Application Note

Semi-preparative Separation of Ginsenoside in Ginseng

Introduction

Ginseng is a natural medicine derived from the araliaceae herbaceous perennial more commonly known as Asian ginseng or Korean ginseng. Ginseng has been widely reported as having many benefits including recovery 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 large amount of ginsenoisides - a type of saponin. Ginsenoside Rb1 has central depressant action and ginsenoiside Rg1 has central excitatory action, and their anti-fatigue and sedative action have been reported.

This HPLC application describes the development of a separation method for ginsenoside Rb1 and Rg1 using gradient elution in conventional reverse phase HPLC which can be scaled-up to semi-preparative HPLC.

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Keywords

Analytical separation, Semi-preparative separation, Coptis japonica, Berberine, Neutraceutical, Natural medicine

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Experimental

Equipment

Conventional HPLC	
Eluent Pump:	PU-2089
Autosampler:	AS-2057
Column oven:	CO-2060
Detector:	MD-2018
min(50/50) ->	
min(80/20)	

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
min(50/50) ->	
min(80/20)	
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
	20 min (50/50) -> 20.1
	1 cycle; 40 min
Eluent flow rate:	1.0 mL/min
Column temp.:	25°C
Wavelength:	200 ~ 4 50 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
Eluent flow rate:	15 mL/min
Column temp.:	25°C
Wavelength:	203 nm
Injection volume:	5 mL
Standard sample:	Powdered Ginseng
	(1.0g/50mL in 60% methanol)

Preparation (extraction)

(1) Weigh 1.0 g of powdered ginseng and place in a centrifuge tube.

(2) Add 30 mL of 60% methanol and mix for 15 minutes.

(3) Centrifuge (3,000 rpm, 10mim) and decant the supernatant into a 50 mL measuring flask

(4) Add 20 mL of 60% methanol to the residue and repeat the procedure.

(5) Add 60% methanol to collected supernatant in measuring flask and make up to 50 mL

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Fig. 1 Structural formula of Ginsenosides.

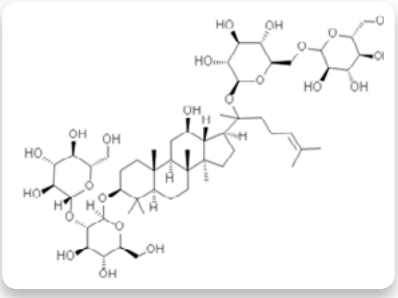

Fig. 1: Ginsenoside Rb1

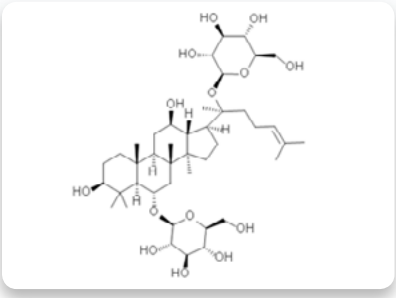

Fig. 1: Ginsenoside Rg1

Result

Fig. 2 Chromatogram and contour plot of extracts from ginseng powder separated using conventional HPLC. Since the retention of ginsenoiside Rg1 and Rb1 are different, the separation conditions have been determined using gradient elution. The MD-2018 PDA detector and spectral comparison was used to optimize the separation of the target compounds from other components; ginsenoisides Rg1 and Rb1 were clearly separated within 16 minutes.

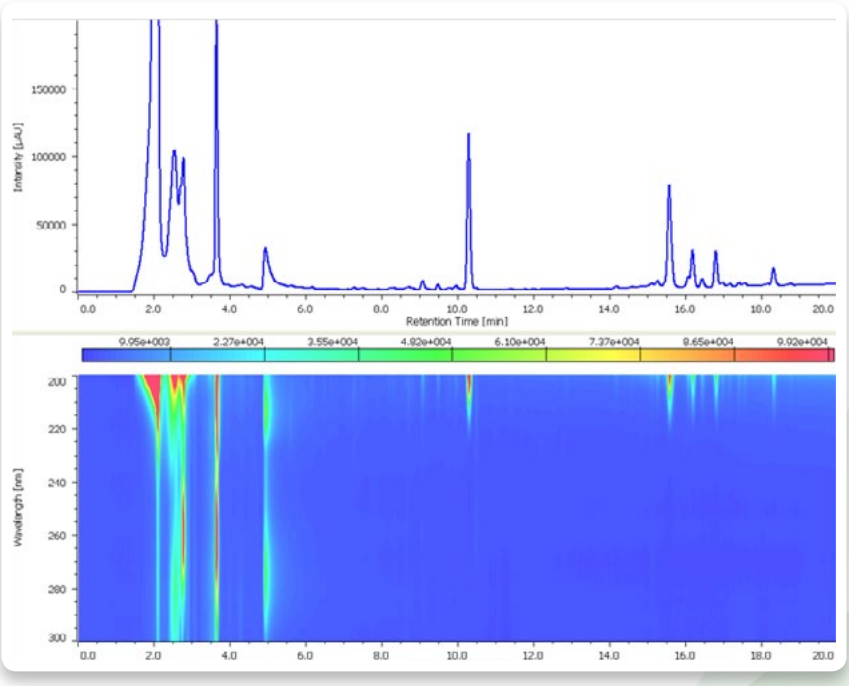

Fig. 2: Chromatogram of the extract from Ginseng powder

Fig. 3. shows the chromatogram of the ginseng powder separated using a semi-preparative HPLC scaled-up from analytical. In order to maximize the recovery of the separated ginsenoisides a 5 mL sample volume was injected. Fig. 4. shows the fraction display in the ChromNAV chromatography data system. The peaks fractions and sample rack position for the target compounds are highlighted in green. Fig. 5. shows chromatograms of a purity check of the recovered fractions analyzed using the same conditions as in Fig. 2. Ginsenoside Rb1 and a minor component were not completely resolved in the semi-prep separation and small contaminant peak can be observed just after the main peak, but it was confirmed that each compound was clearly isolated.

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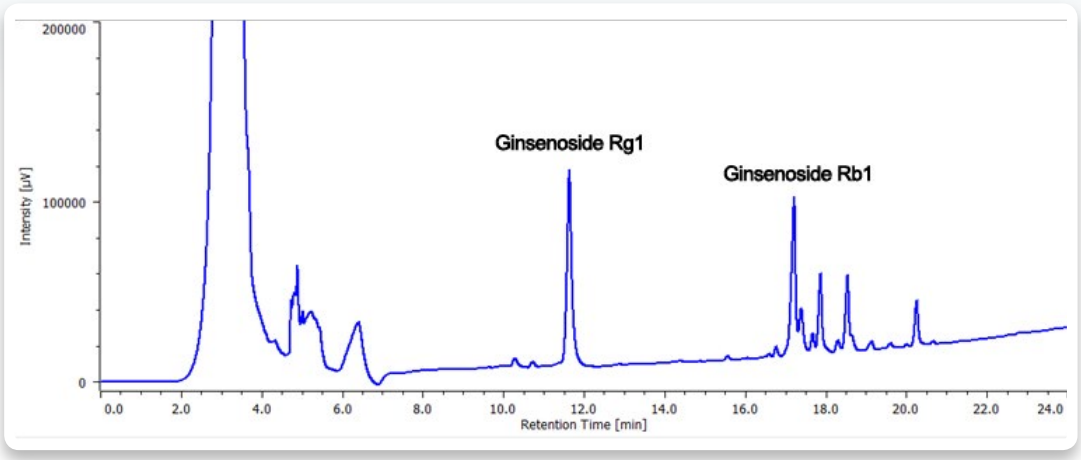

Fig. 3: Semi-preparative chromatogram of an extract from Ginseng powder

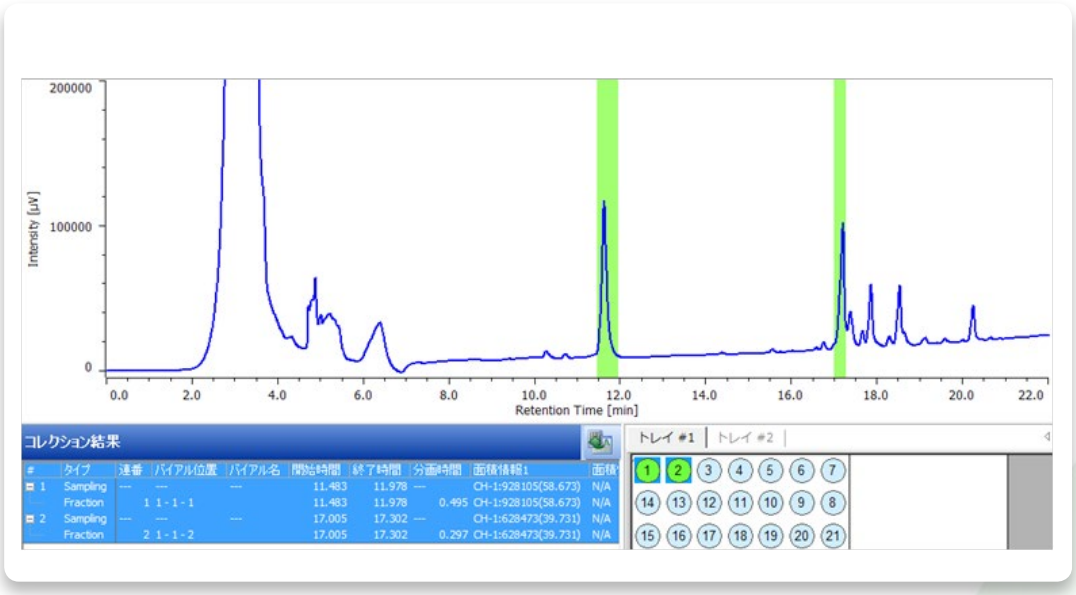

Fig. 4: Peak fraction result of an extract from Ginseng Powder (ChromNAV Screen)

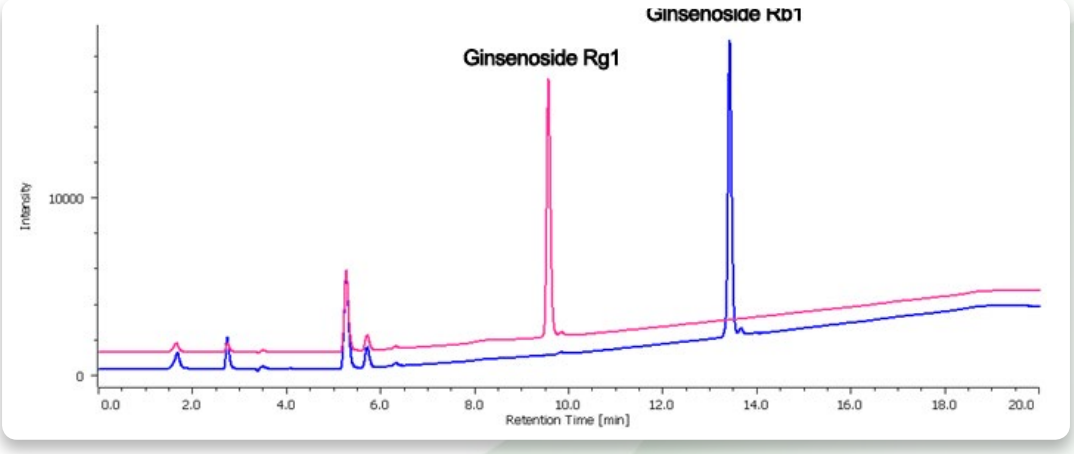

Fig. 5: Chromatogram of the collected fraction (10 µL Injected)

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