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INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY

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DETERMINATION OF MONO- AND DIGLYCERIDES BY CAPILLARY GAS CHROMATOGRAPHY

Results of a collaborative study and the standardized method

Prepared for publication by

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*Membership of the Commission during the period of the collaborative study (1983–87) was as follows:

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Determination of mono- and diglycerides by capillary gas chromatography: results of a collaborative study and the standardized method

Abstract - A method for the determination of mono- and diglycerides by capillary gas chromatography has been elaborated and tested in a collaborative study.
The procedure involves conversion of the mono- and diglycerides into more volatile trimethylsilyl ether derivatives using N,N-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) (3:1) in pyridine. n-Tetradecane was used as internal standard. Capillary gas chromatography with split or on-column injection was applied.
The samples analysed consisted of two commercial mono-, diglyceride emulsifiers, two mixtures of known composition of mono- and diglycerides present in an excess of triglycerides, and of refined sunflower oil spiked with known amounts of mono- and diglycerides.
Besides mono- and diglycerides other components of fats and oils such as glycerol, fatty acids, sterols etc. may be converted into the trimethylsilyl ether derivatives and analysed by the same GC procedure. For the identification of the components coupled gas chromatography/mass spectrometry is advantageous.

INTRODUCTION

Mono- and diglycerides are natural constituents of oils and fats. They are also added as emulsifiers to oils and fats and used as food additives.

According to IUPAC method 2.321 (1), mono-, di- and triglycerides are separated by silica gel column chromatography using solvents of different polarity. 1-monoglycerides can be determined by oxidation with periodic acid solution applying IUPAC method 2.322 (1).

In order to achieve fast and more complete analysis of the different mono- and diglycerides, gas chromatographic methods have been described. The mono- and diglycerides are usually derivatized into the more volatile trimethylsilyl ether derivatives. For this derivatization and replacement of active hydrogen atoms, various silylation reagents have been used. In a number of investigations, Sahasrabudhe (2, 10) used hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS) in pyridine. A'Alonzo (3) applied N,N-bis(trimethylsilyl)trifluoroacetamide (BSTFA). The same reagent was used by Soe (4), who, beside mono- and diglycerides, derivatized other emulsifiers such as acetic acid-, lactic acid- and di-O-acetyltartaric acid esters of monoglycerides. Goh (5) analysed partial glycerides in palm oil preparing the TMS-derivatives using N-trimethylsilylimidazole. Further reagents and GC-conditions have been reported by other authors (6-9).

In a first interlaboratory study, the widely recommended combination of HMDS and TMCS was used for silylation of mono- and diglycerides. These reagents, however, have the disadvantage to form, after reaction, a precipitate of insoluble ammonium chloride. BSTFA gives clear solutions, but it reacts much more slowly. After trials and optimization, the combination BSTFA and TMS (3:1) in pyridine was finally applied. This combination gives fast reactions, clear solutions and better derivatization of substances such as sodium salts of citric- and tartaric acid. Also sulfuric and phosphoric acids are derivatized and detected by GC.

COLLABORATIVE STUDIES

In a first interlaboratory study (1984) two commercial mono-, diglyceride emulsifiers were derivatised with HMDS and TMCS and analysed by GC.

In a second collaborative study (1985), BSTFA and TMCS was used for derivatization and, beside the two above mentioned mono- and diglyceride emulsifiers, additional synthetic compositions with known amounts of mono- and diglycerides in the presence of an excess of triglycerides, as well as refined sunflower oil, spiked with mono- and diglycerides, were analysed. Recovery could thus be calculated. Results were obtained from 8 collaborators.

Samples and standards

The following were provided for the study:

Reference standards: n-tetradecane (internal standard), glycerol, palmitic acid, a mixture of n-tetradecane, 1-palmitate, 1-stearate, 1,2-dipalmitate, 1,3-dipalmitate, 1,2-distearate, (mass-ratio = 1:1:1:1:1:1) (see fig. 1 A).
Samples to be analysed (in brackets, percent mass) were:

1: mono-, diglyceride emulsifier (100) (see fig. 1 B);
2: mono-, diglyceride emulsifier (distilled) (100);
3: 1-palmitate (1.00), 1,2-dipalmitate (1.00), 1,2-distearate (1.00), trimyristate (31.69), tripalmitate (31.65), trioleate (31.67);

4: 1-monopalmitate (1.77), 1-monostearate (2.85), 1,2-dipalmitate (2.06), 1,2-distearate (0.68), trimyristate (25.03), tripalmitate (16.33), trioleate (51.28)
5: 1-monopalmitate (0.80), 1-monostearate (1.54), 1,2-dipalmitate (1.09), 1,3-dipalmitate (1.39), 1,2-distearate (2.18), sunflower seed oil (93.00)
6: identical sample 5.

Instructions

Each laboratory was provided with the method and data sheets to report the gas chromatographic apparatus, the integrator, the column used, the mode of sample injection, the response factors and the percent mass contents found in the samples. Gas chromatograms of the reference standards and of the mono-, diglyceride emulsifier (sample 1), obtained in the co-ordinator's laboratory, were supplied (see fig. 1). These two samples were suggested for practice runs to become familiar with the method and to check GC conditions.

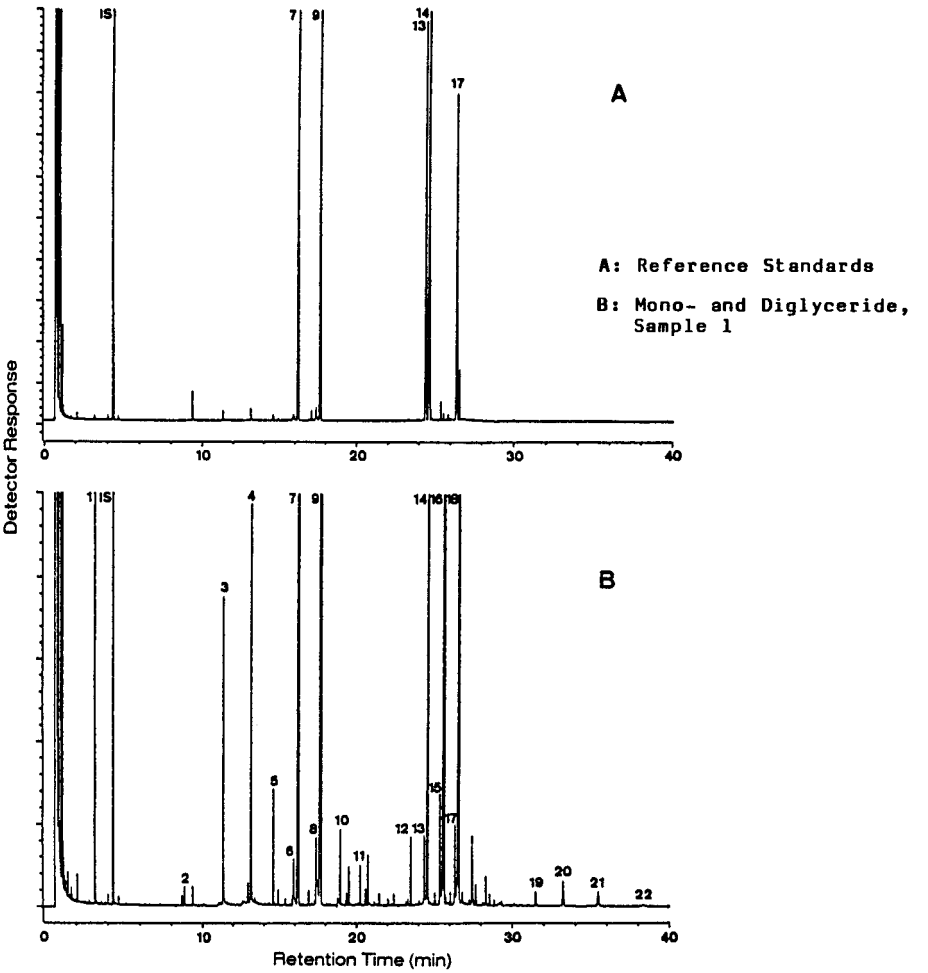

Fig. 1 GAS CHROMATOGRAMS OF TMS-DERIVATIVES OF MONO- AND DIGLYCERIDES

Silylation. Sample: 10 mg; reagents: 0.1ml pyridine containing 1.0 mg n-tetradecane, 0.2ml BSTFA, 0.1ml TMCS; reaction: 20 min at 70 °C.
Column. Fused-silica capillary, 25 m x 0.31 mm (id), film thickness 0.17 μm, 5% phenylmethyl silicon, Ultra # 2 (Hewlett Packard).
Operation conditions: Injector 320 °C, column initial 80 °C, program 10 °C/min, final 360 °C, hold 15 min, detector 350 °C, carrier gas 5 ml He/min (at 80 °C) (see method).
Peak identification. IS: 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 C_{48}, 20: triglyceride C_{50}, 21: triglyceride C_{52}, 22: triglyceride C_{54}.

The response factors of the reference standards vs. n-tetradecane were required to be reported. For the quantification only one concentration level (1 mg/l) had to be used. To calculate the percentage of mass contents of the components in the samples, the response factors of the reference standards vs. n-tetradecane had to be determined first and, after addition of n-tetradecane to the sample and reaction with the silylation reagents, the quantification by GC could be carried out using the formula given in the method. Duplicate reactions with each sample and two injections per reaction had to be carried out. Results had to be reported to the co-ordinator by June 1985.

RESULTS

Operation conditions

Instruments and operating conditions applied by the collaborators are shown in table 1. All laboratories used fused-silica capillary columns. 5 laboratories applied split injection and 3 laboratories on-column injections.

Response factors

Response factors of the reference standards vs. n-tetradecane were determined. A high response factor was reported for glycerol due to derivatization and TMS-groups present in the molecule.

Table 1: **Operating conditions reported by collaborators**

Lab.	Instrument Integrator	Column m x mm (id)	Coating	Temperature Injector	Temperature Initial	Rate °C/min	Final	Carrier Gas	Carrier Gas ml/min at 80 °C	Injection mode (split)	Sample Size
1	Hewlett-Packard 5880 Integrator	Fused silica 25x0.31	Ultra =2	320	80	10	360	He	5	1:20	1 µl
2	Perkin-Elmer Sigma 2000 Integrator	Fused silica 15x0.5	Dimethyl silicon 0.5 µm	320	80	10	340	He	5	1:35	0.1 µl
3	Hewlett-Packard 5890A Integrator 3392A	Fused silica 25x0.31	Ultra =2	350	80	10	360	He	5	1:40	1 µl
4	Hewlett-Packard 5890A Integrator 3392A	Fused silica 15x0.31	Ultra =2	320	80	10	360	He	5	1:40	1 µl
5	Shimadzu GC-9A Integrator C-RZX	Fused silica 15x0.31	Ultra =1	360	80	10	350	He	5	1:50	1 µl
6	Hewlett-Packard 5890 Integrator 3354 Datasystem	Glas 18x0.25	SE30		80	10	340	H_2	0.5	on column	1 µl
7	Carlo-Erba 5160 Spectra Physics 4270	Pyrex 18	SE30 0.07µm		20/60	10	340	H_2	0.75	on column	1 µl
8	Hewlett-Packard 5890A Integrator 3392A	Fused silica 25x0.31	Ultra =2	(350)	60	10	360	He	5	on column	1 µl

Table 2: Results for sample 1, Mono- and Diglycerides (Emulsifiers), expressed as percent by mass of sample

	Glycerol	Palmitic acid	Stearic acid	1-Myristate	1-Palmitate	1-Stearate	1,3-Dipalmitate	1-Palmitate-3-stearate	1,3-Distearate
$\underline{n}$	7	8	8	8	8	8	7	7	7
Mean	2.45	0.67	0.96	0.45	17.08	23.62	6.19	17.42	13.03
$\underline{S}_r$	0.03	0.07	0.07	0.040	0.73	0.81	0.28	0.58	0.41
$\underline{CV}_r$%	1.03	10.40	6.83	8.91	4.25	3.44	4.54	3.33	3.17
$\underline{S}_R$	0.03	0.09	0.13	0.04	1.86	3.33	0.98	4.12	3.02
$\underline{CV}_R$%	1.40	13.02	13.90	9.21	10.90	14.10	15.84	24.16	23.15

Table 3: Results for sample 2, Mono- and Diglycerides (Emulsifiers distilled), expressed as percent by mass of sample

Lab.	Glycerol	Palmitic acid	Stearic acid	1-Myristate	1-Palmitate	1-Stearate	1,3-Dipalmitate	1-Palmitate-3-stearate	1,3-Distearate
Split injection									
1	0.42	0.25	0.72a	1.66	25.24	54.35	0.20	0.70	0.87
	0.43	0.19	0.56a	1.62	25.13	55.60	0.18	0.70	0.88
2	0.45	0.21	0.58	1.77	28.24	65.43	0.31	0.97	1.66
	0.44	0.19	0.54	1.83	20.04	67.40	0.31	1.10	1.66
3	0.44	0.22	0.63	1.67	24.67	52.52	0.18	0.66	0.94
	0.44	0.20	0.62	1.65	26.01	54.20	0.18	0.66	0.95
4	0.43	0.19	0.58	1.55	23.53	50.64	0.17	0.73	0.94
	0.42	0.20	0.62	1.67	25.83	56.64	0.19	0.82	1.08
5	0.41	0.30	0.54	2.38a	30.32	65.10	0.21	0.67	0.96
	0.40	0.23	0.49	1.88a	28.75	66.43	0.20	0.75	1.08
On-column injection									
6	0.50b	0.30	0.76	1.82	27.32	59.75	0.23	0.89	1.19
	0.50b	0.32	0.79	1.81	27.23	59.53	0.23	0.90	1.21
7	0.43	0.12	0.39	1.89	32.01	71.69	0.12	0.71	0.81
	0.40	0.11	0.39	1.77	30.02	66.98	0.12	0.71	0.85
8	0.44	0.26	0.57	1.73	26.57	58.53	0.30a	1.02a	1.32
	0.43	0.39	0.63	1.69	26.06	57.44	0.21a	0.75a	1.10
n	7	8	7	7	8	8	7	7	8
Mean	0.43	0.23	0.58	1.72	27.18	60.14	0.20	0.78	1.09
S_r	0.01	0.04	0.03	0.05	0.89	2.08	0.01	0.05	0.07
CV_r%	2.44	17.92	4.74	2.99	3.28	3.47	4.64	6.05	6.75
S_R	0.02	0.07	0.12	0.10	2.44	6.44	0.06	0.14	0.27
CV_R%	3.48	32.26	20.40	5.70	8.97	10.71	30.03	17.80	24.75

a Results rejected by the Cochran test (95% confidence level)
b Results rejected by the Dixon test (95% confidence level)

Table 4: Results for sample 3, Triglycerides spiked with Mono- and Diglycerides (Emulsifiers), expressed as percent by mass of sample

Lab.	1-Palmitate	1-Stearate	1,2-Dipalmitate	1,3-Dipalmitate	1,2-Distearate	Sum of components analysed
Split injection						
1	1.05	1.06	1.05	1.00	0.96	5.28
	1.10	1.10	1.26	1.00	0.10	
2	0.96	1.03	1.14	1.01	1.07	5.07
	1.01	1.07	1.02	0.97	0.89	
3	0.85	0.84	0.84	0.80	0.92	4.16
	0.84	0.83	0.81	0.77	0.86	
4	0.10	1.01	1.24	0.95	1.00	5.14
	0.96	0.96	1.23	0.90	1.04	
5	0.74	0.68	0.56	0.57	0.69	3.24
	0.78	0.77	0.52	0.53	0.65	
On-column injection						
6	1.02	1.08	0.98	1.19	1.15	5.36
	1.01	1.05	0.96	1.16	1.14	
7	1.15	1.11	1.21	1.03	1.33	5.66
	1.05	1.09	1.15	1.01	1.23	
8	0.97	1.07	0.97	0.98	0.79	4.76
	0.96	1.02	0.95	0.96	0.88	
n	8	8	7	8	8	8
Mean	0.96	0.98	0.97	0.93	0.97	4.83
S_r	0.03	0.03	0.04	0.02	0.06	
CV_r%	3.33	3.37	3.97	2.54	6.21	
S_R	0.12	0.14	0.24	0.19	0.19	0.784
CV_R%	12.02	13.76	24.44	20.22	19.81	16.24
added(%)	1.00	1.00	1.00	1.00	1.00	5.00
recovery(%)	96.0	98.0	97.0	93.0	97.0	96.6

a Results rejected by the Cochran test (95% confidence level)
b Results rejected by the Dixon test (95% confidence level)

Table 5: Results for sample 4, Triglycerides spiked with Mono- and Diglycerides, expressed as percent by mass of sample

	1-Palmitate	1-Stearate	1,2-Dipalmitate	1,2-Distearate	Components analysed
n	8	8	7	8	8
Mean	1.72	2.78	1.98	9.76	7.37
S_r	0.08	0.14	0.06	0.06	
CV_r%	4.81	4.93	2.75	8.02	
S_R	0.23	0.40	0.53	0.20	1.282
CV_R%	13.37	14.48	26.92	26.15	17.40
added(%)	1.77	2.85	2.06	0.68	7.36
recovery(%)	97.2	97.5	96.1	111.8	100.1

Table 6: Results for sample 5, Sunflower seed oil (refined) spiked with Mono- and Diglycerides, expressed as percent by mass of sample

	1-Palmitate	1-Stearate	1,2-Di-palmitate	1,3-Di-palmitate	1,2-Di-stearate	Components analysed
n	7	7	6	6	6	8
Mean	0.75	1.45	1.09	1.37	2.39	7.21
S_r	0.08	0.15	0.02	0.05	0.13	
CV_r%	10.14	10.46	2.18	3.69	5.61	
S_R	0.14	0.29	0.11	0.09	0.74	1.65
CV_R%	18.01	20.04	9.67	6.43	30.82	22.81
added(%)	0.80	1.54	1.09	1.39	2.18	7.00
recovery(%)	93.8	94.2	100.0	98.6	109.6	103.0

Table 7: Results for sample 6, Sunflower seed oil (refined) spiked with Mono- and Diglycerides, expressed as percent by mass of sample

	1-Palmitate	1-Stearate	1,2-Di-palmitate	1,3-Di-palmitate	1,2-Di-stearate	Components analysed
n	6	7	6	6	6	8
Mean	0.72	1.42	0.95	1.34	2.37	6.78
S_r	0.04	1.42	0.96	1.34	2.37	
CV_r%	5.42	10.38	2.81	2.93	11.29	
S_R	0.14	0.29	0.24	0.15	0.76	1.54
CV_R%	19.00	20.52	25.26	11.37	31.93	22.76
added(%)	0.80	1.54	1.09	1.39	2.18	7.00
recovery(%)	90.0	92.2	87.20	96.4	117.4	96.9

The response factor for the fatty acids were between 0.75 and 1. A higher response was found by laboratories after deactivation of the columns by silylation and using on-column injections. One collaborator reported that in order to improve the response, the septum and glass inlet was changed every day. Experience shows, however, that cleaning may have adverse effects, but deactivation with silylating agents improves response. Response may be influenced by the apparatus used. No further comments were made by collaborators despite beeing invited to do so.

Statistical evaluation

Statistical analysis (11) of the collaborative study results are shown in tables 2-7. Samples 3-6 consisted of triglycerides spiked with known amounts of mono- and diglycerides. Recovery could thus be calculated. The recoveries were the following: sample 3: 96.6%, sample 4: 100.1%, sample 5: 103.0%, sample 6: 96.9%.

The results given in tables 2-7 were calculated using the original experimental values with three figures after the decimal point. In the tables only two figures are presented. Values obtained by individual collaborating laboratories are given in table 3 (sample 2) and table 4 (sample 3). These statistical evaluations are also presented in the standardized method. Final statistical results obtained for the samples 1, 4, 5 and 6 after rejection of results using the Cochran and Dixon test, both on the 95% level, are presented in tables 2, 5, 6 and 7.

CONCLUSIONS

On the basis of the results the Commission decided to adopt the method. The text of the standardized procedure is given in the following pages.

2.326 DETERMINATION OF MONO- AND DIGLYCERIDES BY CAPILLARY GAS CHROMATOGRAPHY

1. SCOPE

This standard describes a method for the determination of mono- and diglycerides.

2. FIELD OF APPLICATION

The standard is applicable to mono- and diglyceride concentrates and mono- and diglycerides in fats and oils.

3. PRINCIPLE

Conversion of mono- and diglycerides with N,N-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) in pyridine into more volatile trimethylsilyl ether derivatives and quantitative determination by capillary gas chromatography using an internal standard (n-tetradecane).

4. APPARATUS

4.1 Gas chromatograph, with split injection or on-column injection (note 1), oven temperature programming and flame ionization detector. Recorder and integrator.

4.2 Column, capillary, glass or fused silica, surface fully deactivated by silylation agent (note 2), 15-25 m, 0.25 - 0.35 mm internal diameter, coating SE-54 (or phase with similar polarity), film thickness 0.1 - 0.2 µm.

4.3 Operating conditions, split injection (split ratio 1:10 - 1:50); direct injection (splitless, hold for 1 min); temperatures: injection port 320 °C, or on-column injection 60 °C, column initial 80 °C (or 60 °C, on-column), program 10 °C/min, final 360 °C, hold 15 min, detector 350 °C, carrier gas flow 5 ml He/min (at 80 °C), injection volume 1 - 5 µl. An automatic sampler is advantageous.

4.4 Screw cap vials (2.5 ml) or crimp top vials for auto sampler (e.g. 2.0 ml), with Teflon faced septa.

4.5 Heating device for vials, 70 °C.

5. REAGENTS

5.1 N,N-bis(trimethylsilyl)trifluoroacetamide (BSTFA).
5.2 Trimethylchlorosilane (TMCS).
5.3 Pyridine, p.a., kept over KOH.
5.4 n-Tetradecane, p.a., (internal standard).
5.5 n-Hexane, p.a.
5.6 Reference standards. Glycerol, palmitic acid, 1-O-palmitoylglycerol, 1-O-stearoyl-glycercol, 1,2-di-O-palmitoylglycerol, 1,3-di-O-palmitoylglycerol, 1,2-di-O-stearoyl-glycerol.
5.7 Internal standard solution. Accurately weigh ca. 100 mg n-tetradecane (5.4) into 10 ml volumetric flask and dilute to volume with pyridine (5.3).
5.8 Reference solution. Accurately weigh ca. 100 mg of reference standard (e.g. glycerol, fatty acid, mono- and di-O-acetylglycerol) and accurately weigh ca. 100 mg of n-tetradecane into a 10 ml volumetric flask. Dilute to volume with pyridine. Alternatively, weigh ca. 100 mg of a mixture containing several (e.g. 5) reference standards and n-tetradecane, each component being present in about the same quantities, into a 2 ml volumetric flask and dilute to volume with pyridine.

6. PROCEDURE

6.1 Sample solution

Accurately weigh ca. 10 mg of homogenized 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 0.1 ml of internal standard solution (5.7) containing 1 mg n-tetradecane, 0.2 ml BSTFA and 0.1 ml TMCS to the sample (note 3).

Humidity is strictly excluded. Close vial and shake vigorously. Heat the reaction mixture in heating device at 70 °C for ca. 20 min. Inject 1-5 µl of the reaction mixture into the gas chromatograph showing a stable base line (note 1).
Avoid delay of GC analysis. The reaction is carried out twice and duplicate injections are made per reaction.

6.2 Reference solution

Transfer 0.10 ml of reference solution (5.8) to a vial and add the silylating agents 0.2 ml BSTFA and 0.1 ml TMCS (no internal standard solution is added) and inject as described above (note 1 and 3).
Use a concentration range of reference standards similar to that of the substances to be quantified in sample solution. A plot of response factor vs. concentration of reference standards may be useful to check linearity.
Check response factors periodically. Response factors should be above ca. 0.5. Lower response factors indicate some loss or decomposition. Use concentration range of 0.5 - 10 mg/ml of components in reference and sample solutions.

6.3 Identification

Analyse the reference solution under the same operation conditions as the sample solution. Identify peaks by comparison of retention time with known substances or apply coupled GC/MS.

7. CALCULATION AND EXPRESSION OF RESULTS

7.1 Response factor

Calculate the response factors of the reference substances vs. internal standard using the reference standard chromatogram. The value of the response factor is given by the formula:

$$\underline{R}_x = (\underline{m}_{is}/\underline{m}_x) \times (\underline{A}_x/\underline{A}_{is})$$

where:
$\underline{R}_x$: response factor of reference standard x
$\underline{m}_{is}$: mass, in mg, of internal standard
$\underline{m}_x$: mass, in mg, of reference standard x
$\underline{A}_x$: peak area of reference substance x
$\underline{A}_{is}$: peak area of internal standard

7.2 Calculation of sample component content

Calculate percentage of mass content of component x in the sample by the formula:

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

where:
$\underline{m}'_x$: per cent (m/m) of mass of component x in sample
$\underline{R}_x$: response factor of component x in sample
$\underline{m}'_{is}$: mass, in mg, of internal standard in sample
$\underline{m}'_s$: mass, in mg, of sample
$\underline{A}'_x$: peak area of the component x in sample
$\underline{A}'_{is}$: peak area of the internal standard in sample

8. QUALITY ASSURANCE

8.1 For general principles of analytical quality control see the section on Quality Assurance in the introductory part of the Compendium of the Standard Methods.

8.2 For specific applications of analytical quality control see the Annexe to this standard method.

9. NOTES

1. For on-column injection, or direct injection, dilute 50 µl of reaction mixture (6.1; 6.2) with 1 ml hexane and inject (1 µl).
   In order to lengthen the life time of the columns when applying on-column injections, a pre-column is useful. On-column injection gives better response factors.
2. Use a length of column required to separate 1,2- from 1,3-diglycerides.
3. For automatic samplers with 2 ml crimp top vials, it is convenient to double the amount of sample and reagents.
4. Beside mono- and diglycerides other components such as glycerol, fatty acids, sterols etc. can be analysed by the same method.

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APPENDIX

ANALYTICAL QUALITY CONTROL

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 the tables below, the difference between the two values obtained should not be greater than the repeatability limit (r), which can generally be deduced by linear interpolation from the values in the tables.

2. Reproducibility

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

3. Results of the interlaboratory test

An interlaboratory test carried out at an international level in 1985 by the IUPAC Commission on Oils, Fats and Derivatives, in which 8 laboratories participated, each obtaining two test results for each sample, gave the statistical results (evaluated in accordance with ISO 5725-1986 (11) summarised in the following tables for mono- and diglyceride concentrates (table A) and for mono- and diglycerides in oils (table B).

Table A Statistical Analysis of Results for Mono- and Diglyceride Concentrates (expressed as percent of mass of sample)

	1-Myristate	1-Palmitate	1-Stearate	1,3-Dipalmitate	1-Palmitate-3-stearate	1,3-Distearate
Number of laboratories	8	8	8	8	8	8
Number of results	16	16	16	16	16	16
Number of laboratories retained after elimination of outliers	7	8	8	7	7	8
Number of accepted results	14	16	16	14	14	16
MEAN VALUE (g/100 g sample)	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 relative standard deviation	3.0	3.3	3.5	4.6	6.0	6.8
Repeatability limit (r) [2.83 x S_r]	0.14	2.61	5.91	0.31	0.14	0.20
Reproducibility standard deviation (S_R)	0.1	2.4	6.4	0.06	0.1	0.3
Reproducibility relative standard deviation	5.7	8.9	10.7	30.0	17.8	24.8
Reproducibility limit (R) [2.83 x S_R]	0.3	6.8	18.1	0.2	0.4	0.8

Table B Statistical Analysis of Results for Mono- and Diglycerides in Oil (expressed as percent of mass of sample)

	1-Palmitate	1-Stearate	1,2-Dipalmitate	1,3-Dipalmitate	1,2-Distearate
Number of laboratories	8	8	8	8	8
Number of results	16	16	16	16	16
Number of laboratories retained after elimination of outliers	8	8	8	8	8
Number of accepted results	16	16	16	16	16
MEAN VALUE (g/100 g sample)	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 relative standard deviation	3.3	3.4	4.0	2.5	6.2
Repeatability limit (r) [2.83 x 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 relative standard deviation	12.0	13.8	24.4	20.2	19.8
Reproducibility limit (R) [2.83 x S_R]	0.34	0.40	0.68	0.54	0.54