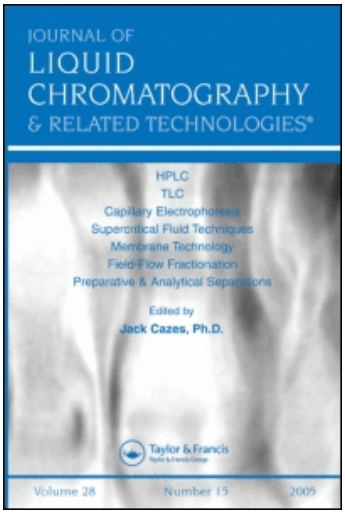

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Microanalysis of Ginseng Saponins by Ion Chromatography with Pulsed Amperometric Detection

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MICROANALYSIS OF GINSENG SAPONINS BY ION CHROMATOGRAPHY WITH PULSED AMPEROMETRIC DETECTION

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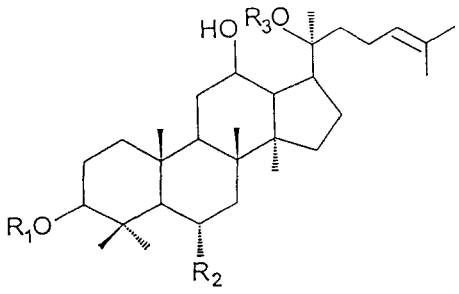
ABSTRACT

Analysis of ginseng saponins by ion chromatographic separation with pulsed amperometric detection(IC/PAD) was examined. Ginseng saponins were separated on Carbopac PA1 or AS4A anion exchange column with 1M NaOH as mobile phase. The measuring potential(E1), oxidation potential(E2), and reduction potential(E3) were 0.0V, +0.6V, and -0.8V, respectively. The dynamic linear range was over three orders and the limit of detection of ginsenoside Re was 0.8 pmol(S/N=2).

INTRODUCTION

Ginseng is an important herbal medicine which has been widely used in the Orient as a tonic, sedative, anti-fatigue, or anti-gastric ulcer drug[1]. Ginseng saponins(TABLE 1) were known as the major components of ginseng and intensive studies on the activity and analysis of them have been undertaken[2-4].

TABLE 1. Structure of ginseng saponins.



R_1=R_2=R_3=H 20(S)-protopaxanadiol
R_1=R_3=H R_2=OH 20(S)-protopaxanatriol

	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	Ara(f)-Glc-
Ginsenoside Rd	Glc-Glc-	H	Glc-
Ginsenoside Rg_3	Glc-Glc-	H	H

The techniques developed so far for the analysis of ginseng saponins include colorimetry[5], TLC-densitometry[6], GC[7,8], HPLC[9], and radioimmunoassay methods[10,11]. Among these, HPLC method is widely adapted[12-14]. However, UV detection at short wavelength, typically at 207nm[15], or refractive index(RI) detection method[16] in HPLC did not provide enough sensitivity for the microscale analysis of ginseng saponins.

Amperometric detector, one of the most sensitive detectors for HPLC, can be used for the microscale detection of the compounds having the functional

groups that can undergo the oxidation-reduction reaction. However, as the oxidation product of analyte accumulates on the surface of the electrode, the detection sensitivity and reproducibility decrease as time goes. Pulsed amperometric detection(PAD) was developed to overcome this drawback by applying oxidation potential(E2) and reduction potential(E3) after the measuring potential(E1) as the cleaning process of the electrode. Detection sensitivity and reproducibility were greatly enhanced by this pulse sequence. Hughes *et al.* reported microscale analysis of sugar alcohol, sugar, aldehyde, or formic acid using amperometric detector with Pt electrode,[17),18)] and Rocklin *et al.* reported the microanalysis of carbohydrate using PAD with Au electrode[19)].

This paper describes the analysis of ginseng saponins by ion chromatographic(IC) separation with PAD.

EXPERIMENTAL

Reagents and Chemicals

Nine kinds of ginseng saponin standards were generous gift from Korean Tobacco and Ginseng Research Institute and 6-year old white ginseng was purchased from the local botanical market in Seoul. Distilled deionized water was prepared with Barnstead ultrapure water system(U.S.A.). Acetonitrile and methanol were HPLC grade and other chemicals were all reagent grade.

Chromatography

Ion chromatograph used was Dionex system 4500i Ion Chromatograph (Dionex, U.S.A.) with 50μl injection loop. Young In 910 pump(Young In Sci. Co., Korea) with Rheodyne Model 7125 injector was used for HPLC.

Carbopac PA1 or HPIC-AS4A column(25cm x 4mm i.d., Dionex, U.S.A.) was used for ion chromatographic separation of ginseng saponins. Zorbax ODS column(8cm x 4mm i.d., 5μm, DuPont, U.S.A.) or Lichrosorb RP-18(25cm x 4mm i.d., 10μm, Merck, Germany) column was used for reverse-phase HPLC. Shimadzu CTO-6A column oven(Japan) was used to control column temperature. For the detection of ginseng saponins, pulsed amperometric detector(Dionex, U.S.A.) with gold-electrode, Hitachi L-4200 UV/Vis detector(Hitachi, Japan) and RID-6A RI detector(Shimadzu, Japan) were used. The chromatogram was recorded using IBM/PC compatible computer with homemade software.

RESULT AND DISCUSSION

Optimization of the condition of IC/PAD

The S/N ratio of ginsenoside Rg_1 and ginsenoside Rf were observed as the three potentials for PAD were changed. When E1 was changed from -0.6 V to 0.0 V, the S/N ratio increased with the increment of potential and showed maximum at 0.0 V. The S/N ratio dropped drastically after 0.0 V and kept constant from 0.2 V to 0.6 V(FIGURE 1). Only a small change in S/N ratio was observed when E2 and E3 were changed. Therefore, the condition used for carbohydrate analysis was adapted for E2 and E3, i.e. +0.6V and -0.8V, respectively[19].

The concentration of NaOH in the mobile phase was changed from 0.1M to 1.0M and ginseng saponin standard solution was injected. In HPIC-AS4A column, the retention time of panaxatriol type saponins increased, while that of panaxadiol type saponins decreased as the concentration of NaOH increased(FIGURE 2). Ginsenoside Rf and ginsenoside Rh_1 eluted at the same time when 0.7M NaOH was used as mobile phase, and the elution

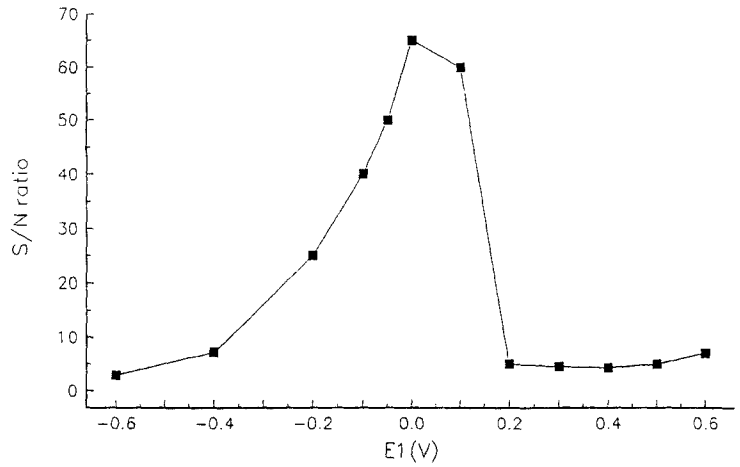


FIGURE 1. Effect of E1 to the S/N ratio of ginsenoside Rg_1.
HPIC-AS4A column, 1M NaOH, 1ml/min, PAD : T1 720ms, T2 120ms (E2 +0.6V), T3 360ms (E3 -0.8V)

order was reversed before and after this concentration. In Carbopac PA1 column, only ginsenoside Rg_1 and ginsenoside Re were eluted(FIGURE 3), and the retention time of these two saponins increased as the concentration of NaOH increased(FIGURE 4). The resolution of ginsenoside Rg_1 and ginsenoside Re showed the maximum at the mobile phase of 1.0M NaOH in both columns.

FIGURE 5 shows the effect of column temperature to the capacity factors of ginseng saponins. The capacity factors increased as the column temperature rises. Ginsenoside Rb_1 and Rb_2 appeared as a single peak over 45°C while ginsenoside Rc and Rd were newly separated. The selectivity factors between each saponins were the greatest at 35°C, but the analysis was carried out at room temperature because the resolution of ginsenoside Rb_1 and Rb_2 got worse at 35°C.

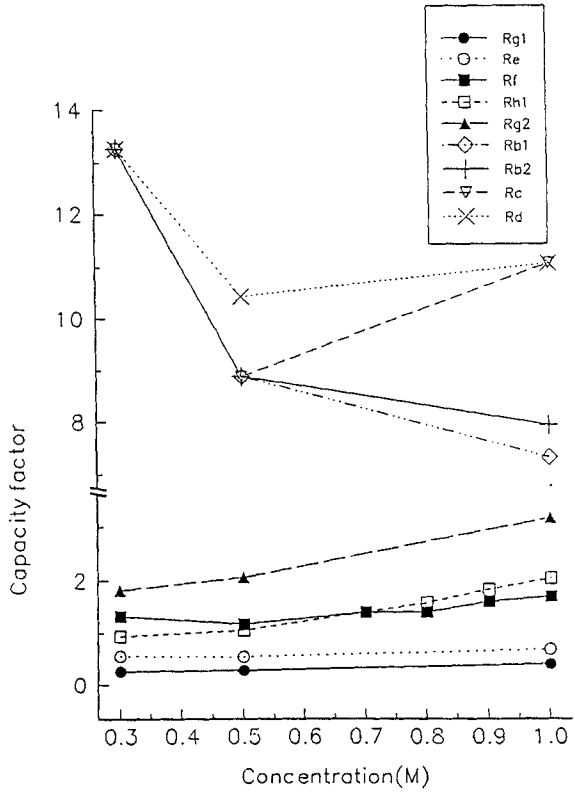


FIGURE 2. Effect of the concentration of NaOH to the capacity factor of ginseng saponins. HPIC-AS4A column, 1ml/min, PAD : 720ms (0.0V), 120ms (+0.6V), 360ms (-0.8V)

Determination of ginseng saponin

Five panaxatriol type saponins were separated well with HPIC-AS4A column, but panaxadiol type saponins were poorly separated (FIGURE 6).

The calibration curves for panaxatriol type saponins showed good linearity in the range of 10ng - 10μg, with the correlation coefficient of 0.996 - 0.999, which suggests the dynamic linear range is over three orders(FIGURE 7).

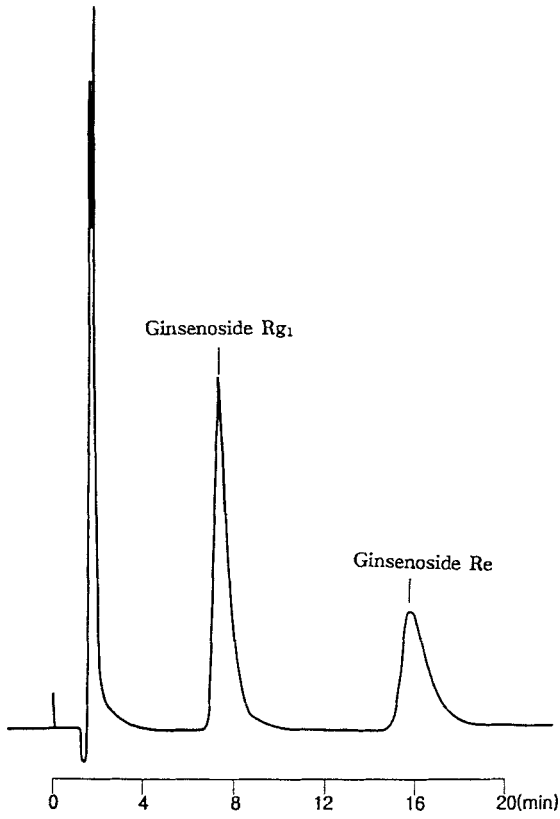


FIGURE 3. Chromatogram of mixture of ginsenoside Rg_1 and Re. Carbopac PA1 column, 1M NaOH, 1ml/min, PAD : 720ms (0.0V), 120ms (+0.6V), 360ms (-0.8V)

Ginsenoside Rg_1 and ginsenoside Re were analyzed by HPLC/UV and HPLC/RI detection methods using ODS column to compare the detection limit with that of IC/PAD method. The detection limits(S/N=2) were 400ng with UV detection method(207nm), and 8μg with RI detection method, which were somewhat higher than the reported data(300ng at 207nm with UV detection)[15].

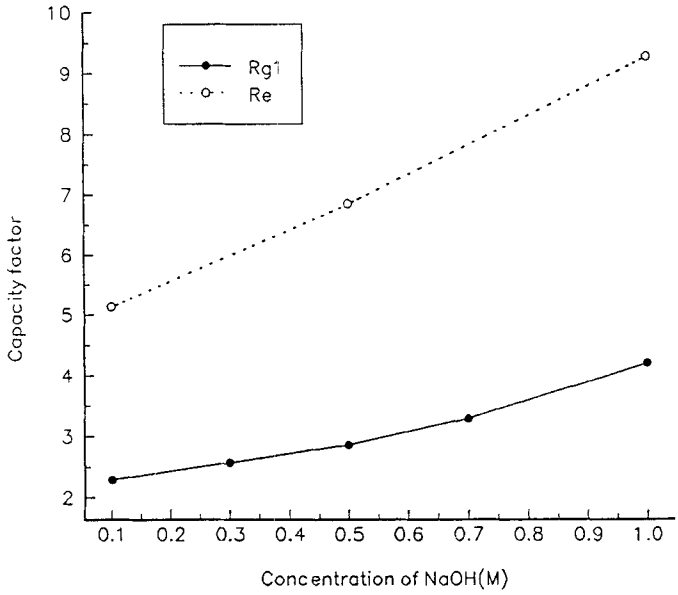


FIGURE 4. Effect of the concentration of NaOH to the capacity factor of ginsenoside Rg_1 and Re.
(Conditions are as in FIGURE 3)

The detection limit by IC/PAD was 0.8ng for ginsenoside Re and 1.0ng for ginsenoside Rg_1. The detection limits of other ginseng saponins are as follows : 1.6ng for ginsenoside Rf, 4.6ng for ginsenoside Rg_2, 2.7ng for ginsenoside Rh_1, 43.3ng for ginsenoside Rb_1, 29.6ng for ginsenoside Rb_2, 50.0ng for ginsenoside Rc, and 97.4ng for ginsenoside Rd. The detection limits of panaxadiol type saponins were greater than those of panaxatriol type saponins because the retention times of the panaxadiol type saponins were longer, which resulted in broad peak shape and low peak height.

This result shows that ginseng saponins could be several hundred times more sensitively analyzed using IC/PAD method than conventional

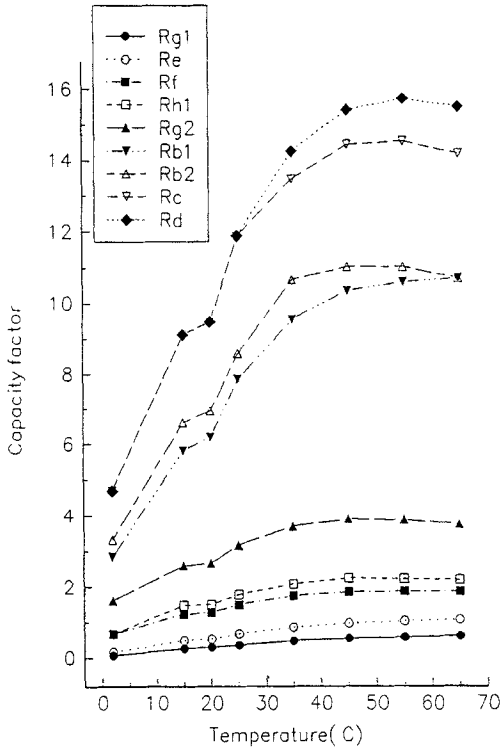


FIGURE 5. Effect of the column temperature to the capacity factor of ginseng saponins. HPIC-AS4A column, 1.0M NaOH, 1ml/min, PAD : 720ms (0.0V), 120ms (+0.6V), 360ms (-0.8V)

HPLC/UV detection method. With the development of a new column that can accomplish effective separation of all ginseng saponins, improvement in detection sensitivity as well as resolution is expected.

CONCLUSION

Ginseng saponins were analyzed by IC/PAD method. The conditions for the analysis of ginseng saponins were optimized with the change of column,

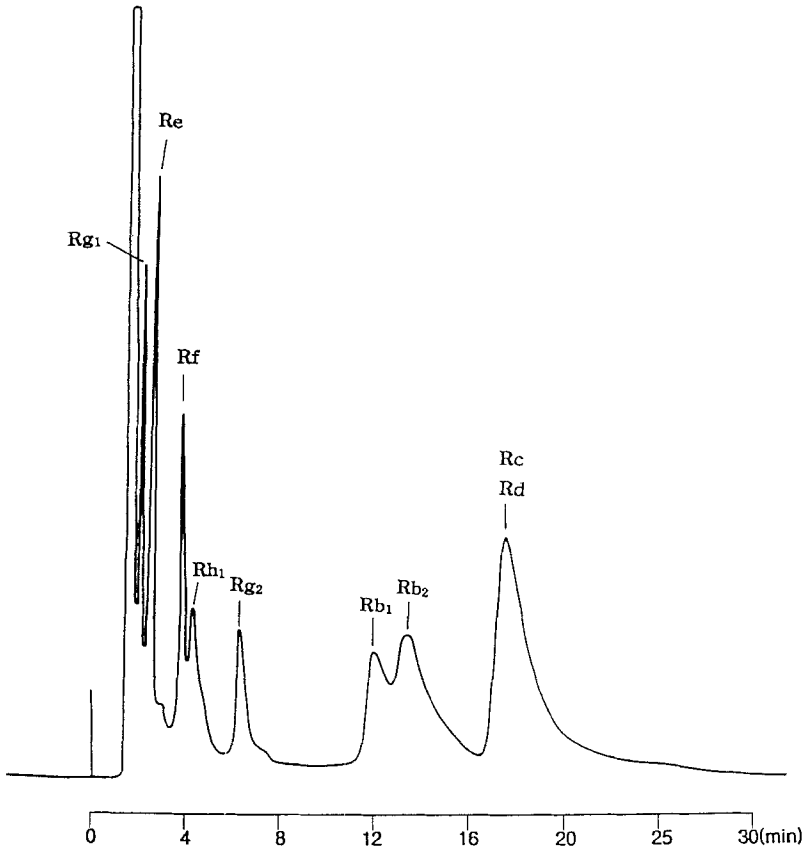


FIGURE 6. Chromatogram of mixture of ginseng saponins.
(Conditions are as in FIGURE 5)

column temperature, the concentration of NaOH in the mobile phase, and potential of E1, E2, E3.

Five panaxatriol type saponins, ginsenoside Re, Rf, Rg_1, Rg_2, Rh_1, were separated on HPIC-AS4A column and detected with PAD. The detection limit of ginsenoside Re was 0.8ng and the dynamic linear range was over 10^3. The correlation coefficient of the calibration curve was better than 0.99 in the range of 10ng - 10μg.

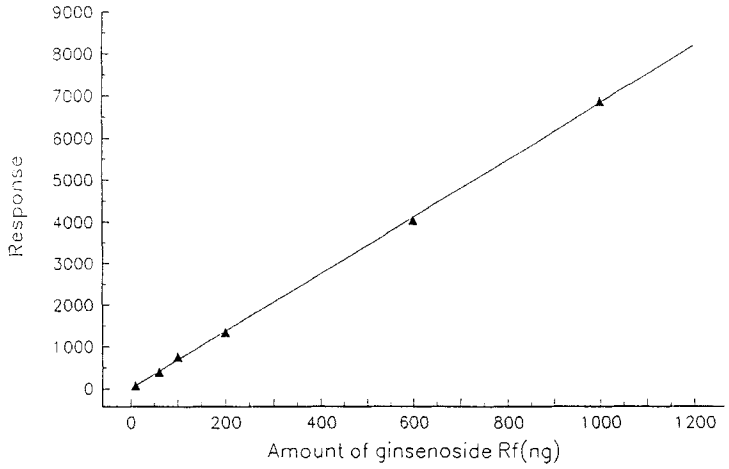


FIGURE 7. Calibration curve of ginsenoside Rf.
(Conditions are as in FIGURE 5)

So far, the analysis of ginseng saponins by IC/PAD has the drawback that the resolution of panaxadiol type saponins is unsatisfactory. However, it is expected that IC/PAD could be a method of choice for the microscale analysis of ginseng saponins premising the development of suitable column which can separate all saponins.

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