
**Animal and vegetable fats and oils —
Preparation of methyl esters of fatty acids**

*Corps gras d'origines animale et végétale — Préparation des esters
méthyliques d'acides gras*



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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 5509 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

This second edition cancels and replaces the first edition (ISO 5509:1978), which has been technically revised.

Annex A forms a normative part of this International Standard. Annex B is for information only.

Introduction

ISO 5509 contains three different procedures to prepare methyl esters.

The general method is the BF_3 method which is applicable to oils and fats and free fatty acids but which is less applicable to caproic acid (C6) and not applicable to butyric acid (C4). The application field is GLC, TLC and IR.

Two alternative methods not involving BF_3 are given using trimethylsulfonium hydroxide and potassium hydroxide in methanol. Both methods are rapid methods for GLC analysis only.

The second method (trimethylsulfonium hydroxide method), which is for GLC analyses only, can be used for all fats and oils including milk fat and milk fat containing blends. In the case of short fatty acids (C4 to C8) the use of an internal standard is recommended.

The third method (trans-esterification method) can be used for neutral oils and fats, and can also be used for the quantitative analysis of oils and fats with short-chain fatty acids down to butyric acid (C4). For the determination of C4 and/or C6, only the internal standard method is maintained.

The principal new approach in this revision is the use of isooctane as solvent instead of hexane or pentane. This is based on references [1] and [2], which showed better results especially for the BF_3 method when using isooctane.

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Animal and vegetable fats and oils — Preparation of methyl esters of fatty acids

1 Scope

This International Standard specifies methods of preparing the methyl esters of fatty acids.

It includes methods for preparing fatty acid methyl esters from animal and vegetable fats and oils, fatty acids and soaps. To cover different requirements, three methylation methods are specified, as follows:

- a) boron trifluoride (BF_3) method (see clause 3);
- b) trimethylsulfonium hydroxide (TMSH) method (see clause 4);
- c) trans-esterification method (see clause 5).

Methyl esters so produced are used in various analytical procedures requiring such derivatives, for example gas-liquid chromatography (GLC), thin-layer chromatography (TLC) and infrared spectrometry (IR).

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

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

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

3 General method using boron trifluoride

WARNING — The method described involves the use of potentially hazardous reagents. Normal precautions shall be taken for eye protection and for protection from the dangers of corrosive chemical burns.

Boron trifluoride is poisonous. For this reason, it is not recommended that the analyst prepare the methanolic solution of boron trifluoride from methanol and boron trifluoride. (See A.1 in annex A.)

3.1 Principle

The glycerides are saponified with methanolic sodium hydroxide. The soaps are converted into methyl esters by reaction with a boron trifluoride/methanol complex.

For analysis of pure fatty acids and soaps, saponification with sodium hydroxide is not necessary and esters can be prepared directly by reaction with boron trifluoride.

3.2 Applicability

This method is to be preferred for most oils, fats and derivatives (fatty acids, soaps) with the exception of milk fats and of fats containing fatty acids with specific groups.

During esterification, compounds containing the following configurations may be totally or partially decomposed:

- keto, epoxy, hydroxy, hydroperoxy groupings;
- cyclopropyl and cyclopropenyl groups;
- acetylenic fatty acids.

If the fatty matter contains such compounds in only very small amounts (e.g. cottonseed oil), the method can be applied; otherwise the method described in clause 4 or 5 should be followed.

For gas chromatography, the optimum recovery of the methyl esters from the reaction mixture is obtained by using isooctane. However, only about 75 % of the methyl caproate (C6) present will be recovered.

3.3 Reagents

Use only reagents of recognized analytical grade.

3.3.1 Water, complying with grade 3 of ISO 3696.

3.3.2 Sodium hydroxide, methanolic solution, approximately 0,5 mol/l.

Dissolve 2 g of sodium hydroxide in 100 ml of methanol containing not more than 0,5 % (mass fraction) of water. If the solution has to be stored for a considerable time, a small amount of white precipitate of sodium carbonate may be formed; this has no effect on the preparation of the methyl esters.

3.3.3 Boron trifluoride (BF₃), methanolic solution, 12 % to 15 % (mass fraction)¹. See A.1.

3.3.4 Isooctane (2,2,4-trimethylpentane), of chromatographic quality. See A.2.

WARNING — Isooctane is flammable and a fire risk. Explosive limits in air are 1,1 % to 6,0 % (volume fraction). It is toxic by ingestion and inhalation. Use a properly operating fume hood when working with this solvent.

3.3.5 Sodium chloride, saturated aqueous solution.

3.3.6 Sodium sulfate, anhydrous.

3.3.7 Nitrogen, having an oxygen content less than 5 mg/kg.

3.3.8 Hexane, of chromatographic quality, for dry methyl esters only. See A.2. Light petroleum, boiling range 40 °C to 60 °C, redistilled and residue-free, with a bromine value less than 1, may be used.

3.3.9 Methyl red, 1 g/l solution in 60 % (volume fraction) ethanol.

¹) 14 %, 20 % (Merck No. 8.01663) and 50 % solutions are available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.

3.4 Apparatus

Usual laboratory equipment and, in particular, the following.

- 3.4.1 Flask**, of capacity 50 ml or 100 ml, with ground neck and fitted with a ground glass stopper.
- 3.4.2 Reflux condenser**, of 20 cm to 30 cm effective length, with a ground joint to fit the flask (3.4.1).
- 3.4.3 Boiling aid**, fat-free.
- 3.4.4 Graduated or automatic pipette**, of capacity at least 10 ml, and fitted with a rubber bulb.
- 3.4.5 Vial**, of capacity 4 ml, with screw cap.
- 3.4.6 Separating funnels**, of capacity 250 ml, for dry methyl esters only.
- 3.4.7 Rotary evaporator**.
- 3.4.8 Analytical balance**, capable of weighing to the nearest 0,001 g.

3.5 Preparation of 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.

3.6 Procedure

WARNING — Because of the toxic character of boron trifluoride, perform the methylation under a ventilated hood. It is essential to wash all glassware with water immediately after use.

3.6.1 Test portion

Use Table 1 to select the appropriate size of flask and quantities of reagents and solvents required to methylate the amount of test portion chosen.

Table 1

Purpose	Test portion mg	Flask (3.4.1) ml	NaOH solution (3.3.2) ml	BF ₃ solution (3.3.3) ml	Solvent (3.3.4) or (3.3.8) ml
GLC	100 to 250	50	4	5	1 to 3
	250 to 500	50	6	7	2 to 5
IR/TLC	500 to 750	100	8	9	4 to 8
	750 to 1 000	100	10	12	7 to 10

3.6.2 Saponification

3.6.2.1 For fats and oils, start the method at 3.6.2.2.

For fatty acids and soaps, start the method at 3.6.2.3.

3.6.2.2 Introduce the test portion into the appropriate flask. See Table 1 and annex A. Add the appropriate amount (see Table 1) of the methanolic sodium hydroxide solution (3.3.2) and a boiling aid (3.4.3). Fit the condenser (3.4.2) to the flask.

If the fatty acids contain more than two double bonds, remove the air from the flask by flushing the flask with dry nitrogen (3.3.7) immediately prior to the reflux for a few minutes.

Boil under reflux until the droplets of fat disappear, swirling the flask gently every 30 s to 1 min to prevent a solid ring of sodium hydroxide forming around the walls of the flask. This usually takes 5 min to 10 min, but in certain exceptional cases it may take longer. See A.3 and A.4. Add the appropriate amount (see Table 1) of the methanolic boron trifluoride solution (3.3.3) through the top of the condenser.

Proceed in accordance with either 3.6.3 or 3.6.4.

3.6.2.3 Introduce the test portion into the appropriate flask (see Table 1). Add the appropriate amount (see Table 1) of the methanolic boron trifluoride solution (3.3.3) into the flask. Fit the condenser (3.4.2) to the flask.

Proceed in accordance with either 3.6.3 or 3.6.4.

3.6.3 Preparation of the methyl esters in isooctane solution (mainly for gas liquid chromatography purposes)

3.6.3.1 Continue boiling for 3 min. In the case of oils with long-chain fatty acids, such as fish oils, continue boiling for 30 min.

3.6.3.2 Add the appropriate amount (see Table 1) of isooctane (3.3.4) to the boiling mixture through the top of the condenser.

3.6.3.3 Remove the flask from the heat source and remove the reflux condenser. IMMEDIATELY, without allowing the flask to cool, add 20 ml of sodium chloride solution (3.3.5). Stopper the flask and shake it vigorously for at least 15 s.

3.6.3.4 Add more of the saturated sodium chloride solution (3.3.5) to bring the liquid level of the mixture into the neck of the flask. Allow the two phases to separate.

3.6.3.5 Transfer 1 ml to 2 ml of the upper isooctane layer into a 4 ml vial (3.4.5) and add a small amount of anhydrous sodium sulfate (3.3.6) to remove any traces of water.

The isooctane solution thus obtained may be injected as follows:

- a) directly onto a packed column for gas-liquid chromatography (see A.5);
- b) after appropriate dilution with isooctane for capillary column systems prior to the injection (see A.6);
- c) after dilution with a lower boiling solvent such as heptane for the special case of capillary on-column injection.

3.6.4 Preparation of dry methyl esters (for TLC or IR spectroscopy)

3.6.4.1 Continue boiling for 3 min.

3.6.4.2 Add the appropriate amount (see Table 1) of hexane (3.3.8) to the boiling mixture through the top of the condenser.

3.6.4.3 Remove the flask from the heat source and remove the reflux condenser. IMMEDIATELY, without allowing the flask to cool, add 20 ml sodium chloride solution (3.3.5). Stopper the flask and shake it vigorously for at least 15 s.

3.6.4.4 Transfer the saline solution and hexane layer to a 250 ml separation funnel (3.4.6). Add about 30 ml of the saturated sodium chloride solution. Allow the two phases to separate. Retain the hexane solution. Extract the saline solution twice with 50 ml portions of hexane (3.3.8).

3.6.4.5 Combine the hexane solution and the two extracts and wash them with 20 ml portions of water (3.3.1) until no free acid is obtained, using the methyl red solution (3.3.9) as indicator.

Dry over anhydrous sodium sulfate (3.3.6). Filter the solution and evaporate the solvent cautiously on a water bath under a stream of nitrogen (3.3.7) or use a rotary evaporator (3.4.7).

If the remaining portion contains a considerable amount of short-chain methyl esters (C₆ to C₁₀), a substantial loss of these can hardly be avoided. For test portions less than 500 mg, it is preferable to reduce proportionally the volumes of sodium chloride solution, solvent and water. See A.6.

4 Trimethylsulfonium hydroxide (TMSH) method

WARNING — The method described involves the use of potentially hazardous reagents. Normal precautions shall be taken for eye protection and for protection from the dangers of corrosive chemical burns. Trimethylsulfonium hydroxide may be poisonous.

4.1 Principle

The test sample is dissolved in *t*-butyl methyl ether and the methyl esters are prepared by trans-esterification with trimethylsulfonium hydroxide (TMSH). Immediate injection in the gas chromatograph at an injector temperature of above 250 °C. In the presence of short-chain fatty acids (4 to 8 carbon atoms), the use of valeric acid methyl ester is recommended as an internal standard (see 4.2).

4.2 Applicability

This rapid method is only for the preparation of methyl esters for GLC. It is applicable to all fats and oils including milk fat and blends containing milk fat. Isomerization of unsaturated fatty acids has not been observed.

The method can be applied to compounds containing the chemical configurations listed in 3.2, but it is not known whether an entire conversion into methyl esters will take place. Also free fatty acids are only esterified by about 70 % to 80 %.

Lipids containing hydroxy groups are partially converted to the corresponding O-methyl ether derivatives which may interfere with fatty acid methyl esters (FAME) in GLC separation. Therefore the TMSH derivatization method is not recommended without limitation for lipids containing hydroxy groups. On the other hand it may be of some diagnostic value for the analysis of such lipids by GLC/mass spectrometry.

The TMSH procedure cannot be applied when cold-on-column injection is used in GLC analysis. Moreover the use of polar stationary phases (cyanopropyl siloxanes) is not recommended.

For the determination of short-chain fatty acids (C₄ to C₈), the use of valeric acid methyl ester (methyl pentanoate) as an internal standard is recommended, provided there is no valeric acid in the sample.

4.3 Reagents

Use only reagents of recognized analytical grade.

4.3.1 *t*-Butyl methyl ether.

4.3.2 Trimethylsulfonium hydroxide (TMSH)²⁾, methanolic solution, 0,2 mol/l.

The solution remains stable for at least 2 months if stored at 4 °C in small quantities in a closed tube.

NOTE Reference [3] gives a method of preparation.

4.3.3 Internal standard stock solution, for butyric and/or caproic acid determination only.

Weigh, to the nearest 0,1 mg, about 250 mg of valeric acid methyl ester into a 50 ml volumetric flask. Use isooctane to dissolve the sample and to dilute to the mark.

4.3.4 Internal standard reference solution, for butyric and/or caproic acid determination only.

Add 10 ml of stock solution (4.3.3) to a 100 ml volumetric flask and dilute to the mark with isooctane. Calculate the concentration of this reference solution. See A.8.

2) Trimethylsulfonium hydroxide (Article 70152) is available from Macherey-Nagel GmbH Co., D-52313 Düren. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

4.3.5 Petroleum ether.

4.3.6 Sodium sulfate, anhydrous.

4.4 Apparatus

Usual laboratory equipment and, in particular, the following.

4.4.1 Test tubes, of capacity 2 ml, with ground glass stoppers (autosampler vials).

4.4.2 Graduated pipettes, of capacity 1 000 μl .

4.4.3 Volumetric flasks, of capacities 50 ml and 100 ml.

4.4.4 Fluted filter paper.

4.4.5 Rotary evaporator.

4.5 Preparation of 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.

4.6 Procedure

4.6.1 Test portion

Weigh into the test tube (4.4.1) $10 \text{ mg} \pm 2 \text{ mg}$ of the test portion.

In the case of samples with higher water content, use a larger test portion.

Melt solid samples carefully at a temperature of approx. 10°C above their melting point and mix. Avoid overheating.

Dissolve samples containing water in petroleum ether (4.3.5) and dry them for 30 min by addition of anhydrous sodium sulfate (4.3.6). Remove the drying agent by filtration through a fluted filter paper and wash the residue carefully with petroleum ether. Remove the solvent with the aid of a rotary evaporator (4.4.5).

4.6.2 Preparation of methyl esters

4.6.2.1 Using a pipette (4.4.2), add 500 μl *t*-butyl methyl ether and dissolve the sample, gently warming if necessary.

For butyric and/or caproic acid determination, add 500 μl of the internal standard reference solution (4.3.4) instead of 500 μl of the *t*-butyl methyl ether.

4.6.2.2 To this solution add, using a pipette (4.4.2), 250 μl of TMSH solution (4.3.2) and shake vigorously for about 30 s. See A.7.

4.6.2.3 The obtained solution (4.6.2.2) is ready for injection in the gas chromatograph. As the methyl esters of free fatty acids are only formed during injection, an injector temperature of at least 250°C is required. See A.2 and A.7.

If it is necessary to dilute the solution prior to injection, use a mixture of *t*-butyl methyl ether (4.3.1) and methanol (9+1) to avoid precipitation of TMSH.

5 Trans-esterification method

WARNING — The method described involves the use of potentially hazardous reagents. Normal precautions shall be taken for eye protection and for protection from the dangers of corrosive chemical burns. Methanolic potassium hydroxide solution is poisonous.

5.1 Principle

The glycerides are dissolved in isooctane and converted to methyl esters by trans-esterification with potassium hydroxide. After the reaction has finished, the potassium hydroxide is neutralized with sodium hydrogen sulfate to prevent saponification of the methyl esters.

5.2 Applicability

This rapid method is applicable to edible fats and oils containing fatty acids down to C₄, having a free fatty acids content (FFA) of not greater than 2 %, and for the determination of butyric acid (C₄) or caproic acid (C₆) by GLC by using an internal standard.

For samples with a higher FFA, an excess of potassium hydroxide should be used. As free fatty acids and soaps are not esterified by potassium hydroxide, the method can be used to obtain methyl esters of the glyceride part of the sample only.

The method can be applied to compounds containing the chemical configurations listed in 3.2 but it is not known whether an entire conversion into methyl esters will take place.

5.3 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

5.3.1 Potassium hydroxide, methanolic solution, approximately 2 mol/l.

Since potassium hydroxide in practice contains about 15 % water, proceed as follows.

Dissolve with gentle heating, 13,1 g of potassium hydroxide in 100 ml of absolute methanol.

Add a quantity of anhydrous sodium sulfate to the solution to dry it. Filter to obtain a clear solution. If the solution has to be stored for a considerable time, a small amount of white precipitate of sodium carbonate may be formed; this has no effect on the preparation of the methyl esters when using the clear supernatant.

5.3.2 Isooctane (2,2,4-trimethylpentane), of chromatographic quality (see A.2).

5.3.3 Sodium hydrogen sulfate monohydrate.

5.3.4 Internal standard stock solution, for butyric and/or caproic acid determination only.

Weigh, to the nearest 0,1 mg, about 250 mg of valeric acid methyl ester (methyl pentanoate) into a 50 ml volumetric flask. Use isooctane (5.3.2) to dissolve the sample and to dilute to the mark.

5.3.5 Internal standard reference solution, for butyric and/or caproic acid determination only.

Add 10 ml of stock solution (5.3.4) to a 100 ml volumetric flask and dilute to the mark with isooctane (5.3.2). Calculate the concentration of this reference solution. See A.8.

5.4 Apparatus

Usual laboratory equipment, and in particular, the following.

5.4.1 Test tube, of capacity 5 ml, with ground glass stopper.

5.4.2 Graduated pipette or dispenser, of capacity 4 ml, and/or a **volumetric pipette** of capacity 4 ml.

5.4.3 Pipette or automatic pipette, of capacity 200 μ l.

5.4.4 Vial, of capacity 4 ml, with screw cap.

5.4.5 Volumetric flasks, of capacities 50 ml and 100 ml.

5.5 Preparation of 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.

5.6 Procedure

5.6.1 Test portion

Weigh into the test tube (5.4.1) about 60 mg of the test portion. For butyric and/or caproic determination, weigh the test portion to the nearest 0,1 mg.

5.6.2 Preparation of methyl esters

5.6.2.1 Add with a pipette or dispenser (5.4.2), 4 ml of isooctane (5.3.2) and dissolve the sample, using gentle warming if necessary.

For butyric and/or caproic acid determination, use a volumetric pipette (5.4.2) to add 4,00 ml of reference solution (5.3.5) instead of isooctane.

5.6.2.2 Add, with a pipette (5.4.3), 200 μ l methanolic potassium hydroxide solution (5.3.1) and stopper the test tube. Shake the mixture vigorously for about 30 s. After an initial cloudiness due to the separation of glycerol, the reaction mixture will become clear.

5.6.2.3 Add about 1 g of sodium hydrogen sulfate monohydrate (5.3.3) to the solution and shake it vigorously to neutralize the potassium hydroxide.

5.6.2.4 After the salt has settled, decant the upper layer containing the methyl esters into the 4 ml vial (5.4.4).

The isooctane solution obtained will contain about 15 mg/ml of methyl esters and may be injected as follows:

- directly onto a packed column for gas-liquid chromatography (see A.5);
- after appropriate dilution with isooctane for capillary column systems prior to the injection (see A.6);
- after dilution with a lower boiling solvent such as heptane for the special case of capillary on-column injection.

6 Precision

Details of an interlaboratory test for comparison of the three different procedures to prepare methyl esters are summarized in annex B. Values for the repeatability and reproducibility were not calculated because these values depend not only on the preparation of the methyl esters but also on the columns used, the GLC conditions and GLC apparatus used.

Annex A (normative)

General analytical procedures

A.1 Preparation of BF_3

If it is absolutely essential to prepare the boron trifluoride methanolic solution, proceed as follows.

WARNING — Operate under a ventilated hood.

Weigh a 2 l flask containing 1 l of methanol. Cool it in a bath of ice-water. Keeping the flask in the bath, bubble BF_3 from a gas cylinder through the glass tube before immersing the latter in the methanol and until it is removed, in order to prevent any liquid from returning to the gas expansion system. The gas should not give off white fumes by escaping too quickly from the flask.

The reagent remains stable for 2 years if stored in a refrigerator.

A.2 Reagents

The reagents shall not produce peaks which interfere with those of the methyl esters of fatty acids during gas-liquid chromatography.

During gas-liquid chromatography of the methyl esters, certain reagents may produce unexpected peaks on the graph. Particularly during long storage, methanolic boron trifluoride generates components which interfere in the C20 to C22 acids region.

Consequently, any new batch of reagent or solvent should be checked by using it to prepare the methyl esters of pure oleic acid and chromatographing them. If any extra peaks appear, the reagent should be rejected.

A.3 Saponification

In the class of oils such as castor oils, which are soluble in methanol, no droplets of oil will be observed.

Therefore clarity of the solution is not proof of completion of the reaction.

A.4 Unsaponifiable matter

Unsaponifiable matter is not removed and, if it is present in substantial amounts, it may interfere with subsequent analysis. If this is the case, it is essential to supplement the method described with the following operations.

Dilute with distilled water the solution obtained after saponification and extract the unsaponifiable matter with diethyl ether, hexane or light petroleum. Acidify the aqueous solution and extract the fatty acids with isooctane or hexane. Prepare the methyl esters from these as described in 3.6.3 or 4.6.2.

A.5 Storage of methyl ester solution

The esters should preferably be analysed as soon as possible. If necessary, the isooctane solution containing the methyl esters may be stored under inert gas in a refrigerator.

For a longer period of storage, it is advisable to protect the methyl esters against autoxidation by adding to the solution an antioxidant in such a concentration as will not interfere with the subsequent analysis, for example a 0,05 g/l solution of BHT (2,6-di-*t*-butyl-4-methylphenol).

Methyl esters containing methyl butyrate shall only be stored in sealed ampoules, and it is essential to take special precautions to prevent any loss by evaporation during filling and sealing of the ampoules.

A.6 Storage of dry methyl esters

The dry methyl esters without solvent should be analysed without delay. If required, they may be kept for 24 h under an inert gas in a refrigerator, or for longer periods under vacuum in a sealed tube in a freezer.

A.7 TMSH method

Free fatty acids react with TMSH to form the corresponding salts, which are transformed to methyl esters and dimethylsulfide in the injector.

To prevent blocking, the capillary of the split vent should have a wide internal diameter. Otherwise it shall be cleaned by heating or flushing with solvent.

If valeric acid methyl ester is used as internal standard, it shall be added directly to the *t*-butyl methyl ether which is used to dissolve the sample (0,5 to 1,0 mg/ml).

A.8 Amount of methyl esters

If fatty acids are to be determined quantitatively by gas-liquid chromatography using internal standard(s), it is essential to weigh the test portion accurately; i.e. to the nearest 0,1 mg. The results will then be expressed as percentages by mass of the fatty acid content in the fat or oil. This does not agree with the results obtained by internal normalization.

Annex B

(informative)

Results of interlaboratory test

An international collaborative study was carried out in 1995. In this test eight laboratories participated and eight samples with different content of free fatty acids were investigated. The aim of this test was to determine the application of the three different methods on the preparation of methyl esters from different kinds of fats and oils and on the content of free fatty acids. The results are listed in Tables B.1 to B.8. The minimum and maximum values are specified as well as the mean values and the standard deviations for the main fatty acids.

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Table B.1 — Refined coconut oil (FFA = 0,03 %)

Boron trifluoride method (7 laboratories)				
FAME	min.	max.	Mean	SD
C 6:0	0,3	0,6	0,5	0,1
C 8:0	5,6	7,8	6,7	0,7
C 10:0	5,3	6,0	5,6	0,3
C 12:0	46,0	47,6	46,8	0,6
C 14:0	17,7	19,1	18,3	0,4
C 16:0	9,0	9,7	9,4	0,3
C 18:0	2,7	3,0	2,9	0,1
C 18:1	7,4	7,9	7,7	0,2
C 18:2	1,9	2,2	2,0	0,1
C 20:0	0,1	0,1	0,1	0,0
C 20:1	0,0	0,1	0,0	0,0
Sum	96,1	104,0	100,0	2,8
Trimethylsulfonium hydroxide method (6 laboratories)				
FAME	min.	max.	Mean	SD
C 6:0	0,5	0,7	0,6	0,1
C 8:0	7,1	8,5	7,8	0,5
C 10:0	5,6	6,3	6,0	0,2
C 12:0	45,8	47,6	46,7	0,7
C 14:0	16,4	17,9	17,4	0,5
C 16:0	8,5	9,4	8,9	0,3
C 18:0	2,5	3,2	2,8	0,2
C 18:1	6,8	7,9	7,4	0,4
C 18:2	1,6	2,6	2,1	0,3
C 20:0	0,1	0,3	0,2	0,1
C 20:1	0,0	0,5	0,1	0,2
Sum	95,0	104,9	100,0	3,7
Trans-esterification method (7 laboratories)				
FAME	min.	max.	Mean	SD
C 6:0	0,5	0,7	0,6	0,1
C 8:0	6,5	9,3	7,4	0,6
C 10:0	5,4	7,1	5,9	0,3
C 12:0	45,8	52,0	46,8	0,7
C 14:0	16,7	18,6	18,0	0,4
C 16:0	6,6	9,5	9,1	0,3
C 18:0	1,7	3,0	2,8	0,2
C 18:1	4,7	8,0	7,5	0,3
C 18:2	1,1	2,2	1,9	0,1
C 20:0	0,0	0,1	0,1	0,0
C 20:1	0,0	0,1	0,0	0,0
Sum	89,1	110,7	100,0	3,1

Table B.2 — Refined soya bean oil (FFA = 0,06 %)

Boron trifluoride method (7 laboratories)				
FAME	min.	max.	Mean	SD
C 14:0	0,0	0,1	0,0	0,0
C 16:0	10,4	11,0	10,6	0,2
C 16:1	0,0	0,1	0,1	0,0
C 17:0	0,1	0,2	0,1	0,0
C 18:0	3,6	3,9	3,7	0,1
C 18:1	20,7	21,0	20,9	0,1
C 18:2	54,5	55,7	55,2	0,4
C 18:3	7,9	9,0	8,4	0,4
C 20:0	0,3	0,5	0,4	0,1
C 20:1	0,2	0,3	0,2	0,1
C 22:0	0,3	0,4	0,4	0,0
C 24:0	0,0	0,2	0,1	0,1
Sum	98,0	102,4	100,0	1,6
Trimethylsulfonium hydroxide method (6 laboratories)				
FAME	min.	max.	Mean	SD
C 14:0	0,0	0,2	0,1	0,1
C 16:0	10,5	11,3	10,7	0,3
C 16:1	0,0	0,1	0,1	0,1
C 17:0	0,0	0,2	0,1	0,1
C 18:0	3,6	3,8	3,7	0,1
C 18:1	20,6	22,5	21,2	0,7
C 18:2	53,4	55,7	54,8	0,9
C 18:3	7,7	9,0	8,3	0,4
C 20:0	0,3	0,6	0,4	0,1
C 20:1	0,1	0,7	0,3	0,2
C 22:0	0,3	0,5	0,4	0,1
C 24:0	0,0	0,2	0,1	0,1
Sum	96,5	104,8	100,0	3,1
Trans-esterification method (8 laboratories)				
FAME	min.	max.	Mean	SD
C 14:0	0,0	0,1	0,1	0,0
C 16:0	10,1	12,1	10,7	0,6
C 16:1	0,0	0,1	0,1	0,0
C 17:0	0,0	0,2	0,1	0,1
C 18:0	3,4	4,0	3,7	0,2
C 18:1	20,4	21,4	20,8	0,3
C 18:2	54,7	56,2	55,2	0,5
C 18:3	8,1	9,0	8,5	0,3
C 20:0	0,3	0,5	0,4	0,1
C 20:1	0,1	0,3	0,2	0,1
C 22:0	0,1	0,4	0,3	0,1
C 24:0	0,0	0,1	0,0	0,0
Sum	97,2	104,3	100,0	2,4

Table B.3 — Refined vegetable oil (FFA = 0,3 %)

Boron trifluoride method (7 laboratories)				
FAME	min.	max.	Mean	SD
C 8:0	0,4	0,6	0,5	0,1
C 10:0	0,4	0,5	0,5	0,0
C 12:0	5,0	5,5	5,3	0,1
C 14:0	3,1	3,2	3,2	0,0
C 16:0	49,2	50,6	50,0	0,5
C 17:0	0,0	0,2	0,1	0,1
C 18:0	37,8	39,6	38,9	0,6
C 18:1	0,1	0,8	0,5	0,3
C 18:2	0,0	0,2	0,1	0,1
C 20:0	0,6	0,7	0,6	0,0
C 22:0	0,3	0,4	0,3	0,1
C 24:0	0,0	0,1	0,1	0,1
Sum	96,9	102,4	100,0	1,9
Trimethylsulfonium hydroxide method (6 laboratories)				
FAME	min.	max.	Mean	SD
C 8:0	0,3	0,9	0,6	0,2
C 10:0	0,4	0,6	0,5	0,1
C 12:0	5,2	7,0	5,7	0,7
C 14:0	3,1	3,3	3,2	0,1
C 16:0	47,8	50,1	49,2	0,8
C 17:0	0,0	1,0	0,3	0,4
C 18:0	37,5	39,2	38,3	0,7
C 18:1	0,2	1,0	0,7	0,3
C 18:2	0,0	0,9	0,3	0,4
C 20:0	0,5	0,7	0,6	0,1
C 22:0	0,3	0,7	0,4	0,2
C 24:0	0,0	0,1	0,0	0,0
Sum	95,2	105,5	100,0	3,8
Trans-esterification method (7 laboratories)				
FAME	min.	max.	Mean	SD
C 8:0	0,5	0,8	0,5	0,1
C 10:0	0,4	0,6	0,5	0,0
C 12:0	5,0	6,6	5,4	0,2
C 14:0	3,1	3,6	3,2	0,1
C 16:0	49,5	51,0	50,2	0,5
C 17:0	0,0	0,2	0,1	0,1
C 18:0	36,7	40,0	38,4	0,6
C 18:1	0,1	0,9	0,6	0,3
C 18:2	0,0	0,2	0,0	0,1
C 20:0	0,5	0,7	0,6	0,0
C 22:0	0,0	0,4	0,4	0,1
C 24:0	0,0	0,1	0,0	0,1
Sum	95,7	105,1	100,0	2,0

Table B.4 — Crude vegetable oil (FFA = 6,4 %)

Boron trifluoride method (7 laboratories)				
FAME	min.	max.	Mean	SD
C 12:0	0,1	0,2	0,1	0,0
C 14:0	1,0	1,1	1,0	0,0
C 16:0	43,3	45,1	43,8	0,6
C 16:1	0,0	0,2	0,1	0,1
C 17:0	0,1	0,1	0,1	0,0
C 18:0	4,5	4,8	4,7	0,1
C 18:1	38,4	39,3	38,9	0,4
C 18:2	9,9	10,8	10,3	0,3
C 18:3	0,2	0,5	0,3	0,1
C 20:0	0,4	0,5	0,4	0,0
C 20:1	0,1	0,2	0,1	0,1
C 22:0	0,0	0,1	0,1	0,0
Sum	97,9	102,9	100,0	1,7
Trimethylsulfonium hydroxide method (6 laboratories)				
FAME	min.	max.	Mean	SD
C 12:0	0,1	1,2	0,5	0,4
C 14:0	1,0	1,3	1,1	0,1
C 16:0	42,6	45,4	43,7	1,0
C 16:1	0,0	0,2	0,1	0,1
C 17:0	0,0	1,5	0,3	0,6
C 18:0	4,4	6,1	4,8	0,6
C 18:1	37,5	38,9	38,4	0,6
C 18:2	9,1	10,9	10,0	0,6
C 18:3	0,2	0,8	0,4	0,2
C 20:0	0,3	0,7	0,5	0,1
C 20:1	0,0	0,3	0,1	0,1
C 22:0	0,0	0,6	0,1	0,2
Sum	95,3	108,0	100,0	4,7
Trans-esterification method (7 laboratories)				
FAME	min.	max.	Mean	SD
C 12:0	0,0	0,2	0,1	0,0
C 14:0	1,0	1,1	1,0	0,0
C 16:0	43,2	45,9	43,7	0,6
C 16:1	0,0	0,2	0,1	0,1
C 17:0	0,0	0,2	0,1	0,1
C 18:0	4,4	4,9	4,7	0,2
C 18:1	38,3	39,6	39,1	0,5
C 18:2	9,9	10,8	10,3	0,3
C 18:3	0,0	0,5	0,3	0,1
C 20:0	0,3	0,5	0,4	0,0
C 20:1	0,0	0,2	0,1	0,0
C 22:0	0,0	0,1	0,0	0,0
Sum	97,0	104,2	100,0	1,9

Table B.5 — Crude fish oil (FFA = 3,8 %)

Boron trifluoride method (7 laboratories)				
FAME	min.	max.	Mean	SD
C 12:0	0,0	1,0	0,2	0,4
C 14:0	7,0	8,7	7,9	0,6
C 14:1	0,0	1,0	0,2	0,3
C 15:0	0,0	0,5	0,4	0,2
C 16:0	15,9	20,4	18,2	1,6
C 16:1	8,2	9,7	8,9	0,5
C 16:2	0,0	2,1	0,8	1,1
C 16:3	0,0	1,7	0,7	0,9
C 16:4	0,0	2,6	1,0	1,3
C 17:0	0,0	1,6	0,8	0,6
C 17:1	0,0	1,6	0,3	0,6
C 18:0	3,3	6,0	4,1	0,9
C 18:1	12,1	13,6	13,0	0,5
C 18:2	1,3	2,4	1,7	0,4
C 18:3	0,6	2,0	1,1	0,4
C 18:4	0,0	3,2	2,2	1,0
C 20:0	0,3	0,9	0,4	0,2
C 20:1	0,0	2,3	1,8	0,8
C 20:2	0,0	0,4	0,2	0,2
C 20:3	0,0	1,3	0,4	0,5
C 20:4	0,8	1,6	1,2	0,4
C 20:5	17,0	18,9	18,2	0,7
C 22:0	0,0	0,9	0,2	0,3
C 22:1	2,1	2,6	2,4	0,2
C 22:2	0,0	0,8	0,2	0,4
C 22:4	0,0	0,8	0,3	0,3
C 22:5	2,2	2,5	2,3	0,1
C 22:6	9,8	10,9	10,4	0,3
C 24:0	0,0	0,3	0,1	0,1
C 24:1	0,0	0,5	0,3	0,3
Sum	80,8	123,0	100,0	16,1

Table B.5 — Crude fish oil (FFA = 3,8 %) (continued)

Trimethylsulfonium hydroxide method (6 laboratories)				
FAME	min.	max.	Mean	SD
C 12:0	0,0	0,2	0,1	0,1
C 14:0	7,4	9,6	8,6	0,9
C 14:1	0,0	0,6	0,2	0,2
C 15:0	0,0	0,6	0,4	0,2
C 16:0	16,2	21,5	18,8	1,9
C 16:1	8,5	10,2	9,2	0,6
C 16:2	0,0	1,8	0,8	0,9
C 16:3	0,0	1,8	0,6	0,9
C 16:4	0,0	2,1	0,4	0,9
C 17:0	0,4	1,6	0,9	0,5
C 17:1	0,0	1,2	0,2	0,5
C 18:0	3,6	6,5	4,3	1,1
C 18:1	12,9	14,3	13,6	0,5
C 18:2	1,3	1,7	1,5	0,2
C 18:3	0,6	2,4	1,3	0,8
C 18:4	0,0	2,6	2,0	1,0
C 20:0	0,2	1,9	0,6	0,6
C 20:1	0,0	2,6	1,8	0,9
C 20:2	0,0	0,7	0,2	0,3
C 20:3	0,0	0,8	0,2	0,3
C 20:4	0,8	1,4	0,9	0,2
C 20:5	16,7	19,4	17,8	1,3
C 22:0	0,0	0,8	0,2	0,3
C 22:1	0,0	3,0	1,9	1,1
C 22:2	0,0	2,3	0,7	0,9
C 22:4	0,0	0,7	0,3	0,3
C 22:5	1,9	2,6	2,3	0,3
C 22:6	8,9	11,1	9,9	0,9
C 24:0	0,0	0,3	0,1	0,1
C 24:1	0,0	0,9	0,2	0,4
Sum	79,4	127,4	100,0	19,1

Table B.5 — Crude fish oil (FFA = 3,8 %) (continued)

Trans-esterification method (8 laboratories)				
FAME	min.	max.	Mean	SD
C 12:0	0,0	0,1	0,1	0,1
C 14:0	6,5	10,7	8,5	1,4
C 14:1	0,0	0,3	0,2	0,1
C 15:0	0,0	0,6	0,4	0,2
C 16:0	15,9	20,8	18,5	1,8
C 16:1	7,6	10,3	9,0	0,9
C 16:2	0,0	2,2	0,6	0,9
C 16:3	0,0	1,8	0,7	0,8
C 16:4	0,0	2,6	0,6	1,2
C 17:0	0,4	1,8	1,1	0,6
C 17:1	0,0	2,7	0,7	1,0
C 18:0	0,0	4,3	3,3	1,4
C 18:1	12,6	13,7	13,2	0,4
C 18:2	1,3	2,7	1,7	0,5
C 18:3	0,6	1,7	0,9	0,4
C 18:4	0,0	3,4	2,3	1,0
C 20:0	0,2	0,8	0,4	0,2
C 20:1	0,0	2,4	1,7	0,8
C 20:2	0,0	0,6	0,2	0,2
C 20:3	0,0	0,9	0,2	0,4
C 20:4	0,8	1,6	1,1	0,3
C 20:5	16,6	20,2	18,8	1,1
C 22:0	0,0	0,7	0,1	0,2
C 22:1	0,0	2,8	1,8	1,0
C 22:2	0,0	2,3	0,5	0,8
C 22:4	0,0	0,8	0,3	0,4
C 22:5	1,9	2,6	2,3	0,2
C 22:6	8,9	15,3	10,7	1,9
C 24:0	0,0	0,3	0,0	0,1
C 24:1	0,0	0,8	0,2	0,3
Sum	73,3	132,1	100,0	20,6

Table B.6 — Oil/fatty acid blend (FFA = 70 %)

Boron trifluoride method (7 laboratories)				
FAME	min.	max.	Mean	SD
C 6:0	0,2	0,4	0,3	0,1
C 8:0	3,0	3,8	3,5	0,3
C 10:0	2,9	3,3	3,1	0,1
C 12:0	27,3	30,7	28,3	1,2
C 14:0	10,1	11,1	10,5	0,3
C 16:0	20,2	21,3	20,8	0,4
C 16:1	0,0	0,4	0,3	0,1
C 18:0	4,9	5,6	5,3	0,2
C 18:1	18,6	21,1	20,2	0,9
C 18:2	5,9	7,0	6,4	0,4
C 18:3	0,2	0,3	0,2	0,0
C 20:0	0,3	0,4	0,3	0,1
C 20:1	0,2	0,5	0,3	0,1
C 22:0	0,0	0,4	0,2	0,1
C 22:1	0,0	0,4	0,2	0,1
Sum	93,9	106,7	100,0	4,6
Trimethylsulfonium hydroxide method (5 laboratories)				
FAME	min.	max.	Mean	SD
C 6:0	0,0	0,6	0,5	0,1
C 8:0	0,3	4,8	4,4	0,4
C 10:0	2,1	3,7	3,5	0,2
C 12:0	10,0	30,2	29,0	0,9
C 14:0	7,7	10,6	10,1	0,4
C 16:0	19,8	26,4	20,5	0,6
C 16:1	0,0	0,3	0,1	0,2
C 18:0	5,0	9,1	5,4	0,5
C 18:1	15,3	32,7	18,5	2,0
C 18:2	5,5	10,2	6,4	0,6
C 18:3	0,2	0,6	0,4	0,2
C 20:0	0,3	0,8	0,4	0,1
C 20:1	0,2	0,5	0,3	0,1
C 22:0	0,0	0,5	0,3	0,1
C 22:1	0,0	0,8	0,3	0,3
Sum	66,4	131,8	100,0	6,7

Table B.7 — Lard

Boron trifluoride method (7 laboratories)				
FAME	min.	max.	Mean	SD
C 10:0	0,0	0,1	0,1	0,0
C 12:0	0,0	0,1	0,1	0,0
C 14:0	1,5	1,7	1,6	0,1
C 16:0	24,2	25,6	24,8	0,5
C 16:1	2,6	3,6	3,0	0,3
C 17:0	0,3	0,4	0,3	0,0
C 17:1	0,3	0,4	0,3	0,0
C 18:0	13,0	13,9	13,4	0,4
C 18:1	41,9	44,3	43,2	0,7
C 18:2	10,3	11,3	10,6	0,3
C 18:3	0,9	1,4	1,0	0,2
C 20:0	0,2	0,8	0,3	0,2
C 20:1	0,5	1,1	0,9	0,2
C 20:2	0,0	0,5	0,4	0,2
C 22:0	0,0	0,1	0,0	0,0
Sum	95,7	105,3	100,0	3,3
Trimethylsulfonium hydroxide method (6 laboratories)				
FAME	min.	max.	Mean	SD
C 10:0	0,0	0,2	0,1	0,1
C 12:0	0,0	1,5	0,3	0,6
C 14:0	1,5	1,8	1,6	0,1
C 16:0	22,1	25,4	24,2	1,2
C 16:1	2,6	3,3	3,0	0,2
C 17:0	0,3	0,5	0,4	0,1
C 17:1	0,2	0,5	0,3	0,1
C 18:0	12,6	13,7	13,3	0,4
C 18:1	41,7	44,9	43,2	1,1
C 18:2	9,8	11,5	10,5	0,6
C 18:3	0,8	2,0	1,2	0,5
C 20:0	0,2	0,8	0,4	0,2
C 20:1	0,2	1,6	1,0	0,5
C 20:2	0,2	0,6	0,5	0,2
C 22:0	0,0	0,1	0,0	0,0
Sum	92,3	108,4	100,0	6,0