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# Rapid qualitative and quantitative analyses of Asian ginseng in adulterated American ginseng preparations by UPLC/Q-TOF-MS

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ABSTRACT

A new method using ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC/Q-TOF-MS) was developed for the rapid qualitative and quantitative analyses of Asian ginseng (*Panax ginseng C.A.Meyer*) in adulterated American ginseng (*Panax quinquefolium* L.) preparations within 2 min. The method was based on the baseline chromatographic separation of isomeric compounds of ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$, two potential chemical markers present in *Panax ginseng C.A.Meyer* and *P. quinquefolium* L. methanolic extracts.

The chromatographic separation was achieved by UPLC, which used a column with 1.7 μm particle packing which enabled the higher peak capacity, greater resolution, increased sensitivity and higher speed of analysis. Ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$ were separated on baseline with retention times of 1.5 and 1.7 min, respectively. Ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$ were identified and conformed unambiguously by accurate mass measurement and their different fragmentation pathways were performed on Q-TOF-MS. Quantitative analysis was carried out under selective ion monitoring (SIM) mode. The limit of detection (LOD) of this UPLC/Q-TOF-MS analysis for ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$ was 0.05 and 0.08 ng, respectively. Ginsenoside Rf was linear over the range of 0.164–16.4 ng with a correlation coefficient ($R^2$) of 0.9997, while 24(R)-pseudoginsenoside $F_{11}$ was linear from 0.243 to 24.3 ng with an $R^2$ of 0.9989. Furthermore, inter-day and intra-day precisions were obtained below 4.0% and the analytical method was fully validated. 12 batches of self-prepared adulterated samples, 11 batches of Asian ginseng, 16 batches of American ginseng and 13 batches of commercial American ginseng preparations were tested.

The method developed is rapid, accurate, reliable and highly sensitive for qualitative and quantitative analyses of Asian ginseng and American ginseng.



## 1. Introduction

American ginseng is the dried root of *Panax quinquefolium* L., which is "cool" and mainly used to tonify "*qi*" and nourish "*yin*", which means removing "heat" and promoting the production of body fluids [1]. Asian ginseng is the dried root of *Panax ginseng C.A.Meyer*, as is known to reinforce "*qi*" and benefiting the spleen and lung. They show different properties and medicinal values in pharmacology, even though the major bioactive ingredients of Asian ginseng and American ginseng are ginsenosides. In the ginseng market, American ginseng is more expensive than Asian ginseng. Asian ginseng recently has been found illegally mixed in American ginseng and its preparations. A rapid method for precise analyzing Asian ginseng mixed in American ginseng is therefore highly desirable.

As characters, morphology and chemical composition of Asian ginseng and American ginseng are very similar, the traditional methods based on morphological, histological and physicochemical characteristics for identification of these two species are not easy [2]. And the contents of $Rg_1$, $Rb_1$ and Re should be quantified both in American ginseng and Asian ginseng in Pharmacopoeia of the People's Republic of China (2005, vol. I) [1]. Since there is no effective standard to control the adulterant situation, the detection of Asian ginseng adulterated in preparations of American ginseng is even more difficult.

Ginsenosides are known to be the major bioactive components of Asian ginseng and American ginseng. Research studies have demonstrated that ginseng can stimulate nervous system and process anti-oxidative, anti-diabetic, and anti-cancer activities [3]. Up to now more than 30 ginsenosides have been isolated and identified according to the reports [4–6]. Ginsenosides $Rb_1$, $Rb_2$, Rc,

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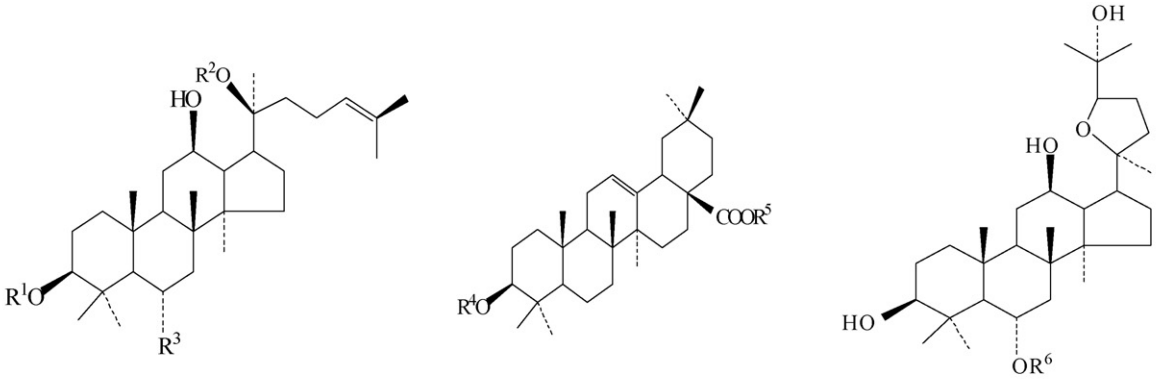


| Ginsenosides | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ | $R^6$ | Formula | MW |
|---|---|---|---|---|---|---|---|---|
| Rb1 | -glc2 1glc | -glc6 1glc | —— | —— | —— | —— | $C_{54}H_{92}O_{23}$ | 1108 |
| Rb2 | -glc2 1glc | -glc6 1ara(p) | —— | —— | —— | —— | $C_{53}H_{90}O_{22}$ | 1078 |
| Rc | -glc2 1glc | -glc6 1ara(f) | —— | —— | —— | —— | $C_{53}H_{90}O_{22}$ | 1078 |
| Rd | -glc2 1glc | -glc | —— | —— | —— | —— | $C_{48}H_{82}O_{18}$ | 946 |
| $Rg_1$ | -H | -glc | -O-glc | —— | —— | —— | $C_{42}H_{72}O_{14}$ | 800 |
| Re | -H | -glc | -O-glc2 1rha | —— | —— | —— | $C_{48}H_{82}O_{18}$ | 946 |
| Rf | -H | -H | -O-glc2 1glc | —— | —— | —— | $C_{42}H_{72}O_{14}$ | 800 |
| Ro | —— | —— | | -glcUA2 1glc | -glc | —— | $C_{48}H_{76}O_{19}$ | 956 |
| $F_{11}$ | —— | —— | —— | —— | —— | -glc2 1rha | $C_{42}H_{72}O_{14}$ | 800 |

glc:β-D-glucos; ara(p):α-L-arabinopyranose; ara(f): α-L-arabinofuranose; rha: α-L-rhamnose; glcUA: β-Dglucuronide

**Fig. 1.** Structure information of ginsenosides.

Rd, $Rg_1$, Re, Rf, Ro and 24(R)-pseudoginsenoside $F_{11}$ (Fig. 1) are the most important compounds in chemical analysis of ginsengs. Among these ginsenosides, ginsenoside Rf can only be found in Asian ginseng, while 24(R)-pseudoginsenoside $F_{11}$ are only present in American ginseng [7–9]. Chromatographic methods like thin layer chromatography (TLC) [10] and high performance liquid chromatography (HPLC) [11–13] for test of ginseng samples have been established based on analysis of the two isomeric compounds. These methods suffer from low resolution, low sensitivity and long analytical time. With its superior performances in sensitivity and selectivity, HPLC-MS-MS has been widely applied for ginsenosides research. Several methods of differentiation of Asia ginseng and American ginseng by LC-MS have been developed [9,14,15]. Since Rf and $F_{11}$ have the same molecular formula, good selectivity of LC-MS/MS method is crucial for separating and distinguishing both ginsenosides. The multiple reaction monitoring (MRM) in triple quadrupole mass spectrometer used for quantitative analysis of $F_{11}$ and Rf can obtain good linear response [16]. However, using liquid chromatography with triple quadrupole mass spectrometry (LC-QqQ-MS) under MRM mode the qualitative information is not enough to elucidate the structure. Though in the full-scan mode this information can be obtained, the lack of sensitivity and the lack of compound databases and mass-spectral libraries may be obstructive to the qualitative analysis. Q-TOF-MS provides several advantages in structural analysis, which employs a time-flight mass spectrometry technology for the accurate mass measurement determination of chemical compounds. Three pairs of ginsenoside isomers (ginsenoside $Rg_2$ and $Rg_3$, $Rg_1$ and 24(R)-pseudoginsenoside $F_{11}$ as well as ginsenoside Rd and Re) were differentiated and identified through accurate mass measurement [17]. The advantages of its higher resolution and the accuracy in mass measurements make Q-TOF-MS a powerful tool for identification of the analyte. In this work the possibility of quantitative analysis by Q-TOF-MS using selective ion monitoring (SIM) was also explored.

A novel approach to chromatographic separation is ultra-performance liquid chromatography (UPLC) [18,19], which is based on the use of columns with smaller packing (1.7 μm particle) and operated at higher pressures (up to 15,000 psi). Compared with traditional HPLC, UPLC provides a higher peak capacity, greater resolution, increased sensitivity and higher speed of analysis. When coupled to orthogonal quadrupole time-of-flight mass spectrometry, it has been introduced in the separation of complicated samples such as the field of metabonomics, drug metabolism and traditional Chinese medicines. For instance, Xie et al. [20] used UPLC-ToF MS to study metabolite profiling of five Panax herbs and 25 saponins were identified by the accurate mass.

The objective of the work was to develop and validate a rapid method for quantitative and qualitative analyses of Asian gin-

**Table 1**
Results of validation of the method.

| Analyte | Results | |
|---|---|---|
| | Ginsenoside Rf | 24(R)-pseudoginsenoside $F_{11}$ |
| Linear range | 0.164–16.4 ng | 0.243–24.3 ng |
| | $y = 8.7416x + 1.8629$ ($R^2 = 0.9997$) | $y = 8.2405x + 2.1062$ ($R^2 = 0.9989$) |
| LOD | 0.05 ng | 0.08 ng |
| LOQ | 0.164 ng | 0.243 ng |
| Intra-precision | RSD = 3.87% ($n = 5$) | RSD = 2.34% ($n = 5$) |
| Inter-precision | RSD = 4.00% ($n = 6$) | RSD = 1.08% ($n = 6$) |
| Recovery | 97.2% (RSD = 4.39%, $n = 6$) | 93.8% (RSD = 4.61, $n = 6$) |

seng mixed in American ginseng preparations by UPLC/Q-TOF. The developed method was later used for analysis of commercial Asian ginseng, American ginseng and their preparations.

## 2. Experimental

### 2.1. Chemicals, standards and samples

Acetonitrile (HPLC grade) and tetrahydrofuran (HPLC grade) were purchased from Fisher (USA); formic acid was obtained from Fluka (Buchs, Switzerland). Ultra-pure water (18.2 MΩ) was prepared with a Milli-Q water purification system (Millipore, France).

Ginsenoside Rf standard was purchased from Extrasynthese (France, batch number 03091112); 24(R)-pseudoginsenoside $F_{11}$ was obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China, batch number 841-9903).

11 batches of Asian ginseng (marked as sample 1–11) and 16 batches of American ginseng (marked as sample 12–27) originated in China, Canada, and the USA were collected from herbal markets. 13 batches of American ginseng preparations (marked as sample 28–40) were purchased from the local drug stores.

### 2.2. UPLC conditions

UPLC was performed on a Waters Acquity UPLC system, equipped with a binary solvent delivery system, an autosampler and a tunable UV (TUV) detector. Chromatographic separation was performed on a ACQUITY UPLC™ BEH $C_{18}$ column (50 mm × 2.1 mm i.d., 1.7 μm, Waters, USA) at 30 °C. The two mobile phases were phase A: water–formic acid (100:0.2, v/v); phase B: acetonitrile–tetrahydrofuran (10:1, v/v), and the proportion of B was kept 30% for 2 min. The flow rate was kept at 0.4 mL/min and 5 μL of sample solution was injected in each run.

### 2.3. Q-TOF MS conditions

The mass spectrometry was performed on a quadrupole orthogonal acceleration time-of-flight tandem mass spectrometer (Waters Q-TOF Premier™). The nebulizer gas was set to 600 L/h at a temperature of 350 °C under negative ion mode. The cone gas was set to a flow rate of 50 L/h, and the source temperature was set to 120 °C. The capillary voltages were set to 2.4 kV, and the cone voltages were set to 15 V. The TOF data being collected between $m/z$ 250 and 1000 with low collision energy of 5 V for quantitative analysis and the MS/MS experiments were performed using high collision energy of 45 V for fragment ion information.

All analyses were acquired using an independent reference spray via the Lock Spray interference to ensure accuracy and reproducibility. The $[M-H]^-$ ion of leucine enkephalin ($m/z$ 554.2615) at a concentration 200 pg/mL at an infusion flow rate of 0.03 mL/min was used as the lock mass. The Lock Spray frequency was set at 10 s, and data for the reference compound were averaged over 10 spectra/min.

The Q-TOF Premier™ was operated in V mode with resolution over 8500 mass with 0.3 s scan time, and 0.02 s inter-scan delay. The accurate mass and composition for the precursor ions and for the fragment ions were calculated using the MassLynx 4.1 software incorporated in the instrument.

### 2.4. Preparation of samples and standard solutions

#### 2.4.1. Sample preparation

0.5 g of dried sample ground powder (0.30–0.45 mm) was accurately weighed into a 100 mL conical flask with stopper, and 70% aqueous methanol 50 mL was accurately added. Sonicate (44 kHz, 250 W) at room temperature for 20 min. Then the same solvent was added to compensate for the lost weight during the extraction. The

**Table 2**
Quantitative test of self-prepared samples ($n = 3$).

| No. | American ginseng, weight (g) ($F_{11}$ 987.7 μg/g) | Asian ginseng, weight (g) (Rf 475.6 μg/g) | Actual proportion (%) (Self-prepared) | Tested pseudo-ginsenoside $F_{11}$ (μg) | Tested ginsenoside Rf (μg) | Tested American ginseng (g) | Tested Asian ginseng (g) | Tested proportion[a] (%) | Absolute deviation (%) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.19820 | 0.00258 | 1.28 | 156.01 | 1.27 | 0.15795 | 0.0027 | 1.66 | 0.38 |
| 2 | 0.19898 | 0.00289 | 1.43 | 159.32 | 2.00 | 0.16130 | 0.0042 | 2.54 | 1.11 |
| 3 | 0.19081 | 0.01087 | 5.39 | 150.07 | 4.93 | 0.15193 | 0.0104 | 6.38 | 0.99 |
| 4 | 0.18983 | 0.01034 | 5.17 | 156.21 | 5.55 | 0.15815 | 0.0117 | 6.87 | 1.71 |
| 5 | 0.18005 | 0.02051 | 10.23 | 142.74 | 9.75 | 0.14452 | 0.0205 | 12.42 | 2.20 |
| 6 | 0.18033 | 0.02123 | 10.53 | 150.46 | 8.44 | 0.15233 | 0.0178 | 10.44 | −0.09 |
| 7 | 0.14030 | 0.05985 | 29.90 | 119.72 | 27.13 | 0.12121 | 0.0570 | 32.00 | 2.10 |
| 8 | 0.14015 | 0.06089 | 30.29 | 119.80 | 28.64 | 0.12128 | 0.0602 | 33.17 | 2.89 |
| 9 | 0.10019 | 0.10078 | 50.15 | 93.14 | 44.68 | 0.09430 | 0.0939 | 49.91 | −0.24 |
| 10 | 0.10087 | 0.09974 | 49.72 | 92.06 | 42.73 | 0.09321 | 0.0898 | 49.08 | −0.64 |
| 11 | 0.05008 | 0.15029 | 75.01 | 52.17 | 63.47 | 0.05282 | 0.1335 | 71.64 | −3.36 |
| 12 | 0.05062 | 0.15209 | 75.03 | 53.47 | 63.59 | 0.05413 | 0.1337 | 71.18 | −3.85 |

[a] Tested proportion = tested Rf(μg)/((tested $F_{11}$(μg)/987.7 + tested Rf(μg)/475.6) × 475.6) × 100%.

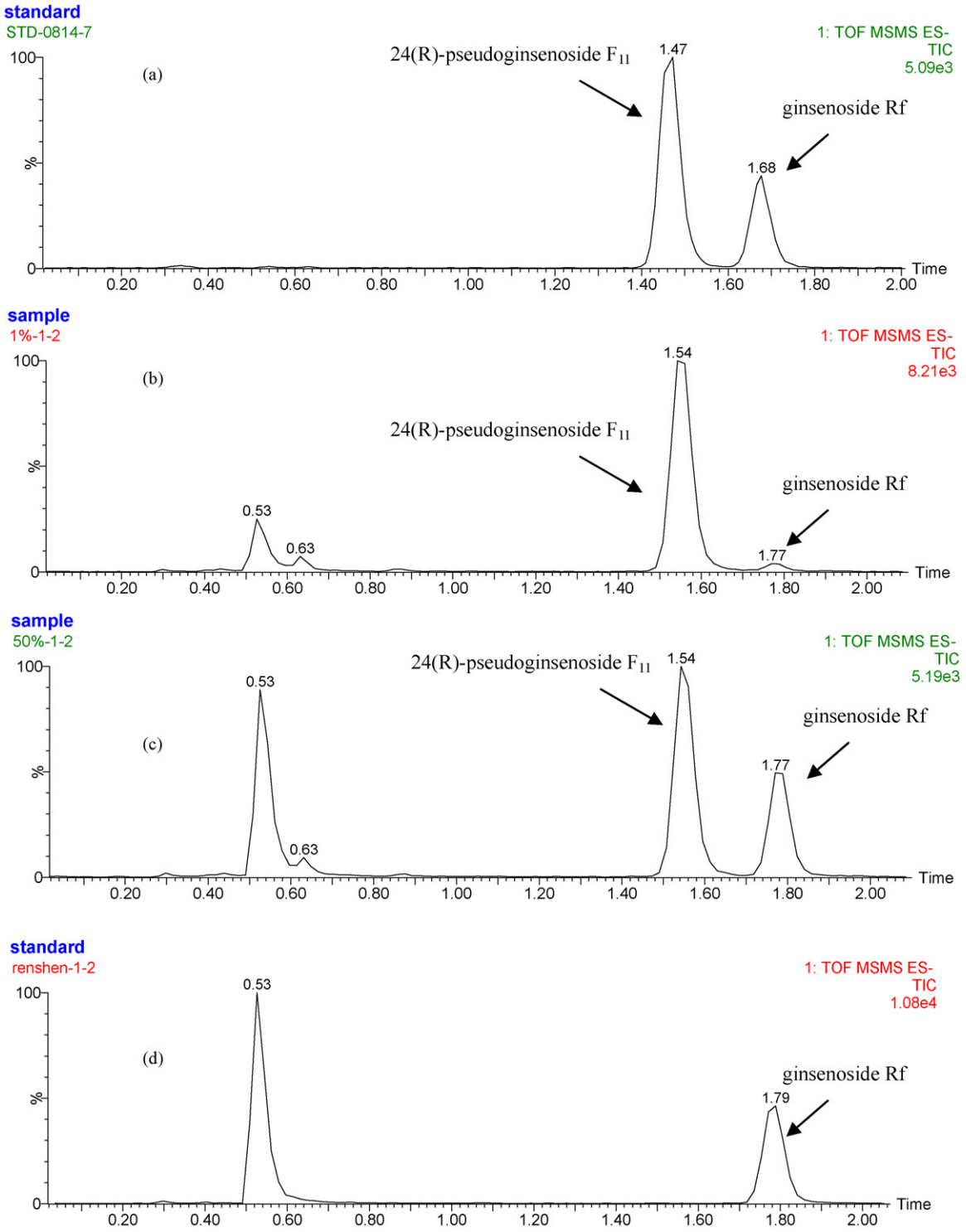


**Fig. 2.** The total ion chromatograms. (a) TIC of ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$ standards (*m/z* 835); (b) TIC of American ginseng preparation adulterated 1% Asian ginseng (*m/z* 835); (c) TIC of American ginseng preparation adulterated 50% Asian ginseng (*m/z* 835); (d) TIC of Asian ginseng (*m/z* 835).

fluid was filtered through a 0.22 μm membrane and injected into the UPLC/Q-TOF-MS system for analysis.

### 2.4.2. Reference solution

The standard stock solutions containing ginsenoside Rf at 164 μg/mL and 24(R)-pseudoginsenoside $F_{11}$ at 243 μg/mL were prepared in methanol. The stock solutions were appropriately diluted to prepare a series of standard working solutions, and then stored at −4 °C. The solutions were brought to room temperature and filtered through a 0.22 μm membrane filter before UPLC/Q-TOF-MS analysis.

## 2.5. Validation of the method

### 2.5.1. Calibration curves

External calibration method was used for the quantitative analysis. Methanol standard stock solution of mixture containing ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$ was prepared and

diluted to appropriate concentrations for the establishment of calibration curves. Each concentration of the mixed-standard solution was injected and analyzed by UPLC/Q-TOF-MS. All measurements were repeated in triplicate and data were processed by MassLynx software (Version 4.1). The calibration curves were constructed by plotting peak areas versus concentrations. The linearity for the investigated compounds can be seen in Table 1.

*2.5.2. Limits of detection and quantification*

The standard stocks were diluted with methanol to appropriate concentrations, and 5 μL of the diluted solutions were injected into UPLC/Q-TOF-MS for analysis. The LOD and LOQ for each analyte were determined at a signal-to-noise ratio (S/N) of about 3 and 10, respectively. Table 1 shows the chromatograms of LOD and LOQ.

*2.5.3. Precision and accuracy*

The precision of the UPLC/Q-TOF-MS method was evaluated by intra- and inter-day variations. 0.5 g of dried sample powder was extracted and analyzed as described in Sections 2.2 and 2.3. The intra-day precision was performed by analysis of the standard solution at five times within one day, while the inter-day precision was determined by repeated analysis of the sample for consecutive six days. Table 1 shows the precision variations.

The recovery test was carried out to evaluate the accuracy of the method. Accurate amounts of ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$ were spiked into 0.5 g of American ginseng preparation sample powders and then extracted and analyzed as described in Sections 2.2 and 2.3. The average recoveries were determined by the following formula: Recovery (%) = (Observed amount − Original amount)/Spiked amount × 100%, RSD (%) = (SD/mean) × 100%.

The mean recoveries of ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$ were 97.2% and 93.8%, respectively (Table 1). The results indicated that this UPLC/Q-TOF-MS method was rapid, precise, accurate and sensitive for quantitative determination of ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$ in American ginseng preparations.

*2.5.4. Quantitative test of self-prepared samples*

Once the method has been validated, a series of self-prepared samples with different contents were used to test the method. The self-prepared samples were prepared by spiking the Asian ginseng powder (containing ginsenoside Rf c.a. 475.62 μg/g) into the American ginseng powder (containing pseudoginsenoside $F_{11}$ c.a. 987.72 μg/g) by various proportions. The samples with Asian ginseng proportion of 1%, 5%, 10%, 30%, 50% and 75% were extracted and analyzed as described above. The tested contents of ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$ in samples were obtained and the proportions were calculated accordingly. Comparing the tested proportion of Asian ginseng with the real proportion of the mixed sample, it can be concluded that the quantitative method in our study is applicable from the results in Table 2. The total ion chromatograms were shown in Fig. 2.

## 3. Results and discussion

### 3.1. Optimization of UPLC conditions

The chromatographic separation was provided by UPLC, which is based on the use of column (ACQUITY UPLC™ BEH $C_{18}$, 50 mm × 2.1 mm i.d.) with 1.7 μm particle packing. According to Van Deemter equation that describes the relationship between linear velocity and column efficiency the optimal operating linear velocity for 2.1 mm column can be achieved at flow rates of 400–1000 μL/min [21]. Meanwhile, considering the best electrospray ionization status and ion suppression which negatively affects several analytical merits, such as detection capability, sensitivity, precision, and accuracy, the ultimate flow rate was optimized at 0.4 mL/min in this study.

Several previous studies using HPLC method [12–16] suggested that acetonitrile–water or acetonitrile–ammonium acetate as mobile phases could be a good choice. In this study, a series of preliminary experiments was carried on different mobile phases including acetonitrile/water, methanol/water, acetonitrile/ammonium acetate and methanol/0.2% formic acid. The best separation of ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$ was obtained when using 30% of 0.2% formic acid and 70% acetonitrile/tetrahydrofuran (10:1, v/v) by isocratic elution. Under the selected UPLC conditions, the resolution between ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$ was more than 1.5 (Fig. 2).

The tetrahydrofuran (THF)/acetonitrile mobile phase system can offer different selectivities. According to Snyder and Kikland [22], THF was categorized as a Type II solvent on reversed phase chromatography while both acetonitrile and methanol were Type I solvents. Austin et al. [23] used THF/water mobile phases and achieved target selectivity for compounds with diverse nature of physicochemical properties. In this study, the effects of THF with various percentages in acetonitrile were evaluated. It is found that with the proportion of THF less than 10%, ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$ were not completely separated; while, when the proportion of THF more than 10%, good resolution was again not obtained and analysis time was prolonged. Thus in this work tetrahydrofuran/acetonitrile (1:10, v/v) was used and the run time was 2 min, which was a fortyfold reduction in analysis time compared to conventional HPLC (80 min run) [12].

### 3.2. Selection of MS conditions

All factors related with MS performance including ionization mode, collision energy, gas flow, desolvation temperature and mobile phase additives have been experimented. The infusion of reference compounds ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$ showed that the negative ion mode was more sensitive than positive ion mode. The desolvation gas flow was set at 600 L/h and desolvation temperature at 350 °C, respectively. In addition, the effect of acid modifier (e.g., formic acid) on the ionization of ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$ was also investigated. It is found that if the concentration of formic acid is too high the peak intensity will quickly decrease and 0.2% (v/v) formic acid in mobile phase B was finally used to improve ionization of chemicals.

In this study selective ion monitoring (SIM) was used for quantitative analysis and the intensity of the molecular ion peak was further considered. The test of cone voltage with the range from 15 to 45 V was performed and the collision energy was set at 5 V. The results indicated that a cone voltage of 15 V was sufficient to produce molecular ions in ESI-mode for ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$. The fragmentation MS/MS, however, need a higher collision energy to perform collision induced dissociation (CID) of parent ions of ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$ ($m/z$ 835) and acquired daughter ions for structure interpretations purposes. A higher collision energy of 45 V was selected to obtain higher response intensities of product ions $m/z$ 653, $m/z$ 491 from 24(R)-pseudoginsenoside $F_{11}$ ($m/z$ 835) and product ions $m/z$ 637, $m/z$ 475 from ginsenoside Rf ($m/z$ 835).

### 3.3. Q-TOF confirmation

One of superiorities of Q-TOF is to make mass measurement with great accuracy feasible. It easily gets accuracy level below 5 ppm for small molecules. All data were acquired using leucine–enkephalin ($m/z$ = 554.2615) as reference ions via the Lock Spray interface. The

**Table 3**
Accurate mass measurement and product ions of the ginsenosides.

| Compound | Experimental mass (*m*/*z*) | Theoretical mass (*m*/*z*) | Error (ppm) | Product ions (*m*/*z*) |
|---|---|---|---|---|
| Ginsenoside Rf | 835.4633 | 835.4611 | 2.6 | 637[M-Glc-H]-<br>475[M-2Glc-H]- |
| 24(R)-pseudoginsenoside $F_{11}$ | 835.4637 | 835.4611 | 3.1 | 653[M-Rha-H]-<br>491[M-Rha-Glc-H] |

**Table 4**
Contents of Rf in Asian ginseng and $F_{11}$ in American ginseng.

| Sample NO. | Purchase from | Rf (μg/g) | Sample No. | Producing area | $F_{11}$ (μg/g) |
|---|---|---|---|---|---|
| 1 | Tongren Tang | 631.93 | 12 | Canada | 1021.84 |
| 2 | Jinyu Jilin | 267.89 | 13 | Wendeng | 1062.42 |
| 3 | Huanren Liaoning | 529.14 | 14 | Beijing, China | 827.28 |
| 4 | Fusong Jilin | 266.51 | 15 | Tonghua, Jilin | 727.51 |
| 5 | Zengfuwang | 611.65 | 16 | Yedian, Jilin | 689.71 |
| 6 | Jian Jilin | 520.09 | 17 | Laiyang, Shandong | 1313.61 |
| 7 | Yurensheng | 416.32 | 18 | China | 1100.11 |
| 8 | Antu Jilin | 563.20 | 19 | Ontario, Canada | 984.76 |
| 9 | Fusong Jilin | 485.64 | 20 | China | 978.62 |
| 10 | Dongfanghong | 770.44 | 21 | Wisconsin, America | 1171.11 |
| 11 | Beijing | 475.62 | 22 | Wisconsin, America | 1096.64 |
| Mean content | | 503.49 | 23 | Ontario, Canada | 1211.66 |
| | | | 24 | Wisconsin, America | 987.72 |
| | | | 25 | British, Columbia, Canada | 862.27 |
| | | | 26 | Wisconsin, America | 1097.65 |
| | | | 27 | British, Columbia, Canada | 787.06 |
| | | | Mean content | | 995.00 |

**Table 5**
Results of Asian ginseng in adulterated American preparations.

| Sample No. | Bathes No. | $F_{11}$ (μg/g) | Rf (μg/g) | Asian ginseng (%)[a] |
|---|---|---|---|---|
| 28 | 071201 | 50.45 | 10.01 | 16.56 |
| 29 | 080103 | 487.93 | 23.05 | 4.51 |
| 30 | 20070501 | 509.81 | 18.63 | 6.73 |
| 31 | 20071201 | 557.09 | 12.36 | 4.20 |
| 32 | 070709 | 369.47 | 0 | 0 |
| 33 | 070224 | 181.40 | 0 | 0 |
| 34 | 20071022 | 360.63 | 36.58 | 16.70 |
| 35 | 0711011 | 1123.53 | 213.53 | 27.30 |
| 36 | 20071218 | 298.10 | 7.80 | 4.92 |
| 37 | 1040371 | 611.44 | 0 | 0 |
| 38 | 311027 | 1241.58 | 0 | 0 |
| 39 | 07AA01 | 407.22 | 177.24 | 46.24 |
| 40 | 20060902 | 177.77 | 0 | 0 |

[a] Asian ginseng (%) = Rf (μg)/((($F_{11}$(μg)/995.00 + Rf(μg)/503.49) × 503.49) × 100%.

raw data files were then processed by MassLynx 4.1, which provided the elemental compositions and the mass errors. Generally, a mass error below 5 ppm is regarded as acceptable certainty. Molecular formula $C_{42}H_{72}O_{14}Cl$ was then determined as seen in Table 3. These results showed that the molecular ion containing $[M+Cl]^-$ adducts.

Once the same molecular formula was confirmed, the further structure elucidation of the pair of ginsenoside isomer (ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$) was needed to carry out. CID mass spectra of $[M+Cl]^-$ (*m*/*z* 835.46) provided different fragment ion informations of ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$, which can be seen in Table 3.

The main fragment ions of ginsenoside Rf were seen at *m*/*z* 637 and *m*/*z* 475. The peak of *m*/*z* 637 corresponds to the loss of a glucosyl group while the *m*/*z* 475 corresponds to the consecutive loss of two glucoses. Under the same analytic conditions two major product ions of 24(R)-pseudoginsenoside $F_{11}$ at *m*/*z* 653 and *m*/*z* 491 were also detected. The fragment of *m*/*z* 653 was considered to derive from the loss of a rhamnosyl whilst *m*/*z* 491 generated from the loss of both rhamnosyl and glucosyl. The clear MS/MS spectral confirmed ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$ unambiguously due to their different fragmentation pathways.

### 3.4. Quantitative analysis of Q-TOF-MS

Quantitative analysis of Q-TOF-MS was carried out using the SIM at *m*/*z* 835 $[M+Cl]^-$ with collision energy of 5 V. Ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$ were separated with retention times of 1.5 and 1.7 min, respectively, as seen in Fig. 2. Harald and Michael [24] indicated that the linearity of Q-TOF-MS at the high concentration range is better than at lower concentrations because signal intensities level off. Nevertheless in this study the calibration curve for ginsenoside Rf was linear from 0.164 to 16.4 ng with a correlation coefficient of 0.9997, while 24(R)-pseudoginsenoside $F_{11}$ was linear over the range 0.243–24.3 ng with a correlation coefficient of 0.9989. The limit of detection of this UPLC/Q-TOF-MS analysis for ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$ was 0.05 and 0.08 ng, respectively, which can be seen in Table 1. Compared with the quantitative analysis using HPLC/QqQ-MS working in MRM mode, whose LOD of ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$ was 0.12 ng [19], Q-TOF decrease the limit by 50%. It is approving

that good sensitivity combined with accurate mass measurement makes UPLC/Q-TOF-MS a powerful tool in analysis of Asian ginseng in adulterated American ginseng preparations.

### 3.5. Application to analysis of American ginseng preparations

Considering it is difficult to know the growing areas, ages and cultivated conditions of the material in the finished products, we hardly determine the accurate percentage of adulterant Asian ginseng. In this study a solution to estimate the adulterant proportion by using the reference average was provided. According to the average contents of Rf of 11 batches of Asian ginseng samples and $F_{11}$ of 16 batches of American ginseng from different areas (Table 4), 'reference Asia ginseng' and 'reference American ginseng' were used to estimate the general content of adulterant Asian ginseng in American ginseng products (Table 5). Despite the contents were not so exact, the quantitative expression of the adulterant can facilitate the comparison of the general situation of adulterant between different markets (such as China, US, or EU) or the changes of a marking product in a period. For example, two samples (sample No.34 and sample No.40) from the same factory with different batch numbers were tested. The sample No.40 with batch number 20060902 was not adulterated, but the adulterated proportion in sample No.34 with batch number 20071022 was up to 16.70%.

The developed and validated method was then applied to 11 batches of Asian ginseng samples, 16 batches of American ginseng samples and 13 batches of various commercial American ginseng preparations. These samples were acquired from different markets in China, Canada and America. The results showed the contents of ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$ were disparate in the different origins of Asian ginseng and American ginseng. As Table 5 shows, in the 13 batches of commercial American ginseng preparations, which were labeled pure American ginseng, Asian ginseng was detected in 8 samples, and not in other 5 samples. The percentage of adulteration in the 13 commercial samples was up to 62%, and the adulterated proportion of Asian ginseng in one sample was even up to 50%.

## 4. Conclusions

A UPLC/Q-TOF-MS method has been developed and validated for rapid qualitative and quantitative analyses of Asian ginseng in adulterated American ginseng preparations. The UPLC offered better chromatographic resolution, shorter chromatographic run time, and higher signal-to-noise ratio. They greatly saved the analysis time, cost, and improved the analytical performance. By adding THF in the mobile phase, a better chromatographic resolution was obtained on the isomeric compounds ginsenoside Rf and 24(R)-pseudoginsenoside $F_{11}$ in 2 min. The advantages of hybrid Q-TOF mass spectrometry not only include quality detection capability, sensitivity, but also include accurate measurement, reliable chemical fragmentation, which make the structure elucidations easier. The UPLC/Q-TOF-MS method for qualitative and quantitative analyses of Asian ginseng and American ginseng should be helpful in the quality control of American ginseng preparations.

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