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Application News

Supercritical Fluid Chromatograph Nexera™ UC / ELSD-LT III

Quantitative Analysis of Glucosylceramide in Commercial Supplement

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User Benefits

- Compared with normal phase chromatography, SFC analysis of glucosylceramide is less toxic because chloroform is not used as mobile phase.
- High speed analysis of glucosylceramide can be carried out as well as normal phase chromatography, resulting in high sensitivity detection.
- Reduction of running cost can be expected because carbon dioxide is less expensive than organic solvents used in HPLC.

■ Introduction

Glucosylceramide, which is contained in rice, corn, and konjak (also known as devil's tangue), is a kind of glycosphingolipids. Glucosylceramide is often included in cosmetics because of its positive effect to moisturize the skin of human being.

Glucosylceramide cannot be detected by UV-VIS spectrophotometric detector due to its very limited UV adsorption. Evaporative light scattering detector(ELSD) is a highly universal detector that detects scattering light from the target compounds after nebulization and evaporation of the mobile phase. ELSD can detect a lot of compounds regardless of their UV absorbing power.

In general, a determination of the total amount of various molecular entities of glucosylceramide is carried out by using chloroform as mobile phase in order to elute them within a single peak. Supercritical Fluid Chromatography (SFC) employs carbon dioxide, which is low polar, as the mobile phase instead of chloroform. Therefore, SFC analysis of glucosylceramide is less toxic compared with normal phase chromatography.

In this article, glucosylceramide originated from rice was analyzed by SFC-ELSD without using chloroform as mobile phase.

■ Analysis of Glucosylceramide in Standard Solution

Fig. 1 shows the chromatogram of standard glucosylceramide originated from rice. Table 1 shows the analytical conditions. Glucosylceramide was able to be eluted within 2 minutes by SFC.

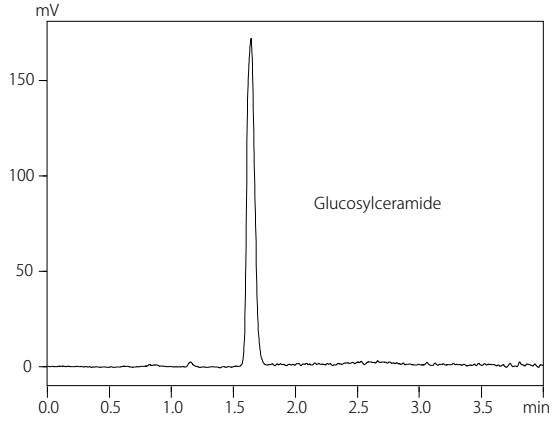

Fig. 1 Chromatogram of Standard Glucosylceramide Originated from Rice (50 mg/L)

Table 1 Analytical Conditions

System	: Nexera UC
Column	: Shim-pack™ UC Sil*1 (150 mm x 4.6 mm I.D., 5.0 µm)
Mobile Phase	: A) CO_2 B) Methanol
Flow Rate	: 3.0 mL/min
Time Program	: B. Conc. 20% (0.0 min)→40% (3.0 - 4.0 min) →20% (5.0 – 8.0 min)
Column Temp.	: 40 ℃
Injection Vol.	: 5 µL
Vial	: LabTotal Vial for LC 1.5 mL, Glass*2
BPR Temp.	: 70 ℃
BPR Pressure	: 10 MPa
Detection	: ELSD-LTIII
Gain	: Wide
Filter	: 1 sec
Drift Tube Temp.	: 40 ℃
Nebulizer Gas	: N_2
Gas Pressure	: 350 kPa

*1 P/N: 227-30415-01、 *2 P/N: 227-34001-01

■ Calibration Curve

The calibration curve of glucosylceramide was created using five different concentrations of 25, 50, 100, 150, and 300 mg/L on the log-log graph because the logarithm of ELSD response is in portion to the logarithm of the concentration.

Fig. 2 shows the calibration curve. The calibration curve showed good linearity. The coefficient of determination (r^2) was 0.999 or more.

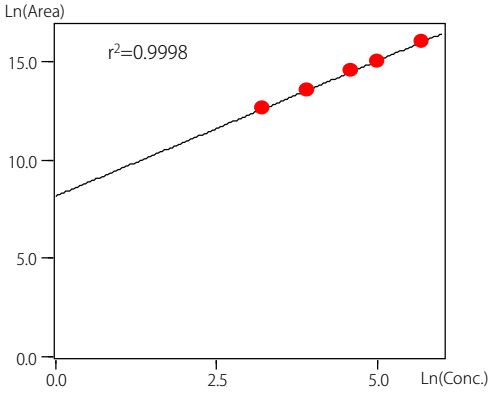

Fig. 2 Calibration Curve

■ Repeatability

Table 2 shows the repeatability. The repeatability was confirmed by repeated analyses of the standard glucosylceramide solution at 50 mg/L (n=6). The repeatability of retention time and area showed good results. The relative standard deviation (%RSD, n=6) of retention and peak area were 0.26 and 3.76 respectively.

Table 2 Repeatability (50 mg/L Standard Solution)

Compound	Retention Time (%RSD, n=6)	Area (%RSD, n=6)
Glucosylceramide	0.26	3.76

■ Analysis of Glucosylceramide in commercial Supplement

Fig. 4 shows the chromatogram of the commercial supplement treated by the procedures showed in Fig. 3 obtained under the analytical conditions showed in Table 1. The Amount of glucosylceramide contained in the supplement was 3.1 mg calculated using the calibration curve in Fig. 2. As is explained in the introduction, glucosylceramide consists of many molecular species. Even the rice-originated standard glucosylceramides may not be identical to each other due to the differences of the production process such as the extraction and the purification procedures. Therefore, it is strongly recommended that the standard glucosylceramide for creating calibration curve should be identical to that contained in the sample for improving accuracy, if such standard is available.

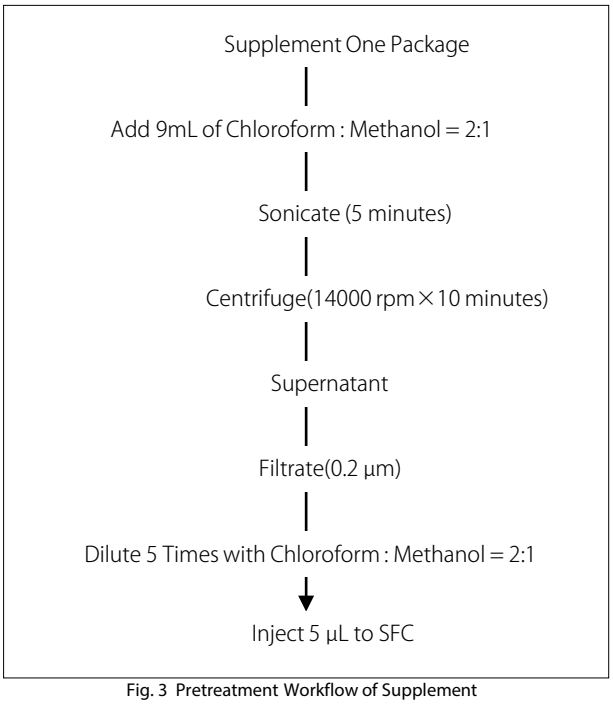

Fig. 3 Pretreatment Workflow of Supplement

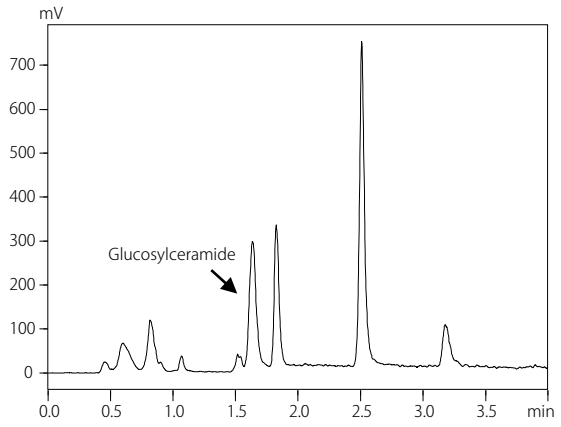

Fig. 4 Chromatogram of Supplement

■ Conclusion

The quantitative determination of glucosylceramide originated from rice contained in a supplement was carried out by SFC and ELSD. Glucosylceramide was able to be eluted within 2 minutes safely by SFC. Glucosylceramide was able to be analyzed with good repeatability and high sensitivity.

In addition, SFC uses carbon dioxide as mobile phase instead of highly harmful chloroform. Moreover, the running cost of the analysis can be reduced because carbon dioxide is less expensive than organic solvents such as chloroform and the expensive waste solvent treatment is not required.

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