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Fat and oil derivatives — Fatty Acid Methyl Esters (FAME) — Determination of ester and linolenic acid methyl ester contents

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National foreword

This British Standard is the UK implementation of EN 14103:2011. It supersedes BS EN 14103:2003 which is withdrawn.

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 ester and linolenic acid methyl ester contents

Produits dérivés des corps gras - Esters méthyliques d'acides gras (EMAG) - Détermination de la teneur en ester et en ester méthylique de l'acide linolénique

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

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Foreword

This document (EN 14103:2011) 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of an identical text or by endorsement, at the latest by October 2011, and conflicting national standards shall be withdrawn at the latest by October 2011.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 14103:2003.

The main modifications of the standard are intended:

- to enlarge the scope of the method to FAME from animal fat, by changing the internal standard (FAME C19 instead of FAME C17);

- to verify the purity of the internal standard as it was proven that some lots may have a purity not good enough;

- to improve the precision data by modifying the preparation of the test portion.

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1 Scope

The purpose of this document is to describe a procedure for the determination of the ester content in fatty acid methyl esters (FAME) intended for incorporation into diesel oil. It also allows determining the linolenic acid methyl ester content. It allows verifying that the ester content of FAME is greater than 90 % (*m*/*m*) and that the linolenic acid content is between 1 % (*m*/*m*) and 15 % (*m*/*m*).

This method is suitable for FAME which contains methyl esters between *C*6 and *C*24.

NOTE For the purposes of this European Standard, the terms "% (*m*/*m*)" and "%(*v*/*v*)" are used to represent respectively the mass and volume fractions.

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

Determination of the percentage of total methyl esters of fatty acids and the percentage of linolenic acid methyl ester present in the sample, by gas chromatography according to a procedure using internal calibration (nonadecanoic acid methyl ester).

3 Reagents

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

3.1 Toluene, analytical grade.

3.2 Nonadecanoic acid methyl ester (FAME C19), purity, min. 99,5 % (*m*/*m*)[1)].

NOTE Standard should be kept in a dry storage in order to limit its water absorption. Its water content should be verified by Karl-Fischer when a new lot of standard is open.

3.3 Carrier gas, hydrogen or helium.

3.4 Auxiliary gases:

— air;

— hydrogen.

4 Apparatus

Usual laboratory apparatus and, in particular, the following.

1) Nonadecanoic FAME with acceptable quality is available from Fluka - Ref: 74208 (www.sigmaaldrich.com) or from Nu Chek Prep - Ref: N-19-M (www.nu-chekprep.com) or Dr. Ehrenstorfer GmbH – ref 15 622 360 (www.analytical-standards.com). This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of these products.

4.1 Gas chromatograph, equipped with a variable split flow injector or equivalent device, a temperature programmable oven and a flame ionization detector.

4.2 Capillary column, coated with polyethylene glycol (Carbowax 20M) stationary phase, the following characteristics have been found suitable: length: 30 m, internal diameter: 0,25 mm, film thickness: 0,25 μm.

NOTE Stationary phase other than polyethylene glycol should be tested first before being selected as co-elution between the internal standard (FAME C19) and other fatty acid methyl esters may exist. Indeed, there is a co-elution between FAME C19 and linoleic acid methyl ester (FAME C18:2) when using a column with a stationary phase such as 70 % cyanopropyl-polysilphenyl-siloxane.

4.3 Glass mono-use tubes equipped with plastic mono-use stopper, 10 ml capacity.

4.4 Pipette, 10 ml capacity.

4.5 Analytical balance, accuracy ± 0,1 mg.

5 Sampling

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

6 Procedure

6.1 Operating conditions

The chromatographic analysis conditions will be chosen taking into account the characteristics of the column being used and the type of carrier gas (hydrogen or helium).

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

— column temperature: 60 °C hold for 2 min, programmed at 10 °C.min^{-1} up to 200 °C, programmed at 5 °C.min^{-1} up to 240 °C, final temperature hold for 7 min;

— injector temperature & detector temperature: 250 °C;

— carrier gas flow rate 1-2 ml.min^{-1}, a minimum flow rate of 1 ml.min^{-1} shall be warranted when operating at the maximum temperature;

— injected volume: 1 μl;

— hydrogen pressure = 70 KPa;

— split flow = 100 ml.min^{-1}.

6.2 Internal standard purity determination

Prepare a solution of nonadecanoic acid methyl ester (3.2) in toluene (3.1) approximately at 10 mg.ml^{-1}. Analyse 1 μl of this solution by gas chromatography according to the conditions described in (6.1), but the final temperature should be held for 20 min at 240 °C, instead of 7 min. Calculate the purity of the nonadecanoic acid methyl ester taking into account all the peak eluted in the chromatogram, excepted the solvent peak. The purity of nonadecanoic acid methyl ester should be at least 99,5 % (*m*/*m*). If the purity is lower than 99,5 % (*m*/*m*) do not use this standard for this determination.

NOTE No correction of the purity of the internal standard is performed for the calculation of the FAME content.

6.3 FAME sample preparation and analysis

Accurately weigh approximately 100 mg (accuracy ± 0,1 mg) of homogenized sample in a 10 ml tube (4.3), and approximately 100 mg (accuracy ± 0,1 mg) of nonadecanoic acid methyl ester (3.2), and dilute with 10 ml of toluene (3.1).

NOTE Standard and samples should be let at ambient temperature, in their container closed, at least 3 h prior being weighed, in order to limit the water absorption during weighting.

Analyse 1 µl of this solution by gas chromatography according to the conditions described in (6.1).

For each sample, two test portions are prepared and give rise, each one, to two chromatographic analyses.

6.4 Identification

The chromatographic conditions (injected quantity, oven temperature, carrier gas pressure and split flow rate) shall be adjusted so as to correctly visualize the methyl ester peaks of the lignoceric (C24:0) and nervonic (C24:1) acids.

The integration shall be carried out as from the hexanoic acid methyl ester (C6:0) peak up to that of the nervonic acid methyl ester (C24:1) taking all the peaks identified as fatty acid methyl esters into consideration. In order to identify properly the fatty acid methyl esters, some commercial solutions may be used [2)].

NOTE If some unknown thin peaks are found (others than saturated and mono-unsaturated FAME) between the linolenic acid methyl ester (C18:3) and the nervonic acid methyl ester (C24:1), presence of fish oil in the sample can be suspected.

As a general rule, the separation is done according to carbon atom chain length, unsaturated FAME are eluted after the corresponding saturated ones.

7 Expression of results

7.1 Determination of ester content

The ester C content, expressed as a mass percentage, is calculated using the following equation:

$$C = \frac{\sum A - A_{EI}}{A_{EI}} \times \frac{W_{EI}}{W} \times 100 \quad (1)$$

where

ΣA is the total peak area from the methyl ester in *C*6:0 to that in *C*24:1;

A_{EI} is the peak area corresponding to nonadecanoic acid methyl ester;

W_{EI} is the weight, in milligrams, of the nonadecanoic acid methyl ester being used as internal standard;

W is the weight, in milligrams, of the sample.

NOTE 1 In the case of vegetable oils, the result of the calculation based on relative areas is considered to represent a percentage by mass.

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2) Commercial solutions of mixtures of fatty acid methyl esters are available from Sigma – Ref. 18918-1AMP (www.sigmaaldrich.com) or from Nu Chek Prep – Ref.17a (www.nu-chekprep.com).

This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of these products.

NOTE 2 If the average of two determinations is higher than 100 % then discard the results and verify the experimental conditions as well as the purity of the internal standard by using this method to determine the ester content of a commercial or prepared mixture, and the water content of the internal standard by Karl Fischer determination.

7.2 Determination of linolenic acid methyl ester

The linolenic acid methyl ester content *L*, expressed as a mass percentage, is calculated using the following equation:

$$L = \frac{A_{\mathrm{L}}}{A_{\mathrm{EI}}} \times \frac{W_{\mathrm{EI}}}{W} \times 100 \qquad (2)$$

where

A_{L} is the peak area corresponding to linolenic acid methyl ester;

A_{EI} is the peak area corresponding to nonadecanoic acid methyl ester;

W_{EI} is the weight, in milligrams, of the nonadecanoic acid methyl ester being used as internal standard;

W is the weight, in milligrams, of the sample.

7.3 Expression of results

Ester content and linolenic acid methyl ester content are expressed in percentage (m/m), to the nearest 0,1 %.

8 Precision

8.1 Interlaboratory test

An interlaboratory test organized in 2009 at European level with the participation of 18 laboratories, each having carried out two determinations on each sample, gave the statistical results indicated in Annex B.

8.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short time interval, shall not be greater more than once out of 20 determinations than the values given in Table 1:

Table 1

For ester content	1,01 % (*m*/*m*)
For linolenic acid methyl ester	$r = 0{,}0283 + 0{,}0175 \cdot X$
X being the mean value of the two results in question *r* in % (*m*/*m*).	

8.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories by different operators using different equipment, shall not be greater more than once out of 20 determinations than the values given in Table 2:

Table 2

For ester content	4,16 % (*m*/*m*)
For linolenic acid methyl ester	$R = 0{,}3872 + 0{,}0285 \cdot X$
X being the mean value of the two results in question R in % (*m*/*m*).	

9 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)

Chromatograms of a FAME samples - Determination of ester content - Summary of the analysis conditions

Capillary column coated with a polyethylene glycol stationary phase (Carbowax 20M, DBwax, CPwax, etc.):

— length: 30 m;

— internal diameter: 0,25 mm;

— film thickness: 0,25 μm.

Variable flow split injector:

— split flow rate: 100 $ml.min^{-1}$;

— temperature: 250 °C.

Carrier gas: hydrogen or helium:

— pressure: 30 kPa to 100 kPa;

— flow: 1 $ml.min^{-1}$ at 2 $ml.min^{-1}$ (depending on characteristics of column being used).

Flame ionization detector:

— temperature: 250 °C.

Oven

— temperature: 60 °C hold for 2 min, programmed at 10 $°C.min^{-1}$ up to 200 °C, programmed at 5 $°C.min^{-1}$ up to 240 °C, final temperature hold for 7 min.

These conditions apply to all chromatograms given as examples in Annex A.

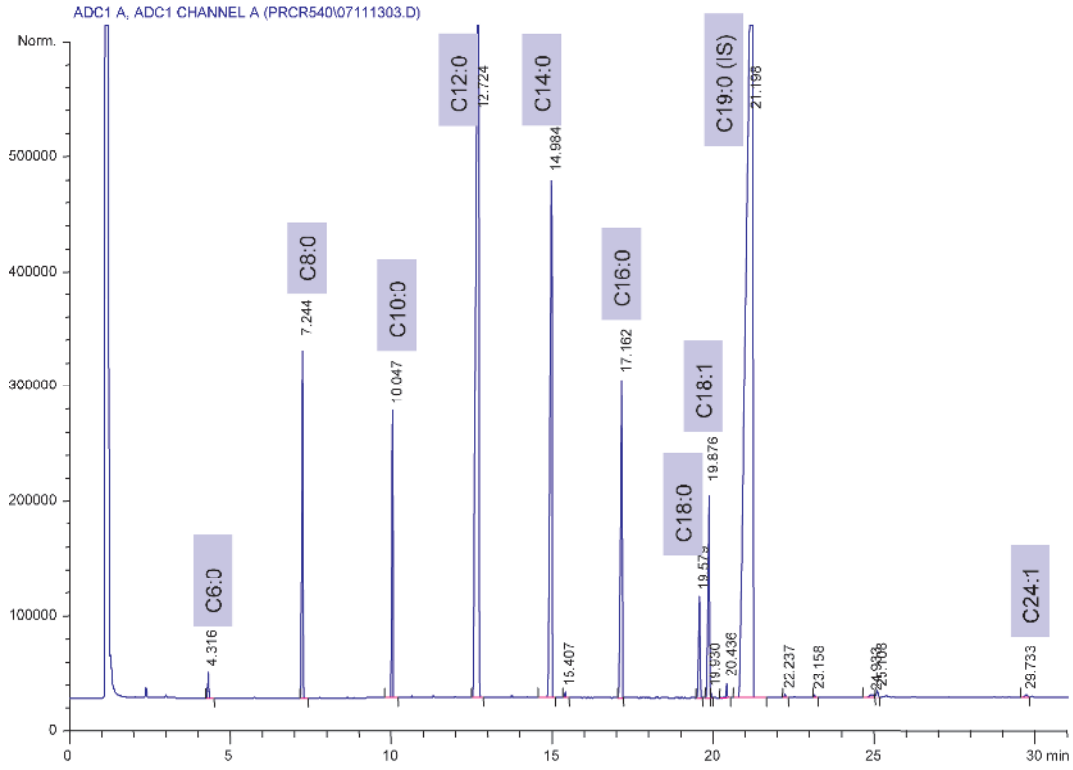

Figure A.1 — Coconut oil FAME

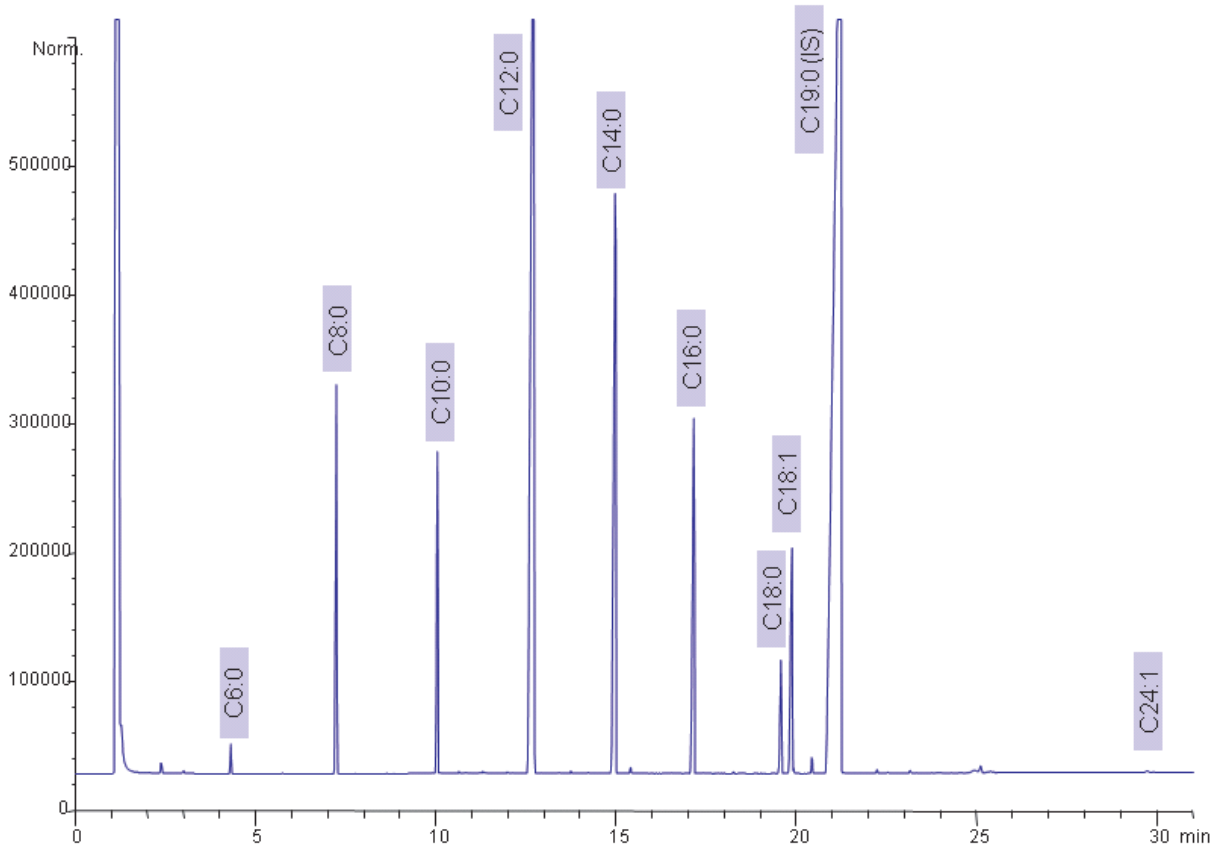

Figure A.2 — Blend of soya & coconut oil FAME (90/10)

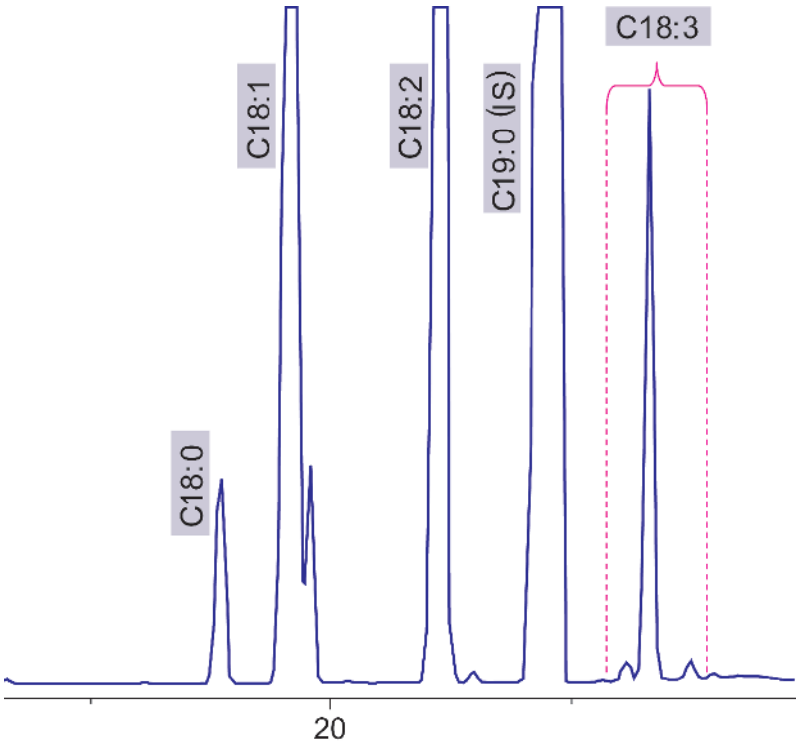

Figure A.3 — Detail of a chromatogram showing linolenic acids

Annex B
(informative)

Results of an interlaboratory trial

A European collaborative test involving 18 laboratories in 6 countries was carried out on 7 samples. Samples used for the collaborative test contain a full range of FAME mentioned in the scope of the standard.

The test was organized by CEN/TC19/TC 307/JWG in 2009 and the results obtained were subjected to statistical analysis in accordance with EN ISO 4259 [2] to give the precision data shown in Tables B.1 and B.2.

Table B.1 — Ester content

Sample	1	2	3	4	5	6	7
N° of participating laboratories	18	18	18	18	18	18	18
N° of participating laboratories after eliminating outliers	15	14	16	15	15	16	13
Mean value, % (*m/m*)	96,88	96,91	97,62	96,59	97,05	96,19	99,83
Repeatability limit (*r*)	0,81	0,65	1,21	0,82	1,17	1,39	1,17
Reproducibility limit (*R*)	3,21	2,61	4,23	3,80	5,24	5,87	2,16

Table B.2 — Linolenic acid methyl ester content

Sample	1	2	3	4	5	6	7
N° of participating laboratories	18	18	18	18	18	18	18
N° of participating laboratories after eliminating outliers	17	18	18	18	17	16	18
Mean value, % (*m/m*)	8,08	8,73	6,12	7,33	0,22	2,08	9,10
Repeatability limit (*r*)	0,13	0,18	0,14	0,11	0,05	0,06	0,26
Reproducibility limit (*R*)	0,56	0,58	0,59	0,75	0,30	0,52	0,58

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 4259:2006)*

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