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Determination of American ginseng saponins and their metabolites in human plasma, urine and feces samples by liquid chromatography coupled with quadrupole time-of-flight mass spectrometry

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Abstract

American ginseng is a commonly consumed herbal medicine in the United States and other countries. Ginseng saponins are considered to be its active constituents. We have previously demonstrated in an *in vitro* experiment that human enteric microbiota metabolize ginseng parent compounds into their metabolites. In this study, we analyzed American ginseng saponins and their metabolites in human plasma, urine and feces samples by liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC-Q-TOF-MS). Six healthy male volunteers ingested 1 g of American ginseng twice a day for 7 days. On day 7, biological samples were obtained and pretreated with solid phase extraction. The ginseng constituents and their metabolites were characterized, including 5 ginseng metabolites in plasma, 10 in urine, and 16 in feces. For the plasma, urine and feces samples, the levels of ginsenoside Rb₁ (a major parent compound) were 8.6, 56.8 and 57.7 ng/mL, respectively, and the levels of compound K (a major metabolite) were 58.4 ng/mL, 109.8 ng/mL and 10.06 µg/mL, respectively. It suggested that compound K had a remarkably high level in all three samples. Moreover, in human feces, ginsenoside Rk₁ and Rg₅, Rk₃ and Rh₄, Rg₆ and F₄ were detected as the products of dehydration. Further studies are needed to evaluate the pharmacological activities of the identified ginseng metabolites.

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Keywords

American ginseng; Ginsenosides; Enteric; Microbiota; Ginsenoside Rb₁; Compound K; LC-Q-TOF-MS

1. Introduction

American ginseng or *Panax quinquefolius* is a commonly consumed herbal medicine in the United States and other countries. Ginseng saponins are considered to be active constituents of this botanical [1]. Until now, nearly a hundred ginsenosides have been isolated and characterized from American ginseng, and many of them have shown pharmacological activities [2, 3]. Yet, compared with studies of other ginseng species, such as *Panax ginseng* and *Panax notoginseng*, analytical studies of American ginseng and its components are relatively limited [4, 5].

The chemical and pharmacological diversity of different ginseng constituents has been investigated. In studies of many parent compounds of ginseng, attention also has been given to their potential structure-activity relationship [6–8]. Reductionist methodology in ginseng research during *in vitro* screening has been applied primarily to the bioactivity of parent compounds [9, 10]. However, the bioavailability of ginseng compounds, an important consideration for their effects *in vivo*, has been overlooked.

Of the commercially available American ginseng products, nearly all are ingested orally. After oral intake, trillions of gut microbiota in the gastrointestinal tract may affect ginseng biotransformation [11]. Metabolic profiles of ginseng by enteric microbiota have been reported in animals [12, 13], and the actions of selected biotransformed metabolites have been elucidated [14]. For example, compound K, a major metabolite in the protopanaxadiols group, has a better bioactivity than its parent compound, ginsenoside Rb₁ [15]. In an *in vitro* study we observed that human enteric microbiota metabolized ginseng parent compounds into 25 metabolites [16]. However, to date, the determination of ginseng metabolites in human biological samples has largely not been carried out. To link the health benefits of ginseng compounds to their effects, we sought to determine the profiles of ginseng and its metabolites after oral administration.

Based on our previous studies, liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC-Q-TOF-MS) can be effectively used to characterize ginseng saponins and their metabolites [16]. This technique provides advanced structural information with high sensitivity, specificity, and versatility in characterizing complex metabolite profiles in matrix-based samples. With this method, all ginseng constituents, even at low concentrations, can be successfully detected.

In a previous study in humans, we analyzed a ginsenoside and its metabolism in plasma after a single oral dose of American ginseng using UPLC-TOF-MS [17]. It was indicated that the metabolite profile might not have been adequately revealed after a single dose regimen in the qualitative study. For the study reported here, we recruited six healthy volunteers to ingest American ginseng for 7 successive days. The 2 g daily ginseng used in this study was

within the commonly used therapeutic dose range. Plasma, urine and feces samples were collected at the end of the 7 days and analyzed by LC-Q-TOF-MS. Special attention was paid to the determination of a major ginseng parent compound, ginsenoside Rb₁, and its predominant metabolite, compound K.

2. Experimental

2.1. Chemicals and reagents

Reference ginsenosides, including ginsenoside Rb₁, Rb₂, Rb₃, Rc, Rd, Re, Rg₁, F₁, F₂, pseudoginsenoside F₁₁, gypenoside XVII, 20S-Rg₂, 20R-Rg₂, 20S-Rg₃, 20R-Rg₃, 20S-Rh₁, 20R-Rh₁, 20S-Rh₂, 20R-Rh₂, compound K, and protopanaxatriol were purchased from Jilin University (Changchun, China). Ginsenoside Rk₃, Rh₄, Rk₁ and Rg₅ were prepared in our laboratory by steaming transformation. The structures are shown in Fig. 1(C), and were elucidated by ¹H NMR, ¹³C NMR and MS. The internal standard (IS) digoxin was obtained from Sigma-Aldrich (St. Louis, USA). The purities of the reference compounds were more than 95%, as determined by HPLC-DAD. Acetonitrile (ACN) and formic acid of HPLC grade were obtained from Merck (Darmstadt, Germany). Deionized water (18 MΩ cm⁻¹) was supplied with a Milli-Q water system (Millipore, Milford, MA, USA). Other reagents were of analytical grade.

2.2. Plant materials

American ginseng roots (*P. quinquefolius* L.) were obtained from Roland Ginseng, LLC (Wausau, WI, USA). The voucher samples were authenticated by Dr. Chun-Su Yuan and deposited at the Tang Center for Herbal Medicine Research at the University of Chicago (Chicago, IL, USA). Air-dried American ginseng was pulverized to powder and passed through a 40 mesh screen to prepare the sample.

2.3. Human subjects and study protocol

With approval from the Institutional Review Board, six healthy male volunteers (ages 18–45 years) were enrolled in the clinical trial. The subjects were screened for drug abuse disorders or medical contraindications for participation in the study. The volunteers underwent a physical examination and completed a health history questionnaire. On day 1, each subject was asked to fast overnight, and their biological samples (blood, urine and feces) were collected as the control. Then all volunteers were instructed to ingest 1 g of American ginseng powder with water twice a day for 7 consecutive days. On day 7, biological samples from these subjects were again obtained for analysis. The blood samples in the heparin tubes were centrifuged at 4000 rpm for 5 min and the plasma was transferred to Eppendorf tubes. All samples were then immediately chilled on ice and stored at –80°C until analysis.

2.4. Sample preparation

To prevent matrix effects and to concentrate the analytes, all the biological samples were pretreated with solid phase extraction (SPE) before LC-Q-TOF-MS. Oasis HLB columns (1 mL, 30 mg, Waters, Milford, MA, USA) were preconditioned with 2 mL of methanol and equilibrated with 2 mL of deionized water.

The plasma samples (200 μ L) were mixed with 20 μ L of 2% acetic acid and vortexed for 30 sec at room temperature. Acetic acid was used to release the protein binding ginsenosides and metabolites to increase the recovery of the ingredients for the measurement. The samples were subsequently diluted with 1 mL of physiological saline. The homogenates were loaded onto the preconditioned SPE columns, washed with 2 mL of deionized water and slowly eluted using 1 mL of methanol. The elution was evaporated to dryness under nitrogen at 20°C, and the residue was dissolved in 100 μ L of methanol and centrifuged at 13,000 rpm for 10 min before further analysis.

The urine samples were centrifuged at 4,000 rpm for 10 min at room temperature and the supernatants (200 μ L) were purified by the preconditioned SPE columns. The cartridge was cleaned with 2 mL of deionized water, and slowly eluted using 1 mL of methanol. The elution was evaporated to dryness under nitrogen at 20°C, and the residue was dissolved in 100 μ L of methanol and centrifuged at 13,000 rpm for 10 min.

The feces samples (0.5 g) were extracted with 3 mL of 80% methanol by ultrasound (30°C, 100 Hz) for 15 min. They were then centrifuged at 4,000 rpm for 10 min at room temperature, and the supernatants (200 μ L) were purified by the preconditioned SPE columns. The cartridge was cleaned with 2 mL of deionized water and slowly eluted using 1 mL of methanol. The elution was evaporated to dryness under nitrogen at 20°C, and the residue was dissolved in 100 μ L of methanol and centrifuged at 13,000 rpm for 10 min.

2.5. LC-MS/MS instrument and conditions

Chromatographic analysis was performed on an Agilent 1290 Series LC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a binary pump, an online degasser, an auto plate-sampler, and a thermostatically controlled column compartment. Sample separation was carried out at 25°C on an Agilent Zorbax Extend-C₁₈ column (4.6 mm $\times$ 250 mm, 5 μ m) with a C₁₈ guard column (4.6 mm, 0.2 μ m). The mobile phase consisted of water (0.1% formic acid, Solvent A) and acetonitrile (0.1% formic acid, Solvent B), using a gradient elution of 21% B at 0–15 min, 21%–30% B at 15–18 min, 30%–33% B at 18–30 min, 33% B at 30–34 min, 33%–45% B at 34–40 min, 45%–60% B at 40–50 min, 60%–80% B at 50–55 min, 80%–100% B at 55–60 min, and 100% B at 60–65 min. The flow rate was kept at 1 mL/min, and the injection volume of plasma, urine, and feces samples was set at 10 μ L, 5 μ L, and 10 μ L, respectively.

Detection was performed by a 6530 Q-TOF mass spectrometer (Agilent) with a Dual electrospray ionization (ESI) source. The operating parameters were optimized as follows: drying gas (N₂) flow rate, 10.0 L/min; drying gas temperature, 320°C; nebulizer, 35 psig; capillary, 3500 V; OCT RFV, 750 V; and fragmentor voltage, 120 V. Mass spectra were recorded across the range m/z 100–3000 in both positive and negative ion modes. The operation and acquisition of data were monitored by Agilent LC-Q-TOF-MS MassHunter Acquisition Software (Version B.04.00). A low flow of calibrant solution A was applied to obtain high accuracy measurements. The calibrant solution A was a reference mass solution of purine and HP-0921, which was used to provide reference ions in the Agilent mass spectrometer. The reference ions applied in different ion modes were distinguishing; 121.0508, 922.0097 (positive ions) and 112.9855, 1033.9881 (negative ions). The dosage of

reference mass solution added in the system was adjusted on the basis of the actual MS response value in LC-Q-TOF-MS, and the signal intensities of the reference ions were expected to be higher than 10000. Thus, the calibrant solution A was imported from reference nebulizer, which was set at 5 psi as default.

2.6. Method validation

The method was validated for selectivity, linearity, limits of detection (LODs), lower limits of quantification (LLOQs), and recovery based on FDA Guidance for Industry-Bioanalytical Method Validation [18]. The selectivity was investigated by comparing the chromatograms of six individual blank human plasma with those of corresponding standard plasma samples spiked with the analytes and IS and plasma samples after oral administration of American ginseng. The linearity was evaluated by analyzing calibration standards at each concentration level over three consecutive days. The calibration curves were constructed by plotting the peak area ratio (y) of analytes to IS versus plasma concentrations (x) of analytes using a $1/x^2$ weighted least-squares linear regression model. LODs were defined as the lowest amount of analytes that could be detected, which was determined as the concentration with a signal to noise ratio (S/N) of 3:1. LLOQs was served as the lowest concentration of the standard curve, giving a S/N of 10:1. The recoveries of Rb₁ and compound K were determined in the plasma at three spiking levels (10, 100 and 1000 ng/mL), by comparing the peak areas obtained from samples of spiked matrices and the peak areas obtained from direct injection of known amounts of standard solutions.

2.7. Data analysis

Mass data were analyzed with Agilent MassHunter Workstation software (Version B.05.00). Accurate mass measurements (error <5 ppm) were obtained by comparing the theoretical mass of molecular ions and/or fragment ions to achieve an empirical molecular formula. The levels of parent ginsenosides and metabolites in individual subjects and their mean $\pm$ S.D. were calculated.

3. Results and discussion

3.1. Analytical condition optimization

In this study, LC-Q-TOF-MS was used for the online structural characterization of American ginseng saponins and their metabolites in human plasma, urine and feces samples.

Considering the chemical complexity of the biological matrixes, the effects of chromatographic conditions, including the column, mobile phases and modifiers, were evaluated. It was shown that Zorbax Extend-C₁₈ column was the most suitable for the separation of ginsenosides and the relevant metabolites. A solvent system of water/acetonitrile with gradient elution was optimized for better resolution in a shorter time. Moreover, a low concentration of formic acid (0.1%) added in the mobile phase improved the peak symmetry and enhanced the ionization efficiency of major analytes, such as ginsenoside Rb₁ and compound K.

In MS analysis both positive and negative ion modes were employed to obtain the abundance information for the structural characterization of complex constituents from the matrix. Due to the presence of formic acid in the mobile phase, the deprotonated molecular ion $[M-H]^-$ and typical solvent adduct ion $[M+HCOO]^-$ were generated in ESI negative ion mode, providing information on the molecular mass. In particular, $[M-H]^-$ (m/z 1107.59) and $[M+HCOOH]^-$ (m/z 1153.60) of ginsenoside Rb₁, and $[M-H]^-$ (m/z 621.44) and $[M+HCOOH]^-$ (m/z 667.44) of compound K were observed in the negative ion mode of the MS spectrum. $[M+H]^+$ (m/z 1131.59) of ginsenoside Rb₁ and $[M+H]^+$ (m/z 645.43) of compound K were detected in the positive ion mode. The negative ion mode gives much lower background noise with less interference, so it was used for the following MS/MS experiments. Meanwhile, the collision energy was adjusted for optimum performance in negative MS/MS, and 50V was ultimately selected.

Under these conditions, 25 reference ginsenosides were well separated. The total ion chromatogram (TIC) of ginseng saponin standards is shown in Fig. 1A in the negative ion mode. Fig. 1B shows the TIC of American ginseng root extract in the negative ion mode. A total of 19 major ginseng saponins were detected and identified, and are listed in accordance with previous publications [19, 20].

3.2. Methodological validation for quantitative analysis

Ginsenoside Rb₁ and compound K in the plasma were quantified by LC-MS/MS. There was no endogenous interference at the retention times of the analytes and IS. The two analytes exhibited good linear regression ($r^2 > 0.995$) within the test ranges of 0.005–5 $\mu\text{g/ml}$. The LODs of Rb₁ and compound K were estimated to be 1.55 and 0.95 ng/mL , and the LLOQs were 3.75 and 2.50 ng/mL . These data showed that the method was sensitive for the quantitative evaluation of the analytes. The mean recoveries in plasma at three different concentrations (10, 100 and 1000 ng/mL) for Rb₁ were 83.2%, 87.5% and 88.3%, respectively, and for compound K were 85.1%, 89.4% and 89.8%, respectively. These results suggested that this method is acceptable for quantification of ginsenoside Rb₁ and compound K in the biological samples.

3.3.Characterization of American ginseng constituents and their metabolites in plasma

After oral administration, American ginseng saponins inevitably come into contact with different kinds of gut microbiota and are transformed before they are absorbed from the gastrointestinal tract. In this study, human plasma, orally administered with American ginseng, was analyzed by the established LC-Q-TOF-MS method. To differentiate the constituent-related peaks from endogenous effects, blank plasma (Fig. 2A) was compared with a typical plasma sample collected 7 days after oral ginseng administration (Fig. 2B) in the analysis.

A total of 15 peaks were identified as the main components in human plasma, including 10 original ginsenosides and 5 metabolites of American ginseng. Table 1 shows the retention time, experimental and calculated mass m/z , ppm error, and fragment ions in the MS/MS stage of these compounds in the negative ion mode, which were identified by comparing retention times and MS data with those of reference compounds. We observed that 20S-

ginsenoside Rg₂, 20*R*-ginsenoside Rg₂, ginsenoside F₂, 20*R*-ginsenoside Rg₃, and compound K were the major metabolites detected in the plasma of the six subjects. The relative abundance of the compounds measured by signal intensity (peak area) in the extracted ion chromatograms (EICs) is also shown in Table 1. From the data, the concentrations of only 5 detected metabolites in the plasma were relatively low. Since we used sensitive LC-Q-TOF-MS for analysis, some low-level parent ginsenosides were also detected in the present study.

Our previous research data showed that compound K is a primary metabolite of American ginseng root [21]. A narrow mass window of 0.01 Da to restructure the EICs of ginsenoside Rb₁ and compound K of the typical plasma sample is shown in Fig. 2C and 2D, respectively. The relative abundance of ginsenoside Rb₁ and of compound K was measured by the signal intensity in the plasma of six individual subjects for semi-quantitation (Fig. 2E). For ginsenoside Rb₁, subject CW had the highest relative abundance and subject MB had the lowest. Meanwhile, for compound K, subject MM had the highest relative abundance and subject CY had the lowest. Fig. 2F shows that the average intensity of ginsenoside Rb₁ and compound K in the six subjects was $(0.1118 \pm 0.0591) \times 10^4$ and $(1.1069 \pm 0.0710) \times 10^4$, respectively. The calculated average plasma levels for Rb₁ and compound K were 8.6 and 58.4 ng/mL, respectively. In addition, if the plasma level of the major compound, compound K, was set as 100%, the percentage for Rb₁ was 14.7%. It was suggested that the concentration of ginsenoside Rb₁ decreased after oral ginseng administration and it was transferred to compound K before entering the systemic circulation.

3.4. Characterization of American ginseng constituents and their metabolites in urine

The TIC of a typical urine sample collected at 7 days (Fig. 3B) was analyzed and compared with a blank urine sample (Fig. 3A). We identified 20 peaks as the main components in urine, including 10 original ginsenosides and 10 metabolites.

Table 2 shows the retention time, experimental and calculated mass *m/z*, ppm error, and fragment ions in the MS/MS stage of these compounds in six urine samples in the negative ion mode. These were identified by comparing retention times and MS data with those of reference compounds. 20*S*-ginsenoside Rg₂, 20*R*-ginsenoside Rg₂, 20*S*-ginsenoside Rh₁, 20*R*-ginsenoside Rh₁, ginsenoside F₁, ginsenoside F₂, 20*R*-ginsenoside Rg₃, compound K, 20*S*-ginsenoside Rh₂ and 20*R*-ginsenoside Rh₂ were major metabolites detected in urine, more than those found in plasma. The relative abundance of metabolites measured by signal intensity in the EICs are also listed.

The presence of 20*S*-ginsenoside Rh₂ and 20*R*-ginsenoside Rh₂ in urine was discovered for the first time, especially considering that the pair compounds have the same elemental composition and similar MS/MS spectrums, but generate different pathways with compound K. We speculate that 20*S*-ginsenoside Rh₂ and 20*R*-ginsenoside Rh₂ detected in urine may be caused by hepatoenteric circles and other factors.

EICs of ginsenoside Rb₁ and compound K in a typical urine sample are shown in Fig. 3C and 3D, respectively. The results of signal intensity in the urine of six individual subjects

showed that for ginsenoside Rb₁ and compound K, subject MM had the highest relative abundance and subject ZZ had the lowest (Fig. 3E). The average intensity of ginsenoside Rb₁ and compound K in the six subjects was $(0.7720 \pm 0.1245) \times 10^4$ and $(2.1352 \pm 0.1195) \times 10^4$, respectively (Fig. 3F), and these values were calculated as 56.8 ± 9.2 and 109.8 ± 6.1 ng/mL, respectively [17, 22].

3.5. Characterization of American ginseng constituents and their metabolites in feces

The TICs of a blank feces sample and a typical feces sample collected at 7 days are compared in Fig. 4A and 4B. Surprisingly, 36 peaks were identified in human feces, including 10 original ginsenosides and 16 metabolites. Table 3 shows the retention time, experimental and calculated mass m/z, ppm error, and fragment ions in the MS/MS stage of these compounds identified in feces samples in the negative ion mode. Some of the compounds were identified with corresponding reference compounds based on their retention times and MS data. Some were assigned based on a comparison of data obtained from previous publications [23, 24].

The results indicate that the total number of compounds detected is highest in feces. It is possible that some of these compounds were not absorbed by the gastrointestinal tract or that the absorbed ones had levels below our detection limit. Interestingly, the major metabolites detected in the feces of our six subjects were mostly consistent with those found in our previous *in vitro* studies of the biotransformation of American ginseng by human intestinal microflora [16]. Specifically, metabolites Rk₁ and Rg₅, Rk₃ and Rh₄, Rg₆ and F₄ could be produced by intestinal microflora via dehydration. The relative abundance of the metabolites measured in the EICs is listed in Table 3, which also showed the highest of all of the biological samples.

EICs of ginsenoside Rb₁ and compound K in the typical feces sample are shown in Fig. 4C and 4D, respectively. The results of signal intensity in the feces showed that for ginsenoside Rb₁ and compound K (Fig. 4E), subject MM had the highest relative abundance and CW had the lowest. The average intensity of ginsenoside Rb₁ and compound K in the feces samples was $(0.007843 \pm 0.003891) \times 10^6$ and $(1.964307 \pm 0.187727) \times 10^6$, respectively (Fig. 4F). These values were calculated as 57.7 ± 28.6 ng/mL and 10.06 ± 0.96 µg/mL, respectively [17, 22].

Essentially, there was no compound absorption in the colon, and microbiota continuously biotransformed Rb₁ into compound K. Thus, it is reasonable to assume that very high compound K levels and low Rb₁ levels would be found in feces after the oral administration of American ginseng. Fig. 5 summarizes the biotransformation pathways of protopanaxadiol ginsenosides of American ginseng from parent compounds to metabolites by human enteric microbiota.

4. Conclusion

In this study, we tested American ginseng saponins and their metabolites in plasma, urine and feces samples from six human subjects. A total of 5, 10, and 16 metabolites were detected in the plasma, urine and feces, respectively, using LC-Q-TOF-MS technology. Out

of all the metabolites, compound K was found at the highest levels in these biological samples. It is likely that ginsenoside Rb₁ was biotransformed into compound K via gastrointestinal microbiota. This biotransformation appears to contribute to the reported pharmacological effects of American ginseng and compound K [25, 26]. The high number of metabolites detected in feces from this study is consistent with our previous *in vitro* human intestinal microflora investigation [16]. In addition, the dehydration process affects the transformation, and metabolites Rk₁ and Rg₅, Rk₃ and Rh₄, Rg₆ and F₄ are generated. The ginseng metabolites reported in this study should be further evaluated for their biological effects.

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Highlights

- American ginseng saponins and their metabolites in human plasma, urine and feces samples after oral administration by LC-Q-TOF-MS
- The ginseng constituents and their metabolites were characterized, including 5 ginseng metabolites in plasma, 10 in urine, and 16 in feces
- The levels of ginsenoside Rb₁ and compound K were determined, and compound K had remarkably high level in all three kinds of biological samples
- Ginsenosides Rk₁ and Rg₅, Rk₃ and Rh₄, Rg₆ and F₄ were detected as the products of dehydration in human feces

Figure 1.

Ginsenosides detected by LC-Q-TOF-MS in the negative ion mode. (A) Total ion chromatogram (TIC) of ginseng saponin standards; (B) TIC of American ginseng root extract; (C) Chemical structures of 45 American ginseng saponins and their metabolites reported in this study.

Figure 2.

LC-Q-TOF-MS analysis of ginsenoside Rb₁ and compound K in human plasma samples. (A) TIC of blank plasma; (B) TIC of a typical plasma sample collected 7 days after oral administration of American ginseng; (C) Extracted ion chromatogram (EIC) of ginsenoside Rb₁ of typical plasma sample; (D) EIC of compound K; (E) Signal intensity of ginsenoside Rb₁ and compound K in six individual subjects representing their relative abundance measured by peak area with a 0.01 Da mass window; (F) Average intensity of ginsenoside Rb₁ and compound K in the six subjects.

Figure 3. LC-Q-TOF-MS analysis of ginsenoside Rb₁ and compound K in human urine samples. (A) TIC of blank urine; (B) TIC of a typical urine sample collected 7 days after oral administration of American ginseng; (C) EIC of ginsenoside Rb₁ of typical urine sample; (D) EIC of compound K; (E) Signal intensity of ginsenoside Rb₁ and compound K in six individual subjects; (F) Average intensity of ginsenoside Rb₁ and compound K in the six subjects.

Figure 4.

LC-Q-TOF-MS analysis of ginsenoside Rb₁ and compound K in human feces samples. (A) TIC of blank feces; (B) TIC of a typical feces sample collected 7 days after oral administration of American ginseng; (C) EIC of ginsenoside Rb₁ of typical feces sample; (D) EIC of compound K; (E) Signal intensity of ginsenoside Rb₁ and compound K in six individual subjects; (F) Average intensity of ginsenoside Rb₁ and compound K in the six subjects.

Figure 5.
Biotransformation pathways of protopanaxadiol ginsenosides by human intestinal microbiota.

LC-MS/MS data in the negative ion mode of compounds identified in plasma samples after oral American ginseng administration (n = 6).

Table 1

No.	Compound	Formula	t _R (min)	Signal Intensity	[M-H] ⁻ or [M+HCOO] ⁻			Fragment ions in the negative mode with the energy 50V CID
					m/z	Calc m/z	Diff (ppm)	
1	Gjnsenoside Rg ₁	C ₄₂ H ₇₂ O ₁₄	15.72	1368	845.4911	845.4904	-0.86	475.3775[M-H-glc-glc] ⁻ , 637.4289[M-H-glc] ⁻ , 799.4854[M-H] ⁻ , 845.4911[M+HCOO] ⁻
2	Gjnsenoside Re	C ₄₈ H ₈₂ O ₁₈	16.01	1643	991.5468	991.5483	1.60	475.3762[M-H-glc-tha-glc] ⁻ , 637.4306[M-H-glc-rha] ⁻ , 783.4892[M-H-glc] ⁻ , 945.5429[M-H] ⁻ , 991.5468[M+HCOO] ⁻
4	Pseudoginsenoside F ₁₁	C ₄₂ H ₇₂ O ₁₄	24.83	1868	845.4896	845.4904	1.01	491.3747[M-H-rha-glc] ⁻ , 653.4282[M-H-rha] ⁻ , 799.4853[M-H] ⁻ , 845.4896[M+HCOO] ⁻
5	Gjnsenoside Rb ₁	C ₃₄ H ₅₂ O ₂₃	26.31	1168	1153.6018	1153.6011	-0.59	459.3818[M-H-glc-glc-glc-glc] ⁻ , 621.4346[M-H-glc-glc-glc] ⁻ , 783.4953[M-H-glc-glc] ⁻ , 945.5435[M-H-glc-glc] ⁻ , 1107.5927[M-H] ⁻ , 1153.6018[M+HCOO] ⁻
7	Ginsenoside Rc	C ₅₃ H ₆₀ O ₂₂	27.97	920	1123.5905	1123.5906	0.07	459.3827[M-H-araf-glc-glc-glc] ⁻ , 621.4335[M-H-araf-glc-glc] ⁻ , 783.4846[M-H-araf-glc] ⁻ , 945.5422[M-H-H] ⁻ , 1123.5905[M+HCOO] ⁻
8	20S-Ginsenoside Rg ₂	C ₄₂ H ₇₂ O ₁₃	28.71	1502	829.4942	829.4955	1.65	475.3714[M-H-rha-glc] ⁻ , 637.4235[M-H-rha] ⁻ , 783.4887[M-H] ⁻ , 829.4942[M+HCOO] ⁻
10	20R-Ginsenoside Rg ₂	C ₄₂ H ₇₂ O ₁₃	29.47	1347	829.4960	829.4955	-0.52	475.3746[M-H-rha-glc] ⁻ , 637.4310[M-H-rha] ⁻ , 783.4896[M-H] ⁻ , 829.4960[M+HCOO] ⁻
11	Gjnsenoside Rb ₂	C ₅₃ H ₆₀ O ₂₂	30.19	830	1123.5921	1123.5906	-1.41	459.3823[M-H-arap-glc-glc-glc] ⁻ , 621.4476[M-H-arap-glc-glc] ⁻ , 783.4954[M-H-arap-glc] ⁻ , 945.5462[M-H-arap] ⁻ , 1077.5878[M-H] ⁻ , 1123.5921[M+HCOO] ⁻
14	Ginsenoside Rb ₃	C ₅₃ H ₆₀ O ₂₂	30.83	547	1123.5933	1123.5906	-2.52	459.3845[M-H-xyt-glc-glc-glc] ⁻ , 621.4252[M-H-xyt-glc-glc] ⁻ , 783.4842[M-H-xyt-glc] ⁻ , 945.5355[M-H-xyt] ⁻ , 1077.5866[M-H] ⁻ , 1123.5933[M+HCOO] ⁻
18	Gjnsenoside Rd	C ₄₈ H ₈₂ O ₁₈	34.96	3383	991.5502	991.5483	-1.99	459.3837[M-H-glc-glc-glc] ⁻ , 621.4441[M-H-glc-glc] ⁻ , 783.4959[M-H-glc] ⁻ , 945.5436[M-H] ⁻ , 991.5502[M+HCOO] ⁻
21	Gypenoside XVII	C ₄₈ H ₈₂ O ₁₈	38.70	2651	991.5471	991.5483	1.29	459.3858[M-H-glc-glc-glc] ⁻ , 621.4347[M-H-glc-glc] ⁻ , 783.4835[M-H-glc] ⁻ , 945.5414[M-H] ⁻ , 991.5471[M+HCOO] ⁻
28	Ginsenoside F ₂	C ₄₂ H ₇₂ O ₁₃	42.77	4990	829.4947	829.4955	1.01	459.3864[M-H-glc-glc] ⁻ , 621.4403[M-H-glc] ⁻ , 783.4882[M-H] ⁻ , 829.4947[M+HCOO] ⁻
33	20S-Ginsenoside Rg ₃	C ₄₂ H ₇₂ O ₁₃	44.75	3864	829.4935	829.4955	2.54	459.3816[M-H-glc-glc] ⁻ , 621.4322[M-H-glc] ⁻ , 783.4874[M-H] ⁻ , 829.4935[M+HCOO] ⁻
34	20R-Ginsenoside Rg ₃	C ₄₂ H ₇₂ O ₁₃	45.06	2771	829.4938	829.4955	2.16	459.3809[M-H-glc-glc] ⁻ , 621.4373[M-H-glc] ⁻ , 783.4862[M-H] ⁻ , 829.4938[M+HCOO] ⁻
42	Compound K	C ₃₆ H ₆₂ O ₈	49.69	11402	667.4420	667.4427	1.08	459.3853[M-H-glc] ⁻ , 621.4362[M-H] ⁻ , 667.4420[M+HCOO] ⁻

All listed compounds are confirmed with reference compounds.

LC-MS/MS data in the negative ion mode of compounds identified in urine samples after oral American ginseng administration (n = 6).

Table 2

No.	Compound	Formula	t _R (min)	Signal Intensity	[M+HCOO] ⁻			Fragment ions in the negative mode with the energy 50V CID
					m/z	Calc m/z	Diff (ppm)	
1	Ginsenoside Rg ₁	C ₄₂ H ₇₂ O ₁₄	15.77	8971	845.4900	845.4904	0.51	475.3736[M-H-glc-glc] ⁻ , 637.4374[M-H-glc] ⁻ , 799.4838[M-H] ⁻ , 845.4900[M+HCOO] ⁻
2	Ginsenoside Re	C ₄₈ H ₈₂ O ₁₈	16.55	14258	991.5470	991.5483	1.39	475.3765[M-H-glc-tha-glc] ⁻ , 637.4357[M-H-glc-rha] ⁻ , 783.4753[M-H-glc] ⁻ , 945.5456[M-H] ⁻ , 991.5470[M+HCOO] ⁻
4	Pseudoginsenoside F ₁₁	C ₄₂ H ₇₂ O ₁₄	24.93	10046	845.4896	845.4904	1.01	491.3739[M-H-rha-glc] ⁻ , 653.4251[M-H-rha] ⁻ , 799.4826[M-H] ⁻ , 845.4896[M+HCOO] ⁻
5	Ginsenoside Rb ₁	C ₅₄ H ₉₂ O ₂₃	26.45	7720	1153.6012	1153.6011	-0.05	459.3826[M-H-glc-glc-glc-glc] ⁻ , 621.4374[M-H-glc-glc-glc] ⁻ , 783.4956[M-H-glc-glc] ⁻ , 945.5421[M-H-glc] ⁻ , 1107.5851[M-H] ⁻ , 1153.6012[M+HCOO] ⁻
7	Ginsenoside Rc	C ₅₃ H ₉₀ O ₂₂	28.15	3103	1123.5878	1123.5906	2.58	459.3842[M-H-araf-glc-glc-glc] ⁻ , 621.4364[M-H-araf-glc-glc] ⁻ , 783.4881[M-H-araf-glc] ⁻ , 945.5363[M-H-araf] ⁻ , 1077.5866[M-H] ⁻ , 1123.5878[M+HCOO] ⁻
8	20S-Ginsenoside Rg ₂	C ₄₂ H ₇₂ O ₁₃	28.76	9731	829.4959	829.4955	-0.52	475.3726[M-H-rha-glc] ⁻ , 637.4272[M-H-rha] ⁻ , 783.4908[M-H] ⁻ , 829.4959[M+HCOO] ⁻
9	20S-Ginsenoside Rh ₁	C ₃₆ H ₆₂ O ₉	29.27	12439	683.4378	683.4376	-0.33	475.3761[M-H-glc] ⁻ , 637.4329[M-H] ⁻ , 683.4378[M+HCOO] ⁻
10	20R-Ginsenoside Rg ₂	C ₄₂ H ₇₂ O ₁₃	29.55	5628	829.4967	829.4955	-1.54	475.3717[M-H-rha-glc] ⁻ , 637.4297[M-H-rha] ⁻ , 783.4880[M-H] ⁻ , 829.4967[M+HCOO] ⁻
11	Ginsenoside Rb ₂	C ₅₃ H ₉₀ O ₂₂	30.24	2746	1123.5892	1123.5906	1.28	459.3834[M-H-araf-glc-glc-glc] ⁻ , 621.4332[M-H-araf-glc-glc] ⁻ , 783.4855[M-H-araf-glc] ⁻ , 945.5244[M-H-araf] ⁻ , 1077.5853[M-H] ⁻ , 1123.5892[M+HCOO] ⁻
13	20R-Ginsenoside Rh ₁	C ₃₆ H ₆₂ O ₉	30.65	10829	683.4383	683.4376	-1.12	475.3749[M-H-glc] ⁻ , 637.4308[M-H] ⁻ , 683.4383[M+HCOO] ⁻
14	Ginsenoside Rb ₃	C ₅₃ H ₉₀ O ₂₂	30.96	2118	1123.5883	1123.5906	2.11	459.3853[M-H-araf-glc-glc-glc] ⁻ , 621.4322[M-H-araf-glc-glc] ⁻ , 783.4890[M-H-araf-glc] ⁻ , 945.5313[M-H-araf] ⁻ , 1077.5870[M-H] ⁻ , 1123.5883[M+HCOO] ⁻
16	Ginsenoside F ₁	C ₃₆ H ₆₂ O ₉	33.97	11073	683.4373	683.4376	0.45	475.3742[M-H-glc] ⁻ , 637.4314[M-H] ⁻ , 683.4373[M+HCOO] ⁻
18	Ginsenoside Rd	C ₄₈ H ₈₂ O ₁₈	35.41	11832	991.5474	991.5483	0.97	459.3878[M-H-glc-glc-glc] ⁻ , 621.4366[M-H-glc-glc] ⁻ , 783.4907[M-H-glc] ⁻ , 945.5487[M-H] ⁻ , 991.5474[M+HCOO] ⁻
21	Gypenoside XVII	C ₄₈ H ₈₂ O ₁₈	38.85	7721	991.5475	991.5483	0.86	459.3867[M-H-glc-glc-glc] ⁻ , 621.4324[M-H-glc-glc] ⁻ , 783.4837[M-H-glc] ⁻ , 945.5479[M-H] ⁻ , 991.5475[M+HCOO] ⁻
28	Ginsenoside F ₂	C ₄₂ H ₇₂ O ₁₃	42.82	13424	829.4962	829.4955	-0.90	459.3842[M-H-glc-glc] ⁻ , 621.4383[M-H-glc] ⁻ , 783.4889[M-H] ⁻ , 829.4962[M+HCOO] ⁻
33	20S-Ginsenoside Rg ₃	C ₄₂ H ₇₂ O ₁₃	44.76	8910	829.4958	829.4955	-0.39	459.3844[M-H-glc-glc] ⁻ , 621.4374[M-H-glc] ⁻ , 783.4909[M-H] ⁻ , 829.4958[M+HCOO] ⁻
34	20R-Ginsenoside Rg ₃	C ₄₂ H ₇₂ O ₁₃	45.08	8627	829.4968	829.4955	-1.66	459.3864[M-H-glc-glc] ⁻ , 621.4336[M-H-glc] ⁻ , 783.4943[M-H] ⁻ , 829.4968[M+HCOO] ⁻
42	Compound K	C ₃₆ H ₆₂ O ₈	49.77	21352	667.4426	667.4427	0.12	459.3838[M-H-glc] ⁻ , 621.4374[M-H] ⁻ , 667.4426[M+HCOO] ⁻
43	20S-Ginsenoside Rh ₂	C ₃₆ H ₆₂ O ₈	50.83	8236	667.4419	667.4427	1.24	459.3851[M-H-glc] ⁻ , 621.4348[M-H] ⁻ , 667.4419[M+HCOO] ⁻
44	20R-Ginsenoside Rh ₂	C ₃₆ H ₆₂ O ₈	51.14	7150	667.4418	667.4427	1.4	459.3848[M-H-glc] ⁻ , 621.4342[M-H] ⁻ , 667.4418[M+HCOO] ⁻

All listed compounds are confirmed with reference compounds.

LC-MS/MS data in the negative ion mode of compounds identified in feces samples after oral American ginseng administration (n = 6).

Table 3

No.	Compound	Formula	t _R (min)	Signal Intensity	[M-H] ⁻ or [M+HCOO] ⁻			Fragment ions in the negative mode with the energy 50V CID
					m/z	Calc m/z	Diff (ppm)	
1	Ginsenoside Rg ₁ ^a	C ₄₂ H ₇₂ O ₁₄	15.88	15449	845.4900	845.4904	0.51	475.3790[M-H-glc-glc] ⁻ ,637.4356[M-H-glc] ⁻ ,799.4848[M-H] ⁻ ,845.4900[M+HCOO] ⁻
2	Ginsenoside Re ^a	C ₄₈ H ₈₂ O ₁₈	16.80	14817	991.5504	991.5483	-2.20	475.3779[M-H-glc-rha-glc] ⁻ ,637.4304[M-H-glc-glc] ⁻ ,783.4816[M-H-glc] ⁻ ,945.5442[M-H] ⁻ ,991.5504[M+HCOO] ⁻
3	Pseudoginsenoside F ₁₁ ^a	C ₄₂ H ₇₂ O ₁₄	24.90	7721	845.4888	845.4904	2.01	491.3763[M-H-rha-glc] ⁻ ,653.4298[M-H-rha] ⁻ ,799.4861[M-H] ⁻ ,845.4888[M+HCOO] ⁻
5	Ginsenoside Rb ₁ ^a	C ₅₄ H ₉₂ O ₂₃	26.44	7843	1153.6021	1153.6011	-0.86	459.3829[M-H-glc-glc-glc-glc] ⁻ ,621.4370[M-H-glc-glc-glc] ⁻ ,783.4956[M-H-glc-glc] ⁻ ,945.5383[M-H-glc] ⁻ ,1107.5965[M-H] ⁻ ,1153.6021[M+HCOO] ⁻
7	Ginsenoside Rc ^a	C ₅₃ H ₉₀ O ₂₂	28.17	11400	1123.5897	1123.5906	0.81	459.3897[M-H-araf-glc-glc-glc] ⁻ ,621.4403[M-H-araf-glc-glc] ⁻ ,783.4724[M-H-araf-glc] ⁻ ,945.5477[M-H-araf] ⁻ ,1077.5869[M-H] ⁻ ,1123.5897[M+HCOO] ⁻
8	20S-Ginsenoside Rg ₂ ^a	C ₄₂ H ₇₂ O ₁₃	28.75	15908	829.4957	829.4955	-0.26	475.3783[M-H-rha-glc] ⁻ ,637.4246[M-H-rha] ⁻ ,783.4904[M-H] ⁻ ,829.4957[M+HCOO] ⁻
9	20S-Ginsenoside Rh ₁ ^a	C ₃₆ H ₆₂ O ₉	29.27	17974	683.4375	683.4376	0.14	475.3728[M-H-glc] ⁻ ,637.4319[M-H] ⁻ ,683.4375[M+HCOO] ⁻
10	20R-Ginsenoside Rg ₂ ^a	C ₄₂ H ₇₂ O ₁₃	29.47	5564	829.4932	829.4955	2.93	475.3767[M-H-rha-glc] ⁻ ,637.4303[M-H-rha] ⁻ ,783.4836[M-H] ⁻ ,829.4932[M+HCOO] ⁻
11	Ginsenoside Rb ₂ ^a	C ₅₃ H ₉₀ O ₂₂	30.33	4529	1123.5880	1123.5906	2.39	459.3847[M-H-arap-glc-glc-glc] ⁻ ,621.4454[M-H-arap-glc-glc] ⁻ ,783.4951[M-H-arap-glc] ⁻ ,945.5495[M-H-arap] ⁻ ,1077.5842[M-H] ⁻ ,1123.5880[M+HCOO] ⁻
130	20R-Ginsenoside Rh ₁ ^a	C ₃₆ H ₆₂ O ₉	30.56	6334	683.4372	683.4376	0.61	475.3751[M-H-glc] ⁻ ,637.4318[M-H] ⁻ ,683.4372[M+HCOO] ⁻
14	Ginsenoside Rb ₃ ^a	C ₅₃ H ₉₀ O ₂₂	31.00	11280	1123.5933	1123.5906	-2.52	459.3858[M-H-xyI-glc-glc-glc] ⁻ ,621.4272[M-H-xyI-glc-glc] ⁻ ,783.4911[M-H-xyI-glc] ⁻ ,945.5347[M-H-xyI] ⁻ ,1077.5870[M-H] ⁻ ,1123.5933[M+HCOO] ⁻
16	Ginsenoside F ₁ ^a	C ₃₆ H ₆₂ O ₉	33.93	6027	683.4387	683.4376	-1.74	475.3799[M-H-glc] ⁻ ,637.4332[M-H] ⁻ ,683.4387[M+HCOO] ⁻
18	Ginsenoside Rd ^a	C ₄₈ H ₈₂ O ₁₈	35.43	22428	991.5476	991.5483	0.76	459.3862[M-H-glc-glc-glc] ⁻ ,621.4374[M-H-glc-glc] ⁻ ,783.4892[M-H-glc] ⁻ ,945.5422[M-H] ⁻ ,991.5476[M+HCOO] ⁻
21	Gypenoside XVII ^a	C ₄₈ H ₈₂ O ₁₈	38.83	6173	991.5471	991.5483	1.29	459.3844[M-H-glc-glc-glc] ⁻ ,621.4358[M-H-glc-glc] ⁻ ,783.4846[M-H-glc] ⁻ ,945.5415[M-H] ⁻ ,991.5471[M+HCOO] ⁻
23	Compound Mc-1	C ₄₇ H ₈₀ O ₁₇	40.02	12339	961.5400	961.5378	-2.45	459.3781[M-H-araf-glc-glc] ⁻ ,621.4344[M-H-araf-glc] ⁻ ,783.4760[M-H-araf] ⁻ ,915.5333[M-H] ⁻ ,961.5400[M+HCOO] ⁻
25	Compound O	C ₄₇ H ₈₀ O ₁₇	40.44	7322	961.5360	961.5378	1.91	459.3812[M-H-arap-glc-glc] ⁻ ,621.4434[M-H-arap-glc] ⁻ ,783.4992[M-H-arap] ⁻ ,915.5314[M-H] ⁻ ,961.5360[M+HCOO] ⁻
26	Gypenoside IX	C ₄₇ H ₈₀ O ₁₇	40.96	7806	961.5373	961.5378	0.50	459.3847[M-H-xyIp-glc-glc] ⁻ ,621.4425[M-H-xyIp-glc] ⁻ ,783.4960[M-H-xyIp] ⁻ ,915.5321[M-H] ⁻ ,961.5373[M+HCOO] ⁻
27	Ginsenoside Rg ₆	C ₄₂ H ₇₀ O ₁₂	42.62	5318	811.4854	811.4849	-0.61	457.3683[M-H-rha-glc] ⁻ ,619.4237[M-H-rha] ⁻ ,765.4799[M-H] ⁻ ,811.4854[M+HCOO] ⁻
28	Ginsenoside F ₂ ^a	C ₄₂ H ₇₂ O ₁₃	42.80	28340	829.4965	829.4955	-1.28	459.3863[M-H-glc-glc] ⁻ ,621.4354[M-H-glc] ⁻ ,783.4913[M-H] ⁻ ,829.4965[M+HCOO] ⁻
29	Ginsenoside F ₄	C ₄₂ H ₇₀ O ₁₂	43.14	13384	811.4840	811.4849	1.21	457.3713[M-H-rha-glc] ⁻ ,619.4178[M-H-rha] ⁻ ,765.4784[M-H] ⁻ ,811.4840[M+HCOO] ⁻
30	Ginsenoside Rk ₃ ^a	C ₃₆ H ₆₀ O ₈	43.72	7882	665.4276	665.4270	-0.93	457.3774[M-H-glc] ⁻ ,619.4224[M-H] ⁻ ,665.4276[M+HCOO] ⁻

No.	Compound	Formula	t _R (min)	Signal Intensity	[M-H] ⁻ or [M+HCOO] ⁻			Fragment ions in the negative mode with the energy 50V CID
					m/z	Calc. m/z	Diff (ppm)	
31	Zingibroside R ₁	C ₄₂ H ₆₆ O ₁₄	44.26	23676	793.4371	793.4380	1.11	455.3586[M-H-glc-glc-acid] ⁻ ,631.4345[M-H-glc] ⁻ ,793.4371[M-H] ⁻
322	Ginsenoside Rh ₄ ^a	C ₃₆ H ₆₀ O ₈	44.31	22888	665.4281	665.4270	-1.74	457.3763[M-H-glc] ⁻ ,619.4224[M-H] ⁻ ,665.4281[M+HCOO] ⁻
33	20S-Ginsenoside Rg ₃ ^a	C ₄₂ H ₇₂ O ₁₃	44.74	93907	829.4978	829.4955	-2.94	459.3885[M-H-glc-glc] ⁻ ,621.4336[M-H-glc] ⁻ ,783.4913[M-H] ⁻ ,829.4978[M+HCOO] ⁻
34	20R-Ginsenoside Rg ₃ ^a	C ₄₂ H ₇₂ O ₁₃	45.08	50189	829.4970	829.4955	-1.92	459.3845[M-H-glc-glc] ⁻ ,621.4380[M-H-glc] ⁻ ,783.4909[M-H] ⁻ ,829.4970[M+HCOO] ⁻
35	Compound Mc	C ₄₁ H ₇₀ O ₁₂	45.95	96372	799.4862	799.4849	-1.68	459.3871[M-H-araf-glc] ⁻ ,621.4367[M-H-araf] ⁻ ,753.4807[M-H] ⁻ ,799.4862[M+HCOO] ⁻
36	Compound Y	C ₄₁ H ₇₀ O ₁₂	46.28	16440	799.4841	799.4849	1.10	459.3852[M-H-arap-glc] ⁻ ,621.4384[M-H-arap] ⁻ ,753.4788[M-H] ⁻ ,799.4841[M+HCOO] ⁻
37	Compound Mx	C ₄₁ H ₇₀ O ₁₂	46.43	43523	799.4875	799.4849	-3.41	459.3850[M-H-ylp-glc] ⁻ ,621.4544[M-H-ylp] ⁻ ,753.4818[M-H] ⁻ ,799.4875[M+HCOO] ⁻
38	Protopanaxatriol ^a	C ₃₀ H ₅₂ O ₄	47.69	200054	521.3852	521.3848	-0.92	475.3798[M-H] ⁻ ,521.3852[M+HCOO] ⁻
39	Calenduloside E	C ₃₆ H ₅₆ O ₉	47.78	16112	631.3847	631.3852	0.72	455.3558[M-H-glc-acid] ⁻ ,631.3847[M-H] ⁻
40	Ginsenoside Rk ₁ ^a	C ₄₂ H ₇₀ O ₁₂	48.75	19950	811.4861	811.4849	-1.53	441.3746[M-H-glc-glc] ⁻ ,603.4284[M-H-glc] ⁻ ,765.4808[M-H] ⁻ ,811.4861[M+HCOO] ⁻
41	Ginsenoside Rg ₅ ^a	C ₄₂ H ₇₀ O ₁₂	49.20	54581	811.4862	811.4849	-1.66	441.3782[M-H-glc-glc] ⁻ ,603.4275[M-H-glc] ⁻ ,765.4810[M-H] ⁻ ,811.4862[M+HCOO] ⁻
42	Compound K ^a	C ₃₆ H ₆₂ O ₈	49.77	1964307	667.4429	667.4427	-0.37	459.3841[M-H-glc] ⁻ ,621.4375[M-H] ⁻ ,667.4429[M+HCOO] ⁻
43	20S-Ginsenoside Rh ₂ ^a	C ₃₆ H ₆₂ O ₈	50.79	14763	667.4431	667.4427	-0.69	459.3832[M-H-glc] ⁻ ,621.4378[M-H] ⁻ ,667.4431[M+HCOO] ⁻
444	20R-Ginsenoside Rh ₂ ^a	C ₃₆ H ₆₂ O ₈	51.10	10737	667.4435	667.4427	-1.33	459.3844[M-H-glc] ⁻ ,621.4381[M-H] ⁻ ,667.4435[M+HCOO] ⁻
45	Protopanaxadiol	C ₃₀ H ₅₂ O ₃	62.57	349343	505.3904	505.3898	-1.20	459.3850[M-H] ⁻ ,505.3904[M+HCOO] ⁻

^aConfirmed with reference compounds. Others were tentatively identified based on previous reports [23, 24].