Application Note

742026H

Semi-preparative Separation of Ginsenoside in Ginseng

Introduction

Ginseng is a kind of natural medicine of araliaceae herbaceous perennial which is also called as Asian ginseng or Korean ginseng. It is said that ginseng has many positive effects on recovering from fatigue, pyretolysis, blood pressure control (low- and high- blood pressure), anti-inflammatory /antibacterial action (gastric and duodenal ulcer), hemostasis, cardiotonic action, anti-tumor action (anti-cancer action), diabetes care (blood-sugar level control and insulin secretagogue). Ginseng contains a lot of ginsenoisides which are a kind of saponin. Ginsenoiside Rb1 has central depressant action and ginsenoiside Rg1 has central excitatory action, and their anti-fatigue and sedative action have been reported.

In this LC application data, after studying the separation of ginsenoside Rb1 and Rg1 using conventional HPLC by gradient elution method, the separation using scaled up semi-preparative HPLC will be reported.

Keyword: Semi-preparative separation, Ginseng, Asian ginseng, Ginsenoiside Rb1 and Rg1.

Experimental

[Equipment]

<Conventional HPLC>

Eluent Pump:	PU-2089
Autosampler:	AS-2057
Column oven:	CO-2060
Detector:	MD-2018

<Semi-Preparative HPLC>

Eluent Pump:	PU-2086 (x2)
Mixer:	MX-2080-32 (with 10 mL chamber)
Autosampler:	AS-2058
Column oven:	CO-2060
Detector:	MD-2018 (with semi-prep. cell)
Chromatography data system:	ChromNAV
Fraction collector:	ADVANTEC SCF 122SC
Fraction collector controller:	FC-2088-30

[Conditions]

<Conventional HPLC>

Column:	YMC-PACK Pro C18 (4.6 mm ID x 250 mmL, 5 µm)
Eluent:	A; Water, B; Acetonitrile, linear gradient
Gradient condition:	(A/B), 0 min(80/20) -> 15 min(50/50) -> 20 min(50/50) -> 20.1 min(80/20) 1 cycle; 40 min
Eluent flow rate:	1.0 mL/min
Column temp.:	25 °C
Wavelemgth:	200 ~ 450 nm, 203 nm
Injection volume:	20 µL
Standard sample:	Powdered Ginseng (1.0g/50mL in 60% methanol)

<Semi-Preparative HPLC>

Column:	YMC-PACK Pro C18 (20 mm ID x 250 mmL, 5 µm)
Eluent:	A; Water, B; Acetonitrile, linear gradient
Gradient condition:	(A/B), 0 min(80/20) -> 15 min(50/50) -> 20 min(50/50) -> 20.1 min(80/20) 1 cycle; 40 min
Eluent flow rate:	15 mL/min
Column temp.:	25 °C
Wavelemgth:	203 nm
Injection volume:	5 mL
Standard sample:	Powdered Ginseng (1.0g/50mL in 60% methanol)

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[Preparation (extraction)]
(1) Weigh precisely 1.0 g of powdered ginseng and put into the centrifuge tube.
(2) Add 30 mL of 60% methanol and mix them for 15 minutes.
(3) Apply centrifugation (3,000 rpm, 10mim) and put the supernatant into 50 mL measuring flask.
(4) Add 20 mL of 60% methanol to the residue and implement the same procedure.
(5) Add 60% methanol to collected supernatant in measuring flask to be 50 mL.

Fig. 1 shows the structural formula of ginsenosides.

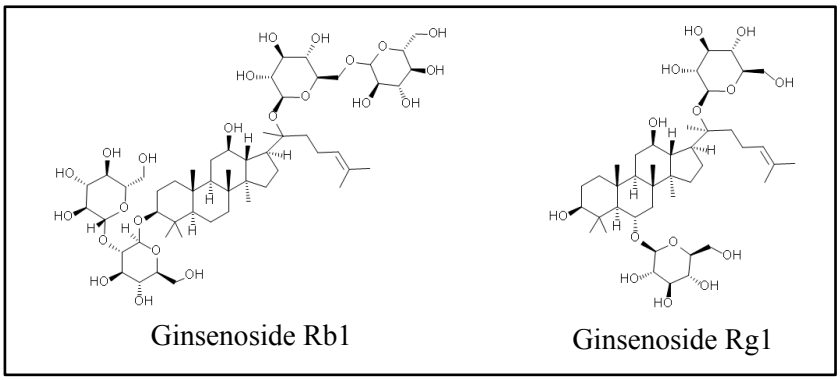

Fig. 1: Structual Formula of Ginsenosides

Result

Fig. 2 shows the chromatogram and contour plot of the extracts from ginseng powder by using conventional HPLC. Since the retention volumes of ginsenoiside Rg1 and Rb1 are different, the separation condition has been determined using gradient elution procedure. Using PDA detector and by comparing spectra for improving the separation of the target from other components, ginsenoiside Rg1 and Rb1 were clearly separated within 16 minutes.

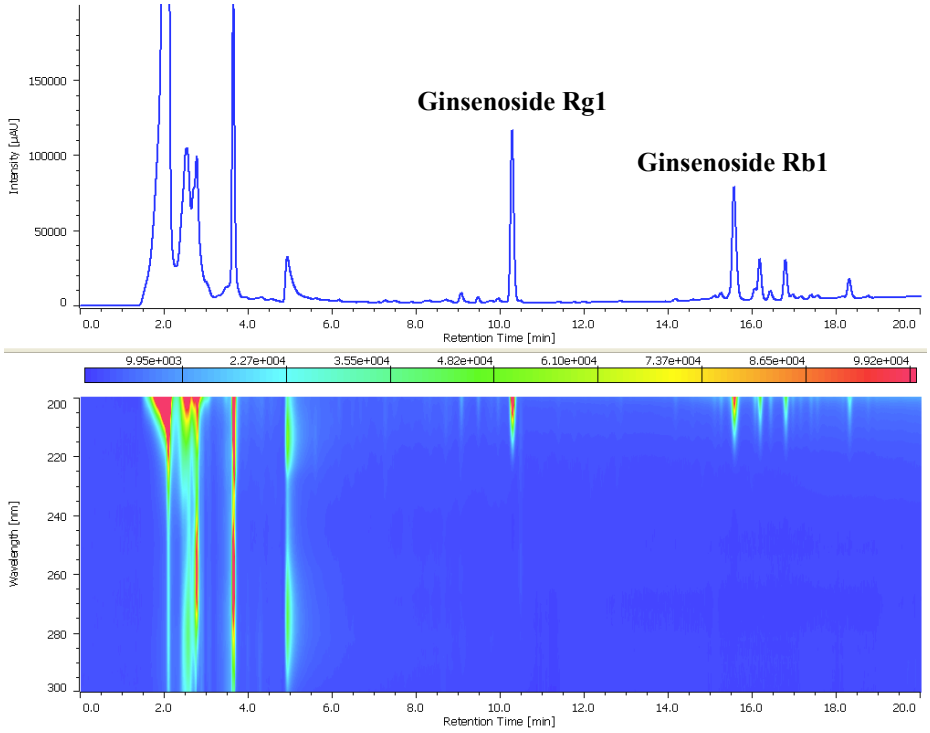

Fig. 2: Chromatogram of the Extracts from Ginseng Powder

Fig. 3 shows the chromatogram of ginseng powder obtained by using semi-preparative separation HPLC scaled up from conventional HPLC. In order to obtain separated ginsenoisides as much as possible, 5 mL of sample was injected. Fig. 4 shows the fraction display in ChromNAV, JASCO chromatography data system. The fractionated peaks and sample rack position for the targets are highlighted with green color. Fig. 5 shows chromatogram of each fraction obtained under the same condition as in Fig. 2. Since ginsenoiside Rb1 and the other components were not separated completely in Fig. 3, the small peaks were still seen just after ginsenoiside Rb1, but it was confirmed that each compound was clearly isolated.

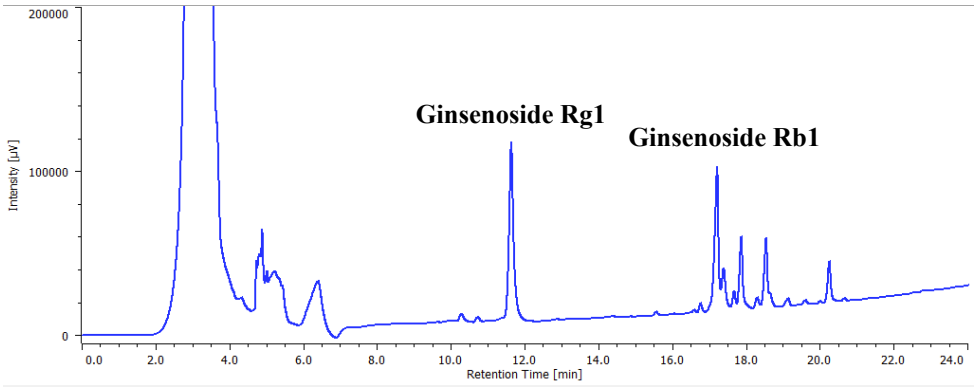

Fig. 3: Semi-preparative Chromatogram of the Extracts from Ginseng Powder

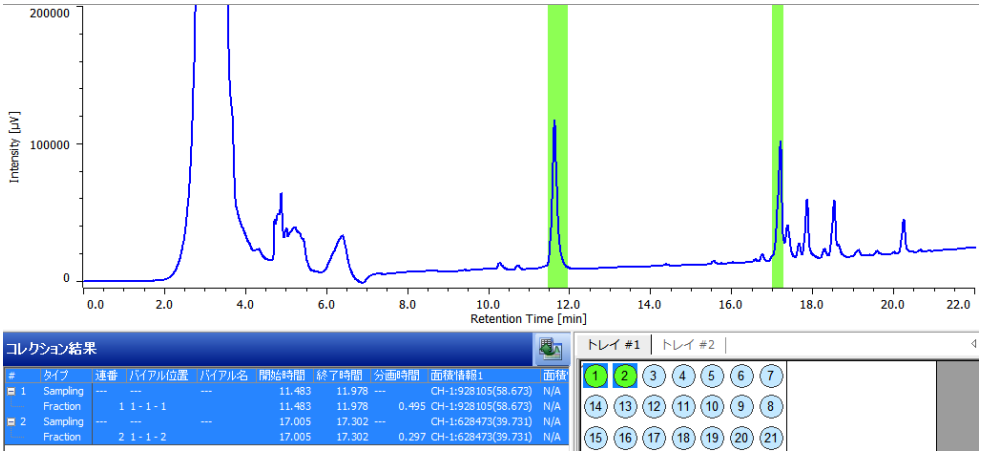

Fig. 4: Fractionation Result of the Extracts from Ginseng Powder (ChromNAV Display)

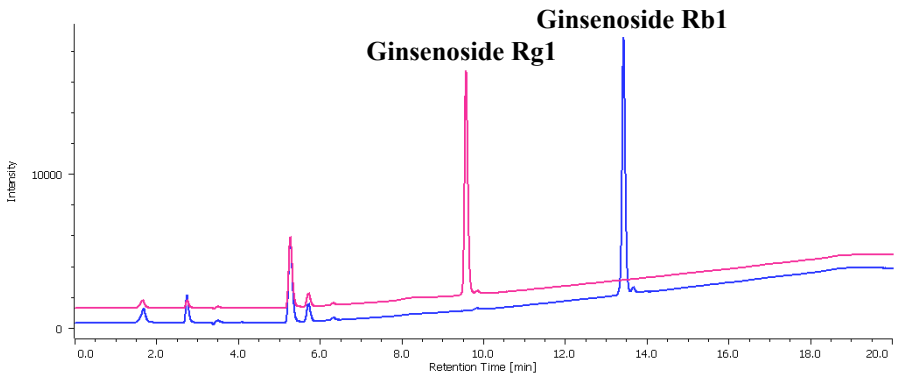

Fig. 5: Chromatogram of Fractionated Compounds (Blue: Ginsenoside Rb1, Red: Ginsenoside Rg1)

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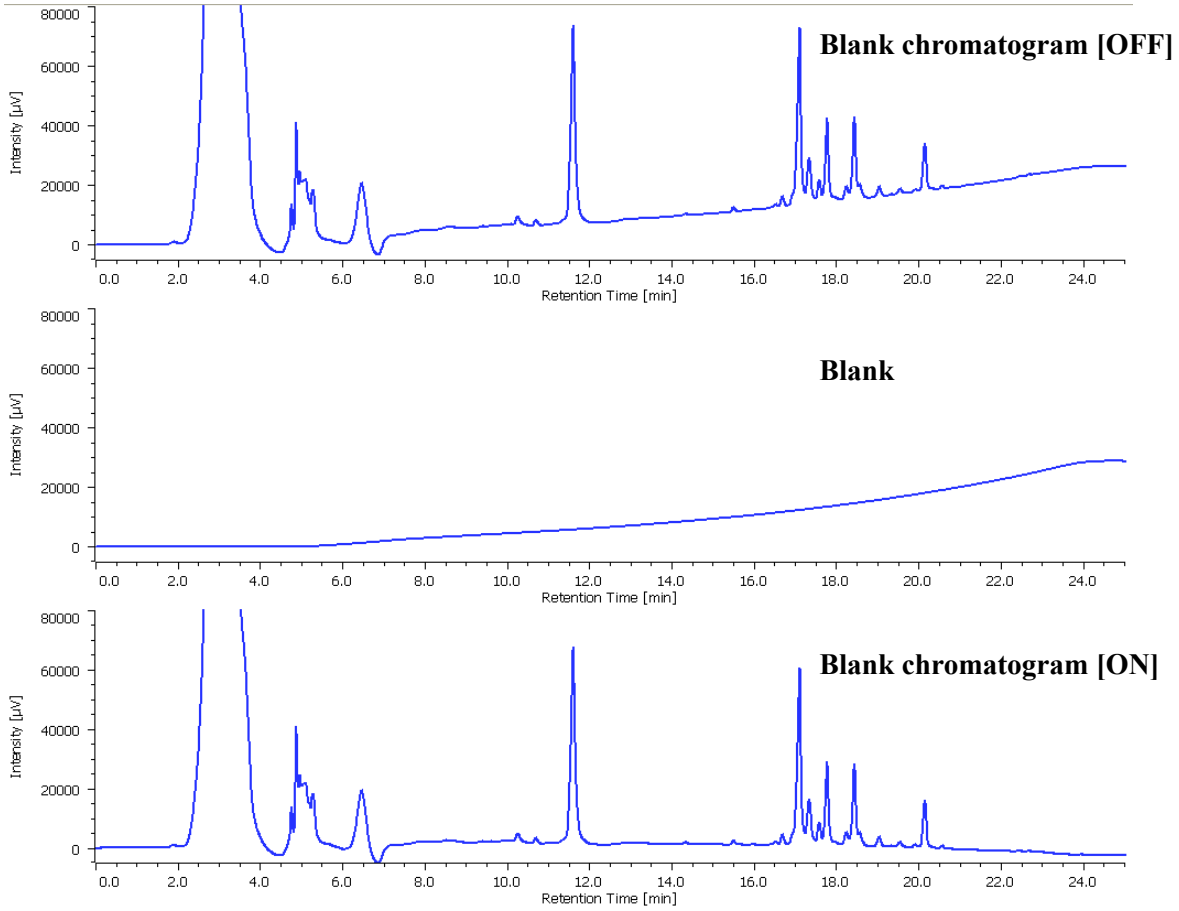
Hint!

<Blank chromatogram>

In the case of fractionation by gradient elution method, when the base line is fluctuated, it is difficult to judge the level slope correctly. For instance, the target has UV absorption only in shorter wavelength range and the eluent used for gradient elution also has UV absorption in the same range. JASCO chromatography data system ChromNAV has “blank chromatogram” function which enables to record the blank chromatogram measured in advance and show the chromatogram in which such baseline is subtracted, for judgment for fractionation. In this analysis of ginseng, since the peaks are detected at 203 nm and methanol which has UV absorption has been used in gradient, this function was employed. Figure 4 shows chromatogram with baseline fluctuation, but in practice for fractionation, the peaks were isolated under baseline corrected chromatogram. Figure 6 shows chromatogram with and without “blank chromatogram” function.



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