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Ultra-high-performance liquid chromatography–quadrupole/time of flight mass spectrometry based chemical profiling approach to rapidly reveal chemical transformation of sulfur-fumigated medicinal herbs, a case study on white ginseng

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ABSTRACT

Sulfur-fumigation may induce chemical transformation of medicinal herbs. Development of rapid method to reveal potential sulfur-fumigation induced chemical transformation of herbs is a very important issue for efficacy and safety of herb application. In present study, a new strategy was proposed to rapidly reveal chemical transformation of sulfur-fumigated herbs by ultra-high-performance liquid chromatography–quadrupole/time of flight mass spectrometry (UHPLC–QTOF-MS/MS) based chemical profiling approach. The non-fumigated herb was water-wetted and further treated with burning sulfur to get sulfur-fumigated herb. Then the chemical fingerprints of both non-fumigated and sulfur-fumigated samples were compared by UHPLC–QTOF-MS/MS analysis. The identities of all detected peaks, in particular those newly generated in sulfur-fumigated samples were confirmed by comparing the mass spectra and retention times of peaks with that of reference compounds, and/or tentatively assigned by matching empirical molecular formula with that of published compounds, and/or elucidating quasi-molecular ions and fragment ions referring to available literature information. The identification could be rationalized through deducing possible reactions involved in the generation of these newly detected compounds. The proposed strategy was extensively investigated in the case of white ginseng. Total 82 components were detected in non-fumigated and sulfur-fumigated white ginseng samples, among them 35 sulfur-containing compounds detected only in sulfur-fumigated white ginseng and its decoction were assigned to be sulfate or sulfite derivatives of original ginsenosides, and were deduced to be generated via reactions of esterification, addition, hydrolysis and dehydration during sulfur-fumigation and decocting of white ginseng. The established approach was applied to discriminate sulfur-fumigated white ginseng among commercial samples from America, Canada, and Hong Kong SAR, Macau SAR and Mainland of China, which indicated that the proposed approach is rapid and specific, and should also be useful for investigation of potential chemical transformation of other sulfur-fumigated medicinal herbs.

1. Introduction

In the near decades, sulfur-fumigation has been employed in post-harvest handling of some medicinal herbs to keep moist, preserve color and freshness, and prevent against insects and moulds [1]. As a matter of fact, sulfur-based preservatives have been used around the world for centuries in food industry to inhibit oxidation ("browing") of light-coloured fruits or vegetables [2]. However, sulfur-fumigation was recently reported to cause chemical transformation of original bioactive components in herbs or its extracts

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[2–5], and consequently altering bioactivities [5], pharmacokinetics [6], or even toxicities [our unpublished data] of herbs. Thus, whether the constituents of a sulfur-fumigated herb changed or not is a very important issue for not only the efficacy but also the safety of the herb application. Development of a rapid and specific approach to determine the potential chemical changes is the key to the quality evaluation of sulfur-fumigated herbs.

Conventional phytochemical approaches which isolate and identify individual components one by one could be used to investigate chemical transformation in sulfur-fumigated herbs, but this strategy is time-consuming and can easily miss potential newly generated artifacts [2,3]. Ultra-high-performance liquid chromatography–quadrupole/time of flight mass spectrometry (UHPLC–QTOF-MS/MS) is a powerful hyphenated technique, and has been used for the rapid holistic chemical profiling studies of medicinal herbs [3,7,8].

Asian ginseng (briefly ginseng), derived from the root and rhizome of *Panax ginseng*, is a commonly used tonic and panacea herb in China, Korea, Japan and other countries or regions [9,10]. Modern chemical, pharmacological and clinical studies indicated that ginsenosides were the major components with many bioactivities responsible for the panacea effects of ginseng [11–19]. The "adaptogenic" actions of ginseng have been clarified to be related to the compositional ratio between individual ginsenosides with opposite activities [20].

Ginseng has been traditionally post-harvest handled in two different ways, i.e., directly dried to get white ginseng, or steamed and dried to get red ginseng [10]. White ginseng was recently reported being sulfur-fumigated by some herbal farmers or wholesalers during post-harvest handling and storage [21,22]. However, to the best of our knowledge, there has been no report about the influence of sulfur-fumigation on bioactive components of white ginseng.

In present study, using white ginseng as a model herb, a new strategy to rapidly reveal chemical transformation of sulfur-fumigated herbs by UHPLC–QTOF-MS/MS analysis was proposed. The protocol was shown in Fig. 1. The non-fumigated white ginseng was water-wetted and further treated with burning sulfur to get sulfur-fumigated white ginseng. Then the chemical fingerprints of both non-fumigated and sulfur-fumigated samples were compared by an improved UHPLC–QTOF-MS/MS analysis. The identities of all detected peaks, in particular those newly generated in sulfur-fumigated samples were confirmed by comparing the mass spectra and retention times with that of available reference compounds, and/or tentatively assigned by matching empirical molecular formula with that of published compounds, and/or elucidating quasi-molecular ions and fragment ions referring to the available literature information. The identification can be rationalized through deducing possible reactions involved in the generation of these newly detected compounds.

The established approach was applied to rapidly discriminate sulfur-fumigated white ginseng among commercial samples from America, Canada, and Hong Kong SAR, Macau SAR and Mainland of China.

2. Experimental

2.1. Chemicals, reference compounds and samples

HPLC–MS grade acetonitrile from TEDIA Company Inc. (Fairfield, USA), MS grade formic acid from Sigma–Aldrich (Steinheim, Germany), chemical pure sulfur from Shanghai Lingfeng Chemical Reagent Co. Ltd. (Shanghai, China) were purchased. Other solvents and chemicals were of analytical grade. Ultra-pure water was prepared using Milli-Q SP system (Millipore, Bedford, MA, USA).

The reference compounds pesudoginsenoside F_{11}, ginsenoside Rf, Re, Rg_1, Rb_1, Rb_2, Ro, and Rd from Shanghai Institute for Food and Drug Control (Shanghai, China), Rg_2, Rc, 20(*R*)-Rg_3, Rb_3 and Rh_2 from Shanghai Yuanye Biotech Co. Lit. (Shanghai, China), were purchased. Their purity was higher than 95.0% by HPLC analysis.

The reference white ginseng samples (JSPACM-03-1 and JSPACM-03-2) were collected from Jilin province, the indigenous cultivating region of ginseng. The commercial samples of white ginseng were purchased from different herbal shops in Jilin, Nanjing, Guangzhou, Hong Kong SAR and Macau SAR of China, Totonto and Edmonton of Canada, and San Francisco and Chicago of America (Table 1). The identities of all white ginseng samples were authenticated to be the dried root and rhizome of *P. ginseng* by morphological and histological methods according to monograph of Chinese Pharmacopoeia (version 2010) [10] by Prof. Song-Lin Li. The voucher specimens were deposited in Department of Metabolomics and Pharmaceutical Analysis, Jiangsu Province Academy of Chinese Medicine.

2.2. Liquid chromatography

Liquid chromatography was performed with a Waters Acquity UPLC core system (Waters Corp., MA, USA), equipped with a binary solvent delivery system, auto-sampler, and a PDA detector. The column was a Waters Acquity HSS T3 (2.1 mm × 100 mm, I.D., 1.8 μm). The mobile phase consisted of (A) 0.1% formic acid in water and (B) ACN containing 0.1% formic acid. The UPLC elution condition was optimized as follows: 5–15% B (0–1 min), 15–60% B (1–22 min), 60–95% B (22–23 min), 95% B (23–24 min), 95–5% B (24–26 min) and isocratic at 5% B (26–27 min). The flow rate was at 0.6 ml/min. The column and auto-sampler were maintained at 35 and 10 °C respectively, and the injection volume of reference compounds and samples was 2 μl.

2.3. Mass spectrometry

Mass spectrometry was performed on a Waters Synapt G2 Q-TOF (Micromass MS Technologies, Manchester, UK) equipped with electrospray ionization (ESI) source operating in negative mode. The nebulization gas was set to 900 l/min at temperature of 450 °C, the cone gas set to 40 l/min. The capillary voltage and cone voltage were set at 2500 V and 30 V respectively. The Q-TOF acquisition rate was 0.2 s and the inter-scan delay was 0.02 s. Argon was employed as the collision gas at a pressure of 7.066×10^{-3} Pa.

The energies for collision-induced dissociation (CID) were set at 5 and 45 eV respectively for the fragmentation information.

2.4. Accurate mass measurement

All MS data were acquired using the LockSpray to ensure mass accuracy and reproducibility. The molecular masses of the precursor ion and of product ions were accurately determined with leucine-enkephalin (m/z 554.2615) in negative electrospray ionization mode at the concentration of 50 pg/μl and the infusion flow rate was 10 μl/min. Centroided data were acquired for each sample from 100 to 1500 Da and dynamic range enhancement was applied in the MS experiment to ensure accurate mass measurement over a wide dynamic range.

2.5. Sample preparation

2.5.1. Sulfur-fumigated ginseng samples

Two sulfur-fumigated ginseng samples (JSPACM-03-3 and JSPACM-03-4) were prepared respectively from two reference white ginseng samples (JSPACM-03-1 and JSPACM-03-2) following the modified procedures similar to that performed by farmers

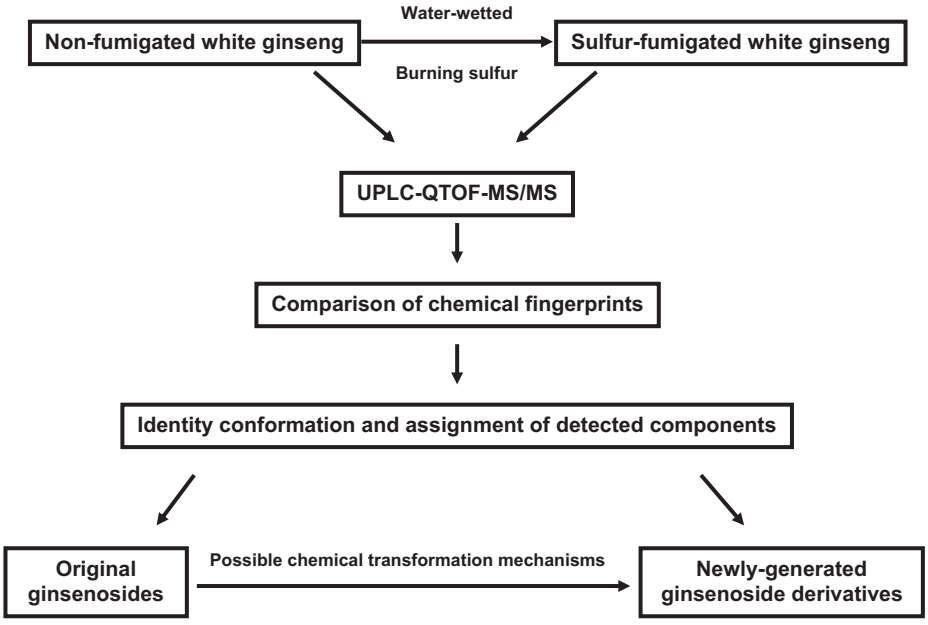

Fig. 1. Strategy proposed for rapidly revealing sulfur-fumigation induced chemical transformation of white ginseng by UHPLC–QTOF-MS/MS based chemical profiling analysis.

Table 1
Detection of ginsenoside sulfates or sulfites in commercial white ginseng samples.

Code no.	Name	Location	Ginsenoside sulfates or sulfites
JSPACM-03-1	White ginseng slice	Jilin, China	–
JSPACM-03-2	White ginseng slice	Jilin, China	–
JSPACM-03-3	White ginseng slice	Sulfur-fumigated product of sample JSPACM-03-1	**1, 2, 3, 4, 5, 6, 7, 10, 23, 25, 26, 29, 32, 35**
JSPACM-03-4	White ginseng slice	Sulfur-fumigated product of sample JSPACM-03-2	**1, 2, 3, 4, 5, 6, 7, 10, 23, 25, 26, 29, 32, 35**
JSPACM-03-5	White ginseng	Jilin, China	–
JSPACM-03-6	White ginseng slice	Nanjing, China	**2, 4 (+)**
JSPACM-03-7	White ginseng slice	Nanjing, China	**2, 4 (+)**
JSPACM-03-8	White ginseng slice	Nanjing, China	–
JSPACM-03-9	White ginseng slice	Nanjing, China	**2, 4 (+)**
JSPACM-03-10	White ginseng slice	Nanjing, China	**2, 4 (+)**
JSPACM-03-11	White ginseng slice	Nanjing, China	**2, 4 (+)**
JSPACM-03-12	White ginseng slice	Nanjing, China	**2, 4 (+)**
JSPACM-03-13	White ginseng	Nanjing, China	**2, 4 (+)**
JSPACM-03-14	White ginseng	Hong Kong, SAR China	**2, 4 (+)**
JSPACM-03-15	White ginseng slice	Hong Kong, SAR China	–
JSPACM-03-16	White ginseng	Hong Kong, SAR China	**2, 4 (+)**
JSPACM-03-17	White ginseng	Hong Kong, SAR China	–
JSPACM-03-18	White ginseng	Hong Kong, SAR China	–
JSPACM-03-19	White ginseng	Hong Kong, SAR China	–
JSPACM-03-20	White ginseng	Hong Kong, SAR China	**2, 4 (+)**
JSPACM-03-21	White ginseng	Hong Kong, SAR China	–
JSPACM-03-22	White ginseng slice	Guangzhou, China	**2, 4 (+)**
JSPACM-03-23	White ginseng slice	Guangzhou, China	**2, 4 (+)**
JSPACM-03-24	White ginseng slice	Guangzhou, China	**2, 4 (+)**
JSPACM-03-25	White ginseng slice	Guangzhou, China	–
JSPACM-03-26	White ginseng slice	Guangzhou, China	**2, 4 (+)**
JSPACM-03-27	White ginseng	Toronto, Canada	**2, 4 (+)**
JSPACM-03-28	White ginseng	Toronto, Canada	–
JSPACM-03-29	White ginseng slice	Toronto, Canada	–
JSPACM-03-30	White ginseng	Edmonton, Canada	–
JSPACM-03-31	White ginseng	Edmonton, Canada	–
JSPACM-03-32	White ginseng	Macau, SAR China	–
JSPACM-03-33	White ginseng	Macau, SAR China	–
JSPACM-03-34	White ginseng	Macau, SAR China	–
JSPACM-03-35	White ginseng	Macau, SAR China	–
JSPACM-03-36	White ginseng	Macau, SAR China	–
JSPACM-03-37	White ginseng	Jilin, China	–
JSPACM-03-38	White ginseng	San Francisco, USA	–
JSPACM-03-39	White ginseng	San Francisco, USA	**2, 4 (+)**
JSPACM-03-40	White ginseng	San Francisco, USA	–
JSPACM-03-41	White ginseng	Chicago, USA	**2, 4 (+)**
JSPACM-03-42	White ginseng	Chicago, USA	**2, 4 (+)**

+: detected with at least 25-hydroxyl-Re sulfate (**2**) and 25-hydroxyl-Rg_1 sulfite (**4**); –: detected without ginsenoside sulfates or sulfites.

Table 2
Components identified from non-fumigated and sulfur-fumigated white ginseng samples and their decoctions.

Peak no.	Identity	t_R (min)	Mean measured mass (Da)	Theoretical exact mass (Da)	Mass accuracy (ppm)	Empirical formula and proposed CID fragment ions	$[M-2H]^{2-}$	Reference literatures
1	25-Hydroxyl-Rg_1 sulfate	1.55	897.4552	897.4518	3.8	$C_{42}H_{73}O_{18}S$ $[M-H]^-$	–	
			879.4415	879.4412	0.3	$C_{42}H_{71}O_{17}S$ $[M-H-H_2O]^-$		
			717.3883	717.3884	0.1	$C_{36}H_{61}O_{12}S$ $[M-H-H_2O-(Glc-H_2O)]^-$		
			699.3762	699.3778	−2.3	$C_{36}H_{59}O_{11}S$ $[M-H-2H_2O-(Glc-H_2O)]^-$		
			537.3244	537.3250	−1.2	$C_{30}H_{49}O_6S$ $[M-H-2H_2O-2(Glc-H_2O)]^-$		
2	25-Hydroxyl-Re sulfate	1.62	1043.5250	1043.5249	0.1	$C_{48}H_{83}O_{22}S$ $[M-H]^-$	–	
			897.4517	897.4518	−0.1	$C_{42}H_{71}O_{17}S$ $[M-H-(Rha-H_2O)]^-$		
			879.4420	879.4412	0.9	$C_{42}H_{71}O_{17}S$ $[M-H-(Rha-H_2O)-H_2O]^-$		
			717.3901	717.3884	2.4	$C_{36}H_{61}O_{12}S$ $[M-H-(Rha-H_2O)-H_2O-(Glc-H_2O)]^-$		
			699.3794	699.3778	2.3	$C_{36}H_{59}O_{11}S$ $[M-H-(Rha-H_2O)-2H_2O-(Glc-H_2O)]^-$		
			537.3245	537.3250	−0.9	$C_{30}H_{49}O_6S$ $[M-H-(Rha-H_2O)-2H_2O-2(Glc-H_2O)]^-$		
3	Re sulfate	1.72	1025.5177	1025.5144	3.2	$C_{48}H_{81}O_{21}S$ $[M-H]^-$	512.2398	
			863.4462	863.4463	−0.1	$C_{42}H_{71}O_{16}S$ $[M-H-(Glc-H_2O)]^-$		
			845.4360	845.4357	0.4	$C_{42}H_{69}O_{15}S$ $[M-H-(Glc-H_2O)-H_2O]^-$		
			699.3802	699.3778	3.4	$C_{36}H_{59}O_{11}S$ $[M-H-(Glc-H_2O)-H_2O-(Rha-H_2O)]^-$		
			537.3234	537.3250	−3.0	$C_{30}H_{49}O_6S$ $[M-H-2(Glc-H_2O)-H_2O-(Rha-H_2O)]^-$		
4	25-Hydroxyl-Rg_1 sulfite	1.78	881.4577	881.4568	1.0	$C_{42}H_{73}O_{17}S$ $[M-H]^-$	–	
			863.4461	863.4463	−0.2	$C_{42}H_{71}O_{16}S$ $[M-H-H_2O]^-$		
			701.3940	701.3935	0.7	$C_{36}H_{61}O_{11}S$ $[M-H-H_2O-(Glc-H_2O)]^-$		
			539.3426	539.3406	2.0	$C_{30}H_{49}O_6S$ $[M-H-H_2O-2(Glc-H_2O)]^-$		
5	25-Hydroxyl-Re sulfite	1.93	1027.5184	1027.5183	0.1	$C_{48}H_{81}O_{20}S$ $[M-H]^-$		
			881.4542	881.4568	−2.9	$C_{42}H_{73}O_{17}S$ $[M-H-(Rha-H_2O)]^-$		
			847.4515	847.4514	0.1	$C_{42}H_{71}O_{15}S$ $[M-H-(Glc-H_2O)-H_2O]^-$		
6	Rg_1 sulfate	2.15	879.4417	879.4412	0.6	$C_{42}H_{71}O_{17}S$ $[M-H]^-$	–	
			717.3908	717.3884	3.3	$C_{36}H_{61}O_{12}S$ $[M-H-(Glc-H_2O)]^-$		
			555.3353	555.3356	−0.2	$C_{30}H_{51}O_7S$ $[M-H-2(Glc-H_2O)]^-$		
7	Rg_1 sulfate isomer	2.27	879.4420	879.4412	0.9	$C_{42}H_{71}O_{17}S$ $[M-H]^-$	439.2119	
			717.3867	717.3884	−2.8	$C_{36}H_{61}O_{12}S$ $[M-H-(Glc-H_2O)]^-$		
			555.3359	555.3356	0.2	$C_{30}H_{51}O_7S$ $[M-H-2(Glc-H_2O)]^-$		
8	25-Hydroxyl-Rh_1 sulfate	2.62	735.3967	735.3589	−3.0	$C_{36}H_{63}O_{13}S$ $[M-H]$-	–	
			717.3885	717.3884	0.1	$C_{36}H_{61}O_{12}S$ $[M-H-H_2O]^-$		
9	Rh_1 sulfate	2.87	717.3890	717.3884	0.8	$C_{36}H_{61}O_{12}S$ $[M-H]^-$	–	
			699.3808	699.3778	3.6	$C_{36}H_{59}O_{11}S$ $[M-H-H_2O]^-$		
			537.3212	537.3250	−4.9	$C_{30}H_{49}O_6S$ $[M-H-H_2O-(Glc-H_2O)]^-$		
10	25-Hydroxyl-Rg_1 sulfite isomer	2.98	881.4576	881.4568	0.9	$C_{42}H_{73}O_{17}S$ $[M-H]^-$	440.2159	
			863.4461	863.4463	−0.2	$C_{42}H_{71}O_{16}S$ $[M-H-H_2O]^-$		
			719.4042	719.4040	0.2	$C_{36}H_{63}O_{12}S$ $[M-H-(Glc-H_2O)]^-$		
11	25-Hydroxyl-Rh_1 sulfate isomer	3.03	735.3963	735.3989	−3.5	$C_{36}H_{63}O_{13}S$ $[M-H]^-$	–	
12	25-Hydroxyl-Rh_2 sulfate	3.21	719.4041	719.4040	0.1	$C_{36}H_{63}O_{12}S$ $[M-H]^-$	–	
13	Unknown	3.39	865.4657	865.4619	4.4	$C_{42}H_{73}O_{16}S$ $[M-H]^-$	–	
14	25-Hydroxyl-Rh_2 sulfate isomer	3.51	719.4019	719.4040	−2.9	$C_{36}H_{63}O_{12}S$ $[M-H]^-$	–	
			539.3428	539.3406	4.1	$C_{30}H_{51}O_{16}S$ $[M-H-Glc]^-$		
15	Rg_2-sulfate	3.59	863.4471	863.4463	0.9	$C_{42}H_{71}O_{16}S$ $[M-H]^-$	431.2114	
			717.3899	717.3884	2.1	$C_{36}H_{61}O_{12}S$ $[M-H-(Rha-H_2O)]^-$		
			555.3360	555.3356	0.7	$C_{30}H_{51}O_5S$ $[M-H-(Rha-H_2O)-(Glc-H_2O)]^-$		
16	Rh_1 sulfate isomer	3.61	717.3913	717.3884	4.0	$C_{36}H_{61}O_{12}S$ $[M-H]^-$	–	
17	25-Hydroxyl-Rh_2 sulfate isomer	3.66	719.4044	719.4040	0.6	$C_{36}H_{63}O_{12}S$ $[M-H]^-$	–	
18	Pk_3 sulfate	3.87	699.3765	699.3778	−1.9	$C_{36}H_{59}O_{11}S$ $[M-H]^-$	–	
19	25-Hydroxyl-Rh_2 sulfate isomer	3.95	719.4044	719.4040	0.6	$C_{36}H_{63}O_{12}S$ $[M-H]^-$	–	
20	Rh_1 sulfate	4.05	717.3891	717.3884	1.0	$C_{36}H_{61}O_{12}S$ $[M-H]^-$	–	
21	Rh_1 sulfate isomer	4.30	717.3889	717.3884	0.7	$C_{36}H_{61}O_{12}S$ $[M-H]^-$	–	

Table 2 (*Continued*)

Peak no.	Identity	t_R (min)	Mean measured mass (Da)	Theoretical exact mass (Da)	Mass accuracy (ppm)	Empirical formula and proposed CID fragment ions	$[M-2H]^{2-}$	Reference literatures
22	Rh_4 sulfate	4.45	699.3751	699.3778	−3.9	$C_{36}H_{59}O_{11}S$ $[M-H]^-$	–	
23	Rb_1 sulfate	4.63	1187.5753	1187.5766	−1.1	$C_{54}H_{91}O_{26}S$ $[M-H]^-$	593.2644	
			1025.5144	1025.5144	0	$C_{48}H_{81}O_{21}S$ $[M-H-(Glc-H_2O)]^-$		
			863.4431	863.4463	−3.7	$C_{42}H_{71}O_{16}S$ $[M-H-2(Glc-H_2O)]^-$		
			845.4391	845.4357	4.0	$C_{42}H_{69}O_{15}S$ $[M-H-2(Glc-H_2O)-H_2O]^-$		
			683.3836	683.3829	1.0	$C_{36}H_{59}O_{10}S$ $[M-H-3(Glc-H_2O)-H_2O]^-$		
			521.3307	521.3301	1.2	$C_{30}H_{49}O_5S$ $[M-H-4(Glc-H_2O)-H_2O]^-$		
24	F_4/Rg_6 sulfate	4.75	845.4332	845.4357	−3.0	$C_{42}H_{69}O_{15}S$ $[M-H]^-$	–	
			699.3764	699.3778	−2.0	$C_{36}H_{59}O_{11}S$ $[M-H-(Rha-H_2O)]^-$		
25	$Rc/Rb_2/Rb_3$ sulfate	4.89	1157.5664	1157.5719	−4.8	$C_{53}H_{89}O_{25}S$ $[M-H]^-$	579.2586	
			995.5071	995.5114	−4.3	$C_{47}H_{79}O_{20}S$ $[M-H-(Glc-H_2O)]^-$		
			845.4337	845.4357	−2.9	$C_{42}H_{69}O_{15}S$ $[M-H-(Glc-H_2O)-Ara\ (or\ Xyl)]^-$		
			683.3800	683.3829	−4.2	$C_{36}H_{59}O_{10}S$ $[M-H-2(Glc-H_2O)-Ara\ (or\ Xyl)]^-$		
			521.3318	521.3301	3.3	$C_{30}H_{49}O_5S$ $[M-H-3(Glc-H_2O)-Ara\ (or\ Xyl)]^-$		
26	Ma-Rb_1 sulfate	5.10	1273.5919	1273.5871	3.8	$C_{57}H_{93}O_{29}S$ $[M-H]^-$	614.2708	
			1229.5935	1229.5965	−2.1	$C_{56}H_{93}O_{27}S$ $[M-H-CO_2]^-$		
			1187.5773	1187.5766	0.6	$C_{54}H_{91}O_{26}S$ $[M-H-CO_2-C_2H_2O]^-$		
			1205.5131	1205.5144	−0.7	$C_{48}H_{81}O_{21}S$ $[M-H-CO_2-C_2H_2O-(Glc-H_2O)]^-$		
			863.4426	863.4463	−4.3	$C_{42}H_{71}O_{16}S$ $[M-H-CO_2-C_2H_2O-2(Glc-H_2O)]^-$		
			845.4332	845.4357	−3.0	$C_{42}H_{69}O_{15}S$ $[M-H-CO_2-C_2H_2O-2(Glc-H_2O)-H_2O]^-$		
			683.3840	683.3829	1.6	$C_{36}H_{59}O_{10}S$ $[M-H-CO_2-C_2H_2O-3(Glc-H_2O)-H_2O]^-$		
			521.3303	521.3301	0.4	$C_{30}H_{49}O_5S$ $[M-H-CO_2-C_2H_2O-4(Glc-H_2O)-H_2O]^-$		
27	Rh_2 sulfate	5.34	701.3900	701.3935	−5.0	$C_{36}H_{61}O_{11}S$ $[M-H]^-$	–	
28	20-glc-Rf	5.41	1007.5565	1007.5541	2.4	$C_{49}H_{83}O_{21}$ $[M-H+HCOOH]^-$	–	[30]
			961.5381	961.5372	0.9	$C_{48}H_{81}O_{19}$ $[M-H]^-$		
			799.4854	799.4844	1.3	$C_{42}H_{71}O_{14}$ $[M-H-(Glc-H_2O)]^-$		
			637.4293	637.4316	−3.6	$C_{36}H_{61}O_9$ $[M-H-2(Glc-H_2O)]^-$		
29	Rb_1 sulfate isomer	5.51	1187.5756	1187.5766	−0.8	$C_{54}H_{91}O_{26}S$ $[M-H]^-$	593.2604	
			1025.5128	1025.5144	−2.4	$C_{48}H_{81}O_{21}S$ $[M-H-(Glc-H_2O)]^-$		
			863.4428	863.4463	−4.1	$C_{42}H_{71}O_{16}S$ $[M-H-2(Glc-H_2O)]^-$		
			845.4380	845.4357	2.7	$C_{42}H_{69}O_{15}S$ $[M-H-2(Glc-H_2O)-H_2O]^-$		
			683.3845	683.3829	2.3	$C_{36}H_{59}O_{10}S$ $[M-H-3(Glc-H_2O)-H_2O]^-$		
			521.3298	521.3301	−0.6	$C_{30}H_{49}O_5S$ $[M-H-4(Glc-H_2O)-H_2O]^-$		
30	Rh_1 sulfite	5.60	701.3932	701.3935	−0.4	$C_{36}H_{61}O_{11}S$ $[M-H]^-$	–	
31	R_1	5.73	977.5284	977.5321	−3.8	$C_{48}H_{81}O_{20}$ $[M-H+HCOOH]^-$	–	[16]
			931.5278	931.5266	1.3	$C_{47}H_{79}O_{18}$ $[M-H]^-$		
			799.4852	799.4844	1.0	$C_{42}H_{71}O_{14}$ $[M-H-(Xyl-H_2O)]^-$		
			637.4319	637.4316	0.5	$C_{36}H_{61}O_9$ $[M-H-(Glc-H_2O)-(Xyl-H_2O)]^-$		
			475.3808	475.3787	4.4	$C_{30}H_{51}O_4$ $[M-H-2(Glc-H_2O)-(Xyl-H_2O)]^-$		
32	Ma-Rb_1 sulfate isomer	5.92	1273.5912	1273.5871	3.2	$C_{57}H_{93}O_{29}S$ $[M-H]^-$	614.2676	
			1229.5951	1229.5965	−1.1	$C_{56}H_{93}O_{27}S$ $[M-H-CO_2]^-$		
			1187.5718	1187.5766	−4.0	$C_{54}H_{91}O_{26}S$ $[M-H-CO_2-C_2H_2O]^-$		
			1025.5125	1025.5144	−1.9	$C_{48}H_{81}O_{21}S$ $[M-H-CO_2-C_2H_2O-(Glc-H_2O)]^-$		
			863.4456	863.4463	−0.8	$C_{42}H_{71}O_{16}S$ $[M-H-CO_2-C_2H_2O-2(Glc-H_2O)]^-$		
			845.4390	845.4357	3.9	$C_{42}H_{69}O_{15}S$ $[M-H-CO_2-C_2H_2O-2(Glc-H_2O)-H_2O]^-$		
			683.3842	683.3829	1.9	$C_{36}H_{59}O_{10}S$ $[M-H-CO_2-C_2H_2O-3(Glc-H_2O)-H_2O]^-$		
			521.3294	521.3301	−1.3	$C_{30}H_{49}O_5S$ $[M-H-CO_2-C_2H_2O-4(Glc-H_2O)-H_2O]^-$		
33	$Rg_1{}^{\Delta}$	6.28	845.4882	845.4899	−2.0	$C_{43}H_{73}O_{16}$ $[M-H+HCOOH]^-$	–	[30]
			799.4852	799.4844	1.0	$C_{42}H_{71}O_{14}$ $[M-H]^-$		
			637.4327	637.4316	1.7	$C_{36}H_{61}O_9$ $[M-H-(Glc-H_2O)]^-$		
			475.3788	475.3787	0.2	$C_{30}H_{51}O_4$ $[M-H-2(Glc-H_2O)]^-$		

Table 2 (*Continued*)

Peak no.	Identity	t_R (min)	Mean measured mass (Da)	Theoretical exact mass (Da)	Mass accuracy (ppm)	Empirical formula and proposed CID fragment ions	$[M-2H]^{2-}$	Reference literatures
34	Re$^{\Delta}$	6.28	991.5507	991.5478	3.2	$C_{49}H_{83}O_{20}$ $[M-H+HCOOH]^-$	–	[16]
			945.5446	945.5391	2.4	$C_{48}H_{81}O_{18}$ $[M-H]^-$		
			799.4870	799.4844	3.3	$C_{42}H_{71}O_{14}$ $[M-H-(Rha-H_2O)]^-$		
			783.4866	783.4895	−3.7	$C_{42}H_{71}O_{13}$ $[M-H-(Glc-H_2O)]^-$		
			637.4316	637.4316	−4.4	$C_{36}H_{61}O_9$ $[M-H-(Rha-H_2O)-(Glc-H_2O)]^-$		
			475.3801	475.3787	3.1	$C_{30}H_{51}O_4$ $[M-H-2(Glc-H_2O)-(Rha-H_2O)]^-$		
35	F_2 sulfate	6.77	863.4467	863.4463	0.5	$C_{42}H_{71}O_{16}S$ $[M-H]^-$	431.2092	
			701.3946	701.3935	1.6	$C_{36}H_{61}O_{11}S$ $[M-H-(Glc-H_2O)]^-$		
			539.3416	539.3406	1.9	$C_{30}H_{51}O_6S$ $[M-H-2(Glc-H_2O)]^-$		
36	Ma-Rg_1	6.86	885.4868	885.4848	2.3	$C_{45}H_{73}O_{17}$ $[M-H]^-$	–	[30]
			841.4988	841.4949	4.6	$C_{44}H_{73}O_{15}$ $[M-H-CO_2]^-$		
37	Ma-Rg_1 isomer	7.32	885.4876	885.4848	3.2	$C_{45}H_{73}O_{17}$ $[M-H]^-$	–	[30]
			841.4944	841.4949	−0.6	$C_{44}H_{73}O_{15}$ $[M-H-CO_2]^-$		
38	Ma-Re	7.41	1031.5420	1031.5427	−0.8	$C_{51}H_{83}O_{21}$ $[M-H]^-$	–	[16]
			987.5547	987.5545	0.2	$C_{50}H_{83}O_{19}$ $[M-H-CO_2]^-$		
39	Rg_3 sulfate isomer	7.82	863.4470	863.4463	0.8	$C_{42}H_{71}O_{16}S$ $[M-H]^-$	431.2080	
			701.3945	701.3935	1.4	$C_{36}H_{61}O_{11}S$ $[M-H-(Glc-H_2O)]^-$		
			539.3429	539.3406	4.3	$C_{30}H_{51}O_6S$ $[M-H-2(Glc-H_2O)]^-$		
40	Rg_3 sulfate isomer	7.92	863.4478	863.4463	1.7	$C_{42}H_{71}O_{16}S$ $[M-H]^-$	431.2060	
			701.3946	701.3935	1.6	$C_{36}H_{61}O_{11}S$ $[M-H-(Glc-H_2O)]^-$		
			539.3390	539.3406	−3.0	$C_{30}H_{51}O_6S$ $[M-H-2(Glc-H_2O)]^-$		
41	Rg_3 sulfate isomer	8.24	863.4458	863.4463	−0.6	$C_{42}H_{71}O_{16}S$ $[M-H]^-$	431.2094	
			701.3948	701.3935	1.9	$C_{36}H_{61}O_{11}S$ $[M-H-(Glc-H_2O)]^-$		
			539.3416	539.3406	1.9	$C_{30}H_{51}O_6S$ $[M-H-2(Glc-H_2O)]^-$		
42	Rk_1 sulfate	8.69	845.4370	845.4357	1.5	$C_{42}H_{69}O_{15}S$ $[M-H]^-$	422.2037	
			683.3828	683.3829	−0.1	$C_{36}H_{59}O_{10}S$ $[M-H-(Glc-H_2O)]^-$		
			521.3284	521.3301	−3.3	$C_{30}H_{49}O_5S$ $[M-H-2(Glc-H_2O)]^-$		
43	Rf$^{\Delta}$	9.18	845.4935	845.4899	4.3	$C_{43}H_{73}O_{16}$ $[M-H+HCOOH]^-$	–	[30]
			799.4863	799.4844	2.4	$C_{42}H_{71}O_{14}$ $[M-H]^-$		
			637.4305	637.4316	−1.7	$C_{36}H_{61}O_9$ $[M-H-(Glc-H_2O)]^-$		
			475.3809	475.3787	4.6	$C_{30}H_{51}O_4$ $[M-H-2(Glc-H_2O)]^-$		
44	24(*S*)-Pseudoginsenoside $F_{11}{}^{\Delta}$	9.36	845.4884	845.4899	−1.8	$C_{43}H_{73}O_{16}$ $[M-H+HCOOH]^-$	–	[16]
			799.4822	799.4844	−2.1	$C_{42}H_{71}O_{14}$ $[M-H]^-$		
			653.4276	653.4265	1.7	$C_{36}H_{61}O_{10}$ $[M-H-(Rha-H_2O)]^-$		
			635.4149	635.4133	2.5	$C_{36}H_{59}O_9$ $[M-H-(Rha-H_2O)-H_2O]^-$		
			491.3723	491.3733	−2.1	$C_{30}H_{51}O_5$ $[M-H-(Rha-H_2O)-(Glc-H_2O)]^-$		
45	Rg_5 sulfate	9.41	845.4375	845.4357	2.1	$C_{42}H_{69}O_{15}S$ $[M-H]^-$	422.2067	
			683.3806	683.3829	−3.4	$C_{36}H_{59}O_{10}S$ $[M-H-(Glc-H_2O)]^-$		
46	Notoginsenoside R_2	9.68	815.4818	815.4793	3.1	$C_{42}H_{71}O_{15}$ $[M-H+HCOOH]^-$	–	[24]
			769.4774	769.4738	4.7	$C_{41}H_{69}O_{13}$ $[M-H]^-$		
			637.4312	637.4316	−0.6	$C_{36}H_{61}O_9$ $[M-H-(Xyl-H_2O)]^-$		
			475.3774	475.3787	−2.7	$C_{30}H_{51}O_4$ $[M-H-(Xyl-H_2O)-(Glc-H_2O)]^-$		
47	$Rg_2{}^{\Delta}$	10.23	829.4962	829.4949	1.6	$C_{43}H_{73}O_{15}$ $[M-H+HCOOH]^-$	–	[26]
			783.4898	783.4895	0.4	$C_{42}H_{71}O_{13}$ $[M-H]^-$		
			637.4289	637.4316	−4.2	$C_{36}H_{61}O_9$ $[M-H-(Rha-H_2O)]^-$		
			475.3797	475.3787	2.1	$C_{30}H_{51}O_4$ $[M-H-(Rha-H_2O)-(Glc-H_2O)]^-$		
48	20 (*R*)-Rh_1/F_1	10.31	683.4389	683.3829	2.8	$C_{37}H_{63}O_{11}$ $[M-H+HCOOH]^-$	–	[30]
			637.4313	637.4316	−0.5	$C_{36}H_{61}O_9$ $[M-H]^-$		
			475.3774	475.3774	−2.7	$C_{30}H_{51}O_4$ $[M-H-(Glc-H_2O)]^-$		
49	Ra_1/Ra_2	10.52	1255.5914	1255.5900	1.1	$C_{59}H_{99}O_{28}$ $[M-H+HCOOH]^-$	–	[31]
			1209.6298	1209.6268	2.5	$C_{58}H_{97}O_{26}$ $[M-H]^-$		
			1077.5889	1077.5846	4.0	$C_{53}H_{89}O_{22}$ $[M-H-(Xyl-H_2O)]$		

Table 2 (*Continued*)

Peak no.	Identity	t_R (min)	Mean measured mass (Da)	Theoretical exact mass (Da)	Mass accuracy (ppm)	Empirical formula and proposed CID fragment ions	$[M-2H]^{2-}$	Reference literatures
50	$Rb_1{}^{\Delta}$	10.68	1153.5936	1153.5912	2.1	$C_{55}H_{92}O_{25}$ $[M-H+HCOOH]^-$	–	[30]
			1107.5958	1107.5951	0.3	$C_{54}H_{91}O_{23}$ $[M-H]^-$		
			945.5425	945.5423	0.2	$C_{48}H_{81}O_{18}$ $[M-H-(Glc-H_2O)]^-$		
			783.4888	783.4849	2.3	$C_{42}H_{71}O_{13}$ $[M-H-2(Glc-H_2O)]^-$		
			621.4355	621.4366	−1.8	$C_{36}H_{61}O_8$ $[M-H-3(Glc-H_2O)]^-$		
51	Ma-Rb_1	11.89	1193.5987	1193.5955	1.7	$C_{57}H_{93}O_{26}$ $[M-H]^-$	–	[25]
			1149.6065	1149.6049	1.4	$C_{56}H_{93}O_{24}$ $[M-H-CO_2]^-$		
52	Ro^{Δ}	10.94	955.4889	955.4903	−1.5	$C_{48}H_{75}O_{19}$ $[M-H]^-$	–	[16]
			793.4357	793.4357	−2.1	$C_{42}H_{65}O_{14}$ $[M-H-(Glc-H_2O)]^-$		
53	Rc^{Δ}	11.09	1123.5861	1123.5828	2.9	$C_{54}H_{91}O_{24}$ $[M-H+HCOOH]^-$	–	[16]
			1077.5860	1077.5846	1.3	$C_{53}H_{89}O_{22}$ $[M-H]^-$		
			945.5453	945.5423	3.2	$C_{48}H_{81}O_{18}$ $[M-H-(Ara(f)-H_2O)]^-$		
			915.5276	915.5317	−4.5	$C_{47}H_{79}O_{17}$ $[M-H-(Glc-H_2O)]^-$		
			783.4883	783.4895	−1.5	$C_{42}H_{71}O_{13}$ $[M-H-(Ara(f)-H_2O)-(Glc-H_2O)]^-$		
			621.4354	621.4366	−1.9	$C_{36}H_{61}O_8$ $[M-H-(Ara(f)-H_2O-2(Glc-H_2O)]^-$		
54	Ma-Rc	11.31	1163.5825	1163.5849	−2.1	$C_{56}H_{91}O_{25}$ $[M-H]^-$	–	[25]
			1119.5944	1119.5929	1.3	$C_{55}H_{91}O_{23}$ $[M-H-CO_2]^-$		
55	Ma-Rb_1/isomer	11.41	1193.5923	1193.5955	−2.5	$C_{57}H_{93}O_{26}$ $[M-H]^-$	–	[25]
			1149.6073	1149.6049	2.1	$C_{56}H_{93}O_{24}$ $[M-H-CO_2]^-$		
56	$Rb_2{}^{\Delta}$	11.51	1123.5860	1123.5828	3.3	$C_{54}H_{91}O_{24}$ $[M-H+HCOOH]^-$	–	[27]
			1077.5889	1077.5846	3.7	$C_{53}H_{89}O_{22}$ $[M-H]^-$		
			945.5455	945.5423	3.3	$C_{48}H_{81}O_{18}$ $[M-H-(Ara(p)-H_2O)]^-$		
			915.5362	915.5317	3.8	$C_{47}H_{79}O_{17}$ $[M-H-(Glc-H_2O)]^-$		
			783.4911	783.4895	4.7	$C_{42}H_{71}O_{13}$ $[M-H-(Ara(p)-H_2O)-(Glc-H_2O)]^-$		
57	$Rb_3{}^{\Delta}$	11.62	1123.5842	1123.5828	2.1	$C_{54}H_{91}O_{24}$ $[M-H+HCOOH]^-$	–	[27]
			1077.5840	1077.5846	−0.6	$C_{53}H_{89}O_{22}$ $[M-H]^-$		
			945.5457	945.5423	3.5	$C_{48}H_{81}O_{18}$ $[M-H-(Xyl-H_2O)]^-$		
			915.5357	915.5317	1.0	$C_{47}H_{79}O_{17}$ $[M-H-(Glc-H_2O)]^-$		
			783.4910	783.4895	4.5	$C_{42}H_{71}O_{13}$ $[M-H-(Xyl-H_2O)-(Glc-H_2O)]^-$		
58	Ma-Rb_2	11.70	1163.5812	1163.5849	−3.0	$C_{56}H_{91}O_{25}$ $[M-H]^-$	–	[25]
			1119.5945	1119.5929	1.6	$C_{55}H_{91}O_{23}$ $[M-H-CO_2]^-$		
59	Ma-Rc/Rb_2/Rb_3/isomer	11.83	1163.5769	1163.5849	−4.2	$C_{56}H_{91}O_{25}$ $[M-H]^-$	–	[25]
			1119.5951	1119.5929	3.8	$C_{55}H_{91}O_{23}$ $[M-H-CO_2]^-$		
60	Chikusetsusaponin IVa	12.20	793.4370	793.4374	−0.5	$C_{42}H_{65}O_{14}$ $[M-H]^-$	–	[16]
			631.3869	631.3846	3.6	$C_{36}H_{55}O_9$ $[M-H-(Glc-H_2O)]^-$		
61	Ma-Rb_3	12.24	1163.5862	1163.5849	1.1	$C_{56}H_{91}O_{25}$ $[M-H]^-$	–	[25]
			1119.5947	1119.5929	2.3	$C_{55}H_{91}O_{23}$ $[M-H-CO_2]^-$		
62	Rd^{Δ}	12.40	991.5515	991.5478	3.7	$C_{49}H_{83}O_{20}$ $[M-H+HCOOH]^-$	–	[25]
			945.5462	945.5423	4.1	$C_{48}H_{81}O_{18}$ $[M-H]^-$		
			783.4864	783.4895	−4.0	$C_{42}H_{71}O_{13}$ $[M-H-(Glc-H_2O)]^-$		
			621.4382	621.4366	2.6	$C_{36}H_{61}O_8$ $[M-H-2(Glc-H_2O)]^-$		
63	Ma-Rd	12.61	1031.5465	1031.5427	2.8	$C_{51}H_{83}O_{21}$ $[M-H]^-$	–	[25]
			987.5551	987.5505	4.7	$C_{50}H_{83}O_{19}$ $[M-H-CO_2]^-$		
64	Rg_6	14.13	811.4877	811.4840	4.5	$C_{43}H_{71}O_{14}$ $[M-H+HCOOH]^-$	–	[27]
			765.4766	765.4789	−3.0	$C_{42}H_{69}O_{12}$ $[M-H]^-$		
			619.4238	619.4210	4.5	$C_{36}H_{59}O_8$ $[M-H-(Rha-H_2O)]^-$		
65	F_4	14.47	811.4812	811.4840	−3.8	$C_{43}H_{71}O_{14}$ $[M-H+HCOOH]^-$	–	[27]
			765.4783	765.4789	−0.8	$C_{42}H_{69}O_{12}$ $[M-H]^-$		
			619.4234	619.4210	3.9	$C_{36}H_{59}O_8$ $[M-H-(Rha-H_2O)]^-$		
66	Rk_3	14.68	665.4252		−2.0	$C_{37}H_{61}O_{10}$ $[M-H+HCOOH]^-$	–	[27]
			619.4217	619.4210	0.4	$C_{36}H_{59}O_8$ $[M-H]^-$		
67	Rh_4	15.07	665.4250	665.4265	−2.3	$C_{37}H_{61}O_{10}$ $[M-H+HCOOH]^-$	–	[27]
			619.4199	619.4210	−2.1	$C_{36}H_{59}O_8$ $[M-H]^-$		
68	Zingibroside R_1	15.31	793.4377	793.4374	0.4	$C_{42}H_{65}O_{14}$ $[M-H]^-$	–	[16]
			631.3895	631.3846	−0.3	$C_{36}H_{55}O_9$ $[M-H-(Glc-H_2O)]^-$		

Table 2 (*Continued*)

Peak no.	Identity	t_R (min)	Mean measured mass (Da)	Theoretical exact mass (Da)	Mass accuracy (ppm)	Empirical formula and proposed CID fragment ions	$[M-2H]^{2-}$	Reference literatures
69	20(*S*)-Rg_3	16.41	829.4920	829.4949	−3.5	$C_{43}H_{73}O_{15}$ $[M-H+HCOOH]^-$	–	[27]
			783.4896	783.4895	0.1	$C_{42}H_{71}O_{13}$ $[M-H]^-$		
			621.4364	621.4366	−0.3	$C_{36}H_{61}O_8$ $[M-H-(Glc-H_2O)]^-$		
			459.3842	459.3838	0.9	$C_{30}H_{51}O_3$ $[M-H-2(Glc-H_2O)]^-$		
70	Acetyl-Rg_1/isomer	16.58	825.4968	825.5000	−3.9	$C_{44}H_{73}O_{14}$ $[M-H]^-$	–	[27]
			783.4886	783.4895	−1.1	$C_{42}H_{71}O_{13}$ $[M-H-C_2H_2O]^-$		
			621.4396	621.4366	4.8	$C_{36}H_{61}O_8$ $[M-H-C_2H_2O-(Glc-H_2O)]^-$		
			459.3821	459.3838	−3.7	$C_{30}H_{51}O_3$ $[M-H-C_2H_2O-2(Glc-H_2O)]^-$		
71	20(*R*)-Rg_3	16.69	829.4963	829.4959	0.5	$C_{43}H_{73}O_{15}$ $[M-H+HCOOH]^-$	–	[27]
			783.4910	783.4895	1.9	$C_{42}H_{71}O_{13}$ $[M-H]^-$		
			621.4362	621.4366	−0.6	$C_{36}H_{61}O_8$ $[M-H-(Glc-H_2O)]^-$		
			459.3838	459.3838	−1.0	$C_{30}H_{51}O_3$ $[M-H-2(Glc-H_2O)]^-$		
72	Acetyl-Rg_1/isomer	16.87	825.4966	825.5000	−4.1	$C_{44}H_{73}O_{14}$ $[M-H]^-$	–	[27]
			783.4868	783.4895	−3.4	$C_{42}H_{71}O_{13}$ $[M-H-C_2H_2O]^-$		
			621.4348	621.4366	−2.9	$C_{36}H_{61}O_8$ $[M-H-C_2H_2O-(Glc-H_2O)]^-$		
			459.3819	459.3838	−4.1	$C_{30}H_{51}O_3$ $[M-H-C_2H_2O-2(Glc-H_2O)]^-$		
73	20(*S*) acetyl-Re_1	17.24	825.4990	825.5000	−1.2	$C_{44}H_{73}O_{14}$ $[M-H]^-$	–	[32]
			783.4906	783.4895	1.4	$C_{42}H_{71}O_{13}$ $[M-H-C_2H_2O]^-$		
			621.4373	621.4366	1.1	$C_{36}H_{61}O_8$ $[M-H-C_2H_2O-(Glc-H_2O)]^-$		
			459.3856	459.3838	3.9	$C_{30}H_{51}O_3$ $[M-H-C_2H_2O-2(Glc-H_2O)]^-$		
74	20(*R*) acetyl-Re_1	17.51	825.5003	825.5000	−3.8	$C_{44}H_{73}O_{14}$ $[M-H]^-$	–	[32]
			783.4872	783.4895	−2.9	$C_{42}H_{71}O_{13}$ $[M-H-C_2H_2O]^-$		
			621.4348	621.4366	−2.5	$C_{36}H_{61}O_8$ $[M-H-C_2H_2O-(Glc-H_2O)]^-$		
			459.3825	459.3838	−2.8	$C_{30}H_{51}O_3$ $[M-H-C_2H_2O-2(Glc-H_2O)]^-$		
75	Unknown	17.97	595.2884	595.2907	−3.9	$C_{34}H_{43}O_9$ $[M-H]^-$	–	
			279.2332	279.2324	2.9	$C_{18}H_{31}O_2$		
76	20(*S*)-Rs_3	18.35	871.5042	871.5055	−1.5	$C_{45}H_{75}O_{16}$ $[M-H+HCOOH]^-$	–	[27]
			825.5002	825.5000	2.9	$C_{44}H_{73}O_{14}$ $[M-H]^-$		
			783.48977	783.4895	0.3	$C_{42}H_{71}O_{13}$ $[M-H-C_2H_2O]^-$		
			621.4366	621.4366	0	$C_{36}H_{61}O_8$ $[M-H-C_2H_2O-(Glc-H_2O)]^-$		
			459.3825	459.3838	−2.8	$C_{30}H_{51}O_3$ $[M-H-C_2H_2O-2(Glc-H_2O)]^-$		
77	20(*R*)-Rs_3	18.68	871.5029	871.5055	−3.0	$C_{45}H_{75}O_{16}$ $[M-H+HCOOH]^-$	–	[27]
			825.4986	825.5000	−1.7	$C_{44}H_{73}O_{14}$ $[M-H]^-$		
			783.4881	783.4895	−1.8	$C_{42}H_{71}O_{13}$ $[M-H-C_2H_2O]^-$		
			621.4360	621.4366	−1.0	$C_{36}H_{61}O_8$ $[M-H-C_2H_2O-(Glc-H_2O)]^-$		
			459.3836	459.3838	−0.5	$C_{30}H_{51}O_3$ $[M-H-C_2H_2O-2(Glc-H_2O)]^-$		
78	24 (R)-pseudoginsenoside RT5	18.78	723.3830	723.3803	3.7	$C_{34}H_{59}O_{16}$ $[M-H+HCOOH]^-$	–	[16]
			677.3726	677.3748	−3.2	$C_{33}H_{57}O_{14}$ $[M-H]^-$		
			279.2329	279.2324	1.8	$C_{18}H_{31}O_2$		
79	Rk_1	19.49	811.4877	811.8440	4.1	$C_{43}H_{71}O_{14}$ $[M-H+HCOOH]^-$	–	[27,30]
			765.4756	765.4789	−4.3	$C_{42}H_{69}O_{12}$ $[M-H]^-$		
			603.4277	603.4261	2.7	$C_{36}H_{59}O_7$ $[M-H-(Glc-H_2O)]^-$		
80	Rs_5	19.63	807.4882	807.4895	−1.6	$C_{44}H_{71}O_{13}$ $[M-H]^-$	–	[27]
			765.4791	765.4789	0.3	$C_{42}H_{69}O_{12}$ $[M-H-C_2H_2O]^-$		
			603.4257	603.4261	−0.7	$C_{36}H_{59}O_7$ $[M-H-C_2H_2O-(Glc-H_2O)]^-$		
81	Rg_5	19.86	811.4848	811.8440	0.5	$C_{43}H_{71}O_{14}$ $[M-H+HCOOH]^-$	–	[27,30]
			765.4769	765.4789	−2.6	$C_{42}H_{69}O_{12}$ $[M-H]^-$		
			603.4287	603.4261	4.3	$C_{36}H_{59}O_7$ $[M-H-(Glc-H_2O)]^-$		
82	Rs_4	19.99	807.4924	807.4895	3.6	$C_{44}H_{71}O_{13}$ $[M-H]^-$	–	[27]
			765.4800	765.4789	1.4	$C_{42}H_{69}O_{12}$ $[M-H-C_2H_2O]^-$		
			603.4283	603.4261	3.6	$C_{36}H_{59}O_7$ $[M-H-C_2H_2O-(Glc-H_2O)]^-$		
83	$Rh_2{}^{\Delta}$	20.76	667.4437	667.4421	2.4	$C_{37}H_{63}O_{10}$ $[M-H+HCOOH]^-$	–	[27]
			621.4376	621.4366	1.6	$C_{36}H_{61}O_8$ $[M-H]^-$		
			459.3816	459.3838	−4.8	$C_{30}H_{51}O_3$ $[M-H-(Glc-H_2O)]^-$		

Δ: Identified with reference standard.

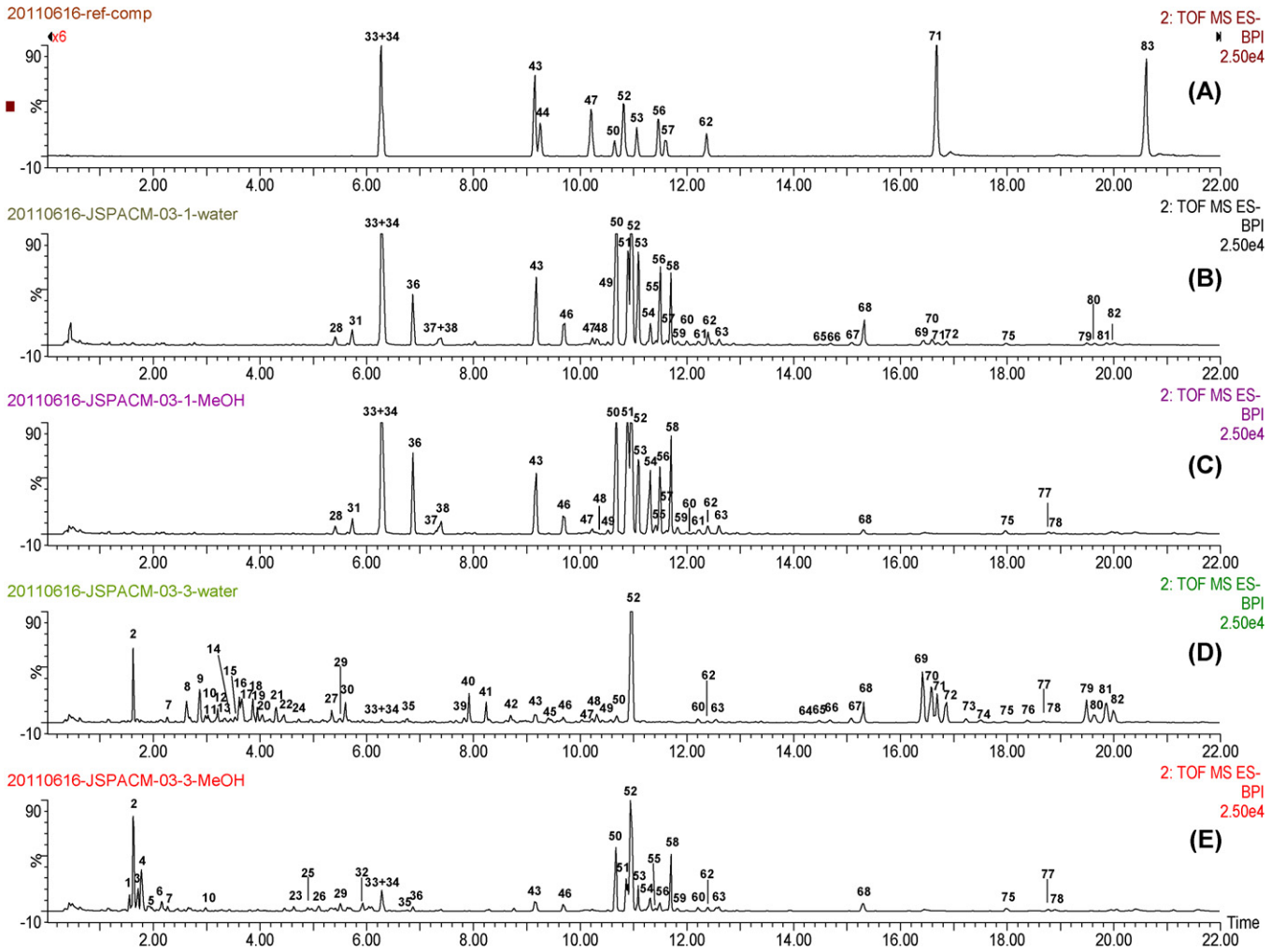

Fig. 2. Representative chromatograms of white ginseng samples and reference compounds. (A) Reference compounds; (B and C) non-fumigated white ginseng (JSPACM-03-1); (D and E) sulfur-fumigated white ginseng (JSPACM-03-3) from JSPACM-03-1; (C and E) 70% aqueous methanol extraction; (B and D) decoction. The peak numbers have the same meanings as in Table 2.

or wholesalers: 100 g of white ginseng slices (thickness 0.1 mm) were wetted with 10 ml water, put to stand for 0.5 h, 10 g of sulfur powder was heated until it burned, then the burning sulfur and the wetted ginseng slices were carefully put into the lower and upper layer of a desiccator respectively. The desiccator was then kept closed for 12 h. After fumigation, the ginseng slices were dried in a ventilated drying oven at 40 °C for 12 h.

2.5.2. Reference compound solutions

Stock solutions: a certain amount of ginsenoside Re, Rg_1, Rf, Rg_2, Rb_1, Ro, Rc, Rb_2, Rb_3, Rd, 20(*R*)-Rg_3, Rh_2 and 24(*S*)-Pseudoginsenoside F_{11} were dissolved with methanol respectively to get thirteen reference compound stock solutions (about 1.0 mg/ml), and were stored under 4 °C.

Reference compounds mixture solution: a certain amount of above thirteen reference compound stock solutions were mixed, and diluted with methanol to get reference compound mixture solution (about 100 ng/ml for each compound), and the solution was filtered by a 0.2 μm PTFE syringe filter before UPLC-Synapt G2 QTOF-MS/MS analysis.

2.5.3. Ginseng sample solutions

Ginseng raw material sample: 0.2 g of ginseng slices were accurately weighed and ultrasonic-extracted with 10.7 ml of 70% methanol for 60 min (ginseng: 70% MeOH = 0.2 g: 10.7 ml). The temperature of the ultrasonic bath was kept consistent (25 ± 1 °C) with running water. The supernatants of the extracts were filtered by a 0.2 μm PTFE syringe filter, and subjected to UPLC-Synapt G2 QTOF-MS/MS analysis. The samples were prepared and analyzed in triplicate.

Ginseng decoction sample: 0.5 g of ginseng slices were accurately weighed and refluxed with 8 ml water for 40 min to get ginseng decoctions. Then the decoctions were added with 18.7 ml of methanol, and ultrasonic-extracted for 60 min (also ginseng: 70% MeOH = 0.2 g: 10.7 ml). The temperature of the ultrasonic bath was kept consistent (25 ± 1 °C) with running water. The supernatants of the extracts were filtered by a 0.2 μm PTFE syringe filter, and subjected to UPLC-Synapt G2 QTOF-MS/MS analysis. The samples were prepared and analyzed in triplicate.

2.6. Establishment of in-house library and generation of empirical molecular formula

By searching from data bases, such as PubMed of the U.S. National Library Medicine and the National Institutes of Health, Scifinder Scholar of American Chemical Society, ScienceDirect of Elsevier and Chinese National Knowledge Infrastructure (CNKI) of Tsinghua University, all components reported in the literatures on *Panax* species were summarized in a Microsoft Office Excel table to establish a in-house library, which includes the name,

molecular formula, molecular weigh, chemical structures and literatures of each published known compound. The "Find" function of Microsoft Office Excel was used to match the empirical molecular formula with that of published known compounds in the library. The empirical molecular formula was deduced from and short listed by comparing the accurately measured mass values to the theoretical exact mass values of putative deprotonated molecular ions $[M-H]^-$ and/or fragment ions at the mass accuracy of less than 5 ppm.

3. Results and discussion

3.1. Optimization of chromatographic and MS conditions

In our previous study, Waters HSS T3 column (100 mm × 2.1 mm, 1.8 μm) designed for retaining more hydrophilic compounds was used, 45 components in white ginseng samples were separated and detected within 18 min when the column was eluted in gradient with acetonitrile and water containing 0.1% formic acid. The previously developed chromatographic conditions were optimized based on the separation of the original major ginsenosides of white ginseng and its decoction [8]. However, under the developed conditions, in a commercial sample, some relatively polar components could not be eluted with baseline separation, two of which were assumed to be sulfur-containing compounds [8]. As there has been no reports on isolation of sulfur-containing compounds from ginseng, this phenomenon remind us of the possibility of commercial white ginseng being sulfur-fumigated, and a new analytical method should be developed to separate and identify the sulfur-containing compounds induced by sulfur-fumigation. Therefore, referring to the previous conditions, the HSS T3 column was employed again,

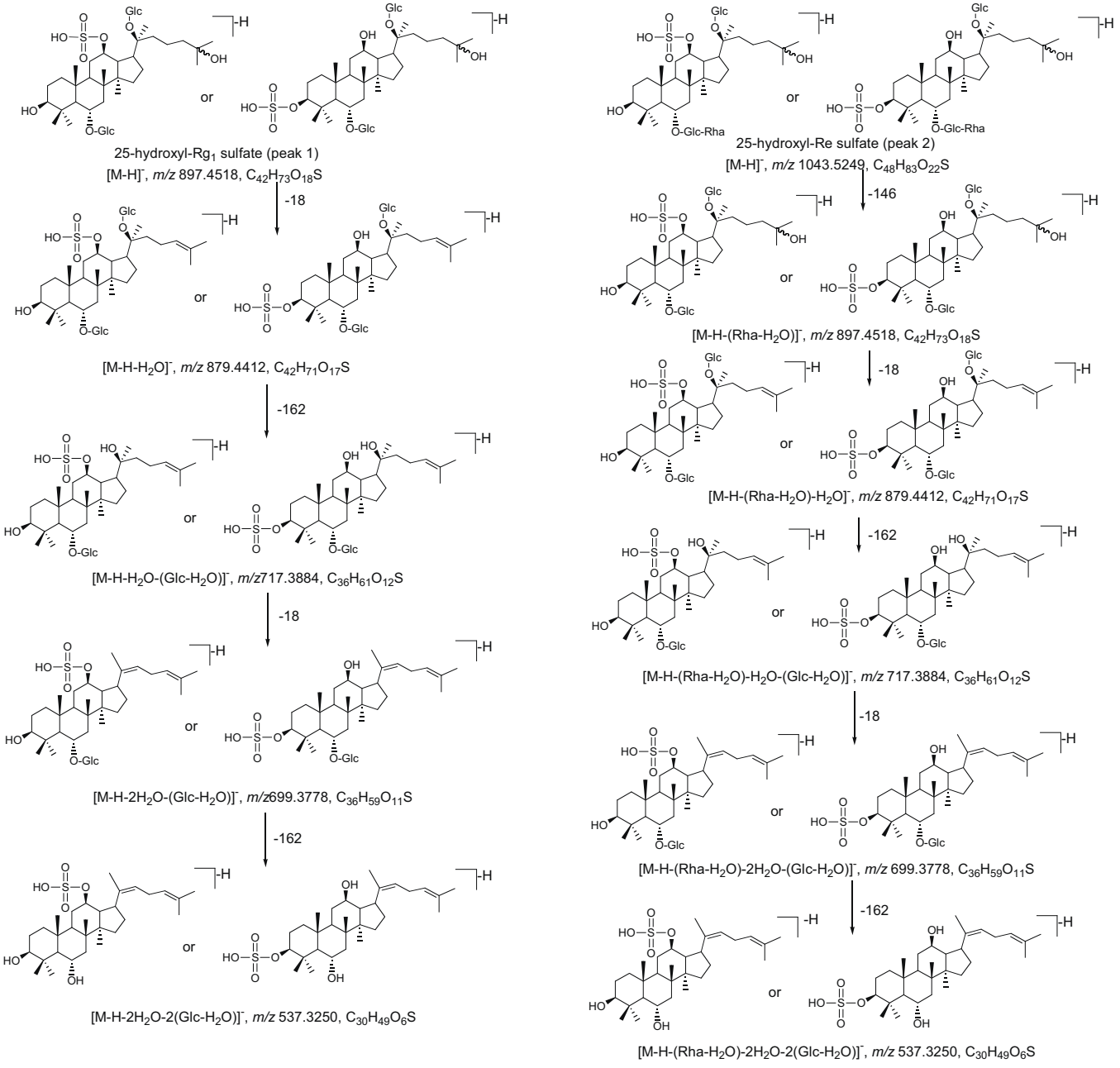

Fig. 3. Proposed ion fragmentation of assumed ginsenoside sulfates and sulfites.

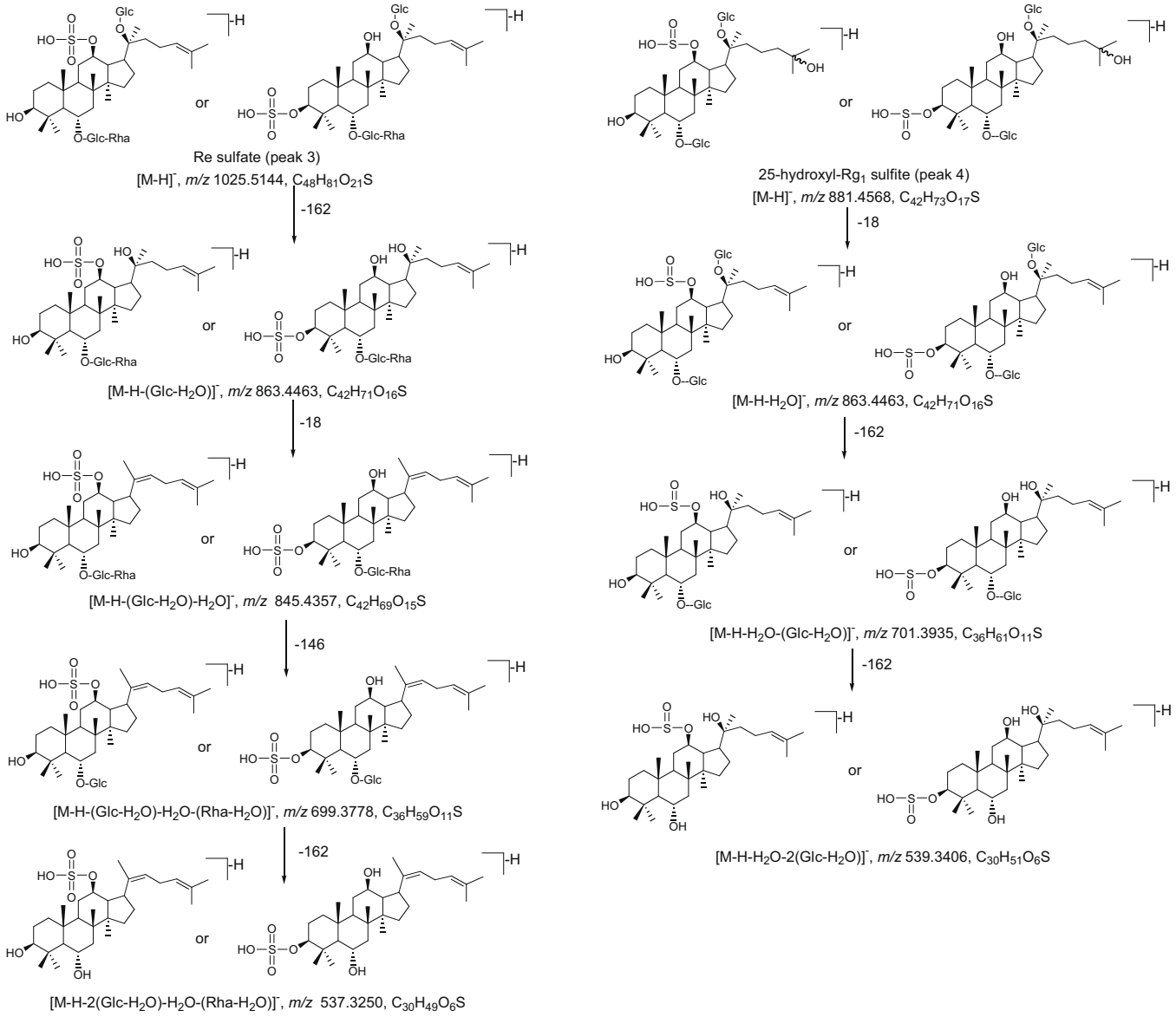

Fig. 3. *Continued.*

but the gradient elution mode of mobile phase was changed, the starting concentration of acetonitrile was optimized from 10% to 5%, the flow rate from 0.5 to 0.6 ml/min, and the whole gradient elution time from 20 to 24 min.

For MS conditions, since the starting concentration of acetonitrile for mobile phase decreased, whereas the flow rate increased, the temperature and flow rate of nebulization gas for ion source were revised from 400 to 450 °C and from 700 to 900 l/h respectively, so that the solvent could be nebulized more easily and quickly. Furthermore, the capillary and cone voltages were revised from 3500 to 2500 V and from 45 to 30 V respectively, so as to balance the ionization of original ginsenosides and the sulfur-containing compounds.

It should be noted that under the newly optimized chromatographic and MS conditions, total 82 components could be separated and detected in white ginseng samples within 22 min, that is 37 compounds more than that detected with our previous method [8]. The representative chromatograms of reference compounds and white ginseng samples were shown in Fig. 2. From Fig. 2, it was found that those more polar components previously co-eluted or eluted closely at retention time from 2 to 4 min [8] were eluted with nearly baseline separation at the retention time from 1.5 to 6 min under the present conditions.

3.2. Comparison of chemical fingerprints of non-fumigated and sulfur-fumigated white ginseng samples

Two batches of non-fumigated ginseng samples (JSPACM-03-1, JSPACM-03-2) and their sulfur-fumigated ones (JSPACM-03-3, JSPACM-03-4) were comparatively analyzed using the newly established UHPLC–QTOF-MS/MS method. Similar results were found for these two pairs of samples, so the results of sample JSPACM-03-1 and JSPACM-03-3 were described and discussed in detail. The representative BPI chromatograms of sample JSPACM-03-1 and JSPACM-03-3 were shown in Fig. 2. It was found that the peak height of those major peaks (peak **33**, **34**, **36**, **43**, **46**, **50**, **51**, **52**, **53**, **54**, **56**, **58**) detected in non-fumigated ginseng sample (Fig. 2C) were obviously decreased in the sulfur-fumigated sample (Fig. 2E). On the other hand, many peaks (peak **1–7**, **10**, **23**, **25**, **26**, **29**, **32**, **35**) which were not detectable in non-fumigated sample (Fig. 2C) were detected as major peaks in sulfur-fumigated sample (Fig. 2E),

suggesting that sulfur-fumigation caused chemical transformation of major components in white ginseng.

Eleven extra peaks (peak **65–67**, **69–72**, **79–82**) were detected in the decoction of non-fumigated ginseng sample (Fig. 2B) when compared with its 70% methanol extract (Fig. 2C), which is in agreement with the findings in our previous study [8]. It was astonished to find that in the decoction of sulfur-fumigated sample, more peaks (peak **8**, **9**, **11–22**, **24**, **30**, **39–42**, **45**) at retention time from 3 to 10 min were detected (Fig. 2D) when compared with the decoction of non-fumigated sample (Fig. 2B). Furthermore, except for peak **52**, the peak height of the original main components (peak **33**, **34**, **36**, **43**, **46–58**) were significantly decreased, or even disappeared in the decoction of sulfur-fumigated sample, whereas the peak height of some components (peak **69–72**, **79–82**) were much higher than that in the decoction of non-fumigated sample (Fig. 2B). All above results suggested that sulfur-fumigation induced the chemical transformation of white ginseng, and consequently altered the chemical
profiles of the decoction of white ginseng.

3.3. Identity elucidation and relationship of detectable components in non-fumigated and sulfur-fumigated ginseng samples

Eighty-two components detected in white ginseng and its decoction were identified to be ginsenosides or its sulfur-containing derivatives, 11 of which were confirmed by comparing the mass spectra and retention times with that of reference compounds (Re, Rg_1, Rf, Rg_2, Rb_1, Ro, Rc, Rb_2, Rb_3, Rd and 20 (*R*)-Rg_3), and the others were tentatively assigned by matching the empirical molecular formula (deduced by matching detected accurate mass values of deprotonated molecular ions with its theoretical

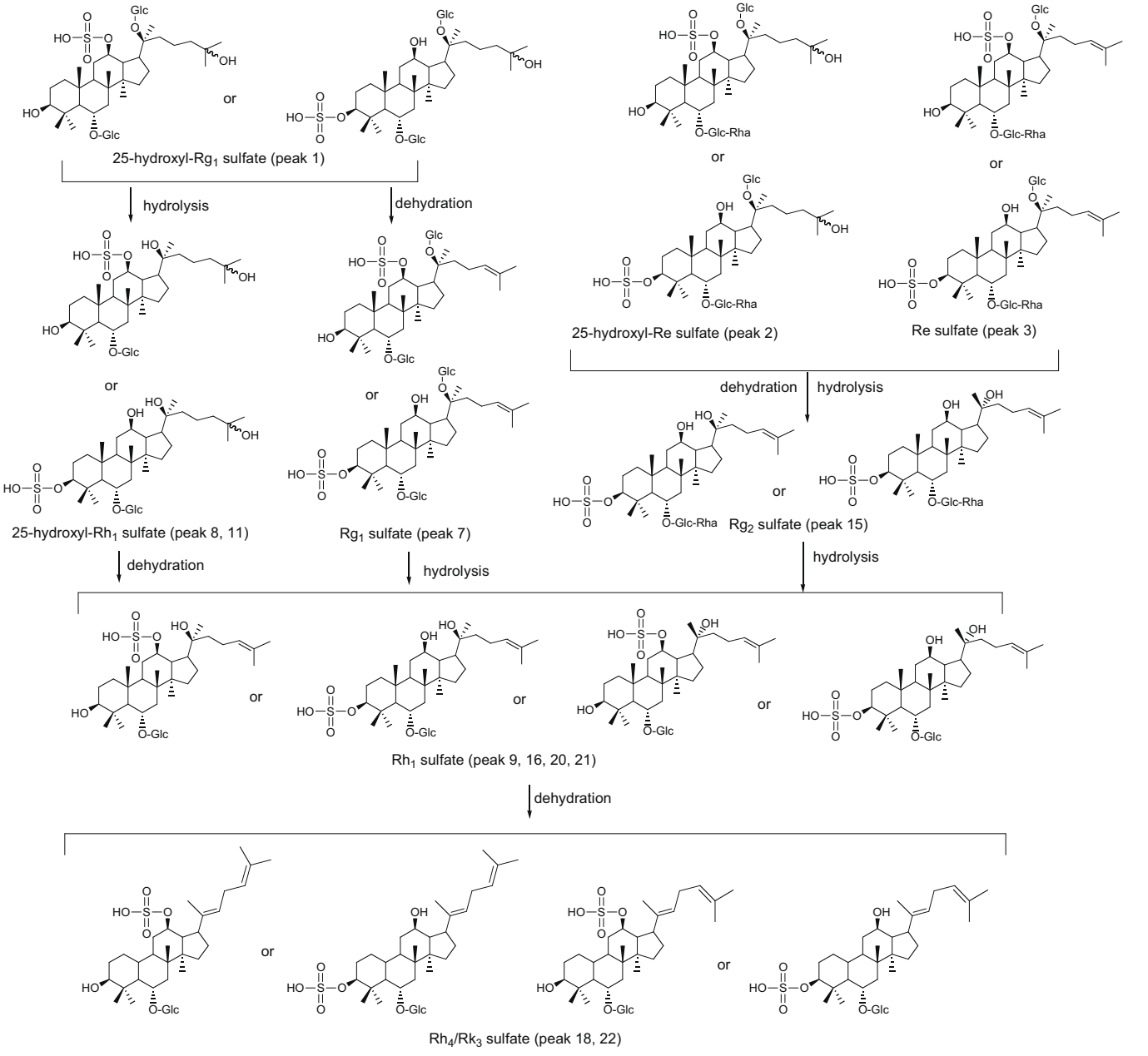

Fig. 4. Possible mechanisms involved in transformation of some ginsenoside sulfates and sulfites during decocting of sulfur-fumigated white ginseng.

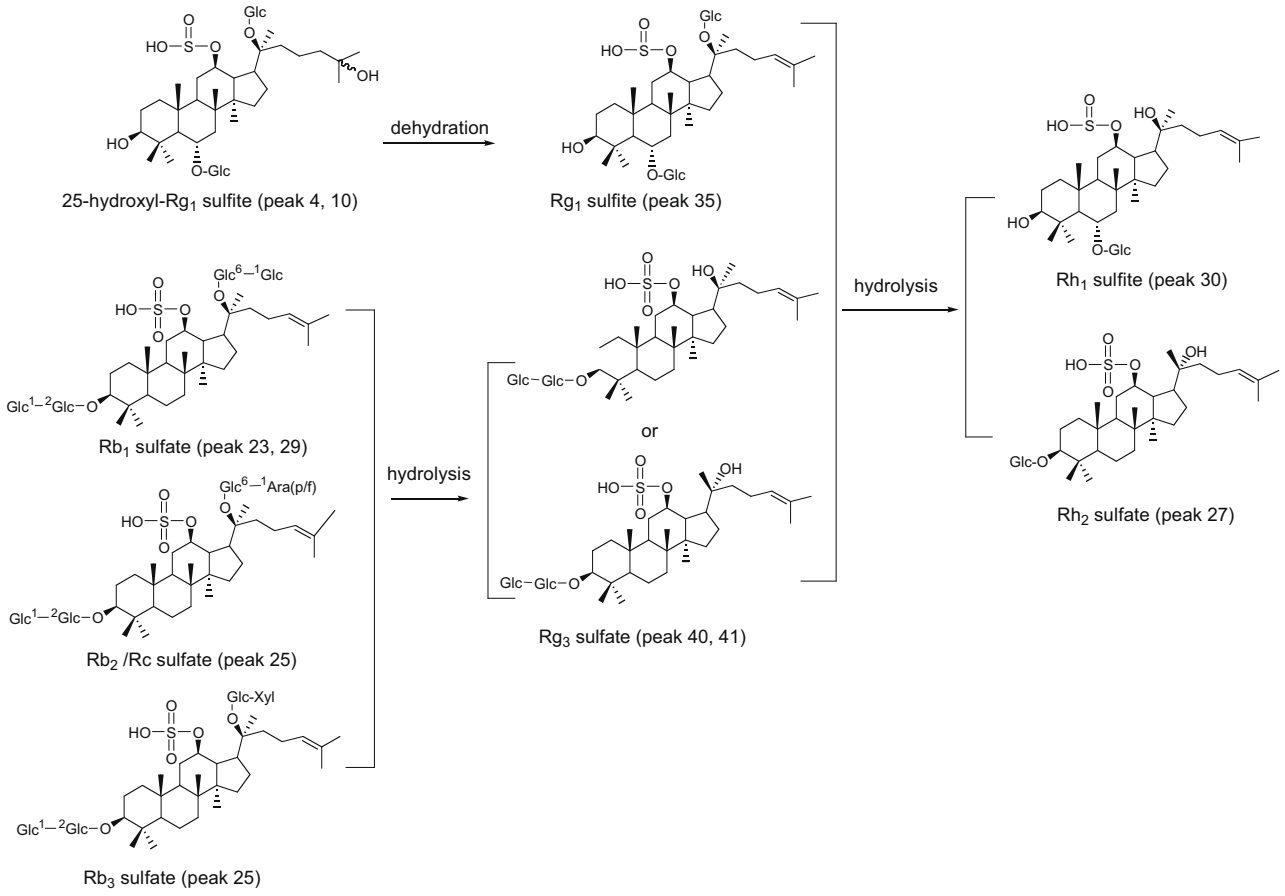

Fig. 4. *Continued.*

values) with that of the published known ginsenosides, and/or further confirmed by elucidating the quasi-molecular ions and fragment ions, in particular for those isomeric ginsenosides [8,23–32] and sulfur-containing ginsenoside derivatives. In addition, the chromatographic behaviors of some ginsenosides in the literatures were considered as complementary data for the identity confirmation of isomers [8,26,27].

From non-fumigated white ginseng and its decoction, totally 43 ginsenosides were identified, 41 of which were reported in our previous study [8], and ginsenoside R1 (**31**) and 24(*R*)-pseudoginsenoside RT5 (**78**) were newly detected under the present conditions. Their identity elucidation and relationship has been reported in our previous study [8], and will not repeat here.

It was interesting to find that in addition to those 43 ginsenosides identified in non-fumigated samples, another 35 sulfur-containing compounds were detected in sulfur-fumigated samples. Fourteen sulfur-containing compounds (**1–7**, **10**, **23**, **25**, **26**, **29**, **32**, **35**) were detected in 70% methanol extract of sulfur-fumigated white ginseng. It was reported that paeoniflorin, a main component of Radix Paeoniae, could be transformed into paeoniflorin sulfite during sulfur-fumigation of Radix Paeoniae [2,3,5]. Paeoniflorin sulfite was much polar than paeoniflorin and consequently eluted with shorter retention time on reversed phase chromatography [3]. This phenomenon reminded us to assume that these sulfur-containing compounds in sulfur-fumigated white ginseng might be the sulfate and sulfite derivatives of original ginsenosides! For example, Compound **3** had accurate mass at *m*/*z* 1025.5177, 80 Da more than that (*m*/*z* 945.5446) of Re (**34**), its empirical molecular formula $C_{48}H_{81}O_{21}S$ showed an exact "SO_3" addition to Re ($C_{48}H_{81}O_{18}$). As demonstrated in Fig. 3 and Table 2, in the low energy CID mass spectrum of this compound, fragment ions at *m*/*z* 863.4462, 845.4560, 699.3802 and 537.3234 were observed, which were proposed to be generated through successive neutral losses of glucosyl moiety, water, rhamnosyl moiety and glucosyl moiety respectively. So compound **3** was tentatively assigned as Re sulfate. Compound **2** had accurate mass at *m*/*z* 1043.5249, 18 Da more than that (*m*/*z* 1025.5177) of Re sulfate (**3**), in its low energy CID mass spectrum, fragment ions at *m*/*z* 897.4518, 879.4412, 717.3884, 699.3778 and 537.3250 were proposed to be generated through successive neutral losses of rhamnosyl moiety, water, glucosyl moiety, water and glucosyl moiety respectively (Fig. 3 and Table 2). Thus compound **2** was assigned to be 25-hydroxyl-Re sulfate. Similarly compound **1** was deduced to be 25-hydroxyl-Rg_1 sulfate. It had accurate mass at *m*/*z* 897.4518, 98 Da more than that (*m*/*z* 799.4852) of Rg_1 (**33**). Its empirical molecular formula $C_{42}H_{73}O_{18}S$ showed an exact "H_2SO_4" addition to Rg_1 ($C_{42}H_{71}O_{14}$). In the low energy CID mass spectrum of this compound, fragment ions at *m*/*z* 879.4412, 717.3884, 699.3778 and 537.3250 were proposed to be generated through successive neutral losses of water, glucosyl moiety, water and glucosyl moiety respectively (Fig. 3 and Table 2). Compound **4** was assigned to be 25-hydroxyl-Rg_1 sulfite. It had accurate mass at *m*/*z* 881.4568, 82 Da more than that (*m*/*z* 799.4852) of Rg_1 (**33**). Its empirical molecular formula $C_{42}H_{73}O_{17}S$ showed an exact "H_2SO_3" addition to Rg_1 ($C_{42}H_{71}O_{14}$). In the low energy CID mass spectrum of this compound, fragment ions at *m*/*z* 863.4463, 701.3935 and 539.3406 were proposed to be generated through successive neutral losses of water, glucosyl moiety and glucosyl moiety respectively (Fig. 3 and Table 2). With the same strategy, compound **5**, **6**, **7**, **10**, **23**, **25**, **26**, **29**, **32** and **35** were tentatively identified to be 25-hydroxyl-Rg_1 sulfate, 25-hydroxyl-Re

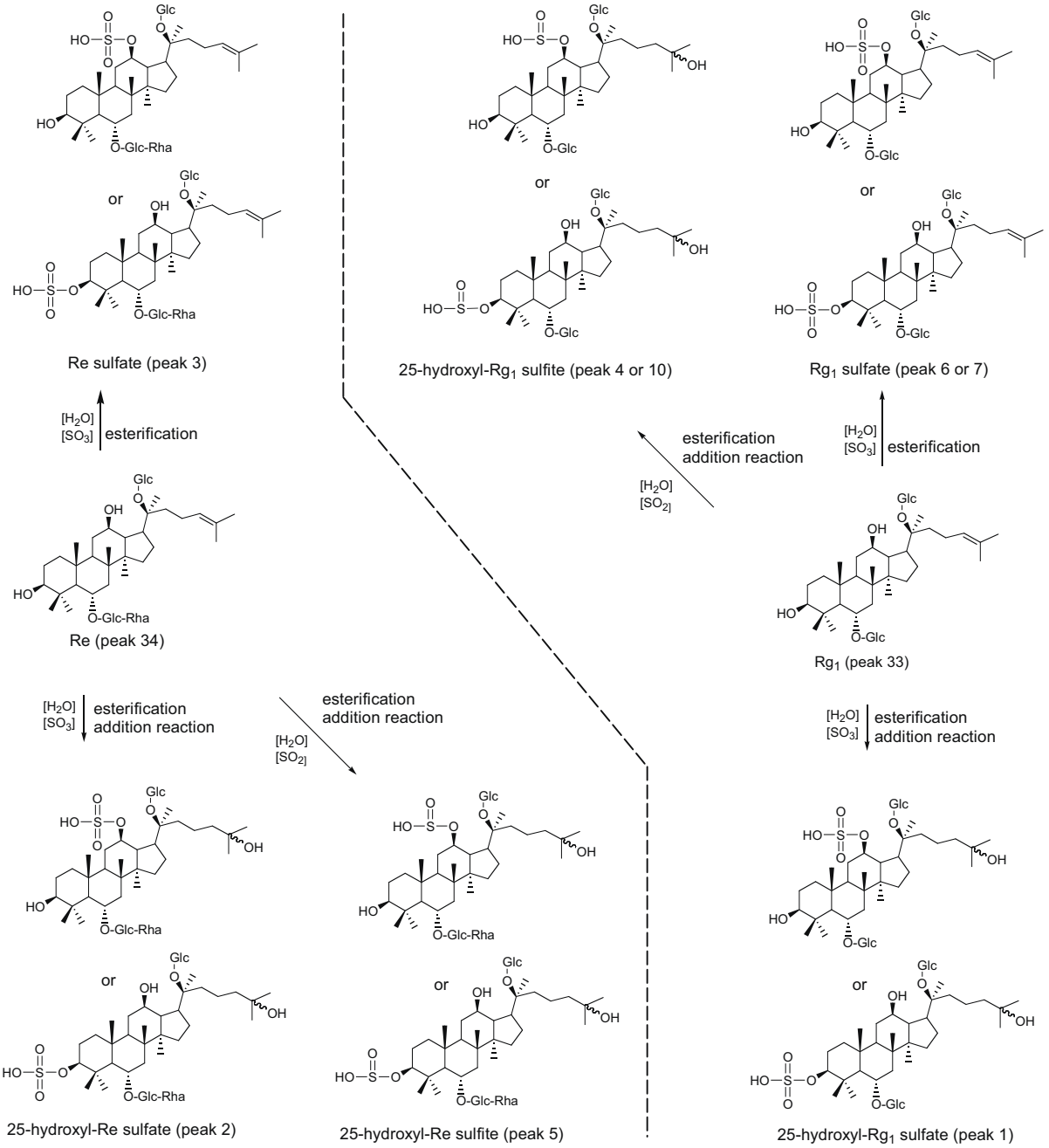

Fig. 5. Possible mechanisms involved in generation of some ginsenoside sulfates and sulfites from original ginsenosides during sulfur-fumigation of white ginseng.

sulfite, Rg_1 sulfate, Rg_1 sulfate isomer, 25-hydroxyl-Rg_1 sulfite isomer, Rb_1 sulfate, $Rc/Rb_2/Rb_3$ sulfate, Ma-Rb_1 sulfate, Rb_1 sulfate isomer, Ma-Rb_1 sulfate isomer and F_2 sulfate respectively (Table 2).

In addition to compound **2**, **7**, **10**, **29** and **32**, another 22 sulfur-containing compounds (**8**, **9**, **11–22**, **24**, **27**, **30**, **39–42**, **45**) were detected in the decoction of sulfur-fumigated white ginseng. Using the same approaches mentioned above, the identities of these sulfur-containing compounds were all tentatively assigned to be sulfate and sulfite derivatives of ginsenosides that reported previously in the decoction of white ginseng [8], all details were summarized in Table 2. It is interesting to note that like those ginsenosides additionally detected in the decoction of white ginseng, which were assumed to be generated from hydrolysis, dehydration, decarboxylation and addition reaction of original ginsenosides in white ginseng [8], these sulfur-containing compounds newly detected in decoction of sulfur-fumigated white ginseng seemed also generated from hydrolysis, dehydration and addition reaction of compound **1–7**, **10**, **23**, **25**, **26**, **29**, **32** and **35** during decocting of sulfur-fumigated white ginseng. Fig. 4 demonstrated possible mechanisms involved in production of some ginsenoside sulfates and sulfites during decocting of sulfur-fumigated white ginseng.

3.4. Possible mechanisms involved in transformation of ginsenosides induced by sulfur-fumigation

Previous studies found that sulfur-fumigation can induce transformation of paeoniflorin into its sulfite [2,3,5], and Patricia Y H et al. assumed that such sulfites might be easily formed from hemiacetals

radical mostly presented in many sugars and bioactive compounds, including saponins [33]. From present study, it seems that both sulfate and sulfite derivatives of ginsenosides could be formed during sulfur-fumigation of white ginseng, and the reaction should not happen at hemiacetals radical of sugars, since in all mass spectra of sulfur-containing compounds identified, the neutral losses of glucosyl, rhamnosyl, arabinosyl (or xylosyl) moieties could be found, and the daughter ions without sugar moieties still contain sulfur element, indicating that the sulfate or sulfite derivatives should be formed from the hydroxyl groups at position 3 or 12 of ginsenosides. The chemical reactions involved might be esterification and/or addition reaction of original ginsenosides and sulfur dioxide or sulfur trioxide formed by sulfur burning with water existed. The possible mechanisms involved in transformation of some ginsenoside sulfates and sulfites were demonstrated in Fig. 5.

It was also interesting to find that unlike those main original components which belongs to the protopanaxadiol, protopanaxatriol and ocotillol type ginsenosides, the peak height of the main component Ro (peak **52**) which belongs to the oleanolic acid type ginsenoside was not significantly decreased in decoctions of non-fumigated (Fig. 2B) and sulfur-fumigated ginseng (Fig. 2D) when compared with the 70% methanol extracts (Fig. 2C and E). The possible reasons might be that there are no free hydroxyl groups at position 3 or 12 of this compound, thus the sulfate or sulfite could not be formed during sulfur-fumigation, and that this compound might not easily undergo degradation during the decoction of non-fumigated and sulfur-fumigated ginseng.

3.5. Quality evaluation of commercial white ginseng samples

The newly established method was used to evaluate commercial white ginseng samples collected from America, Canada, Hong Kong, Macau and mainland China (Table 1). Total 38 commercial white ginseng samples were tested, by comparing their fingerprints with that of sample JSPACM-03-3 or JSPACM-03-4 together with ion extraction of two main sulfur-containing artifacts **2** and **4**, it was found that ginsenoside sulfates or sulfites were detected in 18 samples (Table 1), suggesting that there are nearly 47.4% commercial white ginseng samples analyzed being sulfur-fumigated. Situations were even worse of white ginseng samples in mainland China, as 7 of 8 from Nanjing, 4 of 5 from Guangzhou were sulfur-fumigated, although all 4 from Jilin, the indigenous cultivating region of white ginseng, were not. It was also surprised to find that 2 of 6 from Hong Kong, 1 of 5 from Canada and 3 of 5 from America were also detected with ginsenoside sulfates and sulfites, indicating that sulfur-fumigated white ginseng have been exported overseas.

As sulfur-fumigation can cause chemical transformation of white ginseng, the bioactivities and toxicities of sulfur-fumigated white ginseng need further investigation.

4. Conclusion

In present study, an improved UHPLC–QTOF-MS/MS based chemical profiling approach was developed to reveal chemical transformation of ginsenoside in sulfur-fumigated white ginseng. Thirty-five sulfur-containing compounds were identified for the first time in sulfur-fumigated white ginseng and its decoction, and were deduced to be sulfate or sulfite derivatives of original ginsenosides, which were assumed to be generated through reactions of esterification and/or addition of original ginsenosides with sulfur dioxide or sulfur trioxide formed during sulfur-fumigation. The established method was successfully applied to the rapid identification of sulfur-fumigated white ginseng in commercial ginseng samples. The proposed strategy should be also useful for investigation of potential sulfur-fumigation induced chemical transformation of other medicinal herbs.

Acknowledgements

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