BRITISH STANDARD

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14106:2003

Fat and oil derivatives — Fatty Acid Methyl Esters (FAME) — Determination of free glycerol content

The European Standard EN 14106:2003 has the status of a British Standard

ICS 67.200.10

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, which has the responsibility to:

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English version

Fat and oil derivatives - Fatty Acid Methyl Esters (FAME) - Determination of free glycerol content

Produits dérivés des corps gras - Esters méthyliques d'acides gras (EMAG) - Détermination de la teneur en glycérol libre

Erzeugnisse aus pflanzlichen und tierischen Fetten und Ölen - Fettsäure-Methylester (FAME) - Bestimmung des Gehaltes an freiem Glycerin

This European Standard was approved by CEN on 2 January 2003.

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Management Centre: rue de Stassart, 36 B-1050 Brussels

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Foreword

This document (EN 14106:2003) 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of an identical text or by endorsement, at the latest by October 2003 and conflicting national standards shall be withdrawn at the latest by October 2003.

This document has been prepared under Mandate M/245 on Fatty Acid Methylester (FAME) given to CEN by the European Commission and the European Free Trade Association.

Annex A is informative.

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

1 Scope

This European Standard specifies a gas chromatographic method for the determination of free glycerol content in Fatty Acid Methyl Esters (FAME) in the range of 0,005 % to 0,070 %, hereinafter referred as FAME.

This method aims to evaluate the FAME quality, in terms of transesterification by-products content such as glycerol, whose concentration may affect the fuel behaviour.

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.

NOTE In the context of this method (*m/m*) and (*v/v*) can be used.

2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text, and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies (including amendments).

EN ISO 661, *Animal and vegetable fats and oils - Preparation of test sample (ISO 661:1989).*

3 Terms and definitions

For the purposes of this European Standard, the following term and definition apply.

3.1
free glycerol content
is the residual glycerol remaining in FAME after the vegetable oil transesterification reaction and the separation of so obtained glycerol

4 Principle of method

Ethyl alcohol, water and hexane and a known amount of internal standard are added to a known quantity of sample. The addition of these solvents causes the formation of two phases and free glycerol is quantitatively transferred into the lower one.

The gas chromatographic analysis of lower phase allows to quantify the concentration of free glycerol.

5 Reagents

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

5.1 Hexane.

5.2 1,4 Butanediol, 99 % minimum.

5.3 Ethyl alcohol, 95 % minimum.

5.4 Formic acid, 99 % minimum.

5.5 Glycerol, 99 % minimum.

5.6 Carrier gas : helium, GLC grade.

5.7 Auxiliary gases:

— hydrogen, purity 99 % minimum, free from moisture and organic compounds;

— air, free from organic compounds.

5.8 Internal Standard Solution : weigh approximately 80 mg (accuracy ± 0,0001 g) of 1,4 Butanediol in a volumetric flask, 100 ml capacity. Dissolve in some ml of distilled water, add 1 ml of formic acid and fill up to the mark using distilled water. The so prepared solution is stable for 24 h if stored at ambient temperature.

6 Apparatus

6.1 Gas chromatograph equipped by the following devices:

6.1.1 Thermostatic oven for column, able to maintain the set temperature with an accuracy of ± 1 °C.

6.1.2 Thermostatic injection port, equipped for capillary column in split-splitless mode or, alternatively, for packed column.

6.1.3 Flame ionisation detector (FID) and converter/amplifier device.

6.1.4 Recorder/integrator, capable to run with the converter/amplifier with a response time of 1 s maximum and variable chart speed.

6.1.5 Capillary column, type PoraPLOT Q, length = 10 m, diameter 0,32 mm, film thickness = 10 µm (Note) or, alternatively packed column filled with CHROMOSORB 101, diameter 4 mm, length = 1 m.

NOTE Some problems and discrepancies on capillary column behaviour from different suppliers have been noticed. Some Authors refer also the possibility to use capillary columns coated with FFAP (Free Fatty Acid Phase) or polyethyleneglycol phases instead of the suggested stationary phase. Nevertheless the choice of column should be based on the following criteria:

- the separation between glycerol and internal standard peaks should be complete (baseline nearly to zero between two peaks);
- the analysis time should not exceed 15 min;
- columns with response factor for glycerol, calculated such as 9.3, higher than 2,5 are not suitable.

6.1.6 Flow controllers, for carrier and auxiliary gases.

6.2 Microsyringe for gas-chromatography, 5 µl or 10 µl.

6.3 Volumetric flasks, 50 ml and 100 ml capacity.

6.4 Two mark precision pipettes, 1 ml capacity.

6.5 Volumetric pipettes, 5 ml capacity.

6.6 Analytical balance, accuracy ± 0,0001 g.

6.7 Glass conical test tubes, 10 ml capacity.

6.8 Centrifugal machine, able to run at the speed of 2000 revolutions per minute.

7 Sampling

Sampling is not part of the method specified in this European Standard. A recommended sampling method is given in EN ISO 5555 [1].

Sampling procedures shall take into account that free glycerol contained in FAME has a strong affinity for glass. Therefore samples shall be collected and stored avoiding the use of glass.

8 Preparation of test sample

Prepare the test sample in accordance with EN ISO 661. The test sample shall not be heated and/or filtered.

9 Procedure

9.1 Gas chromatographic conditions

*T*he following general operating conditions have been shown to be satisfactory.

— Oven temperature : 210 °C;

— injector temperature : 230 °C;

— detector temperature : 250 °C;

— split ratio : approximately 50:1;

— carrier gas flow : 1 ml/min to 2 ml/min.

Conditions for packed column :

— oven temperature : 200 °C;

— injector temperature : 230 °C;

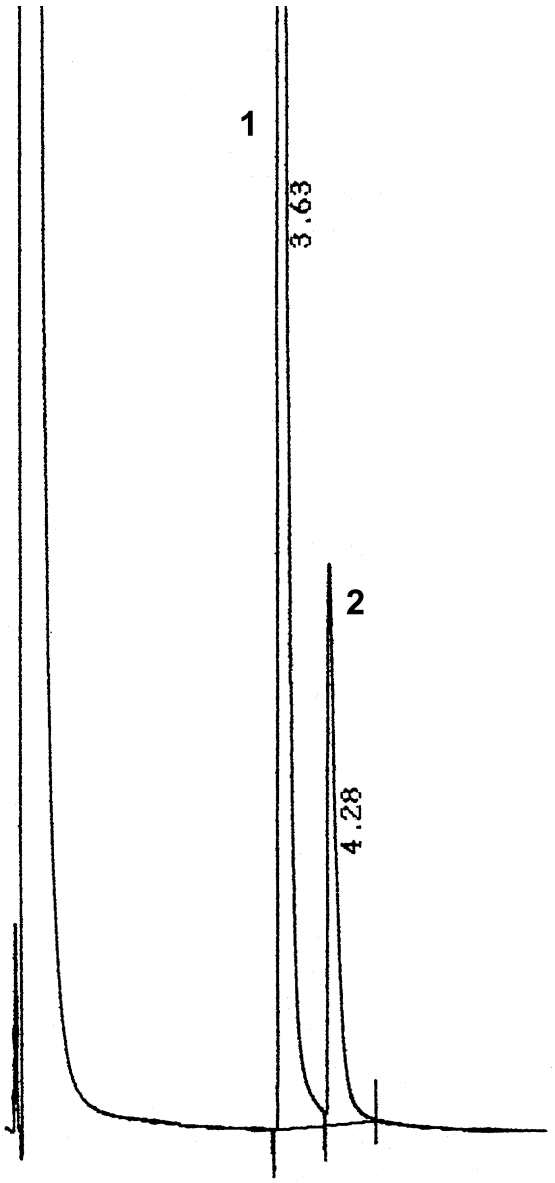
— detector temperature : 250 °C;

— carrier gas flow : 20 ml/min to 30 ml/min.

9.2 Peaks identification

The identification of peaks belonging to internal standard and glycerol can be achieved by retention time comparison of suitable standards analysed under the same analytical conditions. An illustrative chromatogram is reported below.

GLC Analysis



Key

1 1,4 Butanediol
2 Glycerol

Figure 1 — Chromatogram of a synthetic solution used for response factor determination - Determination of free glycerol content

9.3 Determination of response factor

Weigh approximately 100 mg ± 0,1 mg of glycerol and 100 mg ± 0,1 mg of 1,4 butanediol in a 100 ml volumetric flask. Dissolve in 50 ml of ethyl alcohol (5.3) and fill up to the mark with water. Consider the heating and the volume diminution due to the water/alcohol mixing. Inject at least three times in the chromatograph 1 µl of this solution using the experimental conditions listed above, in order to calculate the response factor. The solution is stable for some weeks even if stored at room temperature. Using the obtained chromatograms the response factor F_r can be calculated as follows :

$$\text{Response factor } (F_r) = \frac{A_1 / M_1}{A_2 / M_2} \quad (1)$$

where

A_1 is the peak area of internal standard;

A_2 is the peak area of glycerol;

M_1 is the mass of 1,4 butanediol in response factor solution, expressed in mg;

M_2 is the mass of glycerol in response factor solution, expressed in mg.

The response factor F_r, such as calculated above shall be 2,5 or lower (see note in 6.1.5).

9.4 Quantitative analysis

9.4.1 Sample preparation

Weigh approximately 3,5 g ± 0,0001 g of sample (corresponding to about 4 ml) into a 10 ml test tube. Add 1 ml of ethyl alcohol (5.3) and gently shake to ensure mixing. Add exactly 1 ml of internal standard solution (5.8) and 4 ml of hexane. Tightly plug and shake vigorously the tube for five minutes. Centrifuge the sample for 15 min. Use the lower phase for gas chromatographic analysis.

9.4.2 Gas chromatography analysis

Take approximately 1 µl of the lower phase using the GC micro-syringe and draw enough air to empty the needle ("hot needle" technique). Carefully clean body and needle of syringe using a paper towel. Introduce the needle through the membrane of injection port, wait for 5 s, then rapidly inject the sample. After 5 s draw off the needle. Take note of injection moment on chromatogram. Continue elution some minutes after the complete detection of glycerol peak.

10 Results

Calculate, by means of integrator, the areas of peaks belonging to the unknown sample. The percent content of free glycerol in sample is given by the following formula :

$$\text{Free glycerol, \% (m/m)} = \frac{(A_2/A_1) \times F_r \times m_1}{m} \times 100$$

where

A_1, A_2, F_r have the same meaning as in formula (1);

m_1 is the mass of internal standard in sample (milligrams);

m is the mass of sample (milligrams).

The result shall be expressed as a mass fraction in percent, rounded to two digits..

11 Precision

11.1 Interlaboratory test

Details of interlaboratory test are given in annex A. The values derived from these tests may not be applicable to concentration ranges and matrices other than those given.

11.2 Repeatability

The difference between two results obtained by the same operator, with the same apparatus under constant operating conditions on the same sample, will be higher than the repeatability limit (r) as calculated from the formulae in following table, only in one case in twenty.

11.3 Reproducibility

The absolute difference between two independent single test results, obtained by two different operators working in different laboratories using the same method on identical test material within a short interval of time will be higher than the reproducibility limit (R) as calculated from the formulae in following table, only in once out of 20 determinations.

Table 1 — Repeatability and reproducibility limits (r and R)

Free glycerol in FAME, in % (*m/m*)	r = 0,4664 X – 0,0012	R = 0,7812 X + 0,0032
X corresponding to free glycerol concentration value.		

12 Test report

The test report shall specify:

— all information necessary for the complete identification of the sample;

— the sampling method used if known;

— the test method used, with reference to this European standard;

— 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);

— the test result(s) obtained, or if the repeatability has been checked, the final quoted result obtained.

Annex A
(informative)

Results of an interlaboratory trial

A European collaborative test involving 7 laboratories in 5 countries was carried out on 5 samples:

Sample 1 : FAME prepared from sunflower oil;

Sample 2 : FAME prepared from rapeseed oil;

Sample 3 : FAME prepared from rapeseed oil and sunflower oil (75:25);

Sample 4 : FAME prepared from rapeseed oil;

Sample 5 : FAME prepared from rapeseed oil and sunflower oil (25:75).

The test was organised by CEN/TC307/WG1 in 1999 and the results obtained were subjected to statistical analysis in accordance with EN ISO 4259 [2] to give the precision data shown in Table A.1.

Table A.1

Sample	**1**	**2**	**3**	**4**	**5**
N° of participating laboratories	7	7	7	7	7
N° of participating laboratories after eliminating outliers	6	6	6	6	6
Mean value % (*m/m*)	**0,018**	**0,014**	**0,032**	**0,001**	**0,048**
Repeatability standard deviation % (*m/m*)	0,001	0,001	0,005	0,000	0,006
Reproducibility standard deviation % (*m/m*)	0,003	0,004	0,007	0,002	0,012
Repeatability limit, r % (*m/m*)	0,004	0,004	0,018	0,001	0,020
Reproducibility limit R % (*m/m*)	0,011	0,015	0,025	0,006	0,042

Bibliography

[1] EN ISO 5555, *Animal and vegetable fats and oils - Sampling (ISO 5555:2001).*

[2] EN ISO 4259, *Petroleum products - Determination and application of precision data in relation to methods of test (ISO 4259:1992/Cor 1:1993).*

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