

---

---

एकल और कुल स्टेरोल की मात्रा का  
निर्धारण — गैस क्रोमैटोग्राफिक पद्धति

भाग 1 पशु और वनस्पति वसा और तेल

**Determination of Individual and  
Total Sterols Contents — Gas  
Chromatographic Method**

**Part 1 Animal and Vegetable Fats and  
Oils**

ICS 67.200.10

© BIS 2024

© ISO 2014



भारतीय मानक ब्यूरो

BUREAU OF INDIAN STANDARDS  
मानक भवन, 9 बहादुर शाह ज़फर मार्ग, नई दिल्ली - 110002

MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG  
NEW DELHI - 110002

[www.bis.gov.in](http://www.bis.gov.in) [www.standardsbis.in](http://www.standardsbis.in)

## NATIONAL FOREWORD

This Indian Standard (Part 1) which is identical to ISO 12228-1 : 2014 'Determination of individual and total sterols contents — Gas chromatographic method — Part 1: Animal and vegetable fats and oils' issued by the International Organization for Standardization (ISO) was adopted by the Bureau of Indian Standard on recommendation of the Oils and Oilseeds Sectional Committee and approval of the Food and Agriculture Division Council.

This standard has been published into two parts. The other part in this series is:

**Part 2 Olive oil and olive pomace oils**

The text of ISO standard has been approved as suitable for publication as an Indian Standard without deviations. Certain conventions are, however, not identical to those used in Indian Standards. Attention is particularly drawn to the following:

- a) Wherever the words 'International Standard' appear referring to this standard, they should be read as 'Indian Standard'; and
- b) Comma (,) has been used as a decimal marker while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

In this adopted standard, reference appears to the following International Standard for which Indian Standard also exists. The corresponding Indian Standard which is to be substituted in its place is listed below along with its degree of equivalence for the edition indicated.

<i>International Standard</i>	<i>Corresponding Indian Standard</i>	<i>Degree of Equivalence</i>
ISO 661 Animal and vegetable fats and oils — Preparation of test sample	IS 17563 : 2021/ISO 661 : 2013 Animal and vegetable fats and oils — Preparation of test sample	Identical

The Committee has reviewed the provisions of the following International Standard referred in this adopted standard and has decided that it is acceptable for use in conjunction with this standard:

<i>International Standard</i>	<i>Title</i>
ISO 3696	Water for analytical laboratory use — Specification and test method

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 2022 'Rules for rounding off numerical values (*second revision*)'.

# Contents

Page

<b>1</b>	<b>Scope</b> .....	<b>1</b>
<b>2</b>	<b>Normative references</b> .....	<b>1</b>
<b>3</b>	<b>Terms and definitions</b> .....	<b>1</b>
<b>4</b>	<b>Principle</b> .....	<b>1</b>
<b>5</b>	<b>Reagents</b> .....	<b>2</b>
<b>6</b>	<b>Apparatus</b> .....	<b>3</b>
<b>7</b>	<b>Sample</b> .....	<b>3</b>
	7.1 Sampling.....	3
	7.2 Preparation of the test sample.....	3
<b>8</b>	<b>Procedure</b> .....	<b>4</b>
	8.1 Preparation of the aluminium oxide column.....	4
	8.2 Test portion.....	4
	8.3 Extraction of the unsaponifiable matter.....	4
	8.4 Thin-layer chromatography.....	4
	8.5 Isolation of the sterols.....	4
	8.6 Preparation of sterol trimethylsilyl ethers.....	5
	8.7 Gas chromatography.....	5
<b>9</b>	<b>Expression of results</b> .....	<b>5</b>
	9.1 Identification of sterols.....	5
	9.2 Composition of sterols.....	5
	9.3 Determination of the total sterol content.....	6
<b>10</b>	<b>Precision</b> .....	<b>7</b>
	10.1 Interlaboratory test.....	7
	10.2 Repeatability limit, <i>r</i> .....	7
	10.3 Reproducibility limit, <i>R</i> .....	7
<b>11</b>	<b>Test report</b> .....	<b>7</b>
	<b>Annex A (informative) Figures</b> .....	<b>8</b>
	<b>Annex B (informative) Interlaboratory trial</b> .....	<b>15</b>
	<b>Bibliography</b> .....	<b>24</b>



*Indian Standard*

# DETERMINATION OF INDIVIDUAL AND TOTAL STEROLS CONTENTS — GAS CHROMATOGRAPHIC METHOD

## PART 1 ANIMAL AND VEGETABLE FATS AND OILS

### 1 Scope

This part of ISO 12228 specifies a procedure for the gas chromatographic determination of the content and composition of sterols in animal and vegetable fats and oils. However, the determination of the contents and composition of sterols in olive and olive pomace oils is to be carried out using ISO 12228-2.

### 2 Normative references

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

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

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

### 3 Terms and definitions

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

#### 3.1

##### **composition of sterols**

composition of individual sterols in the sample, beginning with cholesterol and ending with  $\Delta^7$ -avenasterol (see [Table 1](#)) under the conditions specified in this part of ISO 12228

Note 1 to entry: The composition is expressed as a percentage of all peak areas, normalized to 100 %.

#### 3.2

##### **total sterol content**

mass fraction of the sum of all individual sterols, as determined in accordance with the method specified in this part of ISO 12228, beginning with cholesterol and ending with  $\Delta^7$ -avenasterol (see [Table 1](#)), divided by the mass of the test portion

Note 1 to entry: The content is expressed in milligrams per kilogram.

### 4 Principle

A test portion is saponified by boiling under reflux with an ethanolic potassium hydroxide solution. The unsaponifiable matter is isolated by solid-phase extraction on an aluminium oxide column. The aluminium oxide column is used to retain the fatty acid anions; sterols pass through the column. The sterol fraction from the unsaponifiable matter is separated by thin-layer chromatography. The qualitative and quantitative compositions of the sterol fraction are determined by gas chromatography using cholestanol or betulin as the internal standard.

## 5 Reagents

**WARNING** — Attention is drawn to the regulations which specify the handling of hazardous substances. Technical, organizational, and personal safety measures shall be followed.

Use only reagents of recognized analytical grade, unless otherwise stated, and water complying with grade 3 of ISO 3696[1].

**5.1 Potassium hydroxide (KOH)**, ethanolic solution, molar concentration  $c(\text{KOH})$  approximately 0,5 mol/l.

Dissolve 3 g of potassium hydroxide in 5 ml of water and dilute to 100 ml with ethanol (5.3). The solution should be colourless or straw-coloured.

**5.2 Internal standard solution**, cholestanol (5 $\alpha$ -cholestan-3 $\beta$ -ol) or betulin, volume fraction of 1,0 mg/ml solution in ethanol (see note to 5.10).

NOTE In case of hydrogenated oils, which may contain cholestanol, the use of betulin (peak 17 in Table 1) is recommended.

**5.3 Ethanol**, of minimum volume fraction  $\varphi = 95 \%$ .

**5.4 Aluminium oxide**, neutral, particle size 0,063 mm to 0,200 mm, activity grade I (water content = 0 %).

**5.5 Diethyl ether**, freshly distilled, free from peroxides and residue.

**WARNING** — Diethyl ether is highly flammable and can form explosive peroxides. Explosive limits in air are 1,7 % to 48 % (volume fraction). Take special precautions when using it. Keep away from heat sources and sunlight.

**5.6 Silica gel thin-layer chromatography (TLC) plates**, commercially available, dimensions 20 cm  $\times$  20 cm, thickness of layer 0,25 mm.

**5.7 Developing solvent**, hexane/diethyl ether.

Volume fraction of each solvent is 50 ml/100 ml.

**5.8 Standard solution for thin-layer chromatography**, volume fraction of 1,0 mg/ml cholesterol/cholestanol in acetone or 5,0 mg/ml betulin in acetone.

NOTE 1 Cholesterol and cholestanol have the same  $R_f$  value (0,35) in TLC while the  $R_f$  value for betulin is 0,30 (see Figure A.1).

NOTE 2 In case of hydrogenated oils, which may contain cholestanol, the use of betulin (peak 17 in Table 1) is recommended.

**5.9 Spraying reagent**, methanol.

**5.10 Silylating reagent**, prepared by adding 50  $\mu\text{l}$  of 1-methyl imidazole to 1 ml of N-methyl-N-(trimethylsilyl)-hepta-fluorobutyramide (MSHFBA).

NOTE Ready-to-use solutions are commercially available. Other silylation reagents, e.g. bis trimethylsilyl trifluoroacetamide with 1 % trimethylchlorosilane, are also available and can be used when cholestanol is used as internal standard. However for betulin special precautions are taken to ensure that both hydroxyl groups of betulin are silylated. If not, betulin may show two peaks in the chromatogram.

## 6 Apparatus

Usual laboratory apparatus and, in particular, the following.

- 6.1 **Round-bottomed flasks**, of 25 ml and 50 ml capacity, with ground neck.
  - 6.2 **Reflux condenser**, with ground glass joint to fit a flask (6.1).
  - 6.3 **Glass column**, with polytetrafluoroethylene (PTFE) stopper, sintered glass filter and reservoir for 100 ml, length 25 cm, internal diameter 1,5 cm.
  - 6.4 **Rotary evaporator**, attached to a vacuum pump and water bath maintained at 40 °C.
  - 6.5 **Developing tank**, made of glass, with a ground glass lid, suitable for use with plates of dimensions 20 cm × 20 cm.
  - 6.6 **Microsyringe**, to deliver 100 µl.
  - 6.7 **Oven**, maintained at 105 °C ± 3 °C.
  - 6.8 **Desiccator**, containing an efficient desiccant, for storing the plates.
  - 6.9 **Reaction vials**, of 0,3 ml to (1,0 to 1,5) ml capacity, with screw caps and PTFE-lined seals, for preparation of sterol derivatives.
  - 6.10 **Gas chromatograph**, for capillary columns, with split injector, flame ionization detector and suitable recorder.
  - 6.11 **Capillary column**, made of fused silica or glass, length 25 m to 60 m, internal diameter 0,2 mm to 0,25 mm, stationary phase SE-54 (or equivalent non-polar phase with a temperature limit of at least 280 °C to 300 °C); film thickness about 0,1 µm.
- NOTE A better resolution of the peaks is obtained with a film thickness of 0,1 µm.
- 6.12 **Microsyringe for gas chromatography**, for injecting volumes of 1 µl.
  - 6.13 **Analytical balance**, capable of weighing to the nearest 0,001 g and displaying 0,000 1 g.

## 7 Sample

### 7.1 Sampling

Sampling is not part of the method specified in this part of ISO 12228. A recommended sampling method is given in ISO 5555[1].

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

### 7.2 Preparation of the test sample

Prepare the test sample in accordance with ISO 661.

## 8 Procedure

### 8.1 Preparation of the aluminium oxide column

Suspend 10 g of aluminium oxide (5.4) in 20 ml of ethanol (5.3) and pour the slurry into the glass column (6.3). Allow the aluminium oxide to settle and let the solvent run out of the column until the level of the solvent reaches the top level of the aluminium oxide layer.

### 8.2 Test portion

Weigh, to the nearest 1 mg, about 250 mg of the test sample into a 25 ml flask (6.1), proceed with 8.3.

For fats and oils with a low sterol content (for example less than 2 000 mg per kilogram) or for other reasons, proceed using a threefold amount of the test sample. Adjust reagents and apparatus accordingly.

### 8.3 Extraction of the unsaponifiable matter

Add exactly 1,00 ml of internal standard solution (5.2) to the test portion (8.2). Add 5 ml of ethanolic potassium hydroxide solution (5.1) and a few anti-bumping granules. Attach the reflux condenser (6.2) to the flask and keep the contents gently boiling for 15 min. Stop heating. Immediately dilute the contents of the flask while still hot with 5 ml of ethanol (5.3) and swirl or shake to homogenize.

Pipette 5 ml of this solution onto the prepared aluminium oxide column (8.1). Collect the eluate in a 50 ml round-bottom flask (6.1) and allow the column to run off until the solvent level has reached the top level of the aluminium oxide layer. Elute the unsaponifiable matter first with 5 ml of ethanol (5.3) and then with 30 ml of diethyl ether (5.5), with a flow rate of about 2 ml/min. Remove the solvents from the flask by means of the rotary evaporator (6.4).

**WARNING — The aluminium oxide column is essential for this procedure. It shall not be replaced by silica or other columns or by solvent extraction.**

### 8.4 Thin-layer chromatography

Dissolve the unsaponifiable matter obtained in 8.3 in a small amount (approximately 0,5 ml) of diethyl ether (5.5). Apply the solution as a line at a distance of 2 cm from the lower edge onto a TLC plate (5.6) using the microsyringe (6.6). Leave a gap of at least 3 cm from each side edge of the plate. Apply a spot of 5 µl of the TLC standard solution (5.8) at 1,5 cm from the edge. Fill the developing tank (6.5) with about 100 ml of developing solvent (5.7). Place the plate into the tank and develop it until the solvent reaches the upper edge. Remove the plate from the tank and allow the solvent to evaporate in a fume cupboard.

NOTE Quantitative transfer of the material (8.3) to the TLC plate is not necessary in this step. Automatic apparatus for applying streaks may be used. Saturation of the chamber is not required.

### 8.5 Isolation of the sterols

Spray the plates with methanol (5.9) until the sterol (and betulin) zones appear white on a translucent (darker) background. The cholestanol is part of the  $\Delta^5$ -sterol zone (see Figure A.1). Mark the zones at the height of the standard spots 2 mm above and 4 mm below the visible zones (see Figure A.1). Scratch off this part of the layer completely using a spatula and quantitatively collect the silica in a small beaker.

NOTE 1 The wider margin at the lower edge of the visible zones (4 mm vs. 2 mm at the upper edge) is a precaution to avoid partial loss of betulin in this step. Sunflower seed oil may show three bands ( $\Delta^5$ -sterols,  $\Delta^7$ -sterols and betulin).

NOTE 2 Betulin, if used as internal standard, appears slightly below the sterol zone (see Figure A.1).

Add 0,5 ml of ethanol to the collected silica gel. Extract the silica gel in the beaker three times with 5 ml of diethyl ether (5.5) and filter into a flask (6.1). Reduce the combined ether extracts to about 1 ml in



the rotary evaporator (6.4) and transfer the remaining solution into the reaction vial (6.9). Blow off the solvent in the reaction vial with a stream of nitrogen.

## 8.6 Preparation of sterol trimethylsilyl ethers

Add 100 µl of the silylation reagent (5.10) to the reaction vial (6.9) containing the isolated sterols. Seal and heat the vial for 15 min in the oven set at (105 ± 3) °C. Allow the reaction vial to cool to room temperature and inject the solution directly into the gas chromatograph (6.10).

## 8.7 Gas chromatography

Optimize the temperature programme and the carrier gas flow rate so that chromatograms similar to Figures A.2 to A.7 are obtained. Test the separation with silylated sterol fractions obtained from known oils, as shown in Figures A.2 to A.7.

NOTE 1 The following parameters were tested and found useful (see chromatograms in Annex A): GC column: SE-54, 50 m length, 0,25 mm internal diameter, 0,10 µm film thickness; carrier gas H<sub>2</sub>, carrier gas flow rate 36 cm/s, split 1:20, detector/injector 320° C, temperature programme 245 °C to 265 °C at 5 °C/min, 40 min isothermal at 265 °C; injection volume 1 µl. Capillary columns of equivalent quality can be used.

NOTE 2 A standard solution containing cholesterol, campesterol, stigmasterol, and sitosterol may be used to check the retention times. Use a blank run to test for possible contamination (e.g. cholesterol) from solvents, glass walls, filter, fingerprints, etc.

## 9 Expression of results

### 9.1 Identification of sterols

To identify the sterols present in the test sample, determine the relative retention times (RRT) by dividing the retention time (RT) of the sterol in question by the RT of cholesterol and/or betulin. Table 1 shows the RRT of the various sterols corresponding to cholesterol (RRT<sub>C</sub>) and betulin (RRT<sub>B</sub>), with SE-54 stationary phase.

NOTE The RRT in Table 1 (determined under the conditions of note 1 in 8.7) are mentioned as an aid for the identification of the individual sterols only, and to illustrate the elution sequence (cf. also Figure A.1). The actual RRT found may deviate slightly from the RRT given in Table 1 because the RRT depends on the experimental conditions (type and length of GLC column, temperature programme, and quality of stationary phase).

### 9.2 Composition of sterols

Calculate the mass fraction  $w_i$ , of the individual sterol  $i$ , in g/100 g (percent), according to the following equation:

$$w_i = \frac{A_i}{\sum A} \cdot 100 \quad (1)$$

where

$A_i$  is the area of the peak of sterol  $i$ ;

$\sum A$  is the sum of the peak areas of all sterols (peaks 1, 3 to 16 or 1 to 16, if betulin is used).

**Table 1 — Gas chromatographic peak identification of individual sterols and betulin by RRT (SE-54 stationary phase)**

Peak No	Common names of sterols	Systematic names of sterols	RRT <sub>C</sub>	RRT <sub>B</sub>
1	Cholesterol	Cholest-5-en-3 $\beta$ -ol	1,00	0,44
2	Cholestanol	5 $\alpha$ -Cholestan-3 $\beta$ -ol	1,02	0,45
3	Brassicasterol	[24S]-24-Methyl cholesta-5,22-dien-3 $\beta$ -ol	1,09	0,48
4	24-Methylene cholesterol	24-Methylene cholesta-5,24-dien-3 $\beta$ -ol	1,21	0,53
5	Campesterol	[24R]-24-Methyl cholest-5-en-3 $\beta$ -ol	1,23	0,54
6	Campestanol	[24R]-24-Methyl cholestan-3 $\beta$ -ol	1,25	0,55
7	Stigmasterol	[24S]-24-Ethyl cholesta-5,22-dien-3 $\beta$ -ol	1,31	0,57
8	$\Delta$ 7-Campesterol	[24R]-24-Methyl cholest-7-en-3 $\beta$ -ol	1,38	0,59
9	$\Delta$ 5,23-Stigmastadienol	[24R,S]-24-Ethyl cholesta-5,23-dien-3 $\beta$ -ol	1,40	0,60
10	Clerosterol	[24S]-24-Ethyl cholesta-5,25-dien-3 $\beta$ -ol	1,42	0,62
11	Sitosterol	[24R]-24-Ethyl cholest-5-en-3 $\beta$ -ol	1,47	0,64
12	Sitostanol	[24R]-24-Ethyl cholestan-3 $\beta$ -ol	1,50	0,65
13	$\Delta$ 5-Avenasterol	[24Z]-24(28)-Ethylidene cholest-5-en-3 $\beta$ -ol	1,52	0,66
14	$\Delta$ 5,24-Stigmastadienol	[24R,S]-24-Ethyl cholesta-5,24-dien-3 $\beta$ -ol	1,59	0,69
15	$\Delta$ 7-Stigmastenol	[24R,S]-24-Ethyl cholest-7-en-3 $\beta$ -ol	1,65	0,72
16	$\Delta$ 7-Avenasterol	[24Z]-24(28)-Ethylidene cholest-7-en-3 $\beta$ -ol	1,70	0,74
X	(Erythrodiol)		2,03	0,88
Y	(Uvaol)		2,17	0,95
17	Betulin	Lup-20[29]-ene-3 $\beta$ ,28-diol	2,30	1,00

RRT<sub>C</sub>: relative retention time based on cholesterol = 1,00  
RRT<sub>B</sub>: relative retention time based on betulin = 1,00  
NOTE Sitosterol may coelute together with  $\alpha$ -spinasterol and  $\Delta$ 7,22,25-stigmastatrienol. [24R]-24-Ethylcholesta-7,25(27)-dien-3 $\beta$ -ol is present in sterols of sunflower and pumpkin seed oil and may coelute with peak 14 ( $\Delta$ 5,24-stigmastadienol).

### 9.3 Determination of the total sterol content

For the purposes of this method, it is assumed that the response factors of all sterols and of betulin are equal.

NOTE In several tests silylated sterols and silylated betulin in equal amounts gave the same detector response using an FID detector under these conditions.

Calculate the total sterol content  $w$ , in milligrams per kilogram of fat, according to Formula (2):

$$w = \frac{\sum(A) \cdot m_{IS} \cdot 1\,000}{A_{IS} \cdot m} \quad (2)$$

where

$m_{IS}$  is the mass of the internal standard (cholestanol) in milligrams;

$\sum(A)$  is the sum of the peak areas of all sterols (peaks 1, 3 to 16 or 1 to 16, if betulin is used);

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

$m$  is the mass of the test sample, in grams.

For calculation of the total sterol content, consider all peaks of sterols beginning with cholesterol and ending with  $\Delta^7$ -avenasterol (peak 16), but without erythrodiol and uvaol (peaks X and Y).

## 10 Precision

### 10.1 Interlaboratory test

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

### 10.2 Repeatability limit, $r$

The repeatability limit ( $r$ ) is the value less than or equal to which the absolute difference between two test results obtained under repeatability conditions may be expected to be with a probability of 95 %.

Repeatability conditions are conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time.

### 10.3 Reproducibility limit, $R$

The reproducibility limit ( $R$ ) is the value less than or equal to which the absolute difference between two test results obtained under reproducibility conditions may be expected to be with a probability of 95 %.

Reproducibility conditions are conditions where independent test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment within short intervals of time.

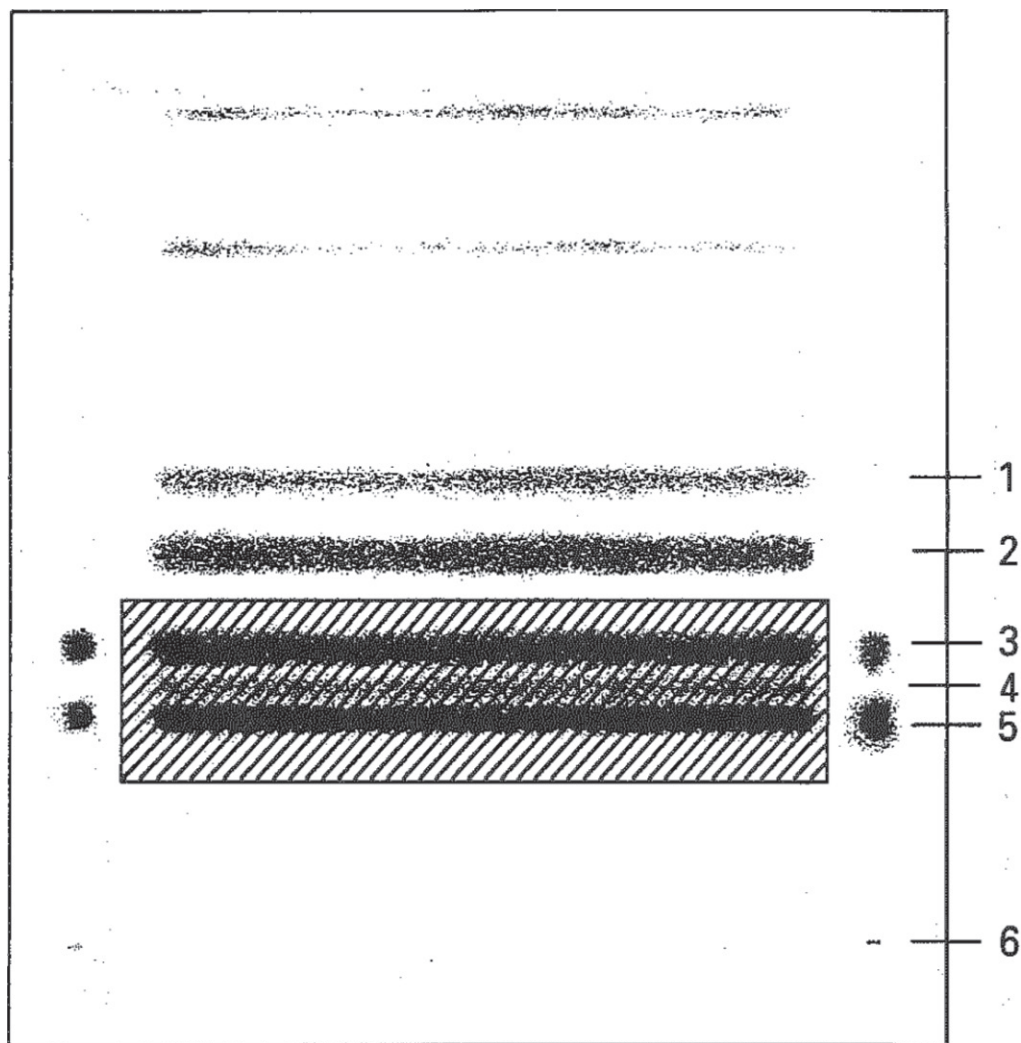
## 11 Test report

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 test method used, together with reference to this part of ISO 12228 (i.e. ISO 12228-1);
- 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; or if the repeatability has been checked, the final quoted result obtained.

## Annex A (informative)

### Figures

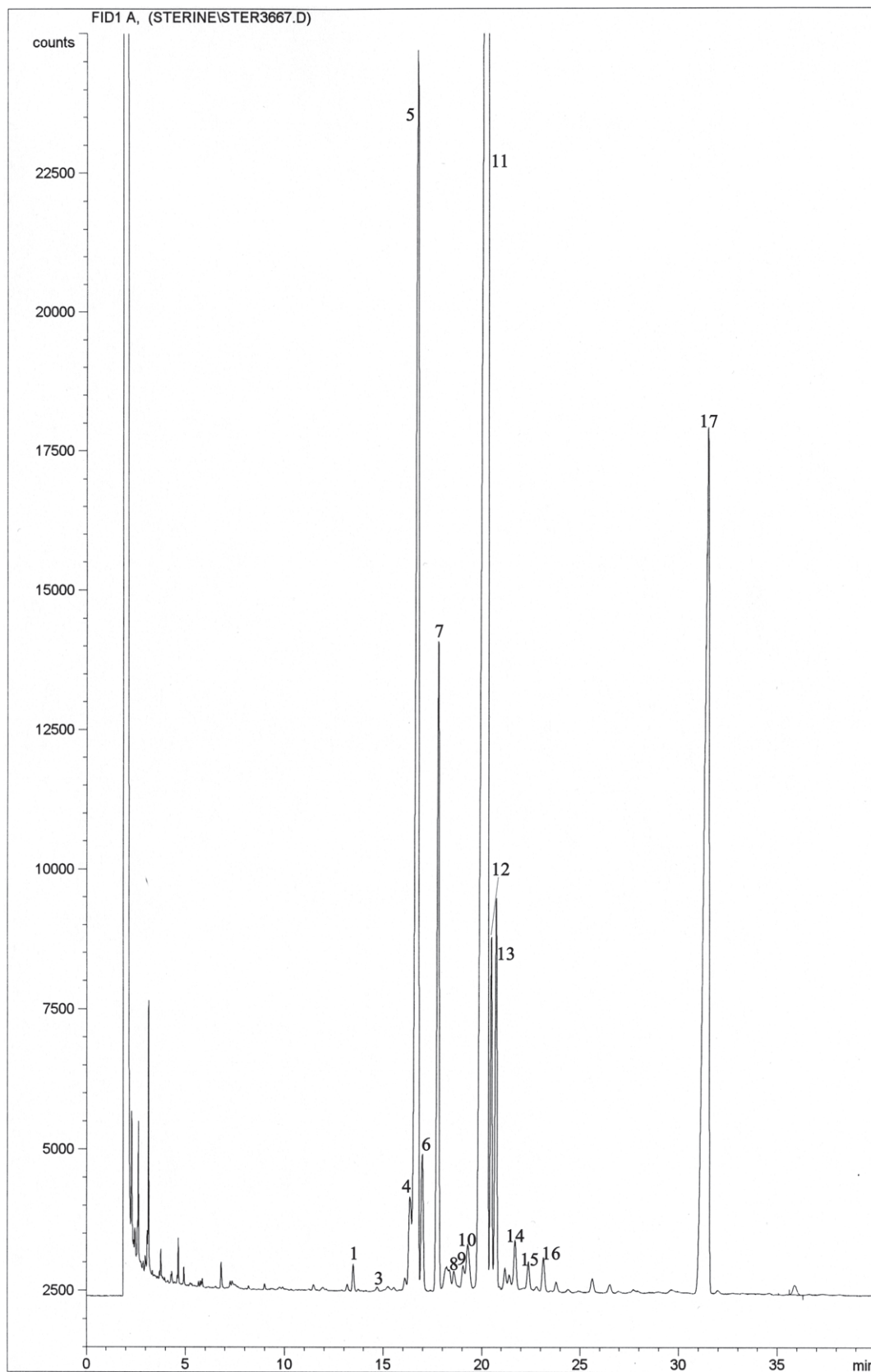


#### Key

- 1 triterpenes
- 2 methyl sterols
- 3 cholestanol and  $\Delta$ 5-sterols
- 4  $\Delta$ 7-sterols
- 5 betulin
- 6 start

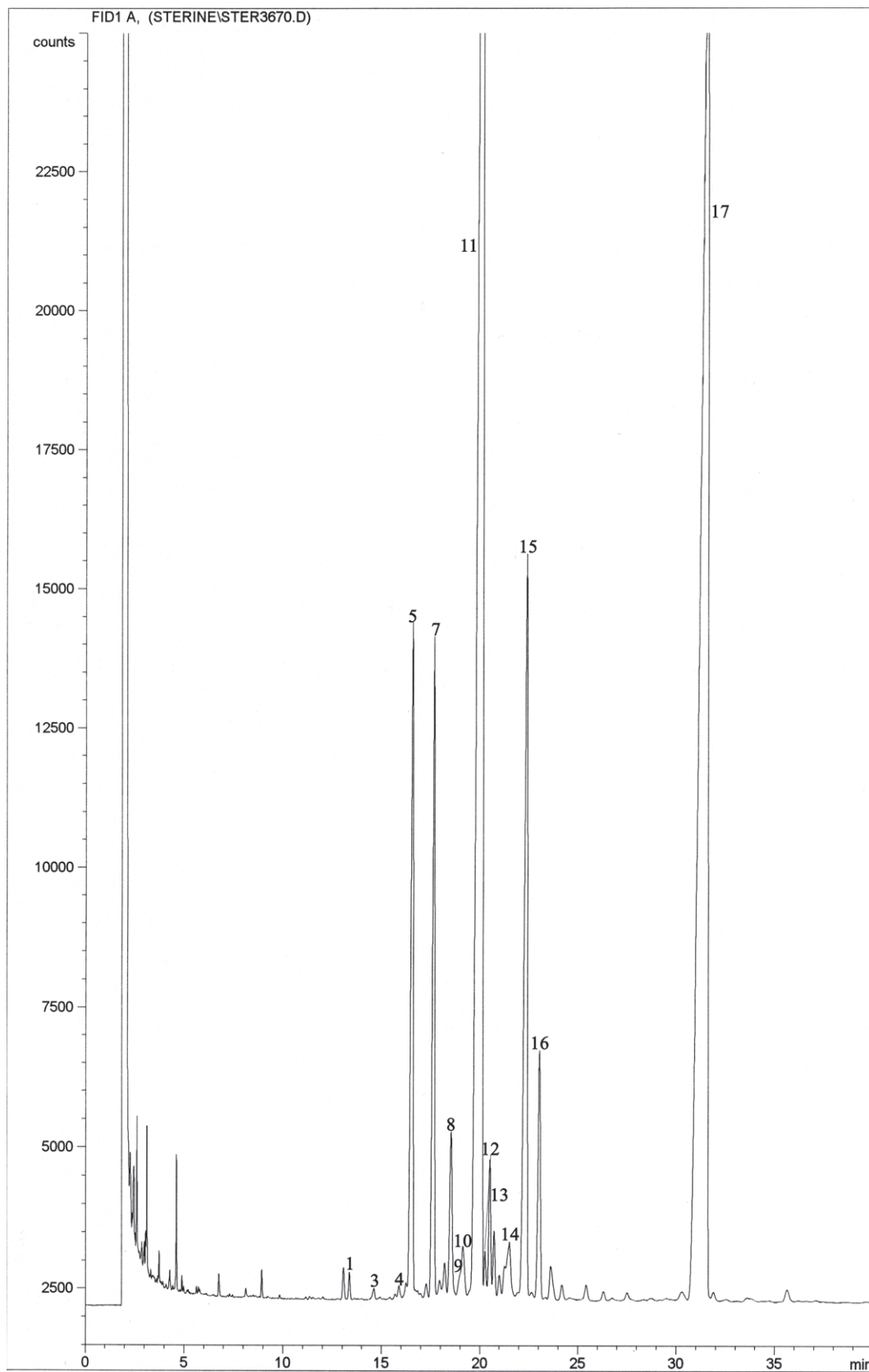
NOTE Zones appear white on a transparent background. The hatched area is scratched off; note wider margin at the bottom (4 mm vs. 2 mm at the top). Rf values of the bands: betulin 0,30;  $\Delta$ 7-sterols 0,33;  $\Delta$ 5-sterols 0,35; methyl sterols 0,45; triterpenes 0,53.

Figure A.1 — TLC isolation of sterols from unsaponifiable matter (steps 8.3 and 8.4)



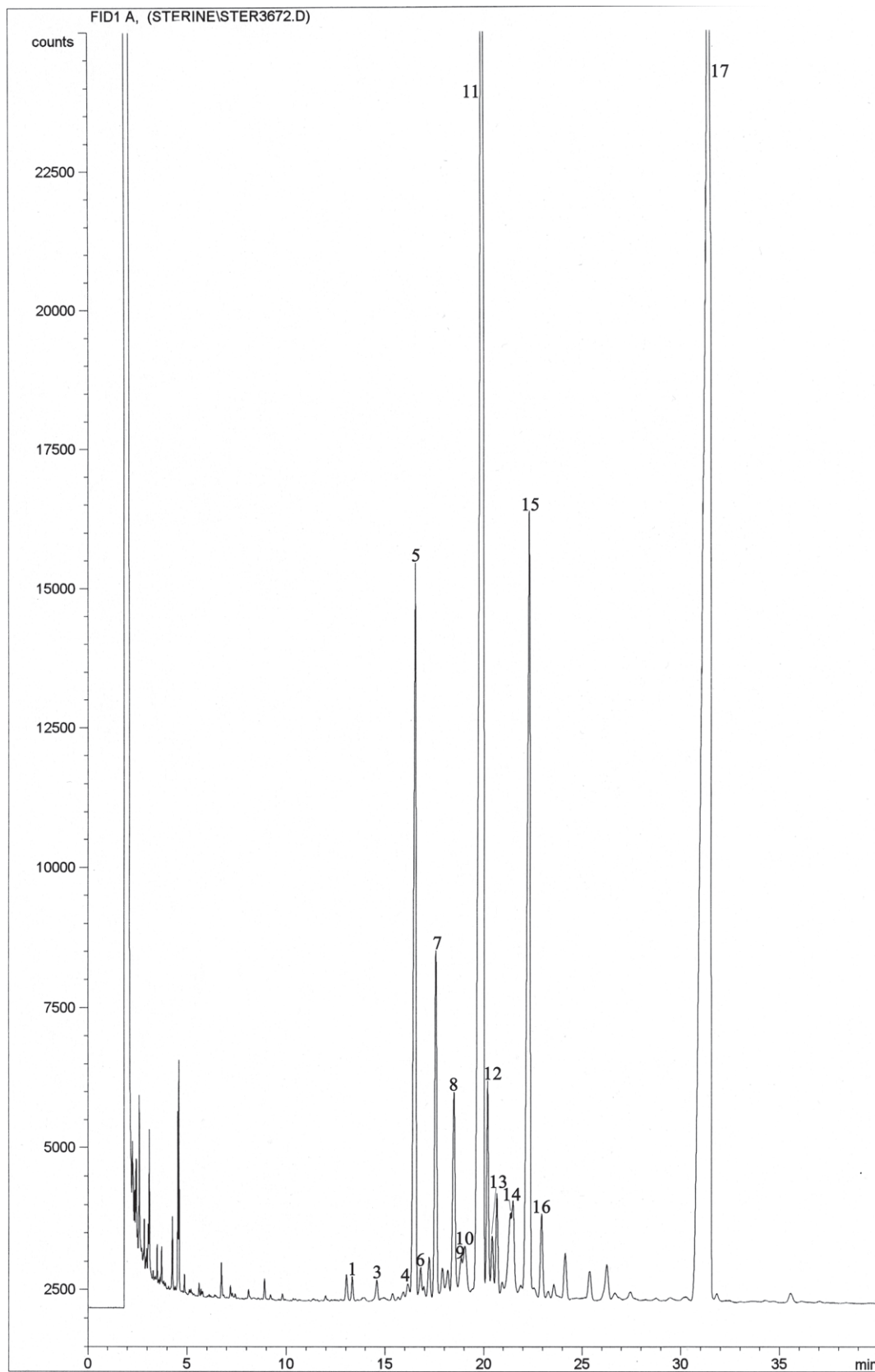
NOTE Identification of the peak numbers is according to [Table 1](#). Conditions are as in NOTE 1 of [8.7](#).

**Figure A.2 — GLC of sterols from corn oil (Sample A)**



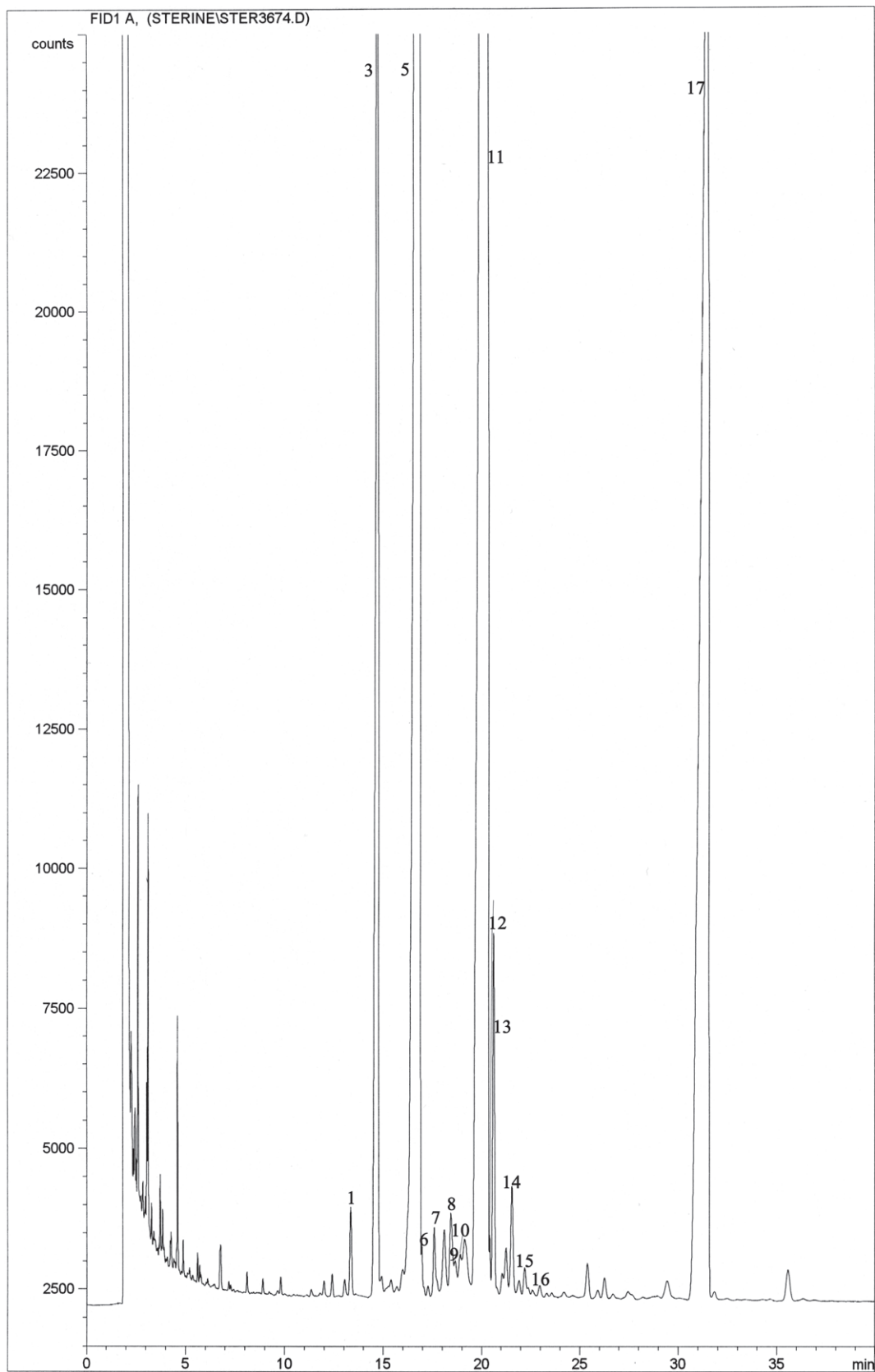
NOTE Identification of the peak numbers is according to [Table 1](#). Conditions are as in NOTE 1 of [8.7](#).

**Figure A.3 — GLC of sterols from refined sunflower seed oil (Sample B)**



NOTE Identification of the peak numbers is according to [Table 1](#). Conditions are as in NOTE 1 of [8.7](#).

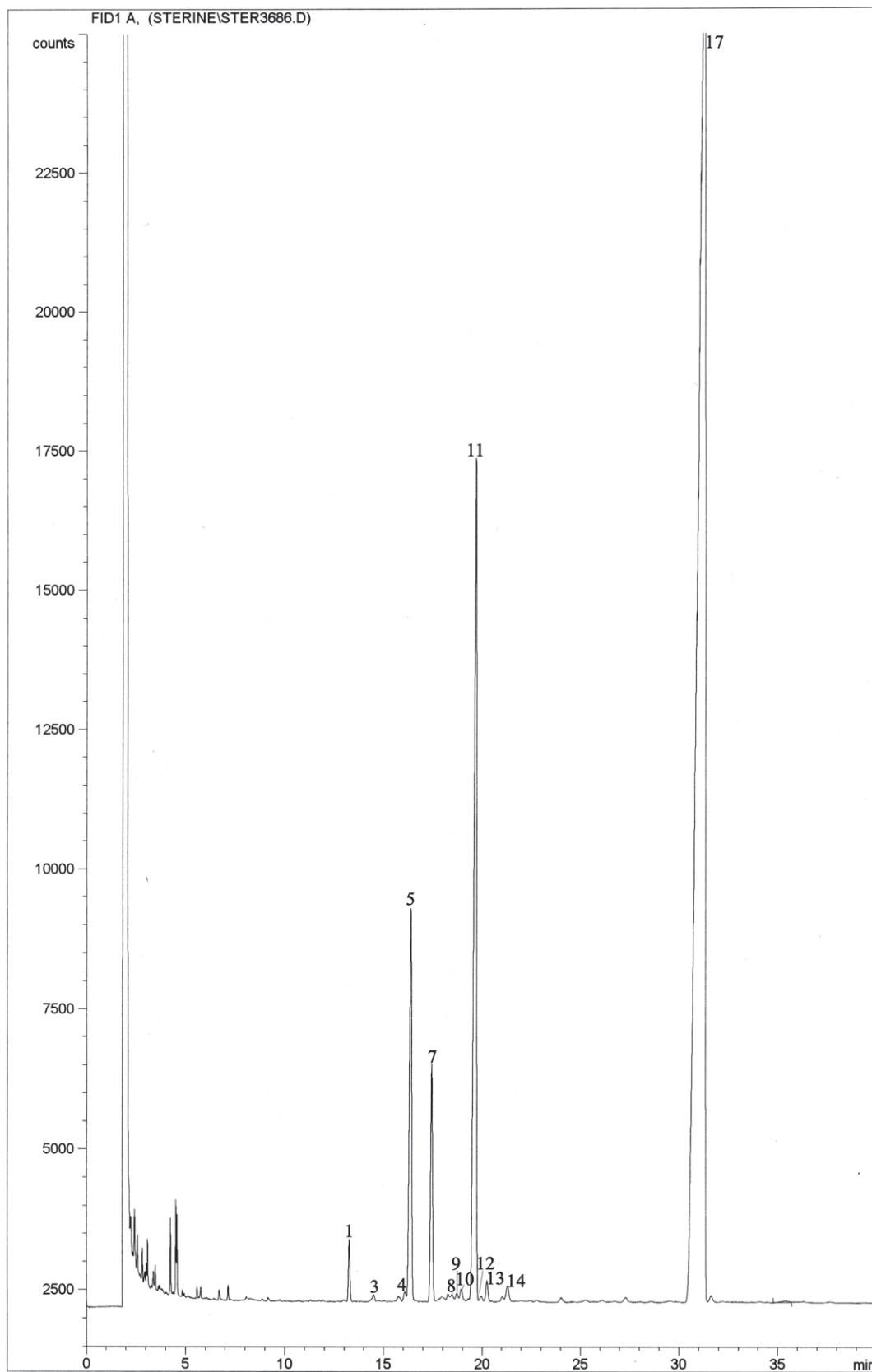
**Figure A.4 — GLC of sterols from refined safflower seed oil (Sample C)**



NOTE Identification of the peak numbers is according to [Table 1](#). Conditions are as in NOTE 1 of [8.7](#).

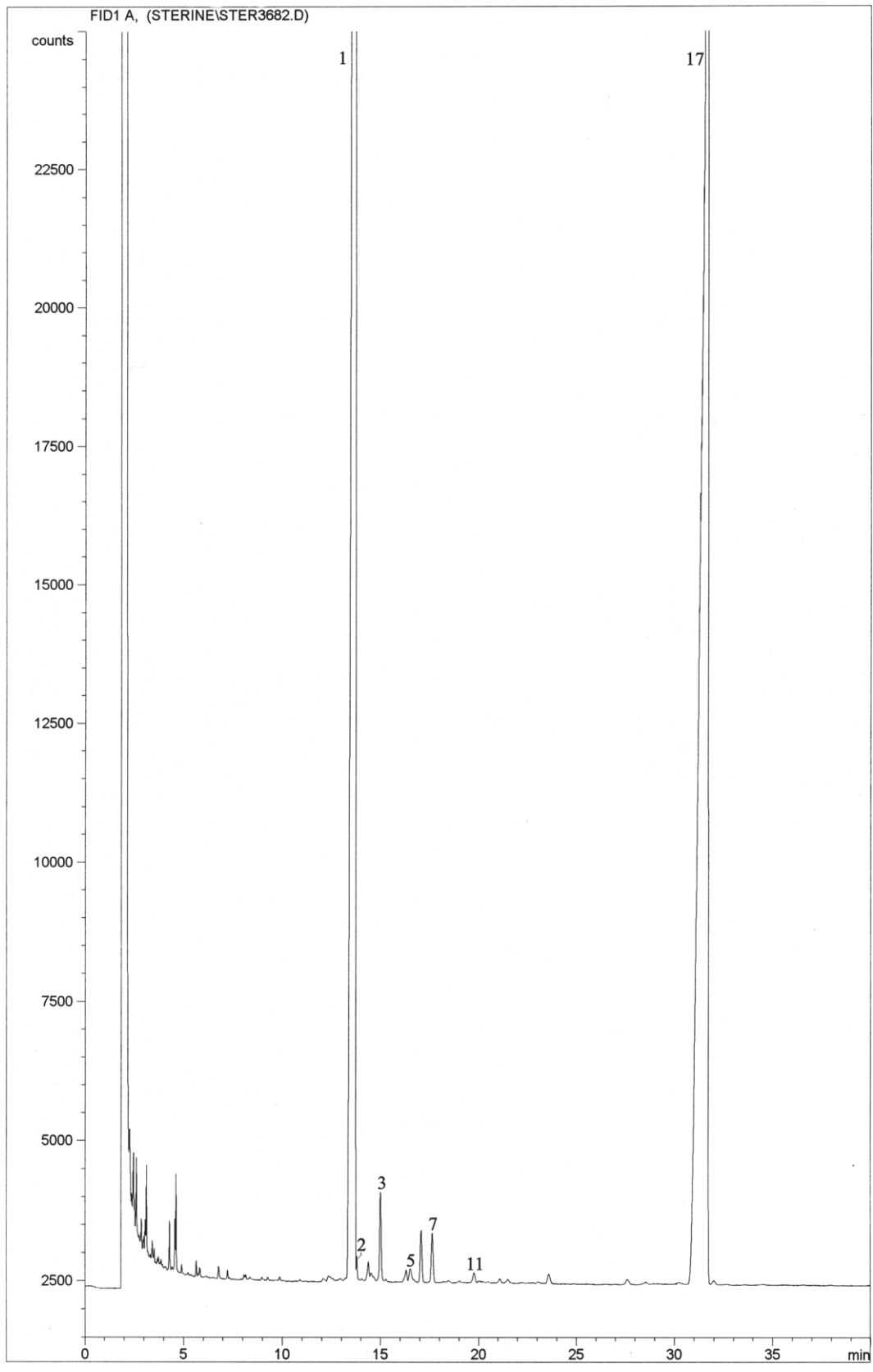
**Figure A.5 — GLC of sterols from refined rapeseed oil (Sample D)**





NOTE Identification of the peak numbers is according to [Table 1](#). Conditions are as in NOTE 1 of [8.7](#).

**Figure A.6 — GLC of sterols from palm oil (Sample E)**



NOTE Identification of the peak numbers is according to [Table 1](#). Conditions are as in NOTE 1 of [8.7](#).

**Figure A.7 — GLC of sterols from butter fat (Sample F)**

## Annex B (informative)

### Interlaboratory trial

The precision of the method has been established by two international interlaboratory tests organized by Germany in 2011/2012 and 1996/1997. The statistical evaluation was carried out in accordance with ISO 5725-1 and ISO 5725-2.

Sample identification:

- |  |                              |
|--|------------------------------|
| A Refined corn oil                           | B Refined sunflower seed oil |
| C Refined safflower seed oil                 | D Refined rapeseed oil       |
| E Refined deodorized bleached palm oil (RDB) | F Butter fat                 |

**Table B.1 — Statistical results for the total sterol content (in mg/kg)**

Collaborative trial organized in	2011/2012						1996/1997	
Sample	A	B	C	D	E	F	B	D
Number of participating laboratories	14	14	14	14	13	14	14	14
Number of laboratories retained after eliminating outliers	10	12	12	11	12	12	14	13
Number test results in all laboratories	20	24	24	22	24	24	28	26
<b>Mean value (<i>m</i>), mg/kg</b>	<b>8 771,2</b>	<b>3 355,5</b>	<b>2 490,3</b>	<b>7 365,2</b>	<b>607,0</b>	<b>2 551,8</b>	<b>3 785</b>	<b>7 585</b>
Standard deviation of repeatability ( <i>s<sub>r</sub></i> )	98,8	49,3	68,0	154,6	32,1	117,5	165,6	174,6
Coefficient of variation of repeatability, <i>CV<sub>r</sub></i> , %	1,1	1,5	2,7	2,1	5,3	4,6	4,4	2,3
<b>Repeatability limit (<i>r</i>)</b>	<b>276,6</b>	<b>138,1</b>	<b>190,5</b>	<b>432,9</b>	<b>89,8</b>	<b>328,9</b>	<b>463,7</b>	<b>489,0</b>
Standard deviation of reproducibility ( <i>s<sub>R</sub></i> )	1 328,1	317,7	282,5	418,5	84,0	434,6	542,4	762,7
Coefficient of variation of reproducibility <i>CV<sub>R</sub></i> , %	15,1	9,5	11,3	5,7	13,8	17,0	14,3	10,1
<b>Reproducibility limit (<i>R</i>)</b>	<b>3 718,7</b>	<b>889,6</b>	<b>791,1</b>	<b>1 171,7</b>	<b>235,3</b>	<b>1 216,9</b>	<b>1 518,7</b>	<b>2 135,5</b>

**Table B.2 — Statistical results for the individual sterol content: Cholesterol (in %)**

Collaborative trial organized in	2011/2012						1996/1997	
Sample	A	B	C	D	E	F	B	D
Number of participating laboratories	14	14	14	14	13	14	14	14
Number of laboratories retained after eliminating outliers	12	13	13	11	11	13	12	14
Number test results in all laboratories	24	26	26	22	22	26	24	28
<b>Mean value (m), %</b>	<b>0,22</b>	<b>0,28</b>	<b>0,57</b>	<b>0,36</b>	<b>3,34</b>	<b>97,69</b>	<b>0,40</b>	<b>0,40</b>
Standard deviation of repeatability ( $s_r$ )	0,04	0,10	0,06	0,03	0,33	0,25	0,05	0,08
Coefficient of variation of repeatability, $CV_r$ , %	16,0	34,7	10,9	8,3	9,8	0,3	11,5	18,3
<b>Repeatability limit (r)</b>	<b>0,10</b>	<b>0,27</b>	<b>0,17</b>	<b>0,08</b>	<b>0,92</b>	<b>0,71</b>	<b>0,13</b>	<b>0,22</b>
Standard deviation of reproducibility ( $s_R$ )	0,05	0,17	0,39	0,10	1,57	1,07	0,21	0,15
Coefficient of variation of reproducibility $CV_R$ , %	23,3	60,6	68,1	28,2	47,1	1,1	52,4	34,1
<b>Reproducibility limit (R)</b>	<b>0,14</b>	<b>0,47</b>	<b>1,09</b>	<b>0,29</b>	<b>4,41</b>	<b>2,99</b>	<b>0,58</b>	<b>0,41</b>

**Table B.3 — Statistical results for the individual sterol content: Brassicasterol (in %)**

Collaborative trial organized in	2011/2012					1996/1997	
Sample	A	B	C	D	E	B	D
Number of participating laboratories	14		12	14		14	14
Number of laboratories retained after eliminating outliers	14		12	14		13	13
Number test results in all laboratories	28		24	28		26	26
<b>Mean value (m), %</b>	<b>0,04</b>		<b>0,18</b>	<b>10,04</b>		<b>0,09</b>	<b>10,80</b>
Standard deviation of repeatability ( $s_r$ )	0,04		0,04	0,15		0,068	0,14
Coefficient of variation of repeatability, $CV_r$ , %	88,2		20,1	1,5		0,190	0,38
<b>Repeatability limit (r)</b>	<b>0,11</b>		<b>0,10</b>	<b>0,43</b>		<b>80,289</b>	<b>1,26</b>
Standard deviation of reproducibility ( $s_R$ )	0,05		0,12	0,36		0,089	0,26
Coefficient of variation of reproducibility $CV_R$ , %	118,6		64,7	3,5		104,9	2,4
<b>Reproducibility limit (R)</b>	<b>0,14</b>		<b>0,33</b>	<b>1,00</b>		<b>0,25</b>	<b>0,72</b>

**Table B.4 — Statistical results for the individual sterol content: Sum of 24-Methylene cholesterol and Campesterol (in %)**

Collaborative trial organized in	2011/2012					1996/1997	
Sample	A	B	C	D	E	B	D
Number of participating laboratories	14	14	14	14	13	14	14
Number of laboratories retained after eliminating outliers	14	14	11	14	10	11	13
Number test results in all laboratories	28	28	22	28	20	22	26
<b>Mean value (m), %</b>	<b>21,64</b>	<b>9,13</b>	<b>12,09</b>	<b>35,39</b>	<b>22,33</b>	<b>9,0</b>	<b>37,50</b>
Standard deviation of repeatability ( $s_r$ )	0,12	0,21	0,09	0,25	0,19	2,3	0,19
Coefficient of variation of repeatability, $CV_r$ , %	0,5	2,3	0,7	0,7	0,9	0,6	0,5
<b>Repeatability limit (r)</b>	<b>0,33</b>	<b>0,58</b>	<b>0,24</b>	<b>0,70</b>	<b>0,53</b>	<b>0,58</b>	<b>0,54</b>
Standard deviation of reproducibility ( $s_R$ )	0,74	0,64	0,44	0,57	0,69	0,26	0,35
Coefficient of variation of reproducibility $CV_R$ , %	3,4	7,1	3,6	1,6	3,1	2,9	0,9
<b>Reproducibility limit (R)</b>	<b>2,06</b>	<b>1,80</b>	<b>1,23</b>	<b>1,60</b>	<b>1,92</b>	<b>0,72</b>	<b>0,97</b>

**Table B.5 — Statistical results for the individual sterol content: Campesterol (in %)**

Collaborative trial organized in	2011/2012					1996/1997	
Sample	A	B	C	D	E	B	D
Number of participating laboratories	14	14	14	14	13		
Number of laboratories retained after eliminating outliers	13	14	12	14	10		
Number test results in all laboratories	26	28	24	28	20		
<b>Mean value (m), %</b>	<b>20,67</b>	<b>8,99</b>	<b>11,95</b>	<b>34,86</b>	<b>22,33</b>		
Standard deviation of repeatability ( $s_r$ )	0,11	0,18	0,08	0,25	0,19		
Coefficient of variation of repeatability, $CV_r$ , %	0,5	2,0	0,7	0,7	0,9		
<b>Repeatability limit (r)</b>	<b>0,32</b>	<b>0,51</b>	<b>0,23</b>	<b>0,71</b>	<b>0,53</b>		
Standard deviation of reproducibility ( $s_R$ )	0,44	0,59	0,64	0,74	0,69		
Coefficient of variation of reproducibility $CV_R$ , %	2,1	6,6	5,4	2,1	3,1		
<b>Reproducibility limit (R)</b>	<b>1,23</b>	<b>1,65</b>	<b>1,80</b>	<b>2,07</b>	<b>1,92</b>		

**Table B.6 — Statistical results for the individual sterol content: Campestanol (in %)**

Collaborative trial organized in	2011/2012					1996/1997	
Sample	A	B	C	D	E	B	D
Number of participating laboratories	14	14	14	14	13	14	14
Number of laboratories retained after eliminating outliers	11	9	13	12	8	10	12
Number test results in all laboratories	22	18	26	24	16	20	24
<b>Mean value (m), %</b>	<b>1,36</b>	<b>0,09</b>	<b>0,41</b>	<b>0,14</b>	<b>0,00</b>	<b>0,1</b>	<b>0,2</b>
Standard deviation of repeatability ( $s_r$ )	0,03	0,03	0,04	0,03	0,00	0,03	0,06
Coefficient of variation of repeatability, $CV_r$ , %	2,2	37,5	10,7	20,4	0,00	35,1	32,8
<b>Repeatability limit (r)</b>	<b>0,08</b>	<b>0,09</b>	<b>0,12</b>	<b>0,08</b>	<b>0,00</b>	<b>0,09</b>	<b>0,16</b>
Standard deviation of reproducibility ( $s_R$ )	0,17	0,09	0,20	0,11	0,00	0,13	0,18
Coefficient of variation of reproducibility $CV_R$ , %	12,4	96,1	47,7	79,3	0,00	147,5	106,0
<b>Reproducibility limit (R)</b>	<b>0,47</b>	<b>0,24</b>	<b>0,55</b>	<b>0,31</b>	<b>0,00</b>	<b>0,37</b>	<b>0,50</b>

**Table B.7 — Statistical results for the individual sterol content: Stigmasterol (in %)**

Collaborative trial organized in	2011/2012					1996/1997	
Sample	A	B	C	D	E	B	D
Number of participating laboratories	14	14	14	14	13	14	14
Number of laboratories retained after eliminating outliers	14	13	12	13	12	13	13
Number test results in all laboratories	28	26	24	26	24	26	26
<b>Mean value (m), %</b>	<b>7,05</b>	<b>7,84</b>	<b>5,22</b>	<b>0,30</b>	<b>11,28</b>	<b>8,10</b>	<b>0,30</b>
Standard deviation of repeatability ( $s_r$ )	0,07	0,10	0,14	0,04	0,30	0,11	0,05
Coefficient of variation of repeatability, $CV_r$ , %	0,9	1,3	2,7	13,1	2,7	1,4	15,5
<b>Repeatability limit (r)</b>	<b>0,18</b>	<b>0,28</b>	<b>0,39</b>	<b>0,11</b>	<b>0,85</b>	<b>0,31</b>	<b>0,15</b>
Standard deviation of reproducibility ( $s_R$ )	0,18	0,61	0,29	0,08	0,95	0,25	0,10
Coefficient of variation of reproducibility $CV_R$ , %	2,5	7,7	5,5	25,3	8,4	3,0	30,9
<b>Reproducibility limit (R)</b>	<b>0,49</b>	<b>1,70</b>	<b>0,81</b>	<b>0,21</b>	<b>2,66</b>	<b>0,69</b>	<b>0,29</b>

**Table B.8 — Statistical results for the individual sterol content:  $\Delta^7$ -Campesterol (in %)**

Collaborative trial organized in	2011/2012					1996/1997	
Sample	A	B	C	D	E	B	D
Number of participating laboratories	14	14	14	14	13	14	14
Number of laboratories retained after eliminating outliers	14	13	14	14	10	14	14
Number test results in all laboratories	28	26	28	28	20	28	28
<b>Mean value (<i>m</i>), %</b>	<b>0,39</b>	<b>2,28</b>	<b>3,77</b>	<b>0,71</b>	<b>0,13</b>	<b>1,60</b>	<b>0,30</b>
Standard deviation of repeatability ( <i>s<sub>r</sub></i> )	0,06	0,16	0,20	0,08	0,09	0,15	0,05
Coefficient of variation of repeatability, <i>CV<sub>r</sub></i> , %	15,5	7,0	5,2	11,00	37,1	9,4	17,4
<b>Repeatability limit (<i>r</i>)</b>	<b>0,17</b>	<b>0,45</b>	<b>0,55</b>	<b>0,2</b>	<b>0,09</b>	<b>0,43</b>	<b>0,15</b>
Standard deviation of reproducibility ( <i>s<sub>R</sub></i> )	0,20	0,35	0,50	0,29	0,12	0,91	0,15
Coefficient of variation of reproducibility <i>CV<sub>R</sub></i> , %	51,1	15,3	13,3	40,5	137,9	56,2	50,1
<b>Reproducibility limit (<i>R</i>)</b>	<b>0,55</b>	<b>0,98</b>	<b>1,40</b>	<b>0,81</b>	<b>0,35</b>	<b>2,56</b>	<b>0,43</b>

**Table B.9 — Statistical results for the individual sterol content:  $\Delta^5,23$ -Stigmastadienol (in %)**

Collaborative trial organized in	2011/2012					1996/1997	
Sample	A	B	C	D	E	B	D
Number of participating laboratories	14	14	14	14	13	14	14
Number of laboratories retained after eliminating outliers	14	12	13	14	12	14	14
Number test results in all laboratories	28	24	26	28	24	28	28
<b>Mean value (<i>m</i>), %</b>	<b>0,35</b>	<b>0,24</b>	<b>0,76</b>	<b>0,25</b>	<b>0,17</b>	<b>1,2</b>	<b>0,5</b>
Standard deviation of repeatability ( <i>s<sub>r</sub></i> )	0,03	0,04	0,07	0,04	0,04	0,14	0,06
Coefficient of variation of repeatability, <i>CV<sub>r</sub></i> , %	9,4	14,6	8,6	16,7	20,7	11,6	11,9
<b>Repeatability limit (<i>r</i>)</b>	<b>0,09</b>	<b>0,10</b>	<b>0,18</b>	<b>0,12</b>	<b>0,10</b>	<b>0,39</b>	<b>0,16</b>
Standard deviation of reproducibility ( <i>s<sub>R</sub></i> )	0,16	0,18	0,29	0,11	0,23	0,94	0,15
Coefficient of variation of reproducibility <i>CV<sub>R</sub></i> , %	44,9	73,8	37,5	44,3	132,6	78,5	31,7
<b>Reproducibility limit (<i>R</i>)</b>	<b>0,44</b>	<b>0,50</b>	<b>0,80</b>	<b>0,31</b>	<b>0,63</b>	<b>2,62</b>	<b>0,42</b>

**Table B.10 — Statistical results for the individual sterol content: Clerosterol (in %)**

Collaborative trial organized in	2011/2012					1996/1997	
Sample	A	B	C	D	E	B	D
Number of participating laboratories	14	14	14	14	13	14	14
Number of laboratories retained after eliminating outliers	14	14	14	12	12	10	12
Number test results in all laboratories	28	28	28	24	24	20	24
<b>Mean value (m), %</b>	<b>0,76</b>	<b>0,92</b>	<b>1,10</b>	<b>0,55</b>	<b>0,48</b>	<b>0,07</b>	<b>0,2</b>
Standard deviation of repeatability ( $s_r$ )	0,08	0,08	0,17	0,04	0,05	0,022	0,03
Coefficient of variation of repeatability, $CV_r$ , %	9,9	8,5	15,0	7,4	9,5	34,4	16,5
<b>Repeatability limit (r)</b>	<b>0,21</b>	<b>0,22</b>	<b>0,46</b>	<b>0,11</b>	<b>0,13</b>	<b>0,063</b>	<b>0,08</b>
Standard deviation of reproducibility ( $s_R$ )	0,14	0,15	0,34	1,40	0,42	0,101	0,20
Coefficient of variation of reproducibility $CV_R$ , %	18,1	16,5	31,1	25,1	88,1	156,0	114,6
<b>Reproducibility limit (R)</b>	<b>0,39</b>	<b>0,42</b>	<b>0,96</b>	<b>0,39</b>	<b>1,18</b>	<b>0,284</b>	<b>0,56</b>

**Table B.11 — Statistical results for the individual sterol content:  $\beta$ -Sitosterol (in %)**

Collaborative trial organized in	2011/2012					1996/1997	
Sample	A	B	C	D	E	B	D
Number of participating laboratories	14	14	14	14	13	14	14
Number of laboratories retained after eliminating outliers	10	13	12	14	12	13	14
Number test results in all laboratories	20	26	24	28	24	26	28
<b>Mean value (m), %</b>	<b>58,84</b>	<b>57,42</b>	<b>48,91</b>	<b>49,11</b>	<b>56,96</b>	<b>57,0</b>	<b>44,3</b>
Standard deviation of repeatability ( $s_r$ )	0,16	0,55	0,35	0,33	0,35	0,41	0,22
Coefficient of variation of repeatability, $CV_r$ , %	0,3	1,0	0,7	0,7	0,6	0,7	0,5
<b>Repeatability limit (r)</b>	<b>0,45</b>	<b>1,53</b>	<b>0,99</b>	<b>0,93</b>	<b>0,99</b>	<b>1,15</b>	<b>0,63</b>
Standard deviation of reproducibility ( $s_R$ )	0,60	1,56	2,11	0,73	2,59	1,42	0,73
Coefficient of variation of reproducibility $CV_R$ , %	1,0	2,7	4,3	1,5	4,6	2,5	1,7
<b>Reproducibility limit (R)</b>	<b>1,69</b>	<b>4,36</b>	<b>5,92</b>	<b>2,05</b>	<b>7,26</b>	<b>3,99</b>	<b>2,05</b>



**Table B.12 — Statistical results for the individual sterol content: Sitostanol (in %)**

Collaborative trial organized in	2011/2012					1996/1997	
Sample	A	B	C	D	E		
Number of participating laboratories	14	14	14	14	13		
Number of laboratories retained after eliminating outliers	12	12	10	12	9		
Number test results in all laboratories	24	24	20	24	18		
<b>Mean value (m), %</b>	<b>2,84</b>	<b>0,52</b>	<b>3,95</b>	<b>0,25</b>	<b>0,28</b>		
Standard deviation of repeatability ( $s_r$ )	0,07	0,11	0,09	0,04	0,00		
Coefficient of variation of repeatability, $CV_r$ , %	2,4	20,4	2,9	16,3	0,0		
<b>Repeatability limit (r)</b>	<b>0,19</b>	<b>0,30</b>	<b>0,24</b>	<b>0,11</b>	<b>0,00</b>		
Standard deviation of reproducibility ( $s_R$ )	0,42	0,26	0,49	0,19	0,24		
Coefficient of variation of reproducibility $CV_R$ , %	14,7	49,6	16,6	76,2	85,9		
<b>Reproducibility limit (R)</b>	<b>1,17</b>	<b>0,72</b>	<b>1,37</b>	<b>0,53</b>	<b>0,67</b>		

**Table B.13 — Statistical results for the individual sterol content:  $\Delta$ 5-Avenasterol (in %)**

Collaborative trial organized in	2011/2012					1996/1997	
Sample	A	B	C	D	E	B	D
Number of participating laboratories	14	14	14	14	13	14	14
Number of laboratories retained after eliminating outliers	12	13	10	13	9	12	13
Number test results in all laboratories	24	26	20	26	18	24	26
<b>Mean value (m), %</b>	<b>4,46</b>	<b>2,12</b>	<b>1,12</b>	<b>1,65</b>	<b>1,18</b>	<b>4,7</b>	<b>4,9</b>
Standard deviation of repeatability ( $s_r$ )	0,13	0,07	0,12	0,15	0,09	0,13	0,11
Coefficient of variation of repeatability, $CV_r$ , %	3,0	3,5	11,2	9,3	8,0	2,70	2,20
<b>Repeatability limit (r)</b>	<b>0,37</b>	<b>0,21</b>	<b>0,35</b>	<b>0,43</b>	<b>0,26</b>	<b>0,36</b>	<b>0,30</b>
Standard deviation of reproducibility ( $s_R$ )	0,31	0,77	0,34	0,22	0,24	0,68	0,33
Coefficient of variation of reproducibility $CV_R$ , %	7,0	36,4	30,7	13,5	20,4	14,43	6,75
<b>Reproducibility limit (R)</b>	<b>0,88</b>	<b>2,16</b>	<b>0,96</b>	<b>0,63</b>	<b>0,67</b>	<b>1,90</b>	<b>0,93</b>

**Table B.14 — Statistical results for the individual sterol content:  $\Delta 5,24$ -Stigmastadienol (in %)**

Collaborative trial organized in	2011/2012					1996/1997	
Sample	A	B	C	D	E	B	D
Number of participating laboratories	14	14	14	14	13	14	14
Number of laboratories retained after eliminating outliers	11	10	11	12	11	11	13
Number test results in all laboratories	22	20	22	24	22	22	26
<b>Mean value (<math>m</math>), %</b>	<b>0,78</b>	<b>1,02</b>	<b>3,33</b>	<b>0,76</b>	<b>0,85</b>	<b>1,40</b>	<b>0,50</b>
Standard deviation of repeatability ( $s_r$ )	0,07	0,13	0,16	0,08	0,07	0,06	0,08
Coefficient of variation of repeatability, $CV_r$ , %	9,1	13,2	4,7	11,0	8,7	4,1	15,9
<b>Repeatability limit (<math>r</math>)</b>	<b>0,20</b>	<b>0,38</b>	<b>0,44</b>	<b>0,24</b>	<b>0,21</b>	<b>0,16</b>	<b>0,23</b>
Standard deviation of reproducibility ( $s_R$ )	0,08	0,2,1	0,97	0,10	0,54	0,25	0,16
Coefficient of variation of reproducibility $CV_R$ , %	9,7	20,8	29,0	12,8	64,0	17,7	31,2
<b>Reproducibility limit (<math>R</math>)</b>	<b>0,21</b>	<b>0,60</b>	<b>2,70</b>	<b>0,27</b>	<b>1,51</b>	<b>0,69</b>	<b>0,46</b>

**Table B.15 — Statistical results for the individual sterol content:  $\Delta 7$ -Stigmastenol (in %)**

Collaborative trial organized in	2011/2012					1996/1997	
Sample	A	B	C	D	E	B	D
Number of participating laboratories	14	14	14	14	13	14	14
Number of laboratories retained after eliminating outliers	9	12	12	12	8	11	12
Number test results in all laboratories	18	24	24	24	16	22	24
<b>Mean value (<math>m</math>), %</b>	<b>0,37</b>	<b>13,30</b>	<b>16,59</b>	<b>0,16</b>	<b>0,03</b>	<b>10,9</b>	<b>0,1</b>
Standard deviation of repeatability ( $s_r$ )	0,04	0,24	0,32	0,04	0,03	0,12	0,09
Coefficient of variation of repeatability, $CV_r$ , %	11,0	1,8	1,9	25,8	80,0	1,1	79,1
<b>Repeatability limit (<math>r</math>)</b>	<b>0,11</b>	<b>0,66</b>	<b>0,90</b>	<b>0,11</b>	<b>0,07</b>	<b>0,33</b>	<b>0,25</b>
Standard deviation of reproducibility ( $s_R$ )	0,11	1,50	1,45	0,08	0,06	0,21	0,11
Coefficient of variation of reproducibility $CV_R$ , %	29,6	11,3	8,8	53,3	198,3	2,0	96,3
<b>Reproducibility limit (<math>R</math>)</b>	<b>0,31</b>	<b>4,21</b>	<b>4,07</b>	<b>0,24</b>	<b>0,17</b>	<b>0,60</b>	<b>0,30</b>

**Table B.16 — Statistical results for the individual sterol content:  $\Delta^7$ -Avenasterol (in %)**

Collaborative trial organized in	2011/2012					1996/1997	
Sample	A	B	C	D		B	D
Number of participating laboratories	14	14	14	14		14	14
Number of laboratories retained after eliminating outliers	14	13	13	14		12	13
Number test results in all laboratories	28	26	26	28		24	26
<b>Mean value (<i>m</i>), %</b>	<b>0,56</b>	<b>3,82</b>	<b>1,45</b>	<b>0,07</b>		<b>4,9</b>	<b>0,1</b>
Standard deviation of repeatability ( <i>s<sub>r</sub></i> )	0,07	0,24	0,10	0,03		0,14	0,04
Coefficient of variation of repeatability, <i>CV<sub>r</sub></i> , %	12,7	6,2	7,0	43,4		2,8	31,9
<b>Repeatability limit (<i>r</i>)</b>	<b>0,20</b>	<b>0,66</b>	<b>0,28</b>	<b>0,09</b>		<b>0,38</b>	<b>0,11</b>
Standard deviation of reproducibility ( <i>s<sub>R</sub></i> )	0,25	0,66	0,56	0,05		0,35	0,10
Coefficient of variation of reproducibility <i>CV<sub>R</sub></i> , %	45,4	17,2	38,5	77,7		7,1	78,6
<b>Reproducibility limit (<i>R</i>)</b>	<b>0,71</b>	<b>1,84</b>	<b>1,56</b>	<b>0,15</b>		<b>0,93</b>	<b>0,27</b>

## Bibliography

- [1] ISO 5555:2001, *Animal and vegetable fats and oils — Sampling*
- [2] ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*
- [3] 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*



## Bureau of Indian Standards

BIS is a statutory institution established under the *Bureau of Indian Standards Act, 2016* to promote harmonious development of the activities of standardization, marking and quality certification of goods and attending to connected matters in the country.

### Copyright

BIS has the copyright of all its publications. No part of these publications may be reproduced in any form without the prior permission in writing of BIS. This does not preclude the free use, in the course of implementing the standard, of necessary details, such as symbols and sizes, type or grade designations. Enquiries relating to copyright be addressed to the Head (Publication & Sales), BIS.

### Review of Indian Standards

Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the website-[www.bis.gov.in](http://www.bis.gov.in) or [www.standardsbis.in](http://www.standardsbis.in).

This Indian Standard has been developed from Doc No.: FAD 13 (22569).

### Amendments Issued Since Publication

Amend No.	Date of Issue	Text Affected

## BUREAU OF INDIAN STANDARDS

### Headquarters:

Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi 110002

Telephones: 2323 0131, 2323 3375, 2323 9402

Website: [www.bis.gov.in](http://www.bis.gov.in)

### Regional Offices:

	Telephones
Central : 601/A, Konnectus Tower -1, 6 <sup>th</sup> Floor, DMRC Building, Bhavbhuti Marg, New Delhi 110002	{ 2323 7617
Eastern : 8 <sup>th</sup> Floor, Plot No 7/7 & 7/8, CP Block, Sector V, Salt Lake, Kolkata, West Bengal 700091	{ 2367 0012 2320 9474
Northern : Plot No. 4-A, Sector 27-B, Madhya Marg, Chandigarh 160019	{ 265 9930
Southern : C.I.T. Campus, IV Cross Road, Taramani, Chennai 600113	{ 2254 1442 2254 1216
Western : 5 <sup>th</sup> Floor/MTNL CETTM Technology Street, Hiranandani Gardens, Powai, Mumbai - 400076	{ 25700030 25702715

**Branches :** AHMEDABAD, BENGALURU, BHOPAL, BHUBANESHWAR, CHANDIGARH, CHENNAI, COIMBATORE, DEHRADUN, DELHI, FARIDABAD, GHAZIABAD, GUWAHATI, HARYANA (CHANDIGARH), HUBLI, HYDERABAD, JAIPUR, JAMMU, JAMSHEDPUR, KOCHI, KOLKATA, LUCKNOW, MADURAI, MUMBAI, NAGPUR, NOIDA, PARWANOO, PATNA, PUNE, RAIPUR, RAJKOT, SURAT, VIJAYAWADA.