SHIMADZU

Application News

Liquid Chromatography Mass Spectrometry No.C60A

Fractionation of Anthocyanins by Preparative LC-MS System

Anthocyanins are the flavonoid color pigments present in all tissues of higher plants, including the petals and leaves.
Besides their use as food dyes, anthocyanins have recently attracted attention due to their antioxidant properties.
In particular, blackcurrant, also known as “cassis”, is known to contain cyanidin and 4 types of anthocyanins derived from glycosides of delphinidin, a type of anthocyanidin.

When researching substances like anthocyanins that have a large variety of analogs, purification of constituents is necessary to grasp the effects and characteristics of each substance individually.
This Application News introduces the fractionation of anthocyanins in blackcurrant using the prepLCMS-2010EV.
For reference, please refer to Application News No. L340A for an example of analysis of anthocyanins by HPLC.

■ Preparative Isolation of Anthocyanins in Blackcurrant

Fig.1 shows the structures of anthocyanins present in a commercial blackcurrent extract.
The contents of 1 capsule (about 0.5 g) was dissolved in 2 mL water, and after ultrasonic mixing, the mixture was filtered through a 0.45 µm membrane filter, and then submitted to the preperative LC-MS system.

The fractionation results are shown in Fig.2. Positive ion electrospray ionization (ESI) was used because anthocyanins exist as positive ions under acidic conditions. Fractionation was conducted using M^+ as the target for each constituent. The threshold was set high to increase the purity of the isolated constituents.

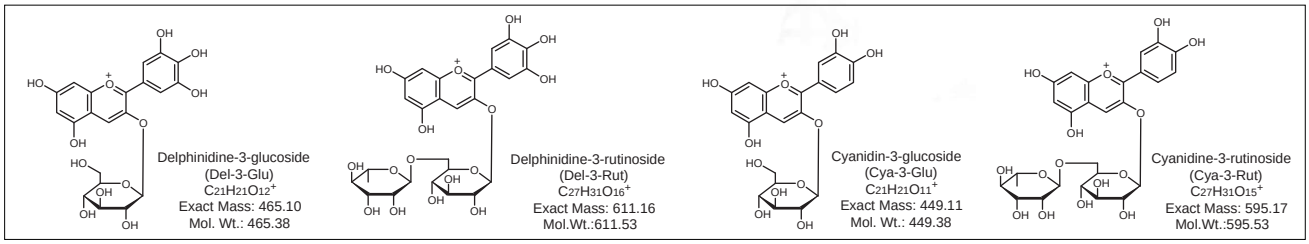

Fig.1 Chemical Structures of Four Anthocyanins

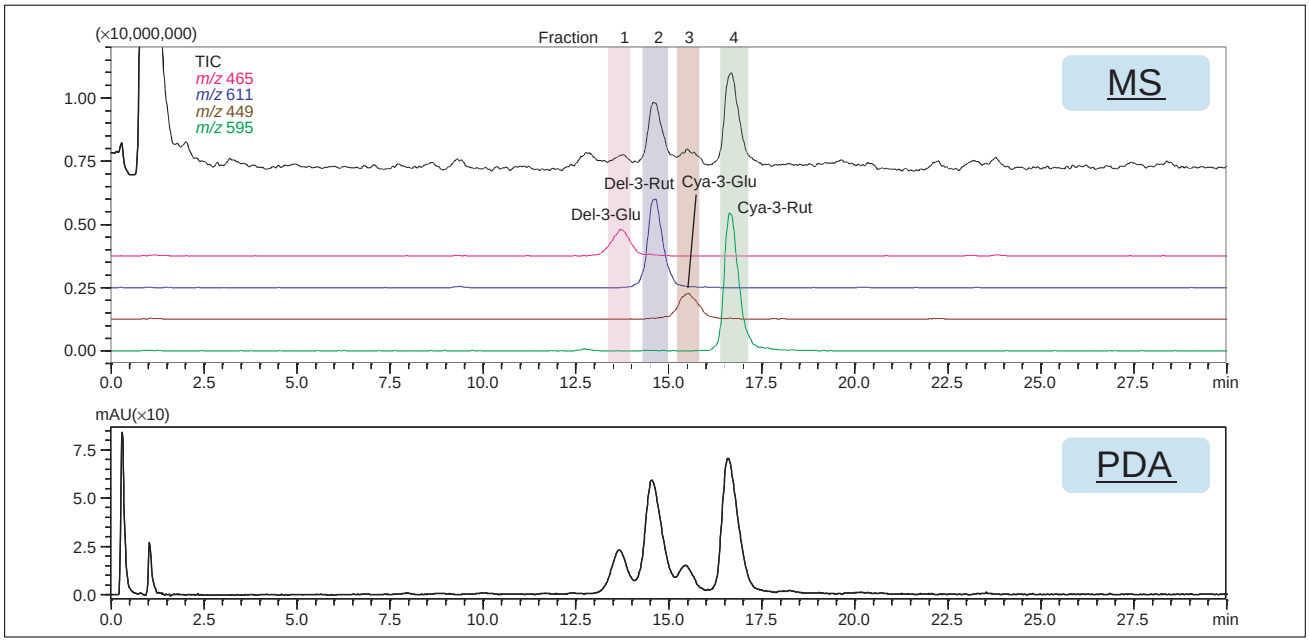

Fig.2 Preparative Isolation of Anthocyanins in Commercial Capsules of Blackcurrant Extract

■ Purity Confirmation of Anthocyanins in Fractions by Ultra Fast LC-MS

Fig.3 shows the results of ultra fast analysis of each fraction using the Prominence UFLC with the LCMS-2010EV. The results confirmed high purities of 92 to 99% in the respective fractions. Even in the cases of nearby chromatographic components, as shown in Fig.2, high-purity fractionation was achieved by setting the threshold value appropriately.

Table 1 Preparative Conditions

[LC Condition]	
Column	: Gemini 5 µm C18 Axia packed (21.2 mm I.D. × 50 mmL.)
Mobile Phase A	: Water containing 0.1 % trifluoroacetic acid
Mobile Phase B	: Acetonitrile containing 0.1 % trifluoroacetic acid
Time Program	: 5 %B (0.00 min) - 20 %B (30.00 min) - 95 %B (30.01 to 35.00 min) - 5 %B (35.01 min) - STOP (40.00 min)
Make-up Flow	: Methanol (0.2 mL/min)
Split Ratio	: 1/550
Flow Rate	: 22 mL/min
Injection Volume	: 200 µL
Column Temp.	: Room temperature
[MS Condition]	
Probe Voltage	: +4.5 kV (ESI-positive mode)
Nebulizing Gas Flow	: 1.5 L/min
Drying Gas Pressure	: 0.1 MPa
CDL Temp.	: 250˚C
Block Heater Temp.	: 200˚C
CDL Voltage	: Using default values
Q-array Voltage	: Using default values
Scan Range	: *m/z* 100-1000 (1.0 sec)
Trigger Ions	: *m/z* 465 for delphinidine -3- glucoside *m/z* 611 for delphinidine -3- rutinoside *m/z* 449 for cyanidine -3- glucoside *m/z* 595 for cyanidine -3- rutinoside

Table 2 Analytical Conditions

[LC Condition]	
Column	: Shim-pack XR-ODS (2.0 mm I.D. × 50 mmL.)
Mobile Phase A	: Water containing 0.1 % trifluoroacetic acid
Mobile Phase B	: Acetonitrile containing 0.1 % trifluoroacetic acid
Time Program	: 5 %B (0.00 min) - 25 %B (6.00 min) - 95 %B (6.01 to 8.00 min) - 5 %B (8.01 min) - STOP(10.00 min)
Flow Rate	: 0.5 mL/min
Injection Volume	: 2 µL
Column Temp.	: 40˚C
[MS Condition]	
Probe Voltage	: +4.5 kV (ESI-positive mode)
Nebulizing Gas Flow	: 1.5 L/min
Drying Gas Pressure	: 0.1 MPa
CDL Temp.	: 250˚C
Block Heater Temp.	: 200˚C
CDL Voltage	: Using default values
Q-array Voltage	: Using default values
Monitoring Ions	: *m/z* 465 for delphinidine -3- glucoside *m/z* 611 for delphinidine -3- rutinoside *m/z* 449 for cyanidine -3- glucoside *m/z* 595 for cyanidine -3- rutinoside

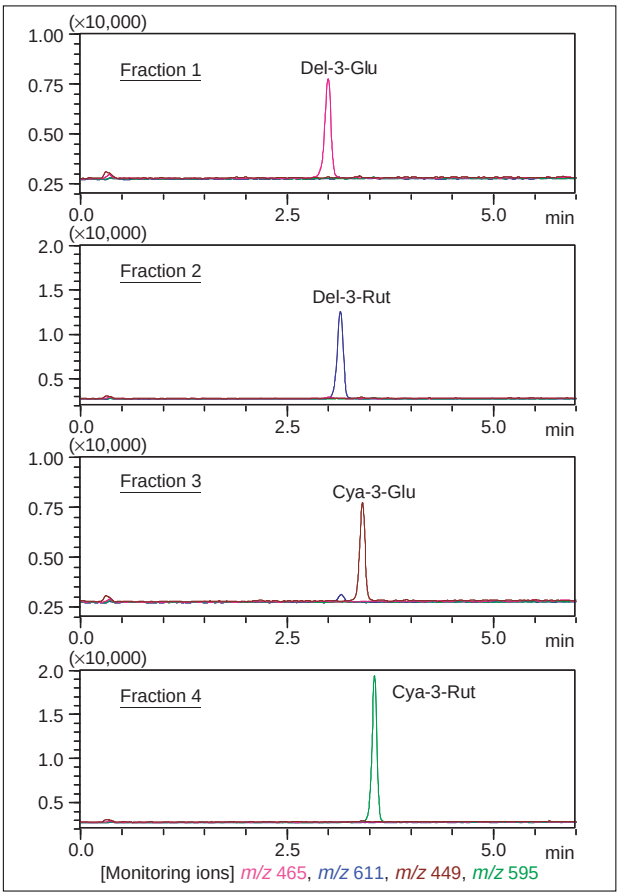

Fig.3 Ultra Fast Analysis of Anthocyanins in each Fraction

■ Preperative Isolation with High Recovery

There are times when high recovery is more important than absolute purity when conducting fractionation. Fig.4 shows an example of fractionation in which the settings reflect a greater emphasis on recovery yield. Here, *m/z* 611 only was used as the indicator to ensure a higher recovery of delphinidin -3-rutinoside, and fractionation was conducted using a lower threshold. With this approach, the entire peak was isolated, enabling high-level recovery fractionation.

Using MS to control fraction collection allows flexibility in fractionation that can be used to emphasize either purity or recovery, depending on the objective.

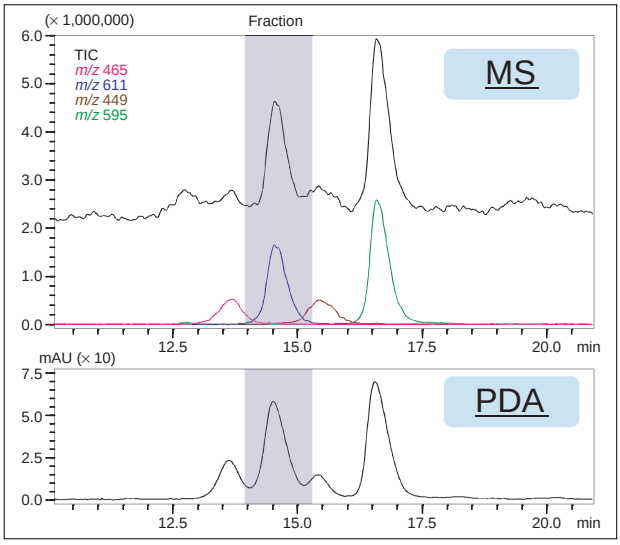

Fig.4 Preparative Isolation with High Recovery

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 3295-06805-10A-IK