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Determination of 24(*R*)-pseudoginsenoside F_{11} in North American ginseng using high performance liquid chromatography with evaporative light scattering detection

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Abstract

A gradient liquid chromatographic method with evaporative light scattering detection (ELSD) for the determination of 24(*R*)-pseudoginsenoside F_{11} in North American ginseng is described. Samples are analyzed by means of a reverse-phase column (Waters Spherisorb ODS-2, C_{18}) using acetonitrile and water under gradient conditions as the mobile phase over 20 min. The evaporative light scattering detector (ELSD) used, was set at an evaporating temperature of 35°C and nitrogen gas pressure of 3.4 bar. The detection limit ($S/N > 5$) of 24(*R*)-pseudoginsenoside F_{11} is 53 ng on column.

Keywords: 24(R)-pseudoginsenoside F_{11}; North American ginseng; High performance liquid chromatography; Evaporative light scattering detection

1. Introduction

North American ginseng (*Panax quinquefolius*) originates from the northern region of the United States and Canada. Native North Americans used ginseng root as part of their traditional medicine. The international trade in North American ginseng began early in the 1700s, when it was discovered that North American ginseng roots possessed properties similar to those of the ginseng from China (*Panax ginseng*) [1,2][1]. Successful cultivation of North American ginseng was begun in the 1800s [1] and is now dominated by growers in Wisconsin, USA, Ontario and British Columbia, Canada. In the recent years, North American ginseng has become one of the most popular medicinal plants, commercially available as an herbal medicine or nutritional supplement. It is

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[1] Any medical claims for 'ginseng' referenced in references [1] and [2] have not been substantiated by appropriate clinical trials, however they are excellent sources for the history and current use of ginseng products.

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also known to be different from Asian ginseng in some properties and indications for use in ethnopharmacology [3]. In the ginseng market, it is 5–10 times more expensive than that of Asian ginseng [4]. The active constituents of North American ginseng are similar to those of Asian ginseng, and also commonly referred to as ginsenosides. The most abundant ginsenosides present in North American ginseng are Rb_1, Rb_2, Rc and Rd with 20(*S*)-protopanaxadiol as the aglycone; and Rg_1 and Re with 20(*S*)-protopanaxatriol as the aglycone [5–8].

Many attempts have been made for the QA/QC of North American ginseng by HPLC for past 20 years [9–16]. Although most ginsenosides, including Rg_1, Re, Rb_1, Rc, Rb_2 and Rd, have weak UV-absorption, they are detectable and quantifiable at 201–205 nm with minimum detectable concentration in the range of 0.2–0.3 μg on column, and as a result, these ginsenosides have been quantitated in routine QA/QC of North American ginseng. However, there are some minor ginsenosides, e. g. 24(*R*)-pseudoginsenoside F_{11} (Fig. 1), reported as a promising compound for improving memory performance, in North American ginseng [17]. Their poor UV absorption and low concentration limits the application of the HPLC method using an UV detector. Although there has been a TLC method indicating that 24(*R*)-pseudoginsenoside F_{11} is detectable in North American ginseng [18], the TLC method for quantification of the compound in North American ginseng root extracts presents a challenge for selectivity and sensitivity. Compared to TLC, LC/MS or LC/MS/MS provide a higher selectivity and sensitivity and give unambiguous identification of the compound in extracts [19,20]. Our recent LC/MS/MS analysis revealed that North American ginseng contains more than 0.1% 24(*R*)-pseudoginsenoside F_{11}, 1000 times higher than that in Asian ginseng (less than 0.0001%) [20]. While LC/MS or LC/MS/MS method may work well for the identification and quantification of the wide range of 24(*R*)-pseudoginsenoside F_{11} levels to be tested, the equipment is relatively expensive and may not be available in every laboratory. It is useful to develop an alternative HPLC method using the relative inexpensive evaporative light scattering detector (ELSD) for the routine analysis of 24(*R*)-pseudoginsenoside F_{11} in North American ginseng.

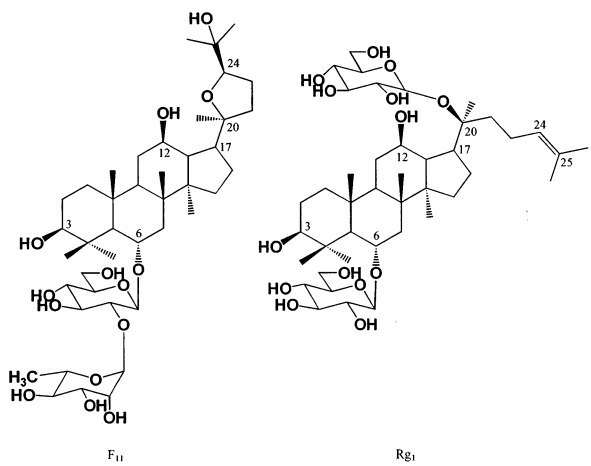

Fig. 1. Structure of 24(*R*)-pseudoginsenoside F_{11} and ginsenoside Rg_1.

Table 1
Solvent gradient conditions

Final time	Flow rate (ml/min)	A% (water)	B% (acetonitrile)
Initial	1.6	75	25
20	1.6	70	30
21	1.6	10	90
30	1.6	10	90
31	1.6	75	25
40	1.6	75	25

Evaporative light scattering detection (ELSD) is a mass detection method which is based on LC column effluent nebulization into droplets by the nebulizing gas, the resulting vapor enters a temperature-controlled evaporator tube, which causes the evaporation of mobile phase [21–24]. The resulting non-volatile analyte particles are then directed towards a narrow light beam. Light is scattered by residual particles and measured using a photomultiplier or photodiode. The signal intensity is related to the mass of the analyte in the scattering chamber and the signal is indicative of

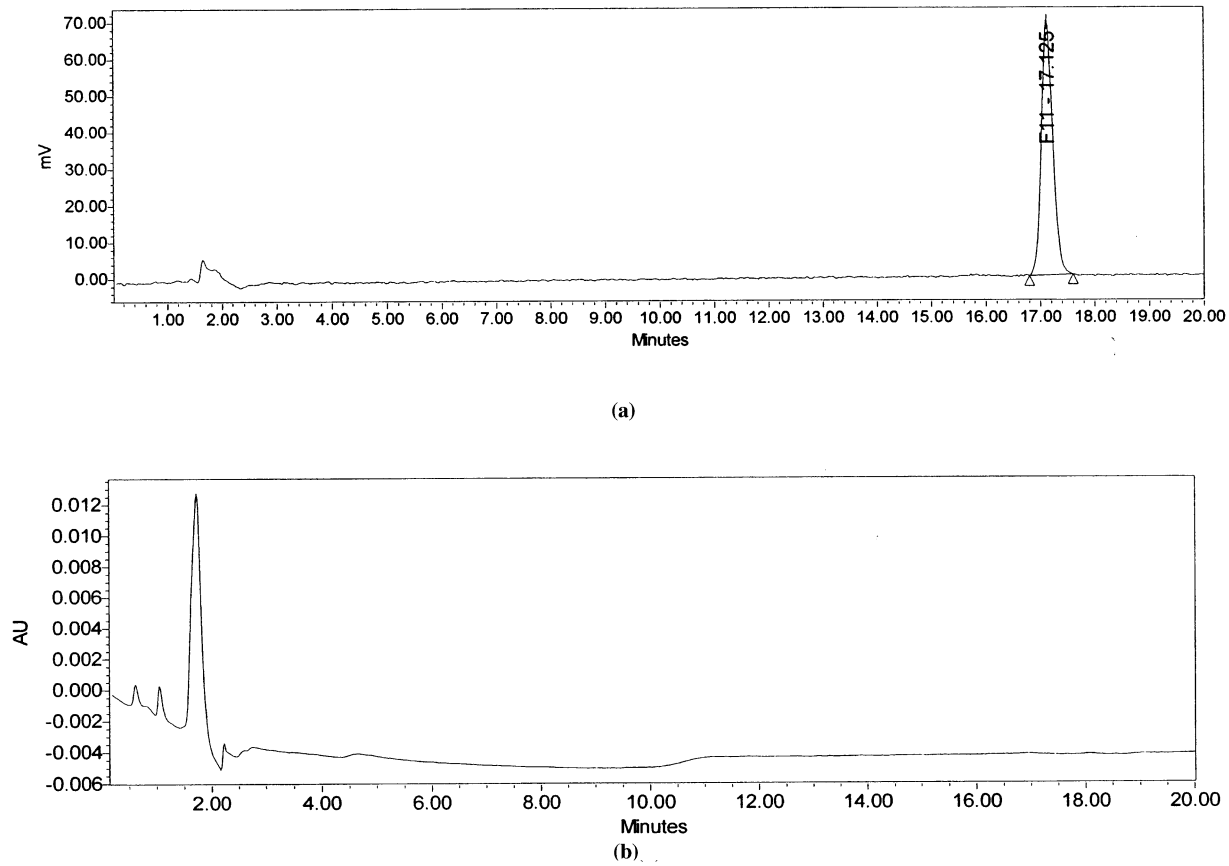

Fig. 2. A comparison of HPLC chromatogram of 24(*R*)-pseudoginsenoside F_{11} (1060 ng) with ELSD detection (a) and UV detection at 203 nm (b).

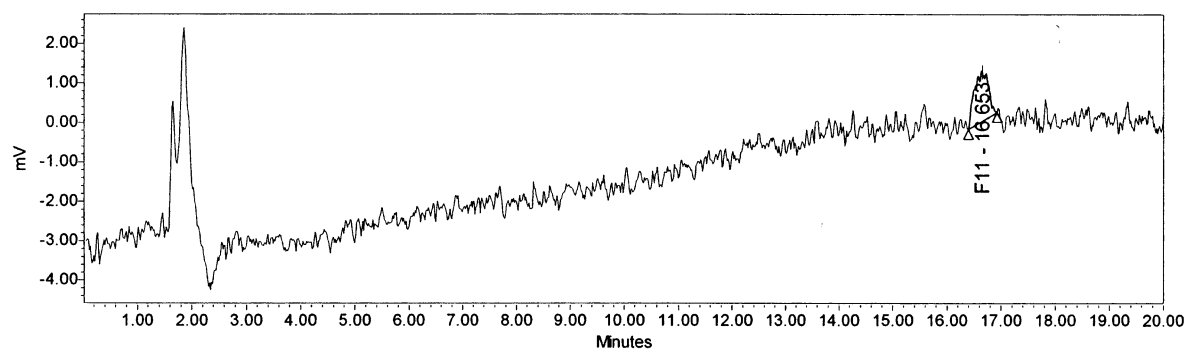

Fig. 3. A typical HPLC/ELSD chromatogram of 24(*R*)-pseudoginsenoside F_{11} with minimum detectable concentration of 53 ng.

molecular size and shape, but not the chemical identity of the residual particles passing through the light beam. The ELSD can provide a stable baseline even with gradient elution and has been successfully applied to the analysis of ginsenosides in Asian ginseng. In 1996, Park, et al. first introduced ELSD techniques for the identification of ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_3 and Rh in white and red Asian ginseng (*Panax ginseng*) with detection limit ranging from 35 to 155 ng on column, although all of the above ginsenosides could easily be detected with HPLC–

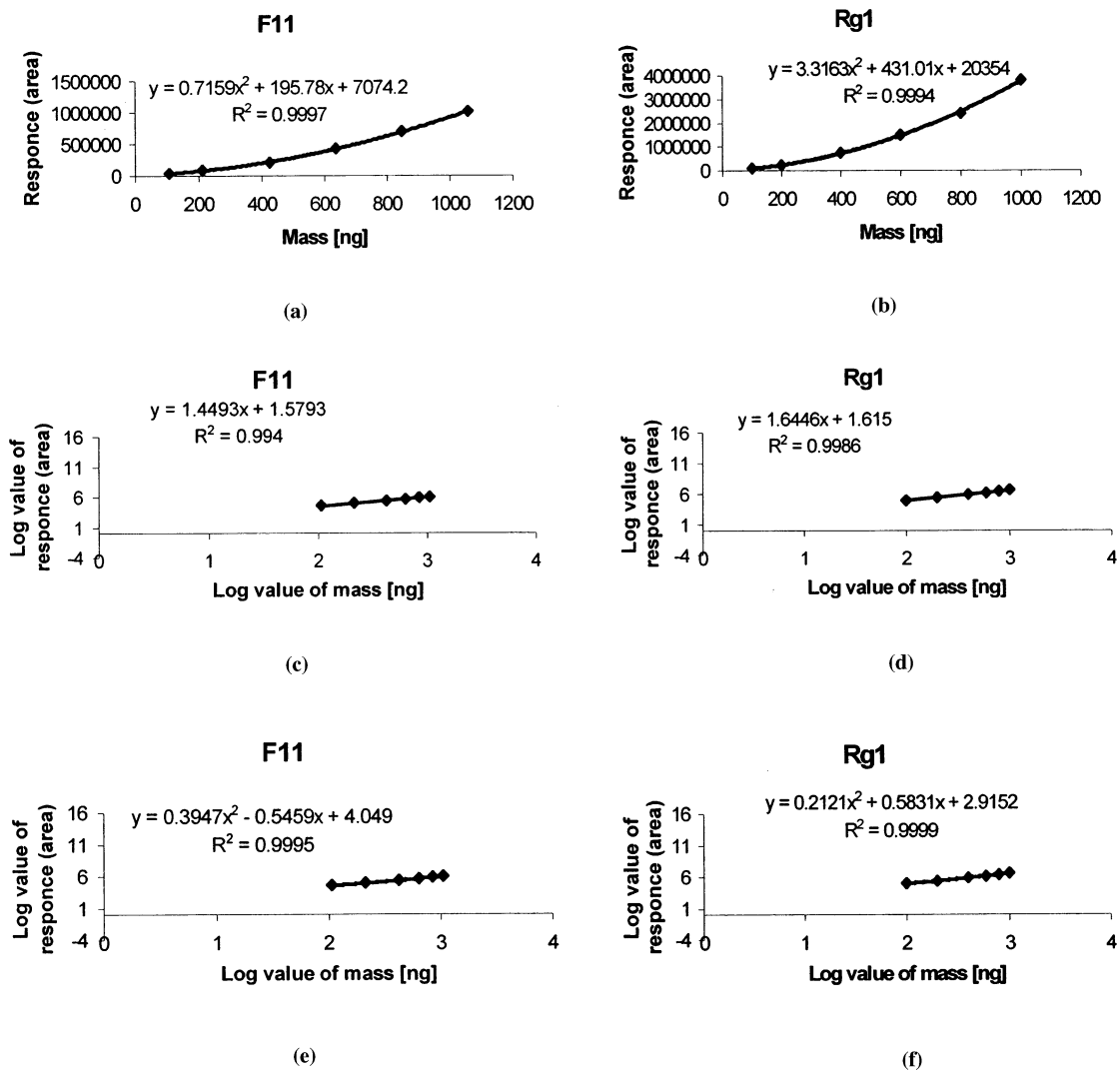

Fig. 4. Calibrations of 24(*R*)-pseudoginsenoside F_{11} and a reference compound, ginsenoside Rg_1, with (a) and (b) showing the second-order polynomial calibration curves ($Y = aX^2 + bX + c$); (c) and (d), the log-transformed linear calibration curves, and (e) and (f), the log-transformed second-order polynomial calibration curves.

Table 2
Reproducibility over three consecutive days

Group	Spiked concentration (μg ml^{-1})	Days	Measured concentration (mean ± S.D., $n = 3$, μg ml^{-1})	Coefficient of variance (%)	Relative error (%)
QC-1	10.6	Day 1	11.60 ± 0.13	1.09	9.40
		Day 2	10.99 ± 0.33	2.96	3.73
		Day 3	10.79 ± 0.30	2.80	1.75
QC-2	53	Day 1	48.54 ± 0.60	1.24	−8.42
		Day 2	46.82 ± 0.88	1.89	−11.65
		Day 3	45.54 ± 1.05	2.31	−14.08
QC-3	106	Day 1	111.30 ± 0.96	0.87	5.00
		Day 2	110.74 ± 1.30	1.17	4.47
		Day 3	110.99 ± 1.22	1.10	4.71

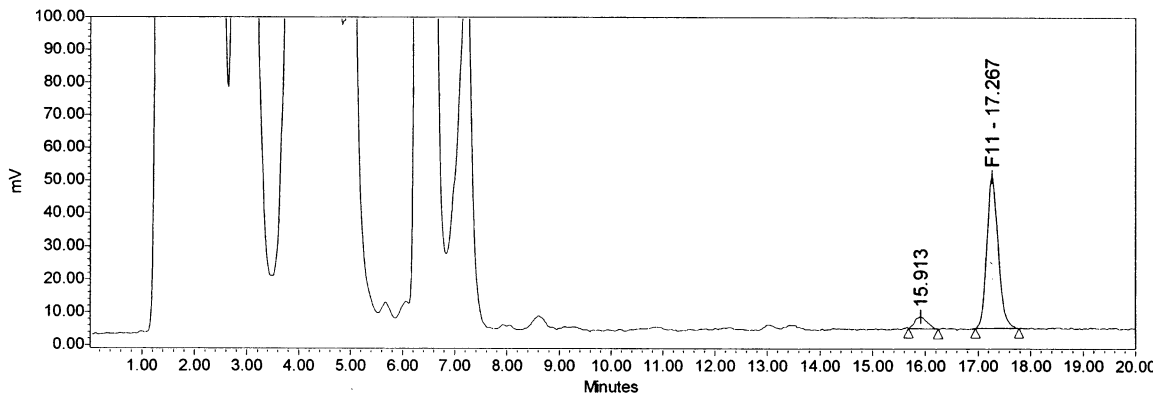

Fig. 5. A typical HPLC/ELSD chromatogram of North American ginseng with the retention time of 24(R)-pseudoginsenoside F_{11} of 17.27 min.

UV techniques [25]. In 1999, Fuzzati, et al. reported an HPLC–ELSD method to detect 25 ginsenosides in Asian ginseng (*Panax ginseng*) extracts, confirming that the ELSD has an advantage over UV detection in providing a stable flat baseline when buffer was used in the mobile phase with gradient elution [26].

This paper describes for the first time a quantitative analysis of the minor ginsenoside 24(R)-pseudoginsenoside F_{11} in North American ginseng in a single run by HPLC/ELSD using gradient elution.

2. Experimental

2.1. Reagents and chemicals

The 24(R)-pseudoginsenoside F_{11} was isolated from North American ginseng by Dr Hongjie Zhang in the Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago [20]. The North American ginseng root samples used in this study were from Wisconsin, USA, British Columbia and Ontario, Canada. Methanol and acetoni-

trile (HPLC grade) were purchased from Fisher Scientific Co. (Fair Lawn, NJ, USA). DI water was obtained with an in-house Nano-pure® water system (Barnstead, Newton, MA, USA).

2.2. Chromatographic conditions

A Waters 2690 Alliance HPLC system (Milford, MA, USA), equipped with a 996 photodiode array UV detector, an on-line degasser and an autosampler, was used for solvent delivery and detection. The measurements were carried out on a Waters Spherisorb ODS-2 C_{18} column (250 × 4.6 mm, 5 μm particle size, Serial No. 0123391941L, USA) protected by a Waters Delta-Pak C_{18} guard column (Waters Technology Ireland, Ltd., Wexford, Ireland) and set at room temperature. The solvents used for separation were water, solvent A; and acetonitrile, solvent B. Solvent gradient conditions are reported in Table 1. All injections were 10 μl in volume. After the UV detector, the column effluent was directed to a Sedex 75 evaporative light scattering detector (ELSD) (Cedex 94141, Alfortville, France). Nebulization of the eluent in the ELSD was provided by a stream of pressured nitrogen at 3.4 bar. The nebulization was performed at room temperature, and the nebulized effluents were evaporated at 35°C. The detector output was interfaced, using a SATIN box, to the Waters Millennium 2000® chromatographic manager system (Waters, Milford, MA) loaded on a Compaq 6400X/10000/CDS computer (Houston, TX) for data handling and chromatogram generation.

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.

2.3. Preparation of standard solution and samples

In a clean, dry 10-ml volumetric flask, a sample of 24(*R*)-pseudoginsenoside F_{11} (approx. 1 mg) was accurately weighed and dissolved in HPLC grade methanol to make a stock solution. Calibration standard working solutions were prepared by diluting the stock solution with methanol in appropriate quantities. Three controls were also prepared so as to lie in the lowest, middle and highest regions of the calibration curve, i.e. 10.6, 53 and 106 μg ml^{-1}. All working solutions were stored at −20°C and brought to room temperature before use. Finely pulverized North American ginseng roots or powders were weighed (1.0 g) into a PTFE-stopped 20-ml sample vial. Methanol (Fisher, HPLC grade, 18 ml) was added, and the mixture was shaken and then sonicated at 25–30°C for 30 min. After cooling, the mixture was filtered through filter paper (Whatman No. 40) into a 250-ml round-bottom flask, and the residue was returned to the sample vial. Another 18 ml of methanol was added and the mixture was sonicated at 25–30°C for 30 min. The extract was filtered through filter paper (Whatman No. 40) into the same round-bottom flask. The above extraction procedure was repeated once more before washing the residue with methanol (3 × 15 ml) while on the filter. The combined methanol extracts were evaporated under reduced pressure at 35°C. The residue was re-dissolved and transferred with methanol to a 10-ml volumetric flask and made up to volume with methanol. The sample solution was filtered through 0.2 μm Whatman hydrophilic membrane filter (Whatman Inc., Clifton, NJ) into HPLC sample vial just before HPLC/UV/ELSD analysis.

2.4. Optimization of ELSD parameters

In order to obtain minimum noise and maximum detection signal in ELSD, three basic parameters, nebulizer gas flow rate (pressure), evaporating temperature and gain were varied to optimize the detection of 24(*R*)-pseudoginsenoside F_{11}. Nitrogen (99.999% purity, AGA Specialty Gas, Cleveland, OH) was used as the driving gas for nebulization and the carrier gas for analyte transport. The carrier gas was passed though filter frits prior to entering the detector to minimize extraneous particles from being introduced by the gas.

2.5. Reproducibility

The precision and accuracy of the method were assessed by within and between run validations. The variation was evaluated by injecting three sets of controls (10.6, 53, 106 μg ml^{-1}, $n = 3$) on three separate days. By substituting the peak-area into the calibration curve equation from the same run the measured concentrations were obtained. By comparing calculated and theoretical concentrations, the relative errors were obtained. The coefficient of variance was calculated by comparing the measured concentrations.

3. Results and discussion

3.1. Chromatography

Several combinations of acetonitrile and water were evaluated for use as the mobile phase in order to improve the resolution and sensitivity. After trying several types of gradients and varying duration, an optimum solvent system was found as described under Section 2.

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

Fig. 2 shows a typical HPLC/UV and HPLC/ELSD chromatograms of 24(*R*)-pseudoginsenoside F_{11} with retention time of about 17 min, within a 20-min gradient elution. Because the compound has no double bond in the molecule, it shows very poor UV absorption, even when the injection amount was increased to 1060 ng on column. This further verifies the advantage of ELSD over normal UV in the detection of some minor non-double bond containing ginsenosides.

The detection limit ($S/N > 5$) of the described method was observed for of 24(*R*)-pseudoginsenoside F_{11} at 53 ng on the column in the current assay (Fig. 3).

3.2. Calibration

In ELSD, a second-order polynomial calibration (peak area against amount) was obtained with a regression coefficient ($r^2 = 0.9997$; Fig. 4a) in the range of 106–1060 ng on column. Although we could find documentation of a similar calibration curve for lipids [27], we could find no prior report of this type of calibration curve for 24(*R*)-pseudoginsenoside F_{11} or other ginsenosides. After log-transformed, the data provided a linear function for 24(*R*)-pseudoginsenoside F_{11} following the equation: $Y = a + bX$ with Y being the log value of the peak area, X the log value of sample amount, a the intercept and b the slope with a regression coefficient of 0.994 (Fig. 4c), which was consistent with the previous results that ELSD provided an exponential linear response for ginsenosides Rg_1, Rd, Re and Rg_3 [25,28] and Rb_1, Ro, Rc, Rb_2 [28]. It is interesting that a log-transformed second-order polynomial regression might be more suitable for the calibration curve here, following the equation: $Y = aX^2 + bX + c$, with Y being log value of the peak area, X the log value of the amount of sample injected, and a, b and c the numerical coefficients. The coefficient was found to be 0.9995 (Fig. 4e). To test this finding, ginsenoside Rg_1 (Fig. 1) was analyzed for linearity at the amount range of 100–1000 ng on column using both UV detector at 203 nm and ELSD with the same chromatography conditions as described under Experimental. With UV detection, a

linear calibration curve was obtained with a regression coefficient of 0.9992 (curve not shown). In ELSD, the same second-order polynomial calibration curve between peak area and amount injected ($r^2 = 0.9994$) as 24(*R*)-pseudoginsenoside F_{11} was observed (Fig. 4b). Also, its log-transformed data fit the second-order polynomial calibration ($r^2 = 0.9999$) better than the exponential one ($r^2 = 0.9986$; Fig. 4d and 4f). But, more ginsenosides, including those reported in the literature [25,28], must be tested over multiple days in order to verify this finding. In the current study, a log-transformed linear (exponential) calibration curve was used.

3.3. Reproducibility

The reproducibility of the method was evaluated by analyzing a set of three controls (10.6, 53, 106 μg ml^{-1}; $n = 3$) on three separate days ($n = 3$) and calculating the RSD% and relative errors (%). As shown in the Table 2, the RSD% and the relative errors (%) were found to be less than 2.96 and 14.08%, respectively.

3.4. Sample analysis

A typical HPLC/ELSD chromatogram is shown in Fig. 5. Two sets of samples were analyzed according to the method described above. The average content of 24(*R*)-pseudoginsenoside F_{11} in North American ginseng, originating from Wisconsin, USA, British Columbia and Ontario, Canada, was found to be 0.146, 0.091 and 0.162%, respectively.

4. Conclusions

A high-performance liquid chromatography method has been developed for the detection and quantitation of 24(*R*)-pseudoginsenoside F_{11} using an evaporative light scattering detector. With this method, 24(*R*)-pseudoginsenoside F_{11} was successfully quantitated, using a calibration curve, with detection limit of 53 ng on the column. Although the sensitivity of HPLC/ELSD does not compare with that of LC/MS/MS analysis, it is prior to the HPLC/UV method. The HPLC/ELSD method was found to be rapid, relatively inexpensive and straight-forward. Moreover, in principle it can be used to quantitate any ginsenoside of interest.

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