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Chemical Differentiation of Two Taste Variants of *Gynostemma pentaphyllum* by Using UPLC–Q-TOF-MS and HPLC–ELSD

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ABSTRACT: To differentiate the sweet and bitter taste variants of a Chinese medicinal tea *Gynostemma pentaphyllum* (GP), a method for the quantitative analysis of ginsenosides Rb_1, Rb_3, Rd, and F_2 in GP by using UPLC–Q-TOF-MS was developed. According to the different contents of the four ginsenosides, chemical differentiation of the two taste variants of GP was achieved by principal component analysis (PCA). A supplementary quantitative analysis method of using HPLC–ELSD for determination of 20(*S*)-panaxadiol in the hydrolysates of GP was also developed. Similarly, chemical differentiation based on different amounts of 20(*S*)-panaxadiol was established and the result was well consistent with that based on the analysis of the four ginsenosides. It was found that the amounts of the four ginsenosides and 20(*S*)-panaxadiol in the sweet taste variant were significantly higher than those in the bitter one. The significant difference between the sweet and bitter taste variants of GP was easily visualized in 3D-PCA score plots. The PCA loading plot also indicated the contributions among the four ginsenosides (Rd > Rb_3 > F_2 > Rb_1) for distinguishing the two taste variants. This is the first report to describe the use of these two quantitative methods (UPLC–Q-TOF-MS and HPLC–ELSD) for the accurate authentication and quality control of GP.

KEYWORDS: *Gynostemma pentaphyllum, taste variant, UPLC–Q-TOF-MS, HPLC–ELSD, ginsenosides, 20(S)-panaxadiol*

INTRODUCTION

Gynostemma pentaphyllum (Thunb.) Makino (also called "Jiao-gu-lan" in Chinese) is a perennial liana herb of the Cucurbitaceae family, which is mainly distributed in Southern China, Japan, Korea, and Southeast Asian countries. The book "Herbs for Famine" published in the Chinese Ming Dynasty (1368–1644 A.D.) described that this herb was used as vegetable, food, and medicinal tea.[1]

Various studies have shown that the crude extract and/or the triterpene saponins of *Gynostemma pentaphyllum* (GP) exhibit antioxidant effects[2,3] and a variety of biological activities, such as strengthening the immune system,[4] anti-inflammation,[5] treating chronic bronchitis and gastritis,[6−8] lowering blood cholesterol,[9] treating hypoglycemia,[10−12] and reducing risk of cardiovascular diseases[13,14] with minimal toxicity.[15] Many health products, functional foods, and beverages based on GP, such as "Fuzhenghuayu" tablets, total Jiao-gu-lan saponin tablets, and Jiao-gu-lan tea, were developed in China since the early 1990s.[16] However, it is known that the chemical constituents of GP planted in different areas are very different in composition.[17,18] Furthermore, there are two taste variants, sweet and bitter, for GP herbs, which have different clinical application in folk medicine in China.[18−21] Therefore, it is important to differentiate these two variants for the quality control of GP. As it is very difficult to differentiate these two variants accurately by taste or morphological characteristics, a more reliable differentiation approach is thus desired. Recently, we reported the authentication of these taste variants of GP by means of LC–MS fingerprinting profiles of the triterpenoid saponins and the ITS sequences of rDNA.[22] In this paper, we established a more efficient, accurate, and specific quantitative method for differentiation of the sweet and bitter taste variants of GP.

The most abundant components in GP are triterpene saponins,[23,24] named gypenosides or gynosaponins, which have been considered as the main bioactive components for the medicinal properties of GP.[1] The compositions and/or the contents of total saponins have been found to be different within GP samples from different places or between the sweet and bitter taste variants of GP.[17,18,22,25,26] To date, approximately 169 gypenosides from GP have been reported.[27] Of these, eight gypenosides were identified as ginsenosides Rb_1, Rb_3, Rc, Rd, F_2, Rg_3, malonyl-Rb_1, and malonyl-Rd, which are commonly found in *Panax ginseng* C.A. Meyer; these ginsenosides make up around 25% of the total saponins in GP herb and all of them belong to the protopanaxadiol type.[1] Moreover, 20(*S*)-protopanaxadiol, the prototype of 20(*S*)-panaxadiol, was found to be the main aglycon obtained by hydrolysis of the gypenosides of GP.[23,26] In this case, protopanaxadiol-type ginsenosides were considered as the constituents that result in chemical difference between the two taste variants of GP.

In the present study, ginsenosides Rb_1, Rb_3, Rd, and F_2, which were regarded as the bioactive saponins of GP and are commercially available, were selected as markers for quantitative

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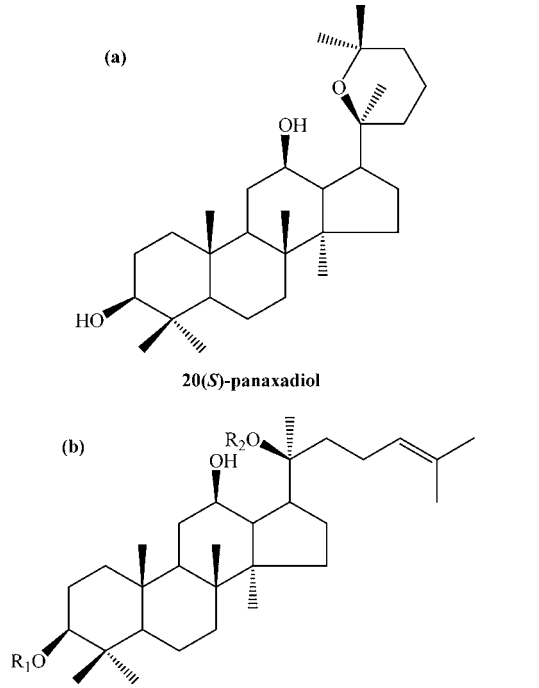
differentiation of the two taste variants of GP by using UPLC−MS. Based on the different contents of ginsenosides Rb_1, Rb_3, Rd, and F_2 in GP, chemical differentiation between the two taste variants of GP purchased from different places in China and Japan was assessed by using multivariate statistical approaches, such as principal component analysis (PCA). In addition, a supplementary quantitative method by using HPLC−ELSD was also developed for determination of 20(*S*)-panaxadiol, the major acid-hydrolyzed aglycon of protopanaxadiol-type saponins in GP.[28] Similarly, chemical differentiation based on different amounts of 20(*S*)-panaxadiol in GP was established and the result was well consistent with that based on the four ginsenosides. These quantitative data provided solid evidence for the accurate authentication and quality control of GP.

Table 1. Twenty-Three Batches of GP Samples from China and Japan

code	taste	plant sources
S1	sweet	Liuzhou, Guangxi Province
S2	sweet	Liuzhou, Guangxi Province
S3	sweet	Guangxi Province
S4	sweet	Guangxi Province
S5	sweet	Guangxi Province
S6	sweet	Guangxi Province
S7	sweet	Liuzhou, Guangxi Province
S8	sweet	Guangxi Province
S9	sweet	Guilin, Guangxi Province
S10	sweet	Guilin, Guangxi Province
S11	sweet	Guigang, Guangxi Province
S12	sweet	Guangxi Province
S13	sweet	Sichuan Province
B1	bitter	Shuicheng, Guizhou Province
B2	bitter	Liuzhou, Guangxi Province
B3	bitter	Liuzhou, Guangxi Province
B4	bitter	Ruijin, Jiangxi Province
B5	bitter	Nara, Japan
B6	bitter	Sichuan Province
B7	bitter	Guangxi Province
B8	bitter	Guangxi Province
B9	bitter	Anhui Province
B10	bitter	Guangxi Province

MATERIALS AND METHODS

Chemicals and Materials. Twenty-two batches of *Gynostemma pentaphyllum* were purchased from different places in China, and one batch was acquired from Japan. All of them were taxonomically identified by one of the authors (Z.-Z.Z.), and they were grouped on the basis of the taste of the herbs before analysis (Table 1). Each herbal sample was first homogenized, pulverized in a mill, and passed through a 24-mesh sieve before analysis. Marker compounds 20(*S*)-panaxadiol and ginsenosides Rb_1, Rb_3, and Rd (purity >98%) were purchased from



Ginsenosides	R_1	R_2	Formula	M.W.
Rb_1	-glc[2→1]glc	-glc[6→1]glc	$C_{54}H_{92}O_{23}$	1109.2966
Rb_3	-glc[2→1]glc	-glc[6→1]xyl	$C_{53}H_{90}O_{22}$	1079.2706
Rd	-glc[2→1]glc	-glc	$C_{48}H_{82}O_{18}$	947.1558
F_2	-glc	-glc	$C_{42}H_{72}O_{13}$	785.0149

Figure 1. Chemical structures of 20(*S*)-panaxadiol (a) and ginsenosides Rb_1, Rb_3, Rd, and F_2 (b); glc, glucose; xyl, xylose.

Table 2. Validation Results of UPLC−MS Quantitative Analysis for Ginsenosides Rb_1, Rb_3, Rd, and F_2

ginsenosides	Rb_1	Rb_3	Rd	F_2
linearity study				
calibration curves	$y = 6265.4x + 2040.7$	$y = 33887.5x + 4358.0$	$y = 86667.5x + 9608.3$	$y = 84252.6x + 6851.4$
R^2	0.993	0.993	0.993	0.994
linear range (μg/mL)	0.0154−7.813	0.0154−7.813	0.0080−7.813	0.0061−6.250
LOD (μg/mL)	0.017	0.007	0.006	0.003
LOQ (μg/mL)	0.087	0.041	0.019	0.012
precision RSD[a] (%)				
intraday ($n = 5$)	3.2	1.6	0.6	1.0
interday ($n = 5$)	1.4	0.6	1.8	0.3
repeatability ($n = 5$)				
mean (μg/g)	70.61	690.54	793.74	134.97
RSD (%)	2.4	4.9	2.0	2.7
recovery ($n = 5$)				
mean (%)	90.5	94.3	99.1	94.5
RSD (%)	2.8	2.2	3.3	2.3

[a]RSD: relative standard deviation.

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Table 3. Validation Results of HPLC−ELSD Quantitative Analysis for 20(*S*)-Panaxadiol

					repeatability (n = 5)		recovery (n = 5)	
analyte	calibration curve	R^2	linearity (μg/mL)	precision RSD[a] (%)	mean (μg/g)	RSD (%)	mean (%)	RSD (%)
20(*S*)-panaxadiol	$y = 1.6441x - 0.4945$	0.9992	8.0−500.0	0.6	2348.22	1.92	100.22	4.19

[a]RSD: relative standard deviation.

Table 4. LOD and LOQ of HPLC−ELSD Quantitative Analysis for 20(*S*)-Panaxadiol

			recovery study of LOQ				
						recovery (n = 5)	
analyte	MDL (μg/mL)	LOQ (μg/mL)	eq	R^2	concn (μg/mL)	mean (%)	RSD[a] (%)
20(*S*)-panaxadiol	4	12	$y = 1.7010x - 0.6797$	0.996	6.4−64.0	96.0	4.16

[a]RSD: relative standard deviation.

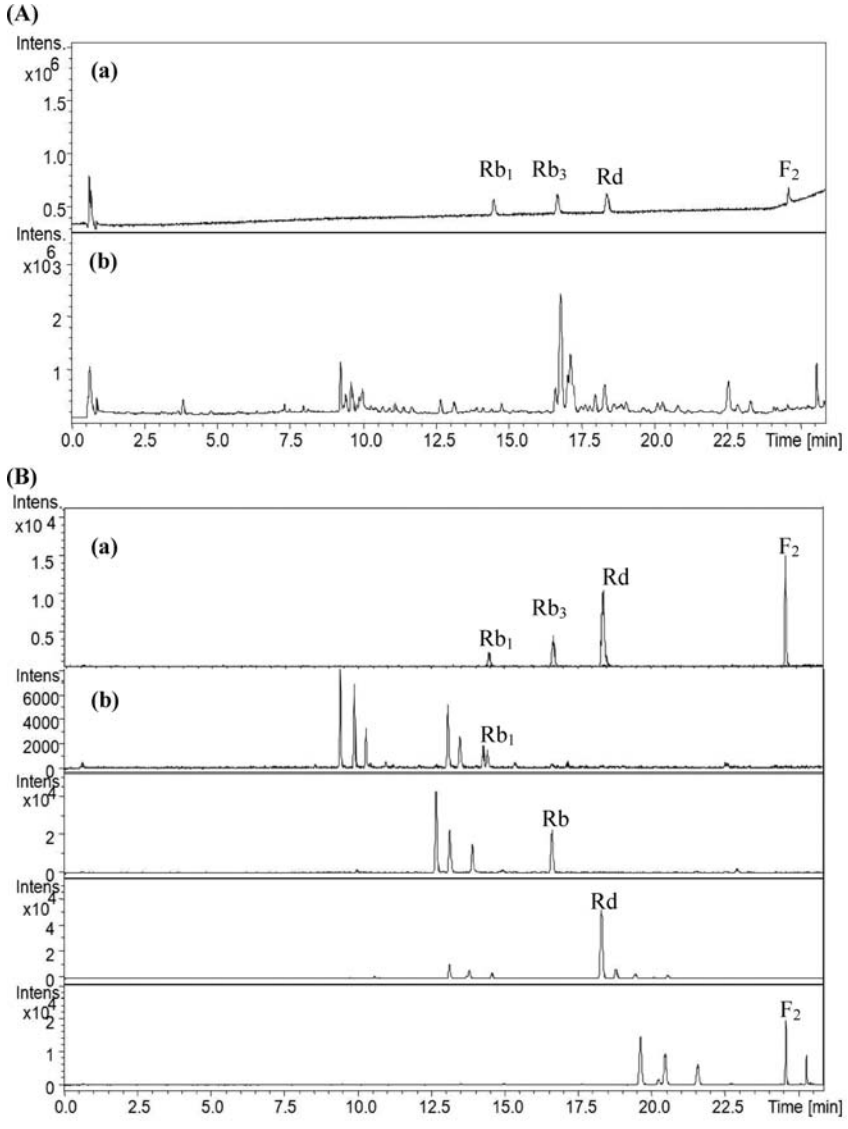

Figure 2. (A) UPLC−MS total ion chromatograms (TICs). (a) Mixed standard of four ginsenosides (Rb_1 0.25 μg/mL, Rb_3 0.25 μg/mL, Rd 0.25 μg/mL, and F_2 0.1 μg/mL). (b) Typical GP sample (S7, 4 mg/mL). (B) UPLC−MS extracted ion chromatograms (EICs). (a) Mixed standard of four ginsenosides (Rb_1 0.25 μg/mL, Rb_3 0.25 μg/mL, Rd 0.25 μg/mL, and F_2 0.1 μg/mL). (b) Typical GP sample (S7, 4 mg/mL).

the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China); ginsenoside F_2 (purity >98%) was purchased from Mustbio-Tech (Chengdu, China) (Figure 1). Acetonitrile and methanol of HPLC grade were purchased from Merck (Darmstadt, Germany). HPLC-grade formic acid was purchased from Fluka (Buchs, Switzerland). Ultrapure water was purified with a Milli-Q system (Millipore, Billerica, MA, USA).

Preparation of Standard and Sample Solutions for UPLC−MS Analysis. A mixed standard stock solution for ginsenosides Rb_1 (1.0 μg/mL), Rb_3 (1.0 μg/mL), Rd (1.0 μg/mL), and F_2 (0.4 μg/mL)

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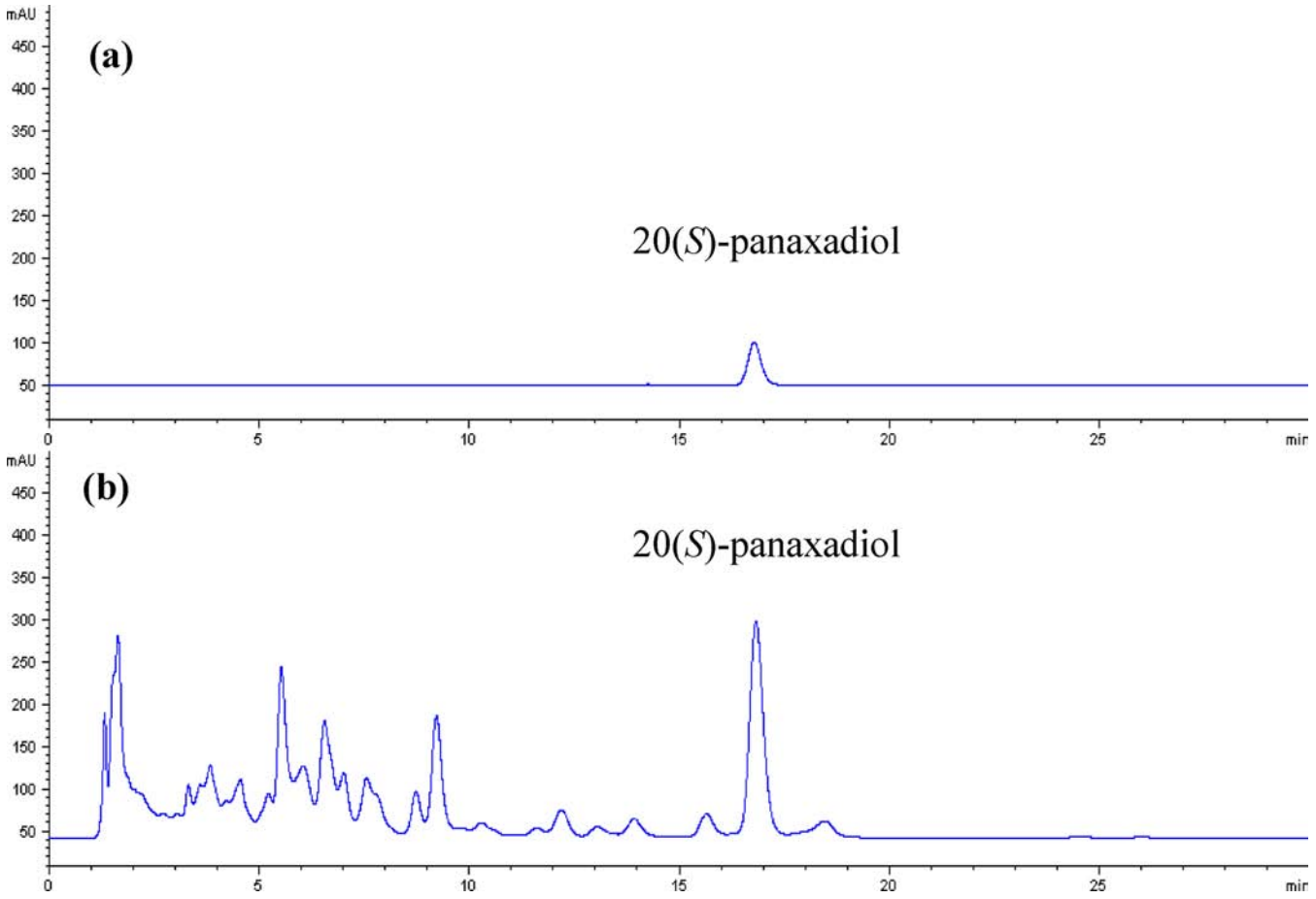

Figure 3. HPLC−ELSD chromatograms. (a) 20(*S*)-Panaxadiol (80 μg/mL). (b) Typical GP sample (S7, 100 mg/mL).

was prepared in 70% methanol (v/v). Working standard solutions for calibration curves were prepared by diluting the mixed standard stock solution with 70% methanol to different concentrations within the following ranges: 0.0625−1.0 μg/mL for Rb_1, Rb_3, and Rd; 0.025−0.4 μg/mL for F_2. The standard solutions were stored at 4 °C before analysis.

Powdered herbal sample (0.5 g) was accurately weighed into a 50 mL centrifugal tube, and then 10 mL of 70% methanol was added. The mixture was sonicated for 30 min with occasional shaking and then centrifuged at 1800*g* for 5 min. The residual herbal sample was extracted two more times using 8 and 6 mL of 70% methanol. The three supernatants were combined into a 25 mL volumetric flask, and then 70% methanol was added to a final volume of 25 mL. The mixture solution was filtered through a 0.22 μm PTFE filter and then diluted 5- and 50-fold (4 mg/mL and 0.4 mg/mL) separately with 70% methanol prior to UPLC−MS analysis.

Preparation of Standard and Sample Solutions for HPLC−ELSD Analysis. 20(*S*)-Panaxadiol was dissolved in methanol at 1000 μg/mL to be used as a standard stock solution. Working standard solutions were prepared by diluting the standard stock solution with methanol to different concentrations within the range of 16−500 μg/mL. The standard solutions were stored at 4 °C before analysis.

Powdered herbal sample (1.0 g) was accurately weighed into a 50 mL centrifugal tube, and then 15 mL of methanol was added. The mixture was sonicated for 30 min with occasional shaking and then centrifuged at 1800*g* for 5 min. The residual herbal sample was extracted two more times using 15 mL of methanol. The three supernatants were combined into a 100 mL round-bottomed flask and evaporated at reduced pressure in a rotary evaporator (50 °C) to obtain the final extract.

The extract in 20 mL of 3 mol/L HCl solution was hydrolyzed in a water bath of 70 °C for 4 h. After cooling down to room temperature, the hydrolyzed sample was transferred into a 125 mL separating funnel and partitioned repeatedly with 30 mL of dichloromethane three times. The organic layer was evaporated to dryness at reduced pressure in a rotary evaporator (30 °C). The residue was dissolved in 10 mL of methanol (100 mg/mL), and the solution was filtered with a 0.45 μm PTFE filter prior to HPLC−ELSD analysis.

UPLC−MS Analysis. UPLC was performed with a Waters ACQUITY UPLC system (Waters Corp., Milford, MA, USA) which was equipped with a binary solvent delivery system and coupled to a micrOTOF-Q mass spectrometer (Bruker) with an ESI source. All the operations and analysis of data were conducted using the Hystar software (Bruker Daltonik GmbH, Germany).

The chromatography was performed on an Acquity BEH C_{18} column (2.1 × 100 mm, 1.7 μm). The mobile phase consisted of 0.1% formic acid in deionized water (A) and 0.1% formic acid in acetonitrile (B). A gradient elution procedure was used: 0−8 min, 12−27% B; 8−23 min, 27−40% B; 23−26 min, 40−74% B; 26−29 min, 100% B; 29−32 min, 12% B. The flow rate was kept at 0.35 mL/min, and the injection volume was 5 μL.

The ESI-MS data were acquired in negative mode, and the conditions of MS analysis were as follows: end plate offset, −500 V; capillary voltage, 4500 V; collision energy, 10 eV; nebulizing gas (N_2) pressure, 2.5 bar; drying gas (N_2) flow rate, 8.0 L/min; drying gas temperature, 180 °C; mass range, *m*/*z* 100−3000; spectra rate, 3.0 HZ.

The peaks in the chromatogram of the herb were identified by comparing retention time values and the mass spectra of reference standards. Extracted ion chromatograms (EICs) at *m*/*z* 1153.60 for the $[M + HCOO]^-$ ion of Rb_1, *m*/*z* 1123.59 for the $[M + HCOO]^-$ ion of Rb_3, *m*/*z* 991.55 for the $[M + HCOO]^-$ ion of Rd, and *m*/*z* 829.50 for the $[M + HCOO]^-$ ion of F_2 were integrated, and the peak areas were used for quantification.

HPLC−ELSD Analysis. An Agilent 1100 series HPLC system (Agilent Technologies, Inc., USA) consisting of a degasser (G1379A), a binary solvent delivery system (G1312A), an online autosampler

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Table 5. Contents of Ginsenosides Rb_1, Rb_3, Rd, and F_2 and 20(*S*)-Panaxadiol in Twenty-Three Batches of GP Samples[a]

code	Rb_1*[b] mean (μg/g)	ADM[c]e (%)	Rb_3* mean (μg/g)	ADM (%)	Rd* mean (μg/g)	ADM (%)	F_2* mean (μg/g)	ADM (%)	sum of ginsenosides mean (μg/g)	ADM (%)	20(*S*)-panaxadiol* mean (μg/g)	ADM (%)
S1	11.9	0.1	120.7	1.9	231.2	0.6	8.4	1.5	374.8	1.0	2523.0	3.2
S2	4.4	0.9	238.9	1.4	443.9	0.3	72.2	0.1	761.9	0.6	4555.3	3.6
S3	59.1	1.8	796.3	0.9	647.9	0.8	74.2	2.0	1581.0	1.8	2173.4	3.6
S4	59.1	0.3	311.9	1.4	613.1	2.2	78.6	3.5	1066.5	1.6	4252.3	0.5
S5	41.3	0.1	55.9	1.0	714.7	2.5	476.2	1.3	1291.6	1.8	2577.6	1.6
S6	65.6	0.3	39.7	1.3	301.3	0.3	22.8	0.6	431.3	0.1	2696.5	9.1
S7	70.9	0.7	692.4	2.9	793.5	2.6	136.9	0.6	1699.8	2.4	2363.4	5.6
S8	34.5	0.1	26.3	3.6	412.4	0.3	66.8	2.8	543.9	0.3	1862.9	7.4
S9	35.5	2.5	20.4	1.0	129.0	2.5	19.3	1.7	210.2	1.1	1880.6	5.1
S10	58.8	3.1	33.4	0.9	310.2	2.3	26.3	0.0	434.9	1.2	2988.4	3.6
S11	51.9	0.8	17.0	0.3	175.8	0.1	45.9	0.7	291.7	0.8	2559.8	2.1
S12	102.2	2.0	81.5	0.9	835.0	2.0	57.3	3.4	1080.7	2.0	4136.9	1.8
S13	126.6	1.6	62.3	1.9	761.2	1.4	40.0	3.1	994.9	1.6	3731.4	1.2
mean	55.5		192.0		489.9		86.5		823.9		2946.3	
B1	0.0		0.0		2.6	0.1	2.5	0.8	5.2	0.5	438.7	1.3
B2	0.0		0.0		33.7	0.3	6.1	3.0	40.1	0.7	434.9	3.9
B3	0.0		0.0		3.6	0.9	0.0		4.5	0.9	0.0	
B4	0.0		0.0		0.0		0.0		0.0		0.0	
B5	0.0		0.0		0.0		0.0		0.0		0.0	
B6	0.0		0.0		14.9	1.5	11.2	1.5	27.6	1.5	564.2	1.2
B7	0.0		0.0		0.0		0.0		0.0		0.0	
B8	0.0		0.0		0.0		0.0		0.0		0.0	
B9	0.0		0.0		0.0		0.0		0.0		129.8	2.2
B10	0.0		0.0		8.3	2.7	0.0		11.0	2.7	337.1	3.0
mean	0.0		0.0		6.3		2.0		8.3		190.5	

[a]S1−S13 represent the sweet taste variant, and B1−B10 represent the bitter one. [b]*$p < 0.05$ for comparison between the sweet and bitter taste variants of GP samples using Student's *t*-test. [c]ADM: the absolute deviation from the mean of duplicate analysis according to the following equation: $ADM = |D_1 - (D_1 + D_2)/2|/[(D_1 + D_2)/2] \times 100\%$, D_1 = concentration of analyte in sample, D_2 = concentration of analyte in sample duplicat.

(G1313A) with a 100 μL sample loop, a column oven controller (G1316A), a multichannel interface unit (35900E), and an ELSD 2000 (Alltech Associates, Inc., USA) was used. The chromatography was performed on an Alltech Alltima C_{18} column (4.6 × 150 mm, 5 μm). An isocratic program was applied with water (A) and methanol (B) (22:88, v/v) at a flow rate of 1.0 mL/min and a column temperature of 30 °C. The detection conditions of ELSD were as follows: drift tube temperature of 75 °C, nebulizer gas (N_2) flow of 2.0 L/min, gain of 2, and impactor in off mode. The injection volume was 20 μL for quantitative analysis. All the operations and analysis of data were conducted using the software of Agilent ChemStation.

Statistical Analysis. To evaluate the differentiation between sweet and bitter taste GP samples, the contents of the four ginsenosides and 20(*S*)-panaxadiol in duplicate samples were analyzed by Student's *t*-test and exported to the SIMCA P+ 11.5 software (Umertrics, Umea, Sweden) for PCA analysis.

RESULTS AND DISCUSSION

Optimization of Sample Preparation and MS Conditions for UPLC−MS Analysis. To achieve optimal extraction conditions, extraction solvents (50%, 60%, 70%, 80%, and 100% methanol) and number of extraction times (three and four times) were investigated. The results demonstrated that 70% methanol could extract the highest amount of four ginsenosides (Rb_1, Rb_3, Rd, and F_2). To optimize the number of extraction times, the amount of four ginsenosides extracted from the first three extractions was compared with that from the fourth extraction. The results showed that three extractions were enough to extract the four ginsenosides completely. For accurate quantification, dilution folds of the sample solution were investigated, which showed that dilutions of sample solution by 5- to 50-fold were appropriate for evaluating different levels of the four ginsenosides in the GP herb.

To obtain the optimal sensitivity, ESI modes (positive and negative) and collision energy (8, 10, 12 eV) were also studied. ESI negative mode with the collision energy of 10 eV was found to provide not only clear information on the molecular ions but also the optimal ratio of signal-to-noise of the molecular ions of the four ginsenosides.

Optimization of Sample Preparation for HPLC−ELSD Analysis. To optimize hydrolysis conditions, the concentration of hydrochloric acid (2, 3, 4, 6 mol/L), temperature (60, 70, 80 °C), and time (2, 4, 6, 8 h) of hydrolysis were investigated. It was demonstrated that 3 mol/L hydrochloric acid and hydrolysis at 70 °C for 4 h were the best conditions for complete hydrolysis of saponins in the crude extract of the herb. According to the hydrophobic property of 20(*S*)-panaxadiol, dichloromethane was chosen as the partition solvent with consideration of its toxicity being lower than that of chloroform. It was proven that partition three times was sufficient for complete extraction of 20(*S*)-panaxadiol from aqueous solution.

Method Validation of UPLC−MS Analysis. A mixed standard stock solution containing ginsenosides Rb_1, Rb_3, Rd, and F_2 was diluted to a series of appropriate standard solutions with 70% methanol for linearity study. The intraday and interday precisions of injection were assessed by injecting five replicate injections of a working standard solution at an intermediate concentration (Rb_1, Rb_3, Rd 0.25 μg/mL each and F_2 0.1 μg/mL)

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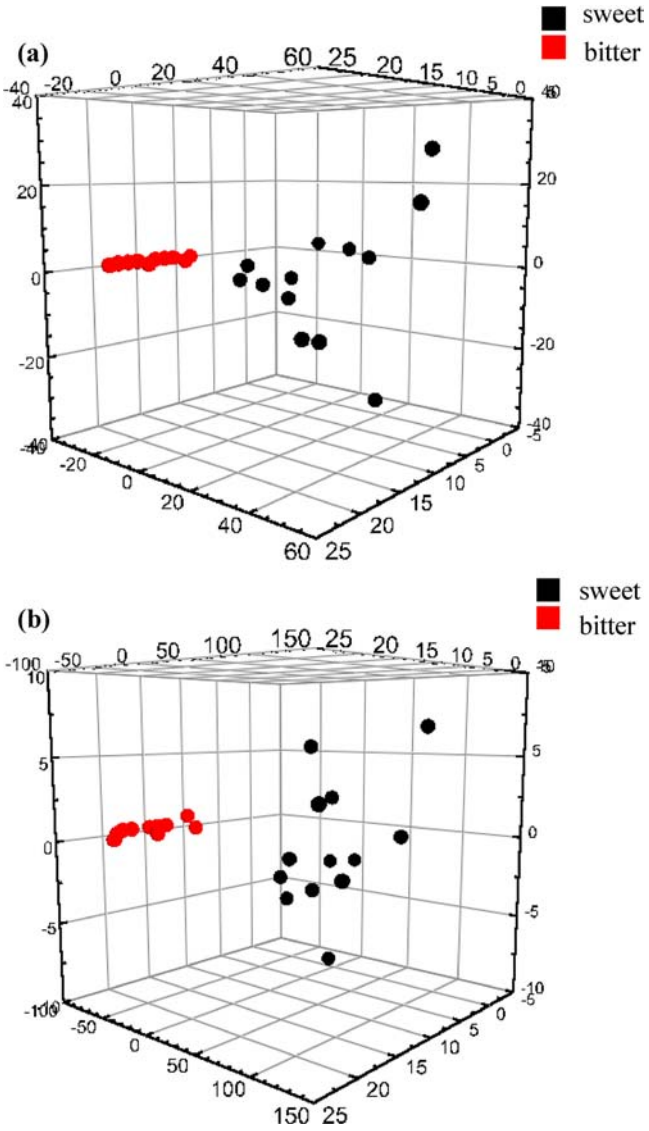

Figure 4. 3-D PCA score plots processed from UPLC−MS data (a) and 3-D PCA from HPLC−ELSD data (b). Dark dots represent sweet taste GP samples, and red dots represent bitter taste GP samples.

in a single day and on five consecutive days, respectively. Repeatability was studied by analyzing one batch of GP sample (S7) five times. The recovery studies were performed by spiking the mixed standard solution (with the same concentration as the sample) into the powder of the GP sample (S7) five times. Limit of detection LOD (S/N = 3) and limit of quantitation LOQ (S/N = 10) of the four ginsenosides were calculated by a five-point calibration curve (low concentration of the standard solutions versus signal-to-noise ratio). All of the validation data for the four ginsenosides are summarized in Table 2.

Method Validation of HPLC−ELSD Analysis. Linearity study was performed on a series of standard solutions of 20(*S*)-panaxadiol. Precision was studied by five replicate injections of a working standard solution at an intermediate concentration of 80 μg/mL. Repeatability was obtained by analyzing one batch of GP sample (S7) five times, and the recovery experiments were carried out by spiking 20(*S*)-panaxadiol standard solution (with the same concentration as the sample) into the powder of the GP sample (S7) five times. LOD of 20(*S*)-panaxadiol in GP was determined as the analyte concentration producing a signal of at least 3.14 times the standard deviation of measurements of seven spiked Poria samples (blank matrix) with 20(*S*)-panaxadiol standard at 0.25 mg. LOQ of 20(*S*)-panaxadiol in GP was estimated as three times the LOD, and then five replicate analyses of the Poria sample spiked with the standard at the above estimated amount were performed to evaluate the recovery of LOQ. All of the validation data for 20(*S*)-panaxadiol were summarized in Tables 3 and 4.

Quantitative Analysis of GP Samples by Using UPLC−MS and HPLC−ELSD. Twenty-three batches of GP samples (13 batches of sweet taste and 10 batches of bitter taste) were quantitatively assessed in duplicate using the developed UPLC−MS method and HPLC−ELSD method. The typical UPLC−MS total ion chromatograms (TICs) and extracted ion chromatograms (EICs) of the mixed standard containing the four ginsenosides and a typical GP sample (S7) are shown in Figures 2A and 2B, respectively. The typical HPLC−ELSD chromatograms of 20(*S*)-panaxadiol and a typical GP sample (S7) are shown in Figure 3. The contents of the four ginsenosides and 20(*S*)-panaxadiol in GP samples are summarized in Table 5, which shows that the amounts of the four ginsenosides and 20(*S*)-panaxadiol in sweet taste GP samples were found to be significantly higher than those in bitter taste GP samples ($p < 0.05$). The results indicate that 20(*S*)-protopanaxadiol-type saponins were more abundant in the sweet taste GP than those in the bitter taste one. This characteristic can be used to distinguish the sweet taste GP from the bitter taste variant of GP.

The significant difference between sweet and bitter taste GP herbs can be easily visualized from principal component analysis (PCA) results. The three-dimensional (3-D) score plots of the PCA results obtained from UPLC−MS and HPLC−ELSD data are shown in Figures 4a and 4b, respectively. The sweet and bitter taste GP samples were well separated in the 3-D space. Furthermore, as shown in Figure 5, the PCA loading plot provided the contributions of the four ginsenosides (Rd > Rb_3 > F_2 > Rb_1) for distinguishing sweet and bitter taste GP samples. These results demonstrated that the selected ginsenosides above are proper markers for the authentication and quality control of the herb. It is worth mentioning that taste variant of the herb was never specified in the pharmacological research on GP herb before. For this reason, the results from our study may attract attention and interest of biologists undertaking bioassay research of GP herb.

In conclusion, this study developed both the UPLC−MS and HPLC−ELSD methods for the differentiation of sweet taste and bitter taste GP samples. UPLC−MS offers important advantages including simplicity in sample preparation, shorter analysis time, and less solvent usage, whereas HPLC−ELSD analysis of 20(*S*)-protopanaxadiol provides a method for quantitative assay of total ginsenosides, solving the problem of shortcomings in colorimetric determination of total saponins in GP.[25] In our study, the selected ginsenoside markers Rb_1, Rb_3, Rd, F_2, and 20(*S*)-panaxadiol were notable, representative, and characteristic, while two quantitative methods were specific, accurate, and complementary. This approach could directly differentiate the two taste variants of GP for rapid, accurate, and practical authentication and quality control of GP herb and its products.

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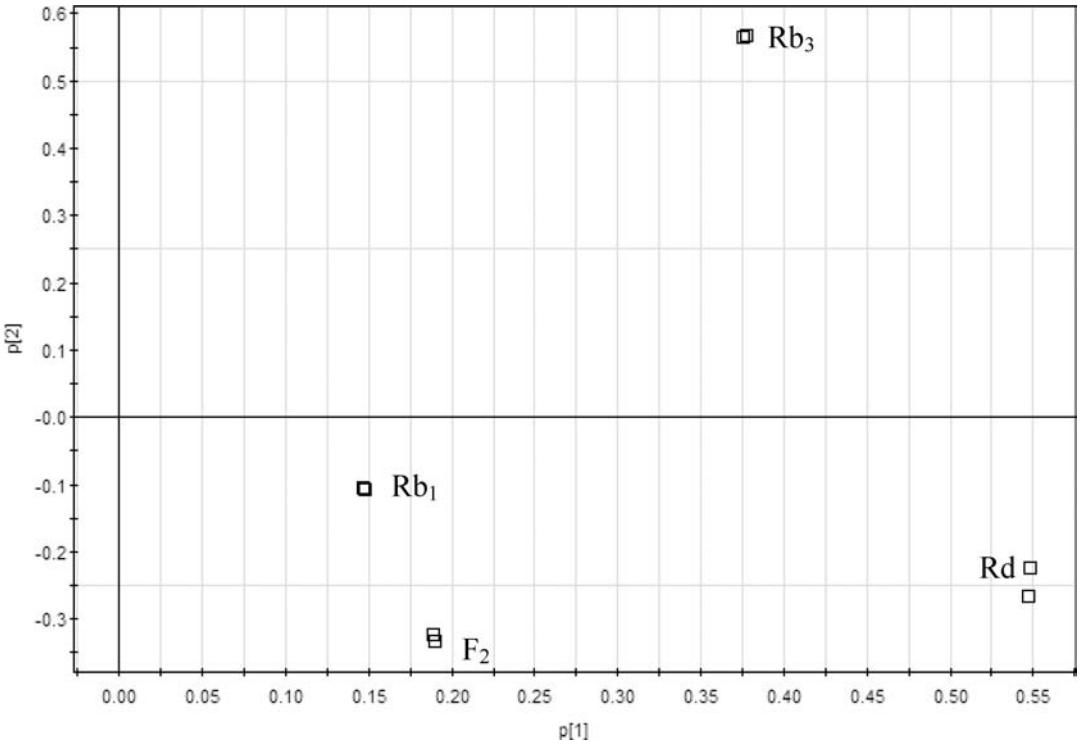

Figure 5. PCA loading plot processed from UPLC−MS data corresponding to the 3-D PCA score plot.

AUTHOR INFORMATION

Notes

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