.0188	1.3	153, 138, 123, 109	$C_7H_6O_4$	Protocatechuic acid
4	2.45	$[M-H]^-$	137.0233	137.0239	−4.4	137, 129, 119, 109, 93, 81, 66, 55	$C_7H_6O_3$	Protocate chualdehyde
5	2.70	$[M-H]^-$	179.0337	179.0344	−3.9	359, 179, 135, 107	$C_9H_8O_4$	Caffeic acid
6	3.10	$[M-H]^-$	313.0714	313.0712	0.6	627, 313, 269, 254, 239, 185, 159, 147, 135, 121, 109	$C_{17}H_{14}O_6$	Salvianolic acid F
7	3.59	$[M-H]^-$	535.1819	535.1816	0.6	535, 355, 339, 295, 269, 197, 179, 119	$C_{26}H_{32}O_{12}$	1-Hydroxy-pinoresinol-1-O-β-D-glucoside
8	3.74	$[M-H]^-$	417.0824	417.0822	0.5	417, 197, 175, 135	$C_{20}H_{18}O_{10}$	Salvianolic acid D
9	3.74	$[M-H]^-$	339.0498	339.0505	−2.1	339, 295, 277, 267, 249, 239, 225, 209	$C_{18}H_{12}O_7$	Salvianolic acid G
10	3.74	$[M-H]^-$	537.1016	537.1033	−3.2	537, 355, 339, 295, 269	$C_{27}H_{22}O_{12}$	Salvianolic acid H/I/J/isomer
11	4.06	$[M-H]^-$	717.1446	717.1456	−1.4	717, 537, 519, 493, 339, 321	$C_{36}H_{30}O_{16}$	Salvianolic acid E
12	4.31	$[M-H]^-$	359.0771	359.0767	3.1	719, 359, 197, 179, 161, 135	$C_{18}H_{16}O_8$	Rosmarinic acid
13	4.52	$[M-H]^-$	537.1024	537.1033	−1.7	537, 339, 313, 295, 197, 185, 179, 135, 109	$C_{27}H_{22}O_{12}$	Lithospermic acid
14	5.21	$[M-H]^-$	717.1464	717.1456	1.1	717, 537, 519, 339, 321	$C_{36}H_{30}O_{16}$	Salvianolic acid B
15	6.07	$[M-H]^-$	717.1459	717.1456	0.4	717, 537, 519, 493, 339, 321	$C_{36}H_{30}O_{16}$	Salvianolic acid B/E/isomer

Table I. (continued)

Peak (No.)	RT (min)	Ion mode	Measured mass *(m/z)*	Calculated mass *(m/z)*	Error (ppm)	MS/MS *(m/z)*	Formula	Identification
16	6.28	[M−H]⁻	493.1154	493.1135	3.9	987, 493, 313, 295, 203, 185, 159, 135, 109	$C_{26}H_{22}O_{10}$	Salvianolic acid A
17	6.59	[M−H]⁻	731.1595	731.1612	−2.3	731, 551, 533, 353, 335	$C_{37}H_{32}O_{16}$	3′-O-Monomethyllithospermic acid B
18	8.10	[M−H]⁻	491.0992	491.0978	2.9	983, 491, 403, 329, 311, 293, 267, 197, 185,179, 135, 109	$C_{26}H_{20}O_{10}$	Salvianolic acid C
19	8.89	[M−H]⁻	327.1219	327.1232	−4.0	655, 327, 299, 284, 253, 239, 227, 201	$C_{19}H_{20}O_5$	Phenanthro[1,2-b]furan-10,11-dione, 1,2,6,7,8,9-hexahydro-7(8 or 9)-hydroxy-1-(hydroxymethyl)-6,6-dimethyl-, (1S)-
20	9.07	[M−H]⁻	317.1750	317.1753	−0.9	635, 317, 299, 286, 273	$C_{19}H_{26}O_4$	2-Butenoic acid, 4-[1,2,3,4-tetrahydro-1-hydroxy-7-methoxy-6-(1-methylethyl)-1-naphthalenyl]-, methyl ester
21	9.18	[M−H]⁻	487.3417	487.3424	−1.4	975, 487, 469	$C_{30}H_{48}O_5$	Tormentic acid
22	9.66	[M−H]⁻	313.1425	313.1440	−4.8	627, 313, 269, 241, 226, 213, 198, 185	$C_{19}H_{22}O_4$	Neocryptotanshinone
23	9.85	[M−H]⁻	355.1903	355.1909	−1.7	711, 355, 340, 295, 280	$C_{22}H_{28}O_4$	1-Phenanthreneacetic acid, 1,2,3,4-tetrahydro-1-hydroxy-6-methoxy-7-(1-methylethyl)-, ethyl ester
24	10.43	[M−H]⁻	281.1186	281.1178	2.8	281, 263, 251, 236, 222, 207	$C_{18}H_{18}O_3$	Unknown
25	10.59	[M−H]⁻	357.2064	357.2066	−0.6	715, 357, 297, 277	$C_{22}H_{30}O_4$	9(1H)-Phenanthrenone, 3-(acetyloxy)-2,3,4,4a,10,10a-hexahydro-6-hydroxy-1,1,4a-trimethyl-7-(1-methylethyl)-, (3S,4aS,10aS)-
26	10.72	[M−H]⁻	373.2025	373.2015	2.7	747, 373, 345, 340, 327, 313, 285	$C_{22}H_{30}O_5$	7α-Acetoxyroyleanone
27	11.44	[M−H]⁻	315.1962	315.1960	0.6	315, 287, 269, 254, 163	$C_{20}H_{28}O_3$	20-Deoxocarnosol
28	12.99	[M−H]⁻	371.1847	371.1858	−3.0	743, 371, 356, 311, 296	$C_{22}H_{28}O_5$	Micranthin B

Table I. (continued)

Peak (No.)	RT (min)	Ion mode	Measured mass *(m/z)*	Calculated mass *(m/z)*	Error (ppm)	MS/MS *(m/z)*	Formula	Identification
29	14.54	$[M-H]^-$	293.2128	293.2117	3.8	293, 265, 249, 209, 197, 185, 167, 149, 141, 135, 125, 113	$C_{18}H_{30}O_3$	9-Oxo-10E,12Z-octadecadienoic acid
30	14.72	$[M-H]^-$	299.2016	299.2011	1.7	299, 281, 253	$C_{20}H_{28}O_2$	Sugiol
31	15.18	$[M-H]^-$	317.2122	317.2117	1.6	635, 317, 299, 286, 281, 179	$C_{20}H_{30}O_3$	1-Naphthalenol, 1-(3-buten-1-yl)-1,2,3,4-tetrahydro-5,7-dimethoxy-2-methyl-6-(1-methylethyl)-
32	15.51	$[M-H]^-$	341.2100	341.2117	−5.0	683, 341, 299, 281	$C_{22}H_{30}O_3$	Anacardic acid
33	15.90	$[M-H]^-$	277.2163	277.2168	−1.8	277, 259, 250, 233, 221, 219, 209	$C_{18}H_{30}O_2$	Linolenic acid
34	16.09	$[M-H]^-$	283.2065	283.2062	1.1	567, 329, 283, 212	$C_{20}H_{28}O$	Retinene
35	16.55	$[M-H]^-$	279.2320	279.2324	−1.4	559, 279, 261	$C_{18}H_{32}O_2$	Linoleic acid
36	17.63	$[M-H]^-$	255.2313	255.2324	−4.3	255, 237, 212, 183, 143, 117	$C_{16}H_{32}O_2$	Palmitic acid
37	8.30	$[M+H]^+$	283.0977	283.0970	2.5	283, 265, 255, 241, 237, 227, 223, 219, 209, 195, 191, 171, 161, 143	$C_{17}H_{14}O_4$	Phenanthro[1,2-b]furan-6,10,11(7H)-trione, 1,2,8,9-tetrahydro-1-methyl-, (R)-
38	8.45	$[M+H]^+$	293.0824	293.0814	3.4	293, 278, 275, 265, 249, 235, 219, 205, 193, 178, 169, 155, 151, 133, 119, 109	$C_{18}H_{12}O_4$	Tanshinol A
39	8.51	$[M+H]^+$	295.0978	295.0970	2.7	611, 295, 277, 267, 261, 249, 233, 221, 207, 197, 193, 179, 169, 165	$C_{18}H_{14}O_4$	Trijuganone A
40	8.89	$[M+H]^+$	311.1278	311.1283	−1.6	643, 333, 311, 293, 283, 275, 267, 265, 251, 247, 237, 225, 207, 185	$C_{19}H_{18}O_4$	Tanshinone IIB

Table I. (continued)

Peak (No.)	RT (min)	Ion mode	Measured mass *(m/z)*	Calculated mass *(m/z)*	Error (ppm)	MS/MS *(m/z)*	Formula	Identification
41	9.22	$[M+H]^+$	341.1401	341.1389	3.5	703, 341, 309, 301, 295, 281, 263, 235, 231	$C_{20}H_{20}O_5$	Trijuganone C
42	9.35	$[M+H]^+$	327.1229	327.1232	−0.9	653, 349, 327, 309, 283, 265, 223	$C_{19}H_{18}O_5$	3-Hydroxtanshinone IIB
43	9.63	$[M+H]^+$	297.1493	297.1491	0.7	297, 279, 270, 253, 241, 238, 225, 211, 199, 183, 169	$C_{19}H_{20}O_3$	Isocryptotanshinone II
44	9.73	$[M+H]^+$	309.1133	309.1127	1.9	331, 309, 293, 281, 278, 263, 261, 247, 235, 211	$C_{19}H_{16}O_4$	Tanshinaldehyde
45	9.98	$[M+H]^+$	311.1295	311.1283	3.9	643, 311, 293, 282, 275, 265, 251, 247, 236, 219, 212, 209	$C_{19}H_{18}O_4$	1-Ketoisocryptotanshinone
46	10.01	$[M+H]^+$	279.1020	279.1021	−0.4	579, 301, 279, 261, 251, 246, 237, 233, 223, 218, 209, 205, 190, 169, 149	$C_{18}H_{14}O_3$	15,16-Dihydrotanshinone I
47	10.49	$[M+H]^+$	281.1187	281.1178	3.2	583, 303, 281, 263, 253, 248, 235, 220, 217, 207, 202, 192, 169	$C_{18}H_{16}O_3$	Trijuganone B
48	10.71	$[M+H]^+$	339.1233	339.1232	0.3	699, 361, 339, 279, 261, 233	$C_{20}H_{18}O_5$	Methyltanshinonate
49	10.87	$[M+H]^+$	295.1339	295.1334	1.7	611, 317, 295, 280, 277, 267, 262, 249, 235, 225, 221, 185	$C_{19}H_{18}O_3$	Isotanshinone IIA
50	11.27	$[M+H]^+$	315.1952	315.1960	−2.5	651, 337, 315, 297, 287, 269, 255, 241, 229, 215, 201, 189, 175, 163, 157, 109	$C_{20}H_{26}O_3$	6,7-Dehydroroyleanone

Table I. (continued)

Peak (No.)	RT (min)	Ion mode	Measured mass *(m/z)*	Calculated mass *(m/z)*	Error (ppm)	MS/MS *(m/z)*	Formula	Identification
51	11.89	$[M+H]^+$	297.1481	297.1491	−3.4	615, 593, 319, 297, 282, 279, 268, 254, 251, 237, 227, 209	$C_{19}H_{20}O_3$	Cryptotanshinone
52	12.34	$[M+H]^+$	277.0870	277.0865	1.8	575, 299, 277, 262, 249, 234, 231, 221, 203, 193, 178, 169	$C_{18}H_{12}O_3$	Tanshinone I
53	13.49	$[M+H]^+$	279.1013	279.1021	−2.9	579, 301, 279, 261, 233, 218, 205, 190	$C_{18}H_{14}O_3$	Dihydrotanshinone I
54	14.08	$[M+H]^+$	293.1174	293.1178	−1.4	607, 315, 293, 278, 275, 265, 260, 251, 247, 229, 219, 204	$C_{19}H_{16}O_3$	1,2-Didehydrotanshinone IIA
55	14.67	$[M+H]^+$	277.2158	277.2168	−3.6	277, 262, 249, 233, 217, 207, 199, 189, 185, 175, 165, 161, 151, 133, 119, 107	$C_{18}H_{28}O_2$	Stearidonic acid
56	15.04	$[M+H]^+$	281.1540	281.1542	−0.7	583, 303, 281, 266, 253, 238, 221, 211, 193, 183, 169, 155, 149	$C_{19}H_{20}O_2$	1,2-Didehydromiltirone
57	15.26	$[M+H]^+$	295.1333	295.1334	−0.3	611, 317, 295, 280, 277, 266, 252, 249, 235, 231, 221, 207	$C_{19}H_{18}O_3$	Tanshinone IIA
58	15.40	$[M+H]^+$	283.1700	283.1698	0.7	587, 283, 268, 265, 254, 240, 237, 223, 208, 195, 181	$C_{19}H_{22}O_2$	Miltirone

Note: RT means retention time; $[M-H]^-$ and $[M+H]^+$ mean the deprotonated and protonated molecular ions in the negative and positive ion modes, respectively; measured and calculated masses were obtained by Masslynx software; MS/MS includes the data of dimers and fragment ions.

the fragmentation ion $[M-H-60]^-$ at *m/z* 311. However, micranthin B first yielded a formation of product ion $[M-H-15]^-$ at *m/z* 356 owing to the disassociation of a methyl (15 Da). The major fragmentation pathways of micranthin B are shown in *Fig. 3*.

9-Oxo-10E,12Z-octadecadienoic acid, an organic acid of unsaturation, contains carboxyl and carbonyl groups. The deprotonated molecular ion $[M-H]^-$ of 9-oxo-10E,12Z-octadecadienoic acid at *m/z* 293.2128 may generate four product ions at *m/z* 265, 249, 197, and 185 after the losses of 28 Da, 44 Da, 96 Da, and 108 Da, respectively. The disconnections of 36 Da and 60 Da were produced further from the deprotonated daughter ion $[M-H-108]^-$ at *m/z* 185, respectively. The major fragmentation pathways of 9-oxo-10E,12Z-octadecadienoic acid are explained in *Fig. 4*.

Fragmentation Mechanisms of Hydrophilic Phenolics and Lipophilic Diterpenoids

Some of hydrophilic phenolics in the ESI-MS-MS spectra have a dimer of $[2M-H]^-$, such as danshensu, salvianolic acid F, and salvianolic acid A. Many other hydrophilic phenolics including salvianolic acid B, salvianolic acid E, and salvianolic acid A exhibited a common fragmentation behavior, which mainly caused the losses of danshensu (198 Da) and caffeic acid (180 Da) molecules. For example, the proposed fragmentation pathways of salvianolic acid B are schematized in *Fig. 5*. Differently, a product ion at *m/z* 537 was firstly caused by the deprotonated molecular ion $[M-H]^-$ of salvianolic acid B at *m/z* 717.1464 after the disconnection of a caffeic acid (180 Da) molecule. Then a product ion at *m/z* 339 was formed due to the disconnection of another caffeic acid (180 Da) molecule from the deprotonated daughter ion $[M-H-198]^-$ at *m/z* 519. The fragment ions of $[M-H-18]^-$, $[M-H-44]^-$, and $[M-H-28]^-$ corresponding to the losses of H_2O, CO_2, and CO molecules, respectively, are also presented in the ESI–MS/MS spectra of hydrophilic phenolics, including danshensu, caffeic acid, and salvianolic acid F. Besides, protocatechuic acid is easy to be ignored due to its low concentration.

On the other hand, most of lipophilic diterpenoids have a dimer of $[2M+Na]^+$, such as tanshinone I, tanshinone IIA, dihydrotanshinone I, and cryptotanshinone. The fragment ions of lipophilic diterpenoids primarily took place in the losses of H_2O (18 Da), CH_3 (15 Da), and CO (28 Da). For instance, the proposed fragmentation pathways of tanshinone I are summarized in *Fig. 6*. The protonated molecular ion $[M+H]^+$ of tanshinone I at *m/z* 277.0870 firstly has two fragmentation directions. One is the disassociation of a methyl (15 Da) and the other is the loss of CO (28 Da). Then, the latter

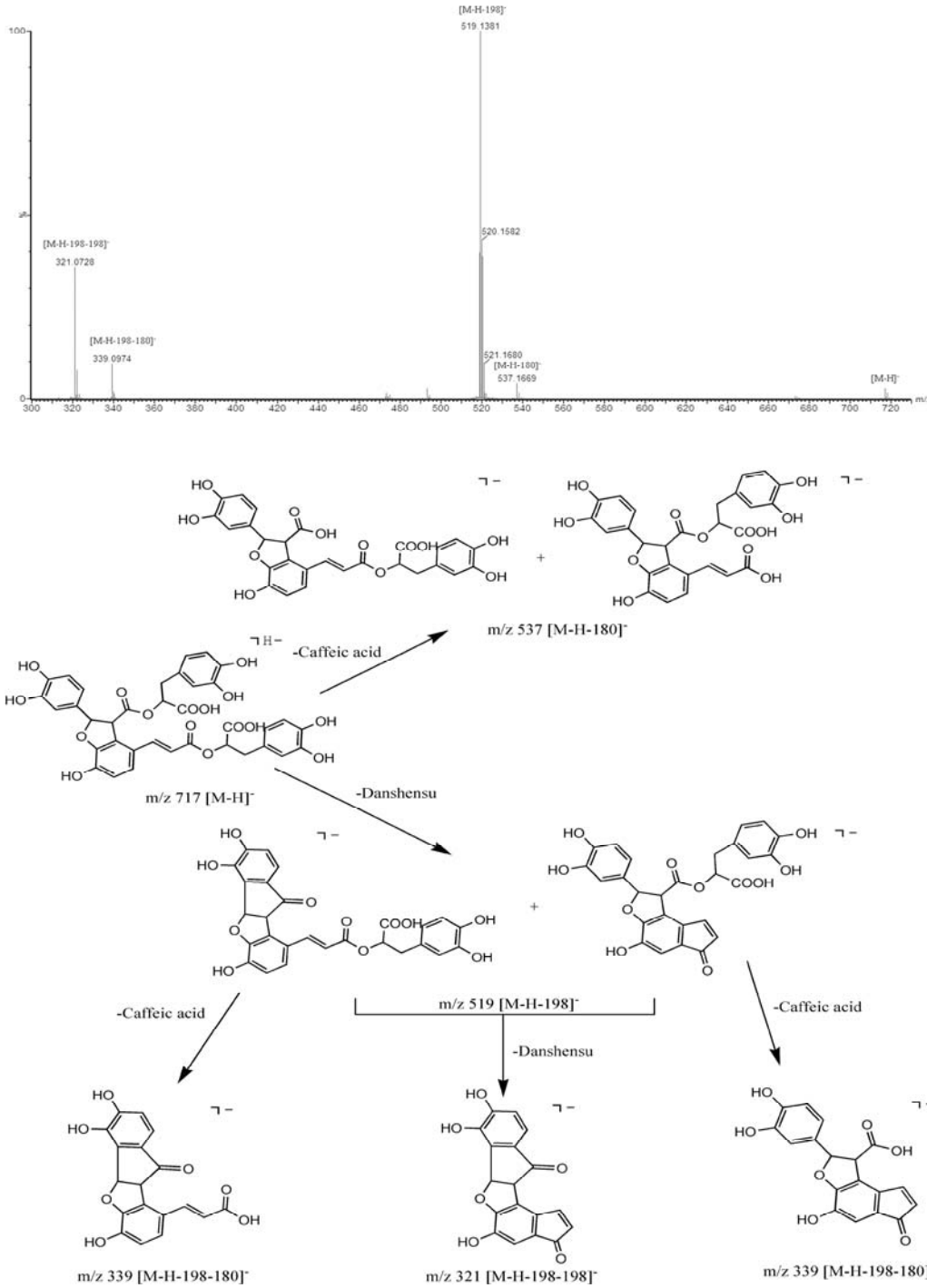

Fig. 5. Fragmentation pathways of salvianolic acid B

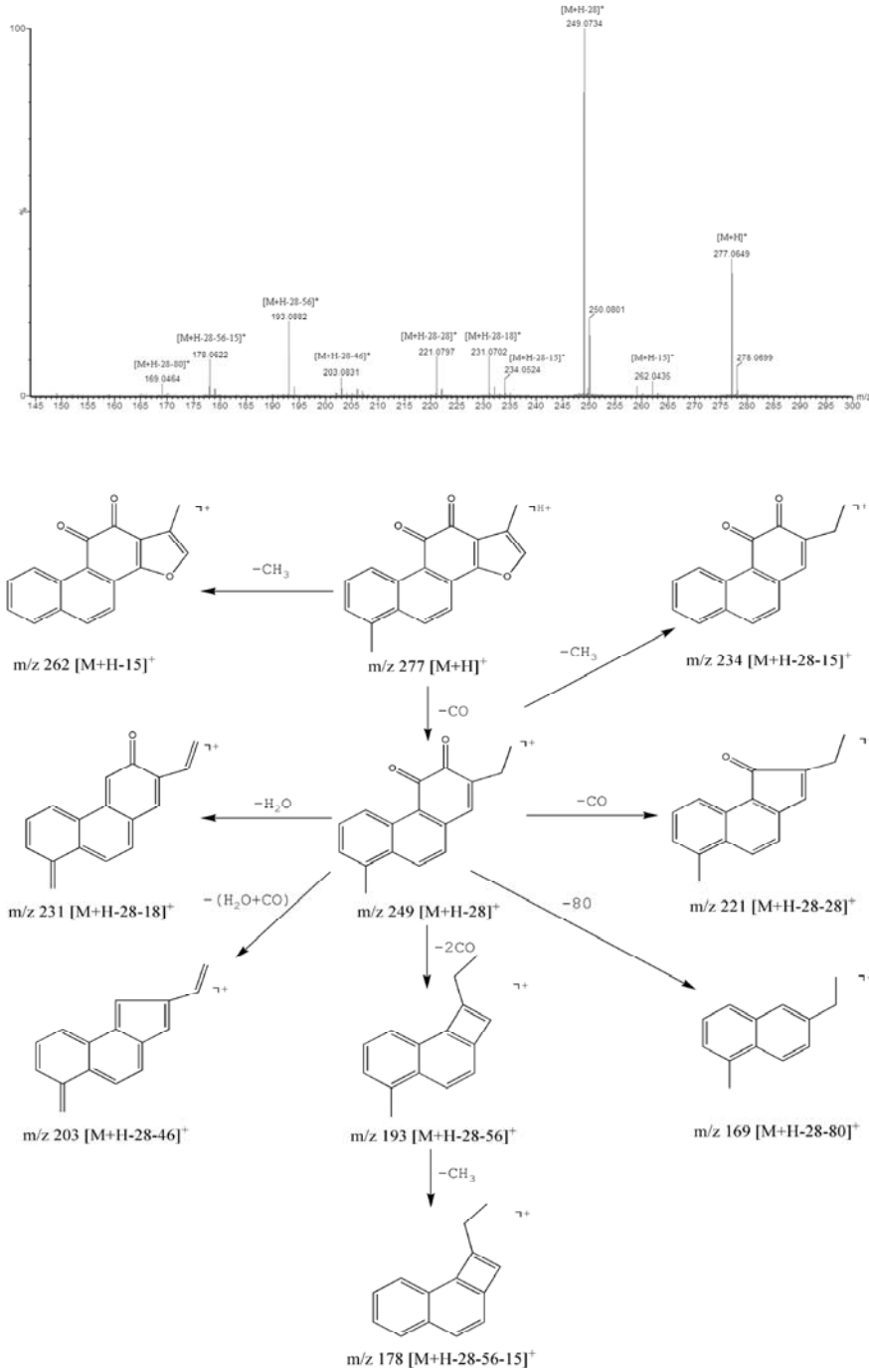

Fig. 6. Fragmentation pathways of tanshinone I

continued to generate six product ions at *m/z* 234, 231, 221, 203, 193, and 169 after the losses of 15 Da, 18 Da, 28 Da, 46 Da, 56 Da, and 80 Da, respectively. Finally, a molecule of methyl (15 Da) was produced further from the product ion $[M+H-28-56]^+$ at *m/z* 193.

Conclusion

UPLC/Q-TOF-MS was proved to be an effective, rapid, and reliable method for the isolation and identification of hydrophilic phenolics and lipophilic diterpenoids from Radix Salviae Miltiorrhizae. A total of 58 compounds were successfully and simultaneously identified within 20 min by this method. Compounds micranthin B and 9-oxo-10E,12Z-octadecadienoic acid were firstly found in RSM. The major fragmentation patterns of micranthin B and 9-oxo-10E,12Z-octadecadienoic acid are listed. Fragmentation mechanisms of hydrophilic phenolics and lipophilic diterpenoids were investigated. In conclusion, the structural identification investigated by UPLC/Q-TOF-MS could support systematic analysis and improve further quality evaluation standard for Radix Salviae Miltiorrhizae.

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