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**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](http://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](http://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 octadecatrenoic ( $\alpha$ -linolenic) acids. Wide ranges of *cis*- and *trans*-octadecenoic methyl ester isomers are available on the market. *Trans*-geometrical isomers of linoleic and  $\alpha$ -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.

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.**

## 5 Apparatus

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<sup>1)</sup>, 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.

**WARNING — Hydrogen, which is used with capillary columns, can double the speed of the analysis (in comparison with helium), but is hazardous. Hydrogen generators and safety devices are available and it is essential that a suitable device be incorporated into the apparatus.**

5.5 **Flame gases**, hydrogen and air, gas chromatography quality, free from organic impurities.

5.6 **Make-up gas**, nitrogen or helium, gas chromatography quality, free from organic impurities.

## 6 Sampling

A representative sample should be sent to the laboratory. It should not be 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.

## 7 Preparation of test sample

Prepare the test sample in accordance with ISO 661.

## 8 Preparation of methyl esters from fats, oils, and fatty acids

The fatty acid methyl esters shall be prepared in accordance with ISO 12966-2 or ISO 12966-3.

**NOTE** Prior to methylation, the internal standard solution, if required, is added to the reaction flask so that after the oil or fat is added, the mass fraction is between 0,05 and 0,10 mg IS/mg oil or fat. Since a solvent is used in the IS, it shall be evaporated from the flask prior to the methylation procedure.

Dissolve the prepared FAMES in *n*-heptane, *n*-hexane, or iso-octane. The mass concentration should be approximately 15 mg/ml to 20 mg/ml for split injection. For on-column injection, the mass concentration should be adapted.

## 9 Procedure

**WARNING — Due to the toxic character of some solvents, a ventilated hood shall be used.**

### 9.1 General

The first sample in an analysis batch shall always be a blank FAME dissolution solvent. No peaks shall be detected in this blank run.

### 9.2 GC conditions

Adapt the temperatures and GC conditions considering the type of fat, oil, or fatty acid analysed and the apparatus used. The following conditions have been proven to be suitable for the separation of FAMES (C4 to C24) on 100 m columns. However, other conditions are also possible and can be used.

Injector temperature	250 °C
Detector temperature:	250 °C
Oven temperature:	120 °C to 240 °C with 4 °C/min, hold for further 7 min at 240 °C
Carrier gas hydrogen:	column head pressure, 220 kPa linear velocity; (30 to 40) cm/s, flow rate approx. 1,0 ml/min



split ratio, 1:100

Injection volume: 1 µl (equivalent to 15 µg to 20 µg FAME)

Examples of chromatograms and alternative conditions are shown in [Annex B](#) and [Annex C](#):

NOTE For the analysis of animal fats, the complete elution of all FAMES can be checked with certified reference standards.

### 9.3 Performance check

Column performance is checked using a suitable mixture of fatty acid methyl esters covering the range of fatty acids under investigation. Since commercial GC designs are different and the separation obtained is not identical to the example chromatograms, small changes in the sample size, sample concentration, or oven temperature may be required. If so, adjust the sample size, sample concentration, or oven temperature until the best separation results are obtained. If the column oven temperature needs to be adjusted, it should be adjusted by small increments, preferably in steps of 1 °C.

NOTE On all cyanopropylsilicone capillary columns, the column temperature has a major effect on the elution pattern of 13t- and 14t-C18:1, 16t-C18:1, 14c-C18:1, 9c,12c,15t-C18:3, 11c-C20:1 and 9c,12c,15c-C18:3.

## 10 Calculations

### 10.1 Qualitative analysis and peak identification

The individual FAMES are identified by their retention times and in comparison with FAME reference standards and reference hydrogenated oil samples.

When unknown peaks are observed, they should be identified using appropriate procedures such as GC-MS, FTIR, silver-ion chromatography, and classical chemical methods. Peaks of unknown identity should not be included in the summation of peak areas when calculating the fatty acid composition, unless they have been confirmed to be fatty acids. It is also possible to summarize unknown peaks as such.

NOTE There is minor co-elution of cis- and trans-fatty acid isomers, particularly in the C18:1 (cis-9-oleic acid) region using this technique. During (high temperature) refining (deacidification and deodorization), only geometrical isomers are formed of the mono- and poly-unsaturated fatty acids, i.e. the double bond(s) remain(s) at the same natural position. During hydrogenation, both positional and geometrical isomers are formed.

### 10.2 Quantitative analysis

#### 10.2.1 Calculation of the composition of fatty acid methyl esters

Calculate the area fraction,  $x_i$ , of the individual fatty acid methyl esters, expressed as a percentage by area of methyl esters, as given by Formula (1):

$$x_i = \frac{A_i}{\sum A} \times 100 \quad (1)$$

where

$A_i$  is the area of the individual fatty acid methyl ester  $i$ ;

$\sum A$  is the sum of areas under all peaks of all individual fatty acid methyl ester.

For most fats and oils, the area fraction of the fatty acid methyl esters is equal to the area fraction of triacylglycerols in grams per 100 g (for certain cases, see [10.2.2](#)).

According to the method AOCS Ce 1h-05, the factors for the conversion of FAMES to TAG equivalents are between 0,9114 (C8:0) and 0,9965 (C24:1) and are therefore negligible. If the chromatographic system

obeys these factors, it can be assumed that the ratio of the peak areas of the FAMES is identical to the ratio of the mass fractions.

The results are expressed in grams per 100 g with one decimal place for values.

### 10.2.2 Calculation of the composition of fatty acid methyl esters using correction factors

In certain cases, for example, when fatty acids with fewer than 16 carbon atoms are present (lauric fats and oils with C10, C12, and C14), the areas should be corrected with specific correction factors ( $F$ ). These factors should be determined for each single instrument. For this purpose, suitable reference materials with certified fatty acid composition in the corresponding range should be used.

According to the requirements of the clients, the correction factor might not be used. However, the utilization (or not) of the correction factor shall be specified on the analysis report.

These correction factors are not identical with theoretical FID correction factors, which are given in [Annex A](#), as they also include the performance of the injection system, etc. However, in the case of bigger differences, the whole system shall be checked for performance.

For the reference mixture, the mass fraction  $w_i$ , in grams per 100 g of FAME,  $i$ , is given by Formula (2):

$$w_i = \frac{m_i}{\sum m} \times 100 \quad (2)$$

where

$m_i$  is the mass of the FAME,  $i$ , in the reference mixture;

$\sum m$  is the total of the masses of the various components, as FAMES of the reference mixture.

From the chromatogram of the reference mixture, calculate the percentage by area for the FAME,  $i$ , as follows:

$$x_i = \frac{A_i}{\sum A} \times 100 \quad (3)$$

where

$A_i$  is the area of the FAME,  $i$ , in the reference mixture;

$\sum A$  is the sum of all areas of all FAMES of the reference mixture.

The correction factor,  $F_i$ , is then:

$$F_i = \frac{m_i \times \sum A}{A_i \times \sum m} \quad (4)$$

For the sample, the mass fraction,  $w_i$ , in grams per 100 g of each FAME,  $i$ , is as given by Formula (5):

$$w_i = \frac{F_i \times A_i}{\sum (F_i \times A_i)} \quad (5)$$

NOTE The calculated value corresponds to the percentage of mass of the individual fatty acid calculated as triacylglycerol per 100 g fat.

### 10.2.3 Calculation of the composition of fatty acid methyl esters using an internal standard

In certain analyses (for example, where not all of the fatty acids are quantified, such as when acids with four and six carbons are present, alongside acids with 16 and 18 carbons, or when it is necessary to determine the absolute amount of a fatty acid in a sample), it is necessary to use an internal standard.

Fatty acids with 15, 17, 19, or 21 carbons are frequently used. The correction factor (if any) for the internal standard should be determined.

The mass fraction in grams per 100 g, of the fatty acid,  $i$ , expressed as methyl ester, is then given by Formula (6):

$$w_i = \frac{m_{IS} \times F_i \times A_i}{m \times F_{IS} \times A_{IS}} \quad (6)$$

where

$A_i$  is the area the FAME,  $i$ ;

$A_{IS}$  is the area of the internal standard;

$F_i$  is the correction factor of the fatty acid,  $i$ , expressed as FAME;

$F_{IS}$  is the correction factor of the internal standard;

$m$  is the mass of the test portion, in milligrams;

$m_{IS}$  is the mass of the internal standard, in milligrams, corrected by its purity (usually 0,99).

The results are expressed with one decimal place.

## 11 Precision

### 11.1 Results of interlaboratory test

Details of an interlaboratory test on the precision of the method are summarized in [Annex D](#). The values derived from this interlaboratory test might not be applicable to concentration ranges and matrices other than those given.

### 11.2 Repeatability

The absolute difference between two independent single test results obtained using the same method on identical test material in the same laboratory, by the same operator, using the same equipment within a short interval of time, will, in not more than 5 % of cases, be greater than  $r$  given in [Tables D.1](#) to [D.3](#).

### 11.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material, in different laboratories, with different operators, using different equipment, will, in not more than 5 % of cases, be greater than  $R$  given in [Tables D.1](#) to [D.3](#).

## 12 Test report

The test report shall specify:

- a) all information necessary for the complete identification of the sample;
- b) the sampling method used, if known, with reference to this part of ISO 12966, i.e ISO 12966-4;
- c) the test method used with reference to this part of ISO 12966;
- d) all operating details not specified in this part of ISO 12966, or regarded as optional, together with details of any incidents which might have influenced the result;
- e) the test result(s) obtained;

- f) indication of the utilization (or not) of correction factors for the calculation of the results.

## Annex A (informative)

### Theoretical flame ionization detector correction factor (TCF) for fatty acid methyl esters (FAMES)

Table A.1

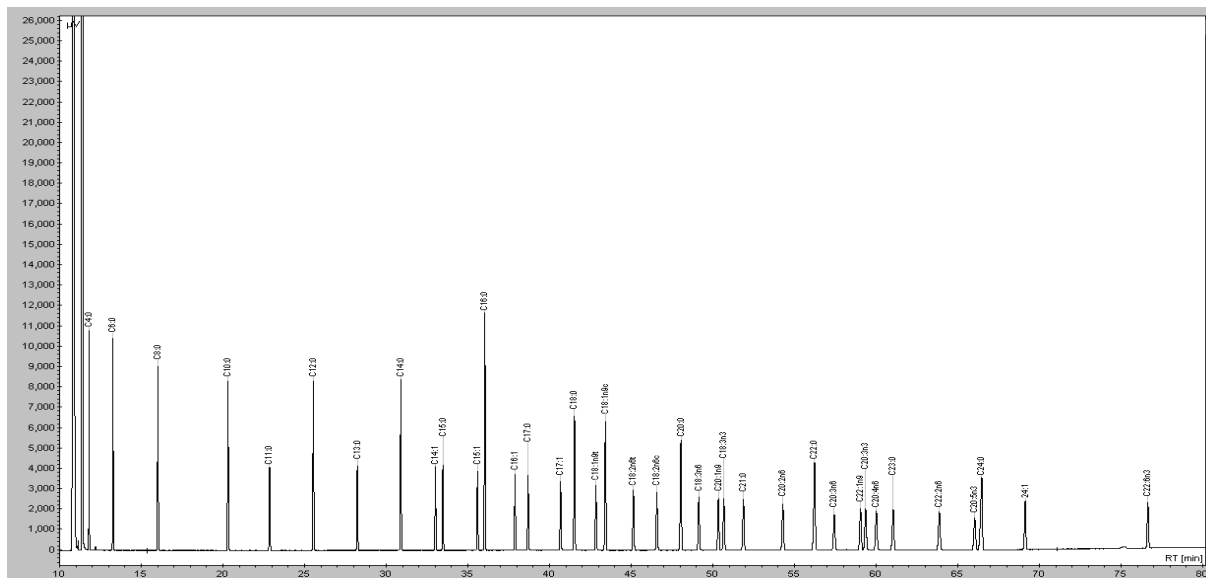
FAME	TCF	FAME	TCF	FAME	TCF	FAME	TCF
<b>C4:0</b>	1,574 2	<b>C14:1</b>	1,058 7	<b>C18:2</b>	1,008 7	<b>C22:1</b>	0,988 1
<b>C5:0</b>	1,432 4	<b>C15:0</b>	1,054 0	<b>C18:3</b>	1,001 7	<b>C22:2</b>	0,982 5
<b>C6:0</b>	1,337 8	<b>C15:1</b>	1,045 7	<b>C18:4</b>	0,994 9	<b>C22:3</b>	0,976 9
<b>C7:0</b>	1,270 2	<b>C16:0</b>	1,042 2	<b>C19:0</b>	1,014 2	<b>C22:4</b>	0,971 3
<b>C8:0</b>	1,219 5	<b>C16:1</b>	1,034 5	<b>C20:0</b>	1,006 7	<b>C22:5</b>	0,965 5
<b>C9:0</b>	1,180 2	<b>C16:2</b>	1,026 7	<b>C20:1</b>	1,000 5	<b>C22:6</b>	0,959 9
<b>C10:0</b>	1,148 6	<b>C16:3</b>	1,018 9	<b>C20:2</b>	0,994 3	<b>C23:0</b>	0,988 2
<b>C11:0</b>	1,122 8	<b>C16:4</b>	1,011 1	<b>C20:3</b>	0,988 0	<b>C24:0</b>	0,983 0
<b>C12:0</b>	1,101 3	<b>C17:0</b>	1,031 8	<b>C20:4</b>	0,981 9	<b>C24:1</b>	0,977 9
<b>C12:1</b>	1,091 0	<b>C17:1</b>	1,024 4	<b>C20:5</b>	0,966 5		
<b>C13:0</b>	1,083 1	<b>C18:0</b>	1,022 5	<b>C21:0</b>	1,000 0		
<b>C14:0</b>	1,067 5	<b>C18:1</b>	1,015 5	<b>C22:0</b>	0,993 9		

NOTE Atomic weights used: carbon 12,011; hydrogen, 1,007 9; and oxygen, 15,994. Factors are relative to 21:0 which has a factor of 1,000 0 by definition. Only one factor is given for all positional and geometric isomers and for branched-chain FAME as the factors are dependent only on the content of carbon to which hydrogen is bonded.

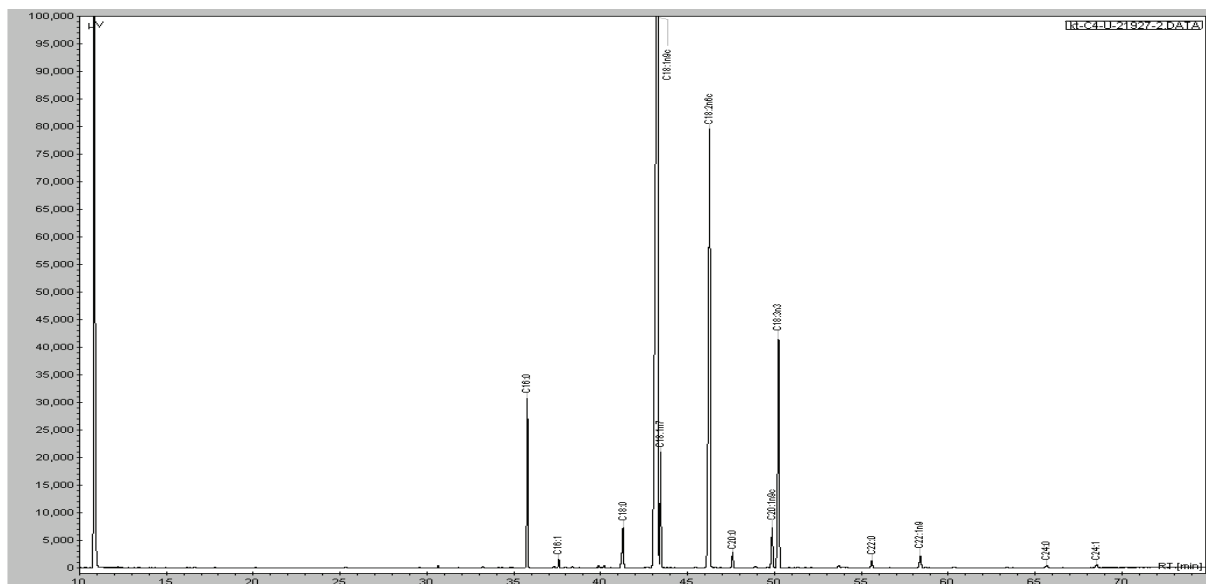
Table according to AOCS method Ce 1h-05.

## Annex B (informative)

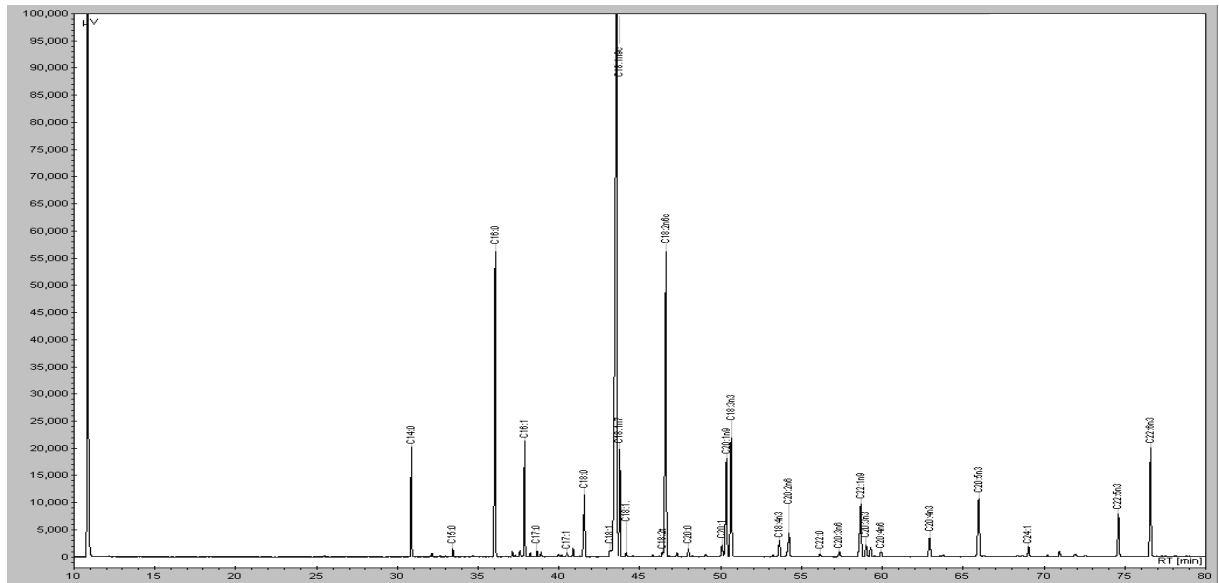
### Examples of chromatograms



**Figure B.1 — Typical chromatogram of FAMES mixture solution (SupelcoR 37 Component FAME Mix, cat. No. 47885-U)**



**Figure B.2 — Chromatogram of FAMES of rapeseed oil**



**Figure B.3 — Chromatogram of FAMES of fish oil**

The chromatographic conditions are as follows:

- Column: SP-2560, 100 m x 0,25 mm I.D., 0,20  $\mu\text{m}$  (Supelco 24056);
- Oven temperature: 110  $^{\circ}\text{C}$  for 7 min, then 3  $^{\circ}\text{C}/\text{min}$  to 190  $^{\circ}\text{C}$ , hold 2 min, 0,5  $^{\circ}\text{C}/\text{min}$  to 205  $^{\circ}\text{C}$ , 5  $^{\circ}\text{C}/\text{min}$  to 230  $^{\circ}\text{C}$ , hold 5 min, 5  $^{\circ}\text{C}/\text{min}$  to 240  $^{\circ}\text{C}$ , hold 5 min;
- Injector: 260  $^{\circ}\text{C}$ , split 1:50;
- Detector (FID): 260  $^{\circ}\text{C}$ ;
- Carrier gas: Helium, 360 kPa;
- Injection volume: 1  $\mu\text{l}$ .

## Annex C (informative)

### Comparison of FAME composition with two different GC columns

Table C.1

Fatty acid methyl esters	Blend of vegetable oils		Refined rapeseed oil		Crude groundnut oil		Margarine	
	BPX 70	CPSIL 88	BPX 70	CPSIL 88	BPX 70	CPSIL 88	BPX 70	CPSIL 88
8:0							0,1	0,1
10:0							0,2	0,1
10:1								
12:0							2,7	2,5
12:1								
13:0								
14:0	0,1	0,0	0,1	0,0	0,0	0,0	1,2	1,2
14:1								
15:0	0,0	0,0	0,0	0,0			0,0	0,0
16:0	4,7	4,6	4,7	4,6	10,8	10,8	11,7	11,8
16:1	0,2	0,2	0,3	0,3	0,1	0,1	0,1	0,2
17:0	0,1	0,0	0,1	0,0	0,1	0,1	0,1	0,1
17:1	0,1	0,0	0,1	0,1	0,0	0,0	0,0	0,0
18:0	2,9	2,8	1,6	1,6	3,8	3,7	3,1	3,1
18:1 <i>trans</i>	1,4	1,4	0,0		0,0	0,0	0,4	0,4
18:1 <i>cis</i>	59,1	59,1	61,4	61,8	56,8	57,1	47,9	48,2
18:2 <i>trans</i>	0,5	0,6	0,1	0,1	0,1	0,1	0,1	0,1
18:2 <i>cis</i> (n-6)	21,0	20,9	19,2	19,2	20,9	20,8	27,4	27,6
18:3 <i>cis</i> (n-6)								
18:3 <i>trans</i>	2,0	2,0	0,5	0,5			0,1	
18:3 <i>cis</i> (n-3)	4,8	4,7	9,0	8,9	0,1	0,1	2,8	2,8
20:0	0,6	0,6	0,6	0,6	1,6	1,6	0,4	0,4
18:2 conj.								
20:1	1,2	1,2	1,2	1,2	1,1	1,1	0,6	0,6
22:0	0,6	0,6	0,3	0,4	2,9	2,8	0,5	0,5
22:1	0,3	0,3	0,3	0,3	0,1	0,1	0,1	0,1
24:0	0,3	0,3	0,1	0,1	1,4	1,3	0,2	0,2
24:1	0,1	0,1	0,1	0,1			0,1	
unidentified	0,3	0,4	0,3	0,3	0,1	0,2	0,2	0,2
Sum of TFA	3,9	4,0	0,6	0,5	0,1	0,2	0,7	0,5

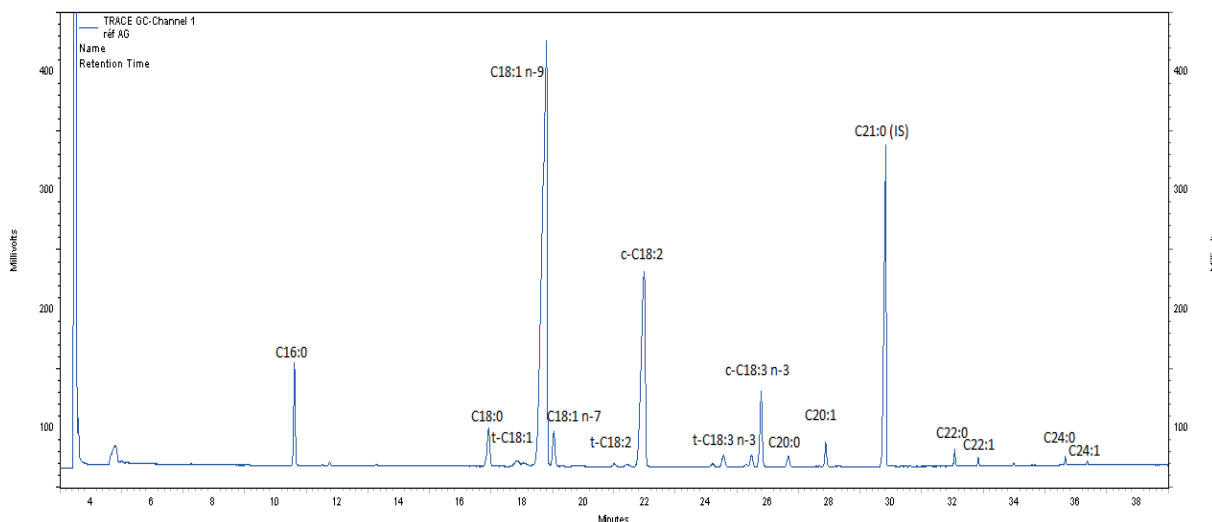


**Analytical conditions:**

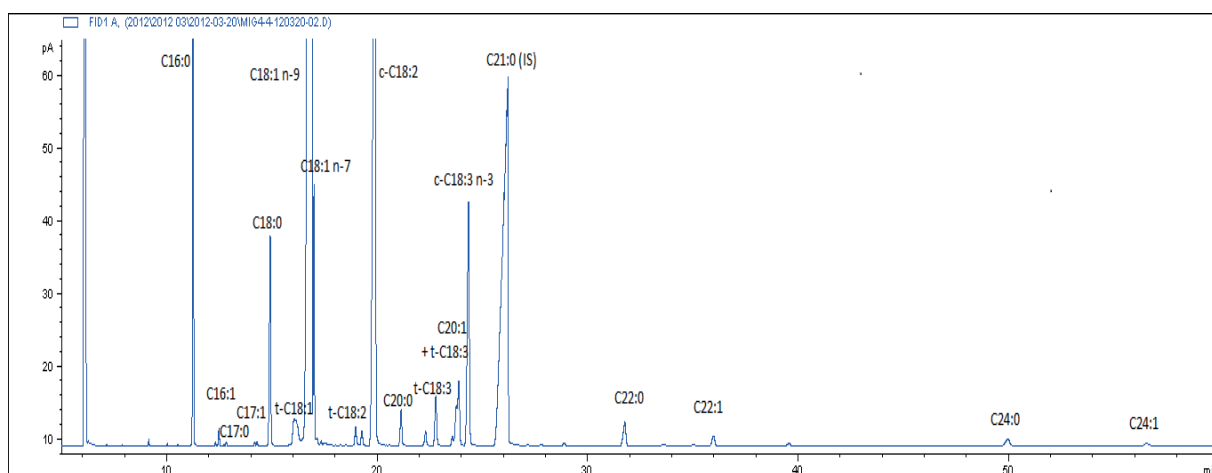
Sample preparation in accordance with ISO 12966-2, C21-FAME as internal standard:

BPX 70 (50 m, 0,22 mm to 0,25 µm): 170 °C, hold 23 min, 5 °C/min, 230 °C, hold 4 min, 0,5 ml/min H<sub>2</sub>, split ratio is 1:100 (see [Figure C.1](#));

CP-Sil 88 (100 m, 0,25 mm to 0,2 µm): 180 °C, hold 90 min, H<sub>2</sub> at 170 kPa, split ratio is 1:100 (see [Figure C.2](#)).



**Figure C.1 — Chromatogram on 50 m BPX 70**



**Figure C.2 — Chromatogram on 100 m CP-Sil 88**

## Annex D (informative)

### Results of an interlaboratory trial

An interlaboratory test on the precision of the method was organized in 2005 by AOCS in which 13 laboratories participated. The values derived from this interlaboratory test might not be applicable to concentration ranges and matrices other than those given.

The following fats and oils were used for the collaborative trial: canola oil, cocoa butter, coconut oil, hydrogenated lard, lard, margarine oil, sunflower oil, vegetable shortening.

The following abbreviated terms are used in [Table D.1](#) to [Table D.3](#):

$s_r$  is the repeatability standard deviation;

$C_{V,r}$  is the relative repeatability, as a percentage (%);

$r$  is the repeatability;

$s_R$  is the reproducibility standard deviation;

$C_{V,R}$  is the relative reproducibility standard deviation, as a percentage (%);

$R$  is the reproducibility.

**Table D.1 — Summary of precision data for *trans*-fatty acids**

Sample	Mean (%)	$s_r$	$C_{V,r}$	$r$	$s_R$	$C_{V,R}$	$R$
Hydrogenated lard	1,00	0,10	10,1	0,28	0,22	21,6	0,61
Margarine oil	11,62	0,16	1,4	0,45	0,25	2,2	0,71
Canola oil	26,55	0,64	2,4	1,78	0,65	2,5	1,82
Lard	0,90	0,07	7,4	0,19	0,20	21,7	0,55
Sunflower oil	0,17	0,03	15,9	0,07	0,10	60,3	0,28
Coconut oil	0,10	0,04	35,4	0,10	0,04	35,9	0,10
Canola oil	26,27	0,51	1,9	1,43	0,78	3,0	2,19
Vegetable shortening	45,01	0,66	1,5	1,84	2,05	4,6	5,74
Cocoa butter	0,06	0,03	53,7	0,09	0,04	69,7	0,12
Coconut oil	0,11	0,01	12,3	0,04	0,02	14,8	0,05

Table D.2 — Precision data from the collaborative study calculated as triacylglycerol content and organized according to the kind of fatty acids (SAFA, MUFA, and PUFA)

Parameter	Mean %	$s_r$	$C_{V,r}$	$r$	$s_R$	$C_{V,R}$	$R$
<b>SAFAs</b>							
<b>C06:0</b>	0,53	0,09	17,2	0,26	0,17	31,7	0,47
<b>C08:0</b>	7,50	0,44	5,8	1,22	0,63	8,4	1,76
<b>C10:0</b>	5,72	0,29	5,1	0,81	0,51	8,9	1,42
<b>C10:0</b>	0,09	0,00	4,5	0,01	0,01	11,8	0,03
<b>C12:0</b>	45,50	1,12	2,4	3,12	4,44	9,8	12,44
<b>C12:0</b>	0,08	0,00	5,3	0,01	0,01	8,2	0,02
<b>C12:0</b>	0,04	0,00	6,2	0,00	0,01	18,8	0,02
<b>C13:0</b>	0,02	0,00	9,9	0,00	0,02	115,7	0,05
<b>C14:0</b>	17,76	0,30	1,7	0,85	0,91	5,1	2,54
<b>C14:0</b>	1,40	0,02	1,3	0,05	0,07	5,1	0,20
<b>C14:0</b>	0,07	0,00	5,2	0,01	0,01	8,3	0,02
<b>C15:0</b>	0,07	0,00	4,7	0,01	0,01	13,6	0,03
<b>C15:0</b>	0,02	0,00	9,5	0,00	0,01	60,7	0,03
<b>C16:0</b>	24,58	0,56	2,3	1,57	1,32	5,4	3,70
<b>C16:0</b>	9,95	0,20	2,0	0,56	0,45	4,5	1,25
<b>C16:0</b>	3,25	0,06	1,8	0,16	0,14	4,2	0,38
<b>C17:0</b>	0,38	0,01	3,2	0,03	0,03	7,9	0,08
<b>C17:0</b>	0,23	0,01	2,1	0,01	0,01	4,5	0,03
<b>C17:0</b>	0,11	0,01	4,0	0,01	0,01	6,8	0,02
<b>C17:0</b>	0,04	0,01	14,9	0,02	0,01	18,1	0,02
<b>C18:0</b>	34,20	0,70	2,1	1,97	1,56	4,6	4,38
<b>C18:0</b>	14,15	0,24	1,7	0,66	0,56	4,1	1,61
<b>C18:0</b>	6,05	0,14	2,4	0,40	0,28	4,5	0,79
<b>C18:0</b>	3,62	0,06	1,7	0,17	0,09	2,4	0,25
<b>C20:0</b>	1,09	0,02	2,1	0,07	0,03	2,9	0,09
<b>C20:0</b>	0,31	0,01	2,2	0,02	0,02	5,6	0,05
<b>C20:0</b>	0,10	0,01	5,1	0,02	0,01	10,7	0,03
<b>C22:0</b>	0,87	0,02	1,9	0,05	0,02	2,6	0,06
<b>C22:0</b>	0,34	0,01	3,4	0,03	0,02	5,5	0,05
<b>C22:0</b>	0,20	0,01	4,1	0,02	0,01	6,1	0,03
<b>C22:0</b>	0,02	0,02	71,5	0,05	0,03	136,5	0,08
<b>MUFAs</b>							
<b>C16:1c</b>	2,19	0,11	5,5	0,32	0,19	8,7	0,53
<b>C16:1c</b>	0,28	0,01	4,3	0,03	0,03	9,0	0,07
<b>C16:1c</b>	0,09	0,01	6,9	0,02	0,01	11,4	0,03
<b>C16:1c</b>	0,02	0,00	15,9	0,01	0,02	74,0	0,05

Average mean, average range, and average  $C_{V,R}$  were calculated, then sorted by mean value to determine if the accuracy was dependent on the concentration or related to the individual FA. Special attention was focused on the identification of the trans FAs. Similar mean values were grouped to compare the average  $C_{V,R}$  values.

Table D.2 (continued)

Parameter	Mean %	$s_r$	$C_{V,r}$	$r$	$s_R$	$C_{V,R}$	$R$
C16:1t	0,16	0,01	7,1	0,03	0,17	107,9	0,47
C16:1t	0,03	0,02	53,4	0,05	0,03	106,0	0,09
C17:1c	0,26	0,01	5,3	0,04	0,02	8,0	0,06
C17:1c	0,05	0,00	6,3	0,01	0,01	17,8	0,03
C17:1c	0,02	0,00	12,4	0,01	0,02	77,9	0,05
C18:1c	84,32	1,16	1,4	3,23	4,18	5,0	11,70
C18:1c	38,30	0,75	2,0	2,11	1,37	3,7	3,83
C18:1c	22,66	0,41	1,8	1,15	0,77	3,4	2,15
C18:1c	5,49	0,08	1,6	0,24	0,14	2,5	0,38
C18:1t	45,39	0,84	1,9	2,35	2,04	4,5	5,71
C18:1t	22,35	0,43	1,9	1,19	0,61	2,7	1,69
C18:1t	10,56	0,17	1,6	0,47	0,18	1,7	0,49
C18:1t	0,67	0,08	11,6	0,22	0,09	13,2	0,25
C18:1t	0,09	0,02	30,9	0,07	0,04	49,4	0,11
C20:1	0,69	0,02	2,2	0,04	0,02	3,2	0,06
C20:1	0,17	0,01	5,2	0,03	0,01	8,2	0,04
C20:1	0,05	0,01	13,4	0,02	0,01	14,3	0,02
C20:1	0,02	0,01	33,6	0,02	0,02	83,6	0,05
C22:1	0,04	0,00	3,8	0,00	0,08	213,2	0,28
PUFAs							
C18:2c	38,92	0,79	2,0	2,21	1,37	3,5	3,84
C18:2c	11,74	0,19	1,6	0,52	0,36	3,1	1,01
C18:2c	4,35	0,09	2,4	0,26	0,17	4,4	0,47
C18:2c	1,35	0,04	2,6	0,10	0,04	3,1	0,12
C18:2c	0,49	0,07	15,0	0,21	0,11	22,4	0,31
C18:2t	5,01	0,19	3,8	0,54	0,35	7,0	0,99
C18:2t	1,40	0,21	15,0	0,59	0,46	32,6	1,28
C18:2t	0,84	0,13	15,8	0,37	0,17	20,1	0,47
C18:2t	0,15	0,04	26,1	0,11	0,06	43,8	0,17
C18:2t	0,05	0,01	13,0	0,02	0,04	77,1	0,11
C18:3c	4,89	0,09	1,9	0,26	0,15	3,1	0,43
C18:3c	0,62	0,03	5,0	0,09	0,04	6,0	0,11
C18:3c	0,20	0,01	6,4	0,04	0,03	13,5	0,08
C18:3t	0,69	0,05	6,9	0,13	0,23	32,6	0,63
C18:3t	0,15	0,02	13,8	0,06	0,05	37,0	0,15
C18:3c	0,05	0,01	19,9	0,02	0,01	47,9	0,03
C18:3t	0,04	0,02	41,0	0,04	0,04	108,5	0,12
C20:2	0,27	0,09	30,7	0,25	0,27	101,2	0,75

Average mean, average range, and average  $C_{V,R}$  were calculated, then sorted by mean value to determine if the accuracy was dependent on the concentration or related to the individual FA. Special attention was focused on the identification of the trans FAs. Similar mean values were grouped to compare the average  $C_{V,R}$  values.

Table D.2 (continued)

Parameter	Mean %	$s_r$	$C_{V,r}$	$r$	$s_R$	$C_{V,R}$	$R$
<b>C20:3</b>	0,03	0,00	4,8	0,00	0,06	193,5	0,17
<b>C20:4</b>	0,07	0,00	3,8	0,01	0,11	150,1	0,30

Average mean, average range, and average  $C_{V,R}$  were calculated, then sorted by mean value to determine if the accuracy was dependent on the concentration or related to the individual FA. Special attention was focused on the identification of the trans FAs. Similar mean values were grouped to compare the average  $C_{V,R}$  values.

Table D.3 — Precision data from the collaborative study. Data are calculated as fatty acid content and organized according to the mean value

Parameter	Mean (%)	$s_r$	$C_{V,r}$	$r$	$s_R$	$C_{V,R}$	$R$
<b>SAFAs</b>							
<b>C06:0</b>	0,48	0,08	17,2	0,23	0,15	31,6	0,42
<b>C08:0</b>	6,89	0,40	5,8	1,12	0,58	8,3	1,61
<b>C10:0</b>	5,32	0,27	5,1	0,76	0,47	8,9	1,32
<b>C10:0</b>	0,08	0,00	4,6	0,01	0,01	11,9	0,03
<b>C12:0</b>	42,77	1,05	2,4	2,93	4,19	9,8	11,73
<b>C12:0</b>	0,08	0,01	7,3	0,02	0,01	8,9	0,02
<b>C12:0</b>	0,03	0,00	5,7	0,01	0,01	18,4	0,02
<b>C13:0</b>	0,02	0,00	9,2	0,00	0,02	115,6	0,05
<b>C14:0</b>	16,81	0,29	1,7	0,81	0,86	5,1	2,41
<b>C14:0</b>	1,33	0,02	1,2	0,05	0,07	5,1	0,19
<b>C14:0</b>	0,07	0,00	4,7	0,01	0,01	9,4	0,02
<b>C15:0</b>	0,07	0,00	5,1	0,01	0,01	13,7	0,03
<b>C15:0</b>	0,02	0,00	9,2	0,00	0,01	60,8	0,02
<b>C16:0</b>	23,42	0,54	2,3	1,50	1,26	5,4	3,53
<b>C16:0</b>	9,48	0,19	2,0	0,54	0,43	4,5	1,19
<b>C16:0</b>	3,10	0,06	1,8	0,16	0,13	4,1	0,36
<b>C17:0</b>	0,37	0,01	3,2	0,03	0,03	7,9	0,08
<b>C17:0</b>	0,22	0,01	2,1	0,01	0,01	4,5	0,03
<b>C17:0</b>	0,10	0,00	4,0	0,01	0,01	6,8	0,02
<b>C17:0</b>	0,04	0,01	14,7	0,01	0,01	17,9	0,02
<b>C18:0</b>	32,73	0,67	2,1	1,89	1,51	4,6	4,23
<b>C18:0</b>	13,55	0,23	1,8	0,64	0,56	4,2	1,57
<b>C18:0</b>	5,79	0,14	2,4	0,38	0,27	4,5	0,76
<b>C18:0</b>	3,13	0,06	1,7	0,16	0,09	2,5	0,24
<b>C20:0</b>	1,05	0,02	2,1	0,06	0,03	2,9	0,09
<b>C20:0</b>	0,30	0,01	2,3	0,02	0,02	5,3	0,04
<b>C20:0</b>	0,10	0,00	3,7	0,01	0,01	11,4	0,03
<b>C22:0</b>	0,84	0,02	1,9	0,04	0,02	2,2	0,05

Average mean, average range, and average  $C_{V,R}$  were calculated, then sorted by mean value to determine if the accuracy was dependent on the concentration or related to the individual FA. Special attention was focused on the identification of the trans FAs. Similar mean values were grouped to compare the average  $C_{V,R}$  values.

Table D.3 (continued)

Parameter	Mean (%)	$s_r$	$C_{V,r}$	$r$	$s_R$	$C_{V,R}$	$R$
C22:0	0,33	0,01	3,3	0,03	0,02	5,2	0,05
C22:0	0,20	0,01	4,1	0,02	0,01	6,2	0,03
C22:0	0,02	0,02	71,6	0,05	0,03	136,5	0,08
C24:0	0,24	0,09	37,4	0,25	0,12	49,5	0,34
C24:0	0,07	0,04	50,8	0,10	0,05	65,8	0,13
MUFAs							
C14:1	0,00	0,01	197,1	0,02	0,01	213,7	0,02
C16:1c	2,08	0,11	5,2	0,30	0,18	8,6	0,50
C16:1c	0,27	0,01	4,3	0,03	0,02	9,0	0,04
C16:1c	0,08	0,01	6,9	0,02	0,01	11,4	0,03
C16:1c	0,02	0,00	15,6	0,01	0,02	74,0	0,04
C16:1t	0,15	0,01	7,0	0,03	0,16	108,4	0,45
C16:1t	0,03	0,01	49,9	0,04	0,03	106,3	0,09
C17:1c	0,25	0,01	5,3	0,04	0,02	8,0	0,06
C17:1c	0,05	0,00	6,3	0,01	0,01	17,7	0,03
C17:1c	0,02	0,00	14,1	0,01	0,02	78,4	0,04
C18:1c	80,66	1,10	1,4	3,09	4,03	5,0	11,29
C18:1c	36,64	0,71	2,0	2,00	1,33	3,7	3,73
C18:1c	21,74	0,40	1,8	1,12	0,78	3,5	2,18
C18:1c	5,25	0,08	1,6	0,23	0,13	2,5	0,37
C18:1t	43,45	0,77	1,8	2,17	2,00	4,6	5,59
C18:1t	21,37	0,42	2,0	1,17	0,59	2,8	1,65
C18:1t	10,11	0,19	1,9	0,54	0,19	1,9	0,54
C18:1t	0,64	0,07	11,6	0,21	0,08	13,2	0,24
C18:1t	0,08	0,02	31,1	0,07	0,04	49,3	0,10
C20:1	0,66	0,01	2,2	0,04	0,02	3,2	0,06
C20:1	0,17	0,01	5,2	0,02	0,01	8,1	0,04
C20:1	0,05	0,01	13,2	0,02	0,01	14,3	0,02
C20:1	0,02	0,01	34,1	0,02	0,02	83,7	0,05
PUFAs							
C18:2c	37,22	0,75	2,0	2,11	1,33	3,6	3,72
C18:2c	11,22	0,18	1,6	0,50	0,35	3,2	0,98
C18:2c	4,16	0,09	2,4	0,25	0,16	4,5	0,45
C18:2c	1,29	0,03	2,6	0,10	0,04	3,2	0,12
C18:2c	0,47	0,07	15,0	0,20	0,11	22,4	0,29
C18:2t	4,79	0,18	3,8	0,51	0,34	7,1	0,95
C18:2t	1,34	0,20	15,0	0,56	0,44	32,5	1,22
C18:2t	0,79	0,14	17,8	0,39	0,19	24,1	0,53

Average mean, average range, and average  $C_{V,R}$  were calculated, then sorted by mean value to determine if the accuracy was dependent on the concentration or related to the individual FA. Special attention was focused on the identification of the *trans* FAs. Similar mean values were grouped to compare the average  $C_{V,R}$  values.

Table D.3 (continued)

Parameter	Mean (%)	$s_r$	$C_{V,r}$	$r$	$s_R$	$C_{V,R}$	$R$
<b>C18:2t</b>	0,14	0,04	26,4	0,10	0,06	44,1	0,16
<b>C18:3c</b>	4,68	0,09	1,9	0,25	0,15	3,2	0,41
<b>C18:3c</b>	0,58	0,02	3,9	0,06	0,02	4,1	0,07
<b>C18:3c</b>	0,19	0,01	6,4	0,03	0,03	13,6	0,07
<b>C18:3c</b>	0,04	0,01	19,8	0,02	0,01	47,6	0,03
<b>C18:3t</b>	0,64	0,11	16,9	0,30	0,25	38,4	0,69
<b>C18:3t</b>	0,14	0,02	14,4	0,06	0,05	37,3	0,15
<b>C18:3t</b>	0,04	0,01	41,9	0,04	0,04	109,3	0,11
<b>C20:2</b>	0,26	0,09	30,7	0,24	0,26	101,2	0,72
<b>C20:3</b>	0,03	0,00	5,0	0,00	0,06	193,6	0,17
<b>C20:4</b>	0,07	0,00	3,8	0,01	0,10	150,1	0,29
<b>SAFA</b>	84,31	1,90	2,3	5,31	4,61	5,5	12,91
<b>SAFA</b>	58,71	0,45	0,8	1,26	2,20	3,7	6,15
<b>SAFA</b>	38,86	0,71	1,9	1,98	1,68	4,3	4,69
<b>SAFA</b>	18,35	0,24	1,4	0,67	0,83	4,5	2,33
<b>SAFA</b>	7,51	0,13	1,7	0,36	0,25	3,3	0,69
<b>PUFA</b>	41,70	1,32	3,2	3,69	1,72	4,1	4,81
<b>PUFA</b>	11,88	0,23	1,9	0,63	0,44	3,7	1,23
<b>PUFA</b>	4,27	0,09	2,5	0,26	0,13	3,2	0,36
<b>PUFA</b>	1,29	0,03	2,6	0,10	0,04	3,2	0,12
<b>PUFA</b>	0,49	0,07	13,7	0,19	0,11	23,1	0,32
<b>trans</b>	45,01	0,66	1,5	1,84	2,05	4,6	5,74
<b>trans</b>	26,41	0,57	2,2	1,61	0,72	2,7	2,00
<b>trans</b>	11,62	0,16	1,4	0,45	0,25	2,2	0,71
<b>trans</b>	0,95	0,08	8,8	0,23	0,21	21,7	0,58
<b>trans</b>	0,11	0,03	29,3	0,08	0,05	45,2	0,14

Average mean, average range, and average  $C_{V,R}$  were calculated, then sorted by mean value to determine if the accuracy was dependent on the concentration or related to the individual FA. Special attention was focused on the identification of the *trans* FAs. Similar mean values were grouped to compare the average  $C_{V,R}$  values.

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