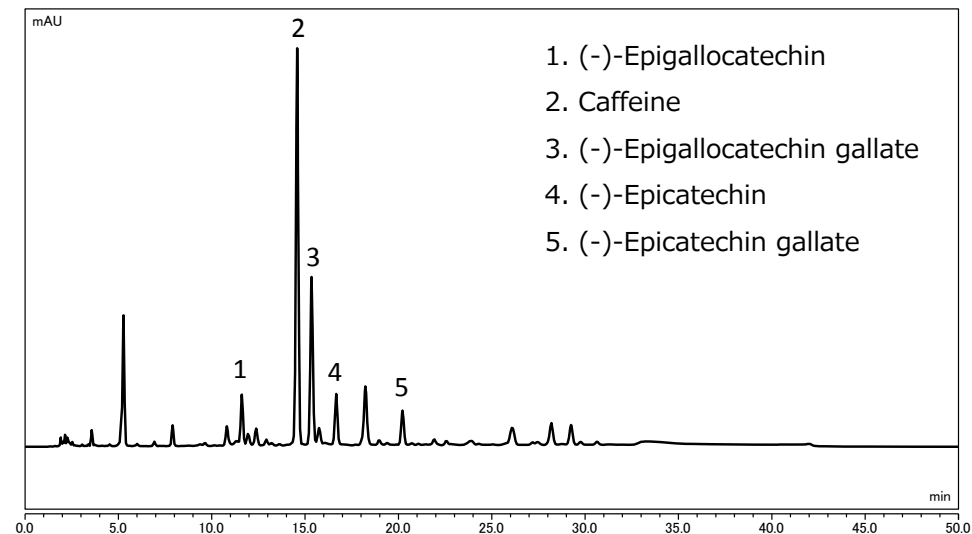


Develosil Application Data

No.102 Analysis of catechins in tea (UHPLC)

● Original method (HPLC)



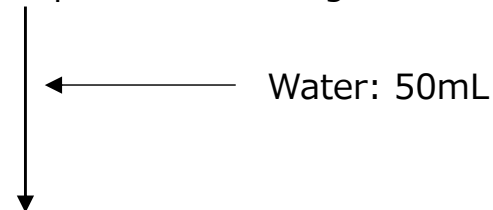
HPLC Conditions:

Column: Develosil HSR AQ C18, 5um (4.6x150mm)
 Mobile phase: A) 0.1% Phosphoric acid B) Methanol
 Flow rate: 1.0mL/min
 Temperature: 40°C (ForcedAir)
 Detection: UV280nm
 Sample: Japanese Tea
 Injection volume: 10uL
 System: Thermo Fisher SCIENTIFIC Vanquish_H
 Mixer volume: 10uL

Gradient:	A	B	Curve
0.0	90	10	5
30.0	50	50	5
40.0	50	50	5
40.1	90	10	5
50.0		End	

Preparation of samples

Japanese Tea: 6.0g



Leave overnight (24hr)

Centrifugation 3,000rpm – 30min

Diluted with Mobile phase

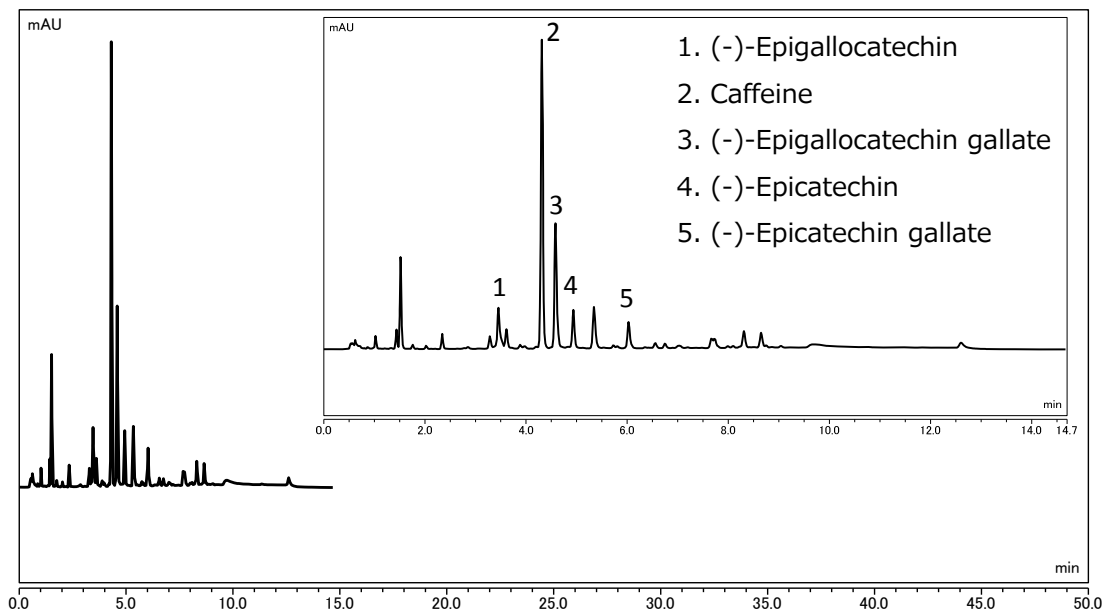
※MeOH/0.1% H₃PO₄=50/50 : 10-fold dilution

Filtration with 0.45um GHP Membrane

Injection

● UHPLC method

Develosil HSR AQ C18, 2um (2.0x100mm)



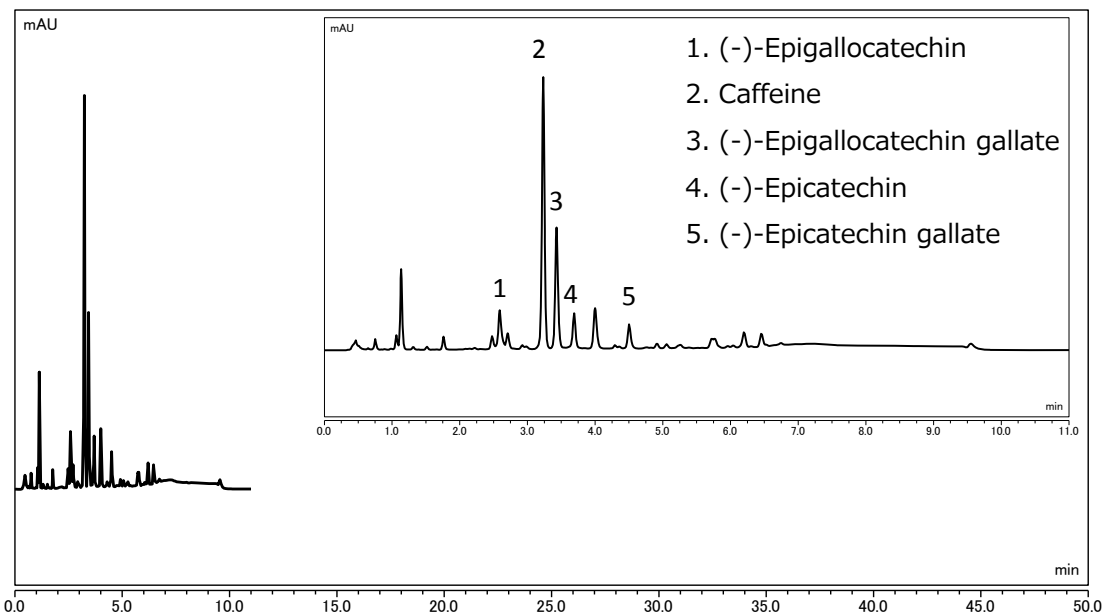
UHPLC Conditions:

Column: Develosil HSR AQ C18, 2um (2.0x100mm)
 Mobile phase: A) 0.1% Phosphoric acid B) Methanol
 Flow rate: 0.474mL/min
 Temperature: 40°C (ForcedAir)
 Detection: UV280nm
 Sample: Japanese Tea
 Injection volume: 1.13uL
 System: Thermo Fisher SCIENTIFIC Vanquish_H
 Mixer volume: 10uL

Gradient:	A	B	Curve
0.0	90	10	5
8.8	50	50	5
11.7	50	50	5
11.8	90	10	5
14.7		End	

Injection volume: 89% cut
 Solvent usage: 86% cut
 Measurement time: 71% cut
 Throughput: x3.4

Develosil HSR AQ C18, 2um (2.0x75mm)



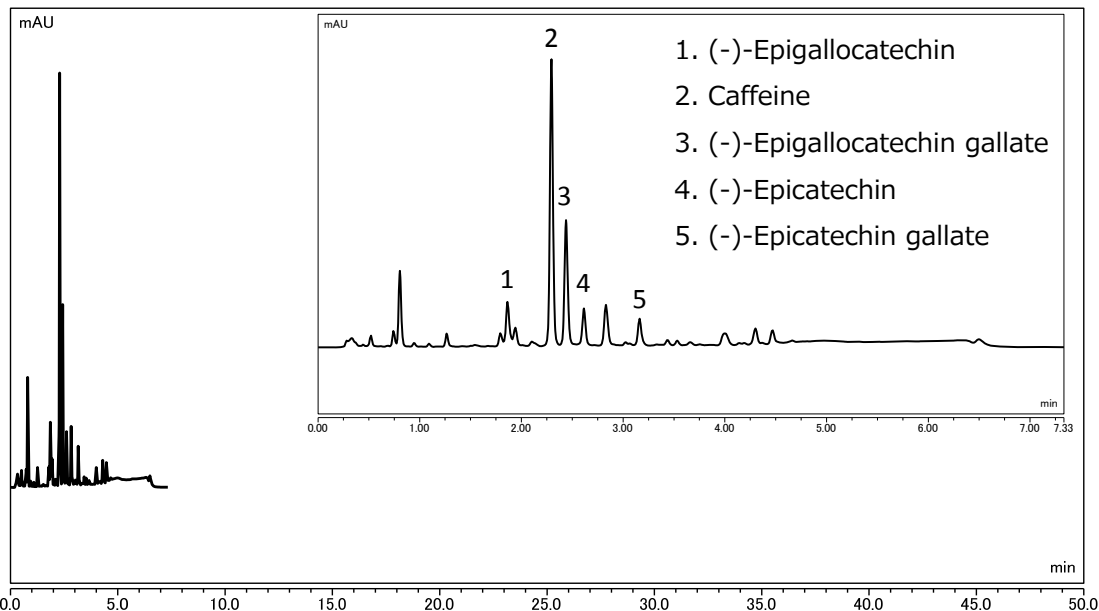
UHPLC Conditions:

Column: Develosil HSR AQ C18, 2um (2.0x75mm)
 Mobile phase: A) 0.1% Phosphoric acid B) Methanol
 Flow rate: 0.474mL/min
 Temperature: 40°C (ForcedAir)
 Detection: UV280nm
 Sample: Japanese Tea
 Injection volume: 0.98uL
 System: Thermo Fisher SCIENTIFIC Vanquish_H
 Mixer volume: 10uL

Gradient:	A	B	Curve
0.0	90	10	5
6.6	50	50	5
8.8	50	50	5
8.82	90	10	5
11.0		End	

Injection volume: 90% cut
 Solvent usage: 90% cut
 Measurement time: 78% cut
 Throughput: x4.5

Develosil HSR AQ C18, 2um (2.0x50mm)



UHPLC Conditions:

Column: Develosil HSR AQ C18, 2um (2.0x50mm)
 Mobile phase: A) 0.1% Phosphoric acid B) Methanol
 Flow rate: 0.474mL/min
 Temperature: 40°C (ForcedAir)
 Detection: UV280nm
 Sample: Japanese Tea
 Injection volume: 0.8uL
 System: Thermo Fisher SCIENTIFIC Vanquish_H
 Mixer volume: 10uL

Injection volume: 92% cut
 Solvent usage: 93% cut
 Measurement time: 85% cut
 Throughput: x6.8

Gradient:	A	B	Curve
0.0	90	10	5
4.4	50	50	5
5.87	50	50	5
5.88	90	10	5
7.3		End	

● Comparison of Separation in each column

Sample	5um, 4.6x150mm	2um, 2.0x100mm	2um, 2.0x75mm	2um, 2.0x50mm
(-)-Epigallocatechin	12.89	12.52	8.89	7.46
Caffeine	3.14	3.53	2.71	2.92
(-)-Epigallocatechin gallate	5.26	4.54	3.50	3.06
(-)-Epicatechin	13.88	13.57	10.72	9.20
(-)-Epicatechin gallate	—	—	—	—