SHIMADZU

Application News

No.C84

Liquid Chromatography Mass Spectrometry

Analysis of 3-MCPD Fatty Acid Diesters in Palm Oil Using a Triple Quadrupole LC/MS/MS [LCMS-8030]

3-MCPD (3-monochloropropane-1,2-diol) is a by-product that is formed in the production of condiments such as soy sauce when hydrochloric acid is used to accelerate the hydrolysis of vegetable proteins such as defatted soybean and wheat gluten. According to the risk assessment of 3-MCPD by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 3-MCPD is not considered to be genotoxic or carcinogenic. However, animal tests have indicated that it adversely affects the kidneys if ingested in large quantities over a long period of time. In Japan, it has been confirmed that there is no 3-MCPD present in *honjozo* (authentically-brewed) soy sauce produced by a traditional method, which accounts for 85 % of the soy sauce produced in Japan. The general dietary intake of 3-MCPD that can be ingested without causing problems is not regulated in Japan. However, measures have been implemented to improve upon production methods and limit the inclusion of 3-MCPD.
Recently, the presence of 3-MCPD fatty acid esters have been reported in many foods containing refined edible oils. The toxicity of 3-MCPD fatty acid esters has not yet been clarified, therefore the analysis of 3-MCPD fatty acid ester is very important. The application of GC/MS following derivatization with phenylboronic acid (DGF Standard methods 2009, Section C-Fats) has traditionally been used for analysis of 3-MCPD fatty acid esters, yet direct analysis by LC/MS/MS without derivatization is gaining attention as an attractive alternative method. Significant amounts of 3-MCPD fatty acid esters are present in numerous natural vegetable oils, and their concentration is particularly high in palm oil. Here, we introduce the quantitative analysis of 3-MCPD fatty acid esters in palm oil using LC/MS/MS. Synthetic 3-MCPD-dipalmitoyl ester and 3-MCPD-dioleoyl ester were used as standard samples. Electrospray ionization (ESI) was used as the ionization method and the 3-MCPD-di-fatty acid esters were detected as NH_4^+ adduct ions due to the addition of ammonium acetate in the mobile phase. The MS/MS spectra obtained using the adduct ions as the precursor are shown in Fig. 1. Varying the collision energies (CE) produced the MS/MS spectra in Fig. 1 with the top, middle, and bottom spectra generated by 10, 30, and 40 V, respectively. As each one of the fatty acids is desorbed, it is detected as a product ion. Fig. 2 shows the MRM chromatograms of the standard samples (1 µg/L).

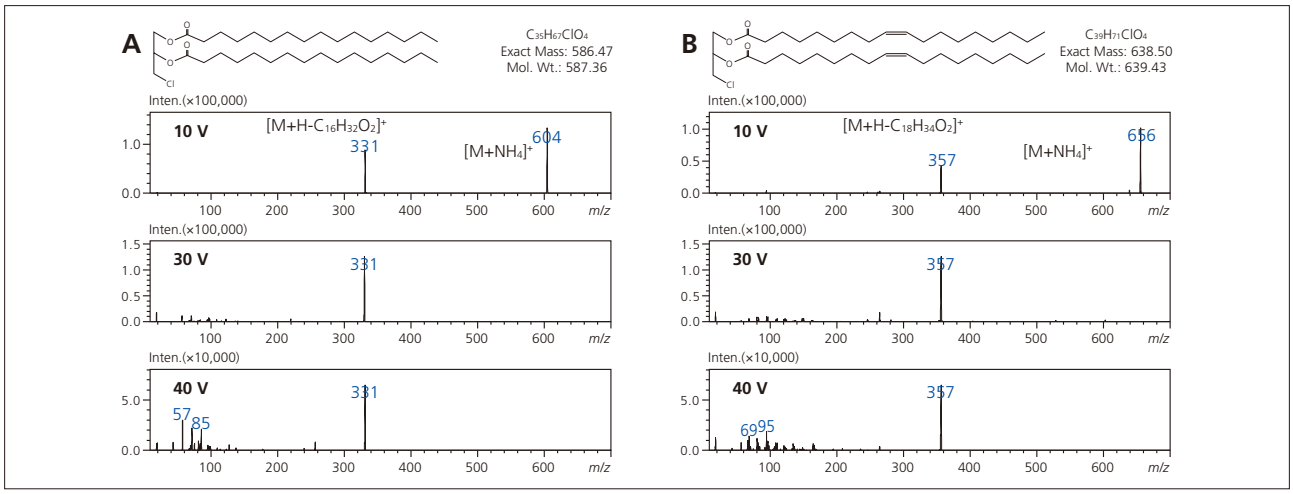

Fig. 1 MS/MS Spectra of the Synthetic Samples (A: 3-MCPD-Dipalmitoyl Ester, B: 3-MCPD-Dioleoyl Ester)

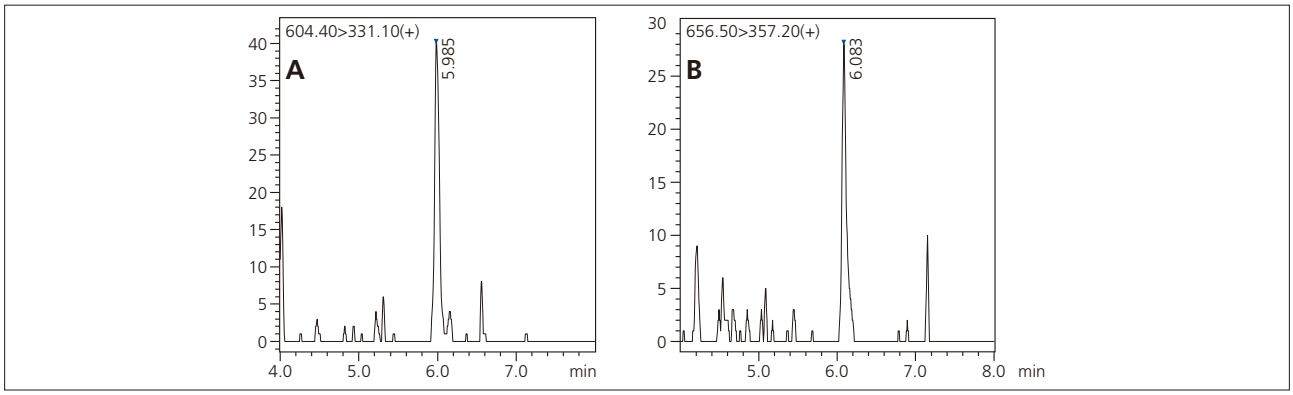

Fig. 2 MRM Chromatograms of the Synthetic Samples (1 µg/L, A: 3-MCPD-Dipalmitoyl Ester, B: 3-MCPD-Dioleoyl Ester)

Next, the calibration curves for 3-MCPD-dipalmitoyl ester and 3-MCPD-dioleoyl ester are shown in Fig. 3A and Fig. 3B, respectively. Excellent linearity was obtained over a wide range from 1–1000 µg/L, with correlation coefficient (R^2) values greater than 0.999.

The repeatability using 6 repeat measurements of 3-MCPD-dipalmitoyl ester and 3-MCPD-dioleoyl ester was 15.47 and 19.64 area %RSD, respectively, at 1 µg/L, and 6.54 and 9.32, respectively, at 10 µg/L.

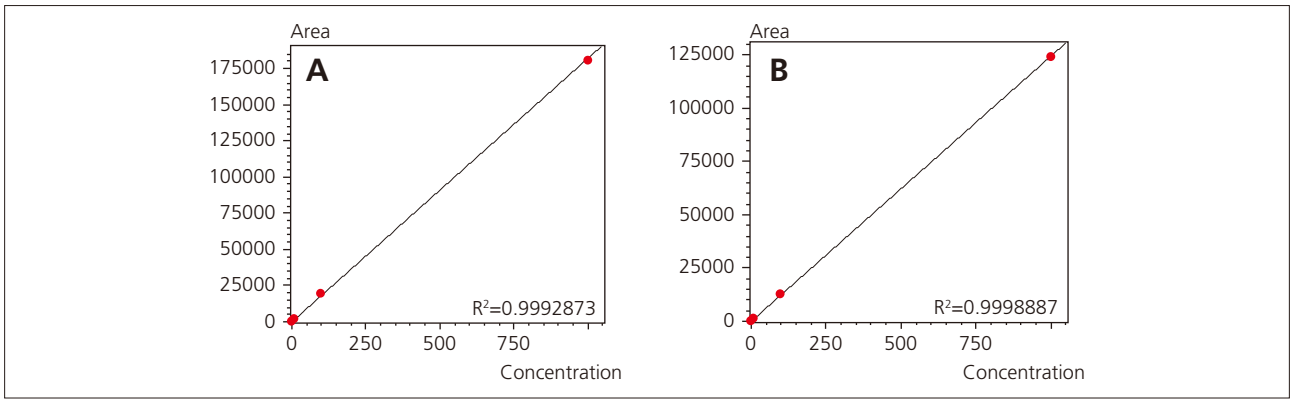

Fig. 3 Calibration Curves (1–1000 µg/L, n = 6)

Next, Fig. 4 shows an example of analysis of palm oil. 169.9 mg of palm oil was weighed out, dissolved in 1 mL of hexane and diluted 100 to 1 with acetone (588.6 times dilution), and then analyzed. 3-MCPD-dipalmitoyl ester and 3-MCPD-dioleoyl ester were detected in this diluted solution at approximately 10 µg/L (Fig. 4A and B). This corresponds to a concentration in palm oil of about 6 mg/L of 3-MCPD-dipalmitoyl ester and 3-MCPD-dioleoyl ester, respectively. Thus, it is possible to use a triple quadrupole mass spectrometer for detection of 3-MCPD fatty acid esters using a simple pretreatment procedure that is limited to sample dilution.

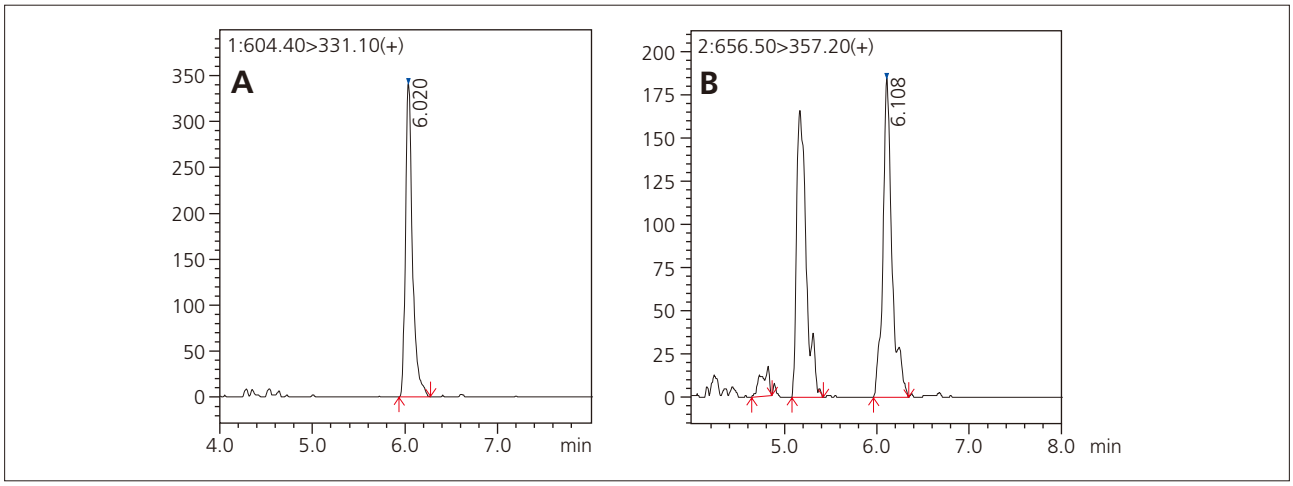

Fig. 4 MRM Chromatograms of 3-MCPD Fatty Acid Diesters in Palm Oil (A: 3-MCPD-Dipalmitoyl Ester, B: 3-MCPD-Dioleoyl Ester)

Table 1 Analytical Conditions

Column	: Shim-pack XR-ODS Ⅱ (75 mmL. × 2.0 mmI.D., 2.2 µm)		
Mobile Phase A	: Methanol with 3 mmol/L Ammonium acetate / Acetonitrile = 9/1		
Mobile Phase B	: Acetone / Methanol with 3 mmol/L Ammonium acetate / Acetonitrile = 8/1/1		
Time Program	: 0 %B (0-2.5 min) → 65 %B (7.5 min) → 100 %B (7.51-10 min) → 0 %B (10.01-15 min)		
Flow Rate	: 0.4 mL/min		
Injection Volume	: 2 µL	Column Temprature	: 40 °C
Probe Voltage	: 4.5 kV (ESI-Positive mode)	Nenulizing Gas Flow	: 1.5 L/min
DL Temperature	: 300 °C	Block Heater Temprature	: 400 °C
DL / Q-array Voltage	: Using default values	Drying Gas Flow	: 20 L/min

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