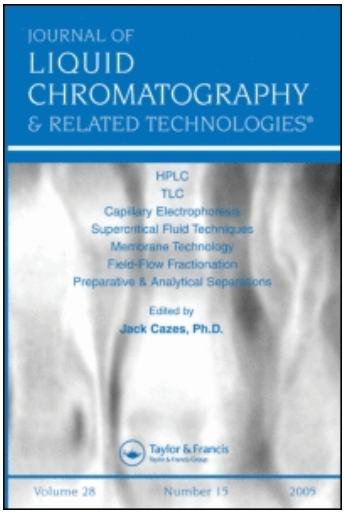


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

HPLC ANALYSIS OF GINSENOSIDES IN THE ROOTS OF ASIAN GINSENG (*PANAX GINSENG*) AND NORTH AMERICAN GINSENG (*PANAX QUINQUEFOLIUS*) WITH IN-LINE PHOTODIODE ARRAY AND EVAPORATIVE LIGHT SCATTERING DETECTION

Wenkui Li[a]; John F. Fitzloff[a]
[a] Functional Food for Health (FFH) Core Analytical Laboratory, Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL, U.S.A.

Online publication date: 01 November 2002

To cite this Article Li, Wenkui and Fitzloff, John F.(2002) 'HPLC ANALYSIS OF GINSENOSIDES IN THE ROOTS OF ASIAN GINSENG (*PANAX GINSENG*) AND NORTH AMERICAN GINSENG (*PANAX QUINQUEFOLIUS*) WITH IN-LINE PHOTODIODE ARRAY AND EVAPORATIVE LIGHT SCATTERING DETECTION', Journal of Liquid Chromatography & Related Technologies, 25: 1, 29 — 41
To link to this Article: DOI: 10.1081/JLC-100108537
URL: http://dx.doi.org/10.1081/JLC-100108537

J. LIQ. CHROM. & REL. TECHNOL., 25(1), 29–41 (2002)

HPLC ANALYSIS OF GINSENOSIDES IN THE ROOTS OF ASIAN GINSENG (*PANAX GINSENG*) AND NORTH AMERICAN GINSENG (*PANAX QUINQUEFOLIUS*) WITH IN-LINE PHOTODIODE ARRAY AND EVAPORATIVE LIGHT SCATTERING DETECTION

Wenkui Li* and John F. Fitzloff

Functional Food for Health (FFH) Core Analytical Laboratory, Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, IL 60612, USA

ABSTRACT

In-line connected ultraviolet (UV) and evaporative light scattering (ELS) detection were compared in the reversed-phase high-performance liquid chromatographic (HPLC) analysis of ginsenosides in ginseng roots. Similar to all the QA/QC literature on ginseng and ginseng products, employing HPLC-UV, the UV detection showed a good linearity in the range of

*Corresponding author. E-mail: wenkuili@uic.edu

100–2000 ng of ginsenosides, with minimum detectable concentration of 10 ng of ginsenosides on the column.

In contrast, the sensitivity of the ELSD was found to be 5 times lower than that obtained with the UV, with minimum detectable concentration of 50 ng of ginsenosides on the column. Further, the calibration curve obtained with the ELSD was exponential linear in the range of 200–2000 ng of ginsenosides. Quantitative evaluation in terms of ginsenosides using both methods of detection showed similar results. But, in view of the better sensitivity and easier handling, the UV is still recommended for the routine analysis of ginseng samples.

INTRODUCTION

Ginseng has been widely used as a tonic and restorative agent in Chinese traditional medicine. (1) During the last decade, the consumption of ginseng and ginseng products have expanded outside of the Far East to the level of a commonly used herbal medicine. Although several compounds in ginseng root extracts may exert biological effects, it is well known that ginsenosides are the compounds mainly responsible for the effects ascribed to ginseng. (2–4) Ginseng and ginseng products are usually standardized and characterized according to the content of ginsenosides. (5) The amount of ginsenosides in ginseng roots, however, vary greatly, depending on the time of harvesting, growing environment, and species. There is, therefore, a real need for a rapid, simple, and reliable methodology to determine the content of ginsenosides in the ginseng roots in order to ensure a constant quality of ginseng products.

Measurement of ginsenosides can be accomplished by means of thin layer chromatography (TLC)-densitometry, (6–7) radioimmunoassay, (8) droplet counter current chromatography (DCCC), (9–10) gas chromatography (GC), (11–13) and high performance liquid chromatography (HPLC), (10,14–30) with HPLC having been studied extensively for the routine assessment of quality assurance (QA) and quality control (QC) of ginseng and ginseng products.

Several detection techniques have been employed in the HPLC analysis, including UV (10,14–24) pulsed amperometry, (25) photo-reduction fluorescence, (26) and refractometry. (27) Among these techniques, UV was utilized in most of the published literature. Evaporative light scattering detection (ELSD) was mainly used for biological molecules such as triglycerides, fatty acid esters, steroids, and sugars, which lacked appropriate chromophores for UV absorbance based detection. It has recently enjoyed renewed interest as an alternative to UV in the saponin analysis; in part because of the dramatic improvements in the design of the instruments, which have made the ELSD more sensitive than before.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

In 1996, Park et al, first reported an HPLC-ELSD methodology to analyze ginsenosides in Asian ginseng (*Panax ginseng*). The minimum detectable concentration was reported to be more than 35 ng of ginsenosides on the column. It was also claimed in the paper that the HPLC-ELSD method is superior to the HPLC-UV method with respect to both sensitivity and separation. (28)

The ELSD is now complementary to, and competitive with the UV in the routine analysis of ginseng because it can provide a stable baseline when gradient elution is employed, even when buffer is used in the mobile phase. (29) However, one concern is the comparability of the technique with the UV in routine analysis. Further, the ELSD might have a lower sensitivity when compared with that of the UV, although it could detect all the compounds in the effluents, especially those without double bonds, e.g., 24(*R*)-pseudoginsenoside F_{11}, a characteristic compound in North American ginseng (*Panax quiquefolius*). (30)

Based on a project aimed at evaluating HPLC-ELSD in routine analysis of biologically active components in herbal extracts, this article reports an in-line comparison of sensitivity, linearity, and reproducibility of UV versus ELSD for the HPLC qualitative and quantitative analysis of ginsenosides in ginseng roots, under identical conditions.

EXPERIMENTAL

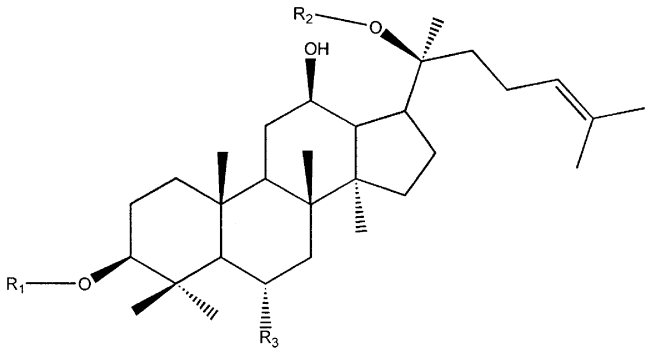
Plant Material and Chemicals

Samples of Asian ginseng (*Panax ginseng*) and North American ginseng (*P. quinquefolius*) roots were obtained from the Institute of Chinese Materia Medica, Chinese Academy of Chinese Traditional Medicine, Beijing, China and ginseng farms located in Wisconsin, USA, respectively, and the voucher specimens are deposited in the herbarium of the University of Illinois at Chicago Pharmacognosy Field Station, Downer's Grove, IL 60515, USA. The samples were ground and mixed thoroughly before use. Ginsenoside standards (Rg_1, Re, Rb_1, Rc, Rb_2 and Rd, Figure 1) were isolated and identified in the Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA.

Instrumentation

All HPLC analyses were performed using a Waters Alliance (Waters, Milford, MA, USA) 2690 model instrument with a model 996 photodiode array detector (PDA). The detection wavelength range of the PDA was set at 195–210 nm. The evaporative light scattering detector (ELSD) model Sedex 75 (Cedex

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016



Ginsenoside	R_1	R_2	R_3
Rg_1	H	glc	O-glc
Re	H	glc	O-glc[2-1]rha
Rb_1	glc[2-1]glc	glc[6-1]glc	H
Rc	glc[2-1]glc	glc[6-1]ara(f)	H
Rb_2	glc[2-1]glc	glc[6-1]ara(p)	H
Rd	glc[2-1]glc	glc	H

(glc: glucose; ara (p): α-L-arabinopyranose; ara (f): α-L-arabinofuranose; rha: rhamnose)

Figure 1. Structures of ginsenosides Rg_1, Re, Rb_1, Rc, Rb_2, and Rd.

94141, Alfortville, France) was connected in series to the PDA and the ELSD was interfaced to the Waters Millennium 2000® chromatographic manager system (Waters, Milford, MA, USA) loaded on a Compaq computer (Houston, TX, USA) using a SATIN box for data handling and chromatogram generation. The HPLC system was cleaned approximately once a month using 30% phosphoric acid, followed by DI water, and 80% methanol in water, consecutively.

Standard Solutions and Calibration Curves of Ginsenosides

The ginsenoside Rg_1, Re, Rb_1, Rc, Rb_2 and Rd were dissolved in HPLC grade methanol (Fisher Scientific Co. Fair Lawn, NJ, USA) to make a stock solution. It should be noted, that this stock solution was found to be stable for more than two months when stored at −20°C. Calibration standards were prepared by diluting the stock solution with methanol in appropriate quantities. A series of calibration standard solutions were made at the concentrations of 10, 20, 40, 80, 120, 160, and 200 μg/mL for each ginsenoside. Three controls were

prepared at 37.5, 62.5, and 125 μg/mL for ginsenoside Rg_1, Re, and Rb_1, respectively. In the UV detection, the calibration curve was generated by plotting the peak area under curve versus the concentration of standards. In the calibration of the ELSD, the calibration curve was generated by plotting log-transformed peak area under curve versus log-transformed concentration of the standards. The regression equation was used to determine the concentration of control and unknown samples.

Preparation of Sample Solutions

An accurately weighed portion of sample (0.5 g) was placed into a tarred 50-mL flask. 15 mL of methanol was added and shaken to mix. The mixture was sonicated at 25–30°C for 30 min, cooled, and filtered through filter paper (Whatman #40) into a 250-mL round-bottom flask, and the residue returned to the flask. Another 15 mL of methanol was added and the mixture was sonicated at 25–30°C for 30 min. The extract was filtered into the same round-bottom flask, and the residue was washed with methanol (3×15 mL) while on the filter. The combined methanol extracts were brought to dryness, *in vacuo*, at 45–50°C. The residue was redissolved with methanol (4×2 mL) and the solution transferred to a 10-mL volumetric flask, and made up to the volume with methanol. The sample solution was filtered directly into a HPLC sample vial, using a 0.2 μm Whatman hydrophilic membrane filter (Whatman Inc., Clifton, NJ, USA) just prior to HPLC analysis.

Chromatography

The chromatographic separations were carried out on a Waters Spherisorb ODS-2 C_{18} column (250×4.6 mm, 5 μm particle size, Serial # 0123391941 L, Waters, Milford, MA, USA) protected by a Waters Delta-Pak C_{18} guard column (Waters Technological Ireland, Ltd, Wexford, Ireland) and set at 20°C. The mobile phase used for the separation consisted of solvent A (water, DI) and solvent B (acetonitrile, Fisher Scientific Co., Fair Lawn, NJ, USA). The elution profile was: 0 → 20 min, 20% B; 20–60 min, 20 → 42% B; 60 → 61 min, 42 → 90% B; 61 → 71 min (wash out), 90% B; 71 → 72 min, 90 → 20% B; 72 → 80 min, 20% B (reconditioning); all gradient steps were linear. The flow rate was set to 1.6 mL/min. The column temperature was fixed at 20°C and the injection volume was chosen to be 10 μL.

The peak identifications were based on retention times, and comparison with injected authentic reference compounds. The peaks were detected, consecutively, with the UV and the ELSD. In the UV, the detection wavelength

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

was set at 203 nm. After the UV detector, the eluent was transferred to the ELSD with a gain of 11, the evaporation chamber temperature at 35°C, and the nebulizing gas pressure at 3.4 bar. Prior to each run, the HPLC-UV-ELSD system was allowed to warm up for 20–30 min and the pumps were primed using the protocol suggested by the manufacturer. Using freshly prepared mobile phase, the baseline was monitored until stable before the samples were run.

Quantification

Asian ginseng (*Panax ginseng*) and North American ginseng (*P. quinquefolius*) were extracted, following the procedure as above, and analyzed in triplicate. Concentrations of ginsenoside Rg_1, Re, Rb_1, Rc, Rb_2, and Rd were interpolated using the calibration graphs made with external standards.

Reproducibility

The reproducibility of the UV and the ELSD were determined by analyzing three quality control solutions containing a known amount of ginsenoside Rg_1, Re, and Rb_1, respectively. Each solution was injected three times and the concentrations of ginsenosides, Rg_1, Re and Rb_1, were calculated using the calibration graphs. The precision was determined by calculating the relative standard deviation (RSD). The accuracy was estimated by comparing the average of measured values and the actual concentration of quality control solutions.

RESULTS AND DISCUSSION

Chromatography and Sensitivity

The main aims of the current study were to compare the ELSD with the UV for the qualitative and quantitative analysis of ginsenosides in ginseng roots.

For the quantitation of ginsenosides in ginseng roots, the present HPLC method was developed, because not only could the ginsenosides be simultaneously determined, but also, the different ginsenosides in each group were well separated in 60 minutes with baseline resolution.

In the UV, the detection was highly sensitive with identical minimum detectable concentrations ($S/N > 3$) of 10 ng on the column for all the ginsenosides analyzed in the current assay.

In ELSD, a constant nebulization process is important for satisfactory repeatability. Several factors have influence on the average diameter of the

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

droplets and their distribution, which include density, viscosity, and liquid surface tension. Among these factors, the nebulizer gas flow rate affects the signal responses most significantly. When the gas flow rate is too low, large droplets are formed, resulting in spikes and random noise. But, when the gas flow rate is too high, the droplets decrease in size, which results in a decreased signal response. The optimum nebulizer gas (nitrogen) pressure in this work was determined to be 3.4 bar. The evaporating temperature is also an important parameter affecting the signal response.

At low temperature, solvent evaporation is not complete, and at high temperature, the detector response is decreased, owing to the decrease in particle size by improper vaporization of the nebulized analytes in the drift tube. The signal–noise ratio (S/N) was improved when the temperature was lowered to 35°C. Also the gain in ELSD was set at 11 in order to obtain the best sensitivity. (31) Compared with the UV, the ELSD showed a lower sensitivity with minimum detectable concentration of 50 ng (S/N > 3) of ginsenosides on the column.

Calibration

As indicated in the literature,(14–24) the calibration curve generated in the UV detector was linear with the peak area against the concentration (r^2 being more than 0.998 for all ginsenosides) in the range of 100–2000 ng. In the ELSD, second-order polynomial calibration curves were observed with peak area against concentration. After log-transformation, the plot of the logarithm of the peak area versus the logarithm of the concentration was a straight line with r^2 more than 0.997 for all ginsenosides.(29–30) Calibration range, detection limits, and correlation coefficient for each ginsenoside in the UV and the ELSD are summarized in Table 1.

Reproducibility

The reproducibility of both detectors was validated on the basis of analysis of three sets of quality control solutions, containing 37.5, 62.5, and 125 μg/mL of ginsenoside Rg_1, Re, and Rb_1, respectively. The results are shown in Table 2. At three different concentrations, the coefficients of variation for the UV were found to be less than 4.24% and the mean relative errors ranged from −1.5 to −5.67%. In the ELSD, the coefficients of variation were found to be less than 9.89% and the mean relative errors ranged from −1.43 to −8.35%, respectively.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 1. Determination of Linearity of Calibration of Ginsenosides Employing UV and ELSD

Ginsenoside	Detection Mode	Regression Curve	Correlation Coefficient (r^2)
Rg_1	UV (10–200 μg/mL)	Y = 326.6287X−5841.978	0.9992
	ELSD (20–200 μg/mL)	Y = 1.6132X+0.709	0.9982
Re	UV (10–200 μg/mL)	Y = 317.7215X−9370.835	0.9987
	ELSD (20–200 μg/mL)	Y = 1.793X+0.248	0.9976
Rb_1	UV (10–200 μg/mL)	Y = 230.0347X−3038.414	0.9987
	ELSD (20–200 μg/mL)	Y = 1.6675X+0.8587	0.9998
Rc	UV (10–200 μg/mL)	Y = 259.1406X−2888.536	0.9992
	ELSD (20–200 μg/mL)	Y = 1.668X+0.9248	0.9992
Rb_2	UV (10–200 μg/mL)	Y = 281.5289X−5719.268	0.9992
	ELSD (20–200 μg/mL)	Y = 1.7153X+0.8117	0.9997
Rd	UV (10–200 μg/mL)	Y = 205.1927X−3691.178	0.9992
	ELSD (20–200 μg/mL)	Y = 1.6385X+0.7542	0.9997

Sample Analysis

Both techniques gave reliable and reproducible results, both in qualitative and quantitative terms. Table 3 compared the contents (percentage of dry weight) and relative standard deviations of six ginsenosides in Asian ginseng (*Panax ginseng*, Figure 2) and North American ginseng (*Panax quinquefolius*, Figure 3). The reported values are the means of three separate extractions of each sample. The results with the two techniques are comparable for all ginsenosides tested herein, although, most of the ELSD% values and standard deviations are, in all cases, slightly higher than those obtained with the UV.

Table 2. Relative Standard Deviation (RSD, %) and Accuracy (RE, %) of Integration Results for Ginsenosides Rg_1, Re and Rb_1[a]

	Rg_1			Re			Rb_1		
	RSD (%)[b]	RE (%)[b]	Concentration (μg/mL)	RSD (%)[b]	RE (%)[b]	Concentration (μg/mL)	RSD (%)[b]	RE (%)[b]	Concentration (μg/mL)
LC-UV	4.24	−1.53	37.5	4.03	2.39	62.5	2.93	−5.67	125
LC-ELSD	9.89	−1.43	37.5	4.88	9.35	62.5	4.23	−8.35	125

[a]The amount of the compounds (ng) are reported in parentheses.
[b]n = 3.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 3. Concentration of Ginsenoside Rg_1, Re, Rb_1, Rc, Rb_2, and Rd in the Roots of Asian Ginseng (*Panax ginseng*) and North American Ginseng (*P. quinquefolius*) by HPLC-UV and HPLC-ELSD

	Rg_1	Re	Rb_1	Rc	Rb_2	Rd
Asian ginseng (%, w/w)[a]						
LC-UV	0.32 ± 0.005	0.34 ± 0.025	0.80 ± 0.013	0.53 ± 0.012	0.40 ± 0.010	0.39 ± 0.007
LC-ELSD	0.32 ± 0.029	0.38 ± 0.004	0.85 ± 0.030	0.58 ± 0.026	0.41 ± 0.008	0.42 ± 0.002
North American ginseng (%, w/w)[a]						
LC-UV	0.17 ± 0.003	1.04 ± 0.027	2.74 ± 0.352	0.32 ± 0.007	0.054 ± 0.001	0.63 ± 0.003
LC-ELSD	0.20 ± 0.007	1.07 ± 0.058	3.22 ± 0.162	0.39 ± 0.016	0.050 ± 0.002	0.66 ± 0.053

[a]Values are the mean of 3 samples $\pm$ error for the calculated concentration.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

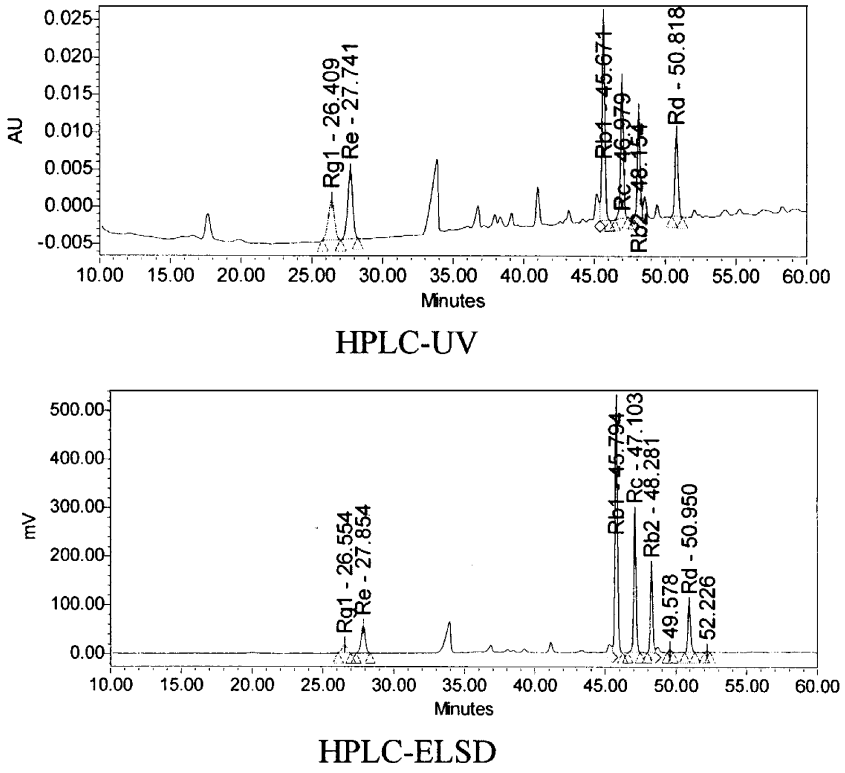


Figure 2. The typical HPLC-UV and HPLC-ELSD chromatograms of Asian ginseng (*Panax ginseng*) methanolic extract.

As expected, the HPLC-UV analysis is much easier in setting the detector parameters than that of HPLC-ELSD; using HPLC-ELSD, it is necessary to optimize three important parameters, e.g., nebulizing gas pressure, evaporating temperature, and gain. But, as long as these parameters have been optimized, they could be set for routine analysis.

CONCLUSIONS

An in-line comparison of ultraviolet (UV) and evaporative light scattering (ELS) detections was carried out in the reverse-phase high performance liquid chromatographic (HPLC) analysis of ginsenosides in ginseng roots. These two detectors were found to be comparable with regard to sensitivity, calibration, and

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

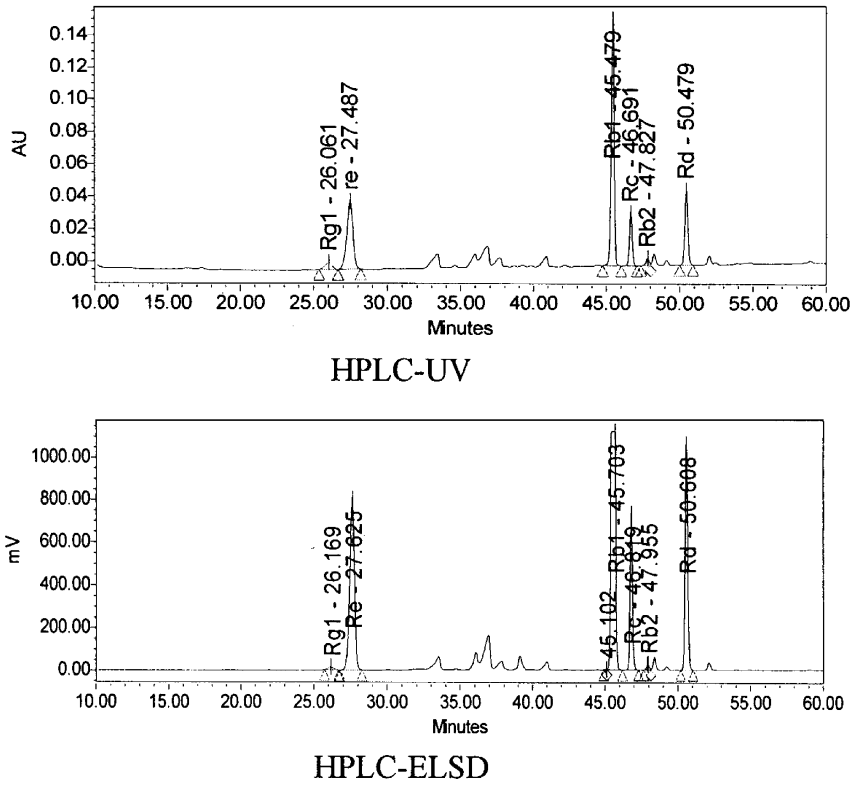


Figure 3. The typical HPLC-UV and HPLC-ELSD chromatograms of North American ginseng (*Panax quinquefolius*) methanolic extract.

reproducibility. Similar results were obtained for the quantitation of six ginsenosides in Asian ginseng (*Panax ginseng*) and North American ginseng (*P. quinquefolius*). Further studies to find applications of HPLC-ELSD and HPLC-UV-ELSD in ginseng analysis are presently under way.

REFERENCES

1. Liu, C.X.; Xiao, P.G. J. Ethnopharmacol. **1992**, *36*, 27–38.
2. Attele, A.S.; Wu, J.A.; Yuan, C.S. Biochem. Pharmacol. **1999**, *58*, 1685–1693.
3. Gillis, C.N. Biochem. Pharmacol. **1997**, *54*, 1–8.

4. Shibata, S.; Tanaka, O.; Shoji, J.; Saito, H. Chemistry and Pharmacology of *Panax*. In *Economic and Medicinal Plant Research*; Wagner, H., Hikino, H., Farnsworth, N.R., Eds.; Academic Press: Orlando, 1985; Vol. I, 218–284.
5. Soldati, F. *Panax ginseng*: Standardization and Biological Activity. In *Biologically Active Natural Products: Pharmaceutics*; Cutler, S.J., Cutler, H.G., Eds; CRC Press: Orlando, FL. 1999; 209–232.
6. Corthout, J.; Naessens, T.; Apers, S.; Vlietinck, A.J. J. Pharm. Biomed. Anal. **1999**, *21*, 187–192.
7. Vanhaelen-Fastre, R.T.; Faes, M.L.; Vanhaelen, M.H. J. Chromatogr. A **2000**, *86*, 269–276.
8. Yoon, S.R.; Nah, J.J.; Kim, S.K.; Kim, S.C.; Nam, K.Y.; Jung, D.W.; Nah, S.Y. Chem. Pharm. Bull. **1998**, *46*, 114–147.
9. Otsuka, H.; Morita, Y.; Ogihara, Y.; Shibata, S. Planta Med. **1977**, *32*, 9–17.
10. Yip, T.T.; Lau, C.N.B.; But, P.P.H.; Kong, Y.C. Am. J. Chin. Med. **1985**, *13*, 77–88.
11. Bombardelli, E.; Bonati, A.; Gabetta, B.; Martinelli, E.M. J. Chromatogr. **1980**, *196*, 121–132.
12. Cui, J.F.; Garle, M.; Lund, E.; Bjorkhem, I.; Eneroth, P. Anal. Biochem. **1993**, *210*, 411–417.
13. Cui, J.F.; Bjorkhem, I.; Eneroth, P. J. Chromatogr. B **1997**, *689*, 349–355.
14. Ma, Y.C.; Luo, M.; Lynn, M.; Monique, D. Chin. J. Med. Chem. **1996**, *6*, 11–21.
15. Chuang, W.C.; Wu, H.K.; Sheu, S.J.; Chiou, S.H.; Chang, H.C.; Chen, Y.P. Planta Med. **1995**, *61*, 459–465.
16. Chuang, W.C.; Sheu, S.J. J. Chromatogr. **1994**, *685*, 243–251.
17. Court, W.A.; Hendel, J.G.; Elmi, J. J. Chromatogr. **1996**, *755*, 11–17.
18. Kanazawa, H.; Nagata, Y.; Matsushima, Y.; Tomoda, M.; Takai, N. J. Chromatogr. **1993**, *630*, 408–414.
19. Li, T.S.C.; Mazza, G.; Cottrel, A.C.; Gao, L. J. Agric. Food Chem. **1996**, *44*, 717–720.
20. Pietta, P.; Mauri, P.; Rava, A. J. Chromatogr. **1986**, *356*, 212–219.
21. Samukawa, K.I.; Yamashita, H.; Matsuda, H.; Kubo, M. Chem. Pharm. Bull. **1995**, *43*, 137–141.
22. Soldati, F.; Sticher, O. Planta Med. **1980**, *38*, 348–357.
23. Yamaguchi, H.; Kasai, R.; Matsuura, H.; Tanaka, O.; Fuwa, T. Chem. Pharm. Bull. **1988**, *36*, 3468–3473.
24. Yamaguchi, H.; Matsuura, H.; Kasai, R.; Tanaka, O.; Satake, M.; Kohda, H.; Izumi, H.; Nuno, M.; Katsuki, S.; Isoda, S.; Shoji, J.; Goto, K. Chem. Pharm. Bull. **1988**, *36*, 4177–4181.
25. Park, M.K.; Park, J.H.; Lee, M.Y.; Kim, S.J.; Park, I.J. J. Liq. Chromatogr. **1994**, *17*, 1171–1182.

26. Park, M.K.; Kim, B.K.; Park, J.H.; Shin, Y.G.; Cho, K.H. J. Liq. Chromatogr. **1995**, *18*, 2077–2088.
27. Zhou, Z.H.; Zhang, G.D. Acta Pharm. Sin. **1988**, *23*, 137–141.
28. Park, M.K.; Park, J.H.; Han, S.B.; Shin, Y.G.; Park, H.I. J. Chromatogr. **1996**, *736*, 77–81.
29. Fuzzati, N.; Gabetta, B.; Jayakar, K.; Pace, R.; Ramaschi, G.; Villa, F. J. AOAC Internatl. **2000**, *83*, 820–829.
30. Li, W.K.L; Fiotzloff, J.F.. J. Pharm. Biomed. Anal. **2001**, *25*, 257–265.
31. Hsu, B.H.; Orton, E.; Tang, S.Y.; Carlton, R.A. J. Chromatogr. B **1999**, *725*, 103–112.

Received June 8, 2001
Accepted July 5, 2001
Manuscript 5608

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Request Permission or Order Reprints Instantly!