SLOVENSKI STANDARD
SIST EN ISO 12966-4:2026

01-maj-2026

Nadomešča:
SIST EN ISO 12966-4:2015

Živalske in rastlinske maščobe ter olja - Plinska kromatografija metilnih estrov maščobnih kislin - 4. del: Določanje s kapilarno plinsko kromatografijo (ISO 12966-4:2026)

Animal and vegetable fats and oils - Gas chromatography of fatty acid methyl esters - Part 4: Determination by capillary gas chromatography (ISO 12966-4:2026)

Tierische und pflanzliche Fette und Öle - Gaschromatographie von Fettsäuremethylestern - Teil 4: Bestimmung mittels Kapillargaschromatographie (ISO 12966-4:2026)

Corps gras d'origines animale et végétale - Chromatographie en phase gazeuse des esters méthyliques d'acides gras - Partie 4: Détermination par chromatographie capillaire en phase gazeuse (ISO 12966-4:2026)

Ta slovenski standard je istoveten z: **EN ISO 12966-4:2026**

ICS:

67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products
67.200.10	Rastlinske in živalske maščobe in olja	Animal and vegetable fats and oils

SIST EN ISO 12966-4:2026 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN ISO 12966-4

March 2026

ICS 67.200.10

Supersedes EN ISO 12966-4:2015

English Version

Animal and vegetable fats and oils - Gas chromatography of fatty acid methyl esters - Part 4: Determination by capillary gas chromatography (ISO 12966-4:2026)

Corps gras d'origines animale et végétale - Chromatographie en phase gazeuse des esters méthyliques d'acides gras - Partie 4: Détermination par chromatographie capillaire en phase gazeuse (ISO 12966-4:2026)

Tierische und pflanzliche Fette und Öle - Gaschromatographie von Fettsäuremethylestern - Teil 4: Bestimmung mittels Kapillargaschromatographie (ISO 12966-4:2026)

This European Standard was approved by CEN on 1 September 2025.

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. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of 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, Türkiye and United Kingdom.

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 ISO 12966-4:2026 E

Contents

Page

European foreword

This document (EN ISO 12966-4:2026) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with 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 September 2026, and conflicting national standards shall be withdrawn at the latest by September 2026.

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 ISO 12966-4:2015.

Any feedback and questions on this document should be directed to the users’ national standards body/national committee. A complete listing of these bodies can be found on the CEN website.

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, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye and the United Kingdom.

Endorsement notice

The text of ISO 12966-4:2026 has been approved by CEN as EN ISO 12966-4:2026 without any modification.

International Standard

ISO 12966-4

Second edition
2026-03

Animal and vegetable fats and oils — Gas chromatography of fatty acid methyl esters —

Part 4: Determination by capillary gas chromatography

Corps gras d'origines animale et végétale — Chromatographie en phase gazeuse des esters méthyliques d'acides gras —

Partie 4: Détermination par chromatographie capillaire en phase gazeuse

Reference number
ISO 12966-4:2026(en)

COPYRIGHT PROTECTED DOCUMENT

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 307, *Oilseeds, vegetable and animal fats and oils and their by-products - Methods of sampling and analysis*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces the first edition (ISO 12966-4:2015), which has been technically revised.

The main changes are as follows:

— the Scope has been extended to the separation of fatty acid methyl esters from C4 to C24;

— ruminant fat has been added to the Scope,

— quantification by area (%) or by mass (g/100 g) using internal standards and corrections factors calculated with a quantitative fatty acid methyl esters standard mixture containing *cis* and *trans* fatty acid methyl esters from C4:0 to C22:6; has been added

— quantification of total *trans* fatty acid methyl esters by mass (g/100 g) has been added;

— the use of 100 m, 0,25 mm ID, 0,20 µm film thickness columns are now required to separate most C18:1 *trans*- and *cis*-isomers;

— a method has been added for determination of the composition of fatty acid methyl esters expressed by area % in liquid vegetable oils.

A list of all parts in the ISO 12966 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user’s national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This document is one part of a series of four International Standards for the preparation and determination of fatty acid methyl esters (FAMEs) by gas chromatography in animal and vegetable fats and oils. The ISO 12966 series is applicable to crude, refined, partially hydrogenated, or fully hydrogenated fats, oils, and fatty acids derived from animal and vegetable sources, and fats extracted from foodstuff.

The ISO 12966 series is not suitable for milk and milk products (or fat coming from milk and milk products), or products supplemented with conjugated linoleic acid (CLA). Furthermore, it is not intended to be applied to polymerized and oxidized fats and oils.

This document gives the conditions for the analysis of FAMEs by capillary gas chromatography, while ISO 12966-2 and ISO 12966-3 cover the preparation of FAMEs by different methods. ISO 12966-1 is a guideline to the modern gas chromatography of FAMEs.

Animal and vegetable fats and oils — Gas chromatography of fatty acid methyl esters —

Part 4: Determination by capillary gas chromatography

1 Scope

This document specifies a method for the determination of fatty acid methyl esters (FAMEs) derived by transesterification or esterification from fats, oils, and fatty acids by capillary gas chromatography (GLC). FAMEs from C4 to C24 can be separated using this document including saturated FAMEs, *cis*- and *trans*-monounsaturated FAMEs, and *cis*- and *trans*-polyunsaturated FAMEs.

This document is applicable to crude, refined, partially hydrogenated or fully hydrogenated fats, oils and fatty acids derived from animal and vegetable sources, and fats extracted from foodstuff.

This document does not apply to milk and milk products (or fat coming from milk and milk products) or products supplemented with conjugated linoleic acid (CLA).

This document does not apply to di-, tri-, polymerized, hydroxylated and oxidized fatty acids, and fats and oils.

A method for the determination of the composition of FAMEs expressed by area % in liquid vegetable oils is proposed in Annex E.

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.

ISO 661, *Animal and vegetable fats and oils — Preparation of test sample*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 6353-2, *Reagents for chemical analysis — Part 2: Specifications — First series*

ISO 6353-3, *Reagents for chemical analysis — Part 3: Specifications — Second series*

ISO 12966-2:2017, *Animal and vegetable fats and oils — Gas chromatography of fatty acid methyl esters — Part 2: Preparation of methyl esters of fatty acids*

ISO 12966-3, *Animal and vegetable fats and oils — Gas chromatography of fatty acid methyl esters — Part 3: Preparation of methyl esters using trimethylsulfonium hydroxide (TMSH)*

3 Terms and definitions

No terms and definitions are listed in this document.

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

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

4 Principle

Using capillary gas chromatography, FAMEs are separated on a highly polar stationary phase with respect to their chain length, degree of (un)saturation, and geometry and position of the double bonds. Peaks are identified by comparison with the retention time of pure standards and quantified as fatty acids methyl esters by reference to internal standards and instrument correction factors.

5 Reagents and materials

Unless otherwise stated, use only reagents as specified in ISO 6353-2 and ISO 6353-3 (if listed there). If not, then use reagents of recognized analytical grade and water of at least grade 3, as defined in ISO 3696.

WARNING — Attention is drawn to the regulations which specify the handling of dangerous matter. Technical, organizational and personal safety measures shall be followed.

5.1 Reference standards.

5.1.1 Reference FAMEs.

Methyl esters of pure fatty acids, in particular, *cis*- and *trans*-isomers of octadecenoic (oleic), *trans*-isomers of octadecadienoic (linoleic), and octadecatrienoic (α-linolenic) acids. Wide ranges of *cis*- and *trans* methyl ester isomers are available on the market. The following are examples of suitable products available commercially.

5.1.1.1 Octadecenoic acid methyl esters,*cis* and *trans* isomers mixture of C18:1 with *trans*-4 to *trans*-16 octadecenoic (all isomers) and principal *cis* isomers. Concentration 2,5 mg/ml in iso-octane (5.3) or MTBE (5.4) or *n*-heptane (5.6) or *n*-hexane (5.5) or dichloromethane (5.8).

NOTE This standard is commercially available from Supelco Inc, brand of Sigma-Aldrich (Cat. 40495-U)[1)].

5.1.1.2 Linoleic acid methyl esters,*cis* and *trans* isomers mixture of C18:2 with *trans*-9, *trans*-12-octadecadienoic acid (approximately 50 %), *cis*-9,*trans*-12-octadecadienoic acid (approximately 20 %), *trans*-9,*cis*-12-octadecadienoic acid (approximately 20 %) and *cis*-9,*cis*-12-octadecadienoic acid (approximately 10 %). Concentration 10 mg/ml in iso-octane (5.3) or MTBE (5.4) or *n*-heptane (5.6) or *n*-hexane (5.5) or dichloromethane (5.8).

NOTE This standard is commercially available from Supelco Inc, brand of Sigma-Aldrich (Cat. 47791)[1)].

5.1.1.3 Linolenic acid methyl esters, *cis* and *trans* isomers mixture of C18:3 with:

— *cis*-9,*cis*-12,*cis*-15-octadecatrienoic acid methyl ester (approximately a mass fraction of 3 %);

— *cis*-9,*cis*-12,*trans*-15-octadecatrienoic acid methyl ester (approximately a mass fraction of 7 %);

— *cis*-9,*trans*-12,*cis*-15-octadecatrienoic acid methyl ester (approximately a mass fraction of 7 %);

— *cis*-9,*trans*-12,*trans*-15-octadecatrienoic acid methyl ester (approximately a mass fraction of 15 %);

— *trans*-9,*cis*-12,*cis*-15-octadecatrienoic acid methyl ester (approximately a mass fraction of 7 %);

— *trans*-9,*cis*-12,*trans*-15-octadecatrienoic acid methyl ester (approximately a mass fraction of 15 %);

— *trans*-9,*trans*-12,*cis*-15-octadecatrienoic acid methyl ester (approximately a mass fraction of 15 %);

1) Supelco Inc., brand of Sigma Aldrich, is an example of suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by either ISO or IDF of the product named. Equivalent products may be used if they can be shown to lead to the same results.

— *trans*-9,*trans*-12,*trans*-15-octadecatrienoic acid methyl ester (approximately a mass fraction of 30 %).

Concentration 10 mg/ml in iso-octane (5.3) or MTBE (5.4) or *n*-heptane (5.6) or *n*-hexane (5.5) or dichloromethane (5.8).

NOTE This standard is commercially available from Supelco Inc, brand of Sigma Aldrich (Cat. 47792)[1)]. This standard contains all *trans* isomers of C18:3 (eight in total) but their abundance and ratio are different to those observed in refined/deodorized oils and fats.

5.1.1.4 Methyl octadecadienoate conjugated acids (CLA), mixture of C18:2 *cis*-9,*trans*-11 and *cis*-10,*trans*-12-octadecadienoate conjugated acids, of purity ≥ 99 % mass fraction.

NOTE This standard is commercially available from Supelco Inc, brand of Sigma Aldrich (Cat. O5507)[1)]. This standard contains the two principal CLA isomers, but isomer ratio can vary from lot to lot.

5.1.2 Fats and oils with certified fatty acid composition.

Fats and oils with certified fatty acid composition (e.g. external reference materials or certified reference material). This type of sample is accompanied with a certificate that provides mean values and its associated uncertainty for each fatty acid. These products are often, for example, commercially available from proficiency testing programmes (BIPEA, AOCS, FAPAS, JRC, etc).

5.1.3 Quantitative FAME standard mixture containing *cis* and *trans* FAMEs from C4:0 to C22:6.

This type of FAME mixture is commercially available[2)]. It is also possible to prepare the FAME standard mixture from individual and pure FAME standards, but the purchasing of individual FAME standards is more expensive and the preparation is time consuming and requires high precision.

The amount of each FAME standard present in the mixture is necessary for determining the correction factor (area/amount) for each FAME (see Figure B.1).

5.1.4 Calibration FAME standard solution at 2 mg/ml for the calculation of the correction factors.

Allow quantitative FAME standard mixture (5.1.3) to come to room temperature in the dark without heating. Using a Pasteur pipet, rapidly transfer the content of the vial into a 50 ml volumetric flask, and dilute to the mark with iso-octane (5.3), or MTBE (5.4), or *n*-heptane (5.6), or *n*-hexane (5.5). Dilute accordingly to the type of injector used.

5.2 Internal standards.

For the quantification of the fatty acids, in grams per 100 g, the use of a FAME as an internal standard (IS) is mandatory.

If it is necessary to check the recovery and the effectiveness of the derivatization method, then either or both a triacylglycerol (TAG) and a FAME internal standard should be used. While the TAG-IS is added to the sample prior to the FAME preparation, the FAME-IS is added before or after the FAME preparation. The FAME-IS is used to calculate the recovery of the FAME from the TAG-IS and therefore, the efficiency of the derivatisation procedure. In this case, a different chain length of the standards is required.

For the quantification of all fatty acids in vegetable oils, animal fats or extracted fats, C21:0 FAME or C19:0-FAME are the recommended internal standards, depending on the risk of coelutions such as C21:0/C18:2 conjugated.

For the quantification of all fatty acids in fish oil, C23:0 FAME is the recommended internal standard.

For the quantification of butyric acid (C4:0) and caproic acid (C6:0) only, in fat containing short chain fatty acids, C5:0 FAME is the recommended internal standard.

2) Examples of suitable products available commercially: Nu-Check-Prep, Cat. No. GLC 36 or GLC 37 (including C23:0 FAME). This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products. Equivalent products may be used if they can be shown to lead to the same results.