
Derivati maščob in olj - Metil estri maščobnih kislin (FAME) - Določevanje prostega in celotnega glicerola ter mono-, di- in trigliceridov

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

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

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

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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
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Ölen - Fettsäure-Methylester (FAME) - Bestimmung
des Gehaltes an freiem und Gesamtglycerin und Mono-,
Di- und Triglyceriden

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European foreword

This document (prEN 14105:2017) 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 document is currently submitted to the CEN Enquiry.

This document will supersede EN 14105:2011.

In comparison with the previous edition, the following technical modifications have been made:

- a) document revised editorially;
- b) improvement of the quality of the figures;
- c) addition of figures in Annex A for clarification.

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1 Scope

This document specifies a method to determine the free glycerol and residual mono-, di- and triglyceride contents in fatty acid methyl esters (FAME). 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 1 For the purposes of this document, the term “% (*m/m*)” is used to represent the mass fraction.

NOTE 2 Under the common EN 14105 GC conditions squalene can coelute with alpha glycerol monostearate. If the presence of squalene is suspected, EN 17057 can be used to discriminate between squalene and glycerol monostearate.

WARNING — The use of this Standard can involve hazardous materials, operations and equipment. This Standard does not purport to address all of the safety problems associated with its use. It is the responsibility of users of this standard to take appropriate measures to ensure the safety and health of personnel prior to application of the standard, and fulfil statutory and regulatory requirements for this purpose.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 5555, *Animal and vegetable fats and oils — Sampling*

EN ISO 3170, *Petroleum liquids — Manual sampling*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at <http://www.electropedia.org/>
- ISO Online browsing platform: available at <http://www.iso.org/obp>

4 Principle

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

The sample after silylation is analysed 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.

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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.

5 Reagents

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

5.1 N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA).

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

Pyridine silyl grade (5.10) can also be used.

5.3 Tetrahydrofurane (THF).

5.4 n-Heptane.

5.5 Glycerol.

5.6 1,2,4-Butanetriol.

5.7 1-Glyceryl mononadecanoate (Mono C19)¹.

5.8 1-3 Glyceryl dinadecanoate (Di C38)².

5.9 Glyceryl trinadecanoate (Tri C57)³.

5.10 Pyridine, silyl grade.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

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

6.2 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.

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

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

³ Trinadecanoic acid available from Larodan, ref. 33-1900-13 (www.larodan.com), or from Sigma, ref. T4632-1G (www.sigmaaldrich.com).

- 6.3 Volumetric flask**, 50 ml capacity, Grade A.
- 6.4 Volumetric flasks**, 20 ml capacity, Grade A.
- 6.5 Volumetric flasks**, 10 ml capacity, Grade A.
- 6.6 Screw-cap vials with PTFE-faced septa**, 10 ml capacity.
- 6.7 Precision pipette**, 1 ml capacity.
- 6.8 Precision pipette or syringe**, 100 µl capacity.
- 6.9 Precision pipette or syringe**, 500 µl capacity.
- 6.10 Graduated cylinder**, 10 ml capacity.
- 6.11 Analytical balance**, with a weighing accuracy of ± 1 mg or better and a readability of $\pm 0,1$ mg or better.
- 6.12 Carrier gas**, hydrogen or helium.
- 6.13 Auxiliary gases**, such as air, hydrogen and nitrogen.

7 Preparation of solutions

7.1 1,2,4-Butanetriol stock solution, 1 mg/ml

Weigh $(50,0 \pm 0,1)$ mg 1,2,4-butanetriol (5.6) in a 50 ml volumetric flask (6.3) and fill up to the mark with pyridine (5.2 or 5.10).

7.2 Glycerol stock solution, 0,5 mg/ml

Weigh $(50,0 \pm 0,1)$ mg glycerol (5.5) in a 10 ml volumetric flask (6.5) and fill up to the mark with pyridine (5.2 or 5.10). Transfer 1 ml of this solution into a 10 ml volumetric flask (6.5) using a pipette (6.7) and fill up to the mark with pyridine (5.2 or 5.10).

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

For each reference glyceride, monononadecanoate (5.7), dinonadecanoate (5.8) and trionadecanoate (5.9), weigh $(50,0 \pm 0,1)$ mg in a unique 20 ml volumetric flask (6.4) and fill up to the mark with tetrahydrofurane (5.3).

The solution shall be perfectly transparent at ambient temperature. After storage in a refrigerator at 4 °C the solution might show a precipitate that shall 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.

7.4 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.

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Prepare a stock solution of this mixture by weighing approximately 100 mg in a 10 ml volumetric flask (6.5) and fill up to the mark with pyridine (5.2 or 5.10).

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

7.5 Calibration solutions

Prepare four calibration solutions by transferring the volumes given in Table 1 of the glycerol stock solutions (7.2) and of the 1,2,4-butanetriol (7.1) into a series of vials (6.6), using the 100 µl precision pipette (6.8). If using a syringe, make sure that the needle and the body of the syringe are free from air bubbles.

Table 1 — Preparation of calibration solutions

Stock solution	1 (µl)	2 (µl)	3 (µl)	4 (µl)	syringe (µl)
glycerol solution (7.2)	10	40	70	100	100
internal butanetriol solution (5.1)	80	80	80	80	100

8 Sampling

Samples shall be taken in accordance with EN ISO 5555 or EN ISO 3170.

9 Procedure**9.1 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 in Table 2:

Table 2 — Example of analysis conditions

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 or constant flow (about 2 ml/min)
volume injected:	1 µl

9.2 Analysis of the calibration solutions

Using a precision pipette (6.9), add 150 µl of MSTFA (5.1) to each of the four calibration solutions (7.5), close hermetically the vials and shake vigorously. Store 15 min at room temperature, then add 8 ml of n-heptane (5.4) using a graduated cylinder (6.10).

Analyse 1 µl of each reaction mixture by gas chromatography under the conditions defined in 9.1, using only the first part of the temperature programme, stopping the analysis when the temperature of 230 °C has been reached. Samples are stable for some hours after derivatisation.

NOTE The silylated standard solutions are only stable for one day.

9.3 Analysis of the commercial mixture of monoglycerides

Using precision pipette (6.9), transfer 200 µl of commercial mixture of monoglycerides dissolved in pyridine (5.10) and 150 µl of MSTFA (5.1) into a 10 ml vial (6.6). 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 (5.4).

Analyse 1 µl of the reaction mixture by gas chromatography according to the conditions described in 9.1.

9.4 Preparation and analysis of the samples

Weigh $(100,0 \pm 0,1)$ mg of homogenized sample in a 10 ml vial (6.6).

Using precision pipettes or syringes (6.8 and 6.9), add 80 µl of 1,2,4-butanetriol stock solution (7.1), 200 µl of standard glycerides stock solution (7.3), 200 µl of pyridine (5.10) and 200 µl of MSTFA (5.1). 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 (5.4). Analyse 1 µl of the reaction mixture by gas chromatography according to the conditions described in 9.1.

9.5 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 (7.4), the latter having been previously submitted to the derivatisation reaction.

Chromatograms of a rapeseed oil and a palm oil methyl ester sample, obtained under the operating conditions and preparation described in 9.1 is presented in Annex A. In addition a second palm oil methyl ester sample containing fatty acid methyl ester dimers is also presented. Internal glyceride standards may be analysed under the above mentioned chromatographic conditions, after silyl derivatisation.

9.6 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.