Journal of Chromatography A, 1230 (2012) 93–99

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Diagnostic ion filtering to characterize ginseng saponins by rapid liquid chromatography with time-of-flight mass spectrometry

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ARTICLE INFO

Article history:
Received 23 November 2011
Received in revised form 11 January 2012
Accepted 26 January 2012
Available online 4 February 2012

Keywords:
Ginseng
Ginseng saponins
Ginsenosides
Liquid chromatography time-of-flight mass spectrometry
Segmental monitoring
Diagnostic ion filtering

ABSTRACT

As one of the most commonly used medicinal plants, ginseng has been an attractive model herb for study. A wide range of analytical methods has been used to characterize its constituents. However, less effort has been devoted to the rare ginseng saponins, especially their isomers and sugar linkages. In this study, we used segmental monitoring and diagnostic ion filtering to characterize ginseng saponins by rapid liquid chromatography with time-of-flight mass spectrometry (LC-TOF-MS). By using selected diagnostic ions, specific groups of ginseng saponins were readily extracted from the complicated matrix. 20(*R*) and 20(*S*) stereo-saponins were differentiated using the peak abundance ratio of $[M\text{-}H_2O+H]^+$ to $[M\text{-}2H_2O+H]^+$. The fragmentation behavior of ginsenosides was first reported in negative ion mode by MS/MS with high-energy collision-induced dissociation, producing rules to determine sugar numbers, positions and linkages. Using the rules, we identified and compared the nontarget ginseng saponin profiling of raw and steamed American ginseng roots and berries. We characterized 70 saponins in the samples. Our strategy can be extended to screen and characterize other rare ginseng saponins and their metabolites.

1. Introduction

Ginseng has been used for thousands of years in oriental countries [1,2]. Because of its popularity, ginseng has become a model herb for study [3]. The major active constituents in ginseng are triterpene saponins [2]. High performance liquid chromatography (HPLC) continues to be the most commonly used technique in ginseng analytical research [4,5]; however, because of the limited availability of standards for ginseng saponins, only a few ginseng saponins have been described in ginseng roots [5].

Combining liquid chromatography (LC) with mass spectrometry (MS) is a powerful tool for elucidating the structures of ginseng saponins. One limitation of MS-based methods is that ionization is instrument-dependent [3]. The time-of-flight (TOF) mass analyzer measures mass accurately, giving the elemental composition of obtained ions [6]. However, because of the structural complexity of ginseng saponins, especially their sugar linkages, fewer than 30 ginseng saponins have been assayed [5,7]. Information on other ginseng saponins is still unavailable using current analytical methods.

Identification of isomers is another challenge in ginseng saponin analysis. 20(*S*) and 20(*R*) stereoisomers, differing in the position of the C-20 hydroxyl, are common in ginseng-related samples [8,9]. The stereochemistries of the 20(*S*)- and 20(*R*)-ginsenosides produce different pharmacological effects. Yet in most publications, 20(*S*)- and 20(*R*)-ginsenosides have not been distinguished [10,11]. In one study that compared the MS fragmentation rules of 20(*S*)- and 20(*R*)-ginsenosides, only limited data were obtained [5].

Because American ginseng (*Panax quinquefolius* L.) is one of the most commonly used botanicals in the United States [3,12], we proposed segmental monitoring and diagnostic ion filtering to characterize 70 ginseng saponins by rapid LC-TOF-MS. High-speed separation would be achieved by HPLC connected with a fast column.

2. Materials and methods

2.1. Chemicals and reagents

HPLC grade acetonitrile (ACN) and formic acid were obtained from Merck (Darmstadt, Germany). Deionized water (18 MΩ cm^{-1}) was supplied with a Millipore Milli-Q water system (Milford, MA, USA). Other reagents were of analytical purity. Reference ginsenosides, including ginsenosides Rb_1, Rc, Rd, Re, Rg_1, Rg_2, 20(*S*)-Rg_3, 20(*R*)-Rg_3, 20(*S*)-Rh_1, 20(*R*)-Rh_1, 20(*S*)-Rh_2, 20(*R*)-Rh_2, and pseudo-ginsenoside F_{11} were purchased from Jilin University (Changchun,

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doi:10.1016/j.chroma.2012.01.079

China). Their structures were further elucidated in the authors' laboratory by ^{13}C NMR and MS data. The purity of each standard compound was determined to be more than 95% by normalization of the peak areas detected by HPLC-DAD-TOF/MS.

2.2. Plant materials and sample preparation

Roots and fresh berries of American ginseng (*P. quinquefolius* L.) were obtained from Roland Ginseng, LLC (Wausau, WI, USA). All ginseng samples were gathered from 4-year-old plants. The voucher samples were authenticated by Dr. Chong-Zhi Wang and deposited at the Tang Center for Herbal Medicine Research at the University of Chicago (Chicago, IL, USA). The seeds of the berries were removed and lyophilized to obtain dried pulp samples. The ginseng roots and berries were steamed at 120 °C for 4 h, and then air-dried. Before extraction, all the samples were frozen for 2 h and then lyophilized.

The dried American ginseng samples were pulverized into powder. One gram of the powder was extracted twice by heat-reflux with 40 ml 70% ethanol at 90 °C for 4 h each. The combined extract was evaporated under vacuum and lyophilized. The samples were re-diluted in methanol and transferred to a 25-ml volumetric flask to which methanol was added to fill 25 ml. A 0.5 ml solution from the volumetric flask was diluted with 9.5 ml methanol:water (4.5:5, v/v) mixture. The solution was centrifuged at 12,000 rpm for 5 min, and the supernatant was transferred to an autosampler vial for analysis.

2.3. HPLC-TOF-MS analysis

Chromatographic analysis was performed on an Agilent 1100 series (Agilent, Germany) liquid chromatography system, equipped with a binary pump, an online degasser, an auto plate-sampler, and a thermostatically controlled column compartment. Chromatographic separation was carried out at 25 °C on an Agilent Zorbax Extend-C18 column (4.6 mm × 50 mm, 1.8 μm). The mobile phase consisted of 0.2% formic acid water (A) and ACN (B), using 20–30% B at 0–3 min, 30% B at 3–10 min, 30–50% B at 10–17 min, 50–90% B at 17–19.5 min, 90% B at 19.5–24 min, 90–100% B at 24–25 min. The flow rate was kept at 0.5 ml/min, and the sample volume injected was set at 2 μl.

Analysis was performed by an orthogonal G1315C diode array detection (DAD)-G1969A time of flight (TOF)-MS (Agilent Technologies, Santa Clara, CA, USA) equipped with an electrospray ionization (ESI) source. The TOF-MS analysis was performed using full scan mode, and the mass range was set at 120–1800 Da in the positive and negative mode. The conditions of the ESI source were as follows: drying gas (N_2) flow rate, 9.0 l/min; drying gas temperature, 320 °C; nebulizer, 35 psig; capillary voltage, 4000 V; fragmentor voltage, dynamic adjustment from 120 V to 425 V; skimmer voltage, 60 V; octapole RF, 250 V. Accurate mass measurements (error <5 ppm for analytes) were obtained by means of an automated calibrant delivery system using a dual-nebulizer ESI source. The ESI source introduces a low flow (100 μl/min) of a calibrating solution (calibrant solution A, Agilent Technologies), which contains the internal reference masses at *m*/*z* 112.9856, 301.9981, 601.9790, 1033.988, 1333.9689, 1633.9498, 1933.9306, 2233.9115, 2533.8923 and 2833.8731 in negative ion mode, and at *m*/*z* 118.0863, 322.0481, 622.0290, 922.0098, 1221.9115, 1521.9715, 1821.9523, 2121.9332, 2421.9140, and 2721.8948 in positive ion mode. Reference masses consisted of unknown fluorinated compounds furnished by the manufacturer with empirical formulas. The operations were controlled by Agilent LC-MS TOF software version. A.01.00 (Agilent Technologies, USA). The data recorded were processed with Applied Biosystems/MDS-SCIEX Analyst QS software (Frankfurt, Germany).

Diagnostic ion filtering and segmental monitoring: For identification of a non-target ginseng saponin, sensitive negative ion mode was first used to obtain its molecular formula. Then, positive ion mode was employed to get abundant fragment ions. Next, diagnostic ion presence was filtered to locate its ginseng saponin type. Other fragment ions were useful for matching its possible structures based on available databases.

2.4. Quadrupole-TOF-MS analysis

Chromatographic analysis was the same as Section 2.3. Analysis was performed by a G6530 quadrupole (Q)-TOF mass spectrometer (Agilent Technologies, Germany) equipped with an ESI interface. The operating parameters were as follows: drying gas (N_2) flow rate, 5.0 l/min; drying gas temperature, 325 °C; nebulizer, 45 psig; sheath gas temperature, 400 °C; sheath gas flow 12 l/min; capillary, 3500 V; skimmer, 65 V; OCT RF V, 750 V; and fragmentor voltage, 100 V. For MS/MS experiments, the collision energy was set at 70. The ginsenosides were analyzed in negative mode. The mass range was set at *m*/*z* 100–2000. The system was operated under MassHunter workstation software, version B.02.00 (Agilent Technologies).

3. Results and discussion

3.1. Selection of extraction procedure

Ginseng saponins have been extracted from ginseng with different solvents and methods [3]. The conventional method uses heat-reflux, Soxhlet, and ultrasound-assisted extraction. Extraction solvent includes water, methanol and ethanol. Different sample preparation differs in extraction efficiency, affecting the relative abundance of the extractable saponins. In modern herbal drug preparation, heat-reflux with aqueous ethanol is a preferable choice for ginseng extraction. For ginseng-related herbal products in the clinic, ginseng saponins were commonly extracted by heat-reflux with aqueous ethanol. Thus, we selected heat-reflux with 70% aqueous ethanol to extract ginseng and then characterized diverse ginseng saponins.

3.2. Economics of conventional HPLC system with a small particle-size column

The introduction of the UPLC system has revolutionized the analysis of herbal medicines. The high cost of a UPLC system, however, limits its application. When we used a conventional HPLC system with a small 1.8-μm particle-size column, the total analysis time for separation of American ginseng extract was less than 30 min without a compromise in resolution (Fig. 1). This time was approximately three times faster than that for a conventional column packed with 5.0-μm particles [7,13]. The use of smaller particles in the column generated greater backpressure. In contrast to UPLC systems that operate at high pressures, HPLC systems generally operate at pressures not in excess of 300 Pa. Under a low flow rate of 0.5 ml/min, the system pressure is acceptable for the pump (<200 bar). The sampling rate needed for MS met the requirements. Replacement of short connection pipelines improved resolution. With conventional HPLC systems and small particle-size column, the run time and resolution was improved for analysis of ginseng saponins.

3.3. Negative-to-positive switching in single TOF-MS

The fragmentor in single TOF-MS transmits ions that balance sensitivity and fragmentation. In general, a low 100–150 V generates minimal fragmentation and maximum molecular ion intensity

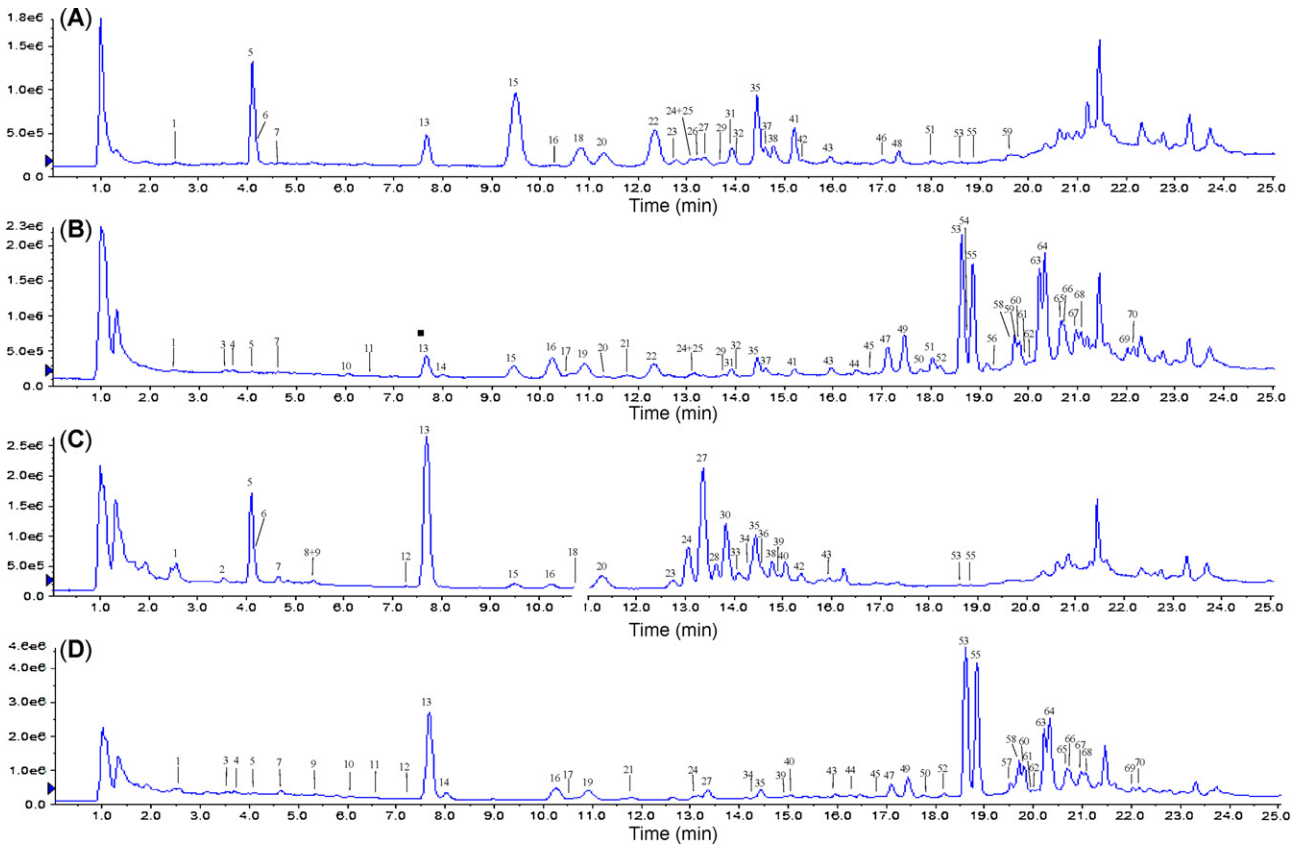

Fig. 1. The total ion chromatograms of American ginseng root (A), steamed root (B), berry (C), and steamed berry (D) by LC-TOF-MS in negative ion mode. Chromatographic conditions, detection parameters, and sample preparation are described in Section 2. Peak numbers of compounds correspond to those in Table 1.

for most natural compounds. In contrast, a 300 or higher voltage induces extensive fragmentation. For ginseng saponins in negative ion mode, typical solvent adducts are generated, such as $[M-H+CH_3COOH]^-$, because of the addition of HCOOH to solvents. Saponins did not fragment even at 425 V. In contrast, in positive mode, rich fragmentations resulted at 100 V. With negative ion mode, ginseng saponins were detected and their molecular ions and corresponding formulas for non-target compounds were obtained.

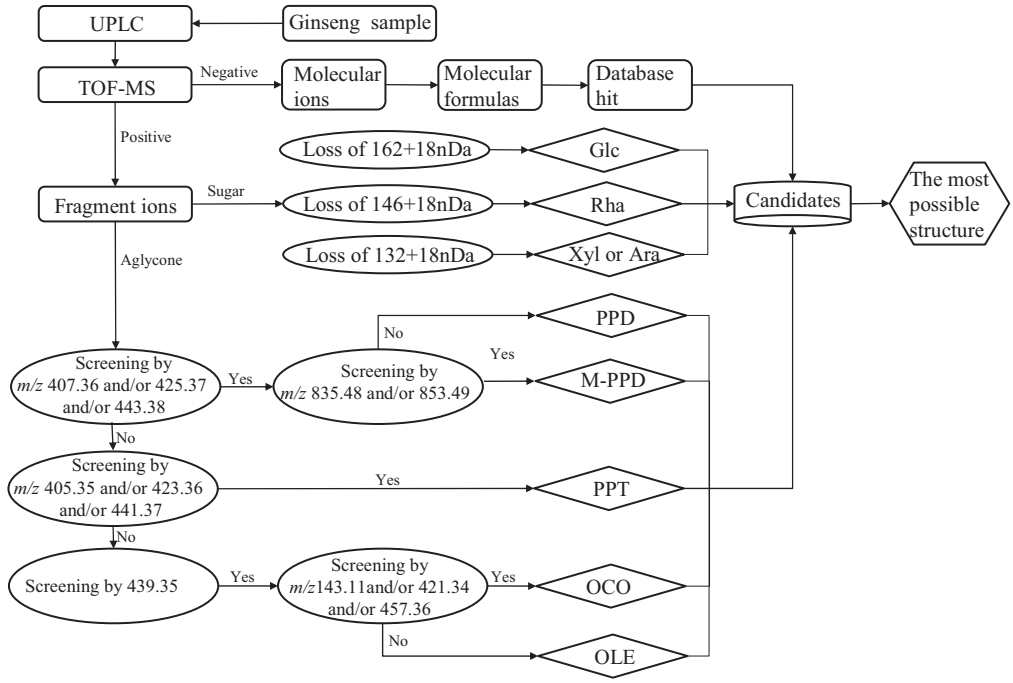

Fig. 2. A diagram for rapid classification and identification of ginsenosides by single TOF-MS. Glc, glucose; Rha, rhamnose; Xyl, xylose; Ara, arabinose; PPD, protopanoxadiol; M-PPD, malonyl-protopanoxadiol; PPT, protopanaxatriol; OCO, ocotillol; OLE, oleanane.

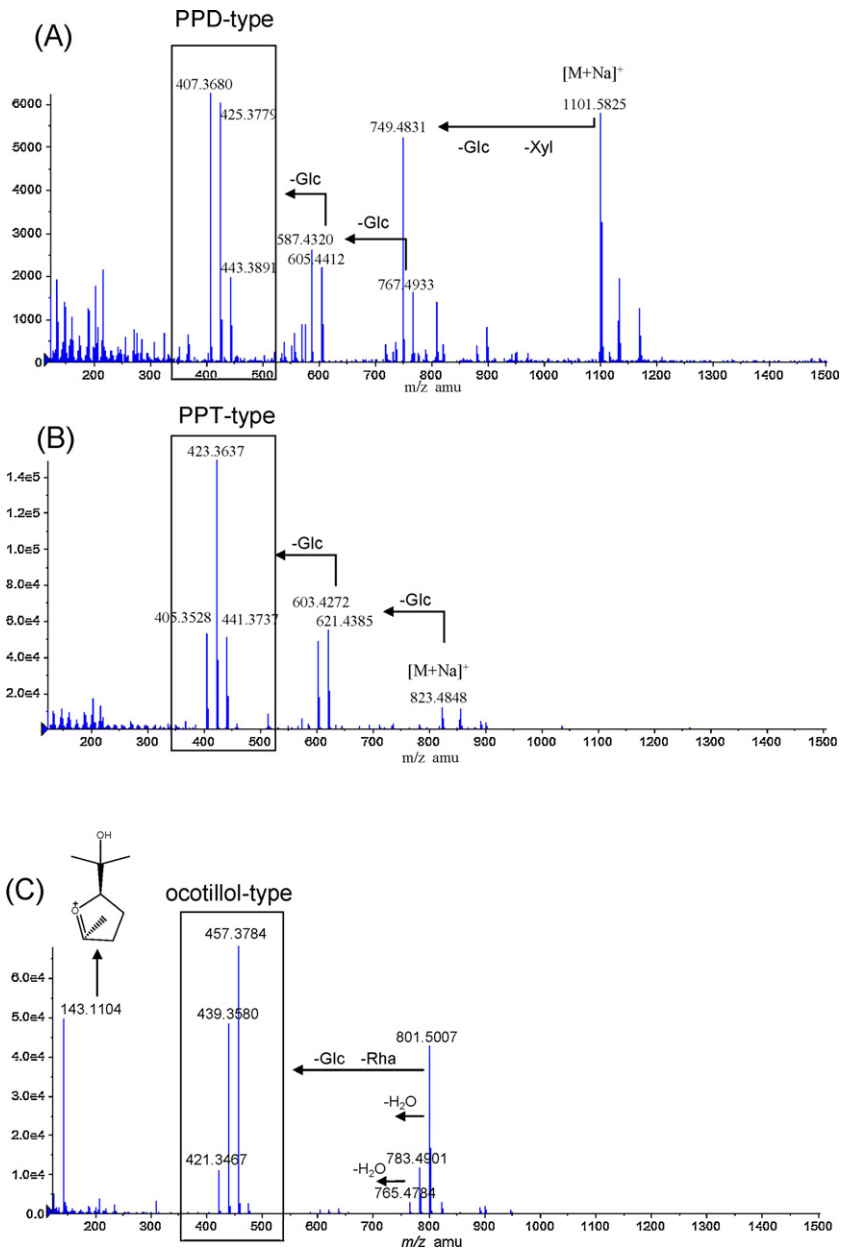

Fig. 3. The typical TOF-MS spectrum and fragmentation pathways of ginsenoside Rb_3 belonging to the PPD class (A); ginsenoside Rg_1, belonging to the PPT class (B); and pseudoginsenoside F_{11} (C). Detection was set in positive ion mode with a 120 V fragmentor.

The ion data gathered from the positive mode was an aid in structural characterization. This result was consistent with results from previous reports describing saponins in *Astragalus membranaceus*, *Lonicera* species and *Glycyrrhiza uralensis* [14–17]. From these reports and our data, we conclude that all saponins, including ginseng saponins, can be analyzed in a negative-to-positive switching mode. Detection is sensitive and fragmentation is abundant.

3.4. Classification and differentiation of diverse ginseng saponins

Ginseng saponins can be divided into several groups. Two major groups are the protopanaxadiol (PPD) group with sugar moieties attached to the C-3 and/or C-20 and the protopanaxatriol (PPT) group with sugar moieties at C-6 and/or at C-20. Another family, the malonyl ginseng saponins, also called the acidic ginseng saponins, has a malonyl group attached at the 6″-position of the glucosyl moiety. Minor groups include the ocotillol (OCO)-type with a five-membered epoxy ring at C-20, and the oleanane (OLE)-type with a nonsteroidal structure [3,12]. Although a number of reports have characterized diverse ginseng saponins, few of them have classified and differentiated saponins. Our differentiation of ginseng saponins is found in Fig. 2. The mother skeleton for saponins can be readily differentiated by a highly abundant aglycone ion and a subsequent series of dehydrated ions. Fragment ions at *m*/*z* 443.3891, 425.3779, and/or 407.3680 correspond to the PPD-type aglycone (Fig. 3A). Fragment ions at *m*/*z* 441.3737, 423.3637, and/or 405.3528 correspond to the PPT-type aglycone (Fig. 3B). Fragment ions at *m*/*z* 457.3784, 439.3580, and/or 421.3467 correspond to the OCO-type aglycone (Fig. 3C). Characteristic sugar fragments can be found by successive or simultaneous losses of 162 Da (-Glc), and/or 146 Da (-Rha), and/or 132 Da (-Ara or -Xyl).

3.5. Differentiation of 20(R) and 20(S) stereo-saponins

20(*S*) and 20(*R*) ginsenosides are stereoisomers of each other that depend on the position of the C-20 hydroxyl. 20(*S*)-OH is

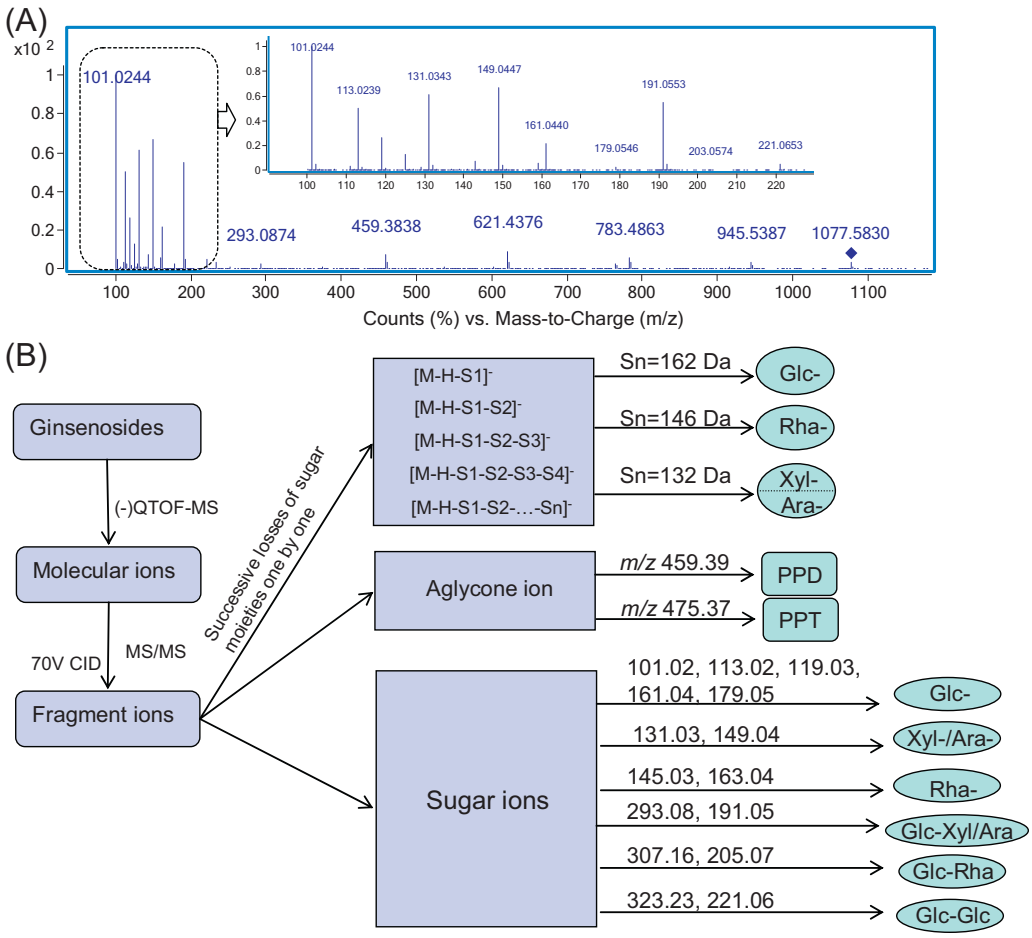

Fig. 4. The typical quadrupole TOF-MS/MS spectrum of the deprotonated ion of ginsenoside Rb_2 in negative ion mode with a high energy 70 V CID (A); a schematic pathway for determining ginsenoside type, sugar numbers, side chain composition and linkage (B).

geometrically close to the C-12 hydroxyl of ginsenosides; 20(*R*)-OH is far from the C-12 hydroxyl [18]. 20(*S*) ginsenosides tend to process the geometrical arrangement of the hydroxyl groups at carbon-12 and -20 [18,19]. The different stereochemistries of the 20(*S*)- and 20(*R*)-ginsenosides produce different pharmacological effects. In many publications, 20(*S*)- and 20(*R*)-ginsenosides are not differentiated [7,10,11].

Most naturally occurring saponins are 20(*S*). By comparing retention times in chromatograms, we found that the retention time of 20(*S*) ginsenosides is slightly shorter than that of the corresponding 20(*R*) ginsenosides. 20(*S*) and 20(*R*) stereoisomers produced similar MS data. Supplementary Fig. 1 shows the MS spectrum of the 20(*S*) and 20(*R*) ginsenoside Rh_1 in positive ion mode. Both of them generated $[M+Na]^+$ and $[2M+H]^+$ molecular ions at *m*/*z* 661.42 and 1277.88. There is an abundance of $[M\text{-}H_2O+H]^+$ and $[M\text{-}2H_2O+H]^+$ ions because of hydroxyl groups in the core moiety. The loss of the glucose substituent group initially yielded the core moiety at *m*/*z* 459.38. As a result of successive losses of H_2O molecules derived from the three hydroxyl groups in the core moiety, fragment ions at *m*/*z* 441.37, 423.36, and 405.35 were produced. One striking spectral feature of stereoisomers is the peak ratio of $[M\text{-}2H_2O+H]^+$ to $[M\text{-}H_2O+H]^+$. As can be seen from the figure, 20(*S*) produced a ratio of about 1:1; 20(*R*) produced a ratio of 0.7:1. 20(*S*) has a higher $[M\text{-}2H_2O+H]^+/[M\text{-}H_2O+H]^+$ ratio than does 20(*R*), indicating the relative difficulty of dehydration at the C-20 hydroxyl group. Tests on other stereo-ginsenosides confirmed our hypothesis.

3.6. Structural characterization of malonyl ginsenosides

Malonyl ginsenosides are a special type of compound, with a malonyl group attached at the 6″-position of the glucosyl moiety. Most malonyl ginsenosides are derived from PPD-type ginsenosides. As shown in Supplementary Fig. 2, like PPD-type ginseng saponins, malonyl ginsenosides have common skeleton ions at *m*/*z* 443.38 and successive dehydrated ions at *m*/*z* 425.37 and 407.37 in positive ion mode. All malonyl ginsenosides produce abundant ions at *m*/*z* 853.49, 835.48 and 817.47 by simultaneous losses of sugar moieties at C-3 and C-20 and successive losses of water molecules. Accurate mass measurements in TOF-MS facilitate screening of target as well as nontarget compounds. The common characteristic ions are useful for rapid screening and identification of malonyl ginsenosides in ginseng samples. Consequently, when we exploited a narrow mass window of 0.01 Da (835.48–835.49) to restructure extracted ion chromatograms, eight major malonyl ginsenosides, namely peaks 18, 23, 28, 30, 33, 36, 38 and 42, were rapidly screened from the complicated matrix. We also observed that malonyl ginsenosides are more abundant in berries than in roots. Since malonyl-Rb_2, Rb_3, and Rc are a group of isomers, their assignment was not determined when reference compounds were lacking. Retention times may provide some information for reference.

It has been widely reported that the presence of a free carboxylic acid group makes malonyl ginsenosides display typical neutral losses of 44 Da (CO_2), 42 Da (CH_2═CO) and 60 Da (CH_3COOH). We did not observe the losses under TOF-MS conditions. We noted that

Table 1
The content of 70 saponins in root (R), steamed root (SR), berry (B), and steamed berry (SB) of American ginseng.

No.	Name	R	SR	B	SB	No.	Name	R	SR	B	SB
1	Neoalsoside A5	+	+	++	++	36	Malonyl-Rb_2/Rb_3/Rc iso	–	–	++	–
2	Notoginsenoside R1	–	–	++	–	37	Chikusetsusaponin IVa	+++	+	–	–
3	Panaxsaponin 1/iso	–	+	–	+	38	Malonyl-Rd/iso	++	–	+++	–
4	Panaxsaponin 1/iso	–	+	–	+	39	Ginsenoside Rs_1/Rs_2/iso	–	–	+	+
5	Ginsenoside Re	+++	+	+++	+	40	Ginsenoside Rs_1/Rs_2/iso	–	–	++	+
6	Ginsenoside Rg_1	+++	–	+++	–	41	Gypenoside XVII	++	+	–	–
7	24(*S*)-Pseudoginsenoside F_{11} iso	+	+	++	++	42	Malonyl-Rd/iso	+	–	++	–
8	Ginsenoside F_5	+	–	+	–	43	Pseudoginsenoside Rc_1	+	+	+	+
9	24(*S*)-Pseudoginsenoside F_{11} iso	–	–	+	+	44	Ginsenoside F_2 *r*/*s*	–	+	–	+
10	Ginsenoside Rg_2 iso	–	+	–	+	45	Ginsenoside F_2 *r*/*s*	–	+	–	+
11	Ginsenoside Rg_2 iso	–	+	–	+	46	Quinquenoside I	+	–	–	–
12	Pseudoginsenoside RT_2	–	–	+	+	47	Ginsenoside Rg_6/Rg_4	–	+++	–	+++
13	Pseudoginsenoside F_{11}	++	++	+++	+++	48	Ginsenoside F_2 iso	+	–	–	–
14	Pseudoginsenoside RT_5	–	+	–	+++	49	Ginsenoside Rg_6/Rg_4	–	+++	–	+++
15	Ginsenoside Rb_1	+++	++	+	–	50	Ginsenoside Rh_4/Rk_3	–	++	–	++
16	20(*S*)-Ginsenoside Rg_2	+	+++	++	+++	51	Zingibroside R_1	+	+++	–	–
17	20(*S*)-Ginsenoside Rh_1	–	+++	–	++	52	Ginsenoside Rh_4/Rk_3	–	++	–	++
18	Malonyl-Rb_1	+++	–	+	–	53	20(*S*)-Ginsenoside Rg_3	+	+++	+	+++
19	20(*R*)-Ginsenoside Rg_2	–	+++	–	+++	54	Saponin Rb-4	–	+	–	–
20	Ginsenoside Rc	+++	+	+++	–	55	20(*R*)-Ginsenoside Rg_3	+	+++	+	+++
21	20(*R*)-Ginsenoside Rh_1	–	++	–	+++	56	Pseudoginsenoside RP_1	–	+++	–	–
22	Ginsenoside Ro	+++	+++	–	–	57	Ginsenoside Rs_3 iso	–	–	–	+
23	Malonyl-Rb_2/Rb_3/Rc	+	–	+	–	58	20(*S*)-Ginsenoside Rs_3	–	+++	–	+++
24	Ginsenoside Rb_2	+	+	+++	++	59	Calenduloside E	+	+	–	–
25	Abrussaponin II	+	+	–	–	60	20(*R*)-Ginsenoside Rs_3	–	+++	–	+++
26	Malonyl-Rb_1 iso	+	–	–	–	61	Ginsenoside Rs_3 iso	–	+	–	+
27	Ginsenoside Rb_3	++	–	+++	++	62	Ginsenoside Rs_3 iso	–	+	–	+
28	Malonyl-Rb_2/Rb_3/Rc	–	–	+++	–	63	Ginsenoside Rg_5/Rk_1	–	+++	–	+++
29	Saponin Rb-2	+	+	–	–	64	Ginsenoside Rg_5/Rk_1	–	+++	–	+++
30	Malonyl-Rb_2/Rb_3/Rc	–	–	+++	–	65	20(*S*)-Ginsenoside Rh_2	–	++	–	++
31	Quinquenoside R_1	++	++	–	–	66	20(*R*)-Ginsenoside Rh_2	–	++	–	++
32	Pseudoginsenoside RT_1	++	+	–	–	67	Ginsenoside Rs_4/Rs_5	–	+++	–	+++
33	Malonyl-Rb_2/Rb_3/Rc iso	–	–	+	–	68	Ginsenoside Rs_4/Rs_5	–	+++	–	+++
34	Ginsenoside Rs_1/Rs_2/iso	–	–	+	+++	69	Ginsenoside Rk_2/Rh_3	–	++	–	++
35	Ginsenoside Rd	+++	++	+++	+++	70	Ginsenoside Rk_2/Rh_3	–	++	–	++

The criteria of the grading levels were defined according to the peak areas after re-constructing extracted ion chromatograms of the target compounds: –, not detected; peak area in the range of $(0–0.5) \times 10^4$ defined as "+", meaning low-level; $(0.5–5) \times 10^4$ defined as "++", meaning moderate-level; peak area $>5 \times 10^4$ defined as "+++", meaning high-abundant.

entire losses of malonyl-glc moieties produced the minor ion at *m*/*z* 605.42 from 835.48. LC–MS ionization is instrument-dependent. Equipment, especially for TOF-MS, should be calibrated accurately and regularly. Generating data depends on the experience and skill of the operators.

3.7. Negative MS/MS by quadrupole-TOF with high collision-induced dissociation

In negative ion mode, saponins did not fragment by single TOF. Negative Q-TOF-MS for multi-step fragmentation was then used to analyze ginseng saponins. High-energy collision-induced dissociation (CID) generated interesting fragmentation behaviors. The MS/MS spectrum of the deprotonated ion at *m*/*z* 1077.5830 for the ginsenoside Rb_2 is shown in Fig. 4A. In the high mass area, successive losses of sugar moieties one by one were observed. The most common sugar residues were hexoses (glucose), deoxyhexoses (rhamnose), and pentoses (arabinose, xylose) linked to an aglycone through the ether glycosidic bonds at the C-3 position (PPD-type), the C-6 position (PPT-type) and the C-25 position. Loss of 162 Da corresponds to Glc-, 146 Da refers to Rha-, and 132 Da was Ara- or Xyl-. Usually, the end sugar moiety at C-3 or C-6 was eliminated first, then the end sugar moiety at C-20, followed by the inner sugar unit at C-3 or C-6, and the inner sugar part at C-20. The characteristic aglycone ion at *m*/*z* 459.39 corresponds to the PPD-type, and 475.37 corresponds to the PPT-type. A series of dehydrated ions from the molecular or aglycone ion that are readily formed in positive ion mode were not found in negative ion mode. Highly abundant low-mass fragment ions in the range of *m*/*z* 100–400 were produced, corresponding to sugar residue ions. Almost all ginseng saponins containing Glc- units produced common highly abundant ions at *m*/*z* 101.02, 113.02, 119.03, 161.04, and 179.05. Saponins containing Rha- units formed common ions at 131.03 and 149.04. Saponins containing Ara- or Xyl- units formed common ions at 145.03 and 163.04. Some saponins with one side chain of Glc-Ara or Glc-Xyl, such as Rb_2 and Rb_3, produced diagnostic ions at 293.08 and 191.05 by a typical loss of 102 Da. Some saponins with one side chain of Glc-Rha, such as Re and Rg_2, produced diagnostic ions at 307.16 and 205.07 by a typical loss of 102 Da. Some saponins with one side chain of Glc-Glc, such as Rb_1 and Rd, produced diagnostic ions at 323.23 and 221.06 by a typical loss of 102 Da. The fragmentation pathways of ginseng saponins in negative ion mode by Q-TOF-MS at high energy CID are depicted in Fig. 4(B). The MS behavior of other groups or sugar compositions of ginseng saponins can be derived from this rule. This method is an alternative to positive ion mode to determine sugar numbers, sugar position and linkage order.

3.8. Chemical composition of ginseng roots and berries

The growth rate of ginseng root is slow. Ginseng berries, also sources of numerous ginsenosides, might be used as an alternative dietary supplement [20,21]. The composition and content of saponins differ in ginseng roots and berries (Fig. 1, Table 1). A total of 31 and 29 ginseng saponins were detected in ginseng roots and berries, respectively. Peaks 2, 8, 9, 12, 28, 30, 33, 34, 36, 39, and

40 were detected in ginseng berries but not in ginseng roots. Peaks 22, 31, 32, 37, 41, 46, 48, 51, and 59 were detected in ginseng roots but not in ginseng berries. The most abundant ginseng saponins in American ginseng root, calculated by peak area, are ginsenosides Rb_1 and Re [22,23]. To our surprise, in berries, ginsenoside Rb_3 and pseudoginsenoside F_{11} were the most abundant. Rb_1, a commonly used marker compound for quality control of ginseng samples, was low in ginseng berries. Fig. 3(A) shows the ESI mass spectrum of ginsenoside Rb_3 in positive ion mode with 120 V fragmentor voltage.

In the herbal market, American ginseng is sometimes adulterated with Asian ginseng. The presence of pseudoginsenoside F_{11} in American ginseng and ginsenoside Rf in Asian ginseng distinguishes them from each other [24]. Ginseng berries have much higher pseudoginsenoside F_{11} content than does ginseng root. Since pseudoginsenoside F_{11} differs structurally from Rb_1 or Rb_3, their pharmacological activities may be different. Because of their different chromatographic fingerprints ginseng berries may be suitable for different medicinal purposes than ginseng root. Because pseudoginsenoside F_{11} and ginsenoside Rf share the same molecular weight and chromatographic fingerprint under most LC conditions [3,24], misidentification of these components is possible. Fig. 3(C) shows the MS/MS spectrum of pseudoginsenoside F_{11} in positive ion mode with 120 V fragmentor voltage. A characteristic fragment ion with high abundance at m/z 143.11 ($C_8H_{15}O_2$) was observed corresponding to a 25-hydroxy-20,24-epoxy residue by the cleavage of the bond between C-17 and C-20. This ion was not observed in Rf. The skeleton fragment ions also were different. Rf, belonging to the PPD group, showed skeleton ions at m/z 405.35, 423.36, 441.37, and 459.38. F_{11}, an OCO-type saponin, showed fragment ions at m/z 421.34, 439.35, 457.37, and 475.38.

3.9. Untreated and steamed ginseng

Processing by heating (*e.g.* steaming) can affect the chemical profile of an herbal compound and change its bioactivities. Ginseng is one such herb. As shown in Fig. 1, the chemical composition of steamed ginseng roots and berries is considerably different from its unsteamed counterparts. Original polar ginsenosides are converted to new less polar compounds during steaming. Structurally, sugar chains are eliminated and the hydroxyl configuration at C-20 of the aglycones is isomerized. The protopanaxadiol (PPD)-type ginsenosides selectively eliminate the C-20 sugar chain to produce Rg_3. Rg_3 is transformed to Rk_1 and Rg_5 by dehydration. Because a small amount of Rh_2 is observed in red ginseng, the elimination of the C-3 sugar residue may be difficult in processing. The protopanaxatriol (PPT)-type ginsenosides first lose the C-20 sugar residue and subsequently their terminal sugar unit at C-6 to form Rg_2 and/or Rh_1. Rh_1 is further converted to Rk_3 and Rh_4 by dehydration at C-20. After steaming, the content of malonyl ginsenosides decreased significantly. Malonyl ginsenosides are easily hydrolyzed in hot alcoholic extracts and are not often observed in commercial extracts [25]. We found that even after hot alcoholic extraction, malonyl ginsenosides were still in our berry extracts. Malonyl ginsenosides vanished after steaming because the process may have released malonic acid.

The retention times, deprotonated ions, ppm errors, common characteristic ions, identified names, formulas, and classifications for 70 major ginseng saponins extracted from American ginseng samples are included in Supplementary Table 1. Their chemical structures are described in Supplementary Fig. 3.

4. Conclusions

In this work, a segmental monitoring and diagnostic ion filtering strategy was developed to characterize ginseng saponins. We propose a new strategy using negative ion mode MS/MS with high energy CID to determine sugar numbers, positions and linkages. We identified 70 ginseng saponins, of which 31, 49, 29, and 43 were identified in the roots, steamed roots, berries, and steamed berries of American ginseng, respectively. We also observed that pseudoginsenoside F_{11}, a representative saponin at low levels in American ginseng root, was found at high levels in the berries. The method developed here can be widely used to screen and identify ginseng saponins in ginseng-contained samples, and set up an example for identification of target and nontarget compounds in complex herbal matrices.

Acknowledgments

We thank Sally Kozlik for editing the manuscript. This work was supported in part by the National Science Foundation of China (Nos. 81173497 and 81130068), NIH/NCCAM Grants AT004418 and AT005362, the National Key Technologies R&D Program of China (No. 2008BAI51B01), and Program for Changjiang Scholars and Innovative Research Teams in Universities (No. IRT0868).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2012.01.079.

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