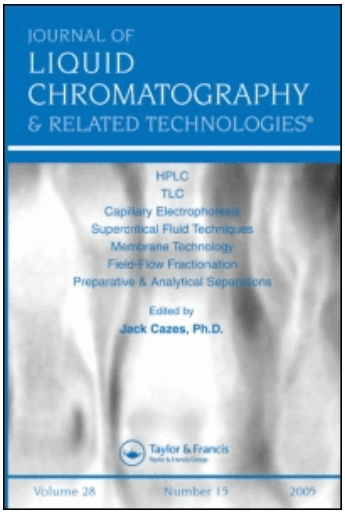

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HPLC WITH EVAPORATIVE LIGHT SCATTERING DETECTION AS A TOOL TO DISTINGUISH ASIAN GINSENG (*PANAX GINSENG*) AND NORTH AMERICAN GINSENG (*PANAX QUINQUEFOLIUS*)

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HPLC WITH EVAPORATIVE LIGHT SCATTERING DETECTION AS A TOOL TO DISTINGUISH ASIAN GINSENG (*PANAX GINSENG*) AND NORTH AMERICAN GINSENG (*PANAX QUINQUEFOLIUS*)

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ABSTRACT

A high performance liquid chromatography-evaporative light scattering detection (HPLC-ELSD) method was developed to identify Asian ginseng (*Panax ginseng* C. A. Meyer) and North American ginseng (*P. quinquefolius* L.), and their products. The method is based on the baseline chromatographic separation of ginsenoside Rf and 24(*R*)-pseudoginsenoside F_{11}, two potential chemical markers present in the methanolic extracts of ginseng roots and their products, and their on-line detection using an evaporative light scattering detector. As a

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result, ginsenoside Rf could be detected only in Asian ginseng and 24(*R*)-pseudoginsenoside F_{11} could only be detected in North American ginseng in the current study.

The method developed is very simple and highly sensitive down to the nanogram level.

INTRODUCTION

The dietary supplements market has been growing exponentially in the past few years with US domestic sales having ballooned to more than $14 billion a year. Topping the list of botanicals is ginseng. (1) Ginseng mainly includes Asian ginseng and North American ginseng. Pharmacognostically, Asian ginseng is the name given to the roots of *Panax ginseng* C. A. Meyer, while North American ginseng is the roots of *Panax quinquefolius* L. Research studies have demonstrated that ginseng possesses antistress, CNS-stimulating, antidiabetes and anticancer activities, and these biological activities have been well documented and reviewed. (2–7) However, Asian ginseng and North American ginseng are well known to have different properties and medical values in ethnopharmacology. (8) The red form of Asian ginseng, produced by steaming, is "warm" and known to replenish the "vital energy", whereas North American ginseng is "cool" and is mainly used for reducing the "internal heat" and promoting the secretion of body fluids. (8–9)

In the ginseng market, North American ginseng is usually 5–10 times more expensive than that of Asian ginseng. (10–11) Since the appearance of the roots of these ginsengs resemble each other, and many commercial ginseng products are in the form of powder, shredded slices, or preparations, it is not uncommon to find incorrect species as well as mixed species in the ginseng products. (9) Therefore, a convenient method for the identification of the origin of ginseng products is highly desirable.

Traditionally, the identification of these two species is based on morphological and histological characteristics, (8) but it is incapable of distinguishing Asian ginseng from North American ginseng in preparations. Recently, the utilization of DNA analysis for the identification of these two species has been reported. (10–12) The technique involves the extraction of DNA, PCR, and data analysis. However, the whole procedure might be time consuming, and the technique might not be available in every QA/QC laboratory. On the other hand, the positive result of DNA analysis relies mainly on sufficient amount of DNA. If template DNA in ginseng or ginseng products is degraded, and if ginseng products are not in the form of roots, slices, powder, but in the form of preparations (e.g., dried extracts, softgel, etc), in which the template

DNA might be absent, it is very difficult to get distinguishable DNA analysis result. This limits the application of the method. (10–12)

Chemically, the isolation and characterization of active constituents in ginseng, i.e., ginsenosides, was started about 100 years ago. (13) More than 30 ginsenosides have been reported by laboratories around the world, (4,13–14) and ginsenosides Rg_1, Re, Rb_1, Rc, Rb_2, Rf, Rd, Rg_2 and 24(*R*)-pseudoginsenoside F_{11} have become commercially available recently. Among these ginsenosides, ginsenoside Rf (Rf, **1**) and 24(*R*)-pseudoginsenoside F_{11} (F_{11}, **2**) (Fig. 1) has aroused more interest than others, because Rf, but not F_{11}, was isolated from Asian ginseng, (15) whereas F_{11}, but not Rf, was obtained from North American ginseng. (16) Methods based on the presence or absence of Rf and F_{11} for the identification of Asian ginseng and North American ginseng samples have been explored, which included TLC, (17) HPLC-MS(9) and HPLC-MS-MS. (18–19) The TLC method is relatively convenient, but its selectivity and sensitivity presents an increasing challenge. Compared to TLC, LC-MS, especially LC-MS-MS with multiple reaction monitoring (MRM), provides a higher selectivity, sensitivity, and reproducibility, and it gives unambiguous identification of the

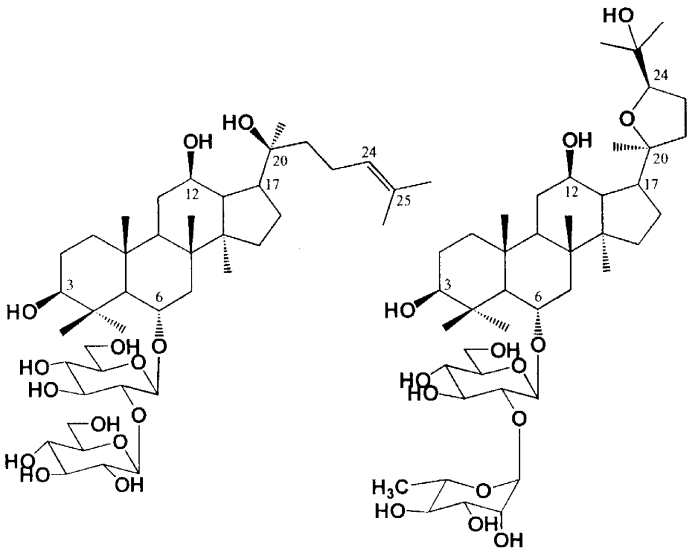

Figure 1. Chemical structures of ginsenoside Rf (**1**) and 24(*R*)-pseudoginsenoside F_{11} (**2**).

compound of interest. (9,18–19) While LC-MS or LC-MS-MS methods may work very well for the identification of Asian ginseng and North American ginseng to be tested, the equipment is relatively expensive and may not be available in every laboratory. Further, F_{11} (**2**) is a very poor chromophore, with a very weak UV absorption at the 201–203 nm range, even when more than 1 μg of standard was injected into HPLC-UV system. (20) This limits the application of the HPLC-UV. It would be useful to develop an alternative HPLC method using the relatively inexpensive evaporative light scattering detector (ELSD) for routine analysis.

The ELSD is a mass detection method. Its response does not depend on chromophores, but on the number and size of analyte particles produced by nebulization of the column effluents and evaporation of the mobile phase. (21) In 1996, Park, et al. first reported an HPLC-ELSD method to analyze ginsenosides in Asian ginseng, with minimum detectable concentration of more than 35 ng of ginsenoside on the column. (22) The ELSD is now complementary to 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. (23) In the current paper, a simple HPLC-ELSD method for the identification of Asian and North American ginseng and their preparations is reported for the first time.

EXPERIMENTAL

Chemicals

HPLC grade methanol and acetonitrile were obtained from Fisher Scientific Co. (Fair Lawn, NJ, USA). Deionized water was generated via an in-home Nanopure® water system (Barnstead, Newton, MA, USA). Ginsenosides Rg_1, Re, Rf, Rb_1, Rc, Rb_2, Rd, and 24(*R*)-pseudoginsenoside F_{11} 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.

Ginseng Samples

Asian ginseng (*Panax ginseng*): (1) Chinese pharmacopeial standard ginseng root powder (#917-9202) was purchased from the Institute for the Control of Pharmaceutical and Biological Products, Beijing, China; (2) Sun-dried white ginseng roots were obtained from the Institute of Chinese Materia Medica, Chinese Academy of Chinese Traditional Medicine, Beijing, China; (3) Heavenly grade red ginseng powder was provided by the Korean Ginseng and Tobacco Institute, Taejon, Korea. North American ginseng (*Panax quinquefolius*): (1) Root samples were obtained from the ginseng farms located in Wisconsin (USA) and

Table 1. HPLC-ELSD Assay Results of Commercial Ginseng Products

Sample	Type	Origin Claimed	Origin Identified
A	capsule	Asian ginseng	Asian ginseng
B	powder	Asian ginseng	Asian ginseng
C	powder	Asian ginseng	Asian ginseng
D	powder	North American ginseng	Mixture of Asian ginseng and North American ginseng
E	softgel	Asian ginseng	Asian ginseng
F	roots	North American ginseng	North American ginseng
G	capsule	North American ginseng	North American ginseng

British Columbia (Canada); (2) Powdered ginseng root sample was obtained from the ginseng farms located in Ontario (Canada).

Commercial ginseng and products, in the form of roots, powder, capsule, and softgel, were obtained though different sources. To protect manufacturers' identity, the samples were labeled A-G (Table 1).

Sample Preparation

1) Ginseng roots, powder, or capsule: a portion of finely pulverized ginseng roots, powder, or the powder from a capsule (~ 0.5 g), was accurately weighed into a 20-mL PTFE capped sample vial and extracted with methanol (3×15 mL) with a sonicator at 25–30°C. The combined extracts were evaporated under reduced pressure at 45–50°C. The residue was dissolved with methanol (4×2 mL) into a 10-mL volumetric flask, and made up to the volume in 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.

2) Ginseng soft gel: the ginseng soft gel was cut with a razor blade and the content was accurately weighted into a 50-mL flask. 10 mL of solvent **I** (hexane : methanol : water, 4 : 3 : 2, upper phase) was added and mixture was sonicated for 3–5 min at 25–30°C. The solution was transferred to a 125-mL separatory funnel. To the residue, 10 mL of solvent **II** (hexane : methanol : water, 4 : 3 : 2, lower phase) was added and the mixture was sonicated for 3–5 min at 25–30°C. The solution was transferred to the same separatory funnel. The above extraction procedure was repeated twice. The resulting extract (~ 60 mL) was

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shaken and left standing until the upper and lower phases were totally separated. The lower phase was collected into a 250-mL round bottom flask. To the separatory funnel, 10 mL of solvent **II** was added and the funnel was thoroughly shaken and left standing. The resulting lower phase was collected into the same flask. This procedure was repeated one more time. The combined lower phase ($\sim$50 mL) was evaporated under reduced pressure at 45–50°C, and the resulting residue was dissolved and transferred with about 5 mL of methanol and about 4 mL of methanol/water (1:1), consecutively, to a 10-mL volumetric flask and made up to the volume with methanol. The sample solution was filtered through a 0.2 μm Whatman hydrophilic membrane filter (Whatman Inc., Clifton, NJ, USA) into a HPLC sample vial immediately before HPLC analysis.

HPLC-ELSD Analysis

Chromatographic separations were performed using a Waters 2690 high performance liquid chromatograph (Waters, Milford, MA, USA) equipped with an auto-sampler and connected to a 996 photodiode array detector (PDA) (Waters, Milford, MA, USA). After the PDA detector, the effluent was directed to a Sedex 75 evaporative light scattering detector (94141 Alfortville Cedex, France). The chromatographic separation was carried out on a Waters Spherisorb ODS-2 RP-18 column (250 × 4.6 mm, 5 μm particle size, Serial #0123391941L-013, USA) protected by a Waters Delta-Pak RP-18 guard column (Waters Technology, Limited, Wexford, Ireland) and set at room temperature. Mobile phases were water, solvent A; acetonitrile, solvent B, with a flow rate of 1.6 mL/min and the following gradient: 0 → 20 min, 20% B; 20–60 min, 20 → 42% B (data acquiring); 60 → 61 min, 42 → 90% B; 61 → 71 min, 90% B (column washing); 71 → 72 min, 90 → 20% B; 72 → 80 min, 20% B (column equilibrating). Samples were introduced by an autosampler with a 10 μL injection volume. The UV-Vis detection wavelength was set at 203 nm. The nebulization of the eluent in the ELSD was provided by a stream of compressed air at 3.4 bar.

The nebulization was performed at room temperature and evaporation chamber temperature was set at 35°C. The gain was set at 11. The ELSD output was interfaced, using a Satin box, to the Waters Millenium 2000® Chromatographic manager system (Waters, Milford, MA, USA) loaded on a Compaq 6400X/10000/CDS computer (Houston, TX, USA) for data handling and chromatogram generation. Authentic ginsenoside standards Rg_1, Re, Rb_1, Rc, Rb_2, Rf (**1**), F_{11} (**2**), and Rd were injected between every three samples for peak identification.

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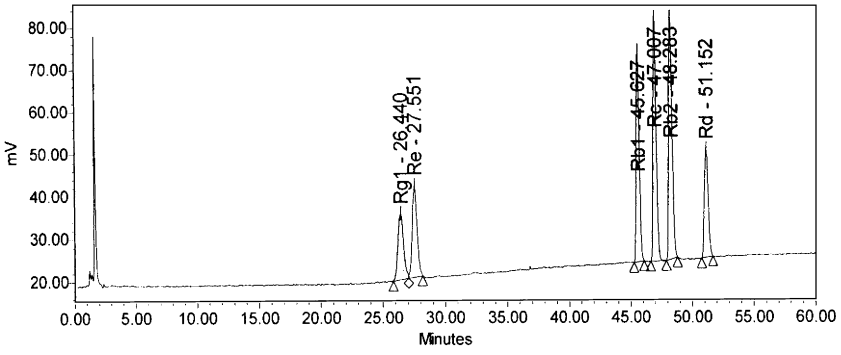
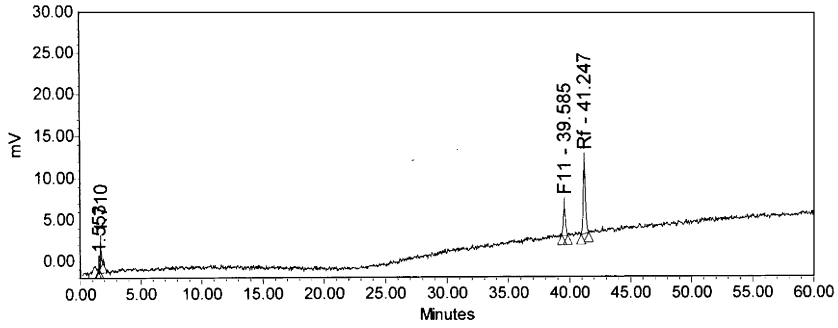
RESULTS AND DISCUSSION

Separation of Ginsenosides

Figures 2 and 3 show the chromatograms of ginsenoside reference standards. Ginsenosides Rg_1, Re, Rf (**1**), F_{11} (**2**), Rb_1, Rc, Rb_2, and Rd were separated on baseline with retention times of 26.44, 27.55, 41.25, 39.59, 45.63, 47.01, 48.28, and 51.15 min, respectively (Fig. 2). As indicated in the figures, F_{11} (**2**) shows a very weak UV absorbance even though 1.06 μg of it was injected into the system (Fig. 3A). In contract, ELSD showed good sensitivity with minimum detectable concentration of 32 ng on the column in current study (Fig. 3B). The detection limits for ginsenosides Rg_1, Re, Rf, Rb_1, Rc, Rb_2, and Rd were observed at 50 ng (each) on the column.



2A



2B

Figure 2. The typical HPLC-ELSD chromatograms of ginsenoside Rg_1, Re, Rb_1, Rc, Rb_2, Rd (2A), Rf, and F_{11} standards (2B).

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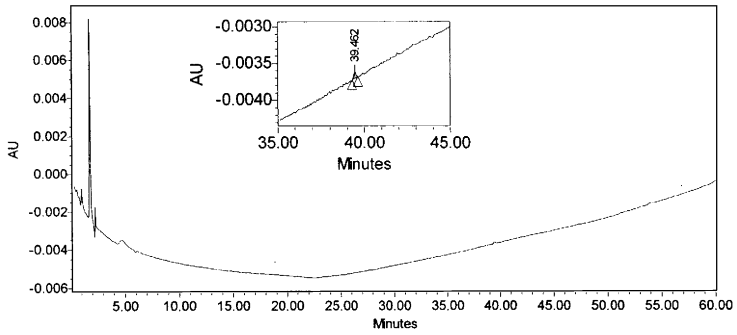
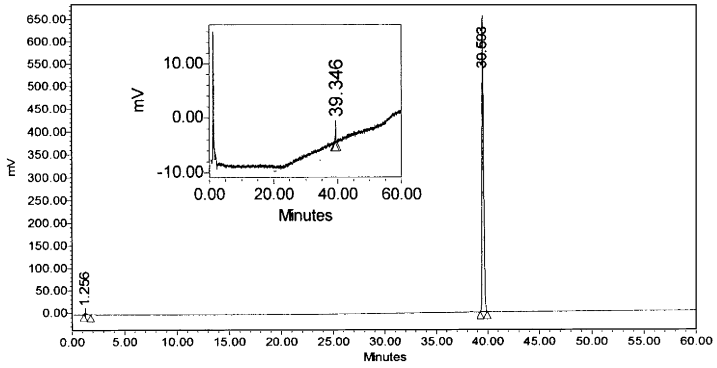


3A

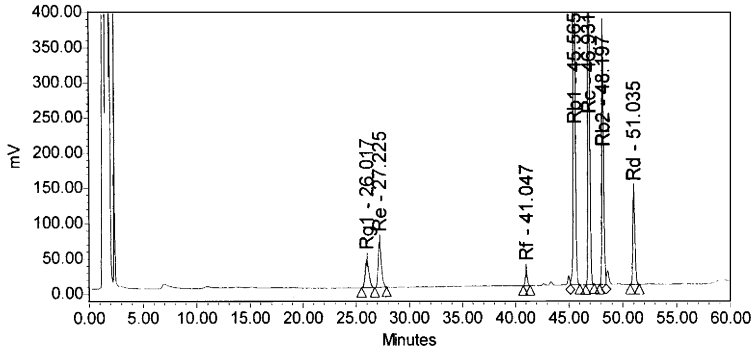


3B

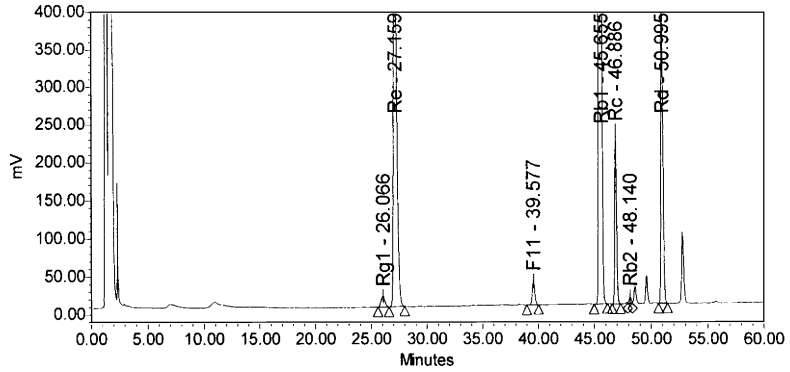
Figure 3. A typical comparison of the UV with detection wavelength of 203 nm (3A, the corresponding peak is shown in the insert) and the ELSD (3B, the minimum detectable limit of 32 ng on column is shown in the insert) for the detection of 1.06 μg of 24(*R*)-pseudoginsenoside F_{11} (**2**).

HPLC-ELSD Identification of Ginseng and Ginseng Products

Figure 4 shows the HPLC-ELSD chromatograms of Asian ginseng and North American ginseng methanolic extracts. Ginsenosides Rg_1, Re, Rb_1, Rc, Rb_2 and Rd could be detected in the extracts of both species (Fig. 4). However, Rf (**1**) can only be detected in Asian ginseng, but not in North American ginseng samples (Fig. 4A). In contract, F_{11} (**2**) can only be detected in North American ginseng, but not Asian ginseng, in the current study (Fig. 4B). Therefore, the HPLC-ELSD method has been established to distinguish Asian ginseng and North American ginseng.



4A



4B

Figure 4. A typical comparison of HPLC-ELSD chromatograms of methanolic extracts of Asian ginseng (4A) and North American ginseng (4B) with the retention times of ginsenoside Rf (**1**) in Asian ginseng and 24(*R*)-pseudoginsenoside F_{11} (**2**) in North American ginseng of 41.1 and 39.6 min, respectively.

Seven commercial ginseng products, including powder, roots, capsule, and soft gel, were analyzed by using the present HPLC-ELSD method. Most of the samples were found to contain ginseng, as described by the manufacturers (Table 1). However, one brand of ginseng powder, which claimed to be made from North American ginseng, actually was a mixture of Asian ginseng and North American ginseng. Figure 5 shows the HPLC-ELSD chromatogram of the methanolic extract of the product. Although the presence of F_{11} (**2**) positively supports the

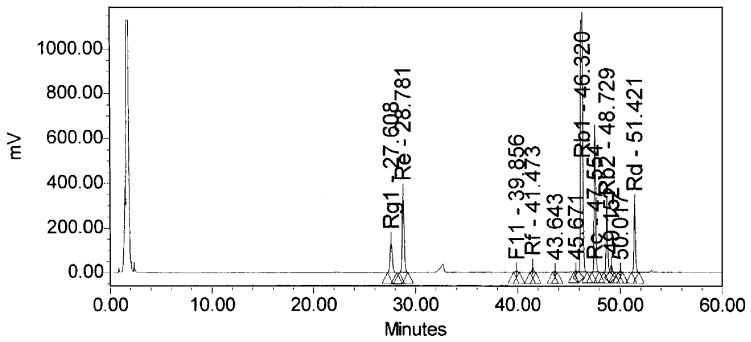


Figure 5. A typical HPLC-ELSD chromatogram of methanolic extract of a North American ginseng product with the retention time of ginsenoside Rf (**1**) and 24(*R*)-pseudoginsenoside F_{11} (**2**) of 41.5 and 39.9 min, respectively, which definitely indicated that the product is a mixture of Asian ginseng and North American ginseng.

identity of North American ginseng, the peak of Rf (**1**) definitely shows the presence of Asian ginseng in the product.

CONCLUSION

The present study demonstrated a simple HPLC-ELSD method for identification of Asian ginseng, North American ginseng, and their commercial products. The identification hinged on the detection of two markers, i.e., ginsenoside Rf and 24(*R*)-pseudoginsenoside F_{11} The limits of detection of the present HPLC-ELSD method were determined. Both ginsenoside Rf and 24(*R*)-pseudoginsenoside F_{11} could be detected at the 32–50 ng on-column level. The method developed is also straightforward and convenient.

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