Technical Report

ASC/LC-107 High performance liquid chromatograph

HITACHI
Inspire the Next

Determination of Scrophularia root

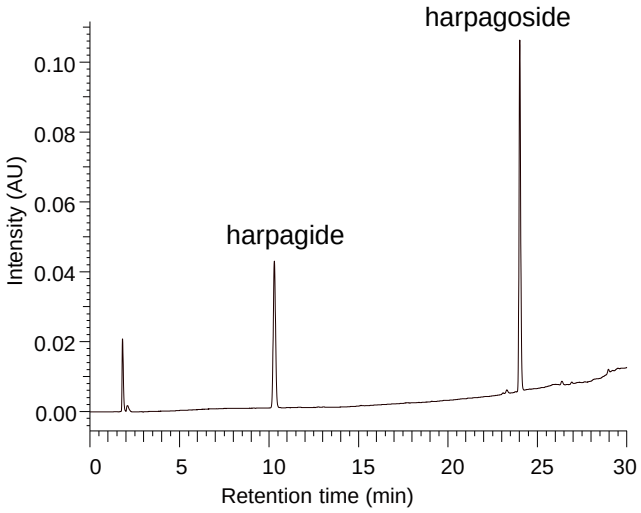
Scrophularia root is the dried root of Scrophularia ningpoensis Hemsl. In this report, the analysis of harpagide and harpagoside in Scrophulariaceae is performed using a Hitachi Primaide HPLC system refers to the first part of the 2020 edition of the Chinese Pharmacopoeia, with a GL Sciences InertSustain AQ-C18 column as the analytical column, acetonitrile-0.03 % phosphoric acid solution as the mobile phase, and the detection is carried out with a UV detector at 210 nm.
The analysis of standard samples and the determination of commercially available samples is measured.

High performance
Liquid Chromatograph

Primaide

Analysis of standard samples

■ Analysis of standard sample



Chromatogram of standard sample
(harpagide：60.0 mg/L，harpagosice: 20.0 mg/L)

■ Analytical conditions

Column : GL Sciences InertSustain AQ-C18, 5 µm,
4.6 mm I.D.× 150 mm

Eluent : A：acetonitrile

B：0.03% phosphoric acid (V / V)

3% A(0 min) →10% A(10 min) →33% A(20 min)
→50% A(25 min) →80% A(30 min-35 min)
→ 3% A(37 min-60 min)

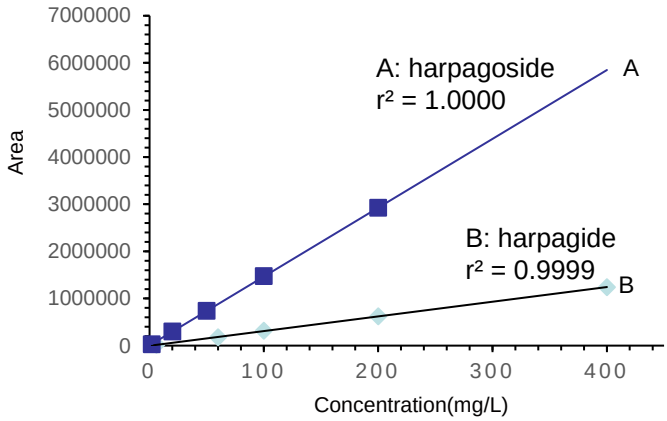
Flow rate : 1.0 mL/min

Column temperature : 25 °C

Detection wavelength : 210 nm

Injection vol. : 10 µL

■ Linearity



■Reproducibility（harpagide：60.0 mg/L，harpagoside: 20.0 mg/L, n=6 ）

	harpagide		harpagoside	
NO.	RT(min)	Area	RT(min)	Area
1	10.305	177771	24.013	299874
2	10.297	178790	24.020	300402
3	10.298	177759	24.018	299956
4	10.300	177666	24.013	299111
5	10.302	177949	24.017	298937
6	10.283	178067	24.007	299558
AVG	10.298	178000	24.015	299640
SD	0.007662	413	0.004676	551
RSD	0.07%	0.23%	0.02%	0.18%

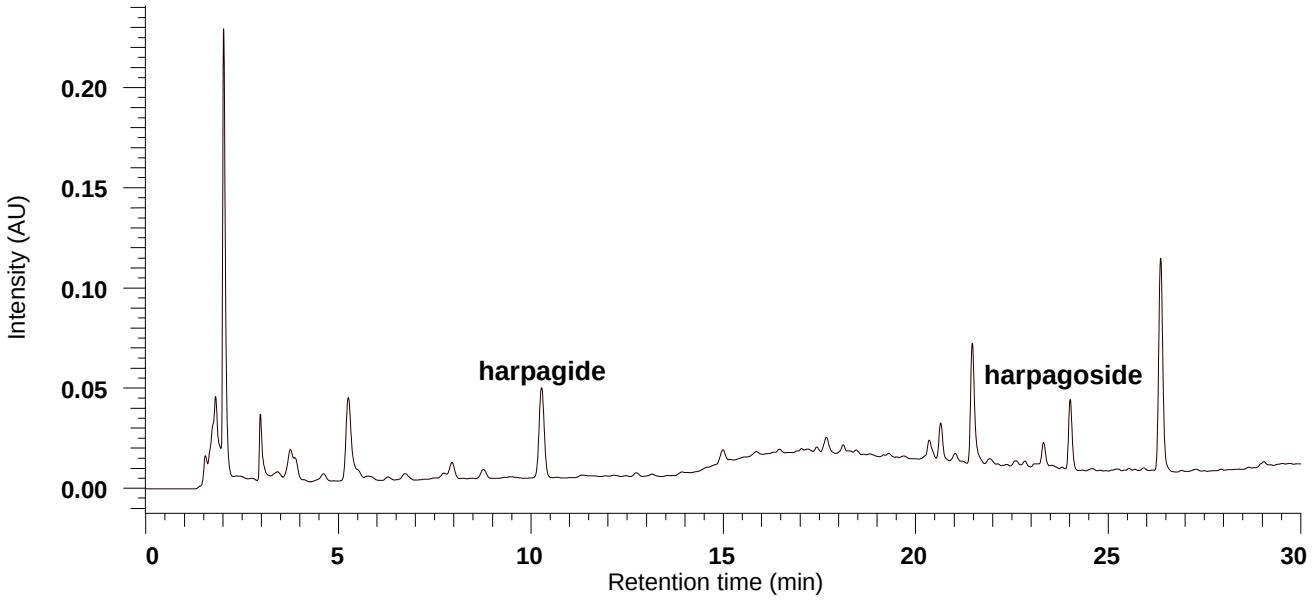
■ System suitability

Component	theoretical plate number in Pharmacopoeia	Measured value of theoretical plate number
harpagide	≥5000	33400
harpagoside	≥5000	386000

The good repeatability of the retention time and area was obtained. Harpagoside and harpagoside has a good linear relationship. The measurement results show that the method meets the requirement of the theoretical plate number specified in the Chinese Pharmacopoeia.

Determination of Scrophulariacease sample

■ Analysis of Scrophulariaceae sample



Chromatogram of Scrophulariaceae sample

■ Result of Quantitative Analysis

Sample name	Component	Measurement result (total content %)	Labeled value (total content %)	Judgement
Scrophulariaceae	harpagide	0.71	≥0.45	OK
	harpagoside			

The contents of harpagide and harpagoside in the commercially available Scrophulariaceae samples were determined, and the determination results met the requirements of the pharmacopoeia.

■ Sample preparation method

Scrophulariaceae sample

↓

Pass through No. 3 sieve after crushing

↓

Get powder

Weigh 0.5g medicine powder

↓ add 50 mL of 50% methanol

Weigh

↓

Soak for 1 hour

↓

Ultrasonic treatment for 45 min

↓

Cool down, weigh it again, and use 50% methanol to make up the lost weight

↓

After shaking, filter with a 0.22 μm hydrophilic PTFE filter and analyze by HPLC

＜System＞ Primaide 1110 Pump, 1210 Autosampler, 1310 Column oven, 1410 UV detector

NOTE: These data are an example of measurement; the individual vales can not be guaranteed.