INTERNATIONAL STANDARD

ISO 12966-3

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 12966-3 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegatable fats and oils*.

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 2: Preparation of methyl esters of fatty acids*

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

The following part is in preparation:

— *Part 4: Determination of* cis-, trans-*, saturated, mono- and polyunsaturated fatty acids in vegetable or non-ruminant oils and fats*

The following part is planned:

— Part 1: Guidelines on gas chromatography

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 including milk fat and blends containing milk fat. 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). As CLA is not correctly analysed, this method is not applicable to the determination of the complete fatty acid composition of milk fat samples.

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 referenced documents are indispensable for the application 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*

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 with ground glass stopper (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 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 contain at least the following information:

a) result of the determination;

b) the method used, together with reference to this part of ISO 12966;

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*

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[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