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Method of Test for Glycidyl Esters in Edible Oils and Fats

1. Scope

This method is applicable to the determination of glycidyl esters (GEs) in edible oils and fats.

2. Method

Using acid catalysis to convert GEs to 3-mono-bromopropanediol monoesters (3-MBPDEs), and followed by extraction, transesterification and derivatization, GEs are determined by gas chromatography/tandem mass spectrometry (GC-MS/MS).

2.1. Equipment

2.1.1. Gas chromatograph/tandem mass spectrometer.

2.1.1.1. Ion source: electron ionization, EI.

2.1.1.2. Column: HP-5MS UI capillary column, 0.25 μm, 0.25 mm × 30 m, or an equivalent product.

2.1.2. Centrifuge: centrifugal force ≥ 5000 ×g.

2.1.3. Vortex mixer.

2.1.4. Ultrasonicator.

2.1.5. Water bath: with temperature control.

2.1.6. Nitrogen evaporator.

2.2. Chemicals

Tetrahydrofuran, GC grade;
Methanol, GC grade;
Acetone, HPLC grade;
Phenylboronic acid, HPLC grade;
Toluene, AR grade;
n-Heptane, AR grade;
Sodium bromide, AR grade;
Sodium hydrogen carbonate, AR grade;
Sodium sulfate anhydrous, AR grade;
Sulfuric acid, AR grade;
Deionized water, resistivity ≥ 18 MΩ·cm (at 25°C);
Glycidyl palmitate (Gly-P), reference standard;
Glycidyl palmitate-d_5 (Gly-P-d_5), isotope-labelled internal standard.

2.3. Apparatus

2.3.1. Centrifuge tube: 15 mL, PP.

2.3.2. Volumetric flask: 5 mL, 10 mL, 20 mL and 100 mL.

2.4. Internal standard solution

Transfer about 1 mg of Gly-P-d_5 isotope-labelled internal standard accurately weighed to a 10-mL volumetric flask, dissolve and dilute to volume with toluene as the internal standard stock solution. Store in the dark under freezing. When to use, mix appropriate volume of the internal standard stock solution, and dilute with tetrahydrofuran to 10 μg/mL as the internal standard solution.

2.5. Standard solution

Transfer about 10 mg of Gly-P reference standard accurately weighed to a 100-mL volumetric flask, dissolve and dilute to volume with toluene as the standard stock solution. Store in dark under freezing. When to use, mix appropriate volume of the standard stock solution and the internal standard solution, dilute with tetrahydrofuran to 5-250 ng/mL (containing 125 ng/mL internal standard) as the standard solutions.

2.6. Reagents

2.6.1. 5% Sulfuric acid

Add 2.5 mL of sulfuric acid slowly into 40 mL of deionized water, and dilute to 50 mL with deionized water.

2.6.2. Sulfuric acid containing 0.3% sodium bromide

Dissolve and dilute 150 mg of sodium bromide with 5% sulfuric acid to 50 mL.

2.6.3. 0.6% Sodium hydrogen carbonate

Dissolve and dilute 3 g of sodium hydrogen carbonate with deionized water to 500 mL.

2.6.4. Methanol containing 1.8% sulfuric acid

Add 9 mL of sulfuric acid slowly into 400 mL of methanol, and dilute with methanol to 500 mL.

2.6.5. 20% Sodium sulfate

Dissolve and dilute 50 g of sodium sulfate with deionized water to 250 mL.

2.6.6. Sodium hydrogen carbonate saturated solution

Add 100 mL of deionized water to 15 g of sodium hydrogen carbonate. Stir and heat until sodium hydrogen carbonate is no longer dissolved. After cooling, take the supernatant as sodium hydrogen carbonate saturated solution.

2.6.7. 95% Acetone

Dilute 95 mL of acetone with deionized water to 100 mL.

2.6.8. Acetone containing 25% phenylboronic acid

Dissolve and dilute 5 g of phenylboronic acid with 95% acetone to 20 mL. Prepare freshly before use.

2.7. Sample solution preparation

Transfer about 0.1 g of the well-mixed sample accurately weighed into a centrifuge tube. Add 25 µL of the internal standard solution, 2 mL of tetrahydrofuran and 30 µL of sulfuric acid containing 0.3% sodium bromide, vortex-mix, incubate for 15 min in a water bath at 50°C, and add 3 mL of 0.6% sodium hydrogen carbonate to stop the reaction. Add 2 mL of *n*-heptane, vortex-mix, centrifuge at 3500 ×g for 1 min, take the upper layer, and evaporate to dryness by gently flushing with a stream of nitrogen at 40°C. Dissolve the residue with 1 mL of tetrahydrofuran, add 1.8 mL of methanol containing 1.8% sulfuric acid, and incubate for 16 hr in a water bath at 40°C. Add 0.5 mL of sodium hydrogen carbonate saturated solution to stop the reaction, and evaporate the organic solvents of the mixture under gently flushing with a stream of nitrogen at 40°C. Add 2 mL of 20% sodium sulfate and 2 mL of *n*-heptane, vortex-mix, centrifuge at 3500 ×g for 1 min, and discard the upper layer. Add 2 mL of *n*-heptane to the lower layer, vortex-mix, centrifuge at 3500 ×g for 1 min, and discard the upper layer. Add 250 µL of acetone containing 25% phenylboronic acid to the lower layer, vortex-mix, and ultrasonicate for 5 min. Add 1 mL of *n*-heptane, vortex-mix, centrifuge at 3500 ×g for 1 min, and collect the upper layer. Add 1 mL of *n*-heptane to the lower layer, repeat the extraction procedure described above once, and combine the upper layers. Evaporate to dryness by gently flushing with a stream of nitrogen at 40°C, dissolve the residue with 0.4 mL of *n*-heptane, centrifuge at 5000 ×g for 10 min, and take the supernatant as the sample solution.

2.8. Calibration standard curve

Separately take 2 mL of the standard solutions, add 30 µL of sulfuric acid

containing 0.3% sodium bromide, vortex-mix, incubate for 15 min in a water bath at 50°C, and follow the procedures described in section 2.7 to obtain the derivatized standard solutions. Operate GC-MS/MS according to the following conditions. Establish the calibration standard curve of GEs by the ratios of the peak area of GEs to that of the internal standard vs. the added concentrations in the range of 25-1250 ng/mL.

GC-MS/MS operating conditions(Note):

Column: HP-5 MS UI capillary column, 0.25 μm, 0.25 mm × 30 m.

Column temperature:

Initial temperature: 50°C, 1 min;

Temperature rising rate: 10°C/min;

Middle temperature: 210°C;

Temperature rising rate:30°C/min;

Final temperature: 300°C, 5 min.

Injector temperature: 250°C.

Inject volume: 1 μL.

Carrier gas and flow rate: helium, 1 mL/min.

Interface temperature: 280°C.

Ion source temperature: 230°C.

Ion source: EI, 70 eV.

Injection mode: splitless.

Detection mode: multiple reaction monitoring (MRM). Detection ion pair and collision energy are shown as follows:

Analyte	Ion pair	Collision energy (eV)
	Precursor ion (*m*/*z*) > product ion (*m*/*z*)	
Glycidyl esters	240 > 147*	5
	242 > 147	5
Glycidyl esters-d_5 (I.S.)	245 > 150	10

*The quantitative ion pair.

Note: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

2.9. Identification and quantification

Accurately inject 1 μL of the sample solution and the derivatized standard solutions into GC-MS/MS separately, and operate according to the conditions described in section 2.8. Identify GEs based on the retention time and the relative ion intensities(Note 1). Calculate the amount of GEs in the sample by the following formula(Note 2):

$$\text{The amount of GEs in the sample (μg/kg)} = \frac{C \times V}{M} \times 0.2371$$

Where,

C: the concentration of GEs in the sample solution calculated by the calibration standard curve (ng/mL)

V: the final make-up volume of the sample solution (0.4 mL).

M: the weight of the sample (g).

0.2371: the conversion factor of Gly-P ester to glycidol.

Note: 1. Relative ion intensities are calculated by peak areas of qualitative ions divided by peak areas of quantitative ions (≤100%). Maximum permitted tolerances of relative ion intensities are as follows:

Relative ion intensity (%)	Tolerance (%)
> 50	± 20
> 20~50	± 25
> 10~20	± 30
≤ 10	± 50

2. The amount of GEs in the sample is expressed as glycidol.

Remark

1. The limit of quantification (LOQ) for GEs (expressed as glycidol) is 25 μg/kg.
2. Further validation should be performed when interfering compounds appear in the samples.

Reference

1. Ermacora, A. and Hrncirik, K. 2013. A novel method for simultaneous monitoring of 2-MCPD, 3-MCPD and glycidyl esters in oils and fats. J. Am. Oil Chem. Soc. 90: 1-8.
2. Dubois, M., Empl, A. M., Jaudzems, G., Basle, Q. and Konings, E. 2019.

Determination of 2- and 3-MCPD as well as 2- and 3-MCPD esters and glycidyl esters (GE) in infant and adult/pediatric nutritional formula by gas chromatography coupled to mass spectrometry method, first action 2018.03. J. AOAC Int. 102: 903-914.

Reference chromatogram

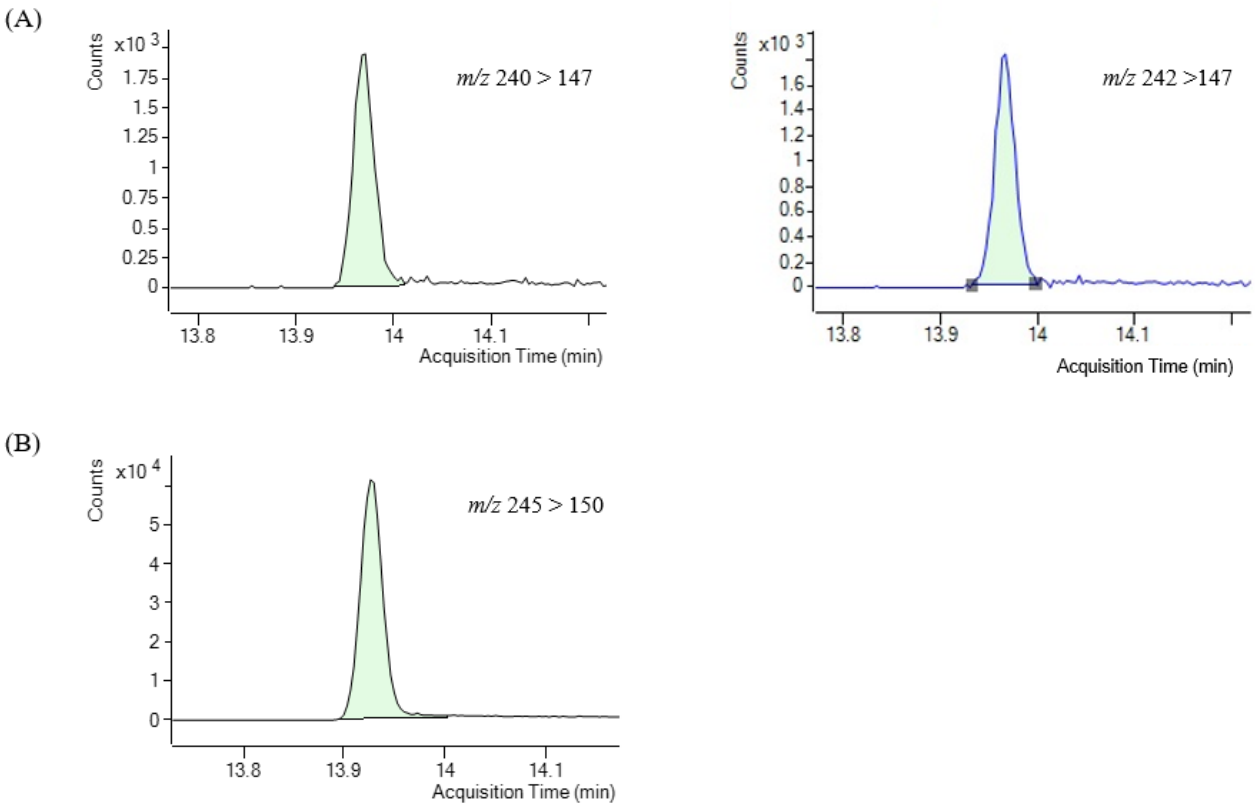

Figure. MRM chromatograms of GEs standard (A) and its isotope-labelled internal standard (B) analyzed by GC-MS/MS.