

Anhydrous milk fat — Determination of sterol composition by gas liquid chromatography (Routine method)

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**Anhydrous milk fat — Determination of
sterol composition by gas liquid
chromatography (Routine method)**

*Matière grasse anhydre du lait — Détermination de la composition
stérolique par chromatographie liquide en phase gazeuse (Méthode de
routine)*



Reference numbers
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Foreword

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

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

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

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

ISO 18252|IDF 200 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 ISO and IDF.

Foreword

IDF (the International Dairy Federation) is a worldwide federation of the dairy sector with a National Committee in every member country. Every National Committee has the right to be represented on 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 the IDF National Committees casting a vote.

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ISO 18252|IDF 200 was prepared by the International Dairy Federation (IDF) and Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. 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. M. Juarez (ES) and Mrs. G. Contarini (IT).

Anhydrous milk fat — Determination of sterol composition by gas liquid chromatography (Routine method)

WARNING — The use of this International Standard may involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this standard to establish safety and health practices and determine the applicability of regulatory limitations prior to use.

1 Scope

This International Standard specifies a routine gas liquid chromatographic method for the determination of the sterol composition in anhydrous milk fat extracted from dairy products directly on the unsaponifiable matter, without purification and derivatization.

The first goal of this International Standard is the quantitative evaluation of cholesterol, which represents about 98 % of the sterol fraction of pure milk fat. Moreover, in the case of analysis of milk fat in a mixture of vegetable fats, the specified procedure allows the evaluation of the most important phytosterols. The procedure has been validated on milk fat samples containing approximately 28 % to 32 % of vegetable fat.

Due to the absence of the purification step, which allows the complete removal of interfering compounds from the unsaponifiable matter, particular care should be taken when applying this method to the verification of the purity of milk fat of unknown origin. In the case of suspicious results, the reference method described in ISO 12078|IDF 159 can be used.

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 3696:1987, *Water for analytical laboratory use — Specification and test methods*

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

sterol composition

mass fraction of substances determined by the procedure specified in this International Standard

NOTE The sterol composition can be expressed either as milligrams per 100 g of fat, or as percent of total sterol content.

4 Principle

5 α -cholestane is added to the test sample as the internal standard. The fat is saponified with methanolic potassium hydroxide. Unsaponifiable matter is extracted by diethyl ether. It is concentrated and determined by capillary gas-liquid chromatography. Individual sterols are identified by comparison with the retention time of the reference standard sample. Sterols are quantified by reference to the internal standard.

5 Reagents

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

5.1 Water, complying with grade 2 in accordance with ISO 3696:1987.

5.2 Ethanol (C₂H₅OH), absolute.

5.3 Methanol (CH₃OH), containing a mass fraction of water of $\leq 0,5$ %.

5.4 Saponification reagent, potassium hydroxide methanolic solution, $c(\text{KOH}) = 2$ mol/l.

Dissolve 11,2 g of KOH in 100 ml of methanol (5.3) and mix well.

5.5 Sodium sulfate (Na₂SO₄), anhydrous.

5.6 Diethyl ether (C₂H₅OC₂H₅), free from peroxides.

5.7 *n*-Hexane [CH₃(CH₂)₄CH₃].

5.8 5 α -Cholestane, of purity 99 %.

5.9 5 α -Cholestane standard solution.

Accurately weigh about 60 mg of 5 α -cholestane (5.8) into a 100 ml one-mark volumetric flask (6.4). Dilute to the 100 ml mark with *n*-hexane(5.7)/ethanol (5.2) with a ratio of 1:10 or *n*-hexane (5.7) and mix.

The 5 α -cholestane standard solution may be stored in a refrigerator for one month.

5.10 Cholesterol, of purity 99 %.

5.11 Cholesterol standard solution.

Accurately weigh about 60 mg of cholesterol (5.10) into a 100 ml one-mark volumetric flask (6.4). Dilute to the 100 ml mark with ethanol (5.2) or *n*-hexane (5.7) and mix.

The cholesterol standard solution may be stored in a refrigerator for one month.

5.12 Campesterol, of purity 65 %.

5.13 Campesterol standard solution.

Accurately weigh about 10 mg of campesterol (5.12) into a 100 ml one-mark volumetric flask (6.4). Dilute to the 100 ml mark with *n*-hexane (5.7) and mix.

The campesterol standard solution may be stored in a refrigerator for one month.

5.14 Stigmasterol, of purity 95 %.

5.15 Stigmasterol standard solution.

Accurately weigh about 10 mg of stigmasterol (5.14) into a 100 ml one-mark volumetric flask (6.4). Dilute to the 100 ml mark with *n*-hexane (5.7) and mix.

The stigmasterol standard solution may be stored in a refrigerator for one month.

5.16 β -Sitosterol, of purity 95 %.

5.17 β -Sitosterol standard solution.

Accurately weigh about 10 mg of β -sitosterol (5.16) into a 100 ml one-mark volumetric flask (6.4). Dilute to the 100 ml mark with *n*-hexane (5.7) and mix.

The β -sitosterol standard solution may be stored in a refrigerator for one month.

NOTE Since the phytosterol standard solutions (5.13, 5.15 and 5.17) are used only for a qualitative evaluation, they may be replaced by sterols prepared from soya oil having campesterol, stigmasterol and β -sitosterol as major components.

6 Apparatus

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

Usual laboratory apparatus and, in particular, the following.

- 6.1 **Drying oven**, capable of being maintained at a temperature of between 50 °C and 70 °C.
- 6.2 **Analytical balance**, capable of weighing to the nearest 1 mg, with a readability of 0,1 mg.
- 6.3 **Round-bottomed flasks**, with ground neck, of capacity 100 ml.
- 6.4 **One-mark volumetric flasks**, of capacity 100 ml.
- 6.5 **Graduated pipettes**, of capacity 10 ml.
- 6.5 **One-mark pipettes**, of capacity 1 ml.
- 6.7 **Water bath**, capable of being maintained at 50 °C \pm 2 °C and of boiling.
- 6.8 **Reflux condenser**, to fit the round-bottomed flask (6.3).
- 6.9 **Separating funnels**, of capacity 100 ml.
- 6.10 **Solvent dispenser** or **graduated cylinders**, of capacities 10 ml and 20 ml.
- 6.11 **Distillation** or **evaporation apparatus** (e.g. rotary vacuum evaporator), for distilling or evaporating solvents, maintaining all temperatures up to boiling.
- 6.12 **Glass funnels**, of diameter 100 mm.
- 6.13 **Dry filter paper**, folded, fast grade, of diameter 200 mm.
- 6.14 **Vials**, cone shaped inside, of capacities 5 ml and 10 ml.
- 6.15 **Nitrogen supply**, with gas purity of at least 99 %.

6.16 Test tube, with PTFE-lined screw cap, of capacity 50 ml.

6.17 Gas liquid chromatography.

6.17.1 Injector.

Maintain the vaporizing type injector (split/splitless), which is equipped with a thermo-stable septum, at a temperature of 30 °C above the maximum oven temperature. In the case of a cold on-column injector, maintain the injector at a temperature several degrees below the boiling point of the solvent.

6.17.2 Oven, capable of being run with the temperature programmed between 50 °C and 320 °C.

6.17.3 Column, fused silica capillary column.

Different types of stationary phase, film thickness, column length, and diameter may be used to successfully obtain sterol separation. In any case, the selected column shall produce both a complete separation between the solvent peak and 5 α -cholestane (i.e. 5 α -cholestane shall not elute in the broadening of the solvent) and a baseline resolution among the cholesterol, campesterol, stigmasterol and β -sitosterol peaks. Moreover, no baseline bleeding shall appear during the whole gas chromatographic (GC) run.

An example of a correct GC profile, obtained by using the operative conditions listed in 6.18.2, is shown in Figure A.1.

NOTE Commercial stationary phases containing dimethylpolysiloxane or dimethylpolysiloxane, together with different percentages of phenyl/cyanopropyl-polysiloxane, are suitable.

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

6.17.5 Carrier gas, nitrogen, helium or hydrogen, of purity at least 99,999 %

6.17.6 Other gases, free from organic impurities (C_nH_m) of below 1 parts per million), nitrogen and hydrogen, of purity at least 99,995 %, and synthetic air.

6.17.7 Injection syringe, of capacity 1 μ l to 10 μ l.

6.17.8 Integration system, preferably computerized.

6.18 Gas chromatographic conditions.

Follow the manufacturer's instructions for the instrument set-up. The oven temperature and the carrier gas flow depend on the column selected and on the injection system adopted. The examples listed below report applicable conditions for split and on-column injection systems.

6.18.1 Split injector.

An example of applicable conditions using a split injector is:

- a) Carrier gas: helium;
- b) Column head pressure: 90 kPa;
- c) Column: fused silica capillary column, of length 25 m, of internal diameter 0,32 mm, of film thickness 0,25 μ m;
- d) Stationary phase: 5 % phenyl and 95 % dimethylpolysiloxane;
- e) Column temperature: isothermal, set at 280 °C;

- f) Detector temperature: set at 310 °C;
- g) Injector temperature: set at 310 °C;
- h) Split ratio: of ratio 1:40;
- i) Sample injected: 0,5 µl.

6.18.2 On-column injector.

An example of applicable conditions using an on-column injector is:

- a) Carrier gas: hydrogen;
- b) Column head pressure: 30 kPa;
- c) Column: fused silica capillary column, of length 30 m, of internal diameter 0,32 mm, of film thickness 0,25 µm;
- d) Stationary phase: 5 % phenyl and 95 % dimethylpolysiloxane;
- e) Column temperature: set initial temperature at 60 °C for 2 min, set first gradient at 40 °C per min, stop temperature increase at 220 °C for 2 min, set second gradient at 5 °C per min, stop temperature increase at 310 °C;
- f) Detector temperature: 330 °C;
- g) Sample injected: 1 µl.

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.

8 Preparation of test sample

Prepare the test sample in accordance with the procedure in ISO 14156|IDF 172. Melt the sample in the drying oven (6.1) set at 50 °C.

9 Procedure

9.1 Sterol standard solutions

9.1.1 Calibrating solution for the determination of cholesterol response factor

Transfer, with a one-mark pipette (6.6), 1 ml of cholesterol standard solution (5.11) to a 5 ml vial (6.14). Add, with another one-mark pipette (6.6), 1 ml of 5 α -cholestane (internal) standard solution (5.9) and mix. Remove the solvents under a gentle stream of nitrogen while warming on the water bath (6.7) set at 50 °C.

9.1.2 Qualitative solution for the determination of sterol retention time

Transfer, with separate one-mark pipettes (6.6), 1 ml of each of the sterol standard solutions prepared in 5.11, 5.13, 5.15 and 5.17, respectively, into a 10 ml vial (6.14). Add, with another one-mark pipette (6.6), 1 ml of 5 α -cholestane (internal) standard solution (5.9) and mix. Remove the solvents under a gentle stream of nitrogen.

When using soya oil instead of pure standards, use the same procedure as described for the test portion in 9.2.

9.2 Test portion

Shake the melted test sample (Clause 8) for 1 min to obtain a homogeneous sample. Weigh, to the nearest 1 mg, about 200 mg of the test sample prepared in this way into a round-bottomed flask (6.3).

9.3 Saponification

Add, with the one-mark pipette (6.6), 1 ml of 5 α -cholestane standard solution (5.9) and 10 ml of saponification reagent (5.4) to the test portion (9.2). Fit the round-bottomed flask to the reflux condenser (6.8). Boil gently on the water bath (6.7) set at 80 °C for 1 h.

NOTE When bumping is expected, any anti-bumping granules may be added.

9.4 Extraction of the unsaponifiable matter

Cool the round-bottomed flask to 35 °C. Transfer the test solution quantitatively to the separating funnel (6.9). Add 10 ml of diethyl ether (5.6) and 20 ml of distilled water. Use a portion of each of the formerly mentioned solvents to rinse the flask in order to avoid sample losses. Shake vigorously while degassing frequently. Allow layers to separate and to clarify completely.

Drain the lower aqueous layer into a second separating funnel (6.9). Extract the obtained soap solution with 10 ml of diethyl ether (5.6) in the same manner as described above. Carry out a third extraction with 5 ml of diethyl ether.

Combine the ether extracts in another separating funnel (6.9). Add 10 ml of water and shake gently (violent shaking at this stage may cause formation of an emulsion). After the separation of layers, drain the water layer. Wash the ether solution twice with 5 ml of water each time. If an emulsion forms during washing, add several drops of ethanol (5.2).

Put a folded filter paper (6.13) filled with 10 g of sodium sulfate (5.5) into a glass funnel (6.12). Filter the ether solution through the sodium sulfate into a round-bottomed flask (6.3). Rinse the separating funnel with 5 ml of diethyl ether (5.6). Remove the solvent with a suitable apparatus (6.11) while gently warming at 50 °C. Add 2 ml of diethyl ether (5.6) with the graduated pipette (6.5) and mix. Quantitatively transfer the unsaponifiable matter to a vial (6.14). Repeat the rinsing of the flask with 1 ml of diethyl ether (5.6). Remove the solvent from the vial under a gentle stream of nitrogen. Dissolve the residue in 0,25 ml of *n*-hexane (5.7) when using the split injector, and in 3 ml of *n*-hexane when using the on-column injector. The test solution obtained in this way is ready for injection into the gas chromatograph.

9.5 Qualitative analysis

Dissolve the dried sterol standard solution (9.1.2) with the same amount of *n*-hexane (5.7) as used in 9.4 and mix. Inject the obtained solution into the gas chromatograph. Record the retention times of the reference sterols. Analyse the test portion (9.2) under the same conditions as for the sterol standard solution.

NOTE 1 The order of elution of the major sterols is the following: cholesterol, campesterol, stigmasterol and β -sitosterol. The internal standard 5 α -cholestane elutes before the cholesterol. The chromatogram given in Figure A.1 is an aid for the identification of these sterols.

NOTE 2 If soya oil is used to identify the phytosterols, analysing the calibrating solution (9.1.1) can permit the determination of the retention time of cholesterol.

Identify the peaks of the test sample by comparison of the retention data obtained with sterol standard solution

9.6 Quantitative analysis

9.6.1 Calculation of response factor

Dissolve the calibrating solution (9.1.1) in the same amount of n-hexane (5.7) as used in 9.4 and mix. Inject the obtained solution into the gas chromatograph. Determine the area of peaks attributable to 5 α -cholestane and cholesterol. Calculate the response factor, F_r , expressed to two decimal places, by using the following equation:

$$F_r = \frac{(w_c \times P_c) \times A_{5\alpha}}{(m_{5\alpha} \times P_{5\alpha}) \times A_c}$$

where

w_c is the mass fraction of cholesterol in the calibrating solution (9.1.1);

$w_{5\alpha}$ is the mass fraction of 5 α -cholestane in calibrating solution (9.1.1);

$A_{5\alpha}$ is the numerical value of the peak area of 5 α -cholestane;

A_c is the numerical value of the peak area of cholesterol;

P_c is the numerical value of the purity of the cholesterol standard (5.10), (e.g. $P_c = 0,99$);

$P_{5\alpha}$ is the numerical value of the purity of the 5 α -cholestane standard (5.8), (e.g. $P_{5\alpha} = 0,99$).

NOTE The response factor calculated for cholesterol will also be applied for the other sterols (campesterol, stigmasterol and β -sitosterol)

9.6.2 Determination of the test portion

Analyse the test portion under the same conditions as used in the calibrating solution. Determine the area of peaks attributable to 5 α -cholestane, cholesterol and other sterols, if detectable.

Repeat the injection of the calibrating solution and the calculation of F_r as described in 9.6.1.

9.6.3 Calculation and expression of results

9.6.3.1 Calculation of sterols as mass fraction

Calculate the average response factor for cholesterol, $F_{r,a}$, the standard deviation and the coefficient of variation between values. A successful determination gives a response factor close to 1 and a coefficient of variation less than 2. Calculate for each sterol the mass fraction, w_i , by using the following equation:

$$w_i = \frac{(w_{5\alpha} \times P_{5\alpha}) \times A_i \times F_{r,a}}{A_{5\alpha} \times m_s} \times 100$$

where

w_i is the mass fraction of each sterol (cholesterol, campesterol, stigmasterol, β -sitosterol) in the sample, in milligrams per 100 g of fat;

$w_{5\alpha}$ is the mass fraction of 5 α -cholestane standard solution added to the test portion, in milligrams (9.3);

$P_{5\alpha}$ is the numerical value of the purity of the 5 α -cholestane standard (5.8) (e.g. $P_{5\alpha} = 0,99$);

A_i is the numerical value of the peak area of each sterol in the test portion (9.6.2);

$F_{r,a}$ is the average value of the cholesterol response factor;

$A_{5\alpha}$ is the numerical value of the peak area of 5 α -cholestane (9.6.2);

m_s is the mass of the test portion, in grams (9.2).

9.6.3.2 Calculation of sterols as percentage of total sterols

Calculate each mass fraction of sterol, w_i , as a percentage of the total mass fraction of sterols by using the following equation

$$w_i = \frac{A_i \times F_{r,a}}{\sum (A_i \times F_{r,a})} \times 100 \%$$

9.6.3.3 Expression of results

Express the results to one decimal place.

10 Precision

10.1 Interlaboratory test

Details of an interlaboratory test, in accordance with ISO 5725-1 and ISO 5725-2, on the precision of the method are summarized in Annex B.

The values for 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.

10.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases be greater than:

- for cholesterol: $r = 14,5$ mg/100 g of fat; $s_r = 5,20$ mg/100 g of fat;
- for campesterol: $r = 1,9$ mg/100 g of fat; $s_r = 0,68$ mg/100 g of fat;
- for stigmasterol: $r = 1,4$ mg/100 g of fat; $s_r = 0,50$ mg/100 g of fat;
- for β -sitosterol: $r = 3,8$ mg/100 g of fat; $s_r = 1,37$ mg/100 g of fat.

10.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than:

- for cholesterol: $R = 32,6$ mg/100 g of fat; $s_R = 11,66$ mg/100 g of fat;
- for campesterol: $R = 6,5$ mg/100 g of fat; $s_R = 2,34$ mg/100 g of fat;
- for stigmasterol: $R = 3,9$ mg/100 g of fat; $s_R = 1,39$ mg/100 g of fat;
- for β -sitosterol: $R = 9,5$ mg/100 g of fat; $s_R = 3,39$ mg/100 g of fat.

11 Test report

The test report shall specify:

- a) all information necessary for the complete identification of the sample;
- b) the sampling method used, if known;
- 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 result obtained.

Annex A
(informative)

Example of the gas-liquid chromatographic analysis

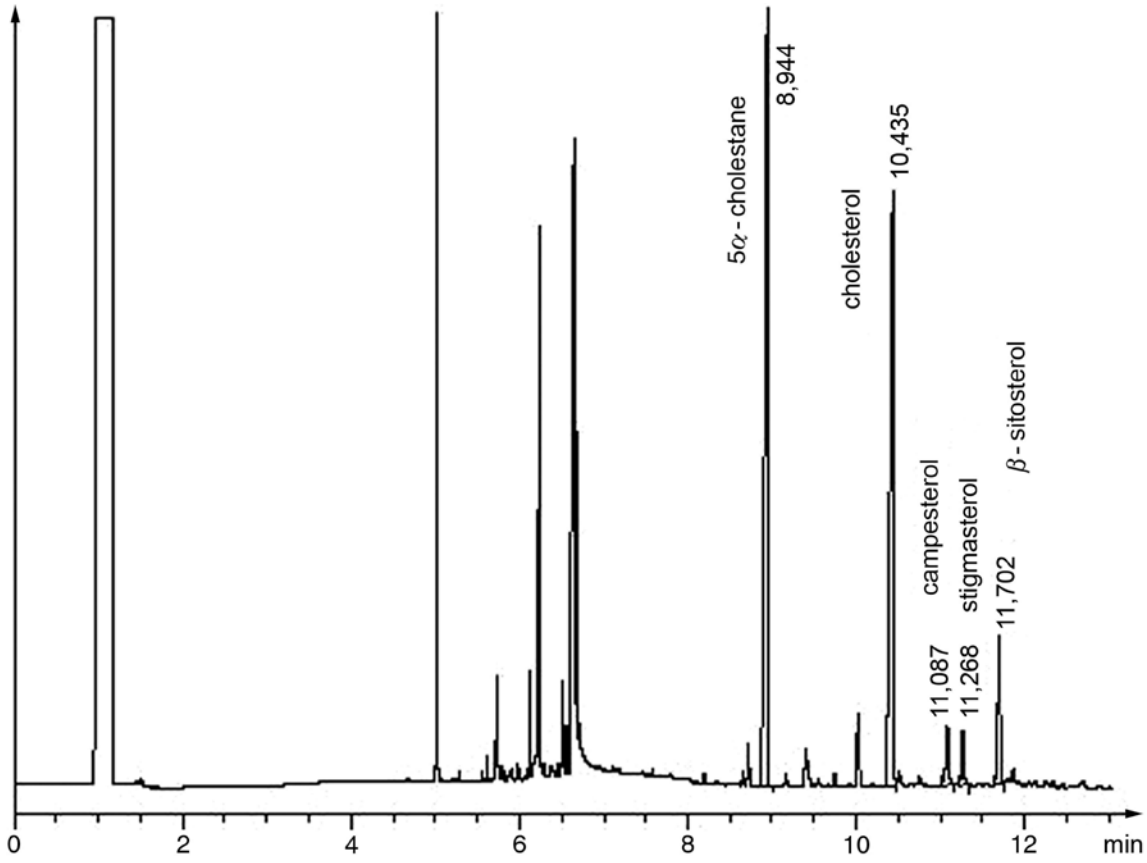


Figure A.1 — Example of a GC profile of sterols obtained by the GC conditions reported in 6.18.2

Annex B (informative)

Results of an interlaboratory trial

An international collaborative test involving 13 laboratories was carried out in accordance with ISO 5725-1 and ISO 5725-2 on three different samples of anhydrous milk fat containing 28 % to 32 % of vegetable fat. The three fat mixtures were divided into six blind duplicated samples.

The test was organized and evaluated by Instituto del Frio (CSIC) (ES) and Instituto Sperimentale Lattiero Caseario (IT). The results for the mass fraction of cholesterol, campesterol, stigmasterol and β -sitosterol were expressed as milligrams per 100 g of fat.

The obtained results were subjected to statistical analysis in accordance with ISO 5725-2 to give the precision data shown in Table B.1.

Table B.1 — Results of interlaboratory test

Cholesterol			
	Sample 1	Sample 2	Sample 3
Number of participating laboratories after eliminating outliers	9	10	10
Mean value (mg/100 g fat)	187,0	179,9	181,5
Repeatability standard deviation, s_r (mg/100 g fat)	1,69	5,87	8,02
Coefficient of variation of repeatability (%)	0,9	3,3	4,4
Repeatability limit, r ($= 2,8 s_r$) (mg/100 g fat)	4,7	16,4	22,5
Reproducibility standard deviation, s_R (mg/100 g fat)	13,67	10,69	10,62
Coefficient of variation of reproducibility (%)	7,3	5,9	5,8
Reproducibility limit, R ($= 2,8 s_R$) (mg/100 g fat)	38,3	29,9	29,7

Campesterol			
	Sample 1	Sample 2	Sample 3
Number of participating laboratories after eliminating outliers	11	10	12
Mean value (mg/100 g fat)	13,1	14,7	15,5
Repeatability standard deviation, s_r (mg/100 g fat)	0,57	0,79	0,69
Coefficient of variation of repeatability (%)	4,4	5,3	4,5
Repeatability limit, r ($= 2,8 s_r$) (mg/100 g fat)	1,6	2,2	1,9
Reproducibility standard deviation, s_R (mg/100 g fat)	1,95	2,29	2,76
Coefficient of variation of reproducibility (%)	14,9	15,6	17,8
Reproducibility limit, R ($= 2,8 s_R$) (mg/100 g fat)	5,5	6,4	7,7

Table B.1 (continued)

Stigmasterol			
	Sample 1	Sample 2	Sample 3
Number of participating laboratories after eliminating outliers	10	8	11
Mean value (mg/100 g fat)	10,1	10,7	12,8
Repeatability standard deviation, s_r (mg/100 g fat)	0,49	0,52	0,48
Coefficient of variation of repeatability (%)	4,9	4,8	3,7
Repeatability limit, r (= 2,8 s_r) (mg/100 g fat)	1,4	1,4	1,3
Reproducibility standard deviation, s_R (mg/100 g fat)	1,41	1,37	1,39
Coefficient of variation of reproducibility (%)	14,0	12,7	10,9
Reproducibility limit, R (= 2,8 s_R) (mg/100 g fat)	3,9	3,8	3,9

β-Sitosterol			
	Sample 1	Sample 2	Sample 3
Number of participating laboratories after eliminating outliers	12	10	8
Mean value (mg/100 g fat)	32,9	35,2	40,8
Repeatability standard deviation, s_r (mg/100 g fat)	1,70	1,19	1,21
Coefficient of variation of repeatability (%)	5,2	3,4	3,0
Repeatability limit, r (= 2,8 s_r) (mg/100 g fat)	4,8	3,3	3,4
Reproducibility standard deviation, s_R (mg/100 g fat)	4,71	4,00	1,45
Coefficient of variation of reproducibility (%)	14,3	11,4	3,6
Reproducibility limit, R (= 2,8 s_R) (mg/100 g fat)	13,2	11,2	4,1

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- [1] ISO 707|IDF 50, *Milk and milk products — Guidance on sampling*
- [2] ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*
- [3] ISO 5725-2, *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*
- [4] ISO 6799, *Animal and vegetable fats and oils — Determination of composition of the sterol fraction — Method using gas chromatography*
- [5] ISO 12078|IDF 159, *Anhydrous milk fat — Determination of sterol composition by gas liquid chromatography (Reference method)*

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