Application Note

742025H

Semi-preparative Separation of Berberine in Coptis Japonica

Introduction

Coptis japonica is a kind of natural medicine of Ranunculaceae and is said to have several positive effects on conjunctivitis and stomatitis as well as on stomachic, intestinal remedy and antidiarrheic. Berberine, one of alkaloids contained in coptis japonica, which has yellow color and bitter taste, is said to have antibacterial and anti-inflammatory effects. This time in this LC application data, after studying the separation of berberine from the powder extract of coptis japonica using conventional HPLC, the separation using scaled-up semi-preparative HPLC will be reported.

Keyword: Semi-preparative separation, Coptis japonica, Berberine

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
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:	0.1% TFA in Acetonitrile / Water (30/70)
Eluent flow rate:	1.0 mL/min
Column temp.:	25 °C
Wavelemgth:	220 ~ 450 nm, 345 nm
Injection volume:	10 µL
Standard sample:	Powdered Coptis japonica (0.5 g/50 mL in methanol / 10% hydrochloric acid (100/1))

<Semi-Preparative HPLC>

Column:	YMC-PACK Pro C18 (20 mm ID x 250 mmL, 5 µm)
Eluent:	0.1% TFA in Acetonitrile / Water (30/70)
Eluent flow rate:	15 mL/min
Column temp.:	25 °C
Wavelemgth:	345 nm
Injection volume:	5 mL
Standard sample:	Powdered Coptis japonica (0.5 g/50 mL in methanol / 10% hydrochloric acid (100/1))

[Preparation (extraction)]

(1) Weigh precisely 0.5 g of powdered coptis japonica and put into the centrifuge tube.
(2) Add 30 mL of methanol/10% hydrochloride mixture (100/1) and mix for 15 minutes.
(3) Apply centrifugation (3,000 rpm, 10mim) and put the supernatant into 50 mL measuring flask.
(4) Add 20 mL of methanol/10% hydrochloride mixture (100/1) to the residue and implement the same procedure.
(5) Add methanol/10% hydrochloride mixture (100/1) to the collected supernatant in measuring flask to be 50 mL.

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Fig. 1 shows the structural formula of berberine.

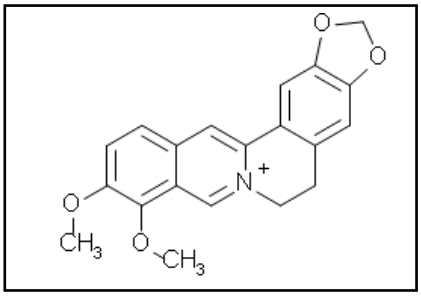

Fig. 1: Structual Formula of Berberine

Result

Fig. 2 shows chromatogram and contour plot of the extracts from coptis japonica powder by using conventional HPLC. Using PDA detector and by comparing spectra for improving the separation of the target from other components, berberine was clearly separated within 15 minutes.

Fig. 3 shows chromatogram of the extracts from coptis japonica powder by using semi-preparative HPLC scaled up from conventional HPLC. In order to obtain separated berberine as much as possible, the injection volume was increased, however, since the elution power by extraction liquid was stronger than by mobile phase, the components containing the target compound was not retained and eluted. Therefore the sample solution was diluted five times by water and then 5 mL of diluted sample was injected. Then the target component was retained as shown in Fig. 3, in which 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 peak and sample rack position for the target are highlighted with green color. Fig. 5 shows chromatogram of this fraction under the same condition as in Fig. 2. It is confirmed that berberine was isolated as single component.

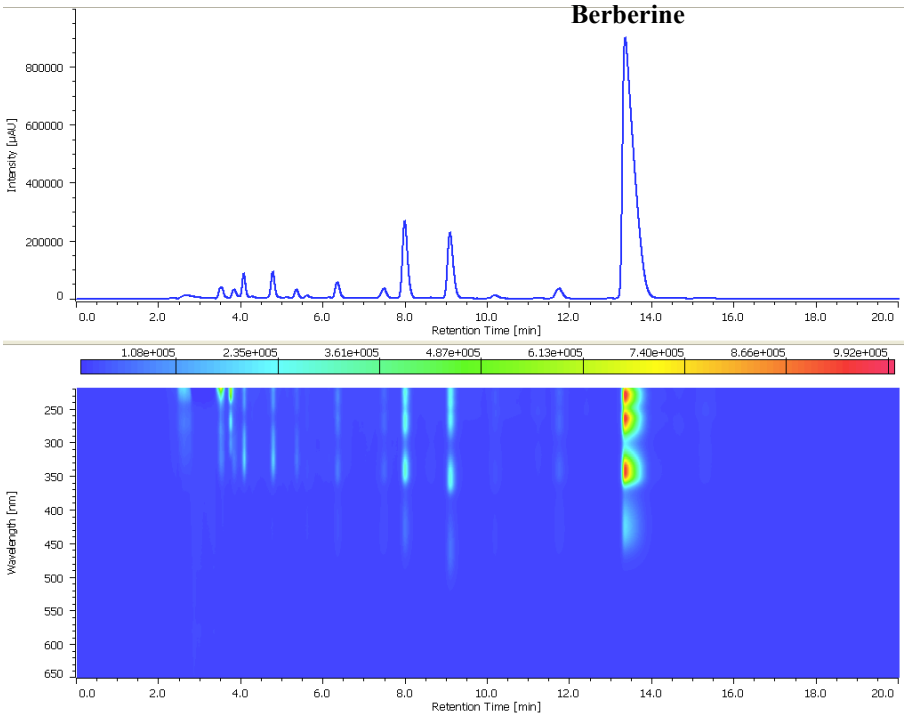

Fig. 2: Chromatogram of the Extract from Coptis Japonica Powder

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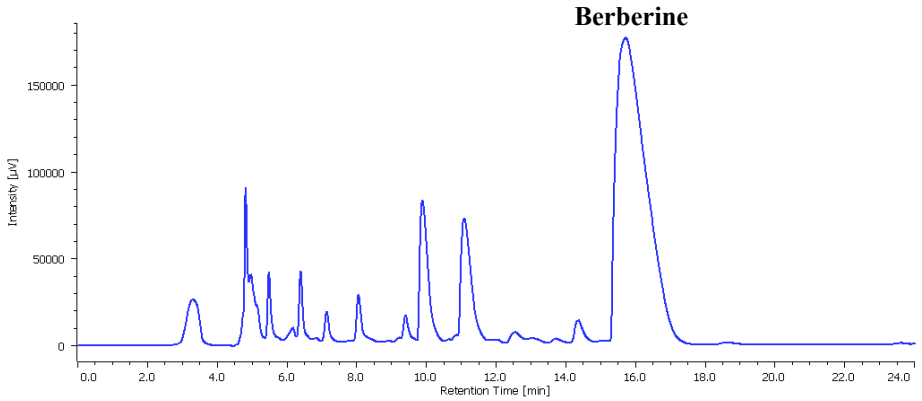

Fig. 3: Semi-preparative Chromatogram of the Extract from Coptis Japonica Powder

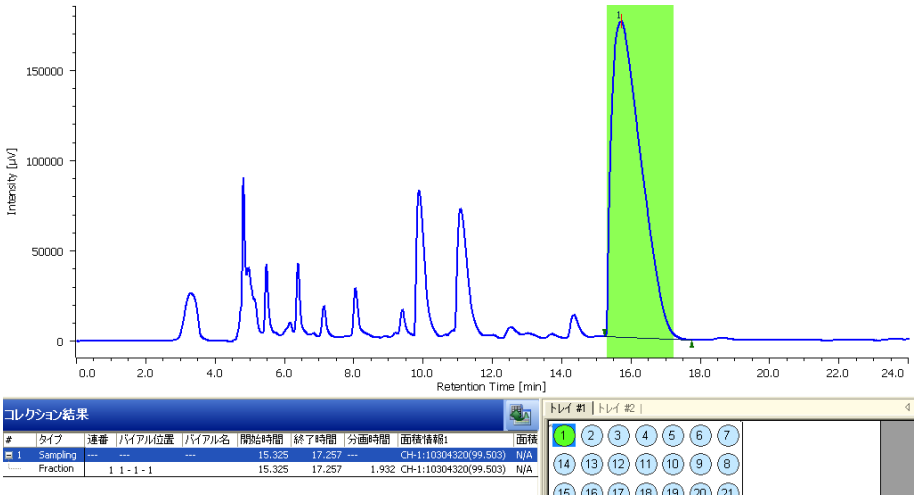

Fig. 4: Fractionation Result of the Extract from Coptis Japonica Powder (ChromNAV Display)

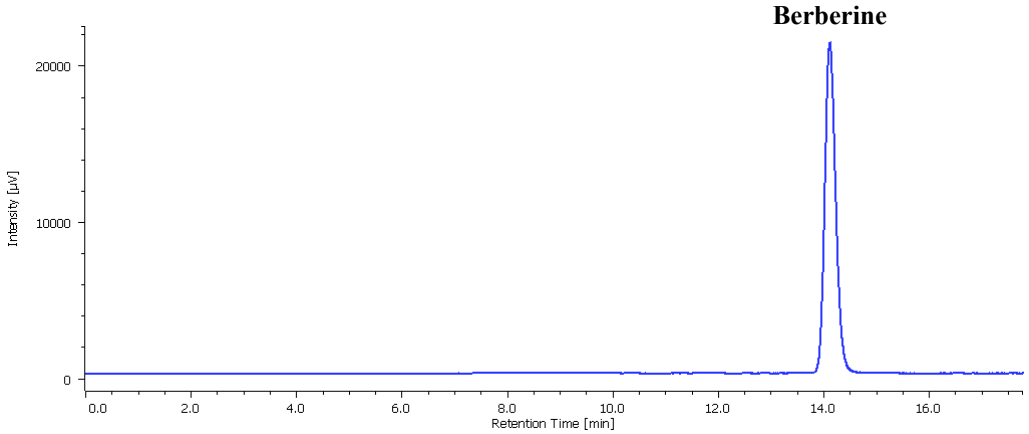

Fig. 5: Chromatogram of Fractionated Compound (10 µL Injected)

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