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Simultaneous Quantification of Ginsenosides in American Ginseng (*Panax quinquefolium*) Root Powder by Visible/Near-Infrared Reflectance Spectroscopy

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Near-infrared reflectance spectroscopy (NIRS) was examined as a possible alternative to high-performance liquid chromatography (HPLC) for the analysis of ginsenosides from American ginseng (*Panax quinquefolium*) root powder ($n = 26$). NIR spectra were collected over 400−2500 nm. For each sample and individual ginsenoside quantified by HPLC, spectral data were regressed against the chemical data to develop prediction equations. The spectral prediction equations produced high correlation coefficient (1-VR) values and low standard errors of cross validation (SECV) values for the determination of individual and total ginsenosides. The contents of individual ginsenosides, Rb_1, Rb_2, Rc, Rd, Re, Rg_1, Ro, m-Rb_1, m-Rb_2, m-Rc, m-Rd, and total ginsenosides (X ± SECV) were (1.29 ± 0.18)%, (0.273 ± 0.096)%, (0.298 ± 0.052)%, (0.091 ± 0.027)%, (1.015 ± 0.114)%, (0.116 ± 0.018)%, (0.25 ± 0.040)%, (0.776 ± 0.116)%, (0.197 ± 0.074)%, (0.239 ± 0.083)%, (0.143 ± 0.042)%, and (4.393 ± 0.283)%, respectively. The (1-VR) values of cross validation were 0.877, 0.872, 0.955, 0.834, 0.899, 0.919, 0.325, 0.849, 0.902, 0.877, 0.871, and 0.963, respectively. Results indicated that the NIRS method could be used for the analysis of the major ginsenosides, Rb_1, Re, and m-Rb_1, as well as the total ginsenosides in American ginseng.

Keywords: *Panax quinquefolium; American ginseng; ginsenosides; quantification; HPLC; visible/NIR spectroscopy*

INTRODUCTION

American ginseng (*Panax quinquefolium*) is an important economic medicinal plant, which is cultivated on a large scale in the northern United States, Canada, and northeastern China. The annual production of dried American ginseng roots is over 1000 tons and valued at about 100 million U.S. dollars. American ginseng products, such as integrity roots and powder, are readily available in health food stores around the world. American ginseng is as famous as Asian ginseng (*Panax ginseng*) in medicinal uses due to its wide spectrum of pharmacological properties and physiological activities. These include cancer-related, antiinflammatory, antiallergic, immunomodulatory, anti-diabetic activities and activities on the cardiovascular system, the central nervous system, and the endocrinal system 1−3 (Lacaille-dubois and Wagner, 1996; Gillis, 1997; Sticher, 1998).

The main active constituents of American ginseng are ginsenosides, which include neutral ginsenosides, malonylginsenosides, and the oleanolic acid-type ginsenoside. Ginsenosides in American ginseng include the ginsenosides Rb_1, Rb_2, Rc, Rd, Re, and Rg_1; malonylginsenosides m-Rb_1, m-Rb_2, m-Rc, and m-Rd; and the oleanolic acid-type ginsenoside Ro (Chuang and Sheu, 1994; Chuang et al., 1995; Ko et al., 1995; William et al., 1996; Ren and Chen, 1999). Ginsenosides are frequently used as a main index for American ginseng evaluation. For meeting this demand, HPLC methods (Yamaguchi et al., 1988; Chuang and Sheu, 1994; Sumukawa et al., 1995; William et al., 1996) have been developed for simultaneously analyzing all the main ginsenosides. The drawback of these methods are that they are time-consuming and expensive.

Near-infrared reflectance spectroscopy (NIRS) is a new analytical technique, which is based on the principle of absorption and reflectance of monochromatic radiation by the surface of samples to be analyzed. It can offer many advantages, such as simple sample preparation and little time required. NIRS has been mainly used in the evaluation of nutritional components in animal feed and human food (Givens et al., 1997). Recently, it has been used for determining natural products in plants and fungus-infected grasses (Molt et al., 1997; Roberts et al., 1997) and also used in pharmaceutical industries (Buchanan et al., 1996, 1998; Roberts et al., 1997).

The aim of this study is to develop a NIRS method for determining ginsenosides in American ginseng root powder.

EXPERIMENTAL PROCEDURES

American Ginseng Roots. Part of the American ginseng roots were obtained from Hong Kong herb stores, and the rest were processed in our laboratory by using fresh American ginseng roots collected from Jilin, P. R. China (Ren and Chen, 1998). The dried roots were cut into small pieces, ground with a blender, and screened through a 200 mesh sieve. A total of 26 American ginseng samples consisting of 2 root fibers and 24 main roots were used. The 2 root fibers and 16 main roots (diameter 4.5−12 mm) were purchased from Hong Kong herb stores. These 18 American ginseng samples were imported from Wisconsin as claimed by the seller. The remaining 8 samples (main roots) were processed by our laboratory using

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Table 1. Ginsenoside Contents (%) in Root Fibers and Main Roots of American Ginseng (*Panax quinquefolium*) by HPLC

no.	root fiber F_1	root fiber F_2	main root R_1	main root R_2	main root R_3	main root R_4	main root R_5
Rb_1	0.76	0.83	0.25	0.25	0.29	1.01	1.72
Rb_2	0.56	0.55				0.17	
Rc	0.99	1.17	0.13	0.15	0.13	0.12	0.33
Rd	0.21	0.30	0.03	0.03	0.01	0.03	0.16
Re	0.97	0.90	0.20	0.18	0.32	0.95	1.40
Rg_1	0.26	0.30	0.14	0.15	0.20	0.04	0.10
Ro	0.26	0.15	0.26	0.28	0.14	0.17	0.27
m-Rb_1	1.04	1.03	0.18	0.17	0.16	0.58	1.11
m-Rb_2	0.90	0.80				0.11	0.13
m-Rc	1.02	0.94	0.11	0.14	0.15	0.10	0.28
m-Rd	0.44	0.53	0.06	0.07	0.03	0.05	0.26
total	7.41	7.52	1.36	1.42	1.43	3.33	5.76

Table 2. Prediction of Ginsenoside Contents (%) in American Ginseng (*Panax quinquefolium*) Powder by NIR Spectroscopy

ginsenosides	no. of samples[a]	range of content (%)	mean (%)	SEC[b] (%)	R^2	SECV (%)	1-VR	RSD (%)
Rb_1	26	0.25−2.36	1.292	0.092	0.968	0.186	0.877	14.4
Rb_2	9	0.04−0.61	0.273	0.019	0.993	0.096	0.872	35.2
Rc	26	0.12−1.18	0.298	0.039	0.975	0.052	0.955	17.4
Rd	26	0.01−0.30	0.091	0.023	0.878	0.027	0.834	29.7
Re	26	0.18−1.65	1.015	0.075	0.955	0.114	0.899	11.2
Rg_1	26	0.04−0.30	0.116	0.012	0.963	0.018	0.919	15.5
Ro	26	0.14−0.33	0.25	0.037	0.418	0.040	0.325	16.0
m-Rb_1	26	0.16−1.24	0.776	0.089	0.910	0.116	0.849	14.9
m-Rb_2	19	0.07−0.90	0.197	0.042	0.968	0.074	0.902	37.6
m-Rc	26	0.06−1.02	0.239	0.051	0.953	0.083	0.877	34.7
m-Rd	26	0.03−0.53	0.143	0.033	0.920	0.042	0.871	29.4
total	26	1.36−7.52	4.393	0.152	0.989	0.283	0.963	6.4

[a] Number of samples containing detectable amounts of ginsenosides out of 26 samples. [b] Standard errors of calibration (SEC).

Table 3. Effects of Scatter Corrections on the Calibration Performance

	without correction		SNVand detrend		modified MSC	
	X ± SECV (%)	1-VR	SECV (%)	1-VR	SECV (%)	1-VR
Rb_1	1.292 ± 0.21	0.839	0.186	0.876	0.189	0.877
Rb_2	0.273 ± 0.115	0.817	0.097	0.869	0.096	0.872
Rc	0.298 ± 0.068	0.923	0.054	0.952	0.052	0.955
Rd	0.091 ± 0.031	0.779	0.027	0.828	0.027	0.834
Re	1.015 ± 0.123	0.881	0.114	0.899	0.116	0.895
Rg_1	0.116 ± 0.020	0.899	0.019	0.916	0.018	0.919
Ro	0.25 ± 0.044	0.169	0.040	0.325	0.040	0.325
m-Rb_1	0.776 ± 0.139	0.777	0.116	0.847	0.116	0.849
m-Rb_2	0.197 ± 0.094	0.844	0.085	0.871	0.074	0.902
m-Rc	0.239 ± 0.097	0.826	0.083	0.877	0.089	0.854
m-Rd	0.143 ± 0.047	0.835	0.042	0.871	0.042	0.869
total	4.393 ± 0.311	0.959	0.283	0.963	0.286	0.962

fresh American ginseng from Jilin, the main production site of American ginseng in China. The diameter of the roots was similar to that of the imported ones.

Data Collection. Near-infrared reflectance spectra of American ginseng powder were measured using an NIR system (model 6500, Perstorp Analytical, Inc, Silver Spring, MD) over a wavelength range of 400−2500 nm and recorded as the logarithm of the reciprocal reflectance, log(1/*R*) at 8 nm intervals.

Data Analysis. The Infrasoft International software, NIRS 2, version 3.0 (Port Matilda, PA), was used to collect and analyze the data, perform the calibration, and cross validation. All 26 American ginseng samples were used for building the calibration equation. The scatter corrections including none, standard normal variance (SNV) and detrend, and modified multiplicative scatter correction (weighted MSC) were tested to reduce the effects of particle size (Table 3).

Extraction of Ginsenosides. Ground ginseng samples (1 g) were transferred to 15-mL centrifuge tubes followed by the addition of 10 mL of methanol−water (7:3). Sample tubes were placed horizontally on a shaker at 100 rpm for 2 h at room temperature. Sample tubes were then centrifuged at 4000 rpm (10 min), and the solvent was decanted. The extraction was repeated two additional times. The extracts were combined and concentrated to less than 10 mL in a vacuum at room temperature. The concentrated extracts were diluted to 10 mL with 70% methanol and stored at 4 °C. Before ginsenoside analysis, the concentrated ginseng extract was centrifuged at 13 000 rpm for 10 min and the supernatant analyzed by HPLC.

High-Performance Liquid Chromatography (HPLC). HPLC was conducted on a Beckman System Gold liquid chromatograph equipped with a 125 Solvent Module pump and a 166 UV−vis detector. The American ginseng extract solution was separated and analyzed (20 μL aliquots) by using a Merck Superspher RP-18 endcapped column (250 × 4.0 mm; 5 μm) at room temperature. The mobile phase consisted of solvent A (acetonitrile) and solvent B (phosphate buffer solution). Solvent B was prepared by dissolving 2.80 g of KH_2PO_4 in 2000 mL of water and adjusting the pH to 5.81 with a concentrated solution of K_2HPO_4 (35 g/100 mL). For the simultaneous separation of neutral ginsenosides and malonylginsenosides, the following gradient procedure was used: 0−15 min, 20−25% A, 80−75% B; 15−37 min, 25−32% A, 75−68% B; 37−50 min, 32−40% A, 68−60% B; 50−52 min, 40−100% A, 60−0%B; and 62−65 min, 100−20%A, 0−80% B. The flow rate was kept constant at 1.0 mL/min. The absorbance was measured at a wavelength of 203 nm to facilitate the detection of ginsenosides. Chromatographic peaks were identified by comparing retention times against known standards or by comparing their retention times with published data (Chuang and Sheu, 1994; Chuang et al., 1995; William et al., 1996). The standard ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rf, and Rg_1 were purchased from Extrasynthese (Genay, France), and the ginsenoside Ro was obtained from the Institute of Special Plants and Wild Animals, Chinese Academy of Agricultural Sciences, Jilin, China. Since standard malonylginsenosides were not available, the contents of m-Rb1, m-Rb2, m-Rc, and m-Rd were determined as Rb_1, which is the richest ginsenoside in American ginseng roots.

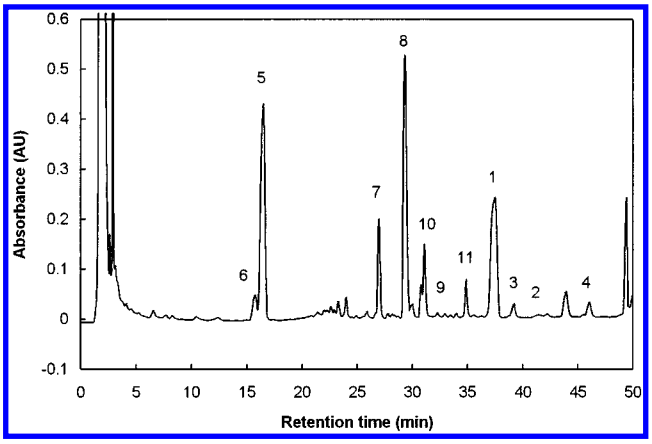

Figure 1. HPLC chromatogram of ginsenosides in American ginseng (*Panax quinquefolium*) roots: Rb_1 (1), Rb_2 (2), Rc (3), Rd (4), Re (5), Rg_1 (6), Ro (7), m-Rb_1 (8), m-Rb_2 (9), m-Rc (10), and m-Rd (11).

RESULTS AND DISCUSSION

Ginsenoside Profile of the Samples. As shown in Figure 1, the chromatographic separation of the neutral ginsenosides, malonylginsenosides, and oleanolic acid-type ginsenoside (Ro) in a single run was achieved by linear gradient elution. The peaks of six neutral ginsenosides, four malonylginsenosides and one oleanolic ginsenoside were well resolved. In agreement with published results, the protopanaxtriol derivatives, Rg_1 and Re, eluted ahead of Ro, m-Rb_1, m-Rc, m-Rb_2, and m-Rd and the protopanaxdiol derivatives, Rb_1, Rb_2, Rc, and Rd, eluted last (Chuang and Sheu, 1994; Chuang et al., 1995; William et al., 1996). Total ginsenosides were the sum of all these individual ginsenosides.

As shown in Table 1, the total ginsenoside content of the root fibers as determined by HPLC was higher than that of the main roots, which was in agreement with a previous report (Smith et al., 1996). Among the main roots, three samples (i.e., R_1, R_2, and R_3) contained less total ginsenosides, less than 2%, while the rest contained 3.33−5.76% (e.g., R_4 and R_5). The big difference in ginsenoside content among the main roots, which was not due to the root size nor due to the production site, was likely due to different preservation times. The total ginsenoside content in the main roots was lower than that in the root fibers. This difference was partially due to the difference in contents of ginsenosides, Rb_2 and Rc, and malonylginsenosides, m-Rb_2 and m-Rc. Very high contents of ginsenoside Rb_2, Rc, m-Rb_2, and m-Rc were found in the two root fibers, while the main roots contained much less Rb_2, Rc, m-Rb_2, and m-Rc. This significant difference may be used for distinguishing root fibers from main roots.

As shown in Table 2, the Rb_2 content in 17 samples and the m-Rb_2 content in 7 samples of the 26 samples were below HPLC detection limits. The major ginsenosides in American ginseng roots were Rb_1, Re, and m-Rb_1, which accounted for about 70% of the total ginsenosides. All ginsenoside content data obtained by the HPLC method were used for building the calibration equation and performing the cross validation. The best calibration equation for each analysis was selected based on the lowest standard error of cross validation (SECV) and highest correlation coefficient of determination (1-VR).

Effect of Scatter Corrections. The calibration performance was improved by using the scatter spectral data. In all cases, the calibration performance using SNV and detrend or weighted MSC was better than that without scatter correction (Table 3).

Table 4. Effects of Derivative Treatment on the Calibration Performance

	first derivative		second derivative		third derivative	
	X ± SECV (%)	1-VR	SECV (%)	1-VR	SECV (%)	1-VR
Rb_1	1.292 ± 0.227	0.815	0.191	0.870	0.186	0.876
Rb_2	0.273 ± 0.096	0.872	0.109	0.836	0.096	0.872
Rc	0.298 ± 0.054	0.952	0.052	0.955	0.060	0.940
Rd	0.091 ± 0.030	0.788	0.027	0.834	0.029	0.806
Re	1.015 ± 0.148	0.829	0.130	0.868	0.114	0.899
Rg_1	0.116 ± 0.020	0.899	0.019	0.916	0.018	0.919
Ro	0.25 ± 0.046	0.097	0.042	0.264	0.040	0.325
m-Rb_1	0.776 ± 0.152	0.736	0.124	0.823	0.116	0.849
m-Rb_2	0.197 ± 0.087	0.864	0.119	0.746	0.074	0.902
m-Rc	0.239 ± 0.097	0.827	0.107	0.791	0.083	0.877
m-Rd	0.143 ± 0.056	0.766	0.050	0.802	0.042	0.869
total	4.393 ± 0.469	0.898	0.387	0.931	0.283	0.963

Table 5. Effects of the Statistical Model on the Calibration Performance

	PCR		PLS		MPLS	
	X ± SECV (%)	1-VR	SECV (%)	1-VR	SECV (%)	1-VR
Rb_1	1.292 ± 0.367	0.516	0.290	0.700	0.186	0.876
Rb_2	0.273 ± 0.134	0.752	0.121	0.798	0.096	0.872
Rc	0.298 ± 0.086	0.876	0.052	0.955	0.060	0.940
Rd	0.091 ± 0.031	0.767	0.027	0.834	0.029	0.808
Re	1.015 ± 0.270	0.731	0.150	0.852	0.114	0.899
Rg_1	0.116 ± 0.019	0.913	0.020	0.899	0.018	0.919
Ro	0.25 ± 0.041	0.294	0.041	0.306	0.040	0.325
m-Rb_1	0.776 ± 0.217	0.459	0.151	0.739	0.116	0.849
m-Rb_2	0.197 ± 0.080	0.886	0.083	0.876	0.074	0.902
m-Rc	0.239 ± 0.148	0.600	0.093	0.840	0.083	0.877
m-Rd	0.143 ± 0.060	0.735	0.047	0.837	0.042	0.871
total	4.393 ± 0.575	0.559	0.379	0.933	0.283	0.963

Effect of Mathematical Treatment. Table 4 shows the statistical results of the calibration equations developed using spectral data (first, second, and third derivatives of log1/*R*). The lowest SECV of the calibration equation for Rb_2 was observed when first and/or third derivatives of log 1/*R* were used. The lowest SECV of the calibration equation for Rc and Rd was observed when second derivatives of log 1/*R* were used. The lowest SECV of the calibration equation for the rest of the ginsenosides and total ginsenosides was observed when third derivatives of log 1/*R* were used.

Effect of Statistical Model. Among the three statistical models (PCR, PLS, and MPLS), PLS was superior to the other two for building calibration equations for Rc and Rd (Table 5). For other individual and total ginsenosides, MPLS was the best.

Best Calibration Equation. The best calibration equation was developed by "try", which refers to evaluating the performance of calibration equations when every combination of derivative treatments, scatter corrections, spectral regions, and statistical models, were tested. The optima for the best calibration equations are present in Table 6. The best calibration equations for the determination of individual ginsenosides and total ginsenosides were chosen based on the lowest SECV and highest 1-VR. The statistical results are summarized in Table 2. As shown in Table 2, the calibration equation of total ginsenosides performed very well with a relative standard deviation (RSD) of only 6.4%. It meant the accuracy of NIRS for total ginsenoside determination is close to that of the HPLC

Table 6. Optima Selected for Building Calibration Equations of Ginsenosides (NIRS Data vs HPLC Value)[a]

ginsenosides	scatter correction	math treatment	max no. of terms	no. of wavelength (nm)	regression method	cross validation groups
Rb_1	SNV[b] and detrend	3,10,10,1[c]	5	400−2500	MPLS[d]	6
Rb_2	weighted MSC[e]	1,10,10,1	4	1100−2500	MPLS	6
		3,10,10,1				
Rc	weighted MSC	2,10,10,1	5	1100−2500	PLS	6
Rd	SNV and detrend	2,10,10,1	4	400−2500	PLS	6
Re	SNV and detrend	3,10,10,1	5	400−2500	MPLS	6
Rg_1	weighted MSC	3,10,10,1	6	1100−2500	MPLS	6
Ro	SNV and detrend or modified MSC	3,10,10,1	5	400−1100	MPLS	6
m-Rb_1	weighted MSC	3,10,10,1	4	400−2500	MPLS	6
m-Rb_2	weighted MSC	1,10,10,1	6	400−2500	MPLS	6
m-Rc	weighted MSC	3,10,10,1	4	400−2500	MPLS	6
m-Rd	SNV and detrend	3,10,10,1	4	400−2500	MPLS	6
total	SNV and detrend	3,10,10,1	6	400−2500	MPLS	6

[a] The selection of optima for building calibration equations was based on the lowest standard error of cross validation (SECV) and highest correlation coefficient of determination (1-VR). [b] Standard normal variance. [c] 3 = third derivative, 10 = the number of data points in the gap over which the derivative is to be calculated, 10 = the number of data points for running averaging, 1 = no smooth for second smooth. [d] Modified partial least-squares regression. [e] Modified multiplicative scatter correction.

method. It was similar to what is reported in Asian ginseng (*Panax ginseng*) (Cho and Lee, 1995). Among the calibration equations for 11 individual ginsenosides, those of the ginsenosides Rb_1, Re, and m-Rb_1 performed relatively well, as judged by the low RSD. values. These three ginsenosides were very rich in American ginseng roots, each individual ginsenoside content was around 1% of dry ginseng roots, the sum of which accounted for 70% of the total ginsenosides. The calibration equation for Rb_2 and m-Rb_2 performed relatively poorly with large RSD values (35.2% and 37.6%, respectively). This might be due to less data used for building the calibration equation. Ro was a very special one, though the 1-VR of its cross validation was the lowest; the RSD of its cross validation was relatively low. This is probably because its concentration changed only very slightly. So the calibration developed here can be used to predict its concentration in American ginseng samples with relatively high accuracy.

Recently the NIRS methods for determining the two natural products (sennoside and agrovalline) have been developed (Molt et al., 1997; Roberts et al., 1997). Together with the work done on ginseng (Corti et al., 1990; Cho and Lee, 1995), three natural products have been determined by NIRS. This suggests that NIRS may have potential in the quality control of herb medicine. Herb medicine has a very complex chemical profile, and authentic standards of its active components are not normally commercially available, which make HPLC methods impractical. NIRS methods can simultaneously predict many components within a short time, and authentic standards are not necessary after the prediction equation is built.

CONCLUSIONS

The first application of NIRS to simultaneously analyze the neutral and malonylginsenosides in American ginseng root powder is reported. Compared with HPLC methods, the NIRS method has many advantages. First, it requires simple sample preparation; the only sample preparation is milling. Second it is fast; analyzing one sample can be finished within several minutes after the calibration equation has been developed. Because it is a spectral procedure, the NIRS procedure requires no chemical reagents, which not only reduces analytical cost, but also provides a safe working environment. However, the NIRS method also has its disadvantages. The precision is lower than that of the HPLC method, but it may be improved by increasing the sample population.

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