



BSI Standards Publication

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

National foreword

This British Standard is the UK implementation of EN 14105:2011. It supersedes BS EN 14105:2003, which is withdrawn.

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

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English Version

**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

This European Standard was approved by CEN on 10 March 2011.

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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of an identical text or by endorsement, at the latest by October 2011, and conflicting national standards shall be withdrawn at the latest by October 2011.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 monoglycerides, 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 mononadecanoate (Mono C19) for monoglycerides;
- glyceryl dinadecanoate (Di C38) for diglycerides;
- glyceryl trinadecanoate (Tri C57) for triglycerides.

3 Reagents

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

3.1 N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA)

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

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

3.3 Tetrahydrofuran (THF)

3.4 n-Heptane

3.5 Glycerol

- 3.6 1,2,4-Butanetriol
- 3.7 1 - Glyceryl mononadecanoate (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 $\pm 0,1$ mg

¹⁾ Mononadecanoic acid available from Larodan, ref. 31-1900-11 (www.larodan.se)

²⁾ 1,3-dinonadecanoic acid available from Larodan, ref. 32-1903-8 (www.larodan.se)

³⁾ Trinonadecanoic acid 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 $\pm 0,1$ 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 $\pm 0,1$ 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 $\pm 0,1$ 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 μ l microsyringes (4.8). Do not use syringe at maximum capacity, but dispense the half volume twice (i.e.: in case of 100 μ l dosing using a 100 μ l syringe, load 50 μ l 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 μ l, fill syringe up to 100 μ l and supply solution up to the 20 μ l mark).