SRI LANKA STANDARD 313 Part 4/ Section 9: 2017

(ISO 12966-3: 2016)

UDC 613.268

METHODS FOR
ANALYSIS OF ANIMAL AND VEGETABLE FATS AND OILS
PART 4 – DETERMINATION OF PRINCIPLE CONSTITUENTS AND NATURAL CONSTITUENTS
Section 9: Gas chromatography of fatty acid methyl esters - Preparation of methyl esters using trimethylsulfonium hydroxide (TMSH)

SRI LANKA STANDARDS INSTITUTION

Sri Lanka Standard
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Sri Lanka

Sri Lanka Standards are subject to periodical revision in order to accommodate the progress made by industry. Suggestions for improvement will be recorded and brought to the notice of the Committees to which the revisions are entrusted.

This Standard does not purport to include all the necessary provisions of a contract.

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NATIONAL FOREWORD

This Sri Lanka Standard was approved by the Sectoral Committee on Food Products and was authorized for adoption and publication as a Sri Lanka Standard by the Council of the Sri Lanka Standards Institution on 2017-12-04.

Considering the experience gained during the years on the subject of testing animal and vegetable fats and oils, it was decided to adopt the Standard with a view of specifying a rapid base-catalysed transesterification method for fats and oils with trimethylsulfonium hydroxide (TMSH) to prepare fatty acid methyl esters. This Standard is applicable to all fats and oils, excluding those coming from milk and milk products.

In order to accommodate large number of test methods within the scope of one Standard, this Standard is published in four parts covering different characteristics as indicated below.

Part 1 Determination of physical characteristic
Part 2 Determination of chemical characteristics
Part 3 Determination of foreign substances and parameters affecting quality and stability
Part 4 Determination of principal constituents and natural constituents

Part 4 of the Standard consists of several sections.
Section 9 of this Standard is identical with **ISO 12966-3: 2016** Animal and vegetable fats and oils – Gas chromatography of fatty acid methyl esters - Preparation of methyl esters using trimethylsulfonium hydroxide (TMSH) published by the International Organization of Standardization (ISO).

Terminology and Conventions:

The text of the International Standard has been accepted as suitable for publication, without deviation, as a Sri Lanka Standard. However, certain terminology and conventions are not identical with those used in Sri Lanka Standards. Attention is therefore drawn to the following:

a) Wherever the words “International Standard” appear referring to this Standard should be interpreted as “Sri Lanka Standard”.

b) The comma has been used throughout as a decimal marker. In Sri Lanka Standards it is the current practice to use the full point on the base line as the decimal marker.

c) Wherever page numbers are quoted, they are ISO page numbers.

The test temperature adopted in Sri Lanka is $27 \pm 2\ ^{o}C$ and relative humidity 65 ± 5 per cent is recommended as per **ISO 554: 1976** Standard atmospheres for conditioning and/ or testing - Specifications.

Cross References

International Standard	Corresponding Sri Lanka Standard
ISO 661, Animal and vegetable fats and oils – Preparation of test sample.	SLS 313: Part 1/ Section 1, Methods for analysis of animal and vegetable fats and oils – Determination of physical characteristics – Preparation of test sample.

SLS 313-4-9: 2017

INTERNATIONAL STANDARD

ISO 12966-3

Second edition
2016-05-15

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

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

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

Partie 3: Préparation des esters méthyliques à l'aide d'hydroxyde de triméthylsulfonium (TMSH)

Reference number
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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 second edition cancels and replaces the first edition (ISO 12966-3:2009), of which it constitutes a minor revision. The scope has been revised to state that the document is not applicable to milk and milk fat products.

ISO 12966 consists of the following part, 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*

INTERNATIONAL STANDARD **ISO 12966-3:2016(E)**

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

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

1 Scope

This part of ISO 12966 specifies a rapid base-catalysed transesterification method for fats and oils with trimethylsulfonium hydroxide (TMSH) to prepare fatty acid methyl esters. The method is exclusively applicable to the preparation of methyl esters of fats and oils for gas liquid chromatographic (GLC) analysis. It is applicable to all fats and oils, but excluding those coming from milk and milk products. Isomerization of unsaturated fatty acids only occurs to a minor extent and isomerized fatty acids are only present at the determination limit. As isomerization takes place, the procedure is not recommended for conjugated linoleic acid (CLA).

Only about 70 % to 80 % of the free fatty acids are esterified. In the case of conjugated cyclopropyl and cyclopropenyl fatty acids, side reactions may occur, but these do not interfere with the determination of the fatty acids.

NOTE This part of ISO 12966 is based upon German Standard Method C-VI 11e (98) (see Reference [8]).

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and 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*

3 Principle

The sample is dissolved in *tert*-butyl methyl ether (TBME) and mixed with a methanolic solution of trimethylsulfonium hydroxide. Glycerides are base-catalysed transesterified and fatty acid methyl esters are formed (see References [4] to [8]). Free fatty acids are converted to salts which are pyrolysed to methyl esters and dimethylsulfide in the injector. Excess reagent is also pyrolysed into methanol and dimethylsulfide. To obtain a complete pyrolytic reaction, a hot injector (split injection) of at least 250 °C is necessary.

For the determination of short-chain fatty acids (C_4 to C_8), valeric acid methyl ester is used as an internal standard. Lipids containing hydroxy groups are partially converted to the corresponding *O*-methyl ether derivatives which may interfere with fatty acid methyl esters in the GLC separation (Reference [9]). In the early part of the chromatogram (region of C_4), peaks may occur, which are from the reagent. These peaks are not taken into account.

4 Reagents

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

During the analysis, unless otherwise stated, use only reagents and solvents of recognized analytical grade.

4.1 ***tert*-Butyl methyl ether (TBME)**.

4.2 **Trimethylsulfonium hydroxide (TMSH)**, methanolic solution, amount of substance concentration $c(Me_3SOH)$ = 0,2 mol/l.

The content of the solution can be determined by acidimetry: dilute 5,0 ml of the solution with 10 ml of methanol, add two drops of phenolphthalein and titrate against 0,1 mol/l HCl. The concentration should be at least 0,15 mol/l.

NOTE The solution remains stable for at least two months when stored at 4 °C in small quantities in closed tubes.

5 Apparatus

Usual laboratory equipment, and in particular:

5.1 **Test tubes**, of capacity 2 ml (autosampler vials).

5.2 **Graduated pipettes**, of capacities 250 µl, 500 µl, and 1 000 µl, ISO 835[1] class A.

6 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

Sampling is not part of the method specified in this part of ISO 12966. A recommended sampling method is given in ISO 5555[3].

7 Preparation of the test sample

The test sample shall be liquid, dry and clear. Proceed in accordance with ISO 661, but heat the sample to just above the melting point.

Solid samples are carefully melted at a temperature of maximum 10 °C above their melting point and mixed. Avoid overheating.

Samples containing water are dissolved in petroleum ether and dried for 30 min by addition of anhydrous sodium sulfate. The drying agent is removed by filtration through a fluted filter paper and the residue is carefully washed with petroleum ether. The solvent is subsequently removed with the aid of a rotary evaporator.

8 Procedure

8.1 Weigh, into a test tube (5.1), (10 ± 2) mg of the test sample.

8.2 Pipette (5.2) 500 µl of TBME (4.1) into the test tube and dissolve the sample, warming gently if necessary.

NOTE For the determination of short-chain fatty acids (C_4 to C_8), valeric acid methyl ester is used as an internal standard. The internal standard solution is used to dissolve the sample.

8.3 Pipette (5.2) 250 µl of TMSH solution (4.2) into the test tube and shake vigorously for about 30 s. After this, the solution is ready for injection into the gas chromatograph. As the methyl esters are formed during injection, an injector temperature of at least 250 °C is required.

If necessary, use a mixture of TBME and methanol (9 volumes + 1 volume) to dilute the solution.

IMPORTANT — Free fatty acids react with TMSH to form the corresponding salts, which are pyrolysed to methyl esters and dimethylsulfide in the injector. Therefore, an injector temperature of 250 °C is necessary. To prevent blocking, the capillary of the split vent shall have an appropriate internal diameter (>1 mm). It should be cleaned by regular heating or flushing with solvent. Moreover, the split vent valve shall be protected with an activated charcoal trap.

9 Test report

The test report shall include the following information:

a) result of the determination;

b) the method used, together with a reference to this part of ISO 12966, i.e. ISO 12966-3;

c) all information necessary for the complete identification of the sample;

d) all operating details not specified in this part of ISO 12966, or regarded as optional, together with details of any incident that may have influenced the result(s).

Bibliography

[1] ISO 835, *Laboratory glassware — Graduated pipettes*

[2] ISO 1042, *Laboratory glassware — One-mark volumetric flasks*

[3] ISO 5555, *Animal and vegetable fats and oils — Sampling*

[4] Butte W.J. Rapid method for the determination of fatty acid profiles from fats and oils using trimethylsulfonium hydroxide for transesterification. *J. Chromatogr. A*. 1983, **261** pp. 142–145

[5] Schulte E., & Weber K. Rapid preparation of fatty acid methyl esters from fats with trimethylsulfonium hydroxide or sodium methylate. *Fat Sci. Technol*. 1989, **91** pp. 181–183

[6] Arens M., Schulte E., Weber K. Fettsäuremethylester, Umesterung mit trimethylsulfoniumhydroxid [Fatty acid methyl esters, transesterification with trimethylsulfonium hydroxide]. *Fat Sci. Technol*. 1994, **96** pp. 67–68

[7] El-Hamdy A.H., & Christie W.W. Preparation of methyl esters of fatty acids with trimethylsulphonium hydroxide — An appraisal. *J. Chromatogr. A*. 1993, **630** pp. 438–441

[8] Deutsche Gesellschaft für Fettwissenschaft. *Deutsche Einheitsmethoden zur Untersuchung von Fetten, Fettprodukten, Tensiden und verwandten Stoffen/German standard methods for the analysis of fats and other lipids*. Wissenschaftliche Verlagsgesellschaft, Stuttgart, 2008

[9] Vosmann K., Schulte E., Klein E, Weber N. Reactions of lipids containing hydroxy groups with trimethylsulfonium hydroxide: Formation of *O*-methyl derivatives. *Lipids*. 1996, **31** pp. 349–352

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SRI LANKA STANDARDS INSTITUTION

The Sri Lanka Standards Institution (SLSI) is the National Standards Organization of Sri Lanka established under the Sri Lanka Standards Institution Act No. 6 of 1984 which repealed and replaced the Bureau of Ceylon Standards Act No. 38 of 1964. The Institution functions under the Ministry of Science & Technology.

The principal objects of the Institution as set out in the Act are to prepare standards and promote their adoption, to provide facilities for examination and testing of products, to operate a Certification Marks Scheme, to certify the quality of products meant for local consumption or exports and to promote standardization and quality control by educational, consultancy and research activity.

The Institution is financed by Government grants, and by the income from the sale of its publications and other services offered for Industry and Business Sector. Financial and administrative control is vested in a Council appointed in accordance with the provisions of the Act.

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All members of the Technical and Sectoral Committees render their services in an honorary capacity. In this process the Institution endeavours to ensure adequate representation of all view points.

In the International field the Institution represents Sri Lanka in the International Organization for Standardization (ISO), and participates in such fields of standardization as are of special interest to Sri Lanka.

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