
**Determination of individual
and total sterols contents — Gas
chromatographic method —**

Part 2:
Olive oils and olive pomace oils

*Détermination de la teneur en stérols individuels et totaux —
Méthode par chromatographie en phase gazeuse —*

Partie 2: Huile d'olive et huile de grignons d'olive



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Foreword

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

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

This first edition of ISO 12228-2 cancels and replaces ISO 12228:1999, which has been technically revised.

ISO 12228 consists of the following parts, under the general title *Determination of individual and total sterols contents — Gas chromatographic method*:

- *Part 1: Animal and vegetable fats and oils*
- *Part 2: Olive oils and olive pomace oils*

Determination of individual and total sterols contents — Gas chromatographic method —

Part 2: Olive oils and olive pomace oils

1 Scope

This part of ISO 12228 specifies a procedure for the gas chromatographic determination of the contents and composition of sterols and triterpene dialcohols in olive and olive pomace oils. For the determination of the contents and composition of sterols in all other animal and vegetable fats and oils, ISO 12228-1 is to be used.

NOTE This part of ISO 12228 is technically identical to IOC Standard COL/T.20/Doc. No. 30 (November 2011).

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*

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 Δ^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 Δ^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.

3.3

composition of triterpene dialcohols

composition of erythrodiol and uvaol in the sample 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, beginning with cholesterol and ending with uvaol (see [Table 1](#)) under the conditions specified in this part of ISO 12228, normalized to 100 %.

4 Principle

A test portion is saponified by boiling under reflux with an ethanolic potassium hydroxide solution. The unsaponifiable matter is extracted with diethyl ether. The sterol and triterpene dialcohol fractions are separated from the unsaponifiable matter by thin-layer chromatography on a basic silica gel plate. The qualitative and quantitative compositions of the sterol and triterpene dialcohol fractions are determined by gas chromatography of the trimethylsilyl ethers using cholestanol as 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, minimum mass fraction $w = 85$ g/100 g.

5.2 Potassium hydroxide, ethanolic solution, amount concentration, c , approximately 2 mol/l.

While cooling, dissolve 130 g of potassium hydroxide (5.1) in 200 ml of distilled water and then make up to 1 l with ethanol (5.9). Keep the solution in well-stoppered dark glass bottles and store for a maximum of 2 d.

5.3 Potassium hydroxide, ethanolic solution, amount concentration, c , approximately 0,2 mol/l.

Dissolve 13 g of potassium hydroxide (5.1) in 20 ml of distilled water and make up to 1 l with ethanol (5.9).

5.4 Diethyl ether, for chromatography.

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.

5.5 Anhydrous sodium sulfate.

5.6 Silica gel thin-layer chromatography (TLC) plates, commercially available, dimensions 20 cm × 20 cm, thickness of layer 0,25 mm, without fluorescence indicator.

5.7 Acetone, for chromatography.

5.8 *n*-Hexane, for chromatography.

5.9 Ethanol 96 %, minimum volume fraction $\varphi = 95$ %.

5.10 Ethyl acetate.

5.11 Reference solution for thin-layer chromatography, cholesterol or mixture of phytosterols, and erythrodiol solution in ethyl acetate (5.10), mass concentration, $\rho = 5$ %.

5.12 2,7-dichlorofluorescein, ethanolic solution, mass concentration, $\rho = 0,2$ %.

Make slightly basic by adding a few drops of 2 mol/l alcoholic potassium hydroxide solution (5.2). Store for a maximum of 1 year.

5.13 α -cholestanol internal standard solution, mass concentration, $\rho = 0,2$ g/100 ml, in ethyl acetate (5.10).

5.14 Phenolphthalein solution, mass concentration, $\rho = 10$ g/l, in ethanol (5.9).

5.15 Carrier gas for gas chromatography, helium or preferably hydrogen.

5.16 Auxiliary gases for gas chromatography, hydrogen, helium, nitrogen, and air.

5.17 Developing solvent, mixture of *n*-hexane and diethyl ether, volume concentrations are: σ (*n*-hexane) = 65 ml/100 ml, σ (diethyl ether) = 35 ml/100 ml.

5.18 Hexamethyldisilazane.

5.19 Trimethylchlorosilane.

5.20 Silylation reagent, mixture of pyridine, hexamethyldisilazane, and trimethylchlorosilane.

Volume concentration σ (pyridine) = 9 ml/13 ml, σ (hexamethyl disilazane) = 3 ml/13 ml, σ (trimethylchlorosilane) = 1 ml/13 ml. Prepare the mixture fresh daily.

NOTE Other silylation reagents can be used, e. g., mixture of N,O-bis-trimethylsilyl-trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS), σ (BSTFA) = 99 ml/100 ml and σ (TMCS) = 1 ml/100ml.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Round-bottomed flasks, of 250 ml, with ground neck.

6.2 Reflux condenser, with ground glass joint to fit the flask (6.1).

6.3 Separating funnel, of 500 ml capacity.

6.4 Developing tank, made of glass, with a ground glass lid, suitable for use with plates of dimensions 20 cm \times 20 cm.

6.5 Ultraviolet lamp, wavelength of 366 nm or 254 nm.

6.6 Microsyringe, to deliver 100 μ l, 500 μ l, and 1000 μ l.

6.7 Cylindrical filter funnel, with sintered-glass filter (G3, porosity 15 μ m to 40 μ m), diameter approximately 2 cm, depth 5 cm, suitable for filtration under vacuum with male ground glass joint.

6.8 Conical flask, for operation under a vacuum, 50 ml with ground glass female joint to fit to the filter funnel (6.7).

6.9 Test tube, 10 ml with a tapering bottom and a sealing glass stopper.

6.10 Gas chromatograph, for capillary columns, with split injector, consisting of:

— **column oven**, capable of maintaining the temperature with an accuracy of $\pm 1^\circ\text{C}$;

- **temperature-controlled injection unit**, with persilanised glass vaporising element and split system;
- **flame ionization detector (FID)**;
- **data acquisition system**.

6.11 Capillary column, made of fused silica, length 20 m to 30 m, internal diameter 0,25 mm to 0,32 mm, coated with 5 % Diphenyl, 95 % Dimethyl polysiloxane (SE-52 or SE-54 or equivalent stationary phase), with a temperature limit of at least 280 °C to 300 °C, film thickness between 0,1 µm and 0,30 µm.

6.12 Microsyringe for gas chromatography, of 1 µl and 10 µl capacity, for gas chromatography, with cemented needle suitable for split injection.

6.13 Desiccator, containing an efficient desiccant, for storing the plates, e.g. calcium dichloride.

6.14 Oven, maintained at 103 °C ± 2 °C.

6.15 Rotary evaporator, attached to a vacuum pump and water bath maintained at 40 °C.

6.16 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.^[2]

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. Dry the samples if necessary by filtration.

8 Procedure

8.1 Test portion

By means of a micro syringe (6.6), fill 500 µl (for olive oils) or 1 500 µl (for olive pomace oils) of the internal standard solution (5.13) in a 250 ml flask (6.1). Evaporate until dryness with a gentle current of nitrogen in a warm water bath and cool the flask.

Weigh, to the nearest 10 mg, about 5 g of olive or olive pomace oil into the same flask and proceed with 8.2.

NOTE Olive (pomace) oils, containing appreciable quantities of cholesterol, might show a peak having a retention time identical to cholestanol. In these cases, the sterol fraction shall be analysed in duplicate with and without the addition of the internal standard.

8.2 Preparation of unsaponifiable matter

8.2.1 Add 50 ml of 2 mol/l ethanolic potassium hydroxide solution (5.2) and some pumice stones, fit the reflux condenser, and heat to gentle boiling until the solution becomes clear (end of saponification).

Continue heating for a further 20 min, add 50 ml of distilled water through the top of the condenser, detach the condenser, and cool the flask to approximately 30 °C.

8.2.2 Transfer the contents of the flask quantitatively into a 500 ml separating funnel (6.3) using several portions of distilled water (50 ml). Add approximately 80 ml of diethyl ether (5.4), shake vigorously for approximately 60 s, periodically releasing the pressure by inverting the separating funnel and opening the stopcock. Allow to stand until the separation of two phases is complete.

Draw off the soap solution as completely as possible into a second separating funnel. Perform two further extractions on the water-alcohol phase in the same way using 60 ml to 70 ml of diethyl ether (5.4).

Emulsions shall be destroyed by adding small quantities of ethanol (5.9) and swirling gently.

8.2.3 Combine the three diethyl ether extracts in one separating funnel containing 50 ml of water. Continue to wash with water (50 ml) until the wash water no longer turns pink on the addition of a drop of phenolphthalein solution (5.14).

After the removal of the wash water, filter through anhydrous sodium sulfate (5.5) into a previously weighed 250 ml flask, washing the funnel and filter with small quantities of diethyl ether (5.4).

8.2.4 Evaporate the solvent with a rotary evaporator at 30 °C under vacuum. Add 5 ml of acetone (5.7) and remove the volatile solvent completely in a gentle current of air. Dry the residue in the oven (6.14) at 103 °C ± 2 °C for 15 min. Cool in a desiccator and weigh to the nearest 0,1 mg.

8.3 Separation of the sterol and triterpene dialcohols (erythrodiol, uvaol) fractions by TLC

8.3.1 Immerse the silica gel plates (5.6) about 4 cm in the 0,2 mol/l ethanolic potassium hydroxide solution (5.3) for 10 s, allow to dry in a fume cupboard for two hours, and place in an oven at 100 °C for 1 h.

Remove the plates from the oven and store in the desiccator (6.13) until use (plates shall be stored for maximum of 15 d).

NOTE When basic silica gel plates are used to separate the sterol fraction, all compounds of an acidic nature (fatty acids and others) are retained on the spotting line and the sterol band is clearly separated from the aliphatic and triterpene alcohols band.

8.3.2 Fill the developing solvent (5.17) to a depth of approximately 1 cm into the development tank (6.4). Close the glass lid and leave for at least half an hour in a cool place to get a liquid-vapour equilibrium. Strips of filter paper dipping into the eluent should be placed on the internal surfaces of the chamber to reduce the developing time by approximately one-third. In addition, it gives a better elution of the components.

The developing solvent shall be replaced for every test, in order to achieve more reproducible elution conditions. It is also possible to use a mixture of *n*-hexane and diethyl ether (volume concentrations $\sigma = 50 \text{ ml}/100 \text{ ml}$) as an alternative developing solvent.

8.3.3 Prepare a 5 % solution of the unsaponifiable (8.2.4) in ethyl acetate (5.10). Using the 100 μl microsyringe, apply 0,3 ml of the solution as a line at a distance of 2 cm from the lower edge onto a TLC plate (5.6). Leave a gap of at least 3 cm from each side edge of the plate. Apply a spot of 5 μl of the TLC reference solution (5.11) at 1,5 cm from the edges.

8.3.4 Place the plate into the tank, prepared according to 8.3.2, and develop it until the solvent reaches approximately 1 cm to 2 cm from the upper edge of the TLC plate. The ambient temperature should be kept constant. Remove the plate from the developing chamber and allow the solvent to evaporate in a fume cupboard.

8.3.5 Spray the plate lightly and uniformly with the 2,7-dichlorofluorescein solution (5.12) and then leave to dry. Observe the plate in the ultraviolet light (6.5) and identify the sterols and triterpene dialcohols bands with the aid of the spots from the reference solution (5.11). Mark the zones at the high of the standard spots with a black pencil (shown in Figure A.1).

8.3.6 Scratch off the marked zone completely using a spatula and quantitatively collect the silica in the filter funnel (6.7). Add 10 ml of hot ethyl acetate (5.10), mix carefully with the metal spatula, and filter under vacuum, collecting the filtrate in a conical flask (6.8.) attached to the filter funnel. Wash the residue in the flask three times with 10 ml of diethyl ether (5.4), and filter in the same flask attached to the funnel. Evaporate the combined ether extracts to a volume of 4 ml to 5 ml, transfer the residual solution to the previously weighed 10 ml test tube (6.9), and blow off the solvent with a gentle stream of nitrogen. Add a few drops of acetone (5.7) and blow off the acetone to dryness. The residue in the test tube contains the sterol and triterpene dialcohol fractions.

8.4 Preparation of the trimethylsilyl ethers

8.4.1 Add the silylation reagent (5.20) into the test tube. Use 50 µl reagent for 1 mg of sterols and triterpene dialcohols.

NOTE Ready-to-use solutions are commercially available. Pyridine can be replaced by acetonitrile.

8.4.2 Stopper the test tube, shake carefully to dissolve completely. Leave to stand for at least 15 min at ambient temperature and then centrifuge for a few minutes. The clear solution is ready for gas chromatographic analysis.

When using hexamethyldisilazane trimethylchlorosilane, a slight opalescence might occur. The formation of a white flock or the appearance of a pink colour indicates the presence of moisture or deterioration of the reagent. In this case, the test shall be repeated.

8.5 Gas chromatographic analysis

8.5.1 GC conditions

Optimize the temperature programme and the carrier gas flow so that chromatograms similar to Figure A.2 and A.3 are obtained. Test the separation with silylated sterol fractions obtained from known oils (see Table 1).

The following parameters were tested and found useful:

- column temperature: (260 ± 5) °C;
- injector temperature: (280 to 300) °C;
- detector temperature: (280 to 300) °C;
- linear velocity of the carrier gas: helium 20 cm/s to 35 cm/s, hydrogen 30 cm/s to 50 cm/s;
- splitting ratio: 1:50 to 1:100;
- injection volume: 0,5 µl to 1,0 µl of TMSE solution;
- retention time for the β -sitosterol shall be (20 ± 5) min;
- campesterol peak of olive oil with a mean content of 3 % shall be (20 ± 5) % of full scale;
- campesterol peak of soybean oil with a mean content of 20 % shall be (80 ± 10) % of full scale;
- all present sterols shall be separated and completely resolved.

NOTE Use a blank run to test for possible contamination (e.g. cholesterol from solvents, glass walls filter, fingerprints, etc.)

Inject 0,5 µl to 1 µl of the sample into the gas chromatograph. An automatic injector can also be used.

Record the chromatograms until the triterpene dialcohols are completely eluted. A horizontal base line with no positive or negative drift shall be obtained.

8.5.2 Peak identification

Identify the individual peaks using relative retention times (RRT) and compare with a mixture of sterols and triterpene dialcohols, prepared and analysed under the same conditions.

The sterols and triterpene dialcohols are eluted according to [Table 1](#), which also gives the RRTs. The RRTs corresponding to β -sitosterol with SE-52 and SE-54 stationary phases are given in [Table 1](#).

Table 1 — GLC peak identification of individual sterols, erythrodiol, and uvaol by RRT for SE-52 and SE-54 stationary phase, based on β -sitosterol

Peak Order	Common names of sterols	Systematic names of sterols	RRT SE-54	RRT SE-52
1	Cholesterol	Cholest-5-en-3 β -ol	0,67	0,63
2	Cholestanol	5 α -Cholestan-3 β -ol	0,68	0,64
3	Brassicasterol	[24S]-24-Methyl cholesta-5,22-dien-3 β -ol	0,73	0,71
*	(Ergosterol)	[24S] 24 Methyl cholesta-5,7,22-trien-3 β ol	0,78	0,76
4	24-Methylene cholesterol	24-Methylene cholesta-5,24-dien-3 β -ol	0,82	0,80
5	Campesterol	[24R]-24-Methyl cholest-5-en-3 β -ol	0,83	0,81
6	Campestanol	[24R]-24-Methyl cholestan-3 β -ol	0,85	0,82
7	Stigmasterol	[24S]-24-Ethyl cholesta-5,22-dien-3 β -ol	0,88	0,87
8	Δ 7-Campesterol	[24R]-24-Methyl cholest-7-en-3 β -ol	0,93	0,92
9	Δ 5,23-Stigmastadienol	[24R,S]-24-Ethyl cholesta-5,23-dien-3 β -ol	0,95	0,95
10	Clerosterol	[24S]-24-Ethyl cholesta-5,25-dien-3 β -ol	0,96	0,96
11	β -Sitosterol	[24R]-24-Ethyl cholest-5-en-3 β -ol	1,00	1,00
12	Sitostanol	[24R]-24-Ethyl cholestan-3 β -ol	1,02	1,02
13	Δ 5-Avenasterol	[24Z]-24(28)-Ethylidene cholest-5-en-3 β -ol	1,03	1,03
14	Δ 5,24-Stigmastadienol	[24R,S]-24-Ethyl cholesta-5,24-dien-3 β -ol	1,08	1,08
15	Δ 7-Stigmastenol	[24R,S]-24-Ethyl cholest-7-en-3 β -ol	1,12	1,12
16	Δ 7-Avenasterol	[24Z]-24(28)-Ethylidene cholest-7-en-3 β -ol	1,16	1,16
17	Erythrodiol	5 α -olean-12-en-3 β ,28-diol	1,41	1,41
18	Uvaol	Δ 12-ursen-3 β ,28-diol	1,52	1,52

NOTE 1 Ergosterol is not considered in the calculation.

NOTE 2 Sitosterol can coelute together with α -spinasterol and Δ 7,22,25-stigmastatrienol. [24R]-24-Ethyl cholesta-7,25(27)-dien-3 β -ol is present in sterols of sunflower and pumpkin seed oil and can coelute with peak 14 (Δ 5,24-stigmastadienol).

9 Expression of results

9.1 Quantitative evaluation

Calculate the areas of the α -cholestanol and the sterol and triterpene dialcohols peaks. Do not take into account peaks which are not listed in [Table 1](#) and ergosterol. For the purposes of this part of ISO 12228, it is assumed that the response factors of all sterols are equal and to be 1.

9.2 Determination of the total sterol content

Calculate the total sterol content, w , in milligrams per kilogram of oil, according to Formula (1):

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

where

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

$\Sigma(A)$ is the sum of the peak areas of the individual sterols present;

A_{IS} is the peak area of the added 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 Δ^7 -avenasterol (peak 16), but without erythrodiol and uvaol (peaks 17 and 18).

Report the total sterol content as a whole number.

9.3 Composition of sterols

Calculate the mass fraction, w_i , of the individual sterol, i , in g/100 g (per cent), according to Formula (2):

$$w_i = \frac{A_i}{\sum A} \cdot 100 \quad (2)$$

where

A_i is the area of the peak of sterol i ;

ΣA is the sum of the peak areas of all sterols (peaks 1 to 16).

NOTE Apparent β -sitosterol is defined as the sum of Δ^5 -23-stigmastadienol, clerosterol, β -sitosterol, sitostanol, Δ^5 -avenasterol, and Δ^5 -24-stigmastadienol.

9.4 Composition of triterpene dialcohols

Calculate the mass fraction, w_{EU} , as a percentage, of the sum of erythrodiol and uvaol according to Formula (3):

$$w_{EU} = \frac{A_E + A_U}{A_E + A_U + \sum A} \cdot 100 \quad (3)$$

where

A_E is the peak area of erythrodiol;

A_U is the peak area of uvaol;

ΣA is the sum of the peak areas of all sterols.

Report the content of the sum of erythrodiol and uvaol with one decimal place.

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 might 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 might 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 might 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 12228-2);
- d) all operating details not specified in this part of ISO 12228, or regarded as optional, together with details of any incidents which might 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)

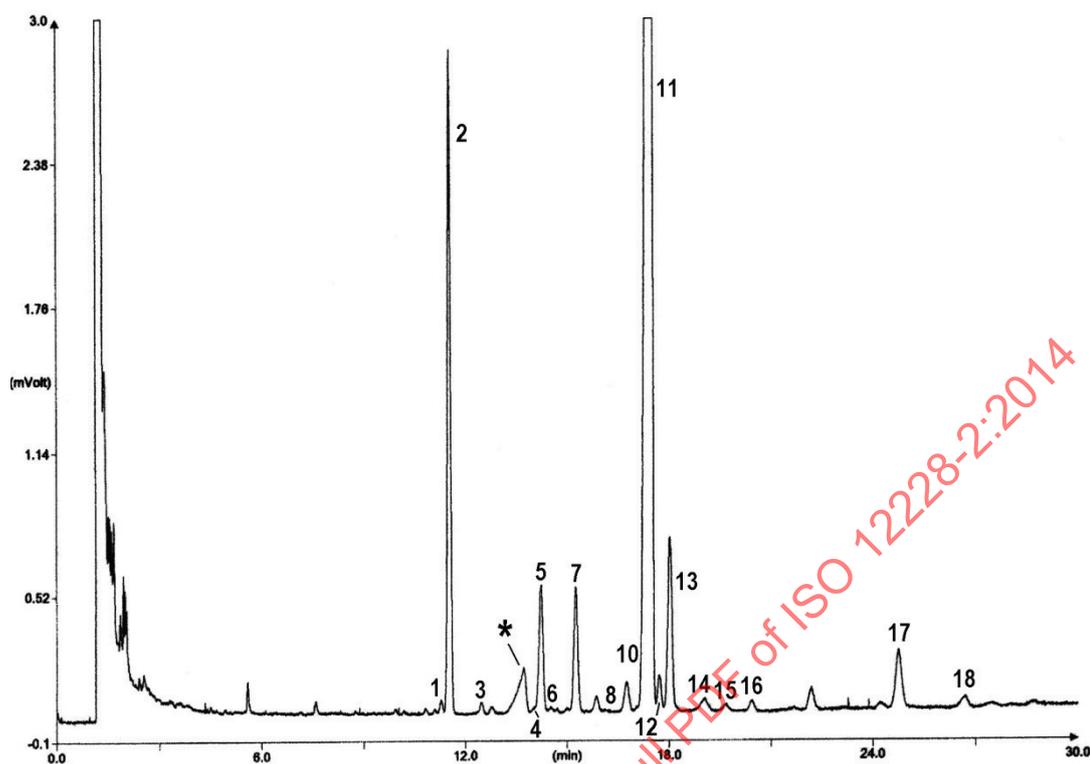
Figures



Key

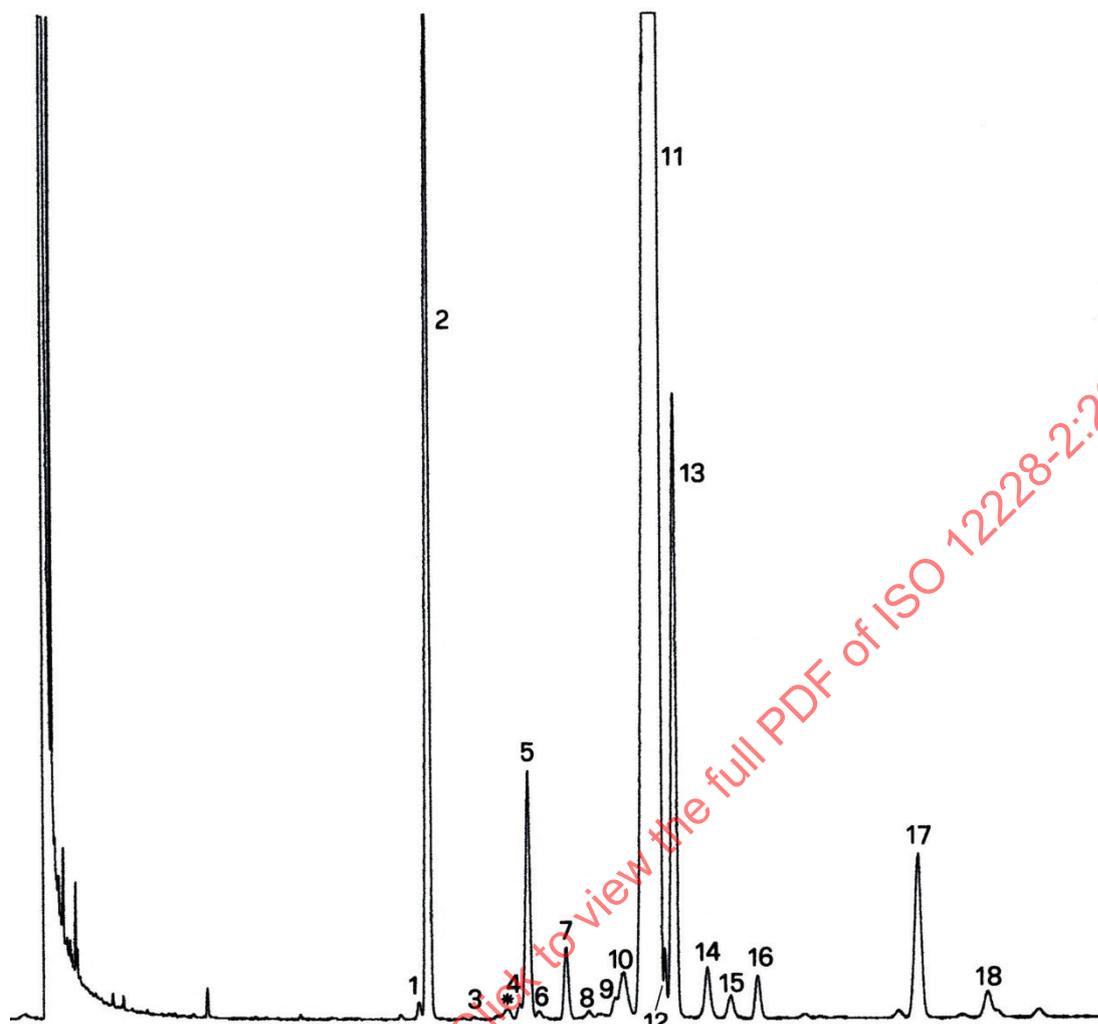
- 1 squalene
- 2 triterpenes and aliphatic alcohols
- 3 sterols and triterpene dialcohols
- 4 start and free fatty acids

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 given in [8.5](#).

Figure A.2 — GLC of sterols and triterpene dialcohols from lampante olive oil



NOTE Identification of the peak numbers is according to [Table 1](#). Conditions are as given in [8.5](#).

Figure A.3 — GLC of sterols and triterpene dialcohols from refined olive oil

Annex B (informative)

Interlaborative trial

The precision of this part of ISO 12228 has been established by an international interlaboratory test organized by The Executive Secretariat of the International Olive Council in 2009, on samples of

- A: crude olive pomace oil,
- B: refined olive pomace oil,
- C: extra virgin olive oil,
- D: blend of 20 % high oleic sunflower oil with 70 % extra virgin olive oil and 10 % rapeseed oil, and
- E: blend of 15 % soybean oil with 85 % olive oil.

The test was carried out in accordance with ISO 5725-1^[3] and ISO 5725-2.^[4]

Table B.1 — Statistical results for the total sterol content

Sample	A	B	C	D	E
Number of participating laboratories	18	18	18	18	18
Number of laboratories retained after eliminating outliers	17	18	18	17	17
Number of test results in all laboratories	34	36	36	34	34
Mean value (<i>m</i>), mg/kg	4 487,0	3 169,8	1 359,8	2 066,5	1 552,1
Standard deviation of repeatability (<i>s_r</i>)	71,8	49,2	45,2	30,1	31,8
Coefficient of variation of repeatability, <i>CV_r</i> , %	1,6	1,6	3,3	1,5	2,1
Repeatability limit (<i>r</i>)	200,9	137,6	126,5	84,4	88,9
Standard deviation of reproducibility (<i>s_R</i>)	378,9	234,7	82,9	131,5	89,5
Coefficient of variation of reproducibility, <i>CV_R</i> , %	8,4	7,4	6,1	6,4	5,8
Reproducibility limit (<i>R</i>)	1 060,9	657,0	232,2	368,1	250,7

Table B.2 — Statistical results for the individual sterol content: Cholesterol

Sample	A	B	C	D	E
Number of participating laboratories	19	19	19	19	19
Number of laboratories retained after eliminating outliers	19	14	16	18	18
Number of test results in all laboratories	38	28	32	36	36
Mean value (<i>m</i>), %	0,13	0,13	0,13	0,16	0,21
Standard deviation of repeatability (<i>s_r</i>)	0,02	0,02	0,02	0,03	0,02
Coefficient of variation of repeatability, <i>CV_r</i> , %	18,8	14,0	12,3	15,1	7,2
Repeatability limit (<i>r</i>)	0,07	0,05	0,04	0,07	0,04
Standard deviation of reproducibility (<i>s_R</i>)	0,04	0,02	0,04	0,05	0,06
Coefficient of variation of reproducibility, <i>CV_R</i> , %	31,9	17,8	29,5	29,1	27,7
Reproducibility limit (<i>R</i>)	0,12	0,07	0,11	0,13	0,16