AOCS Official Method Cd 11b-91
Reapproved 2009

Determination of Mono- and Diglycerides by Capillary Gas Chromatography

DEFINITION

This method is for the determination of mono- and diglycerides by capillary GLC. Mono- and diglycerides are converted with bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorsilane (TMCS) in pyridine into more volatile trimethylsilylether derivatives and determined by capillary GLC.

SCOPE

This method is applicable to mono- and diglyceride concentrates and mono- and diglycerides in fats and oils (see References, 1). Other emulsifiers and components of fats and oils, such as glycerol, fatty acids, sterols, etc., may be converted into the trimethylsilylether derivatives and analyzed by the same GLC procedure. For the identification of the components, coupled GC/MS may be advantageous.

APPARATUS

1. Gas chromatograph—with split injection or on-column injection (see Notes, 1), oven temperature programming and flame-ionization detector.
2. Recording potentiometer.
3. Electronic integrator.
4. Column—capillary, glass or fused silica, surface fully deactivated by silylation agent (see Notes, 2), 15–25 m, 0.25–0.35 mm i.d., coating SE-54 (or other phase with similar polarity), film thickness 0.1–0.2 µm (see Notes, 3).
5. Operating conditions:
   —split injection (split ratio 1:10–1:50)
   —direct injection (splitless, hold for 1 min)
   —injection port 320°C (or for on-column injection 60°C)
   —column initial 80°C (or for on-column 60°C)
   —program rate 10°C/min
   —final temperature 360°C, hold 15 min (see Notes, 3)
   —detector 350°C
   —carrier gas flow 5 mL He/min (at 80°C)
   —injection volume 1–5 µL
6. An automatic sampler is advantageous (see Notes, 4).
7. Screw-cap vials—2.5 mL; or crimp-top vials for auto sampler (*e.g.,* 2.0 mL), with Teflon™-faced septa (see Notes, 3).
8. Heating device for vials—70°C.
9. Balance—analytical, 200-g capacity with ±0.0001 g sensitivity.

REAGENTS

1. *N,N*-bis(trimethylsilyl)trifluoroacetamide (BSTFA).
2. Trimethylchlorsilane (TMCS).
3. Pyridine—analytical reagent grade, kept over KOH (see Notes, *Caution*).
4. n-Tetradecane—analytical reagent grade (minimum 99%).
5. n-Hexane—analytical reagent grade (see Notes, *Caution*).
6. Reference materials—glycerol, palmitic acid, 1-palmitoyl glycerol, 1-stearoyl glycerol, 1,2-dipalmitoyl glycerol, 1,3-dipalmitoyl glycerol, 1,2-distearoyl glycerol.
7. Internal standard solution—prepared by accurately weighing approximately 100 mg internal standard n-tetradecane into 10-mL volumetric flask and diluting to volume with pyridine.
8. Reference solution—prepared by accurately weighing approximately 100 mg of reference substance (*e.g.,* glycerol, fatty acid, mono- and diacyl glycerol) and accurately weighing approximately 100 mg of n-tetradecane into the same 10 mL volumetric flask and diluting to volume with pyridine; or weigh approximately 100 mg of a mixture containing several (*e.g.,* 5) reference materials and n-tetradecane, each component being present in about the same quantity, into a 2 mL volumetric flask and dilute to volume with pyridine (see Notes, 5).

PROCEDURE

1. Test solution—Accurately weigh approximately 10 mg of homogenized test sample of emulsifier concentrates, or 50 mg of oils and fats containing emulsifiers, into a 2.5 mL screw-cap vial with Teflon™-faced septa. Add 0.2 mL BSTFA and 0.1 mL

TMCS, and then 0.1 mL of internal standard solution (Reagents, 7) containing 1 mg n-tetradecane to the test sample (see Notes, 4). Moisture must be strictly excluded. Close vial and shake vigorously. Heat the reaction mixture in heating device at 70°C for approximately 20 min. Inject 1–5 µL of the reaction mixture into the gas chromatograph showing a stable base line. Avoid delay of GC analysis. The reaction is carried out two times, and two injections are made per reaction (see Notes, 1 and 2).

2. Reference solution—Add 0.10 mL of reference solution (Reagents, 8) into vials and add the silylating agents, 0.2 mL BSTFA and 0.1 mL TMCS (no internal standard solution is added). Heat the reaction mixture and inject into the gas chromatograph as described above (see Notes, 4). Use a concentration range of reference standards similar to the range of the components to be quantified in the test solution. A plot of response vs. concentration of reference substances may be useful to check linearity (see Notes, 1 and 2).
3. Response factors—Check response factors periodically. Response factors should be above approximately 0.5. Lower response factors indicate some loss or decomposition. Use a concentration range of 0.5–10 mg/mL of components in both the reference and test solutions. See Calculations, 1, for calculation of response factors.

IDENTIFICATION

1. Analyze reference solution under the same operation conditions as test solution. Identify peaks by comparison of retention time with known substances, or apply coupled GC/MS. See Figure 1.

CALCULATIONS AND EXPRESSION OF RESULTS

1. Response factor—Calculate response factor of the reference substance vs. internal standard using the reference solution chromatogram. The value of the response factor is given by the formula:

$$R_x = (m_{is}/m_x) \times (A_x/A_{is})$$

Where—

R_x = response factor of reference substance x
m_{is} = mass of internal standard, in mg
m_x = mass of reference substance x, in mg
A_x = peak area of reference substance x
A_{is} = peak area of internal standard

2. Calculation of test portion component content—Calculate percentage of mass content of component x in the test portion by the formula:

$$m'_x\,(\%) = 1/R_x \times (m'_{is}/m'_s) \times (A'_x/A'_{is}) \times 100\%$$

Where—

m'_x = percentage of mass of component x in test portion
R_x = response factor of component x in test portion
m'_{is} = mass of internal standard in test portion, in mg
m'_s = mass of test portion, in mg
A'_x = peak area of component x in test portion
A'_{is} = peak area of internal standard in test portion

3. Typical chromatograms—See Figure 1 for typical chromatograms of reference standards and mono- and diglycerides. The silylation procedure, column specifications, operating conditions and peak identification relating to Figure 1 are as follows:
   (a) Silylation—Test sample size: 10 mg; reagents: 0.1 mL pyridine containing 1.0 mg n-tetradecane, 0.2 mL BSTFA, 0.1 mL TMCS; reaction time: 20 min at 70°C.
   (b) Column—Fused silica capillary, 25 m × 0.31 mm (i.d.); film thickness, 0.17 µm, consisting of 5% phenylmethyl silicon, Ultra #2 (Hewlett-Packard, Palo Alto, CA, USA).
   (c) Operating conditions—Injector 320°C, column initial 80°C, program 10°C/min, final 360°C, hold 15 min, detector 350°C; carrier gas, helium at 5 mL/min (at 80°C) (see Apparatus, 5).
   (d) Peak identification—IS (internal standard), tetradecane; 1, glycerol; 2, diglycerol; 3, hexadecanoic acid; 4, octadecanoate acid; 5, glycerol 1-tetradecanoate; 6, glycerol 2-hexadecanoate; 7, glycerol 1-hexadecanoate; 8, glycerol 2-octadecanoate; 9, glycerol 1-octadecanoate; 10, glycerol 1-icosanoate; 11, glycerol 1-docosanoate; 12, glycerol 1-tetradecanoate-3-hexadecanoate; 13, glycerol 1,2-dihexadecanoate; 14, glycerol 1,3-dihexadecanoate; 15, glycerol 1-hexadecanoate-2-octadecanoate; 16, glycerol 1-hexadecanoate-3-octadecanoate; 17, glycerol 1,2-dioctadecanoate; 18, glycerol 1,3-dioctadecanoate; 19, triglyceride C48; 20, triglyceride C50; 21, triglyceride C52; 22, triglyceride C54.

PRECISION

1. Repeatability—When the mean of the values obtained from two single determinations, carried out in rapid succession by the same operator using the same apparatus under the same conditions for the analysis of the same test sample, lies within the range of the mean values cited in Tables 1, 2 and 3, the difference between the two values obtained should not be greater than the repeatability value (r), which can generally be deduced by linear interpolation from Tables 1, 2 and 3 (References, 2).