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BSI Standards Publication

Fat and oil derivatives — Fatty Acid Methyl Esters (FAME) — Determination of free and total glycerol and mono-, di-, triglyceride contents

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National foreword

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The UK participation in its preparation was entrusted to Technical Committee AW/307, Oil seeds, animal and vegetable fats and oils and their by products.

A list of organizations represented on this committee can be obtained on request to its secretary.

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Fat and oil derivatives - Fatty Acid Methyl Esters (FAME) - Determination of free and total glycerol and mono-, di-, triglyceride contents

Produits dérivés des corps gras - Esters méthyliques d'acides gras (EMAG) - Détermination de la teneur en glycérols libre et total et en mono-, di- et triglycérides

Erzeugnisse aus pflanzlichen und tierischen Fetten und Ölen - Fettsäure-Methylester (FAME) - Bestimmung des Gehaltes an freiem und Gesamtglycerin und Mono-, Di- und Triglyceriden

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Management Centre: Avenue Marnix 17, B-1000 Brussels

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Foreword

This document (EN 14105:2011) has been prepared by Technical Committee CEN/TC 307 “Oilseeds, vegetable and animal fats and oils and their by-products - Methods of sampling and analysis”, the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by October 2011, and conflicting national standards shall be withdrawn at the latest by October 2011.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN 14105:2003.

The main modifications of the standard are:

- the utilization of representative internal standards for monoglycérides, diglycerides and triglycerides in order to avoid using calibration solutions for these families of compounds;

- the introduction of a performance criteria for the gas chromatography column calculated with the response factors for the diglyceride and triglyceride internal standards.

The method has been updated to obtain better precision in general, needed for the limits required by European FAME specifications for automotive use [1]. This has been done by introducing separate internal standards for mono- (C19), di- (C38) and triglycerides (C57). Next an improvement of the integration has been incorporated and some evaluation of interference with minor components (i.e. dimers) has been done.

Via a new Round Robin study, improvement of the precision of free glycerol and diglyceride measurement has been proven. The precision statement of the former standard could be confirmed for triglyceride determination, but no improvement was made.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

1 Scope

The purpose of this European Standard is to determine the free glycerol and residual mono-, di- and triglyceride contents in fatty acid methyl esters (FAME) intended for addition to mineral oils. The total glycerol content is then calculated from the obtained results.

Under the conditions described, the quantification limits are 0,001 % (*m/m*) for free glycerol, 0,10 % (*m/m*) for all glycerides (mono-, di- and tri-). This method is suitable for FAME prepared from rapeseed, sunflower, soybean, palm, animal oils and fats and mixture of them. It is not suitable for FAME produced from or containing coconut and palm kernel oils derivatives because of overlapping of different glyceride peaks.

NOTE For the purposes of this European Standard, the term "% (*m/m*)" is used to represent respectively the mass fraction.

WARNING — The use of this method may involve hazardous equipment, materials and operations. This method does not purport to address to all of the safety problems associated with its use, but it is the responsibility of the user to search and establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

2 Principle

Transformation of the glycerol and of the mono- and diglycerides into more volatile and stable silyl derivatives in presence of pyridine and of N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA).

Analysis of the sample after silylation, by gas chromatography on a short capillary column with thin film thickness, with an on-column injector or equivalent device, and flame ionization detection.

After a calibration procedure, the quantification of glycerol is carried out in presence of the internal standard 1,2,4-butanetriol.

Mono-, di- and triglycerides are directly evaluated in presence of an internal standard for each glyceride category:

— glyceryl monononadecanoate (Mono C19) for monoglycerides;

— glyceryl dinonadecanoate (Di C38) for diglycerides;

— glyceryl trinonadecanoate (Tri C57) for triglycerides.

3 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

3.1 N-methyl-N-trimethysilyltrifluoroacetamide (MSTFA)

3.2 Pyridine, max. 0,1 % water, stored on molecular sieve

NOTE Pyridine silyl grade (3.10) can also be used.

3.3 Tetrahydrofurane (THF)

3.4 n-Heptane

3.5 Glycerol

3.6 1,2,4-Butanetriol

3.7 1 - Glyceryl monononadecanoate (Mono C19)[1)]

3.8 1-3 Glyceryl dinonadecanoate (Di C38)[2)]

3.9 Glyceryl trinonadecanoate (Tri C57)[3)]

3.10 Pyridine, silyl grade

4 Apparatus

Usual laboratory apparatus and, in particular, the following.

4.1 Gas chromatograph, equipped with an on-column injector or equivalent device, a temperature-programmable oven and a flame ionization detector.

4.2 Capillary column, capable of being programmed up to 400 °C ("high temperature" type) for which the following characteristics are advised:

— 100 % dimethylpolysiloxane or 95 % dimethyl-5 % diphenylpolysiloxane stationary phase;

— length 15 m;

— internal diameter 0,32 mm;

— film thickness 0,1 μm.

4.3 Volumetric flask, 50 ml capacity

4.4 Volumetric flasks, 20 ml capacity

4.5 Volumetric flasks, 10 ml capacity

4.6 Screw-cap vials with PTFE-faced septa, 10 ml capacity

4.7 Precision pipette, 1 ml capacity

4.8 Microsyringe, 100 μl capacity

4.9 Microsyringe, 500 μl capacity

4.10 Microsyringe, 5 μl or 10 μl capacity specially designed for on-column operation

4.11 Graduated cylinder, 10 ml capacity

4.12 Analytical balance, with an accuracy of ± 0,1 mg

[1)] Monononadecaoin available from Larodan, ref. 31-1900-11 (www.larodan.se)

[2)] 1,3-dinonadecaoin available from Larodan, ref. 32-1903-8 (www.larodan.se)

[3)] Trinonadecaoin available from Larodan, ref. 33-1900-13 (www.larodan.se), or from Sigma , ref. T4632-1G (www.sigmaaldrich.com)

4.13 Carrier gas, hydrogen or helium

4.14 Auxiliary gases, such as air, hydrogen and nitrogen

5 Preparation of solutions

5.1 1,2,4-Butanetriol stock solution, 1 mg/ml

Accurately weigh approximately 50 mg (accuracy ± 0,1 mg) of 1,2,4-butanetriol (3.6) in a 50 ml volumetric flask (4.3) and make up to the mark with pyridine (3.2).

5.2 Glycerol stock solution, 0,5 mg/ml

Accurately weigh approximately 50 mg (accuracy ± 0,1 mg) of glycerol (3.5) in a 10 ml volumetric flask (4.5) and make up to the mark with pyridine (3.2). Using a pipette (4.7), transfer 1 ml of this solution into a 10 ml volumetric flask (4.5) and make up to the mark with pyridine (3.2).

5.3 Standard glycerides stock solution, 2,5 mg/ml

For each reference glyceride, monononadecanoate (3.7), dinonadecanoate (3.8) and trinonadecanoate (3.9), accurately weigh approximately 50 mg (accuracy ± 0,1 mg) in a unique 20 ml volumetric flask (4.4) and make up to the mark with tetrahydrofurane (3.3).

The solution shall be perfectly limpid at ambient temperature. After storage in refrigerator at 4 °C the solution might show a precipitate that must re-dissolve spontaneously when restored at ambient temperature, without any external heating.

NOTE If stored at 4 °C the solution is stable for almost 3 months.

5.4 Commercial mixture of monoglycerides

Made up of mono-palmitoylglycerol (monopalmitin), mono-stearoylglycerol (monostearin) and of mono-oleoylglycerol (monoolein), present in quantities having an identical mass.

Prepare a stock solution of this mixture by weighing approximately 100 mg in a 10 ml volumetric flask (4.5) and make up to the mark with pyridine (3.2).

This solution may be used to locate the relevant peaks in GC paths.

5.5 Calibration solutions

Prepare four calibration solutions by transferring into a series of vials (4.6) the volumes of stock solutions of glycerol (5.2) and of 1,2,4-butanetriol (5.1) given in Table 1, using the 100 μl microsyringes (4.8). Do not use syringe at maximum capacity, but dispense the half volume twice (i.e.: in case of 100 μl dosing using a 100 μl syringe, load 50 μl twice). Make sure that needle and body of the syringe are free from air bubbles, and measure volumes only by difference (i.e.: when dispensing 80 μl, fill syringe up to 100 μl and supply solution up to the 20 μl mark).

Table 1 — Preparation of calibration solutions

Stock solution	1 µl	2 µl	3 µl	4 µl	Syringe µl
glycerol solution (5.2)	10	40	70	100	100
internal butanetriol sol. (5.1)	80	80	80	80	100

6 Sampling

Samples shall be taken in accordance with the requirements of national standards or regulations for the sampling of the product under test. A recommended sampling method is given in EN ISO 5555 [2] or EN ISO 3170 [3].

7 Procedure

7.1 Operating conditions

The chromatographic analysis conditions shall be chosen taking into account the characteristics of the column being used and the type of carrier gas (hydrogen or helium). It is however recommended to observe an analysis time of about 30 min to 35 min to ensure triglycerides elution.

EXAMPLE By way of indication, an example of analysis conditions is described below:

column temperature: 50 °C hold for 1 min, programmed at 15 °C/min up to 180 °C, programmed at 7 °C/min up to 230 °C, programmed at 10 °C/min up to 370 °C, final temperature hold for 15 min;
detector temperature: 380 °C;
carrier gas pressure (hydrogen): 80 kPa;
volume injected: 1 µl.

7.2 Analysis of the calibration solutions

Using a microsyringe (4.10), add 150 µl of MSTFA (3.1) to each of the four calibration solutions (5.5), close hermetically the vials and shake vigorously. Store 15 min at room temperature, then add 8 ml of n-heptane (3.4) using a graduated cylinder (4.11).

Analyse 1 µl of each reaction mixture by gas chromatography under the conditions defined under 7.1, using only the first part of temperature programme, stopping the analysis when the temperature of 230 °C has been reached. Each reaction mixture gives rise to two chromatographic analyses. Samples are stable for some hours after derivatisation.

NOTE The silylated standard solutions are only stable one day.

7.3 Analysis of the commercial mixture of monoglycerides

Using microsyringes (4.10), transfer 200 µl of commercial mixture of monoglycerides dissolved in pyridine (3.10) and 150 µl of MSTFA (3.1) into a 10 ml vial (4.6). Avoid contact with humidity.

Hermetically close the vial and shake vigorously.

Store 15 min at room temperature, and then add 8 ml of n-heptane (3.4).

Analyse 1 µl of the reaction mixture by gas chromatography according to the conditions described under 7.1.

7.4 Preparation and analysis of the samples

Accurately weigh approximately 100 mg (accuracy ± 0,1 mg) of homogenized sample in a 10 ml vial (4.6).

Using precision microsyringes (4.8 and 4.9), add 80 μl of 1,2,4-butanetriol stock solution (5.1), 200 μl of standard glycerides stock solution (5.3), 200 μl of pyridine (3.10) and 200 μl of MSTFA (3.1). Avoid contact with humidity.

Hermetically close the vial and shake vigorously. Store 15 min at room temperature, and then add 8 ml of n-heptane (3.4). Analyse 1 μl of the reaction mixture by gas chromatography according to the conditions described under 7.1.

Carry out the determination in duplicate, by preparing two independent samples.

7.5 Identification

The analysis of the calibration solutions under the same operating conditions as those used for the analysis of the sample allows the identification of the peaks by comparison of the retention times. Due to the overlapping of the elution zones of the methyl esters and of the monoglycerides, it is therefore advised, in order to identify the monoglyceride peaks, to inject the commercial mixture composed of monopalmitin, monosterarin and monoolein (5.4), the latter having been previously submitted to the derivatisation reaction.

A chromatogram of a rapeseed oil methyl ester sample, obtained under the operating conditions and preparation described under 7.1 is presented in Annex A. Internal glyceride standards may be analysed under the above mentioned chromatographic conditions, after silyl derivatisation.

7.6 Calibration

For glycerol only, the study of the variation of weight ratio versus area ratio makes it possible to verify the linearity of the response and to work out a calibration function.

For mono-, di- and triglycerides it is assumed that, within the considered concentration range the detector response is regarded as linear.

7.7 Column performance control

For each analysis, evaluate the relative response factor for glyceryl dinonadecanoate (Di C38) versus glyceryl trinonadecanoate (Tri C57), by using the following equation:

$$RRF = (A_{\mathrm{DiC38}} / M_{\mathrm{DiC38}}) / (A_{\mathrm{TriC57}} / M_{\mathrm{TriC57}}) \quad (1)$$

where

A_{DiC38} is the peak area of internal standard Di C38;

M_{DiC38} is the weight of internal standard Di C38 (mg);

A_{TriC57} is the peak area of internal standard Tri C57;

M_{TriC57} is the weight of internal standard Tri C57 (mg).

The results of the calculation of RRF shall be lower than 1,8. For higher values, the gas chromatography system is not suitable for analysis and shall be verified in order to improve triglyceride detection.

8 Determination of results

8.1 Integration of the peaks

In each family of glycerides, there exist small peaks (see Annex A) which have to be integrated. This method therefore calculates the percentage of mono-, di- and triglycerides (8.4) by summing the area peaks for each family. It is advised to integrate jointly the two diglyceride peaks containing 36 atoms of carbon, major compounds of this family, on account of an insufficient resolution which may induce quantification errors if the two peaks are integrated separately. The presence of a double peak at the level of the glycerol retention time shall lead to the verification of the sylilation stage, which is probably incomplete (presence of water in the samples).

8.2 Glycerol calibration function

The calibration function is given by the following equation, obtained from the experimental data using the linear regression method as in Annex B and according to:

$$M_g / M_{ei} = a_g \left(A_g / A_{ei} \right) + b_g \qquad (2)$$

where

M_g is the weight of glycerol (mg);

M_{ei} is the weight of internal standard 1,2,4-butanetriol (mg);

A_g is the peak area of glycerol;

A_{ei} is the peak area of the internal standard 1,2,4-butanetriol;

a_g , b_g are the regression coefficients of the calibration function for glycerol.

The calibration function shall be regarded as correct only if the correlation coefficient, calculated according to Annex B, is equal or greater than 0,9 (see Annex C for a worked example).

8.3 Free glycerol

Calculate the mass percentage of free glycerol (G) in % (*m/m*) in the sample using the equation:

$$G = \left[a_g \left({}^{A_g}\!/\!_{A_{ei1}} \right) + b_g \right] \times \left({}^{M_{ei}}\!/\!_{m} \right) \times 100 \qquad (3)$$

where

A_{ei1} is the peak area of internal standard 1,2,4-butanetriol;

M_{ei1} is the weight of internal 1,2,4-butanetriol;

m is the weight of sample (mg).

8.4 Glycerides

Calculate the mass percentage of the mono-, di- and triglycerides in % (*m/m*) using the following equations:

$$M = (A_{Mono}/A_{MonoC19}) \times (M_{MonoC19}/m) \times 100 \quad (4)$$

$$D = (A_{Di}/A_{DiC38}) \times (M_{DiC38}/m) \times 100 \quad (5)$$

$$T = (A_{Tri}/A_{TriC57}) \times (M_{TriC57}/m) \times 100 \quad (6)$$

where

M, D, T are the mono-, di- and triglyceride concentration in the sample respectively;

$A_{Mono}, A_{Di}, A_{Tri}$ are the sums of the peak areas of the mono-, di- and triglycerides respectively;

$A_{MonoC19}$ is the peak area of internal standard Mono C19;

$M_{MonoC19}$ is the weight of internal standard Mono C19 (mg);

A_{DiC38} is the peak area of internal standard Di C38;

M_{DiC38} is the weight of internal standard Di C38 (mg);

A_{TriC57} is the peak area of internal standard Tri C57;

M_{TriC57} is the weight of internal standard Tri C57 (mg);

m is the weight of sample (mg).

8.5 Total glycerol

Calculate the percentage of total glycerol in the sample (GT) in % (*m/m*) using the following equation:

$$GT = G + 0{,}255\ M + 0{,}146\ D + 0{,}103\ T \quad (7)$$

9 Expression of results

Free and total glycerol content is expressed to the nearest 0,001 % (*m/m*).

All glyceride contents are each expressed to the nearest 0,01 % (*m/m*).

10 Precision

10.1 Interlaboratory test

An interlaboratory test organized in 2008 at European level with the participation of 16 laboratories, each having carried out two determinations on each sample, gave the statistical results indicated in Annex D.

10.2 Repeatability

The difference between two test results, obtained by the same operator with the same apparatus under constant operating conditions on identical test material would in the long run, in the normal and correct operation of the test method exceed the values given in Table 2 in absolute value in only one case in twenty:

Table 2 — Repeatability

For free glycerol	0,161 5 X + 0,000 3
For monoglycerides	0,078 7 X + 0,005 9
For diglycerides	0,098 9 X + 0,004 2
For triglycerides	0,046 9 X + 0,012 8
For total glycerol	0,109 2 X - 0,003 4
X being the mean value of the two results in question.	

10.3 Reproducibility

The difference between two single and independent test results, obtained by different operators working in different laboratories on identical test material, would in the long run, in the normal and correct operation of the test method, exceed the value given in Table 3 in only one case in twenty.

Table 3 — Reproducibility

For free glycerol	0,183 3 X + 0,006 1
For monoglycerides	0,186 7 X + 0,065 4
For diglycerides	0,188 5 X + 0,028 9
For triglycerides	0,318 0 X + 0,052 0
For total glycerol	0,190 2 X + 0,011 5
X being the mean value of the two results in question.	

11 Test report

The test report shall specify:

a) a reference to this European Standard;

b) the type and complete identification of the product tested;

c) the used method of sampling (see Clause 6);

d) the result of the test (see Clause 9), or if the repeatability has been checked, the final quoted result obtained;

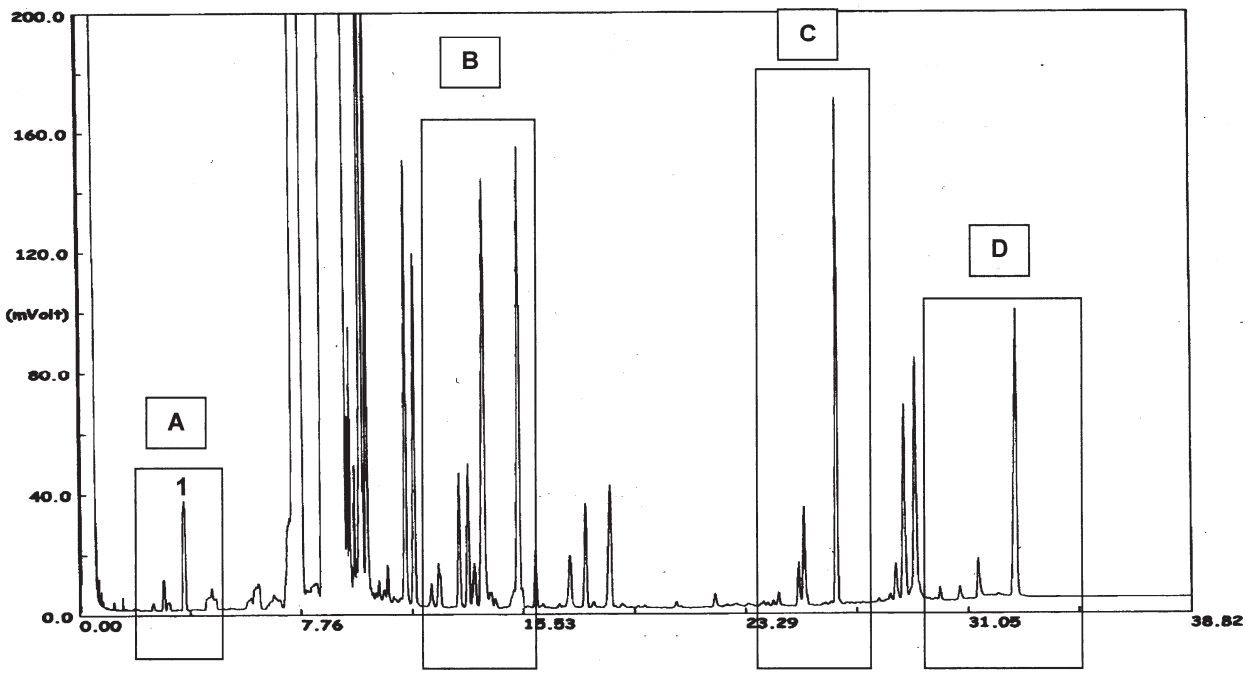
e) all operating details not specified in this European Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

f) the date of the test.

Annex A
(informative)

Sample chromatogram

Figure A.1 shows an example of a chromatogram to determine mono, di-, triglycerides and free glycerol in a FAME sample.

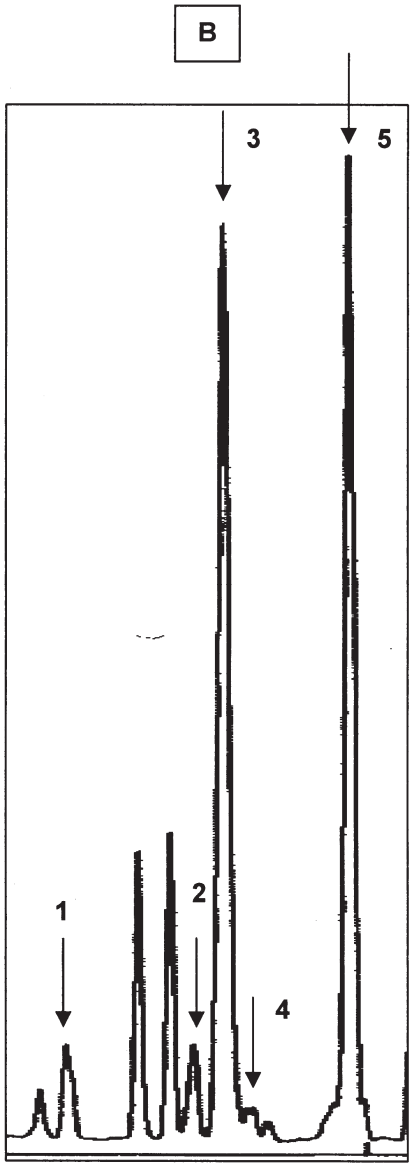


Key

1 1,2,4-Butanetriol
A glycerol zone
B monoglycerides zone
C diglycerides zone
D triglycerides zone

Figure A.1 — FAME sample chromatogram

Figure A.2 shows the specific peaks determining monoglycerides.

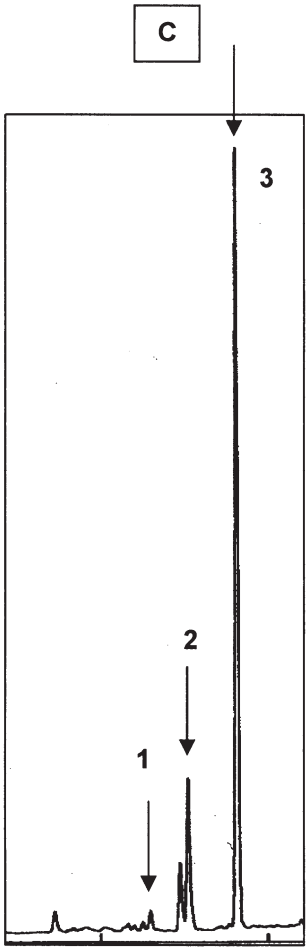


Key

1 mono C 16:0
2 mono C 18:1,2,3
3 mono C 18:1,2,3
4 mono C 18:0
5 mono C 19 – Glyceryl Monononadecanoate (IS)

Figure A.2 — Monoglycerides peaks

Figure A.3 shows the specific peaks determining diglycerides.

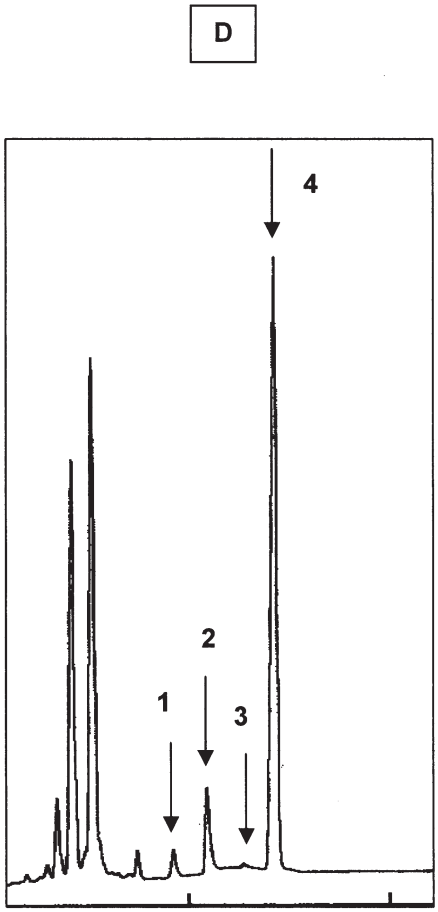


Key

1 Di C 34
2 Di C 36
3 Di C 38 – Glyceryl Dinonadecanoate (IS)

Figure A.3 — Diglycerides peaks

Figure A.4 shows the specific peaks determining triglycerides.



Key

1 Tri C 52
2 Tri C 54
3 Tri C 56
4 Tri C 57 – Glyceryl Trinonadecanoate (IS)

Figure A.4 — Triglycerides peaks

Annex B
(normative)

Calibration function calculation

This annex describes the linear regression method for the calibration function calculation.

After injecting the calibration solutions prepare a table like Table B.1 to fill in the data obtained during the test.

Table B.1 — Experimental data

M_g	M_{is1}	M_g/M_{is1}	A_g	A_{is1}	A_g/A_{is1}

where

M_g is the mass of glycerol (milligrams);

M_{is1} is the mass of IS 1 (milligrams);

A_g is the area of glycerol peak.

A_{is1} is the area of IS 1 peak.

In our regression function X is represented by the term A_g/A_{is1}, while Y is M_g/M_{is1}.

Fill a second table like Table B.2 using X and Y as indicated above:

Table B.2 —Regression function data

X	Y	X^2	Y^2	XY

Then calculate the sum for each row:

ΣX =	ΣY =	ΣX^2 =	ΣY^2 =	ΣXY =

and more:

$(\Sigma X)^2$ = $(\Sigma Y)^2$ = and N = (number of measures)

from obtained data one calculates:

$$a_g = \frac{(N \times \Sigma XY) - (\Sigma X \times \Sigma Y)}{(N \times \Sigma X^2) - (\Sigma X)^2} \quad \text{(B.1)}$$

and:

$$b_g = \frac{\Sigma Y - (a_g \times \Sigma X)}{N} \quad \text{(B.2)}$$

The correlation coefficient r is calculated from the following formula:

$$r = \frac{(N \times \Sigma XY) - (\Sigma X \times \Sigma Y)}{\sqrt{\left(N \times \Sigma X^2 - (\Sigma X)^2\right) \cdot \left(N \times \Sigma Y^2 - (\Sigma Y)^2\right)}} \quad \text{(B.3)}$$

Annex C
(informative)

Worked example

This calculation example is based on glycerol, but other calibration functions may be calculated in the same way from the GC data obtained.

Table C.1 — Experimental data

M_g	M_{is1}	M_g/M_{is1}	A_g	A_{is1}	A_g/A_{is1}
0,007	0,09	0,078	1,263	12,642	0,100
0,007	0,09	0,078	1,264	12,792	0,099
0,028	0,09	0,311	3,113	9,133	0,341
0,028	0,09	0,311	3,065	8,961	0,342
0,049	0,09	0,544	3,788	6,507	0,582
0,049	0,09	0,544	4,150	6,581	0,631
0,070	0,09	0,778	4,725	5,593	0,844
0,070	0,09	0,778	4,531	5,152	0,879

Table C.2 — Regression data

X	Y	X^2	Y^2	XY
0,100	0,078	0,010	0,006	0,008
0,099	0,078	0,010	0,006	0,008
0,341	0,311	0,116	0,096	0,106
0,342	0,311	0,117	0,096	0,106
0,582	0,544	0,339	0,296	0,317
0,631	0,544	0,398	0,296	0,343
0,844	0,778	0,712	0,605	0,657
0,879	0,778	0,773	0,605	0,684

ΣX = 3,818	ΣY = 3,422	ΣX^2 = 2,475	ΣY^2 = 2,006	ΣXY = 2,229

$(\Sigma X)^2$ = 14,577 $(\Sigma Y)^2$ = 11,710 and N = 8

$$a_g = \frac{(8 \times 2{,}229) - (3{,}818 \times 3{,}422)}{(8 \times 2{,}475) - 14{,}577} = 0{,}9127$$

$$b_g = \frac{3{,}422 - (0{,}9127 \times 3{,}818)}{8} = -0{,}008$$

$$r = \frac{(8 \times 2{,}229) - (3{,}818 \times 3{,}422)}{\sqrt{(8 \times 2{,}475 - 14{,}577) \times (8 \times 2006 - 11{,}710)}} = 1{,}001$$

Annex D
(informative)

Results of the interlaboratory trial

A European collaborative test involving 16 laboratories in 5 countries was carried out on 6 FAME samples of different feedstock origin.

The test was organised by CEN/TC 19/TC 307/JWG in January 2008 and the results obtained were subjected to statistical analysis in accordance with EN ISO 4259 [4] to give the precision data shown in Tables D.1 to D.5.

Table D.1 — Monoglycerides

Sample	1	2	3	4	5	6
N° of participating laboratories	16	16	16	16	16	16
N° of participating laboratories after eliminating outliers	14	14	14	15	13	12
Mean value (% *m/m*)	0,426 54	0,433 79	0,615 54	0,761 20	0,303 08	0,810 08
Repeatability standard deviation (% *m/m*)	0,013 25	0,012 72	0,022 08	0,026 85	0,007 92	0,015 77
Reproducibility standard deviation (% *m/m*)	0,0528 6	0,060 11	0,063 55	0,075 16	0,025 64	0,058 69
Repeatability limit, r (% *m/m*)	0,040 09	0,038 49	0,066 83	0,080 88	0,024 20	0,048 62
Reproducibility limit R (% *m/m*)	0,159 99	0,181 93	0,191 42	0,225 33	0,078 33	0,180 94

Table D.2 — Diglycerides

Sample	1	2	3	4	5	6
N° of participating laboratories	16	16	16	16	16	16
N° of participating laboratories after eliminating outliers	15	15	15	13	15	15
Mean value (% *m/m*)	0,142 03	0,159 67	0,172 37	0,305 08	0,184 57	0,080 77
Repeatability standard deviation (% *m/m*)	0,006 19	0,006 03	0,007 13	0,011 27	0,007 67	0,004 14
Reproducibility standard deviation (% *m/m*)	0,021 23	0,018 72	0,019 61	0,029 77	0,018 57	0,015 27
Repeatability limit, r (% *m/m*)	0,018 64	0,018 17	0,021 48	0,034 41	0,023 09	0,012 46
Reproducibility limit R (% *m/m*)	0,063 95	0,056 14	0,058 80	0,090 10	0,055 40	0,046 01

Table D.3 — Triglycerides

Sample	1	2	3	4	5	6
N° of participating laboratories	16	16	16	16	16	16
N° of participating laboratories after eliminating outliers	13	14	14	14	12	14
Mean value (% *m/m*)	0,181 81	0,053 39	0,072 50	0,154 79	0,315 33	0,071 89
Repeatability standard deviation (% *m/m*)	0,007 55	0,005 70	0,005 29	0,007 07	0,008 59	0,004 08
Reproducibility standard deviation (% *m/m*)	0,037 85	0,020 23	0,022 90	0,048 19	0,042 74	0,018 17
Repeatability limit, r (% *m/m*)	0,023 05	0,017 26	0,016 01	0,021 40	0,026 49	0,012 36
Reproducibility limit R (% *m/m*)	0,116 70	0,061 21	0,069 32	0,147 21	0,132 99	0,054 99

Table D.4 — Free glycerol

Sample	1	2	3	4	5	6
N° of participating laboratories	16	16	16	16	16	16
N° of participating laboratories after eliminating outliers	16	16	15	16	15	15
Mean value (% *m/m*)	0,007 44	0,006 00	0,013 93	0,009 31	0,003 53	0,012 10
Repeatability standard deviation (% *m/m*)	0,000 50	0,000 35	0,000 82	0,000 79	0,000 26	0,000 66
Reproducibility standard deviation (% *m/m*)	0,002 51	0,002 31	0,002 53	0,002 58	0,002 20	0,003 21
Repeatability limit, r (% *m/m*)	0,001 50	0,001 06	0,002 46	0,002 37	0,000 78	0,001 98
Reproducibility limit R (% *m/m*)	0,007 54	0,006 95	0,007 58	0,007 75	0,006 66	0,009 67

Table D.5 — Total glycerol

Sample	1	2	3	4	5	6
N° of participating laboratories	16	16	16	16	16	16
N° of participating laboratories after eliminating outliers	13	14	14	13	12	12
Mean value (% *m/m*)	0,154 23	0,145 82	0,202 71	0,261 92	0,139 25	0,238 33
Repeatability standard deviation (% *m/m*)	0,005 20	0,005 34	0,007 81	0,009 22	0,001 71	0,004 92
Reproducibility standard deviation (% *m/m*)	0,014 52	0,014 83	0,017 00	0,022 24	0,010 08	0,015 56
Repeatability limit, r (% *m/m*)	0,015 90	0,016 17	0,023 64	0,028 18	0,005 27	0,015 18
Reproducibility limit R (% *m/m*)	0,043 93	0,044 68	0,050 96	0,067 32	0,031 36	0,047 97

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[4] EN ISO 4259, *Petroleum products — Determination and application of precision data in relation to methods of test (ISO 4259:2006)*

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