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Milk, milk products, infant formula and adult nutritionals — Determination of fatty acids composition — Capillary gas chromatographic method

Lait, produits laitiers, formules infantiles et produits nutritionnels pour adultes — Détermination de la composition en acides gras — Méthode de chromatographie en phase gazeuse sur colonne capillaire

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ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

International Dairy Federation
Silver Building • Bd Auguste Reyers 70/B • B-1030 Brussels

Tel. + 32 2 325 67 40
Fax + 32 2 325 67 41
info@fil-idf.org
www.fil-idf.org

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Forewords

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).

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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 5, *Milk and milk products* and the International Dairy Federation (IDF), in collaboration with AOAC INTERNATIONAL. It is being published jointly by ISO and IDF and separately by AOAC INTERNATIONAL. The method described in this International Standard is equivalent to the AOAC Official Method 2012.13: *Determination of labeled fatty acids content in milk products and infant formula*.

IDF (the International Dairy Federation) is a non-profit private sector organization representing the interests of various stakeholders in dairying at the global level. IDF members are organized in National Committees, which are national associations composed of representatives of dairy-related national interest groups including dairy farmers, dairy processing industry, dairy suppliers, academics and governments/food control authorities.

ISO and IDF collaborate closely on all matters of standardization relating to methods of analysis and sampling for milk and milk products. Since 2001, ISO and IDF jointly publish their International Standards using the logos and reference numbers of both organizations.

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ISO 16958 | IDF 231 was prepared by the IDF Standing Committee on *Analytical Methods for Composition* and the ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee 5 on *Milk and milk products* (ISO/TC 34/SC 5), in collaboration with AOAC INTERNATIONAL. It is being published jointly by ISO and IDF, and separately by AOAC INTERNATIONAL. The method described in this International Standard is equivalent to the AOAC Official Method 2012.13: *Determination of labeled fatty acids content in milk products and infant formula*

All work was carried out by the ISO-IDF Project Group C11 of the Standing Committee on *Analytical Methods for Composition* under the aegis of its project leader, Mr Pierre-Alain Golay (CH).

Milk, milk products, infant formula and adult nutritionals — Determination of fatty acids composition — Capillary gas chromatographic method

1 Scope

This International Standard specifies a method for the quantification of individual and/or all fatty acids in the profile of milk, milk products, infant formula and adult nutritional formula, containing milk fat and/or vegetable oils, supplemented or not supplemented with oils rich in long chain polyunsaturated fatty acids (LC-PUFA). This also includes groups of fatty acids often labelled [i.e. *trans* fatty acids (TFA), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-3, omega-6 and omega-9 fatty acids] and/or individual fatty acids [i.e. linoleic acid (LA), α-linolenic acid (ALA), arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)].

The determination is performed by direct transesterification in food matrices, without prior fat extraction, and consequently it is applicable to liquid samples or reconstituted powder samples with water having total fat ≥ 1,5 % m/m.

The fat extracted from products containing less than 1,5 % m/m fat can be analysed with the same method after a preliminary fat extraction using methods referenced in Clause 2. Dairy products, like soft or hard cheeses with acidity level ≤ 1 mmol/100 g of fat, can be analysed after a preliminary fat extraction using methods referenced in Clause 2. For products supplemented or enriched with PUFA with fish oil or algae origins, the evaporation of solvents should be performed at the lowest possible temperature (e.g. max. 40 °C) to recover these sensitive fatty acids.

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 1042, *Laboratory glassware — One-mark volumetric flasks*

ISO 1735 | IDF 5, *Cheese and processed cheese products — Determination of fat content — Gravimetric method (Reference method)*

ISO 1740 | IDF 6, *Milk fat products and butter — Determination of fat acidity (Reference method)*

ISO 14156 | IDF 172, *Milk and milk products — Extraction methods for lipids and liposoluble compounds*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
fatty acids content
mass fraction of individual or groups of substances determined by the procedure specified in this International Standard

Note 1 to entry: See Table A.1.

Note 2 to entry: The fatty acid content is expressed as a mass fraction in grams (or in milligrams) of the fatty acids per 100 g of product (see Table A.1). Fatty acid results can be converted into other results expression formats (see 10.2).

4 Principle

Addition of the internal standard solution to the sample, preparation of fatty acid methyl esters (FAMEs) by direct transesterification with methanolic sodium methoxide for liquid samples; dissolution (i.e. reconstitution) in water for powder sample and direct transesterification with methanolic sodium methoxide. The same transesterification procedure is applied to fat extracted from various foods (e.g. low fat products, cheeses).

Separation of FAMEs using capillary gas-liquid chromatography. Identification of FAMEs by comparison with the retention time of pure standards and quantification as fatty acids by reference to an internal standard (C11:0 FAME) and instrument response factors. Verification of the transesterification performance using a second internal standard (C13:0 TAG).

5 Reagents

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

5.1 ***n*-Hexane,** [$CH_3(CH_2)_4CH_3$], chromatography grade.

5.2 **Methanol,** [CH_3OH], chromatography grade.

5.3 **Water**, HPLC grade or equivalent purity quality.

5.4 **Sodium methoxide solution**, [CH_3ONa], dissolved in methanol 30 % m/v, or 25 % m/v, depending on local availability.

5.5 **Transesterification solution**, (sodium methoxide solution 5 % m/v in methanol).

Into a 300 ml volumetric flask, pipette 50 ml (or 60 ml) of sodium methoxide solution 30 % m/v (or 25 % m/v) and mix gently with 250 ml of methanol using a magnetic stirrer. Remove the magnetic stirrer, then cool to room temperature and make up to the mark with methanol.

Stored in the dark at 4 °C, this solution is stable for one week. Allow the solution to come to room temperature before use. This solution volume is sufficient to analyse approximately 40 samples. In case of a smaller number of analyses, the reagent volume can be adapted accordingly.

Perform the transesterification reaction at ambient temperature (between 20 °C and 25 °C).

NOTE Value indicated in brackets () corresponds to sodium methoxide solution with 25 % m/v concentration

5.6 **di-Sodium hydrogen citrate sesquihydrate**, [$HOC(COOH)(CH_2COONa)_2.1{,}5\ H_2O$].

5.7 **Sodium chloride**, [NaCl].

5.8 **Neutralization solution**, (di-sodium hydrogen citrate sesquihydrate 10 % m/v, sodium chloride 15 % m/v in water).

Weigh 50,0 g of di-sodium hydrogen citrate sesquihydrate and 75,0 g of sodium chloride in a 500 ml volumetric flask. Dissolve in 450 ml of water using a magnetic stirrer. Remove the magnetic stirrer, then make up to the mark with water.

Stored in the dark at 4 °C, this solution is stable for one month. Presence of salt crystals may appear in the solution during storage, but disappear after shaking.

Allow the solution to come to room temperature before use. This solution volume is sufficient to analyse approximately 40 samples or more. In case of a smaller number of analyses (or single analysis), the mass and volume of solution can be adapted accordingly.

5.9 Tert-butyl methyl ether (MTBE), chromatography grade.

5.10 Methyl undecanoate (C11:0 FAME), of purity ≥ 99 % mass fraction.

5.11 Tritridecanoin (C13:0 TAG), of purity ≥ 99 % mass fraction.

5.12 C11:0 FAME/C13:0 TAG standard solution.

Into a 250 ml volumetric flask, weigh to the nearest 0,1 mg about 500 mg of tritridecanoin and 500 mg of methyl undecanoate. Dissolve and make up to the mark with MTBE.

Stored in the dark at 4 °C, this solution is stable for one week. Allow the solution to come to room temperature before use.

This solution volume is sufficient to analyse approximately 40 samples or more. In case of a smaller number of analyses, standard mass and volume of solvent can be adapted accordingly.

5.13 Octadecenoic acid methyl esters, *cis* and *trans* isomers mixture of C18:1 with *trans*-4 to *trans*-16 octadecenoic (all isomers) and principal *cis* isomers. Concentration 2,5 mg/ml in methylene chloride.

NOTE This standard is commercially available from Supelco Inc, brand of Sigma-Aldrich (Cat. 40495-U)[1)].

5.14 Linoleic acid methyl esters, *cis* and *trans* isomers mixture of C18:2 with *trans*-9,*trans*-12-octadecadienoic acid (approximately 50 %), *cis*-9,*trans*-12-octadecadienoic acid (approximately 20 %), *trans*-9,*cis*-12-octadecadienoic acid (approximately 20 %) and *cis*-9,*cis*-12-octadecadienoic acid (approximately 10 %). Concentration 10 mg/ml in methylene chloride.

NOTE This standard is commercially available from Supelco Inc, brand of Sigma-Aldrich (Cat. 47791)[1)].

5.15 Linolenic acid methyl esters, *cis* and *trans* isomers mixture of C18:3 with

— *cis*-9,*cis*-12,*cis*-15-octadecatrienoic acid methyl ester (approximately 3 % m/m),

— *cis*-9,*cis*-12,*trans*-15-octadecatrienoic acid methyl ester (approximately 7 % m/m),

— *cis*-9,*trans*-12,*cis*-15-octadecatrienoic acid methyl ester (approximately 7 % m/m),

— *cis*-9,*trans*-12,*trans*-15-octadecatrienoic acid methyl ester (approximately 15 % m/m),

— *trans*-9,*cis*-12,*cis*-15-octadecatrienoic acid methyl ester (approximately 7 % m/m),

— *trans*-9,*cis*-12,*trans*-15-octadecatrienoic acid methyl ester (approximately 15 % m/m),

— *trans*-9,*trans*-12,*cis*-15-octadecatrienoic acid methyl ester (approximately 15 % m/m), and

— *trans*-9,*trans*-12,*trans*-15-octadecatrienoic acid methyl ester (approximately 30 % m/m).

Concentration 10 mg/ml in methylene chloride.

NOTE This standard is commercially available from Supelco Inc, brand of Sigma Aldrich (Cat. 47792)[1)]. This standard contains all *trans* isomers of C18:3 (eight in total) but their abundance and ratio are different to those observed in refined/deodorized oils and fats.

5.16 Methyl octadecadienoate conjugated acids, mixture of C18:2 *cis*-9,*trans*-11 and *cis*-10,*trans*-12-octadecadienoate conjugated acids, of purity ≥ 99 % mass fraction.

1) Supelco Inc., brand of Sigma Aldrich, is an example of suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by either ISO or IDF of the product named. Equivalent products may be used if they can be shown to lead to the same results.

NOTE This standard is commercially available from Supelco Inc, brand of Sigma Aldrich (Cat. 05507)[1]. This standard contains the two principal CLA isomers, but isomer ratio may vary from lot to lot.

5.17 Qualitative *cis* and *trans* isomers standard mixture solution

For the retention time (RT) identification of *cis* and *trans* isomers (i.e. C18:1, C18:2, C18:3 and CLA), prepare a qualitative standard solution with the standards listed in 5.13 to 5.16. All standards that are commercially available could be used. Into a 50 ml volumetric flask, add each standard isomer solution in equal proportion. Dissolve and make up to the mark with hexane. Dilute in accordance with the type of injector used.

5.18 FAME standards calibration solution

5.18.1 Preparation with individual FAME standards

5.18.1.1 Individual FAME standards

Purchase individual FAME standards as follows (purity ≥ 99 %):

Butyric acid methyl ester (C4:0), caproic acid methyl ester (C6:0), caprylic acid methyl ester (C8:0), capric acid methyl ester (C10:0), undecanoic acid methyl ester (C11:0), lauric acid methyl ester (C12:0), tridecanoic acid methyl ester (C13:0), myristic acid methyl ester (C14:0), myristoleic acid methyl ester (C14:1 *cis*-9 or n-5), pentadecanoic acid methyl ester (C15:0), *cis*-10-pentadecenoic acid methyl ester (C15:1 *cis*-10 n-5), palmitic acid methyl ester (C16:0), palmitoleic acid methyl ester (C16:1 *cis*-9 or n-7), heptadecanoic acid methyl ester (C17:0), *cis*-10-heptadecenoic acid methyl ester (C17:1 *cis*-10 or n-7), stearic acid methyl ester (C18:0), elaidic acid methyl ester (C18:1 *trans*-9 or n-9), oleic acid methyl ester (C18:1 *cis*-9 or n-9), linolelaidic acid methyl ester (C18:2 all *trans*-9,12 or n-6), linoleic acid methyl ester (C18:2 all *cis*-9,12 or n-6), arachidic acid methyl ester (C20:0), gamma-linoleic acid methyl ester (C18:3 all *cis*-6,9,12 or n-6), *cis*-11-eicosenoic acid methyl ester (C20:1 *cis*-11 or n-9), linolenic acid methyl ester (C18:3 all *cis*-9,12,15 or n-3), heneicosanoic acid methyl ester (C21:0), *cis*-11,14-eicosadienoic acid methyl ester (C20:2 all *cis*-11,14 or n-6), behenic acid methyl ester (C22:0), *cis*-8,11,14-eicosatrienoic acid methyl ester (C20:3 all *cis*-8,11,14 or n-6 *cis*), erucic acid methyl ester (C22:1 *cis*-13 or n-9), *cis*-11,14,17-eicosatrienoic acid methyl ester (C20:3 all *cis*-11,14,17 or n-3), arachidonic acid methyl ester (C20:4 all *cis*-5,8,11,14 or n-6), *cis*-13,16-docosadienoic acid methyl ester (C22:2 all *cis*-13,16 or n-6), lignoceric acid methyl ester (C24:0), *cis*-5,8,11,14,17- eicosapentaenoic acid methyl ester (C20:5 all *cis*-5,8,11,14,17 or n-3), nervonic acid methyl ester (C24:1 *cis*-15 or n-9), *cis*-4,7,10,13,16,19-docosahexaenoic acid methyl ester (C22:6 all *cis*-4,7,10,13,16,19 or n-3).

NOTE Purchasing of individual FAME standards is more expensive than a single FAME standard mixture. In addition, weighing each FAME standard individually could give imprecision and requires high precision of weighing.

5.18.1.2 Stock solution 1 – Saturated

Into a 100 ml volumetric flask, accurately weigh to the nearest 0,1 mg about 25 mg of Lignoceric acid methyl ester (C24:0), 25 mg of behenic acid methyl ester (C22:0), 25 mg of heneicosanoic acid methyl ester (C21:0), 25 mg of arachidic acid methyl ester (C20:0), 25 mg of sacid methyl ester (C18:0), 25 mg of heptadecanoic acid methyl ester (C17:0), 50 mg of palmitic acid methyl ester (C16:0), 25 mg of pentadecanoic acid methyl ester (C15:0), 25 mg of myristic acid methyl ester (C14:0), 25 mg of tridecanoic acid methyl ester (C13:0), 25 mg of lauric acid methyl ester (C12:0), 25 mg of undecanoic acid methyl ester (C11:0), 25 mg of capric acid methyl ester (C10:0), 25 mg of caprylic acid methyl ester (C8:0), 25 mg of caproic acid methyl ester (C6:0) and 25 mg butyric acid methyl ester (C4:0). Make up to the mark with *n*-hexane.

Palmitic acid is weighed in double amount. Short chain fatty acid methyl esters (i.e. C4:0, C6:0 and C8:0) are volatile and shall be weighed at the end of the procedure.

5.18.1.3 Stock solution 2 – Monounsaturated

Into a 100 ml volumetric flask, accurately weigh to the nearest 0,1 mg about 25 mg of nervonic acid methyl ester (C24:1 *cis*-15 or n-9), 25 mg of erucic acid methyl ester (C22:1 *cis*-13 or n-9), 25 mg of *cis*-11-eicosenoic acid methyl ester (C20:1 *cis*-11 or n-9), 25 mg of oleic acid methyl ester (C18:1 *cis*-9 or n-9), 25 mg of elaidic acid methyl ester (C18:1 *trans*-9 or n-9 *trans*), 25 mg of *cis*-10-heptadecenoic acid methyl ester (C17:1 *cis*-10 or n-7), 25 mg of palmitoleic acid methyl ester (C16:1 *cis*-9 or n-7), 25 mg of *cis*-10-pentadecenoic acid methyl ester (C15:1 *cis*-10 or n-5) and 25 mg of myristoleic acid methyl ester (C14:1 *cis*-9 or n-5). Make up to the mark with *n*-hexane.

5.18.1.4 Stock solution 3 – Polyunsaturated

Into a 100 ml volumetric flask, accurately weigh to the nearest 0,1 mg about 25 mg of linolelaidic acid methyl ester (C18:2 all *trans*-9,12 or n-6 *trans*), 25 mg of linoleic acid methyl ester (C18:2 all *cis*-9,12 or n-6), 25 mg of gamma-linoleic acid methyl ester (C18:3 all *cis*-9,12 or n-6), 25 mg of linolenic acid methyl ester (C18:3 all *cis*-12,15 or n-3), 25 mg of *cis*-11,14-eicosadienoic acid methyl ester (C20:2 all *cis*-11,14 or n-6), 25 mg of *cis*-8,11,14-eicosatrienoic acid methyl ester (C20:3 all *cis*-8,11,14 or n-6), 25 mg of *cis*-11,14,17-eicosatrienoic acid methyl ester (C20:3 all *cis*-11,14,17 or n-3), 25 mg of arachidonic acid methyl ester (C20:4 all *cis*-5,8,11,14 or n-6), 25 mg of *cis*-13,16-docosadienoic acid methyl ester (C22:2 *cis*-13,16 or n-6), 25 mg of *cis*-5,8,11,14,17- eicosapentaenoic acid methyl ester (C20:5 all *cis*-5,8,11,14,17 or n-3) and 25 mg of *cis*-4,7,10,13,16,19-docosahexaenoic acid methyl ester (C22:6 *cis*-4,7,10,13,16,19 or n-3). Make up to the mark with *n*-hexane.

5.18.1.5 Preparation of FAME standards calibration solution

Into a 100 ml volumetric flask, pipette 25,0 ml of the calibration standard stock solution 1 (5.18.1.2), 25,0 ml of the calibration standard stock solution 2 (5.18.1.3) and 25,0 ml of the calibration standard stock solution 3 (5.18.1.4). Then make up to the mark with *n*-hexane. Dilute in accordance with the type of injector used.

Stored in the dark at −20 °C, this solution is stable for about six months. To prevent contamination of the standard solution, distribute the solution into different vials (ready to inject) and store them at −20 °C before use. Use each vial once, then discard it.

5.18.2 Preparation from a quantitative FAME standard mixture

5.18.2.1 Quantitative FAME standard mixture

Purchase a quantitative FAME standard mixture: Nu-Check-Prep, Cat. Number GLC- Nestle-36[2)].

The FAME calibration standard mixture is carefully prepared by mass by the supplier. The mass percentage of each component is indicated in the accompanying certificate. Each ampoule contains approximately 100 mg of the FAME calibration standard mix. All individual FAME standards are distributed in equal proportions in the standard mixture, except for palmitic acid methyl ester (C16:0) which is added in double amount.

5.18.2.2 Preparation of FAME standards calibration mixture

Before use, allow the ampoule to come to room temperature (maximum 25 °C) in the dark without heating. Cut the ampoule with a glass knife, using a Pasteur pipette, rapidly transfer the content of the ampoule into a 50 ml pre-tarred volumetric flask, weigh and make up to the mark with *n*-hexane. Dilute in accordance with the type of injector used.

2) Nu-Check-Prep GLC-Nestle36 is an example of suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by either ISO or IDF of the product named. Equivalent products may be used if they can be shown to lead to the same results.

Stored in the dark at −20 °C, this solution is stable for about six months. To prevent contamination of the standard solution, distribute the solution into different vials (ready to inject) and store them at −20 °C before use. Use each vial once, then discard it.

6 Apparatus

WARNING — Since the determination involves the use of volatile flammable solvents, all electrical apparatus employed shall comply with legislation relating to the hazards in using such solvents.

Usual laboratory equipment and, in particular, the following.

6.1 Analytical balance, capable of weighing to the nearest 1 mg, with a readability of 0,1 mg.

6.2 Volumetric flasks, of capacities 50 ml, 100 ml, 250 ml, 300 ml and 500 ml.

6.3 Volumetric pipettes, with one mark, of capacities 2 ml, 5 ml, 10 ml, 25 ml and 50 ml, class AS (ISO 1042).

6.4 Volumetric pipettes, with two marks, of capacities 2 ml and 5 ml, class AS (ISO 1042).

6.5 Micropipette, of capacity 200 µl.

6.6 Dispensers, of capacities 2 ml, 5 ml and 10 ml.

6.7 Test tube, of diameter 26 mm, of length 100 mm, fitted with PTFE-lined screw cap.

6.8 Test tube mixer vortex-genie, or equivalent.

6.9 Laboratory centrifuge, equipped with adapters for test tubes with external diameter of 26 mm.

6.10 Gas-liquid chromatograph, equipped with flame ionization detector and split/splitless or on-column injector. Auto-sampler and integration system preferably computerized.

Use of the cleanest possible glassware and caps is required to avoid impurities in the FAME chromatogram.

6.10.1 Carrier gas, hydrogen or helium, purity ≥ 99,9997 %.

NOTE The use of hydrogen or helium as carrier gas affects principally the chromatography duration (i.e. increase of time between 10 min to 15 min with helium) but does not have significant impact on the chromatographic resolution with optimized conditions.

The other gases necessary for the detector (FID) should be free from organic impurities (i.e. CnHm of below 1 ppm) and have purity at least ≥ 99,995 %. Synthetic air or compressed air can be used. The use of gas generator is also possible.

6.10.2 Capillary column, bonded with cyanopropyl-polysiloxane phase or equivalent (100 m length, 0,25 mm internal diameter, 0,2 micron film thickness), that elutes the FAMEs primarily by carbon chain length and secondarily by the number of double bonds.

Traces of oxygen and humidity will damage the polar phase of the column. If pure gas is not available, use a gas purifying filter device.

6.10.3 Flame ionization detector, capable of being heated to a temperature 50 °C above the final temperature of the column oven.

6.10.4 Split/splitless injector, capable of being heated to a temperature 30 °C above the final temperature of the column oven.

6.10.5 On-column injector, capable of being not heated (cold), or being heated to a temperature 30 °C above the final temperature of the column oven.

NOTE The installation of one single injector (i.e. split/splitless or on-column) on the GC instrument is sufficient.

6.10.6 Injection syringe, capacity 10 µl.

6.10.7 Integration system.

6.11 Gas chromatographic conditions

The oven temperature and the carrier gas flow depend on the column selected, and on the carrier gas used (i.e. hydrogen or helium). In any case, the selected conditions shall produce the separation between *cis* and *trans* zone for C18:1, C18:2, C18:3 and conjugated linoleic acids (CLA), as shown in Annex B, Figures B.1, B.2 and B.3.

The examples in 6.11.1 and 6.11.2 list applicable conditions for a correct separation/identification of *cis* and *trans.*

6.11.1 Example 1 – Split injection mode

— Column: 100 m length, 0,25 mm internal diameter, 0,2 µm film thickness, fused silica capillary column.

— Stationary phase: cyanopropyl-polysiloxane.

— Carrier gas type: helium.

— Column head carrier gas pressure: 225 kPa (175 kPa – 225 kPa).

— Split flow: 25,5 ml/min.

— Split ratio: 10:1.

— Injector temperature: 250 °C.

— Detector temperature: 275 °C.

— Oven temperature programme: initial temperature of 60 °C, maintained for 5 min, raised at a rate of 15 °C min^{-1} up to 165 °C, maintained at this temperature for 1 min and then raised at a rate of 2 °C min^{-1} up to 225 °C for 20 min.

— Amount of sample injected: 1,0 µl.

An example of the full GC profile obtained with these conditions is shown in Annex B, Figure B.4.

6.11.2 Example 2 – On-column injection mode

— Column: 100 m length, 0,25 mm internal diameter, 0,2 µm film thickness, fused silica capillary column.

— Stationary phase: cyanopropyl-polysiloxane.

— Carrier gas type: hydrogen.

— Column head carrier gas pressure: 210 kPa (175 kPa – 225 kPa).

— Injector temperature: cold.

— Detector temperature: 275 °C.

— Oven temperature programme: initial temperature of 60 °C, maintained for 5 min, raised at a rate of 15 °C min^{-1} up to 165 °C, maintained at this temperature for 1 min and then raised at a rate of 2 °C min^{-1} up to 225 °C for 17 min.

— Amount of sample injected: 1,0 µl.

An example of the full GC profile obtained with these conditions is shown in Annex B, Figure B.5.

6.12 Resolution between C18:1 *cis* and *trans*

For the accurate quantification of C18:1 TFA (level ≥ 0,5 g/100 g fat), a sufficient resolution between C18:1 *trans*-13/14 and C18:1 *cis*-9 (oleic acid) is required. The resolution is determined with the injection of the qualitative *cis* and *trans* C18:1 FAME isomers standard mixture solution (5.17).

Inject into the gas chromatograph 1,0 µl of the calibrating solution (5.13). Determine peak width at half height and distance between the left of the chromatogram and the top of peak for C18:1 *trans*-13/14 and C18:1 *cis*-9 (oleic acid methyl ester). The resolution criteria *R* is calculated by using Formula (1):

$$R = 1,18 \times (t_{R2} - t_{R1}) / (W_{\left(\frac{1}{2}\right)1} + W_{\left(\frac{1}{2}\right)2}) \quad (1)$$

where

t_{R1} is the distance, in centimetres, between the left of the chromatogram and the top of peak 1 (C18:1 *trans*-13/14);

t_{R2} is the distance, in centimetres, between the left of the chromatogram and the top of peak 2 (C18:1 *cis*-9);

$W_{\left(\frac{1}{2}\right)1}$ is the peak width, in centimetres, at half height of peak 1 (C18:1 t*rans*-13/14);

$W_{\left(\frac{1}{2}\right)2}$ is the peak width, in centimetres, at half height of peak 2 (C18:1 *cis*-9).

The resolution is sufficient when *R* criterion ≥ 1,00 ± 5 % (see Annex B, Figure B.3).

NOTE In case of insufficient resolution, but with *R* close to the target value, the fine tuning of chromatography conditions (i.e. slight modification of carrier gas pressure/flow, or oven temperature programme) can give an acceptable *R* value.

7 Sampling

It is important that the laboratory receives a sample that is representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707 | IDF 50.

8 Preparation of test sample

8.1 Liquid and powder milk and infant formula with a fat content ≥ 1,5 % m/m

Bring the sample to room temperature and shake vigorously before use. Ensure that the sample is homogeneous (i.e. mix well).

8.2 Liquid and powder milk and infant formula with a fat content < 1,5 % m/m

Bring the sample to room temperature and shake vigorously before use. Ensure that the sample is homogeneous (i.e. mix well).

Extract the fat in accordance with ISO 14156 | IDF 172 taking care to evaporate completely the extraction solvent(s) by heating to a temperature not higher than 40 °C to avoid the degradation of long chain polyunsaturated fatty acids (LC-PUFA).

NOTE See also ISO 1211 | IDF 1, ISO 1737 | IDF 13, ISO 8381 | IDF 123 and ISO 8262-1 | IDF 124-1 for useful guidance on fat extraction methods.

8.3 Cheese

Bring the sample to room temperature. Ensure that the sample is homogeneous (i.e. mix well).

Extract the fat in accordance with ISO 1735 | IDF 5 taking care to remove completely the extraction solvent by heating the fat to a temperature not higher than 60 °C.

Verify the acidity of the fat in accordance with ISO 1740 | IDF 6 (acceptance criteria ≤ 1 mmol/100 g of fat).

NOTE In the presence of methanolic sodium methoxide, free fatty acids are not converted into methyl esters (FAME). In case of higher acidity (i.e. free fatty acids), these fatty acids are not quantified with the others.

9 Procedure

9.1 Test portion

Into a 25 ml centrifuge tube with a screw cap, weigh to the nearest 0,1 mg an equivalent quantity of sample (8.1) in order to have approximately 50 mg of fat in the tube. (For example, for a sample containing 26 g fat per 100 g from a product, the corresponding sample mass is approximately 190 mg.)

NOTE 1 For fatty acid analysis on fat extracted from foods, the same amount of fat sample is required (i.e. approximately 50 mg).

For a powder sample, add 2,0 ml of water using a micropipette. For a liquid sample, water addition is not required. Close the tube, then dissolve gently using a vortex mixer. Wait for 15 min at room temperature.

For the fat extracted from product (8.2 and 8.3), weigh to the nearest 0,1 mg 50 mg of melted fat into a 25 ml centrifuge tube. Water addition is not required for fatty acid analysis of fat sample.

Pipette 5 ml of internal standard solution (5.12). Add with a pipette 5 ml of 5 % (m/v) methanolic sodium methoxide solution (5.5). The transesterification time starts with the addition of the first drop of reagent. Close the tube hermetically and shake well for 10 s using a vortex mixer.

180 s after the start time, open the tube and add 2 ml of hexane. 210 s after the start time add 10 ml of disodium hydrogen citrate and sodium chloride aqueous solution (5.8). The transesterification time stops after the addition of the last drop of neutralization solution. Shake gently using a vortex mixer for 30 s. The transesterification time should not exceed 240 s after the start time.

NOTE 2 It is important to respect the transesterification time (240 s). The number of tubes cannot exceed six tubes at the same time under these conditions. Rapid delivery system (dispenser) can be used to add reagents, but not for the addition of internal standard solution which requires high precision.

Centrifuge the tube at 1 750 min^{-1} (or equivalent to g = 375 ± 25) for 5 min.

Into a 10 ml volumetric flask, pipette 200 µl of the supernatant and make up to the mark with *n*-hexane.

NOTE 3 The dilution factor is calculated for on-column and/or splitless injection only. When using split injection, reduce the dilution to obtain the desired peak responses according to the split ratio used (take care to have a sufficient and accurate detection level for small peaks especially). Stored in the dark at 4 °C, the sample solution after dilution is stable for two days.

NOTE 4 In the sample chromatogram, sometimes a "hill" on the baseline is seen between the solvent peak and the elution of C6:0; this phenomenon is caused by the possible presence of water traces captured by MTBE solvent during the sample preparation. The "hill" can be easily removed from the GC chromatogram with the addition of few mg of $CaCl_2$ in the diluted sample solution before GC injection.

9.2 Quantitative determination

9.2.1 Determination of response factors

Inject three times 1 µl the calibrated solution (see 5.18.1.5 or 5.18.2.2).

9.2.2 Determination of the test portion

Inject 1 µl of the test portion (9.1) into the gas chromatograph applying the same conditions as used with the FAME standards calibrating solution.

9.2.3 Fatty acid identification

Identify the fatty acids in the sample solution chromatogram by comparing their retention times with those of the corresponding peaks in the calibration standard solution (5.18) and in the qualitative standard mixture containing all TFAs and CLA isomers (see 5.13 and 5.17).

C18:1 TFA

Identify and group all *trans* isomers of C18:1 (include also the peak area of C18:1 *trans*-16 eluted in the C18:1 *cis* region of the chromatogram just after the C18:1 *cis*-9 or n-9) in accordance with Annex B, Figures B.1 or B.2.

NOTE 1 When milk fat is present, two *trans* isomers of C18:1 are eluted in the C18:1 *cis* region of the chromatogram (the C18:1 *trans*-15 and C18:1 *trans*-16), but only one isomer is resolved (the C18:1 *trans*-16) with the 100 m length capillary column. The second isomer (C18:1 *trans*-15) is generally overlapped with the oleic acid peak (C18:1 *cis*-9) and its area is only quantifiable using a preliminary separation (i.e. TLC A^+, HPLC A^+,) followed by a capillary GC analysis. According to recent findings, it has been demonstrated that there is no significant difference in total C18:1 TFA amount when the peak area of C18:1 *trans*-15 (the not resolved peak) is excluded from the sum in comparison to the result obtained after preliminary separation techniques followed by a capillary GLC analysis. A part of this phenomenon is explained by the presence of some C18:1 *cis* isomers (i.e. *cis*-6–8), which elute with the C18:1 *trans* region and consequently are added indirectly to the sum of C18:1 TFA. The contribution of these isomers on the sum of C18:1 TFA compensate the fact that C18:1 *trans*-15 is not taken into account.

C18:2 TFA

Identify and group all *trans* isomers of linoleic acid (see Annex B, Figures B.1, B.2 and B.6). For the total TFA of C18:2, include all the *trans* isomers present in milk fat sample as shown in Figures B.1 and B.2.

C18:3 TFA

Identify and group all TFA of linolenic acid (see Annex B, Figures B.1, B.2 and B.6).

NOTE 2 In the presence of milk fat and/or fish oil in the sample, another isomer of C20:1 elutes just before C20:1 *cis*-11 (or n-9). Depending on the column resolution, the retention time of this fatty acid may also correspond to a *trans* isomer of C18:3 (i.e. C18:3 *cis*-9,*trans*-12,*cis*-15 or C18:3 *trans*-9,*cis*-12,*cis*-15). When there is only one peak in the corresponding zone of C18:3 TFA, its correct identification corresponds to a C20:1 isomer. When two, three or four peaks are encountered in the corresponding zone for C18:3 TFA, each peak area is included in the total areas of C18:3 TFA (see elution order and formation rules below). Interferences may also be observed between C18:3 TFA isomers (i.e. C18:3 *cis*-9,*cis*-12,*trans*-15; *cis*-9,*trans*-12,*cis*-15; or *trans*-9,*cis*-12,*cis*-15) and C20:1 *cis*-11 (or n-9). The C20:1 *cis*-11 (or n-9) can elute with C18:3 *cis*-9,*trans*-12,*cis*-15 (the minor C18:3 *trans* isomer), but its contribution to the total C18:3 TFA is negligible. However, if C20:1 *cis*-11 (or n-9) shows interferences with C18:3 *cis*-9,*cis*-12,*trans*-12 or with C18:3 *trans*-9,*cis*-12,*cis*-15 the chromatography condition can be slightly modified to obtain sufficient separation. Interference is also visible when the wrong ratio between C18:3 *cis*-9,*cis*-12,*trans*-15 and C18:3 *trans*-9,*cis*-12,*cis*-15 is observed (the ratio between these isomers is always close to 5:4).

NOTE 3 The kinetics of C18:3 *trans* isomers formation in refined and deodorized oils have been analysed using a highly polar capillary column and are well described in the literature. They could be used as a confirmatory tool to verify the presence of C18:3 *trans* isomers. Most often, a maximum number of four *trans* isomers of C18:3 is encountered.

Case 1 – Absence of C18:3 TFA isomers

No peak (if only one peak is detected; see the comment above regarding the presence of another C20:1 isomer in milk). The presence of single one C18:3 *trans* isomer is not possible.

Case 2 – Presence of two C18:3 TFA isomers (C18:3 *cis*-9,*cis*-12,*trans*-15 and C18:3 *trans*-9,*cis*-12,*cis*-15)

The peak area of C18:3 *trans*-9,*cis*-12,*cis*-15 is approximately 80 % of the peak area of C18:3 *cis*-9,*cis*-12,*trans*-15 (or ratio 5:4). This ratio is always constant when other C18:3 *trans* isomers are present.

Case 3 – Presence of three C18:3 TFA isomers (C18:3 *cis*-9,*cis*-12,*trans*-15; C18:3 *cis*-9,*trans*-12,*cis*-15; and C18:3 *trans*-9,*cis*-12,*cis*-15).

The same as described above for case 2 (two isomers), but with the presence of C18:3 *cis*-9,*trans*-12,*cis*-15. The peak area of this *trans* isomer is always small and sometimes lower than the Limit Of Quantification (LOQ). In case of co-elution of this *trans* isomer with C20:1 *cis*-11 (n-9) or with another C20:1 isomer, its contribution on total C18:3 TFA is negligible.

Case 4 – Presence of four C18:3 TFA isomers (C18:3 *trans*-9,*cis*-12,*trans*-15; C18:3 *cis*-9,*cis*-12,*trans*-15; C18:3 *cis*-9,*trans*-12,*cis*-15; and C18:3 *trans*-9,*cis*-12,*cis*-15).

The same as described above for case 3 (three *trans* isomers), but with the C18:3 *trans*-9,*cis*-12,*trans*-15. This isomer is formed by the partial degradation of C18:3 *cis*-9,*cis*-12,*trans*-15 and C18:3 *trans*-9,*cis*-12,*cis*-15 (the first two C18:3 *trans* isomers occur in deodorized vegetable oils). When its amount is ≥ 50 % of the peak area of C18:3 *cis*-9,*cis*-12,*trans*-15), the presence of other C18:3 *trans* isomers could be suspected and indicate abnormal oil deodorization conditions (i.e. high temperature and/or time). See also Annex B, Figure B.6, which shows a real example of an infant formula sample containing C18:2 TFA and C18:3 TFA originating from deodorized vegetable oils.

The presence of other C18:3 *trans* isomers can be confirmed with the injection of the qualitative standard mixture (5.17).

Use the following terms to express TFA results:

— C18:1 TFA is the sum of *trans* positional isomers from C18:1;

— C18:2 TFA is the sum of *trans* isomers from C18:2 (linoleic acid) in deodorized oils (i.e. C18:2 *trans*-9,*trans*-12, *cis*-9,*trans*-12, and *trans*-9,*cis*-12) and in milk fat (i.e. C18:2 *cis*-9,*trans*-13, C18:2 *trans*-8,*cis*-12 and C18:2 *trans*-11,*cis*-15);

— C18:3 TFA is the sum of *trans* isomers from C18:3 (linolenic acid) in deodorized vegetable oils (*trans*-9,*cis*-12,*trans*-15, *cis*-9,*cis*-12,*trans*-15, *cis*-9,*trans*-12,*cis*-15, and *trans*-9,*cis*-12,*cis*-15);

— total TFA is the sum of C18:1 TFA, C18:2 TFA and C18:3 TFA.

NOTE 4 The method is intended to quantify all TFA in foods (i.e. those originating from ruminants, hydrogenation and/or oil deodorization process). The method is not intended to determine the origin of C18:1 and C18:2 TFAs (i.e. natural TFAs vs. industrial TFAs) in complex foods containing different origins of TFA (i.e. fat from ruminants, hydrogenated and deodorized vegetable oil). An estimation can be made using C18:1 *trans* isomers distribution and/or ratio (i.e. C18:1 *trans*-9 and C18:1 *trans*-11), from the presence of C18:2 TFA and C18:3 TFA originating from oil deodorization process, and also by considering the distribution/abundance of some fatty acids along the full fatty acids profile. The quantification of TFA having different origins is more precise in ingredients.

NOTE 5 Branched-chain fatty acids (i.e. saturated *iso*- and *anteiso*-methyl-branched fatty acids) are also present in milk fats and they elute with other current fatty acids. In pure dairy fats, the branched fatty acids that are most often encountered have 14 to 17 carbon atoms in the chain and represent between 1 % and 2 % of the milk fat profile. Thus, this category of fatty acids is not considered in the standard. Nevertheless, corresponding peak areas of these fatty acids and/or other unidentified fatty acids, can be added into the sum of "other fatty acids" (OFA). A response factor (mean of response factors from C12:0 to C24:0) can be applied for their quantification. For more information, please see Annex B, Figure B.7.

10 Calculation and expression of results

10.1 Calculation

10.1.1 Calculation of response factor

Determine the area of peaks attributable to each fatty acid methyl ester present in the calibration standard mixture injected (9.2.1) and calculate their respective response factors (Rf_i) relative to the internal standard (C11:0) by using Formula (2):

$$Rf_i = \frac{m'_i \times A'_0}{m'_0 \times A'_i} \quad (2)$$

where

m'_i is the mass fraction of $FAME_i$ in the calibration standard solution (5.18.1.5 or 5.18.2.2);

A'_0 is the peak area of C11:0 in the calibration standard solution chromatogram;

m'_0 is the mass of C11:0 in the calibration standard solution (5.18.1.5 or 5.18.2.2);

A'_i is the peak area of $FAME_i$ in the calibration standard solution chromatogram.

The variation between three injections is optimal when coefficients of variation are less than 2 %. An example of the calculation is given in Annex B, Figure B.8.

NOTE The response factors calculated for C18:2 *cis*-9,12 (or n-6) can be applied for C-18:2 CLA (*cis*-9,*trans*-11) and that calculated for C18:3 *cis*-9,12,15 (n-3) can be applied for C18:3 *trans* isomers.

10.1.2 Fatty acids on the product

Calculate the mass fraction of the individual components expressed in g FA_i/100 g product in the test sample by using Formula (3):

$$gFA_i/100\ g\ product = \frac{m_0 \times A_i \times Rf_i \times S_i(FA) \times 100}{A_0 \times m} \quad (3)$$

where

m_0 is the mass of C11:0 internal standard, in milligrams, added to the sample solution;

A_i is the peak area of $FAME_i$ in the sample chromatogram;

Rf_i is the response factor, calculated in accordance with 10.1.1;

$S_i(FA)$ is the stoichiometric factor to convert $FAME_i$ to FA_i (Annex B, Table B.1);

A_0 is the peak area of C11:0 internal standard in the sample chromatogram;

m is the mass of test portion, in milligrams.

An example of the calculation is given in Annex B, Figure B.9.

NOTE 1 In case of fatty acids analysis carried out on fat extracted from foods, the mass of test portion "m" corresponds to fat and not to the product. Consequently fatty acids results are expressed in g FA/100 g fat and not in g FA/100 g product with this equation. Results obtained in g FA/100 g fat can be converted into g FA/100 g product with the fat extraction value (g/100 g) determined with an appropriate validated extraction method. The declared fat value can be imprecise in comparison to fat extraction value and its use is not recommended for the expression of fatty acids on finished products.

NOTE 2 The peak areas corresponding to unidentified fatty acids can be summed-up and reported as the sum of other fatty acids. The contribution of these fatty acids could vary from 0 g /100 g to 5 g/100 g fat (i.e. in milk fat) and thus could contribute to the sum of all fatty acids. The peaks corresponding to impurities (materials and chemicals, samples or having chromatography origin) can never be included in the sum of OFA.

10.1.3 Fatty acids on the total fat

Calculate the mass fraction of the individual components expressed in g FA_i/100 g fat in test sample by using Formula (4):

$$FA_i/100\ \text{g fat} = \frac{\text{g}FA_i/100\ \text{g product} \times 100}{\%\ \text{Fat}} \quad (4)$$

This calculation can be only performed when the fat content is determined with an appropriate validated extraction method. Do not use the declared fat value for the expression of fatty acids on finished products.

10.1.4 Sum of class or group of fatty acids in 100 g product

Calculate the mass fraction of all fatty acids corresponding to a class or a group, in accordance with Annex A, Table A.1, by the simple addition of individual fatty acids results (expressed in g FA/100 g product) by using Formula (5):

$$\sum \text{FA} = \sum_{i=1}^{n} \text{g}\,FA_i/100\ \text{g product} \quad (5)$$

10.1.5 Sum of class or group of fatty acids in 100 g fat

Calculate the mass fraction of all fatty acids corresponding to a class or a group, in accordance with Annex A, Table A.1, by the simple addition of individual fatty acids results (expressed in g FA/100 g fat) by using Formula (6):

$$\sum \text{FA} = \sum_{i=1}^{n} \text{g}\,FA_i/100\ \text{g fat} \quad (6)$$

10.1.6 Performance of the transesterification

Record the areas of the two internal standard peaks (methyl undecanoate and tritridecanoin) in the analysed samples.

The performance of transesterification, *Pt* expressed in %, is calculated from the recovery of the tritridecanoin as second internal standard by using Formula (7):

$$Pt = \frac{m_{c11} \times A_{c13} \times R_{c13} \times S_{c13}(TAG)}{A_{c11} \times m_{c13}} \times 100 \quad (7)$$

where

m_{c11} is the mass, in milligrams, of C-11:0 internal standard added to the solution;

A_{c13} is the peak area of C-13:0 internal standard in the chromatogram;

R_{c13} is the response factor of C13:0 relative to C11:0, calculated according 10.1.1;

S_{c13} is the stoichiometric factor to convert C13:0 FAME into C13:0 TAG (Annex B, Table B.1);

A_{c11} is the peak area of C-11:0 internal standard in the chromatogram;

m_{c13} is the mass, in milligrams, of C13:0 TAG internal standard added to the solution.

The performance of the transesterification indicated by the recovery value of tritridecanoin (C13:0 TAG) should be equivalent to 100,0 % ± 2,0 %. When the performance of the transesterification is > 102,0 % or < 98,0 % the origin of the problem could be the following:

— incomplete transesterification (i.e. problem with reagent/chemical);

— partial degradation of internal standard(s), or problem with their purity/stability;

— sample matrix effect problem.

NOTE The analysis of a reference sample can help to determine if the problem originates from reagents and/or chemicals or with the analysed sample.

10.2 Expression of results

Express to three decimal places the results expressed in g / 100 g and to one decimal place the results expressed in mg / 100 g.

NOTE Fatty acids results expressed in g (or mg)/100 g product can be converted in other results expression format g (or mg) fatty acids /100 g fat:

— g (or mg) fatty acids / 100 g reconstituted powder (i.e. 25 g powder into 200 g water), liquid product (i.e. ready-to-feed), or liquid concentrates diluted 1:1 by mass.

— g (or mg) fatty acids / serving size (according to serving size mass).

11 Precision

11.1 Interlaboratory test

Details of the interlaboratory studies, organized and elaborated in accordance with ISO 5725-1 and ISO 5725-2 on the precision of the method, are summarized in Annex C.

The values for repeatability and reproducibility limit are expressed for the 95 % probability level and may 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, shall in not more than 5 % of cases be greater than *r*, as given in Annex C, Tables C.1 and C.2.

11.3 Reproducibility

The absolute difference between two independent single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, shall in not more than 5 % of cases be greater than *R*, as given in Annex C, Tables C.1 and C.2.

11.4 Limit of detection

Under the described conditions (detector sensitivity, noise, sample dilution, etc.), the estimated detection limit expressed as three times the standard deviation of the background signal (noise) lies around 0,0003 g/100 g of product.

11.5 Limit of quantitation

The limit of quantification, for each fatty acid, is about 0,001 g/100 g of product. The limit of quantification corresponds to the lowest level where the robust repeatability was calculated with satisfactory results.

12 Test report

The test report shall include at least the following information:

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

b) the sampling method used, if known; with reference to this International Standard, i.e. ISO 16958 | IDF 231;

c) the test method used, together with reference to this International Standard;

d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

e) the test result(s) obtained.

Annex A
(normative)

Groups or classes of fatty acids and individual fatty acids

A.1 Group or class of fatty acids

A.1.1 *Trans* fatty acids

Trans fatty acids (TFA) is the sum of fatty acids containing one or more non conjugated double bonds in *trans* configuration (only C18:1, C18:2 and C18:3 *trans* are included in the sum).

NOTE Presence of other *trans* isomers naturally present in milk fat have been reported in the literature (e.g. C16:1 *trans*), but their contributions have no significant influence on the total amount of *trans* fatty acids in milk products. In addition, their identification is rather complex because these isomers are frequently subject to interferences from other isomers of fatty acids (e.g. *cis, iso* and *anteiso*) and thus need a preliminary separation or the use of specific chromatographic conditions.

A.1.2 Conjugated linoleic acids

Conjugated linoleic acids (CLA) is the sum of octadecadienoic acids containing conjugated double bonds in *cis* or *trans* configuration; mainly *cis*-9, *trans*-11 octadecadienoic acid (i.e. rumenic acid). CLA are not included in the sum of TFAs.

A.1.3 Saturated fatty acids

Saturated fatty acids (SFA) is the sum of all fatty acids without double bonds.

A.1.4 Monounsaturated fatty acids

Monounsaturated fatty acids (MUFA) is the sum of all fatty acids containing one double bond in *cis* configuration.

A.1.5 Polyunsaturated fatty acids

Polyunsaturated fatty acids (PUFA) is the sum of all fatty acids containing two or more double bonds in *cis* configuration.

NOTE LC-PUFA is the generic name to describe the long chain polyunsaturated fatty acids. These fatty acids are also included with PUFA (i.e. arachidonic, eicosapentaenoic and docosahexaenoic acids).

A.1.6 Omega-3 fatty acids

Omega-3 fatty acids is the sum of *cis* polyunsaturated fatty acids having the first double bond at carbon n-3 (ω-3) from the terminal methyl group.

A.1.7 Omega-6 fatty acids

Omega-6 fatty acids is the sum of *cis* polyunsaturated fatty acids having the first double bond at carbon n-6 (ω-6) from the terminal methyl group.

A.1.8 Omega-9 fatty acids

Omega-9 fatty acids is the sum of *cis* polyunsaturated fatty acids having the first double bond at carbon n-9 (ω-9) from the terminal methyl group.

A.2 Individual fatty acids

A.2.1 Linoleic acid

Linoleic acid (LA) is an essential fatty acid, an 18 carbon fatty acid containing two double bonds at carbons 9 and 12 (C18:2 all *cis*-9,12) also named C18:2 n-6 (ω-6).

A.2.2 Linolenic acid

Linolenic acid (ALA), also called α-linolenic acid, is an essential fatty acid, an 18 carbon fatty acid containing three double bonds at carbons 9, 12, and 15 (C18:3 all *cis*-9,12,15) also named C18:3 n-3 (ω-3).

A.2.3 Arachidonic acid

Arachidonic acid (ARA) is not an essential fatty acid, a 20 carbon fatty acid containing four double bonds at carbons 5, 8, 11, and 14 (C20:4 all *cis*-5,8,11,14) also named C20:4 n-6 (ω-6).

A.2.4 Eicosapentaenoic acid

Eicosapentaenoic acid (EPA) is a semi-essential fatty acid (essential for pregnant women and infants), a 20 carbon fatty acid containing five double bonds at carbons 5, 8, 11, 14, and 17 (C20:5 all *cis*-5,8,11,14,17) also named C20:5 n-3 (ω-3).

A.2.5 Docosahexaenoic acid

Docosahexaenoic acid (DHA) is a semi-essential fatty acid (essential for pregnant women and infants), a 22 carbon fatty acid containing six double bonds at carbons 4, 7, 10, 13, 16, and 19 (C22:6 all *cis*-4,7,10,13,16,19) also named C22:6 n-3 (ω-3).

Table A.1 — Configuration and groups of fatty acids

Chain length	Configuration and group			Systematic name	Trivial name	Abbreviation
C4:0			SFA	Butanoic	Butyric	
C6:0			SFA	Hexanoic	Caproic	
C8:0			SFA	Octanoic	Caprylic	
C10:0			SFA	Decanoic	Capric	
C12:0			SFA	Dodecanoic	Lauric	
C14:0			SFA	Tetradecanoic	Myristic	
C14:1	ω-5 (or n-5)	*cis*	MUFA	Δ9-Tetradecenoic	Myristoleic	
C15:0			SFA	Pentadecanoic		
C15:1	ω-5 (or n-5)	*cis*	MUFA	Δ10-Pentadecenoic		
C16:0			SFA	Hexadecanoic	Palmitic	
C16:1	ω-7 (or n-7)	*cis*	MUFA	Δ9-Hexadecenoic	Palmitoleic	
C17:0			SFA	Heptadecanoic		
C17:1	ω-7 (or n-7)	*cis*	MUFA	Δ10-Heptadecenoic		
C18:0			SFA	Octadecanoic	Stearic	
C18:1 TFA		*trans*[a]		Sum of C18:1 *trans* isomers	All *trans* 4 to 16 octadecenoic	
C18:1	ω-9 (or n-9)	*cis*	MUFA	Δ9-Octadecenoic	Oleic	

[a] Do not include *trans* fatty acids in MUFA and PUFA sums.

Table A.1 *(continued)*

Chain length	Configuration and group			Systematic name	Trivial name	Abbreviation
C18:2 TFA		*trans*[a]		Sum of C18:2 *trans* isomers	All *trans* 9,12 octadecadienoic in deodorized oils and *trans* originated from milk fat (i.e. C18:2 *cis*-9,*trans*-13, C18:2 *trans*-8,*cis*-12 and C18:2 *trans*-11,*cis*-15)	
C18:2	ω-6 (or n-6)	*cis*	PUFA	Δ9,12-Octadecadienoic	Linoleic	LA
C18:2 CLA	ω-7 (or n-7)	*cis*/*trans*	PUFA	Δ9,11-Octadecadienoic	Rumenic	CLA
C18:3	ω-6 (or n-6)	*cis*	PUFA	Δ6,9,12-Octadecatrienoic	Gamma-linolenic	
C18:3 TFA		*trans*[a]		Sum of C18:3 *trans* isomers	All *trans* 9,12,15 Octadecatrienoic	
C18:3	ω-3 (or n-3)	*cis*	PUFA	Δ9,12,15-Octadecatrienoic	Linolenic	ALA
C20:0			SFA	Eicosanoic	Arachidic	
C20:1	ω-9 (or n-9)	*cis*	MUFA	Δ11-Eicosenoic	Gondoic	
C20:2	ω-6 (or n-6)	*cis*	PUFA	Δ11,14-Eicosadienoic		
C20:3	ω-6 (or n-6)	*cis*	PUFA	Δ8,11,14-Eicosatrienoic	Dihomo-gamma-linolenic (DHGLA)	
C20:3	ω-3 (or n-3)	*cis*	PUFA	Δ11,14,17-Eicosatrienoic		
C20:4	ω-6 (or n-6)	*cis*	PUFA	Δ5,8,11,14-Eicosatetraenoic	Arachidonic	ARA
C20:5	ω-3 (or n-3)	*cis*	PUFA	Δ5,8,11,14,17-Eicosapentaenoic	Eicosapentaenoic	EPA
C21:0			SFA	Heneicosanoic		
C22:0			SFA	Docosanoic	Behenic	
C22:1	ω-9 (or n-9)	*cis*	MUFA	Δ13-Docosenoic	Erucic	
C22:2	ω-6 (or n-6)	*cis*	PUFA	Δ13,16-Docosadienoic		
C22:6	ω-3 (or n-3)	*cis*	PUFA	Δ4,7,10,13,16,19-Docosahexaenoic	Docosahexaenoic	DHA
C24:0			SFA	Tetracosanoic	Lignoceric	
C24:1	ω-9 (or n-9)	*cis*	MUFA	Δ15-Tetracosenoic	Nervonic	

[a] Do not include *trans* fatty acids in MUFA and PUFA sums.

Table A.2 — Abbreviations

FAME	Fatty acid methyl ester	MUFA	Monounsaturated fatty acids
FA	Fatty acid	PUFA	Polyunsaturated fatty acids
GLC	Gas liquid chromatography	LC-PUFA	Long chain polyunsaturated fatty acids
MTBE	Tert-Butyl methyl ester	ω-3 (or n-3)	Omega-3 fatty acids
MeOH	Methanol	ω-6 (or n-6)	Omega-6 fatty acids
R	Resolution factor	ω-9 (or n-9)	Omega-9 fatty acids
RF	Response factor	LA	Linolenic acid (C18:2 all *cis*-9,12 or n-6)
RT	Retention time	ALA	Linolenic acid (C18:3 all *cis*-9,12,15 or n-3) also called α-linolenic acid
TAG	Triacylglycerol	ARA (AA)	Arachidonic acid (C20:4 all *cis*-5,8,11,14,17 or n-6)
TFA	*Trans* fatty acid	EPA	Eicosapentaenoic acid (C20:9 all *cis*-5,8,11,14,17 or n-3)
CLA	Conjugated linoleic acid (C18:2 *cis*-9,*trans*-11, also called rumenic acid	DHA	Docosahexaenoic acid (C22:6 all *cis*-4,7,10,13,16,19 or n-3)
SFA	Saturated fatty acids	OFA	Other fatty acids [sum of unknowns (i.e. unidentified), less important, less abundant or not considered (i.e. branched) fatty acids]. OFA are not included into TFA, SFA, MUFA and PUFA sums.

Annex B
(informative)

Examples of the gas–liquid chromatographic analysis

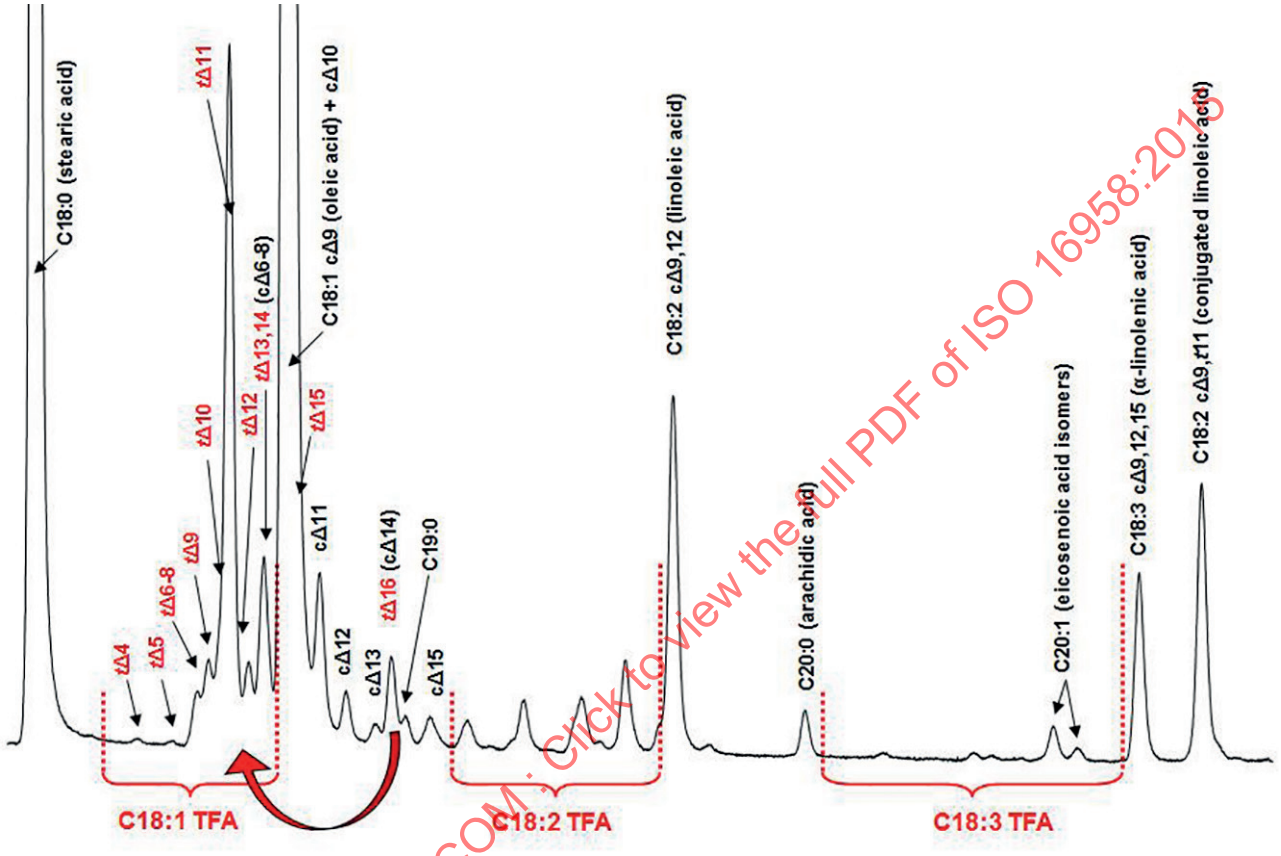


Figure B.1 — Example of GC chromatogram of milk product (enlarged view of C18:1 TFA, C18:2 TFA, C18:3 TFA and CLA) using split injection

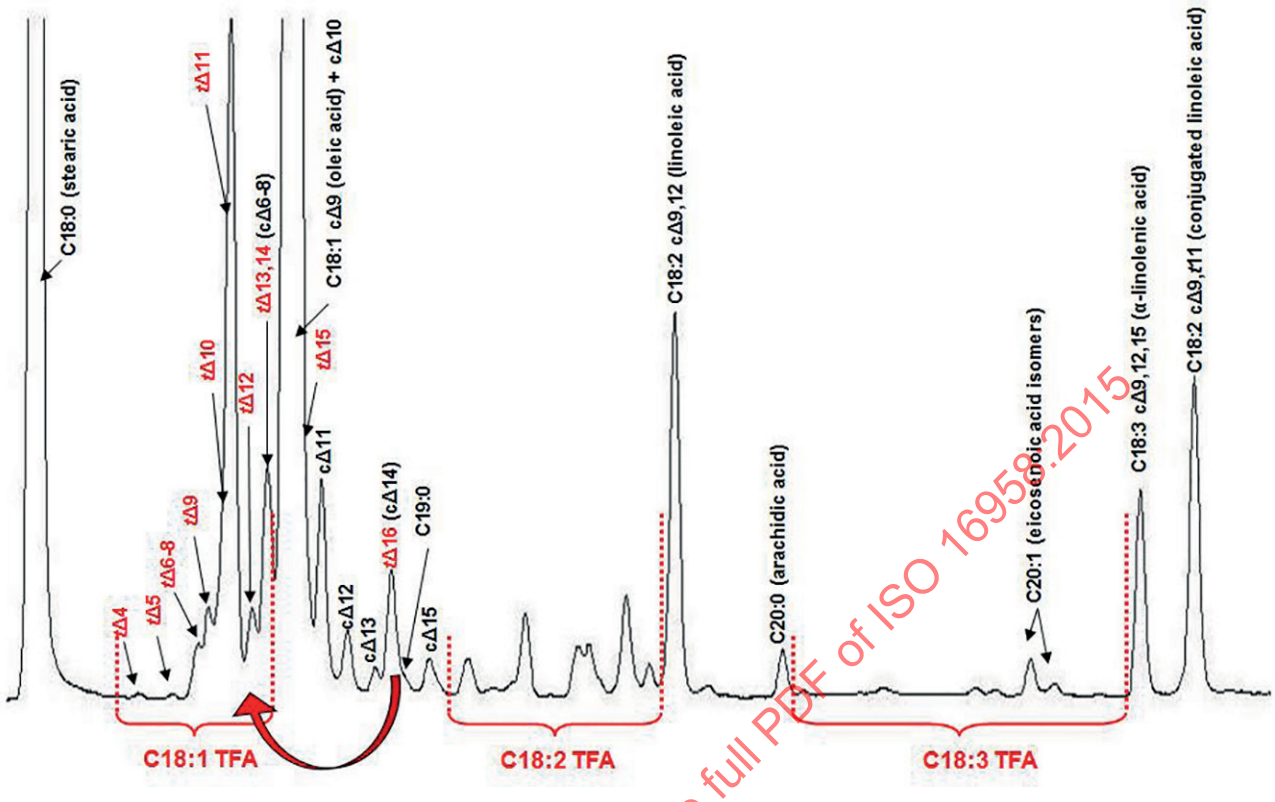


Figure B.2 — Example of GC chromatogram of milk product (enlarged view of C18:1 TFA, C18:2 TFA, C18:3 TFA and CLA) using on-column injection

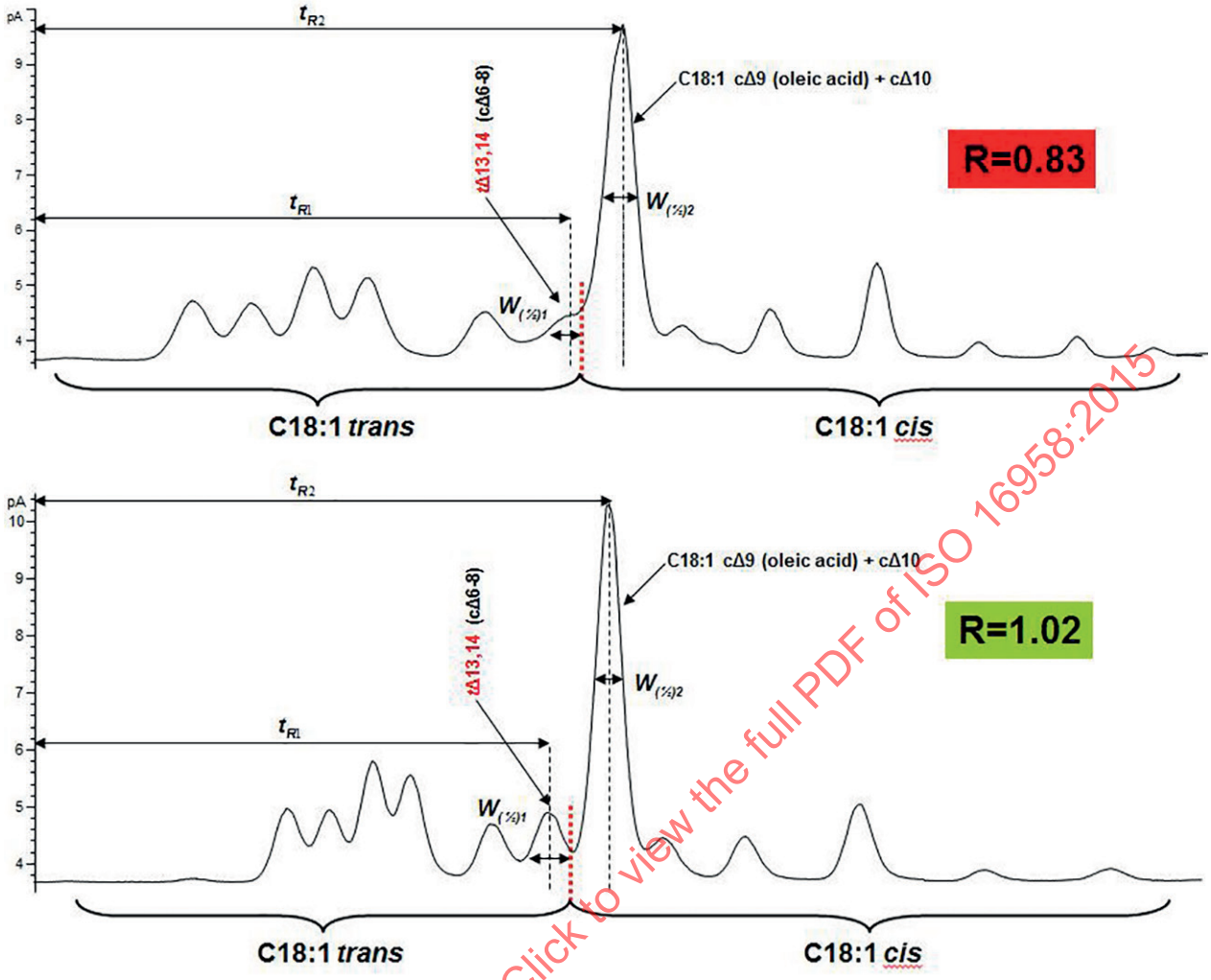


Figure B.3 — Example of GC chromatogram (insufficient and sufficient resolution between C18:1 *cis* and *trans* isomers)

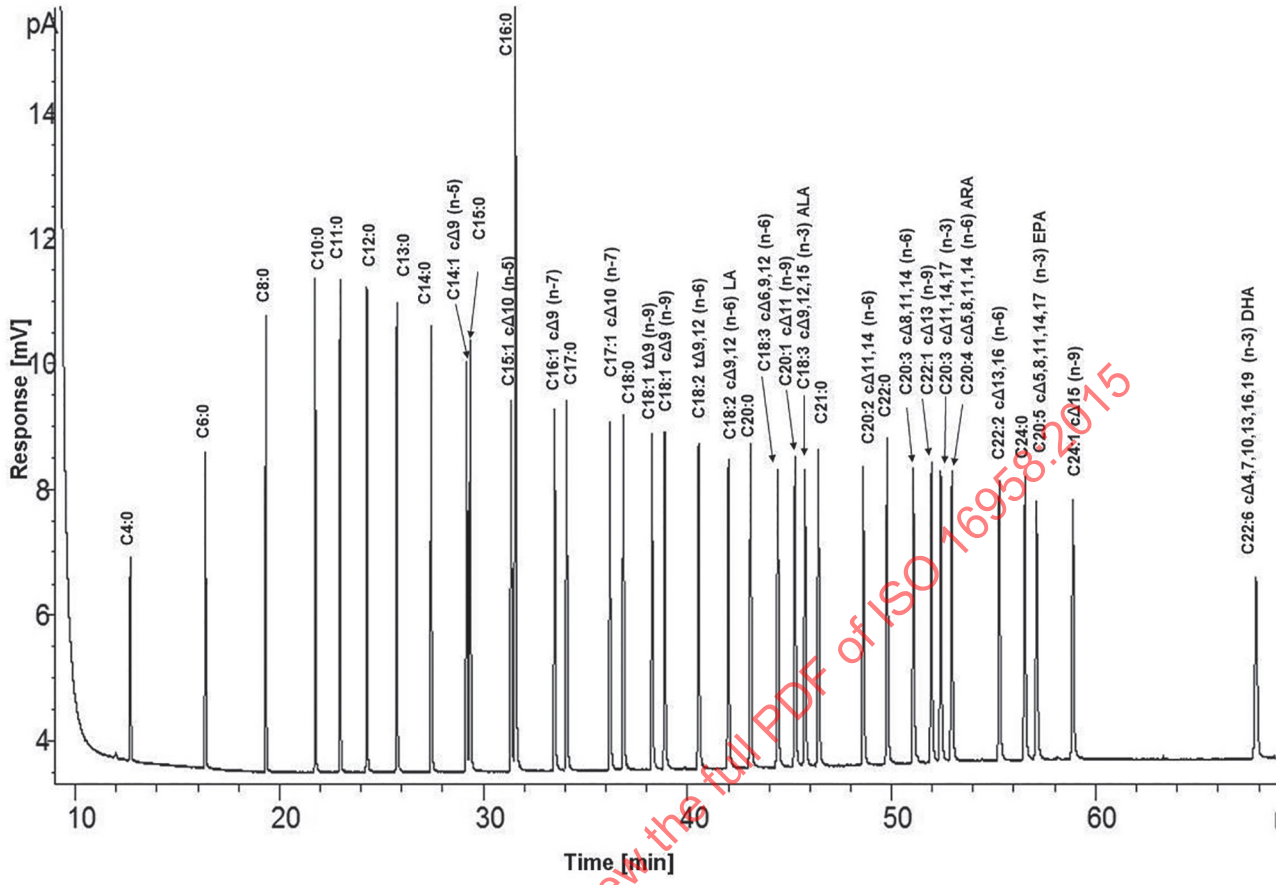


Figure B.4 — Example of GC chromatogram (GLC-Nestle36 standard) using split injection mode

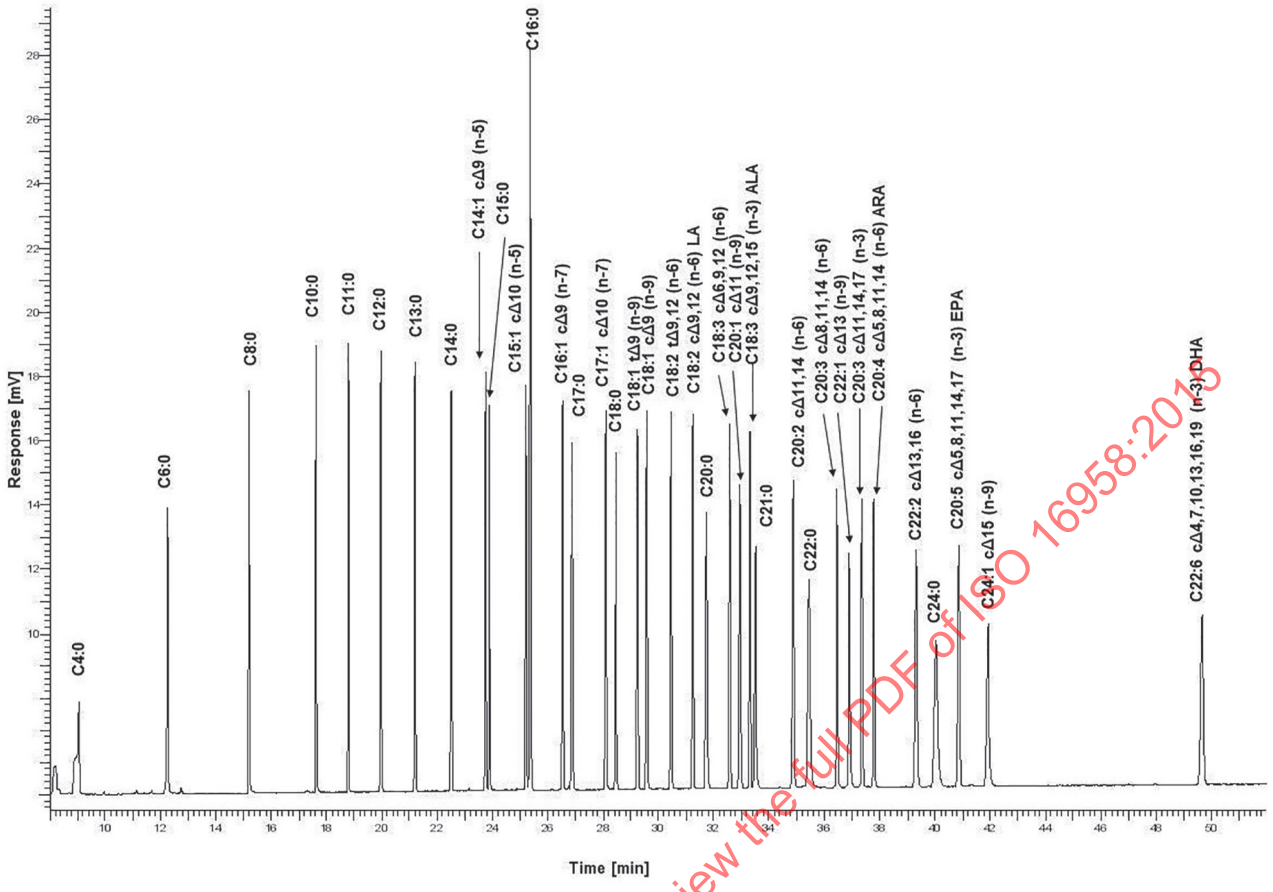
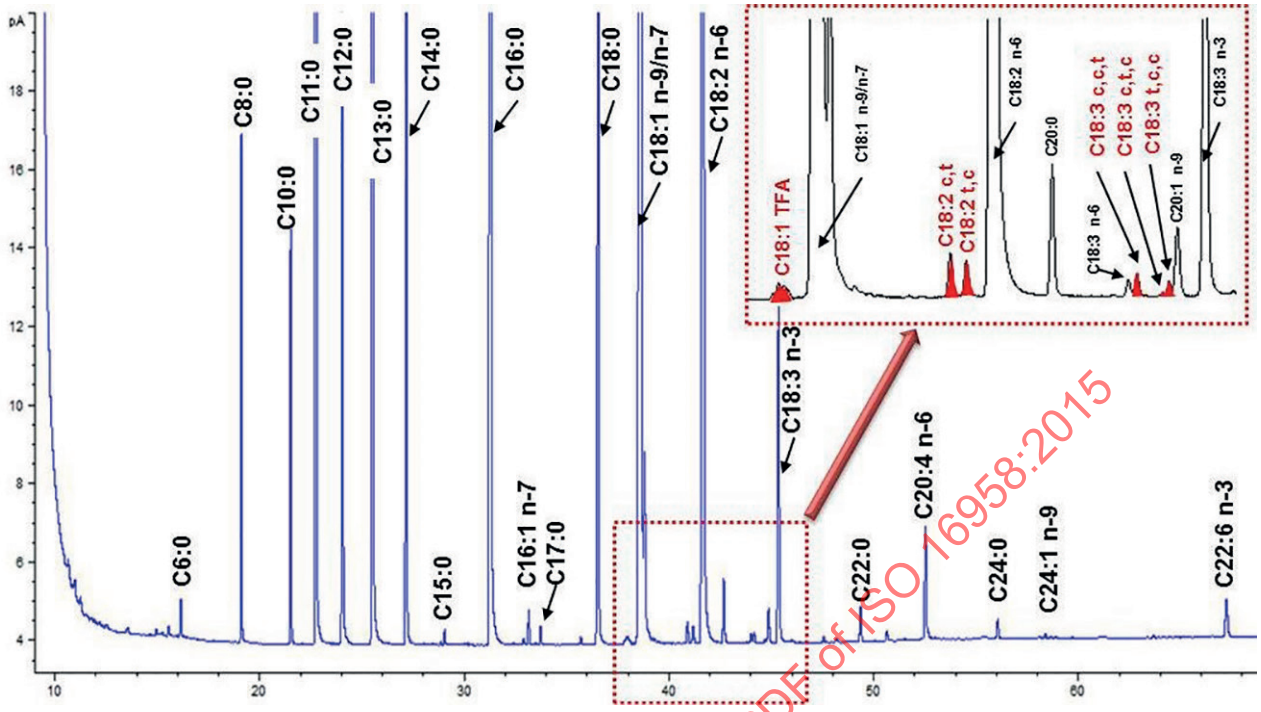


Figure B.5 — Example of GC chromatogram (GLC-Nestle36 standard) using on-column injection mode



Label	g / 100 g[a]	g / 100 g[b]
C18:2 n-6	0,532	4,788
C18:3 n-3	0,055	0,495
C20:4 n-6	0,019	0,171
C22:6 n-3	0,009	0,081
SFA	1,278	11,502
MUFA	1,075	9,675
PUFA	0,620	5,580
TFA	0,009	0,081
ω-3	0,065	0,585
ω −6	0,555	4,995
ω −9	1,069	9,621

[a] Reconstituted product (25 g + 200 g water).

[b] Powder.

NOTE Monounsaturated and polyunsaturated fatty acids are indicated counting from the terminal methyl carbon towards the carbonyl carbon (designated as n or ω).

Figure B.6 — Example of GC chromatogram of infant formula (containing deodorized vegetable oils) using split injection mode

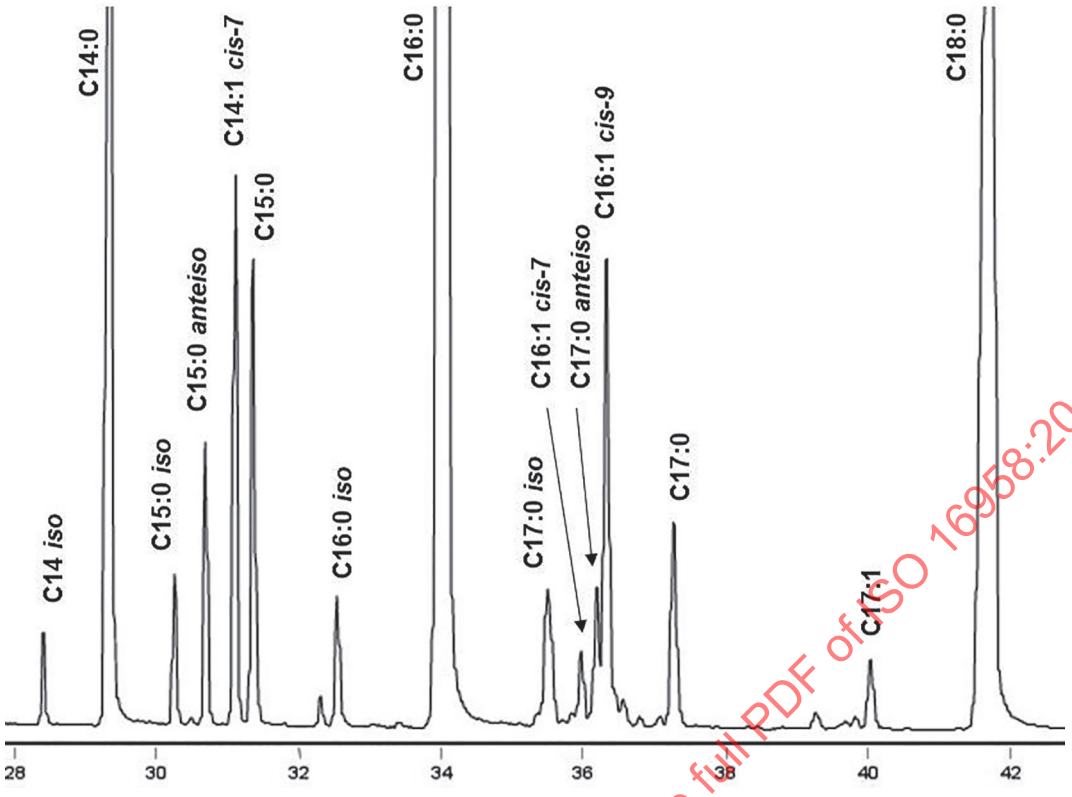


Figure B.7 — Example of GC chromatograms (enlarged view) for branched fatty acids identification in dairy products using split injection mode

Table B.1 — Stoichiometric factors (Si FA) for converting fatty acid methyl esters (FAME) to fatty acids (FA)

Chain length	Configuration and group			Abbreviation	FAME Molecular mass	FA Molecular mass	TAG Molecular mass	Si FA
C4:0			SFA		102,1	88,1	302,4	0,863
C6:0			SFA		130,2	116,2	386,5	0,892
C8:0			SFA		158,3	144,2	470,7	0,911
C10:0			SFA		186,3	172,3	554,9	0,925
C12:0			SFA		214,4	200,3	639,0	0,935
C14:0			SFA		242,4	228,4	723,2	0,942
C14:1	ω-5 (or n-5)	*cis*	MUFA		240,4	226,4	717,1	0,942
C15:0			SFA		256,4	242,4	765,3	0,945
C15:1	ω-5 (or n-5)	*cis*	MUFA		254,4	240,4	759,2	0,945
C16:0			SFA		270,5	256,4	807,3	0,948
C16:1	ω-7 (or n-7)	*cis*	MUFA		268,5	254,4	801,3	0,948
C17:0			SFA		284,5	270,5	849,4	0,951
C17:1	ω-7 (or n-7)	*cis*	MUFA		282,5	268,4	843,4	0,950
C18:0			SFA		298,5	284,5	891,5	0,953
C18:1 TFA		*trans*[a]			296,5	282,5	885,5	0,953
C18:1	ω-9 (or n-9)	*cis*	MUFA		296,5	282,5	885,5	0,953
C18:2 TFA		*trans*[a]			294,5	280,5	879,4	0,952
C18:2	ω-6 (or n-6)	*cis*	PUFA	LA	294,5	280,5	879,4	0,952
C18:2 CLA	ω-7 (or n-7)	*cis/trans*	PUFA	CLA	294,5	280,5	879,4	0,952
C18:3	ω-6 (or n-6)	*cis*	PUFA	GLA	292,5	278,4	873,4	0,952
C18:3 TFA		*trans*[a]			292,5	278,4	873,4	0,952
C18:3	ω-3 (or n-3)	*cis*	PUFA	ALA	292,5	279,4	873,4	0,952
C20:0			SFA		326,6	312,5	975,7	0,957
C20:1	ω-9 (or n-9)	*cis*	MUFA		324,6	310,5	969,6	0,957
C20:2	ω-6 (or n-6)	*cis*	PUFA		322,5	308,5	963,6	0,957
C20:3	ω-6 (or n-6)	*cis*	PUFA	DGLA	320,5	306,5	957,5	0,956
C20:3	ω-3 (or n-3)	*cis*	PUFA		320,5	306,5	957,5	0,956
C20:4	ω-6 (or n-6)	*cis*	PUFA	ARA	318,5	304,5	951,5	0,956
C20:5	ω-3 (or n-3)	*cis*	PUFA	EPA	316,5	302,5	945,4	0,956
C21:0			SFA		340,6	326,6	1 017,8	0,959
C22:0			SFA		354,6	340,6	1 059,9	0,960
C22:1	ω-9 (or n-9)	*cis*	MUFA		352,6	338,6	1 053,8	0,960
C22:2	ω-6 (or n-6)	*cis*	PUFA		350,6	336,6	1 047,8	0,960
C22:6	ω-3 (or n-3)	*cis*	PUFA	DHA	342,5	328,5	1 023,6	0,959
C24:0			SFA		382,7	368,7	1 144,0	0,963
C24:1	ω-9 (or n-9)	*cis*	MUFA		380,7	366,6	1 137,9	0,963

[a] Do not include *trans* fatty acids in MUFA and PUFA sums.

N°	Concentration in m/v (%)	Injection number	1	2	3	Response factor related to	RSD %
		File name	a	b	c		
		Injection date (dd/mm/yy)	x	x	x		
	2.70	Internal standard area	143.09	143.77	144.13	C11:0 FAME	(≤2.0)
1	2.70	C4:0	86.01	87.02	88.25	1.651	0.9
2	2.70	C6:0	106.80	107.88	108.02	1.337	0.3
3	2.70	C8:0	131.75	132.23	132.56	1.088	0.1
4	2.70	C10:0	142.31	142.25	142.88	1.009	0.3
5	2.70	C11:0	143.09	143.77	144.13	1.001	0.0
6	2.70	C12:0	146.70	145.88	147.14	0.981	0.5
7	2.70	C13:0	146.89	147.02	147.98	0.976	0.2
8	2.70	C14:0	147.76	148.45	149.03	0.969	0.1
9	2.70	C-14:1 *cis*-9 (n-5)	144.98	145.06	145.75	0.990	0.2
10	2.70	C-15:0	150.04	150.66	151.13	0.955	0.0
11	2.70	C-15:1 *cis*-10 (n-5)	145.65	146.08	147.06	0.983	0.2
12	5.40	C-16:0	300.12	299.88	301.00	0.957	0.3
13	2.70	C-16:1 *cis*-9 (n-7)	146.32	147.13	147.67	0.978	0.1
14	2.70	C-17:0	148.76	149.25	150.08	0.963	0.2
15	2.70	C-17:1 *cis*-10 (n-7)	147.32	149.01	149.78	0.967	0.5
16	2.70	C-18:0	150.01	148.99	149.09	0.963	0.7
17	2.70	C-18:1 *trans*-9 (n-9)	149.98	147.88	149.99	0.963	0.9
18	2.70	C-18:1 *cis*-9 (n-9)	151.02	149.89	150.08	0.957	0.7
19	2.70	C-18:2 all *trans*-9,12 (n-6)	151.98	150.26	151.77	0.950	0.8
20	2.70	C-18:2 all *cis*-9,12 (n-6)	149.76	150.91	151.25	0.955	0.2
21	2.70	C-18:3 all *cis*-6,9,12 (n-6)	154.67	153.98	154.45	0.932	0.5
22	2.70	C-18:3 all *cis*-9,12,15 (n-3)	149.02	148.78	148.25	0.967	0.6
23	2.70	C-20:0	154.03	156.02	155.54	0.927	0.4
24	2.70	C-20:1 *cis*-11 (n-9)	154.00	153.90	155.36	0.931	0.4
25	2.70	C-20:2 all *cis*-11,14 (n-6)	153.94	152.09	154.03	0.938	0.8
26	2.70	C-20:3 all cis 8,11,14 (n-6)	154.56	153.88	155.09	0.931	0.5
27	2.70	C-20:3 all *cis*-11,14,17 (n-3)	153.45	153.60	154.46	0.935	0.2
28	2.70	C-20:4 all *cis*-5,8,11,14 (n-6)	151.03	151.05	150.99	0.952	0.4
29	2.70	C-20:5 all *cis*-5,8,11,14,17 (n-3)	152.25	153.45	152.00	0.943	0.6
30	2.70	C-21:0	153.45	154.56	154.77	0.932	0.1
31	2.70	C-22:0	152.03	151.88	152.66	0.945	0.3
32	2.70	C-22:1 *cis*-13 (n-9)	154.56	154.89	153.88	0.931	0.6
33	2.70	C-22:2 all *cis*-13,16 (n-6)	154.65	155.05	154.33	0.930	0.5
34	2.70	C-22:6 all *cis*-4,7,10,13,16,19 (n-3)	146.88	147.14	146.99	0.978	0.3
35	2.70	C-24:0	153.40	154.07	155.33	0.932	0.3
36	2.70	C-24:1 *cis*-15 (n-9)	155.99	154.67	155.25	0.926	0.7
	99.99	Total area	5435.16	5438.51	5458.02		

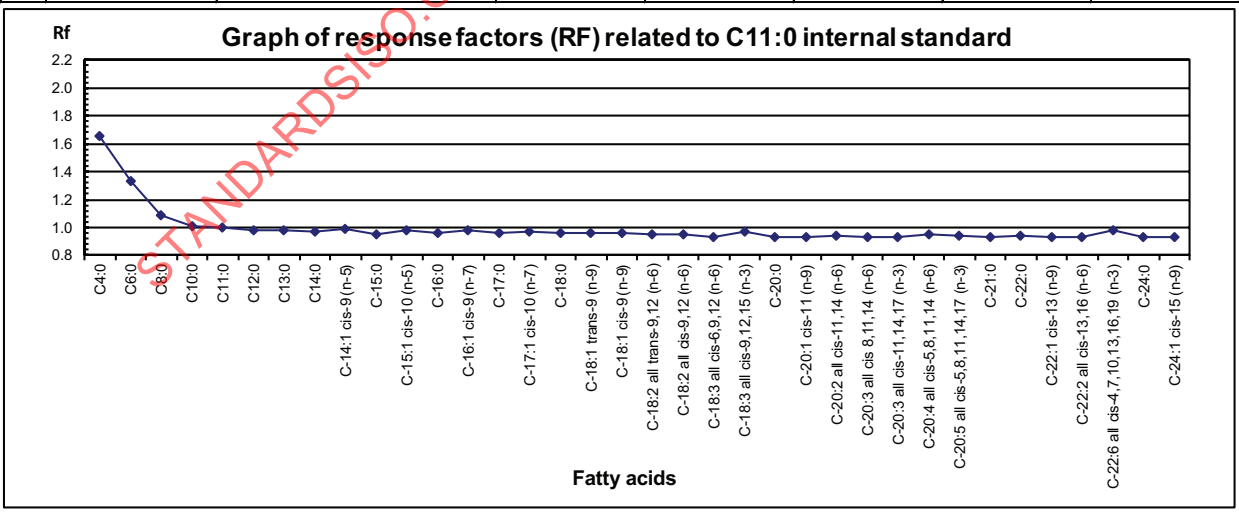


Figure B.8 — Example of calculation of response factors using a spreadsheet programme

Analysis report		Rep 1	Rep2
Sample preparation	Test portion (mg) oil or product	3104.00	3097.00
	Sample preparation date (dd/mm/yy)	x	x
	GC injection date (dd/mm/yy)	x	x
	Injection number	1	2
	File name	x	x

Instrument information	
Capillary column	x
Column size	x
Injection mode	x
Carrier gas	x

Response fators related to C11:0		Rep 1	Rep2
	C-11:0 FAME Internal standard amount (mg)	3.049	3.049
	C-11:0 FAME Internal standard area	147.00	146.55
	C-13:0 (TAG) Internal standard amount (mg)	3.042	3.042
	C-13:0 (TAG) Internal standard area	150.71	149.88
	Preparation date (dd/mm/yy)	x	x

							Product					Total FAT	Total Fatty Acids
							Rep 1	Rep 2	Mean (n=2)	SD	RSD %	Mean (n=2)	Mean (n=2)
1.651	C4:0			butyric			0.000	0.000	0.000	0.000		0.000	0.000
1.337	C6:0			caproic			0.000	0.000	0.000	0.000		0.000	0.000
1.088	C8:0			caprylic			0.000	0.000	0.000	0.000		0.000	0.000
1.009	C10:0			capric			0.000	0.000	0.000	0.000		0.000	0.000
0.981	C12:0			lauric	0.53	0.52	0.000	0.000	0.000	0.000	1.0	0.016	0.018
0.969	C14:0			myristic	10.51	10.46	0.006	0.006	0.006	0.000	0.0	0.313	0.349
0.990	C14:1	n-5 (or ω–5)	Δ9	myristoleic			0.000	0.000	0.000	0.000		0.000	0.000
0.955	C15:0			pentadecanoic	3.16	3.19	0.002	0.002	0.002	0.000	1.0	0.094	0.105
0.983	C15:1	n-5 (or ω–5)	Δ10	pentadecenoic			0.000	0.000	0.000	0.000		0.000	0.000
0.957	C16:0			palmitic	629.98	631.26	0.382	0.385	0.383	0.002	0.5	18.693	20.862
0.978	C16:1	n-7 (or ω–7)	Δ9	palmitoleic	9.21	9.24	0.006	0.006	0.006	0.000	0.6	0.280	0.312
0.963	C17:0			margaric	3.85	3.89	0.002	0.002	0.002	0.000	1.1	0.116	0.129
0.967	C17:1	n-7 (or ω–7)	Δ10	heptadecenoic	1.72	1.79	0.001	0.001	0.001	0.000	3.2	0.053	0.059
0.963	C18:0			stearic	48.62	48.94	0.030	0.030	0.030	0.000	0.8	1.463	1.633
0.963	**C18:1 TFA**			**total trans**	0.95	0.93	0.001	0.001	0.001	0.000	1.1	0.028	0.031
0.957	C18:1	n-9 (or ω–9)	Δ9	oleic & others cis	667.08	673.28	0.406	0.412	0.409	0.004	1.0	19.968	22.286
0.950	**C18:2 TFA**			**total trans**	4.86	4.99	0.003	0.003	0.003	0.000	2.2	0.146	0.163
0.955	C18:2	n-6 (or ω–6)	Δ9,12	linoleic (LA)	1446.38	1458.00	0.878	0.890	0.884	0.008	0.9	43.135	48.141
0.955	C18:2 CLA	n-7 (or ω–7)	Δ9c/11t	conjugated linoleic			0.000	0.000	0.000	0.000		0.000	0.000
0.932	C18:3	n-6 (or ω–6)	Δ6,9,12	gamma-linolenic			0.000	0.000	0.000	0.000		0.000	0.000
0.967	**C18:3 TFA**			**total trans**	1.03	1.09	0.001	0.001	0.001	0.000	4.4	0.032	0.036
0.967	C18:3	n-3 (or ω–3)	Δ9,12,15	alpha-linolenic (ALA)	63.92	64.68	0.039	0.040	0.040	0.000	1.2	1.935	2.160
0.927	C20:0			arachidic	5.37	5.36	0.003	0.003	0.003	0.000	0.2	0.155	0.174
0.931	C20:1	n-9 (or ω–9)	Δ11	eicosenoic	13.52	13.73	0.008	0.008	0.008	0.000	1.5	0.397	0.443
0.938	C20:2	n-6 (or ω–6)	Δ11,14	eicosadienoic	2.06	2.10	0.001	0.001	0.001	0.000	1.7	0.061	0.068
0.931	C20:3	n-6 (or ω–6)	Δ8,11,14	eicosatrienoic (DHGLA)			0.000	0.000	0.000	0.000		0.000	0.000
0.935	C20:3	n-3 (or ω–3)	Δ11,14,17	eicosatrienoic			0.000	0.000	0.000	0.000		0.000	0.000
0.952	C20:4	n-6 (or ω–6)	Δ5,8,11,14	arachidonic	2.44	2.50	0.001	0.002	0.002	0.000	2.1	0.073	0.082
0.943	C20:5	n-3 (or ω–3)	Δ5,8,11,14,17	eicosapentanoic (EPA)	8.38	8.33	0.005	0.005	0.005	0.000	0.0	0.246	0.275
0.945	C22:0			behenic	3.96	3.88	0.002	0.002	0.002	0.000	1.1	0.116	0.130
0.931	C22:1	n-9 (or ω–9)	Δ13	erucic			0.000	0.000	0.000	0.000		0.000	0.000
0.930	C22:2	n-6 (or ω–6)	Δ13,16	docosadienoic			0.000	0.000	0.000	0.000		0.000	0.000
0.978	C22:6	n-3 (or ω–3)	Δ4,7,10,13,16,19	docosahexaenoic (DHA)	30.08	30.29	0.019	0.019	0.019	0.000	0.9	0.926	1.033
0.932	C24:0			lignoceric	3.71	3.72	0.002	0.002	0.002	0.000	0.6	0.109	0.122
0.926	C24:1	n-9 (or ω–9)	Δ15	nervonic			0.000	0.000	0.000	0.000		0.000	0.000
0.953	OFA			other fatty acids	42.69	41.37	0.026	0.025	0.026	0.000	1.8	1.248	1.392
Total					3004.01	3023.54	1.826	1.848	1.837	0.015	0.8	89.601	100.000
Total trans fatty acids							0.004	0.004	0.004	0.000	2.1	0.206	0.230
Transesterification performance							99.7	99.5	99.6	0.2	0.2		

Extracted FAT (%) 2.05 Method used

Figure B.9 — Example of calculation of fatty acids using a spreadsheet programme

Annex C
(informative)

Results of an interlaboratory trial

An interlaboratory test on the precision of the method was organized in 2013 to 2014 by IDF/ISO and AOAC/SPIFAN in which 18 laboratories participated.[16] The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.

More information on the validation of the method can be found at http://standards.iso.org/iso/16958

The following 12 products were used for the collaborative trial:

1. Full cream milk powder (fat 26,27 %);
2. Full cream liquid milk (fat 3,55 %);
3. Full cream (fat 35,27 %);
4. Butter (fat 82,93 %);
5. Soft cheese (fat 13,29 %);
6. Infant formula powder (fat 25,67 %);
7. Adult nutritional milk protein powder (fat 17,44 %);
8. Infant formula partially hydrolyzed soy powder (fat 26,01 %);
9. Infant formula milk powder milk based (fat 28,38 %);
10. Infant formula RTF (liquid) milk based (fat 3,57 %);
11. Adult nutritional RTF (liquid) high protein (fat 3,58 %);
12. Adult nutritional RTF (liquid) high fat (fat 8,61 %).

The following abbreviations are used in the tables:

No. of laboratories	is the number of lab value considered
Mean	is mean value calculated, in g/100 g product
s_r	is the repeatability standard deviation, in g/100 g product
RSD_r	is the relative repeatability standard deviation, in %
r	is the repeatability, in g/100 g products
S_R	is the reproducibility standard deviation, in g/100 g product
RSD_R	is the relative reproducibility standard deviation, in %
R	is the reproducibility, in g/100 g product

Table C.1 contains data from the collaborative study, calculated as g fatty acids/100 g product for the group of labelled fatty acids (*trans* fatty acids (TFA), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-3, omega-6, and omega-9) and individual

fatty acids (linoleic acid (LA), α-linolenic acid (ALA), arachidonic acid (ARA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA)).

Table C.1 — Precision data for the group of labelled fatty acids

Sample no.	Product	No. of laboratories	Mean	s_r	RSD_r	r	s_R	RSD_R	R
***Trans* fatty acids (TFA) total**									
1	Full cream milk powder	17	1,032	0,035	3,4	0,098	0,115	11,2	0,322
2	Full liquid milk	17	0,167	0,005	2,8	0,013	0,015	8,7	0,041
3	Full cream	17	1,624	0,061	3,7	0,170	0,178	11,0	0,500
4	Butter	17	4,235	0,128	3,0	0,357	0,440	10,4	1,233
5	Cheese (extracted fat)	12	5,056	0,174	3,4	0,486	0,562	11,1	1,573
6	IF powder	16	0,073	0,007	9,8	0,020	0,024	32,9	0,067
7	Adult nutritional milk protein powder	15	0,056	0,007	13,0	0,020	0,013	23,5	0,037
8	IF partially hydrolyzed soy powder	18	0,091	0,015	16,6	0,042	0,036	40,0	0,101
9	IF milk based powder	17	0,109	0,007	6,4	0,019	0,032	29,2	0,089
10	IF RTF (liquid) milk based	17	0,027	0,002	8,0	0,006	0,006	21,3	0,016
11	Adult nutritional RTF (liquid) high protein	16	0,009	0,001	5,4	0,001	0,004	38,5	0,010
12	Adult nutritional RTF (liquid) high fat	11	0,010	0,001	10,0	0,003	0,004	42,5	0,012
Saturated fatty acids (SFA)									
1	Full cream milk powder	18	15,116	0,255	1,7	0,713	0,588	3,9	1,646
2	Full milk	17	1,999	0,018	0,9	0,050	0,079	4,0	0,222
3	Full cream	18	20,307	0,657	3,2	1,838	1,161	5,7	3,251
4	Butter	18	48,527	0,938	1,9	2,625	2,431	5,0	6,806
5	Cheese (extracted fat)	11	57,777	1,075	1,9	3,010	3,009	5,2	8,424
6	IF powder	16	7,309	0,106	1,4	0,297	0,174	2,4	0,486
7	Adult nutritional milk protein powder	17	1,753	0,035	2,0	0,097	0,114	6,5	0,319
8	IF partially hydrolyzed soy powder	18	9,841	0,231	2,3	0,646	0,580	5,9	1,623
9	IF milk based powder	16	11,247	0,157	1,4	0,440	0,216	1,9	0,604
10	IF RTF (liquid) milk based	16	1,433	0,018	1,2	0,050	0,033	2,3	0,091
11	Adult nutritional RTF (liquid) high protein	18	1,430	0,051	3,6	0,144	0,072	5,0	0,202
12	Adult nutritional RTF (liquid) high fat	17	1,945	0,060	3,1	0,168	0,085	4,4	0,238
Monounsaturated fatty acids (MUFA)									
1	Full cream milk powder	17	5,411	0,137	2,5	0,385	0,230	4,3	0,644
2	Full liquid milk	17	0,717	0,009	1,2	0,025	0,051	7,1	0,142
3	Full cream	18	7,253	0,265	3,7	0,743	0,638	8,8	1,787
4	Butter	17	17,041	0,535	3,1	1,498	0,881	5,2	2,468
5	Cheese (extracted fat)	11	18,894	0,356	1,9	0,997	1,309	6,9	3,666
6	IF powder	16	11,148	0,236	2,1	0,661	0,629	5,6	1,760
7	Adult nutritional milk protein powder	16	10,574	0,242	2,3	0,678	0,590	5,6	1,653
8	IF partially hydrolyzed soy powder	16	7,230	0,115	1,6	0,323	0,354	4,9	0,990
9	IF milk based powder	17	9,213	0,265	2,9	0,742	0,381	4,1	1,067
10	IF RTF (liquid) milk based	15	1,174	0,014	1,2	0,039	0,055	4,7	0,154
11	Adult Nutritional RTF (liquid) high protein	17	0,966	0,034	3,5	0,094	0,083	8,6	0,234
12	Adult Nutritional RTF (liquid) high fat	15	4,552	0,115	2,5	0,322	0,228	5,0	0,639

Table C.1 *(continued)*

Sample no.	Product	No. of laboratories	Mean	s_r	RSD_r	r	s_R	RSD_R	R
Polyunsaturated fatty acids (PUFA)									
1	Full cream milk powder	14	0,751	0,013	1,7	0,035	0,040	5,4	0,113
2	Full liquid milk	18	0,107	0,004	3,4	0,010	0,007	7,0	0,021
3	Full cream	15	1,040	0,036	3,4	0,100	0,072	6,9	0,201
4	Butter	18	2,775	0,070	2,5	0,195	0,206	7,4	0,576
5	Cheese (extracted fat)	12	2,795	0,070	2,5	0,197	0,312	11,2	0,874
6	IF powder	16	4,292	0,074	1,7	0,206	0,117	2,7	0,328
7	Adult nutritional milk protein powder	17	2,912	0,060	2,1	0,169	0,149	5,1	0,416
8	IF partially hydrolyzed soy powder	18	6,063	0,293	4,8	0,822	0,537	8,9	1,505
9	IF milk based powder	18	5,340	0,160	3,0	0,448	0,245	4,6	0,685
10	IF RTF (liquid) milk based	16	0,639	0,010	1,5	0,027	0,033	5,1	0,091
11	Adult nutritional RTF (liquid) high protein	18	0,692	0,027	3,9	0,076	0,039	5,7	0,110
12	Adult Nutritional RTF (liquid) High fat	17	1,129	0,046	4,0	0,128	0,060	5,3	0,169
Omega-3 fatty acids (ω-3)									
1	Full cream milk powder	18	0,147	0,006	3,9	0,016	0,011	7,3	0,030
2	Full liquid milk	16	0,022	0,000	1,8	0,001	0,001	6,4	0,004
3	Full cream	17	0,235	0,008	3,6	0,024	0,022	9,2	0,061
4	Butter	18	0,637	0,017	2,7	0,049	0,041	6,4	0,114
5	Cheese (extracted fat)	12	0,580	0,011	2,0	0,032	0,068	11,7	0,190
6	IF powder	16	0,524	0,008	1,5	0,022	0,023	4,5	0,066
7	Adult nutritional milk protein powder	17	0,494	0,010	2,0	0,028	0,029	5,8	0,080
8	IF partially hydrolyzed soy powder	17	0,643	0,030	4,6	0,083	0,052	8,1	0,147
9	IF milk based powder	18	0,569	0,022	3,9	0,062	0,030	5,3	0,085
10	IF RTF (liquid) milk based	18	0,059	0,004	7,0	0,012	0,005	8,4	0,014
11	Adult nutritional RTF (liquid) high protein	18	0,121	0,006	4,8	0,016	0,008	6,6	0,022
12	Adult nutritional RTF (liquid) high fat	17	0,110	0,005	4,2	0,013	0,008	7,5	0,023
Omega-6 fatty acids (ω-6)									
1	Full cream milk powder	16	0,387	0,013	3,2	0,035	0,019	5,0	0,054
2	Full liquid milk	18	0,051	0,002	3,8	0,005	0,003	6,6	0,009
3	Full cream	15	0,478	0,024	4,9	0,066	0,037	7,8	0,104
4	Butter	17	1,172	0,029	2,4	0,080	0,074	6,3	0,207
5	Cheese (extracted fat)	11	1,262	0,033	2,6	0,093	0,066	5,2	0,183
6	IF powder	16	3,764	0,071	1,9	0,200	0,108	2,9	0,301
7	Adult nutritional milk protein powder	17	2,414	0,051	2,1	0,144	0,127	5,3	0,357
8	IF partially hydrolyzed soy powder	18	5,419	0,252	4,7	0,706	0,486	9,0	1,360
9	IF milk based powder	18	4,764	0,140	2,9	0,393	0,220	4,6	0,615
10	IF RTF (liquid) milk based	16	0,579	0,008	1,4	0,023	0,029	5,0	0,080
11	Adult nutritional RTF (liquid) high protein	18	0,571	0,022	3,8	0,061	0,033	5,8	0,093
12	Adult nutritional RTF (liquid) high fat	17	1,019	0,041	4,0	0,115	0,054	5,3	0,151
Omega-9 fatty acids (ω-9)									
1	Full cream milk powder	17	4,786	0,135	2,8	0,377	0,211	4,4	0,590
2	Full liquid milk	17	0,631	0,008	1,3	0,024	0,049	7,7	0,136

Table C.1 *(continued)*

Sample no.	Product	No. of laboratories	Mean	s_r	RSD_r	r	s_R	RSD_R	R
3	Full cream	18	6,400	0,242	3,8	0,678	0,578	9,0	1,620
4	Butter	17	15,033	0,416	2,8	1,165	0,782	5,2	2,190
5	Cheese (extracted fat)	11	16,538	0,306	1,9	0,857	1,150	7,0	3,221
6	IF powder	16	11,104	0,238	2,1	0,666	0,629	5,7	1,761
7	Adult nutritional milk protein powder	16	10,542	0,241	2,3	0,676	0,588	5,6	1,646
8	IF partially hydrolyzed soy powder	16	7,195	0,115	1,6	0,323	0,352	4,9	0,985
9	IF milk based powder	17	9,166	0,264	2,9	0,740	0,379	4,1	1,061
10	IF RTF (liquid) milk based	15	1,169	0,014	1,2	0,038	0,055	4,7	0,154
11	Adult nutritional RTF (liquid) high protein	17	0,961	0,034	3,5	0,094	0,083	8,6	0,232
12	Adult nutritional RTF (liquid) high fat	15	4,543	0,115	2,5	0,321	0,228	5,0	0,639
Linoleic acid (LA, C18:2 n-6)									
1	Full cream milk powder	17	0,339	0,009	2,6	0,024	0,021	6,3	0,059
2	Full liquid milk	18	0,044	0,002	3,5	0,004	0,003	7,6	0,009
3	Full cream	16	0,421	0,019	4,6	0,054	0,046	10,9	0,129
4	Butter	18	1,025	0,033	3,3	0,094	0,079	7,8	0,223
5	Cheese (extracted fat)	11	1,036	0,025	2,4	0,071	0,122	11,8	0,343
6	IF powder	16	3,690	0,065	1,8	0,182	0,104	2,8	0,293
7	Adult nutritional milk protein powder	17	2,406	0,051	2,1	0,144	0,127	5,3	0,356
8	IF partially hydrolyzed soy powder	18	5,253	0,239	4,6	0,670	0,446	8,5	1,248
9	IF milk based powder	18	4,584	0,131	2,8	0,366	0,196	4,3	0,550
10	IF RTF (liquid) milk based	16	0,553	0,007	1,2	0,019	0,028	5,0	0,077
11	Adult nutritional RTF (liquid) high protein	18	0,569	0,021	3,7	0,059	0,033	5,8	0,093
12	Adult nutritional RTF (liquid) high fat	17	1,017	0,041	4,0	0,115	0,054	5,3	0,150
α-Linolenic acid (ALA, C18:3 n-3)									
1	Full cream milk powder	18	0,130	0,004	3,2	0,012	0,007	5,6	0,021
2	Full liquid milk	18	0,020	0,001	3,0	0,002	0,002	8,6	0,005
3	Full cream	17	0,210	0,007	3,4	0,020	0,016	7,6	0,044
4	Butter	18	0,574	0,017	2,9	0,047	0,035	6,2	0,099
5	Cheese (extracted fat)	12	0,508	0,009	1,8	0,025	0,048	9,5	0,136
6	IF powder	16	0,457	0,006	1,4	0,018	0,022	4,9	0,063
7	Adult nutritional milk protein powder	17	0,493	0,010	2,0	0,028	0,029	5,8	0,080
8	IF partially hydrolyzed soy powder	15	0,570	0,011	1,9	0,031	0,035	6,2	0,099
9	IF milk based powder	18	0,482	0,015	3,1	0,042	0,023	4,9	0,066
10	IF RTF (liquid) milk based	18	0,048	0,003	6,0	0,008	0,004	7,7	0,010
11	Adult nutritional RTF (liquid) high protein	18	0,121	0,006	4,8	0,016	0,008	6,6	0,022
12	Adult nutritional RTF (liquid) high fat	17	0,109	0,004	3,8	0,012	0,007	6,2	0,019
Arachidonic acid (ARA, C20:4 n-6)									
1	Full cream milk powder	15	0,025	0,001	4,2	0,003	0,006	25,4	0,018
2	Full liquid milk	15	0,003	0,000	3,2	0,000	0,001	19,0	0,002
3	Full cream	15	0,031	0,002	8,0	0,007	0,007	23,9	0,021
4	Butter	16	0,072	0,002	2,7	0,005	0,018	24,6	0,049
5	Cheese (extracted fat)	12	0,089	0,018	20,7	0,051	0,030	33,7	0,084

Table C.1 *(continued)*

Sample no.	Product	No. of laboratories	Mean	s_r	RSD_r	r	s_R	RSD_R	R
6	IF powder	15	0,059	0,004	6,2	0,010	0,006	10,7	0,018
7	Adult nutritional milk protein powder	Not detectable/not evaluated							
8	IF partially hydrolyzed soy powder	15	0,146	0,004	3,0	0,012	0,011	7,3	0,030
9	IF milk based powder	16	0,165	0,006	3,8	0,018	0,010	6,3	0,029
10	IF RTF (liquid) milk based	13	0,023	0,000	2,1	0,001	0,001	3,6	0,002
11	Adult nutritional RTF (liquid) high protein	Not detectable/not evaluated							
12	Adult nRTF (liquid) high fat	Not detectable/not evaluated							
Eicosapentaenoic acid (EPA, C20:5 n-3)									
1	Full cream milk powder	16	0,016	0,002	13,4	0,006	0,004	26,8	0,012
2	Full liquid milk	14	0,002	0,000	6,8	0,000	0,000	10,3	0,001
3	Full cream	14	0,023	0,001	5,0	0,003	0,004	17,3	0,011
4	Butter	15	0,055	0,003	5,5	0,009	0,007	13,4	0,021
5	Cheese (extracted fat)	12	0,069	0,007	10,6	0,020	0,018	25,3	0,049
6	IF powder	11	0,012	0,001	6,8	0,002	0,001	8,3	0,003
7	Adult nutritional milk protein powder	Not detectable/not evaluated							
8	IF partially hydrolyzed soy powder	Not detectable/not evaluated							
9	IF milk based powder	Not detectable/not evaluated							
10	IF RTF (liquid) milk based	Not detectable/not evaluated							
11	Adult nutritional RTF (liquid) high protein	Not detectable/not evaluated							
12	Adult nutritional RTF (liquid) high fat	Not detectable/not evaluated							
Docosahexaenoic acid (DHA, C22:6 n-3)									
1	Full cream milk powder	Not detectable/not evaluated							
2	Full liquid milk	Not detectable/not evaluated							
3	Full cream	Not detectable/not evaluated							
4	Butter	Not detectable/not evaluated							
5	Cheese (extracted fat)	Not detectable/not evaluated							
6	IF powder	16	0,055	0,003	6,0	0,009	0,005	8,5	0,013
7	Adult nutritional milk protein powder	Not detectable/not evaluated							
8	IF partially hydrolyzed soy powder	18	0,070	0,010	13,8	0,027	0,010	14,6	0,029
9	IF milk based powder	17	0,087	0,005	5,5	0,013	0,005	5,5	0,013
10	IF RTF (liquid) milk based	14	0,011	0,000	2,5	0,001	0,001	6,8	0,002
11	Adult nutritional RTF (liquid) high protein	Not detectable/not evaluated							
12	Adult nutritional RTF (liquid) high fat	Not detectable/not evaluated							

Table C.2 contains data from the collaborative study, calculated as g fatty acids/100 g product for all other individual fatty acids (except those given in Table C.1).

Table C.2 — Precision data for all other individual fatty acids

Sample no.	Product	No. of laboratories	Mean	S_r	RSD_r	r	s_R	RSD_R	R
C4:0									
1	Full cream milk powder	16	0,846	0,025	2,9	0,069	0,103	12,2	0,289
2	Full milk	16	0,115	0,002	2,1	0,007	0,013	11,4	0,037
3	Full cream	17	1,215	0,072	5,9	0,202	0,119	9,8	0,334
4	Butter	16	2,934	0,087	3,0	0,243	0,407	13,9	1,139
5	Cheese (extracted fat)	13	3,028	0,161	5,3	0,451	0,451	14,9	1,263
6	IF powder	Not detectable/not evaluated							
7	Adult nutritional milk protein powder	Not detectable/not evaluated							
8	IF partially hydrolyzed soy powder	Not detectable/not evaluated							
9	IF milk based powder	Not detectable/not evaluated							
10	IF RTF (liquid) milk based	Not detectable/not evaluated							
11	Adult nutritional RTF (liquid) high protein	Not detectable/not evaluated							
12	Adult nutritional RTF (liquid) high fat	Not detectable/not evaluated							
C6:0									
1	Full cream milk powder	17	0,500	0,009	1,8	0,025	0,021	4,1	0,058
2	Full liquid milk	17	0,068	0,001	1,1	0,002	0,003	3,8	0,007
3	Full cream	18	0,695	0,025	3,5	0,069	0,040	5,7	0,111
4	Butter	18	1,682	0,041	2,4	0,114	0,088	5,2	0,245
5	Cheese (extracted fat)	12	1,967	0,054	2,8	0,152	0,095	4,9	0,267
6	IF powder	17	0,039	0,003	7,1	0,008	0,004	10,7	0,012
7	Adult nutritional milk protein powder	12	0,005	0,001	12,8	0,002	0,002	30,4	0,004
8	IF partially hydrolyzed soy powder	18	0,033	0,002	5,4	0,005	0,005	14,1	0,013
9	IF milk based powder	18	0,042	0,003	6,1	0,007	0,006	15,1	0,018
10	IF RTF (liquid) milk based	17	0,005	0,000	2,2	0,000	0,001	11,3	0,002
11	Adult nutritional RTF (liquid) high protein	13	0,002	0,000	4,1	0,000	0,000	13,2	0,001
12	Adult nutritional RTF (liquid) high fat	8	0,002	0,000	12,2	0,001	0,001	32,8	0,002
C8:0									
1	Full cream milk powder	17	0,291	0,003	1,1	0,009	0,008	2,8	0,023
2	Full liquid milk	18	0,040	0,000	1,1	0,001	0,001	3,0	0,003
3	Full cream	18	0,403	0,014	3,5	0,039	0,021	5,2	0,058
4	Butter	17	0,972	0,022	2,3	0,061	0,029	3,0	0,081
5	Cheese (extracted fat)	11	1,230	0,019	1,5	0,053	0,049	4,0	0,137
6	IF powder	16	0,446	0,009	2,1	0,026	0,014	3,1	0,039
7	Adult nutritional milk protein powder	17	0,042	0,001	1,4	0,002	0,002	5,5	0,007
8	IF partially hydrolyzed soy powder	16	0,382	0,003	0,8	0,008	0,016	4,1	0,044
9	IF milk based powder	17	0,415	0,008	1,8	0,021	0,020	4,7	0,055
10	IF RTF (liquid) milk based	16	0,051	0,001	1,5	0,002	0,002	3,6	0,005
11	Adult nutritional RTF (liquid) high protein	18	0,708	0,027	3,8	0,076	0,039	5,4	0,108
12	Adult nutritional RTF (liquid) high fat	16	0,821	0,017	2,1	0,048	0,030	3,6	0,083
C10:0									