INTERNATIONAL STANDARD

ISO 12966-4

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

Part 4: Determination by capillary gas chromatography

Sample Document

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).

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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

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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*

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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.

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2 Normative references

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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.

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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.

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Toluene can be used in place of chloroform with the following considerations. Triheneicosanoin is not as soluble in toluene as it is in chloroform. A solution with a mass concentration of 2 mg/ml can be prepared in toluene. It is necessary to warm the solution slightly to get it dissolved, but once in solution, it will stay dissolved if kept at room temperature. If the solution is stored in a refrigerator, it will crystallize out, but can be dissolved again by slight warming of the solution. Care has to be taken so none of the toluene is evaporated during this warming procedure. Also, care has to be taken to prevent the loss of toluene during storage. Solvents other than iso-octane (i.e. chloroform or toluene) have to be removed after the addition of the TAG-IS as these solvents are not used in the derivatization according to ISO 12966-2.

4.3 Iso-octane (2,2,4-trimethyl pentane).

4.4 Methyl *tert*-Butyl ether (MTBE) (2-Methoxy-2-methylpropane).

4.5 Chloroform.

SAFETY PRECAUTIONS — Chloroform is classed as a carcinogenic solvent (Category 3).

4.6 *n*-Hexane.

4.7 *n*-Heptane.

SAFETY PRECAUTIONS — Prolonged exposure through inhalation and swallowing could cause serious damage to health despite limited evidence on the carcinogenic effect (Category 3).

4.8 Toluene.

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5 Apparatus

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Usual laboratory equipment and, in particular, the following.

5.1 Gas chromatograph, equipped with flame ionization detector, split or splitless injector, and data acquisition system.

NOTE The use of on-column and programmable temperature vaporizer (PTV) injectors are also possible.

5.2 Capillary column, fused silica capillary 100 m and 0,25 mm i.d. coated with SP-2560 or CP-Sil 88[1)], 100 % cyanopropylsilicone stationary phase to a thickness of 0,20 µm. Commercially prepared columns are available from different suppliers.

NOTE The use of 100 m, 0,25 mm ID, 0,20 µm film thickness columns with SP-2560 or CP-Sil 88 as the stationary phase are recommended as the separation capacity of these columns is sufficient to separate most C18:1 trans- and cis-isomers. If this separation is not required, a 50 m or 60 m column can also be used. However, some 50 m or 60 m long columns might also achieve this separation mostly for vegetable oils. Other types of columns (BPX70, DB-23, HP-23, Rtx-2330, SP-2330, SP-2380, etc.) are also possible, but a shift in the elution order is possible. For fast GC analysis, short columns are also possible (10 m to 15 m), but with limited information which in certain cases, will not be a problem.

5.3 Micro syringe, for gas chromatography, 10 µl delivery with a hardened needle.

5.4 Carrier gas, hydrogen (recommended) 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.

NOTE Nitrogen gas is not acceptable as a carrier gas for this method.

1) Examples of suitable products available commercially. 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.

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