SLOVENSKI STANDARD
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Nadomešča:
SIST EN ISO 15304:2002
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SIST EN ISO 5508:1996

Živalske in rastlinske maščobe in olja - Plinska kromatografija metilnih estrov maščobnih kislin - 4. del: Določevanje s kapilarno plinsko kromatografsko metodo (ISO 12966-4:2015)

Animal and vegetable fats and oils - Gas chromatography of fatty acid methyl esters - Part 4: Determination by capillary gas chromatography (ISO 12966-4:2015)

Tierische und pflanzliche Fette und Öle - Gaschromatographie von Fettsäuremethylestern - Teil 4: Bestimmung von cis-, trans-, gesättigten, mono- und mehrfach ungesättigten Fettsäuren in pflanzlichen oder nicht-ruminanten Ölen und Fetten (ISO 12966-4:2015)

Corps gras d'origines animale et végétale - Chromatographie en phase gazeuse des esters méthyliques d'acides gras - Partie 4: Détermination des acides gras saturés, mono- et poly-insaturés, cis ou trans, dans les corps gras d'origines végétale ou animale (non ruminant)

Ta slovenski standard je istoveten z: **EN ISO 12966-4:2015**

ICS:

67.200.10	Rastlinske in živalske maščobe in olja	Animal and vegetable fats and oils
71.040.50	Fizikalnokemijske analitske metode	Physicochemical methods of analysis

SIST EN ISO 12966-4:2015 **en**

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EN ISO 12966-4

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English Version

Animal and vegetable fats and oils - Gas chromatography of fatty acid methyl esters - Part 4: Determination by capillary gas chromatography (ISO 12966-4:2015)

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 12966-4:2015)

Tierische und pflanzliche Fette und Öle - Gaschromatographie von Fettsäuremethylestern - Teil 4: Bestimmung mittels Kapillargaschromatographie (ISO 12966-4:2015)

This European Standard was approved by CEN on 16 April 2015.

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

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Foreword

This document (EN ISO 12966-4:2015) 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 December 2015, and conflicting national standards shall be withdrawn at the latest by December 2015.

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 ISO 5508:1995, EN ISO 15304:2002.

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, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Endorsement notice

The text of ISO 12966-4:2015 has been approved by CEN as EN ISO 12966-4:2015 without any modification.

INTERNATIONAL STANDARD

ISO 12966-4

First edition
2015-06-01

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

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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 documents 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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary Information

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

This first edition cancels and replaces ISO 5508:1990 and ISO 15304:2002, which have been technically revised.

ISO 12966 consists of the following parts, under the general title *Animal and vegetable fats and oils — Gas chromatography of fatty acid methyl esters*:

— *Part 1: Guidelines on modern gas chromatography of fatty acid methyl esters*

— *Part 2: Preparation of methyl esters of fatty acids*

— *Part 3: Preparation of methyl esters using trimethylsulfonium hydroxide (TMSH)*

— *Part 4: Determination by capillary gas chromatography*

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

Part 4: Determination by capillary gas chromatography

1 Scope

This part of ISO 12966 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). Fatty acid methyl esters from C8 to C24 can be separated using this part of ISO 12966 including saturated fatty acid methyl esters, *cis*- and *trans*-monounsaturated fatty acid methyl esters, and *cis*- and *trans*-polyunsaturated fatty acid methyl esters.

The method is applicable to crude, refined, partially hydrogenated, or fully hydrogenated fats, oils, and fatty acids derived from animal and vegetable sources.

This method is not suitable for the analysis of dairy, ruminant fats and oils, or products supplemented with conjugated linoleic acid (CLA). Milk and milk products (or fat coming from milk and milk products) are excluded from the scope of this part of ISO 12966.

This part of ISO 12966 is not applicable to di-, tri-, polymerized and oxidized fatty acids, and fats and oils.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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, *Reagents for chemical analysis*

ISO 12966-2, *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 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.

4 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.

4.1 Reference fatty acid methyl esters (FAMEs)

4.1.1 Reference mixtures of pure FAMEs and/or oils with known fatty acid composition should be used for the identification of fatty acids analysed under the test conditions of this method.

4.1.2 Fats and oils with certified fatty acid composition, e.g. certified reference material BCR 162.

4.1.3 Reference fatty acid methyl esters (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*-octadecenoic methyl ester isomers are available on the market. *Trans*-geometrical isomers of linoleic and α-linolenic acids can be prepared in the laboratory with the aid of p-toluenesulfonic acid. In addition to pure compounds, convenient mixtures of FAMEs are also commercially available.

4.2 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 necessary. An external calibration with mixtures of different fatty acids is also possible.

NOTE If it is necessary to check the recovery and the effectiveness of the derivatization method, then either or both a 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.

Depending on the type of fat, different internal standards can be used (C11:0 FAME, C17:0 FAME, C19:0 FAME, C21:0 FAME, C23:0 FAME, etc.). An external calibration with mixtures of different fatty acids is also possible. It is recommended to carry out further analysis of the sample without the addition of the internal standard to check the natural content of the fatty acid which is used as the internal standard. The content shall be considered in the calculation.

IMPORTANT — If the TAG-IS (4.2.2) is hard to dissolve in the cold, a hot methylation procedure, as specified in ISO 12966-2:2011, 4.3, 4.4, and 4.5, shall be used.

The internal standard solutions are stable if precautions are taken to eliminate the loss of solvent and therefore, a change in the concentration of the IS. For example, store the solution in a refrigerator in a well-sealed amber bottle when not in use. Pure standards are available on the market. Purity of the IS shall be confirmed by thin-layer chromatography, high-performance liquid chromatography, gas chromatography analysis, or by any other appropriate technique.

The following are examples of suitable standards (as FAME and TAG):

4.2.1 Fatty acid methyl ester (FAME) as internal standard (IS) solution:

C21:0 FAME – heneicosanoic acid methyl ester (purity >99 %), mass concentration 5,0 mg/ml in iso-octane or MTBE should be used as the internal standard.

4.2.2 Triacylglycerol (TAG) internal standard (IS) solution:

C21:0 TAG - triheneicosanoin (purity >99 %), mass concentration 5,0 mg/ml in chloroform. The TAG internal standard solution is stable if precautions are taken to eliminate the loss of solvent and therefore, a change in the concentration of the IS. For example, store the solution in a refrigerator in a well-sealed amber bottle when not in use. Pure triheneicosanoin is available on the market. Purity of the IS shall be confirmed by thin-layer chromatography, high-performance liquid chromatography, gas chromatography analysis, or by any other appropriate technique.

