INTERNATIONAL STANDARD

ISO 23065

IDF 211

First edition
2009-03-01

Milk fat from enriched dairy products — Determination of omega–3 and omega–6 fatty acid content by gas-liquid chromatography

Matière grasse laitière de produits laitiers enrichis — Détermination de la teneur en acides gras oméga–3 et oméga–6 par chromatographie gaz-liquide

Reference numbers
ISO 23065:2009(E)
IDF 211:2009(E)

© ISO and IDF 2009

Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS

Not for Resale

PDF disclaimer

This PDF file may contain embedded typefaces. In accordance with Adobe's licensing policy, this file may be printed or viewed but shall not be edited unless the typefaces which are embedded are licensed to and installed on the computer performing the editing. In downloading this file, parties accept therein the responsibility of not infringing Adobe's licensing policy. Neither the ISO Central Secretariat nor the IDF accepts any liability in this area.

Adobe is a trademark of Adobe Systems Incorporated.

Details of the software products used to create this PDF file can be found in the General Info relative to the file; the PDF-creation parameters were optimized for printing. Every care has been taken to ensure that the file is suitable for use by ISO member bodies and IDF national committees. In the unlikely event that a problem relating to it is found, please inform the ISO Central Secretariat at the address given below.

COPYRIGHT PROTECTED DOCUMENT

© ISO and IDF 2009

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying and microfilm, without permission in writing from either ISO or IDF at the respective address below.

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

International Dairy Federation
Diamant Building • Boulevard Auguste Reyers 80 • B-1030 Brussels
Tel. + 32 2 733 98 88
Fax + 32 2 733 04 13
E-mail info@fil-idf.org
Web www.fil-idf.org

Published in Switzerland

© ISO and IDF 2009 – All rights reserved

Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

Contents

Page

© ISO and IDF 2009 – All rights reserved

Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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

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

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 23065 | IDF 211 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by ISO and IDF.

© ISO and IDF 2009 – All rights reserved
Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

Foreword

IDF (the International Dairy Federation) is a non-profit organization representing the dairy sector worldwide. IDF membership comprises National Committees in every member country as well as regional dairy associations having signed a formal agreement on cooperation with IDF. All members of IDF have the right to be represented at the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO in the development of standard methods of analysis and sampling for milk and milk products.

Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of IDF National Committees casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. IDF shall not be held responsible for identifying any or all such patent rights.

ISO 23065 | IDF 211 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF). It is being published jointly by IDF and ISO.

All work was carried out by the Joint ISO-IDF Action Team on *Fat*, of the Standing Committee on *Main components in milk*, under the aegis of its project leaders: Mrs. G. Contarini (IT).

© ISO and IDF 2009 – All rights reserved

Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

Milk fat from enriched dairy products — Determination of omega–3 and omega–6 fatty acid content by gas-liquid chromatography

1 Scope

This International Standard specifies a method for the determination of the omega–3 ($\omega-3$) and omega–6 ($\omega-6$) fatty acid content in anhydrous milk fat extracted from dairy products supplemented or naturally enriched with these constituents.

The specified procedure allows the evaluation of the most important $\omega-3$ and $\omega-6$ fatty acids.

NOTE The notations "omega-3", "ω-3" and "ω3" are erroneous, but in common use, they are equivalent to "$\omega-3$". The same is true for "omega-6", "ω-6", and "ω6", which are equivalent to "$\omega-6$".

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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

ISO 15884 | IDF 182, *Milk fat — Preparation of fatty acid methyl esters*

3 Terms and definitions

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

3.1
$\omega-3$ **fatty acid**
omega–3 fatty acid
omega-3 fatty acid (deprecated)
ω-3 fatty acid (deprecated)
ω3 fatty acid (deprecated)
polyunsaturated fatty acid having the first double bond three carbons from the terminal methyl group

3.2
$\omega-6$ **fatty acid**
omega–6 fatty acid
omega-6 fatty acid (deprecated)
ω-6 fatty acid (deprecated)
ω6 fatty acid (deprecated)
polyunsaturated fatty acid having the first double bond six carbons from the terminal methyl group

© ISO and IDF 2009 – All rights reserved

Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

3.3
$\omega-3$ and $\omega-6$ fatty acid content
mass fraction of substances determined by the procedure specified in this International Standard

NOTE The $\omega-3$ and $\omega-6$ fatty acid content is expressed as a mass fraction in milligrams per 100 g of fat of the fatty acids listed in Table 1.

Table 1 — Constituents of $\omega-3$ and $\omega-6$ fatty acid content

Chain length and double bond designation	Systematic name	Trivial name and/or abbreviated term
C18:2$\omega-6$	(9*Z*,12*Z*)-octadeca-9,12-dienoic acid	linoleic acid, LA
C18:3$\omega-3$	(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoic acid	α–linolenic acid, α–LNA
C18:4$\omega-3$	(6*Z*,9*Z*,12*Z*,15*Z*)-octadeca-6,9,12,15-tetraenoic acid	stearidonic acid
C20:5$\omega-3$	(5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-eicosa-5,8,11,14,17-pentaenoic acid	EPA
C22:5$\omega-3$	(7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-docosa-7,10,13,16,19-pentaenoic acid	DPA
C22:6$\omega-3$	(4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-docosa-4,7,10,13,16,19-hexaenoic acid	DHA

4 Principle

An internal standard is added to anhydrous milk fat. Fatty acid methyl esters (FAMEs) are prepared by transesterification. The FAMEs are separated and determined by capillary gas chromatography. Individual $\omega-3$ and $\omega-6$ fatty acids are quantified by reference to the internal standard.

5 Reagents

Use only reagents of recognized analytical grade, and only distilled or demineralized water or water of equivalent purity.

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

5.2 C23:0 (tricosanoic) FAME, of purity 99 % mass fraction.

5.3 C23:0 FAME standard solution.

Accurately weigh approximately 25 mg of C23:0 (5.2) in a 25 ml one-mark volumetric flask (6.3). Dilute to the mark with *n*-hexane (5.1) and mix. The C23:0 standard solution may be stored in a refrigerator for one month.

5.4 C20:5$\omega-3$ (EPA) FAME, of purity 99 % mass fraction.

5.5 C20:5$\omega-3$ FAME standard solution.

Accurately weigh approximately 10 mg of C20:5$\omega-3$ (5.4) in a 10 ml one-mark volumetric flask (6.3). Dilute to the mark with *n*-hexane (5.1) and mix.

5.6 C22:6$\omega-3$ (DHA) FAME, of purity 99 % mass fraction.

5.7 C22:6$\omega-3$ FAME standard solution.

Accurately weigh approximately 10 mg of C22:6$\omega-3$ (5.6) in a 10 ml one-mark volumetric flask (6.3). Dilute to the mark with *n*-hexane (5.1) and mix.

© ISO and IDF 2009 – All rights reserved
Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

5.8 C18:2$\omega-6$ (LA) FAME, of purity 99 % mass fraction.

5.9 C18:2$\omega-6$ FAME standard solution.

Accurately weigh approximately 10 mg of C18:2$\omega-6$ (5.8) in a 10 ml one-mark volumetric flask (6.3). Dilute to the mark with *n*-hexane (5.1) and mix.

5.10 Reference mixture, consisting of $\omega-3$ and $\omega-6$ fatty acid methyl esters, for qualitative evaluation, i.e. identification of retention times.

The reference mixture should contain the FAMEs of the acids listed in Table 1 together with the internal standard C23:0 methyl ester (5.2). It may be obtained by mixing 1 ml of the four standard solutions (5.3, 5.5, 5.7, and 5.9) with 1 ml of the other three solutions prepared by weighing α–linolenic, stearidonic and DPA FAMEs (purity at least 80 % mass fraction) at the same concentration in *n*-hexane (1 mg/ml).

Due to the high cost of some of these $\omega-3$ FAMEs, commercially available mixtures (including other FAMEs) may replace the reference mixture. If a commercial mixture of polyunsaturated FAMEs is used, take into account the concentration indicated and, if necessary, adjust with *n*-hexane. If not already present, add a suitable amount of C23:0 FAME standard solution (5.3).

6 Apparatus

Usual laboratory equipment and, in particular, the following.

6.1 Analytical balance, capable of being read to the nearest 0,1 mg.

6.2 Drying oven, capable of being maintained at 60 °C ± 2 °C.

6.3 One-mark volumetric flasks, of capacities 10 ml, 25 ml and 50 ml, ISO 1042 [4] class A.

6.4 One-mark pipettes, of capacities 1 ml and 5 ml, ISO 648 [1] class A.

6.5 Graduated pipette, of capacity 5 ml, ISO 835 [3] class A.

6.6 Test tube, of capacity 10 ml, fitted with polytetrafluoroethylene-lined screw cap.

6.7 Gas-liquid chromatograph, equipped with flame ionization detector and capillary split injection system or on-column.

6.7.1 Carrier gas, hydrogen or helium of purity grade at least 99,999 % mass fraction.

6.7.2 Other gases, with no detectable trace of organic impurities (hydrocarbon content less than 1 mg/kg), nitrogen and hydrogen, purity at least 99,995 % mass fraction, and synthetic air.

6.7.3 Capillary column, with a stationary phase which has been successfully employed to perform separation of FAMEs.

The use of polyethylene glycol phase capillary columns is suggested, of length 25 m, 30 m or 60 m, of internal diameter 0,25 mm or 0,32 mm and of film thickness 0,12 µm or 0,25 µm. These columns elute the FAMEs primarily by carbon chain length and secondarily by the number of double bonds. It is possible to use more polar phases, such as cyanosilicone stationary phases, but, in this case, particular care should be taken in peak identification; moreover with increasing polarity, a good separation between the C23:0 FAME, adopted as internal standard, and the C20:5$\omega-3$ FAME is increasingly difficult to obtain.

© ISO and IDF 2009 – All rights reserved

Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

6.7.4 Gas chromatographic conditions. The oven temperature and the carrier gas flow depend on the column selected and on the carrier gas adopted. In any case, the selected conditions shall produce separation between C18:4$\omega-3$ and C18:2 conjugated linoleic acid (CLA) and between DPA and DHA (see Figures A.1 and A.2). See Examples 1 and 2 for conditions.

EXAMPLE 1 Using helium as carrier gas and isothermal oven conditions, applicable conditions for correct separation of $\omega-3$ and $\omega-6$ FAMEs are:

Carrier gas:	Helium at a flow rate of 1,8 ml/min	
Column head pressure:	120 kPa	
	Fused silica capillary	
Column:	Length	30 m
	Internal diameter	0,32 mm
	Film thickness	0,25 µm
Stationary phase:	Polyethylene glycol	
Column temperature:	200 °C	
Injector and detector temperature:	260 °C	
Split ratio:	100:1	
Volume of sample injected:	1 µl	

A gas-liquid chromatogram obtained under these conditions is shown in Figure A.1.

EXAMPLE 2 Using hydrogen as carrier gas and programmed oven conditions, applicable conditions for correct separation of $\omega-3$ and $\omega-6$ FAMEs are:

Carrier gas:	Hydrogen at a constant flow rate of 1,2 ml/min	
Column head pressure:	67 kPa	
	Fused silica capillary	
Column:	Length	30 m
	Internal diameter	0,32 mm
	Film thickness	0,25 µm
Stationary phase:	(50 % cyanopropyl)-methyl polysiloxane1	
Column temperature:	Initial temperature of 150 °C maintained for 3 min	
	Raised at a rate of 4 °C/min up to 172 °C	
	Maintained at 172 °C for 1 min	
	Raised at a rate of 8 °C/min up to 210 °C	
Injector and detector temperature:	240 °C	
Split ratio:	100:1	
Volume of sample injected:	1 µl	

A gas-liquid chromatogram obtained under these conditions is shown in Figure A.2.

6.7.5 Flame ionization detector, capable of being heated to a temperature 30 °C above the final temperature of the column oven.

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

NOTE An on-column injection system can be successfully used for this analysis as well. In this case, a suitable dilution of the sample with *n*-hexane is performed before the injection (e.g. 1 ml of the solution prepared as in 9.2 with 9 ml of *n*-hexane).

© ISO and IDF 2009 – All rights reserved
Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

6.7.7 Injection syringes, of capacities 1 µl or 5 µl.

6.7.8 Integration system, preferably computerized.

7 Sampling

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

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707 | IDF 50 [2].

8 Preparation of test sample

Anhydrous milk fat is obtained in accordance with ISO 14156 | IDF 172, taking care to remove the extraction solvent completely by heating the fat to a temperature not higher than 60 °C to avoid the decomposition of polyunsaturated fatty acids.

9 Procedure

9.1 Calibration solution for the determination of LA, EPA and DHA response factor

Pipette (6.4) 1 ml of EPA FAME standard solution (5.5), 1 ml of DHA FAME standard solution (5.7), 1 ml of LA FAME standard solution (5.9) and 1 ml of internal standard C23:0 FAME solution (5.3) into a test tube (6.6) and mix.

9.2 Test portion

Melt the test sample (Clause 8) at 50 °C. Shake the melted sample for 1 min to obtain a homogeneous test sample. Weigh, to the nearest 1 mg, 100 mg of the homogenous sample in a test tube (6.6).

Before preparing FAMEs in accordance with ISO 15884 | IDF 182, pipette (6.4) 1 ml of C23:0 FAME standard solution (5.3). Consequently, add only 4 ml of *n*-hexane instead of the 5 ml specified in ISO 15884 | IDF 182. The amount of internal standard (C23:0 FAME) is about 1 % mass fraction of fat. It is useful to quantify $\omega-3$ and $\omega-6$ fatty acids ranging from 0,5 % mass fraction to 4 % mass fraction (i.e. products supplemented with fish oil).

If the concentration of one or more $\omega-3$ and $\omega-6$ fatty acids is higher than 4 % mass fraction (i.e. products supplemented with flax oil) the concentration of C23:0 FAME has to be increased. In this case, the addition of 5 ml of *n*-hexane specified in ISO 15884 | IDF 182 has to be replaced by the addition of 5 ml of C23:0 FAME solution (5.3).

At the end of the methylation procedure, transfer 2 ml of clear supernatant into a test tube (6.6) or directly into the autosampler vial. This solution is ready for the gas chromatographic analysis or can be stored in a refrigerator for up to 2 days.

9.3 Qualitative determination

Inject 1 µl of the reference mixture (5.10) into the gas chromatograph. Record the retention times of the peaks attributable to C23:0 internal standard, LA, α-LNA, stearidonic acid, EPA, DPA and DHA.

© ISO and IDF 2009 – All rights reserved

Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

9.4 Quantitative determination

9.4.1 Calculation of response factor

9.4.1.1 Inject into the gas chromatograph 1 µl of the calibration solution (9.1). Determine the area of peaks attributable to the FAMEs of C23:0, LA, EPA and DHA.

9.4.1.2 Calculate the response factor, $f_{r\omega}$, for the FAMEs of LA, EPA and DHA, respectively, by using Equation (1):

$$f_{r\omega} = \frac{m_{\omega}\ w_{\omega}\ A_{C23:0}}{m_{C23:0}\ w_{C23:0}\ A_{\omega}} \quad (1)$$

where

$A_{C23:0}$ is the peak area of the C23:0 FAME (9.4.1.1);

A_{ω} is the peak area of the FAME of LA, EPA or DHA (9.4.1.1);

$m_{C23:0}$ is the mass, in milligrams, of C23:0 FAME in the calibration solution (9.1);

m_{ω} is the mass, in milligrams, of the FAME of LA, EPA or DHA in the calibration solution (9.1);

$w_{C23:0}$ is the purity, expressed as a mass fraction in milligrams per milligram, of C23:0 FAME (5.2), e.g. $w_{C23:0}$ = 0,99;

w_{ω} is the purity, expressed as a mass fraction in milligrams per milligram, of the LA, EPA or DHA standard FAME (5.4, 5.6 or 5.8), e.g. w_{ω} = 0,99.

9.4.2 Determination of the test portion

Inject 1 µl of the test portion (9.2) into the gas chromatograph, applying the same conditions as used for the calibration solution. Determine the area of peaks attributable to the FAMEs of the C23:0 internal standard, LA, α-LNA, stearidonic acid, EPA, DPA and DHA.

Repeat the injection of the calibration solution and the $f_{r\omega}$ calculation (9.4.1.2).

9.5 Calculation and expression of results

9.5.1 By using the results of the calibration solution (9.1) injected before and after the analysis of the test portion, calculate the mean response factors, $\overline{f}_{ri}$, for the FAMEs of LA, EPA and DHA, the standard deviation and the coefficient of variation between the values. A successful determination gives coefficients of variation less than 2.

NOTE The response factor calculated for the EPA FAME is also applied to the FAMEs of α-linolenic and stearidonic acid, and that calculated for the DHA FAME is applied to the DPA FAME.

9.5.2 Calculate the mass fraction of each $\omega-3$ and $\omega-6$ fatty acid in the test portion, w_i, in milligrams per 100 g of fat, by using Equation (2):

$$w_i = \frac{m_{C23:0}\ w_{C23:0}\ A_i\ \overline{f}_{ri}}{A_{C23:0}\ m_s} \times 100\ 000 \quad (2)$$

where

© ISO and IDF 2009 – All rights reserved
Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

$A_{C23:0}$ is the peak area of the C23:0 FAME (9.4.2);

A_i is the peak area of each $\omega-3$ and $\omega-6$ FAME in the test portion (9.4.2);

$\overline{f}_{ri}$ is the mean response factor for each $\omega-3$ and $\omega-6$ FAME (9.5.1);

m_s is the mass, in milligrams, of the test portion (9.2);

$m_{C23:0}$ is the mass, in milligrams, of the C23:0 FAME standard solution in the test portion (9.2);

$w_{C23:0}$ is the purity, expressed as a mass fraction in milligrams per milligram, of the C23:0 FAME standard (5.2), e.g. $w_{C23:0}$ = 0,99.

9.6 Expression of results

Express the results to one decimal place.

10 Precision

Details of the interlaboratory test in accordance with ISO 5725-1:1994 [5] and ISO 5725-2:1994 [6] on the precision of the method are shown in Annex B.

The values for the repeatability and reproducibility limits are expressed for the 95 % probability level and may not be applicable to concentration ranges and matrices other than those given.

10.1 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 4,3 % of the mean value for $\omega-3$ and $\omega-6$ fatty acids contents of between 200 mg/100 g fat to 9 000 mg/100 g fat.

10.2 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 25,3 % of the mean value for $\omega-3$ and $\omega-6$ fatty acids contents of between 200 mg/100 g fat to 9 000 mg/100 g fat.

11 Test report

The test report shall contain at least the following information:

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

b) the sampling method used, if known;

c) the method used, 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 that may have influenced the test result(s);

e) the test result(s) obtained or, if the repeatability has been checked, the final quoted results obtained.

© ISO and IDF 2009 – All rights reserved

Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

Annex A
(informative)

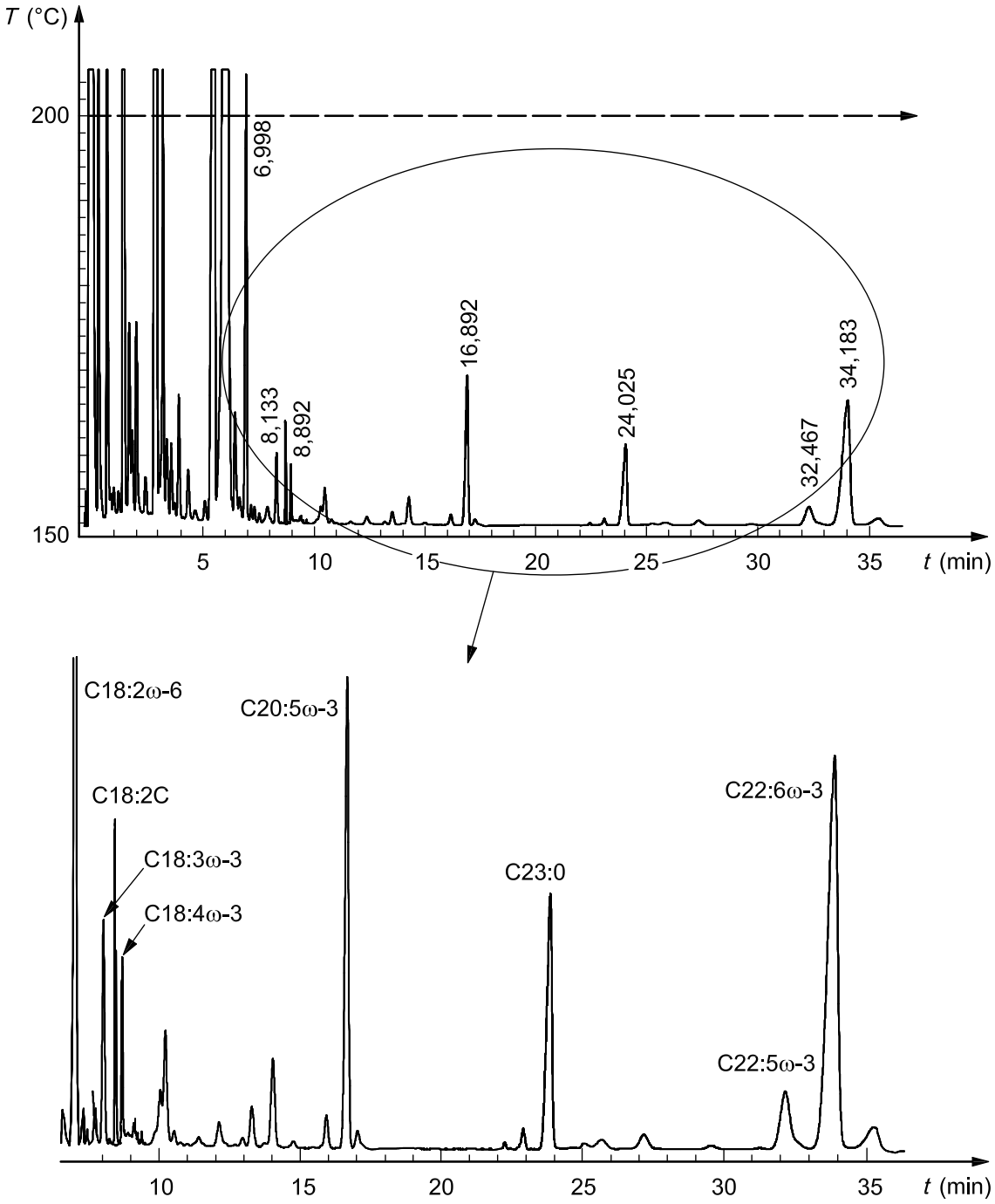
Examples of gas-liquid chromatographic analysis

--`,,```,,,,````-`-`,,`,,`,`,,`---

Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS

© ISO and IDF 2009 – All rights reserved

Not for Resale



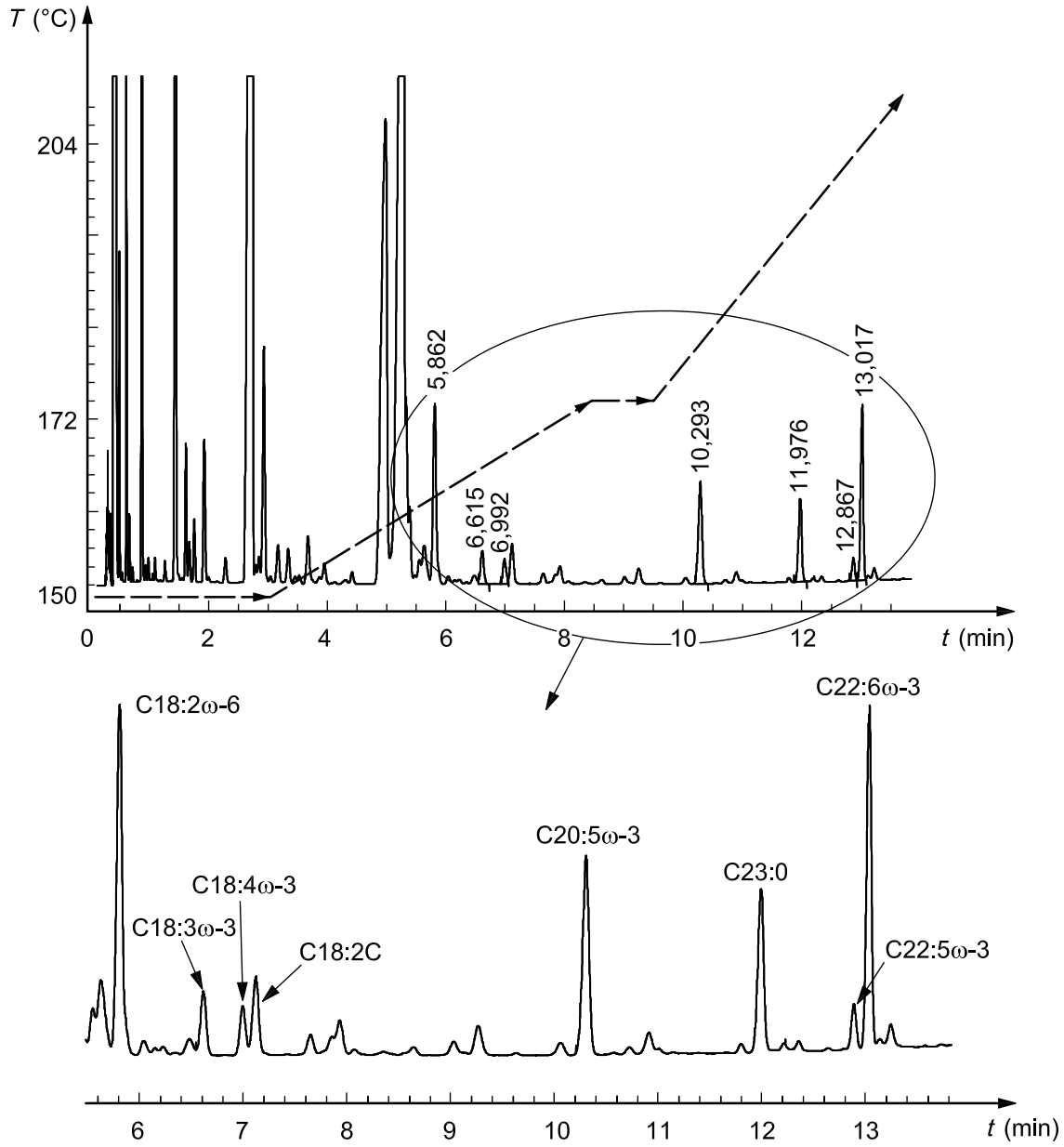
Key

C18:2C	FAME of conjugated linoleic acid
C18:2$\omega-6$	FAME of linoleic acid
C18:3$\omega-3$	FAME of α-linolenic acid
C18:4$\omega-3$	FAME of stearidonic acid
C20:5$\omega-3$	FAME of eicosapentaenoic acid
C22:5$\omega-3$	FAME of docosapentaenoic acid
C22:6$\omega-3$	FAME of docosahexaenoic acid
C23:0	FAME of tricosanoic acid (calibration standard)
T	oven temperature
t	time

Figure A.1 — Gas-liquid chromatogram of FAMEs, including an enlarged view of $\omega-3$, $\omega-6$ and CLA peaks elution zone obtained under the conditions reported in 6.7.4, Example 1

© ISO and IDF 2009 – All rights reserved

Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale



Key

C18:2C	FAME of conjugated linoleic acid
C18:2$\omega-6$	FAME of linoleic acid
C18:3$\omega-3$	FAME of α-linolenic acid
C18:4$\omega-3$	FAME of stearidonic acid
C20:5$\omega-3$	FAME of eicosapentaenoic acid
C22:5$\omega-3$	FAME of docosapentaenoic acid
C22:6$\omega-3$	FAME of docosahexaenoic acid
C23:0	FAME of tricosanoic acid (calibration standard)
T	oven temperature
t	time

Figure A.2 — Gas-liquid chromatogram of FAMEs, including an enlarged view of $\omega-3$, $\omega-6$ and CLA peaks elution zone obtained under the conditions reported in 6.7.4, Example 2

© ISO and IDF 2009 – All rights reserved
Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

Annex B
(informative)

Interlaboratory trial

Six samples of anhydrous fat (three samples in double blind) containing three mass fractions of fish oil of 8 %, 10 % and 12 % and six samples of anhydrous fat (3 samples in double blind) containing three mass fractions of flax oil of 8 %, 12 % and 16 % were prepared and distributed to 17 participating laboratories. The test was organized and evaluated by CRA – FLC Istituto Sperimentale Lattiero Caseario (IT).

Independently of the type of acid, the results were statistically evaluated based on the mass fraction expressed in milligrams per 100 g of fat, and from that, eight different levels were detected.

The results obtained were analysed statistically in accordance with ISO 5725-2:1994 [6] to give the precision data shown in Table B.1.

NOTE Detailed results of both pilot and interlaboratory study appear in Reference [7].

Table B.1 — Results of the interlaboratory test

Level	1	2	3	4	5	6	7	8
Mean value, $\overline{w}_i$	233,4	513,2	1 290,4	2 039,6	3 687,3	4 385,2	6 619,8	8 749,8
Repeatability standard deviation, s_r	7,23	10,61	23,59	43,67	58,94	53,14	130,40	113,93
Coefficient of variation of repeatability, CV(r), %	3,10	2,07	1,83	2,14	1,60	1,21	1,97	1,30
$s_r = b_r \overline{w}_i$	Regression coefficient, b_r = 0,015 3							
Repeatability limit, r (= 2,8 s_r) = (2,8 $b_r \overline{w}_i$)	0,043 $\overline{w}_i$ (4,3 % of mean)							
Reproducibility standard deviation, s_R	20,37	48,82	75,35	180,29	318,12	390,49	625,88	786,17
Coefficient of variation of reproducibility, CV(R), %	8,73	9,51	5,84	8,84	8,63	8,90	9,45	8,98
$s_R = b_R \overline{w}_i$	Regression coefficient, b_R = 0,090 4							
Reproducibility limit, R (= 2,8 s_R) = (2,8 $b_R \overline{w}_i$)	0,253 $\overline{w}_i$ (25,3 % of mean)							

--`,,```,,,,````-`-`,,`,,`,`,,`---
© ISO and IDF 2009 – All rights reserved
Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

Bibliography

[1] ISO 648, *Laboratory glassware — Single-volume pipettes*

[2] ISO 707 | IDF 50, *Milk and milk products — Guidance on sampling*

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

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

[5] ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

[6] ISO 5725-2:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

[7] CONTARINI, G. Determination of omega−3 and omega−6 fatty acid content by gas-liquid chromatography in milk fat from enriched products — Interlaboratory collaborative studies. *Bull. Int. Dairy Fed.* 2008, (428), pp. 1-17

© ISO and IDF 2009 – All rights reserved
Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

ICS 67.100.10

Price based on 12 pages

© ISO and IDF 2009 – All rights reserved
Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale