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

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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).

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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 COI/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: $\sigma(n\text{-hexane}) = 65$ ml/100 ml, $\sigma(\text{diethyl ether}) = 35$ ml/100 ml.

5.18 Hexamethyldisilazane.

5.19 Trimethylchlorosilane.

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

Volume concentration $\sigma(\text{pyridine}) = 9$ ml/13 ml, $\sigma(\text{hexamethyl disilazane}) = 3$ ml/13 ml, $\sigma(\text{trimethylchlorosilane}) = 1$ 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), $\sigma(\text{BSTFA}) = 99$ ml/100 ml and $\sigma(\text{TMCS}) = 1$ 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 $\pm 1^\circ\text{C}$;