SLOVENSKI STANDARD
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Derivati maščob in olj - Metil estri maščobnih kislin (FAME) - Določevanje estra in metil estra linolenske kisline

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

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

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

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67.200.10	Rastlinske in živalske maščobe in olja	Animal and vegetable fats and oils

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English Version

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This draft European Standard is submitted to CEN members for enquiry. It has been drawn up by the Technical Committee CEN/TC 307.

If this draft becomes a European Standard, CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration.

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Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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European foreword

This document (prEN 14103:2018) 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 14103:2011.

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

a) note on natural nonadecanoic acid methyl ester added in the scope;

b) check for nonadecanoic acid methyl ester purity added;

c) GC conditions for internal standard determination added;

d) calculation of results revised by incorporation of theoretical flame ionization detector correction factor (TCF);

e) new ILS conducted and precision adopted;

f) new sample chromatograms recorded and added;

g) calculation of the pattern of fatty acid methyl esters incorporated as informative Annex C;

h) document revised editorially.

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 C6 and C24.

NOTE 1 For the purposes of this document, the terms "% (*m*/*m*)" and "% (*v*/*v*)" are used to represent respectively the mass and volume fractions.

NOTE 2 This method was elaborated for FAME samples from usual raw material. For FAME sample from unidentified raw material, a solution of the test sample is prepared without any internal standard addition, in order to verify the absence of natural nonadecanoic acid methyl ester.

NOTE 3 The distribution off fatty acid methyl esters is given in Annex C.

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 Normative references

There are no normative references in this document.

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

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).

5 Reagents

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

5.1 Toluene, analytical grade.

5.2 Nonadecanoic acid methyl ester (FAME C19), GC purity, min. 99,0 % (*m*/*m*).

Purity of nonadecanoic acid methyl ester shall be checked for every new lot according to the procedure described in 6.2.

Standard shall be kept at room temperature in a dry storage (e. g. desiccator) in order to limit its water absorption. Its water content shall be verified by Karl-Fischer when a new lot of standard is open.

5.3 Carrier gas, hydrogen or helium.

5.4 Auxiliary gases:

- air;
- hydrogen.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

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

6.2 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.

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.

NOTE 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 (BPX70).

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

6.4 Pipette, 10 ml capacity.

6.5 Analytical balance, accuracy ±0,1 mg.

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].

8 Procedure

8.1 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 and 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}.

8.2 Internal standard purity determination

Prepare a solution of nonadecanoic acid methyl ester in toluene approx. at 1 mg/ml. Analyse 1 µl of this solution by gas chromatography on a short capillary column with thin film thickness, with an on-column injector or equivalent device and flame ionization detection, according to the conditions described below. Analyse using the same conditions 1 µl of toluene to verify that the impurities do not come from the solvent. Calculate the purity of the nonadecanoic acid methyl ester taking into account all the peaks eluted in the chromatogram, excluding the solvent peak.

The purity of nonadecanoic acid methyl ester should be taken into consideration for the calculation of the FAME content (see 9.1).

GC conditions for internal standard determination:

- 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 % diphenyl-polysiloxane stationary phase; length 15 m; internal diameter 0,32 mm; film thickness 0,1 µm;
- 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.

8.3 FAME sample preparation and analysis

Accurately weigh approximately 250 mg (accuracy ± 0,1 mg) of homogenized sample in a 10 ml tube (6.3), and approximately 100 mg (accuracy ± 0,1 mg) of nonadecanoic acid methyl ester (5.2), and dilute with 10 ml of toluene (5.1).

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.

For FAME sample from unidentified raw material, a solution of the test sample should be prepared according to 8.3, without any internal standard addition, in order to verify the absence of natural nonadecanoic acid methyl ester. If nonadecanoic acid methyl ester is naturally present, its area should be subtracted to the area of the added internal standard.

Analyse 1 µl of this solution by gas chromatography according to the conditions described in 8.1.

8.4 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 into consideration. In order to identify properly the fatty acid methyl esters, some commercial solutions may be used.

If FAMEs prepared from ruminant animal fats are present some minor additional peaks can be detected (odd chain FAMEs, branched iso and anteiso FAMEs). They represent the normal composition of these animal fats and shall be taken into account even if not identified.

Also the presence of biodiesel prepared from used cooking oils could lead to the presence of unknown peaks, because of the formation of positional and geometric FAME isomers during the thermal treatment of frying. In this case also the related peak areas must be taken into account.

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.

9 Expression of results

9.1 Determination of ester content

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

$$C = \frac{\sum\left(A_X \times R_X\right) - A_{\mathrm{EI}}}{A_{\mathrm{EI}}} \times \frac{W_{\mathrm{EI}}}{W} \times P \times 100 \qquad (1)$$

where

A_x is the peak area of individual methyl ester X in the test sample (from C6:0 to C24:1);

R_x is the theoretical flame ionization detector correction factor (TCF) for FAME X relative to the internal standard (C19:0);

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

P is the purity of the 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.

Theoretical flame ionization detector correction factor (TCF) shall be applied to the analytical data for optimum accuracy.

For unidentified peaks, the TCF of the immediately following identified FAME shall be used.

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

If the result is higher than 101,4 % (100 % + 0,59 R, with R = 2,45 then verify the experimental conditions and check 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 KarlFischer determination.

In the case of nonadecanoic acid methyl ester is naturally present in the FAME sample, the ester C content, expressed as a mass percentage, is calculated using the following formula:

$$C = \frac{\sum\left(A_X \times R_X\right) - \left(A_{EI} - \left(A_E \times \frac{W_2}{W_1}\right)\right)}{A_{\mathrm{EI}} - \left(A_E \times \frac{W_2}{W_1}\right)} \times \frac{W_{\mathrm{EI}}}{W_2} \times P \times 100 \qquad (2)$$

where

A_x is the peak area of individual methyl ester X in the test sample (from C6:0 to C24:1);

R_x is the theoretical flame ionization detector correction factor (TCF) for FAME X relative to the internal standard (C19:0);

A_{EI} is the peak area corresponding to nonadecanoic acid methyl ester when the internal standard is added to the test sample;

A_E is the peak area corresponding to nonadecanoic acid methyl ester without addition of the internal standard to the test sample;

P is the purity of the nonadecanoic acid methyl ester;

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

W_1 is the weight, in milligrams, of the sample in the trial without addition of the internal standard;

W_2 is the weight, in milligrams, of the sample in the trial with addition of the internal standard.

Table 1 — Theoretical flame ionization detector correction factor (TCF) for fatty acid methyl esters (FAME)[a]

FAME	TCF	FAME	TCF	FAME	TCF	FAME	TCF
C6:0	**1,3191**	**C15:0**	**1,0392**	**C18:2**	**0,9946**	**C22:0**	**0,9800**
C7:0	1,2524	**C15:1**	**1,0311**	**C18:3**	**0,9877**	**C22:1**	**0,9743**
C8:0	**1,2024**	**C16:0**	**1,0276**	C18:4	0,9810	C22:2	0,9687
C9:0	**1,1637**	**C16:1**	**1,0200**	C19:0	1,0000	C22:3	0,9632
C10:0	**1,1325**	C16:2	1,0123	**C20:0**	**0,9926**	C22:4	0,9577
C11:0	1,1071	C16:3	1,0046	**C20:1**	**0,9865**	C22:5	0,9520
C12:0	**1,0859**	C16:4	0,9969	C20:2	0,9804	**C22:6**	**0,9465**
C12:1	1,0757	**C17:0**	**1,0174**	C20:3	0,9742	C23:0	0,9744
C13:0	**1,0679**	**C17:1**	**1,0101**	C20:4	0,9682	**C24:0**	**0,9692**
C14:0	**1,0526**	**C18:0**	**1,0082**	**C20:5**	**0,9621**	**C24:1**	**0,9642**
C14:1	**1,0439**	**C18:1**	**1,0013**	C21:0	0,9860		

NOTE 1 The most common FAMEs are marked **bold**.

NOTE 2 The user of this standard does not have to identify all FAMEs indicated in this table.

[a] Atomic weights used: carbon 12,011; hydrogen 1,0079; oxygen 15,994. Factors are relative to 19:0, which has a factor of 1.0000 by definition. Only one factor is given for all positional and geometric isomers and for branched-chain FAME, as the factors are dependent only on the content of carbon to which hydrogen is bonded.

9.2 Determination of linolenic acid methyl ester

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

$$L = \frac{A_{\mathrm{L}} \times R_{\mathrm{L}}}{A_{\mathrm{EI}}} \times \frac{W_{\mathrm{EI}}}{W} \times P \times 100 \qquad (3)$$

where

A_{L} is the peak area corresponding to linolenic acid methyl ester (C18:3);

R_{L} is the theoretical flame ionization detector correction factor (TCF) for linolenic acid methyl ester (C18:3) relative to the internal standard (C19:0);

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

P is the purity of the 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.

9.3 Expression of results

Ester content and linolenic acid methyl ester content are expressed in percentage (w/w), to the nearest 0,1 %. Ester content between 99,0 % and 101,4 % is expressed as "> 99,0 %".

10 Precision

10.1 Interlaboratory test

An interlaboratory test organized in 2017 at European level with the participation of 9 laboratories, each having carried out two determinations on each sample, gave the statistical results indicated in Annex B.

The ILS conditions did not meet all requirements of EN ISO 4259 due to the fact that the samples did not cover the full range of the scope. However, field experience indicate that the precision statement is correct for the full range of the scope. The precision statement will be monitored and verified by field data.

10.2 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 2:

Table 2

For ester content	1,6500 [% m/m]
For linolenic acid methyl ester	0,0092 * (X + 3,9180) [% m/m]
X being the mean value of the two results in question r in % (m/m).	