



# SLOVENSKI STANDARD SIST EN ISO 12228-1:2014

01-oktober-2014

Nadomešča:  
SIST EN ISO 12228:2000

---

**Določevanje posameznih in celotnih sterolov - Plinska kromatografska metoda - 1. del: Rastlinske in živalske maščobe in olja (ISO 12228-1:2014)**

Determination of individual and total sterols contents - Gas chromatographic method - Part 1: Animal and vegetable fats and oils (ISO 12228-1:2014)

Bestimmung der individuellen und der Gesamtsterine - Gaschromatographisches Verfahren - Teil 1: Tierische und pflanzliche Fette und Öle (ISO 12228-1:2014)

Détermination de la teneur en stérols individuels et totaux - Méthode par chromatographie en phase gazeuse - Partie 1: Corps gras d'origines animale et végétale (ISO 12228-1:2014)

**Ta slovenski standard je istoveten z: EN ISO 12228-1:2014**

**ICS:**

67.200.10	Rastlinske in živalske maščobe in olja	Animal and vegetable fats and oils
-----------	--	------------------------------------

**SIST EN ISO 12228-1:2014** en

**iTeh STANDARD PREVIEW**  
**(standards.iteh.ai)**

[SIST EN ISO 12228-1:2014](https://standards.iteh.ai/catalog/standards/sist/779a49c0-acb8-4bbc-a506-7aaa3c18ff1e/sist-en-iso-12228-1-2014)

<https://standards.iteh.ai/catalog/standards/sist/779a49c0-acb8-4bbc-a506-7aaa3c18ff1e/sist-en-iso-12228-1-2014>

EUROPEAN STANDARD  
NORME EUROPÉENNE  
EUROPÄISCHE NORM

**EN ISO 12228-1**

July 2014

ICS 67.200.10

Supersedes EN ISO 12228:1999

English Version

**Determination of individual and total sterols contents - Gas chromatographic method - Part 1: Animal and vegetable fats and oils (ISO 12228-1:2014)**

Détermination de la teneur en stérols individuels et totaux -  
Méthode par chromatographie en phase gazeuse - Partie 1:  
Corps gras d'origines animale et végétale (ISO 12228-  
1:2014)

Bestimmung der individuellen und der Gesamtsterine -  
Gaschromatographisches Verfahren - Teil 1: Tierische und  
pflanzliche Fette und Öle (ISO 12228-1:2014)

This European Standard was approved by CEN on 14 June 2014.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION  
COMITÉ EUROPÉEN DE NORMALISATION  
EUROPÄISCHES KOMITEE FÜR NORMUNG

**CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels**

Contents	Page
Foreword.....	3

**iTeh STANDARD PREVIEW**  
**(standards.iteh.ai)**

[SIST EN ISO 12228-1:2014](https://standards.iteh.ai/catalog/standards/sist/779a49c0-acb8-4bbc-a506-7aaa3c18ff1e/sist-en-iso-12228-1-2014)  
<https://standards.iteh.ai/catalog/standards/sist/779a49c0-acb8-4bbc-a506-7aaa3c18ff1e/sist-en-iso-12228-1-2014>

## Foreword

This document (EN ISO 12228-1:2014) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 307 "Oilseeds, vegetable and animal fats and oils and their by-products - Methods of sampling and analysis" the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by January 2015, and conflicting national standards shall be withdrawn at the latest by January 2015.

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

This document supersedes EN ISO 12228:1999.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

### Endorsement notice

The text of ISO 12228-1:2014 has been approved by CEN as EN ISO 12228-1:2014 without any modification.

**ITeH STANDARD PREVIEW**  
**(standards.iteh.ai)**

SIST EN ISO 12228-1:2014  
<https://standards.iteh.ai/catalog/standards/sist/779a49c0-acb8-4bbc-a506-7aaa3c18ff1e/sist-en-iso-12228-1-2014>

**iTeh STANDARD PREVIEW**  
**(standards.iteh.ai)**

[SIST EN ISO 12228-1:2014](#)

<https://standards.iteh.ai/catalog/standards/sist/779a49c0-acb8-4bbc-a506-7aaa3c18ff1e/sist-en-iso-12228-1-2014>

INTERNATIONAL  
STANDARD

ISO  
12228-1

First edition  
2014-07-15

---

---

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

**Part 1:  
Animal and vegetable fats and oils**

**iTeh STANDARD PREVIEW**  
*Détermination de la teneur en stérols individuels et totaux —  
Méthode par chromatographie en phase gazeuse —  
(standards.iteh.ai)  
Partie 1: Corps gras d'origines animale et végétale*

SIST EN ISO 12228-1:2014

<https://standards.iteh.ai/catalog/standards/sist/779a49c0-acb8-4bbc-a506-7aaa3c18ff1e/sist-en-iso-12228-1-2014>



Reference number  
ISO 12228-1:2014(E)

© ISO 2014

## iTeh STANDARD PREVIEW (standards.iteh.ai)

[SIST EN ISO 12228-1:2014](https://standards.iteh.ai/catalog/standards/sist/779a49c0-acb8-4bbc-a506-7aaa3c18ff1e/sist-en-iso-12228-1-2014)

<https://standards.iteh.ai/catalog/standards/sist/779a49c0-acb8-4bbc-a506-7aaa3c18ff1e/sist-en-iso-12228-1-2014>



### **COPYRIGHT PROTECTED DOCUMENT**

© ISO 2014

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

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](mailto:copyright@iso.org)  
Web [www.iso.org](http://www.iso.org)

Published in Switzerland



# Contents

Page

Foreword.....	iv
<b>1 Scope.....</b>	<b>1</b>
<b>2 Normative references.....</b>	<b>1</b>
<b>3 Terms and definitions.....</b>	<b>1</b>
<b>4 Principle.....</b>	<b>1</b>
<b>5 Reagents.....</b>	<b>2</b>
<b>6 Apparatus.....</b>	<b>3</b>
<b>7 Sample.....</b>	<b>3</b>
7.1 Sampling.....	3
7.2 Preparation of the test sample.....	3
<b>8 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 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 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 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>23</b>

## ISO 12228-1:2014(E)

### 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](http://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](http://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 12228-1, together with 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 content — 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 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.

## ISO 12228-1:2014(E)

## 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 × 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.