SLOVENSKI STANDARD
SIST EN 14103:2020

01-marec-2020

Nadomešča:
SIST EN 14103:2011

Derivati maščob in olj - Metilni estri maščobnih kislin (FAME) - Določevanje estra in metilnega 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

Ta slovenski standard je istoveten z: **EN 14103:2020**

ICS:

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

SIST EN 14103:2020 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 14103

January 2020

ICS 67.200.10

Supersedes EN 14103:2011

English Version

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

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

Ref. No. EN 14103:2020 E

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European foreword

This document (EN 14103:2020) 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of an identical text or by endorsement, at the latest by July 2020, and conflicting national standards shall be withdrawn at the latest by July 2020.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document supersedes 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) new procedure to check nonadecanoic acid methyl ester purity, with new GC conditions, and reduction of the minimum GC purity (99,5 to 99,0 % (m/m));

c) calculation of results revised by incorporation of theoretical flame ionization detector correction factor (TCF), this gives a better accuracy of the calculated contents in case of presence of methyl esters with short chains;

d) new inter-laboratories study (ILS) conducted and precision adopted;

e) new sample chromatograms recorded and added;

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

g) modification of the way of integration in 8.4 by taking all the peaks into consideration whereas in the previous edition all the peaks identified as fatty acid methyl esters were taken into consideration;

h) increase of the FAME sample test portion in 8.3 to 250 mg whereas in the previous edition the sample test portion was 100 mg;

i) document revised editorially.

According to the CEN-CENELEC Internal Regulations, the national standards organisations 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, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

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 methyl ester content is between 1 % (*m*/*m*) and 15 % (*m*/*m*).

The precision was established using FAMEs with an ester content of 95 % (*m*/*m*) and 100 % (*m*/*m*) only, thus covering the range of the limit value. The method is also suitable outside of this range; however, precision for lower concentrations is subject to further work.

This method is suitable for FAME which contains methyl esters between C6 and C24.

NOTE 1 For the purposes of this document, the term "% (*m*/*m*)" is used to represent the mass fractions.

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 or other unknown substances co-eluting with the IS.

NOTE 2 The calculation method of the pattern of 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 mass percentage of total methyl esters of fatty acids and the mass 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, no impurities of toluene eluting after methyl ester C6:0.

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

GC Purity of nonadecanoic acid methyl ester shall be checked for every new lot according to the procedure described in 8.2.

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 below 0,2 % (*m*/*m*) and verified by Karl Fischer determination when a new lot of standard is opened.

If the purity is lower than 99,0 % (*m*/*m*) do not use it for this determination.

5.3 Carrier gas, hydrogen or helium, 99,999 5 % pure or better, gas chromatography quality, dried, oxygen removed by suitable filters (<0,1 mg/kg), free from organic impurities.

5.4 Auxiliary gases, hydrogen and air, gas chromatography quality, free from organic impurities.

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 C19:0) and other fatty acid methyl esters may exist.

NOTE Indeed, there is a co-elution between FAME C19:0 and linoleic acid methyl ester (FAME C18:2) when using a column with a stationary phase such as 70 % cyanopropyl-polysilphenylene-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 up to 200 °C, programmed at 5 °C/min 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, a minimum flow rate of 1 ml/min shall be warranted when operating at the maximum temperature;
- injected volume: 1 µl;
- carrier gas (hydrogen) pressure: 70 kPa;
- split flow: 100 ml/min.

8.2 Internal standard GC purity determination

In order to verify the absence of heavy impurities in the IS, the GC conditions were chosen according to EN 14105.

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 capillary column of 15 m length and capable of being programmed up to 400 °C ("high temperature" type) 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 *P* 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 ester content (see 9.1) and the linolenic acid methyl ester content (see 9.2)..

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

- 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

Standard and FAME 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 weighing.

Accurately weigh approximately 250 mg (accuracy ± 0,1 mg) of homogenized FAME 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).

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 or other substances co-eluting with the IS. If nonadecanoic acid methyl ester or these other substances are naturally present, their area should be subtracted to the area of the added internal standard (see calculation in 9.1).

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) acid methyl esters.

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 FAME 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 shall 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 Calculation

9.1 Determination of ester content

In the case of nonadecanoic acid methyl ester is not naturally present or other substances co-eluting with the IS are not present in the FAME sample, the ester content C, expressed as 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 test 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 or a certified reference material (e.g. ERM-EF001 BIODIESEL[1]), and the water content of the internal standard by Karl Fischer determination.

In the case of nonadecanoic acid methyl ester is naturally present or other substances co-eluting with the IS are present in the FAME sample, the ester content C, expressed as mass percentage, is calculated using the following formula:

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

[1] ERM-EF001 BIODIESEL is an example of a suitable product available commercially from European Commission, Joint Research Centre (JRC), Directorate F - Health, Consumers and Reference Materials. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.