



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 trimethylchlorosilane (TMCS) in pyridine into more volatile trimethylsilyl ether 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 trimethylsilyl ether 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 μ m (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 μ L
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. Trimethylchlorosilane (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:

$$R_x = (m'_i/m'_x) \times (A_x/A_i)$$

Where—

R_x = response factor of reference substance x

m'_i = mass of internal standard, in mg

m'_x = mass of reference substance x, in mg

A_x = peak area of reference substance x

A_i = 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:

$$m'_x (\%) = 1/R_x \times (m'_i/m'_x) \times (A'_x/A'_i) \times 100\%$$

Where—

m'_x = percentage of mass of component x in test portion

R_x = response factor of component x in test portion

m'_i = mass of internal standard in test portion, in mg

m'_x = mass of test portion, in mg

A'_x = peak area of component x in test portion

A'_i = 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, octadecanoic 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 C48; 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).

2. Reproducibility—When the values for the final result, obtained by operators in different laboratories, using different apparatus under different conditions for the analysis of the same laboratory sample, lie within the range of mean values cited in Tables 1, 2 and 3, the difference between the values for the final result obtained by those operators should not be greater than the reproducibility value (R), which can generally be deduced by linear interpolation from Tables 1 and 2.

NOTES

Caution

Pyridine is flammable and a dangerous fire risk. The explosive limits in air are 1.8–12.4%. It is toxic by ingestion and inhalation. The TLV is 5 ppm in air. The danger from crude pyridine is greater than from pure pyridine, the associated homologs and impurities being even more toxic than pyridine.

Hexane is flammable and a dangerous fire risk. The TLV is 50 ppm in air. The Occupational Safety and Health Administration recommends that exposure not exceed 350 ng per cubic meter for a time-weighted average. Hexane vapor causes lung irritation and produces neurotoxic effects. A fume hood should be used at all times when using hexane.

NUMBERED NOTES

1. For on-column injection, or direct injection, dilute 50 μ L of reaction mixture (Procedure, 1 and 2) with 1 mL hexane and inject (1 μ L). In order to lengthen lifetime of the columns, when applying on-column injections, a precolumn is useful. On-column injection gives better response factors.
2. Use length of columns required to separate mono- or diglycerides. Individual unsaturated mono- and diglycerides may not be separated from the saturated or less-unsaturated mono- or diglycerides. TLC on silica gel impregnated with boric acid, immediately prior to derivatization, can be used to resolve 2-mono-glycerides from 1-mono-glycerides (References, 3).
3. The final column temperature of 360°C is not compatible with the maximum operating temperature of 320°C to 325°C recommended for SE-54. The detector should be 10°C to 20°C higher than the maximum column temperature. Therefore, it is recommended to use a maximum column temperature of 325°C to 330°C (apparently this will not affect the separation of the mono- and diglycerides), with a detector temperature of 335°C to 350°C. These proposed changes have not been validated by collaborative study.
4. For automatic samplers with 2-mL crimp-top vials, it is convenient to double the amount of test sample and reagents.
5. One or more reference solutions (Reagents, 8) can also be prepared without adding n-tetradecane to the solution. Silylation of the reference solutions (Procedure, 2) is then carried out as described for the test solution (Procedure, 1) after addition of the internal standard solution (Reagents, 7) and silylating reagents.

REFERENCES

1. *Standard Methods for the Analysis of Oils, Fats and Derivatives*, International Union of Pure and Applied Chemistry, 7th edn., Blackwell Scientific Publications, 1987, IUPAC Method 6.002 (ex. 2.326).
2. *Pure Appl. Chem.* 63:1153 (1991).
3. Christie, W.W., *Lipid Analysis*, 2nd edn., Pergamon Press, New York, 1982, pp. 101, 159.

Table 1
Statistical analysis of results for mono- and diglyceride concentrates (expressed as percent of mass of test portion).

	1-Myristate	1-Palmitate		1-Stearate	1,3-Dipalmitate		1-Palmitate-3-stearate		1,3-Distearate
	8	8	16	8	8	16	8	16	8
No. of laboratories	16			16			16		16
No. of results									
No. of laboratories retained after elimination of outliers ^a	7	8	8	8	7	7	7	7	8
No. of accepted results	14	16	16	16	14	14	14	14	16
Mean value, %	1.7	27.2		60.1	0.2		0.8		1.1
Repeatability standard deviation (S_r)	0.05	0.9		2.1	0.01		0.05		0.07
Repeatability co-efficient of variation (RSD _r)	3.0	3.3		3.5	4.6		6.0		6.8
Repeatability value (v) [$2.83 \times S_r$]	0.14	2.61		5.91	0.031		0.14		0.20
Reproducibility standard deviation (S_R)	0.1	2.4		6.4	0.06		0.1		0.3
Reproducibility co-efficient of variation (RSD _R)	5.7	8.9		10.7	30.0		17.8		24.8
Reproducibility value (R) [$2.83 \times S_R$]	0.3	6.8		18.1	0.2		0.4		0.8

^aEliminated using Dixon and Cochran test methods.

Table 2
Statistical analysis of results for mono- and diglycerides in oil (expressed as percent of mass of test portion).

	1-Palmitate	1-Stearate	1,2-Dipalmitate	1,3-Dipalmitate	1,2-Disearate
No. of laboratories	8	8	8	8	8
No. of results	16	16	16	16	16
No. of laboratories retained after elimination of outliers ^a	8	8	8	8	8
No. of accepted results	16	16	16	16	16
Mean value, %	0.96	0.98	0.97	0.93	0.97
Repeatability standard deviation (S _r)	0.03	0.03	0.04	0.02	0.06
Repeatability co-efficient of variation (RSD _r)	3.3	3.4	4.0	2.5	6.2
Repeatability value (t) [2.83 × S _r]	0.08	0.08	0.11	0.06	0.17
Reproducibility standard deviation (S _R)	0.12	0.14	0.24	0.19	0.19
Reproducibility co-efficient of variation (RSD _R)	12.0	13.8	24.4	20.2	19.8
Reproducibility value (R) [2.83 × S _R]	0.34	0.40	0.68	0.54	0.54

^aEliminated by the Dixon and Cochran test methods.

Table 3
Statistical analysis of results for mono- and diglycerides in sunflower oil (blind duplicates, expressed as percent of mass of test portion).

	1-Palmitate	1-Stearate	1,2-Dipalmitate	1,3-Dipalmitate	1,2-Disearate
No. of laboratories	8	8	8	8	8
No. of results	30	30	30	30	30
No. of laboratories retained after elimination of outliers	7	8	7	7	7
No. of accepted results	26	30	26	26	30
Mean value, %	0.75	1.4	1.1	1.3	2.6
Repeatability standard deviation (S _r)	0.07	0.24	0.09	0.07	0.30
Repeatability co-efficient of variation (RSD _r)	10.0	17.0	8.4	5.3	11.8
Repeatability value (t) [2.83 × S _r]	0.21	0.67	0.25	0.48	0.86
Reproducibility standard deviation (S _R)	0.14	0.33	0.11	0.12	0.94
Reproducibility co-efficient of variation (RSD _R)	18.5	23.4	10.4	9.2	36.3
Reproducibility value (R) [2.83 × S _R]	0.39	0.92	0.32	0.28	2.6

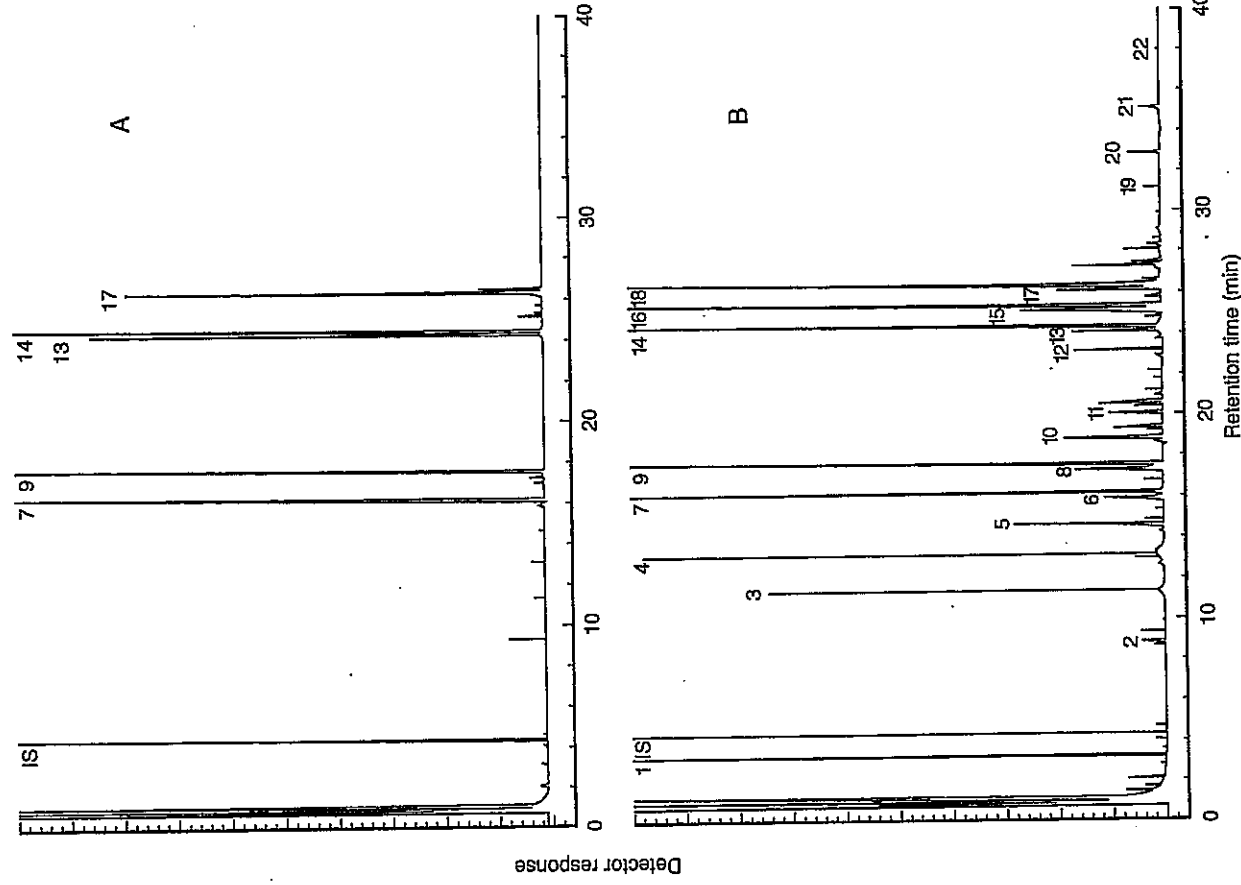


Figure 1. Typical chromatograms of trimethylsilyl ether derivatives of mono- and diglycerides: A, reference standards and B, mono- and diglyceride emulsifier. The silylation procedure, column specifications, operating conditions, and peak identification are as follows: (a) *Silylation*—Test sample size, 10 mg; reagents, 0.1 mL pyridine containing 1.0 mg n-tetradecane, 0.2 mL BSTFA, 0.1 mL TMCS; reaction time, 30 min at 70°C. (b) *Column*—25 m x 0.31 mm i.d. fused silica; 0.17 µm film thickness (5% phenylmethyl silicon, Ultra #2, Hewlett-Packard). (c) *Operating conditions*—Injector, 320°C; hold 15 min; detector, 350°C; carrier gas, He, 5 mL/min, 80°C. (d) *Peak identification*—15, n-tetradecane (internal standard); 1, glycerol; 2, diglycerol; 3, hexadecanoic acid; 4, octadecanoic 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; and 22, triglyceride C54.