

## Semi-preparative Separation of Glycyrrhizic Acid in Glycyrrhiza

### Introduction

Glycyrrhiza known as one of natural medicines belongs to the legume family (Fabaceae) and has excellent positive effects on analgesic, anti-inflammatory, anti-gastralgia, antitussive/expectorant and detoxifying and is also used as a cold medicine. Glycyrrhizic acid contained richly in Glycyrrhiza is several tens to hundred times sweeter than sucrose and is used as natural sweetener.

In this LC application data, after studying the separation of Glycyrrhizic acid from the extract of Glycyrrhiza powder using conventional HPLC system, the separation using scaled-up semi-preparative HPLC will be reported.

**Keyword:** Semi-preparative separation, Glycyrrhiza, Glycyrrhizic acid

### Experimental

#### [Equipment]

<Conventional HPLC>

Eluent Pump: PU-2089

Autosampler: AS-2057

Column oven: CO-2060

Detector: MD-2018

#### [Conditions]

<Conventional HPLC>

Column: YMC-PACK Pro C18  
(4.6 mm ID x 250 mmL, 5  $\mu$ m)

Eluent: Acetonitrile / Water (35/65)

Eluent flow rate: 1.0 mL/min

Column temp.: 25  $^{\circ}$ C

Wavelength: 254 nm

Injection volume: 200  $\mu$ L

Standard sample: Powdered Glycyrrhiza  
(0.5 g/100 mL in 50% ethanol)

<Semi-Preparative HPLC>

Eluent Pump: PU-2086

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

<Semi-Preparative HPLC>

Column: YMC-PACK Pro C18  
(20 mm ID x 250 mmL, 5  $\mu$ m)

Eluent: Acetonitrile / Water (35/65)

Eluent flow rate: 15 mL/min

Column temp.: 25  $^{\circ}$ C

Wavelength: 254 nm

Injection volume: 5 mL

Standard sample: Powdered Glycyrrhiza  
(0.5 g/100 mL in 50% ethanol)

#### [Preparation (extraction)]

- (1) Weigh precisely 0.5 g of powdered glycyrrhiza and put into the centrifuge tube.
- (2) Add 60 mL of the diluted ethanol solution (ethanol/Water (50/50)) and mix for 15 minutes.
- (3) Apply centrifugation (2000 rpm, 10 minutes) and put the supernatant into the 100 mL volumetric flask.
- (4) Add 30 mL of diluted ethanol solution (ethanol/Water (50/50)) to the residue and implement the same procedure.
- (5) Add the diluted ethanol solution to the collected supernatant in the volumetric flask to be 100 mL.

Fig. 1 shows the structural formula of Glycyrrhizic acid.

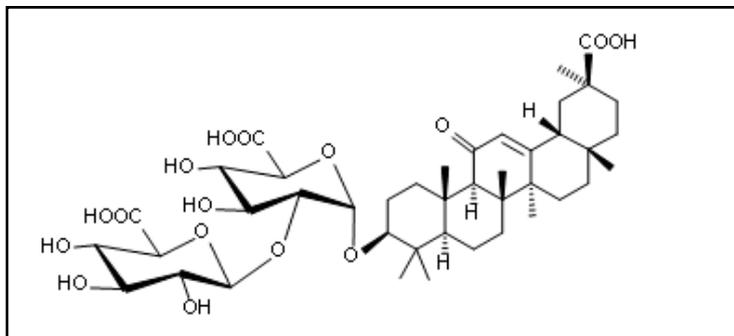


Fig. 1: Structural Formula of Glycyrrhizic Acid

## Result

Fig. 2 shows chromatogram and contour plot of the extracts from Glycyrrhiza powder by using conventional HPLC. Using PDA detector and by comparing spectra for improving the separation of the target from other components, it took 40 minutes for separation, but the good separation result was obtained.

Fig. 3 shows chromatogram using semi-preparative HPLC scaled-up from conventional HPLC. In order to collect the amount of Glycyrrhizic acid as much as possible, 5 mL of sample was injected. As shown in Figure 3, the separation efficiency was increased even at the sacrifice of the peak shape. Fig. 4 shows the fraction display in ChromNAV, JASCO Chromatography data system. The fractionated peaks and sample rack position for the target are highlighted with green color. It is shown that fractionated collection was performed by two vials. Figure 5 shows chromatogram of this fraction under the same condition as in Figure 2. It is confirmed that Glycyrrhizic acid was isolated as single component.

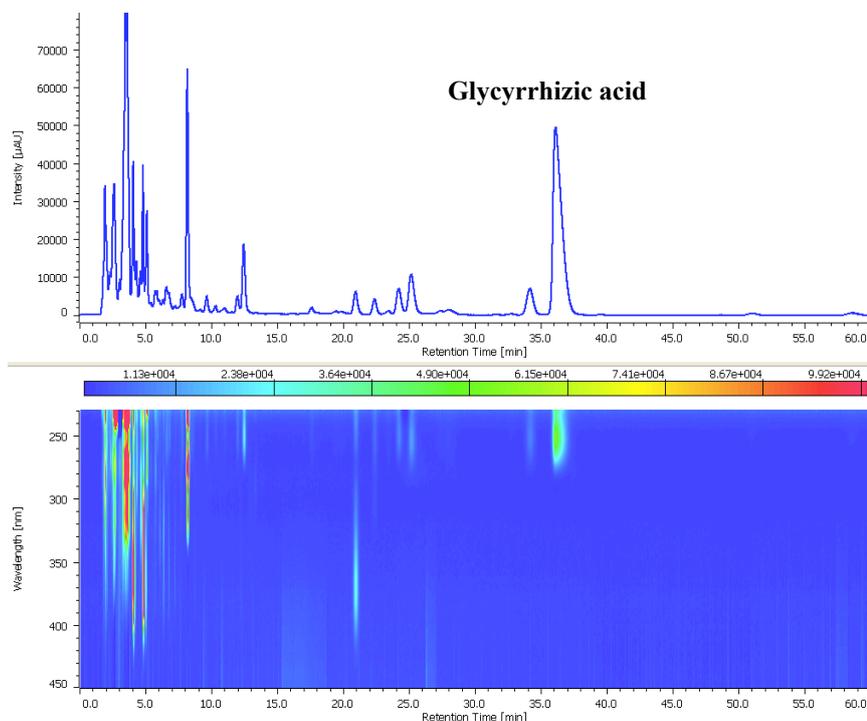


Fig. 2: Chromatogram of the extracts from Glycyrrhiza powder

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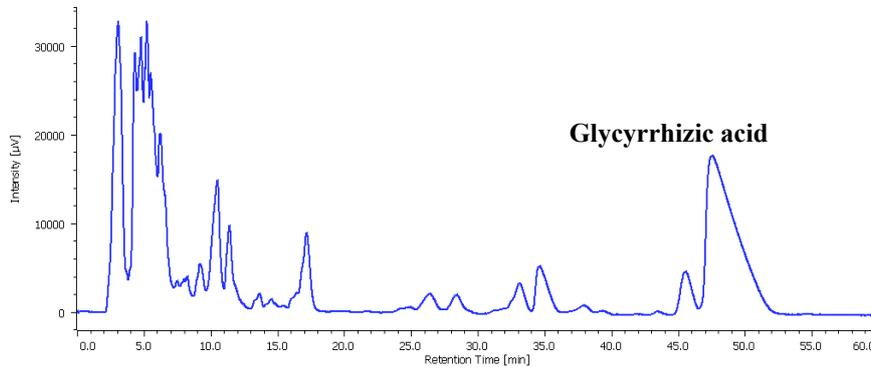


Fig. 3: Semi-preparative chromatogram of the extracts from Glycyrrhiza powder

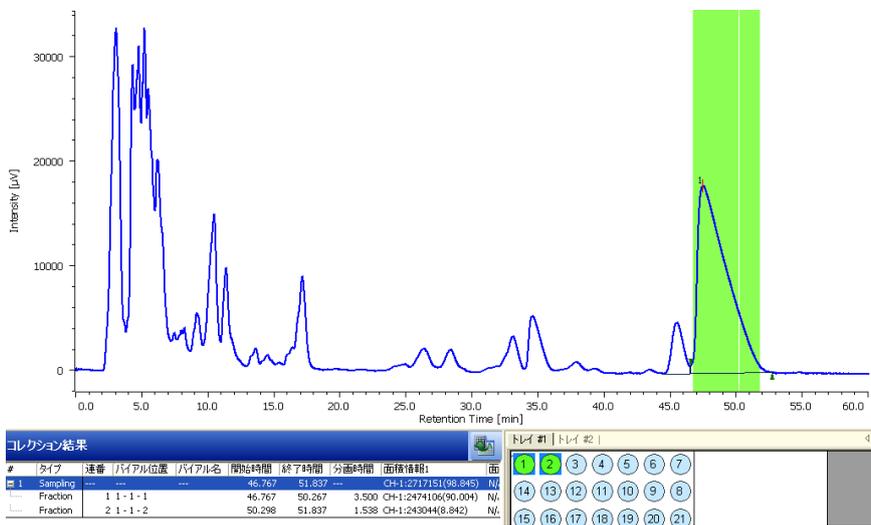


Fig. 4: Fractionation result of the extracts from Glycyrrhiza powder (ChromNAV display)

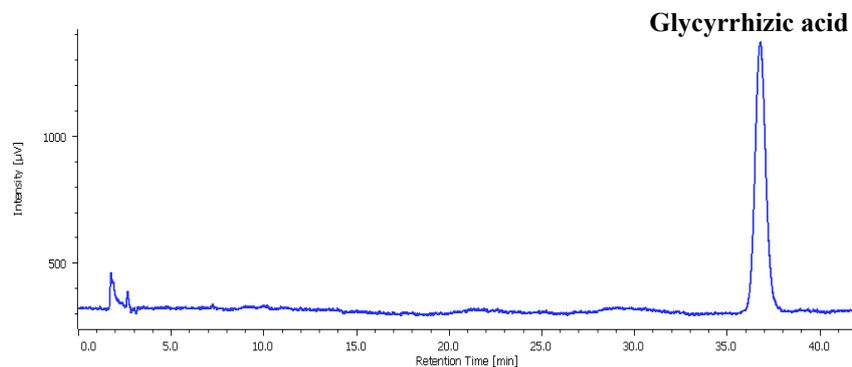


Fig. 5: Chromatogram of fractionated compound (20 μL injected)