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High-performance liquid chromatographic analysis of ginseng saponins using evaporative light scattering detection

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Abstract

Ginseng saponins were analysed using HPLC with evaporative light scattering detection (ELSD). A LiChrosorb NH_2 column was used to separate ginseng saponins in white and red ginseng. The complete separation of ginsenoside Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2, Rg_3 and Rh_1 was achieved within 35 min with an amino-bonded column using an acetonitrile–water–2-propanol gradient system. The ELSD drift tube temperature was set at 145°C and the nitrogen flow was set at 40 mm rotameter units. The detection limits ($S/N = 3$) of the ginsenosides ranged from 35 to 155 ng.

Keywords: Ginseng; Saponins; Ginsenosides

1. Introduction

Ginseng (*Panax ginseng*, Araliaceae) has long been used as a tonic, anti-fatigue, sedative and anti-gastric ulcer drug [1] and many studies have suggested that its pharmacological effects are due to ginseng saponins. Ginseng saponins, often referred to as ginsenosides, are dammarane-type triterpene glycosides which can be classified into two groups possessing either (20*S*)-protopanaxadiol or (20*S*)-protopanaxatriol as the sapogenin (Table 1). More than 30 kinds of ginseng saponins have been reported from ginseng and allied plants.

Many attempts have been made to separate ginseng saponins by GC or HPLC [2–7]. In the GC method, ginsenosides are acid hydrolysed and the resulting panaxadiol (PD) and panaxatriol (PT) are silylated, followed by GC analysis using a non-polar stationary phase and flame ionization detection (FID) [3]. The drawback of the GC method is that the individual ginsenosides cannot be separated, and only two broad groups of ginsenosides, PD and PT groups, can be analysed.

Each ginsenoside can be separated by the HPLC method. However, in HPLC the problem lies in the method of detection. Because ginsenoside is a poor chromopore, it limits the application of the UV detector to only short wavelengths [8]. Consequently, gradient elution is difficult with HPLC–UV detection and so quantification of ginseng saponins in a single run was difficult by conventional detection methods.

Evaporative light scattering detection (ELSD) [9–13] is a mass detection method in which the

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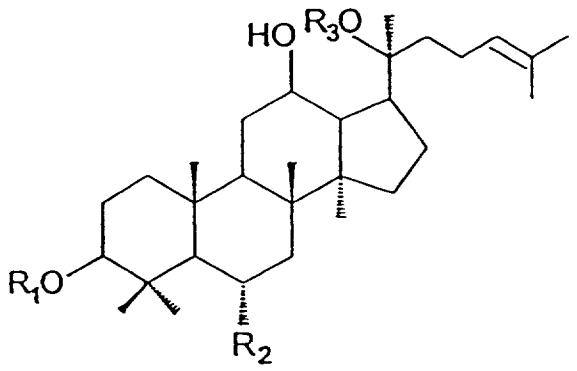
Table 1
Structure of ginseng saponins



	R_1	R_2	R_3
Ginsenoside Rg_1	H	Glc–O–	Glc–
Ginsenoside Rg_2	H	Rha–Glc–O–	H
Ginsenoside Re	H	Rha–Glc–O–	Glc–
Ginsenoside Rf	H	Glc–Glc–O–	H
Ginsenoside Rh_1	H	Glc–O–	H
Ginsenoside Rb_1	Glc–Glc–	H	Glc–Glc–
Ginsenoside Rb_2	Glc–Glc–	H	Ara(p)–Glc–
Ginsenoside Rc	Glc–Glc–	H	Xyl–Glc–
Ginsenoside Rd	Glc–Glc–	H	Ara(f)–Glc–
Ginsenoside Rg_3	Glc–Glc–	H	H

chromatographic eluate is nebulized by a gas stream and the vapour enters a heated tunnel, where the solvent evaporates. The resulting analyte particles pass through a narrow light beam, and the scattered light is collected by a photomultiplier. The ELSD response depends on the number and size of analyte particles. Since ELSD only responds to involatile analytes, it provides a flat baseline even with gradient elution. ELSD has been successfully applied to the analysis of non-volatile compounds such as sugars [14,15], lipids [16–18] and surfactants [19,20].

This paper describes the analysis of major ginseng saponins in a single run by HPLC–ELSD using gradient elution.

2. Experimental

2.1. Reagents and chemicals

Five kinds of ginseng saponin standards were provided by the Korea Ginseng and Tobacco Research Institute (Daejun, South Korea). Other ginsenosides were isolated from ginseng in our laboratory. The Korean white and red ginseng used in this study were purchased at a local market in Seoul. Distilled, deionized water was purified with a Barnstead (Dubuque, IA, USA) ultrapure water system. Solvents were of HPLC grade from Merck (Darmstadt, Germany).

2.2. Preparation of samples and standard ginsenosides

Each of the ginseng saponin standards was dissolved in water or a mixture of water and methanol. White ginseng was refluxed with methanol for 6 h. Solvent was evaporated in vacuo and the residue was partitioned between diethyl ether and water. The water layer was further extracted with water-saturated *n*-butanol. The *n*-butanol fraction was dried in vacuo and the residue was dissolved in methanol, which was subjected to HPLC analysis. Preparation of the *n*-butanol fraction of red ginseng was the same as that of white ginseng. Both the samples and the

ginsenoside standards were filtered through a 0.45-μm membrane filter before injection.

2.3. Chromatographic conditions

The HPLC system consisted of two Model SLC-100 pump (Samsung, Suwon, South Korea), a Model 7125 injector (Rheodyne, Cotati, CA, USA), a Model ELSD IIA detector (Varex, Burtonsville, MD, USA) and a Model C-R4A Chromatopac integrator (Shimadzu, Kyoto, Japan).

The columns used for the separation of ginseng saponins were LiChrosorb NH_2 (250 mm × 4 mm I.D., 5 μm) (Merck) and Capcell Pak NH_2 (250 mm × 4.6 mm I.D., 5 μm) (Shiseido, Tokyo, Japan). The separation was effected by gradient elution, using the eluents (A) CH_3CN–water–2-PrOH (80:5:15) and (B) CH_3CN–water–2-PrOH (80:20:15) according to the following profile: 0–7 min, 80% A–20% B; 7–25 min, linear gradient from 80% A–20% B to 10% A–90% B; 25–40 min, 10% A–90% B. The solvent flow-rate was held constant at 1.0 ml/min at ambient temperature throughout the analysis.

2.4. Optimization of detector parameters

Two basic parameters which determine the ELSD response are the nebulizer gas flow-rate and the drift tube temperature. The optimum conditions were investigated by observing the signal intensity after injection of ginsenoside Rh_1 at various gas flow-rates or drift tube temperatures.

3. Results and discussion

Various mobile phase compositions were studied in order to improve the resolution and sensitivity. Acetonitrile–water–*n*-butanol [21,22] was examined; owing to the high boiling point of *n*-butanol, a very high temperature was necessary to obtain a stable baseline in the drift tube. Therefore, the *n*-butanol was replaced with 2-propanol, which is more volatile. Using the acetonitrile–water–2-propanol gradient system, ELSD can be operated under mild conditions, and also the resolution and sensitivity were improved. After trying several types of gradient shapes and durations, an optimum solvent system was found as described under Experimental.

In ELSD, the nebulizer gas flow-rate affects the signal response significantly. When the gas flow-rate is too low, large droplets are formed, resulting in spikes and noisy signals. On the other hand, when the gas flow-rate is too high, the droplets decrease in size, which results in a decreased signal response [23]. The optimum nebulizer gas (nitrogen) flow-rate in this work was determined to be 40 mm rotameter units.

The drift tube 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 the partial vaporization of the analytes [24,25]. The signal-to-noise ratio (S/N) was improved as the temperature was increased up to 145°C. However, the detector response decreased when the temperature was increased

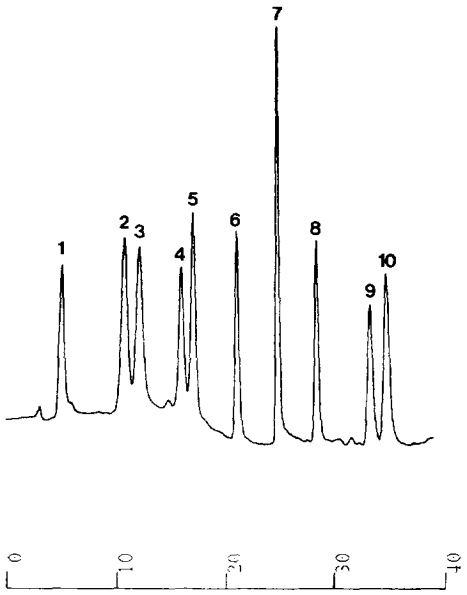


Fig. 1. HPLC–ELSD of standard mixture of ginsenosides on a LiChrosorb NH_2 column (250 mm × 4 mm I.D.). For conditions, see text. Peaks: 1 = Rh_1; 2 = Rg_2; 3 = Rg_1; 4 = Rg_3; 5 = Rf; 6 = Re; 7 = Rd; 8 = Rc; 9 = Rb_2; 10 = Rb_1.

above 150°C. Therefore, the optimum drift tube temperature was determined to be 145°C.

Fig. 1 shows the separation of a standard mixture of ten ginseng saponins on a LiChrosorb NH_2 column. Separation was completed within 35 min with gradient elution. The retention time depended on the sugar moieties of the ginseng saponins. Ginsenoside Rh_1, which has only one glucose, was eluted first. This was followed by ginsenoside Rg_2, Rg_1, Rg_3, Rf, Re, Rd, Rc, Rb_2 and Rb_1. Except for ginsenoside Rg_3, all panaxatriol-type saponins were eluted prior to panaxadiol-type saponins. Fig. 2 shows the chromatograms obtained from a butanol fraction of white and red ginseng.

Two kinds of NH_2 column, LiChrosorb NH_2 and Capcell Pak NH_2, were used. Ten kinds of ginsenosides were well separated on both columns; however, the elution order of ginsenoside Rg_1 and Rg_3 was reversed on the Capcell Pak NH_2 column.

In ELSD, detector response is given by $y = ax^b$, where y is the peak area, x is the sample amount and a and b are numerical coefficients [26,27]. The calibration graphs for four ginsenosides are exponential in the range 0.5–10 μg. Plots of peak area versus sample amount in double logarithmic coordinates are linear for four ginsenosides (Fig. 3). The linear regression correlation coefficients ranged from 0.9985 to 0.9996. The slopes b were in the range 1.21–1.47.

The detection limit ($S/N = 3$) of the described method was dependent on the conditions of the detector. Observed detection limits of ginsenosides were ginsenoside Rb_1 80, Rb_2 85, Rc 45, Rd 40, Re 65, Rf 40, Rg_1 50, Rg_2 155, Rg_3 35 and Rh_1 55 ng.

The described HPLC–ELSD method is su-

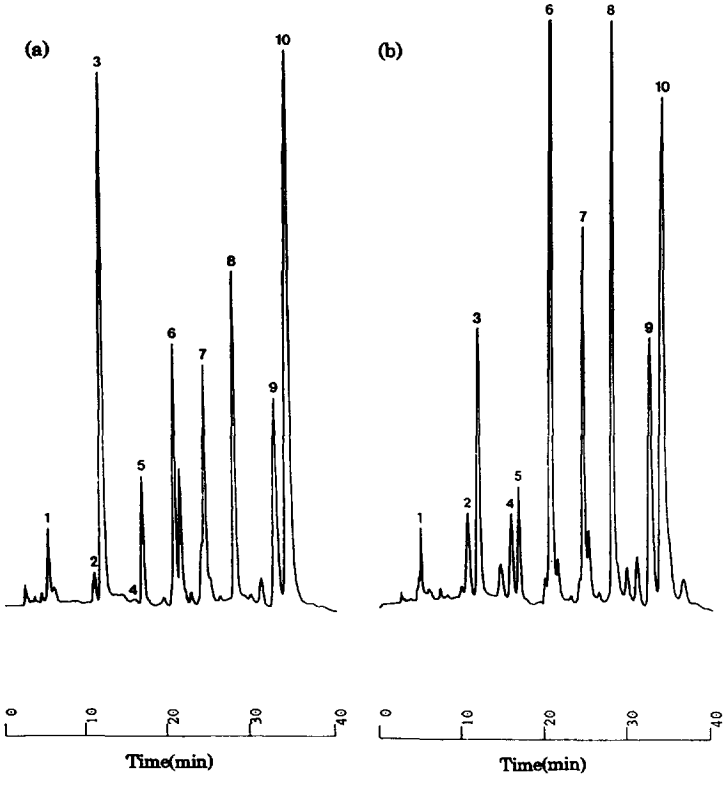


Fig. 2. HPLC–ELSD of (a) white ginseng and (b) red ginseng. Conditions and peak numbers as in Fig. 1.

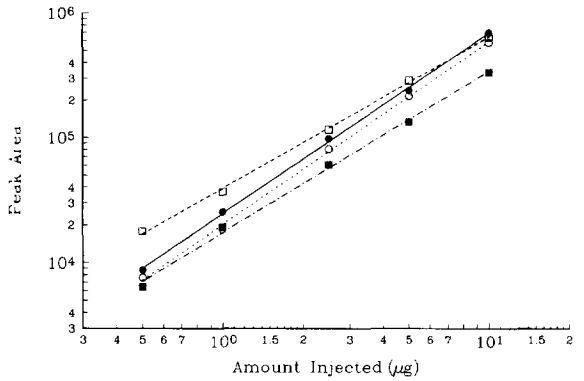


Fig. 3. Log–log plot of the ELSD response against sample amount of four ginsenosides. ○ = Ginsenoside Rg_1; ● = ginsenoside Rd; □ = ginsenoside Rg_3; ■ = ginsenoside Re.

perior to the HPLC–UV method with respect to both sensitivity and separation. The most important advantage of this method is that one can analyse all major ginseng saponins in a single run using gradient elution.

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