SHIMADZU APPLICATION NEWS

LAAN-A-LM-E024

● LIQUID CHROMATOGRAPHY MASS SPECTROMETRY

No.C56

Fractionation by Preparative LC-MS and Fraction Verification by Ultra Fast LC-MS

Mass-directed fractionation is a method based on information obtained from the mass spectrometer. Here, mass spectrometric analysis is conducted for the constituents separated by the column, and fractionation is conducted using the mass chromatogram intensity and slope as indicators. The high LC separation performance coupled with the high MS qualitative analysis performance ensures that the target constituents will be collected with a high degree of certainty.
Furthermore, purity confirmation of the collected fractions is possible by subjecting the fractions to LC-MS analysis. In this case, when large numbers of fractions are obtained, optimization of the purification and fractionation conditions can be conducted with greater efficiency through high-speed analysis.
This Application News introduces an example of LC-MS fractionation of catechins in green tea, as well as an example of purity confirmation using ultra fast analysis by a high-throughput LC-MS system coupled with the Prominence UFLC.

■ Preparative LC-MS System

In the preparative LC-MS system (Fig.1), a very small part of the total flow-rate is split off to be introduced into the MS. The redirected flow passes through a make-up pump to ensure smooth introduction into the MS. In addition, in preparative LC-MS, since the eluate must reach the fraction collector after the peak is detected at the MS, a loop is installed just after the splitter to adjust the arrival time at the fraction collector. At this time, a UV detector is placed in the flow line just before the fraction collector to verify the arrival time at the fraction collector and to check for changes in peak shape due to the loop.

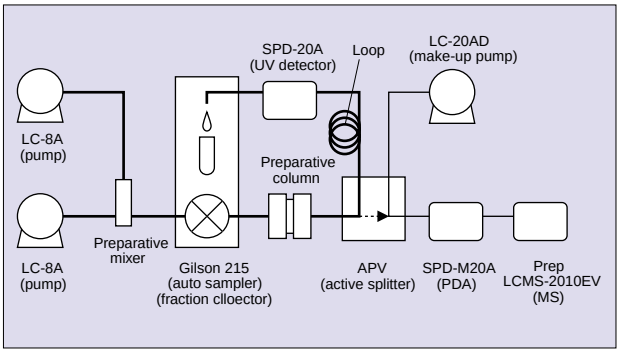

Fig.1 Flow Diagram of Preparative LC-MS System

■ Fractionation of Catechins in Green Tea by Preparative LC-MS

Here we introduce an example of LC-MS fractionation of catechins in green tea.
The fraction results by LC-MS are shown in Fig.2. Deprotonated molecules of each compound is used as an indicator of Fractionation.
The MS results confirm that separation was good for the target compounds. Furthermore, as separation just prior to the fraction collector was also confirmed, this served to verify that the target compounds were correctly fractionated.

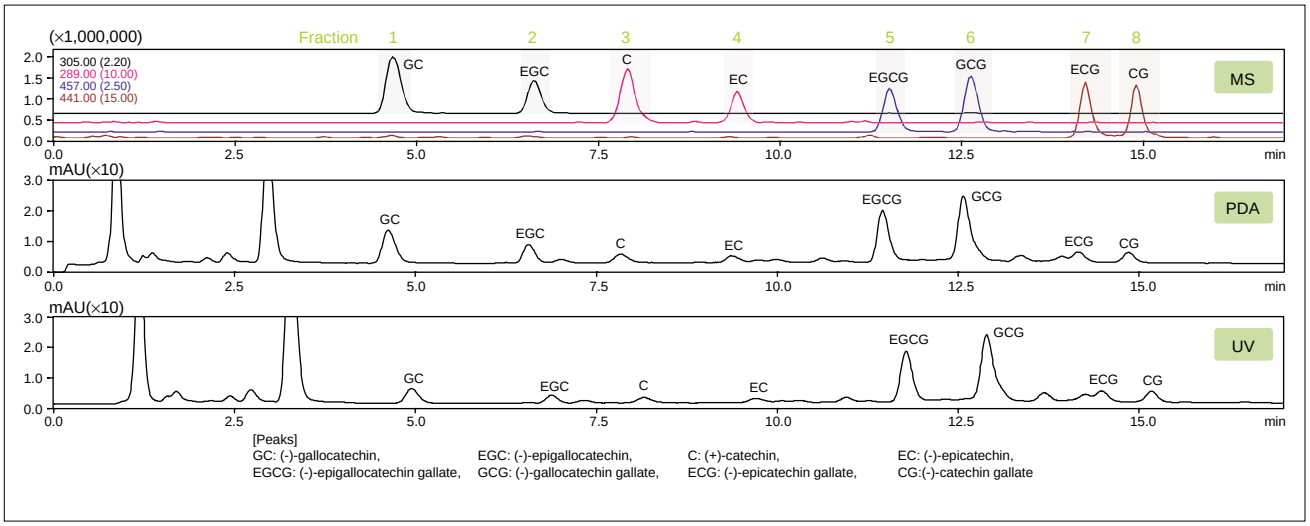

Fig.2 Preparative Isolation of Catechins in Green Tea

■ Confirmation of Fractionated Catechins by Ultra Fast LC-MS

Fig.3 shows the analysis results for each fraction using ultra fast LC-MS. These results confirmed that excellent fractionation was achieved, as the 8 catechins in green tea were detected in each of the respective fractions. However, examination of the magnification figure of Fraction 8 shown in Fig.3 reveals the slight presence of an ECG peak along with the target CG, indicating the necessity to optimize the chromatography conditions for further improvement of fraction purity.

Thus, purity confirmation can be conducted quickly by applying ultra fast analysis to purity confirmation, while also providing for improved efficiency in optimizing purification and fractionation conditions.

For further information regarding ultra fast analysis of catechins, refer to Application News No. C49.

Table 1 Preparative Conditions

[LC condition]	
Column	: Gemini 5 µm C18 Axia packed (21.2 mmI.D. × 50 mmL.)
Mobile phase A	: Water containing 0.1 % formic acid / Tetrahydrofuran = 95/5
Mobile phase B	: Acetonitrile
Timeprogram	: 3 %B (0.00-2.00 min) - 30 %B (15.00 -17.00 min) - 3 %B (17.01 min) - STOP (22.00 min)
Make-up flow	: Water containing 0.1 % formic acid / Acetonitrile = 1 / 1 (0.2 mL/min)
Split ratio	: 1/1000
Flow rate	: 20 mL/min
Injection volume	: 1000 µL
Column temperature	: Room temperature
[MS condition]	
Probe voltage	: -3.5 kV (ESI-negative mode)
Nebulizing gas flow	: 1.5 L/min
Drying gas pressure	: 0.1 MPa
CDL temperature	: 250 ˚C
BH temperature	: 200 ˚C
CDL voltage	: using Default values
Q-array voltage	: using Default values
Scan range	: *m/z* 100-500 (1.0 sec)
Trigger ions	: *m/z* 305 for (-)-gallocatechin, (-)-epigallocatechin *m/z* 289 for (+)-catechin, (-)-epicatechin *m/z* 457 for (-)-epigallocatechin gallate, (-)-gallocatechin gallate *m/z* 441 for (-)-epicatechin gallate, (-)-catechin gallate

Table 2 Analytical Conditions

[LC condition]	
Column	: Shim-pack XR-ODS (2.0 mmI.D. × 50 mmL.)
Mobile phase A	: Water containing 0.1 % formic acid / Tetrahydrofuran = 57/1
Mobile phase B	: Acetonitrile
Time-program	: 5 %B (0.00 min) - 25 %B (2.00 min) - 60 %B (2.01-4.00 min) - 5 %B (4.01 min) - STOP(5.50 min)
Flow rate	: 0.8 mL/min
Injection volume	: 2 µL
Column temperature	: 40 ˚C
[MS condition]	
Probe voltage	: -3.5 kV (ESI-negative mode)
Nebulizing gas flow	: 1.5 L/min
Drying gas pressure	: 0.1 MPa
CDL temperature	: 250 ˚C
BH temperature	: 200 ˚C
CDL voltage	: using Default values
Q-array voltage	: using Default values
Monitoring ions	: *m/z* 305 for (-)-gallocatechin, (-)-epigallocatechin *m/z* 289 for (+)-catechin, (-)-epicatechin *m/z* 457 for (-)-epigallocatechin gallate, (-)-gallocatechin gallate *m/z* 441 for (-)-epicatechin gallate, (-)-catechin gallate

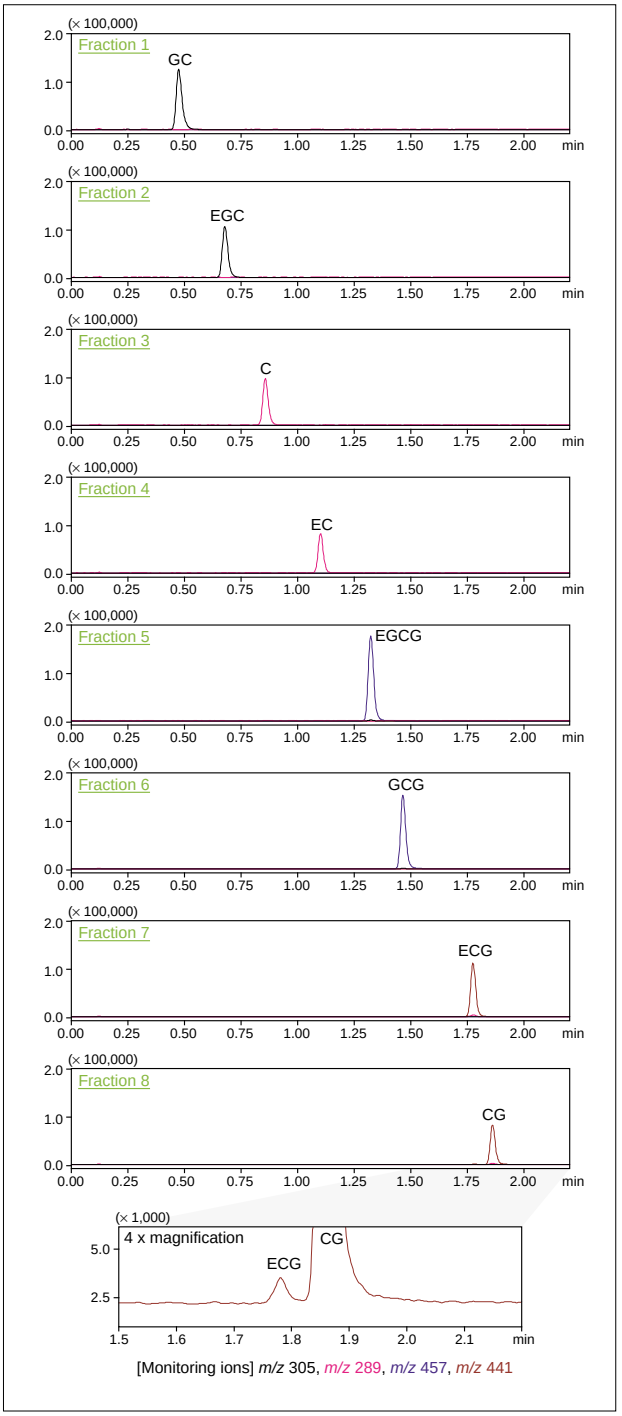

Fig.3 Ultra Fast Analysis of Catechins in Each Fraction

NOTES:
✻This Application News has been produced and edited using information that was available when the data was acquired for each article. This Application News is subject to revision without prior notice.

SHIMADZU

SHIMADZU CORPORATION. International Marketing Division

3. Kanda-Nishikicho 1-chome, Chiyoda-ku, Tokyo 101-8448, Japan Phone: 81(3)3219-5641 Fax. 81(3)3219-5710
Cable Add.:SHIMADZU TOKYO

Printed in Japan 3100-12701-10A-IK