

**Animal and vegetable fats and oils –**  
**Determination of stigmastadienes in vegetable oils –**  
Part 2: Method using high-performance liquid  
chromatography (HPLC) (ISO 15788-2:2003, IDT)

Europastandarden EN ISO 15788-2:2004 gäller som svensk standard. Detta dokument innehåller den officiella engelska versionen av EN ISO 15788-2:2004.

The European Standard EN ISO 15788-2:2004 has the status of a Swedish Standard. This document contains the official English version of EN ISO 15788-2:2004.

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EUROPEAN STANDARD  
NORME EUROPÉENNE  
EUROPÄISCHE NORM

**EN ISO 15788-2**

November 2004

ICS 67.200.10

English version

**Animal and vegetable fats and oils - Determination of  
stigmastadienes in vegetable oils - Part 2: Method using high-  
performance liquid chromatography (HPLC) (ISO 15788-2:2003)**

Corps gras d'origines animale et végétale - Dosage des  
stigmastadiènes dans les huiles végétales - Partie 2:  
Méthode par chromatographie liquide à haute performance  
(CLHP) (ISO 15788-2:2003)

Tierische und pflanzliche Fette und Öle - Bestimmung der  
Stigmastadiene in Pflanzenölen - Teil 2: Verfahren mit  
Hochleistungsflüssigchromatographie (HPLC) (ISO 15788-  
2:2003)

This European Standard was approved by CEN on 13 February 2003.

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 Central Secretariat 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 Central Secretariat has the same status as the official versions.

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



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## Foreword

The text of ISO 15788-2:2003 has been prepared by Technical Committee ISO/TC 34 "Agricultural food products" of the International Organization for Standardization (ISO) and has been taken over as EN ISO 15788-2:2004 by 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 May 2005, and conflicting national standards shall be withdrawn at the latest by May 2005.

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

## Endorsement notice

The text of ISO 15788-2:2003 has been approved by CEN as EN ISO 15788-2:2004 without any modifications.



# Animal and vegetable fats and oils — Determination of stigmastadienes in vegetable oils —

## Part 2: Method using high-performance liquid chromatography (HPLC)

### 1 Scope

This part of ISO 15788 specifies a method for the determination of steradienes, especially stigmastadienes. Steradienes are formed by dehydration of sterols during bleaching and also partially during steam washing and deodorization. The method is also suitable as a screening method to detect the presence of refined vegetable oils in virgin oils such as virgin olive oil.

**NOTE** ISO 15788-1 is the reference method for the determination of stigmastadienes in vegetable oils whilst this part of ISO 15788 can be used as a rapid screening method. In view of the precision of this method (see Annex A), samples of virgin olive oils close to the limit adopted by international regulations (IOOC, EC) can be verified by the GLC method given in ISO 15788-1.

### 2 Normative references

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

ISO 3696:1987, *Water for analytical use — Specification and test method*

ISO 12228, *Animal and vegetable fats and oils — Determination of individual and total sterols content — Gas chromatographic method*

### 3 Terms and definitions

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

#### 3.1

##### **stigmastadienes content**

that part of the stigmastadienes separated by liquid chromatography under the conditions specified in this International Standard

**NOTE** It is expressed in milligrams per kilogram.

#### 3.2

##### **steradienes content**

that part of all the steradienes separated by liquid chromatography under the conditions specified in this International Standard

**NOTE** It is expressed in milligrams per kilogram.

**EN ISO 15788-2:2006 (E)****4 Principle**

Steradienes are separated from the major part of other lipids as non-polar fat compounds, using petroleum ether on a silica gel column. The petroleum ether eluate is concentrated then analysed by RP-18-HPLC and UV-detection at 235 nm. They are quantified, depending on the kind of sample, using an internal or external standard.

**5 Reagents**

**WARNING — Attention is drawn to the regulations which prescribe the handling of dangerous matter. Technical, organizational and personal safety measurements shall be followed.**

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

**5.1 Water**, of at least grade 1 according to ISO 3696:1987.

**5.2 Silica gel 60 for column chromatography**, particle size 0,063 mm to 0,200 mm, or 0,063 mm to 0,100 mm<sup>1)</sup>, with a water content of 2 g per 100 g.

Dry the silica gel in a porcelain dish for 12 h at 160 °C in a drying oven and cool to room temperature in a desiccator. For adjusting the silica gel to a water content of 2 g per 100 g, weigh (to the nearest 1 g) 98 g of the dried silica gel in a conical flask with ground glass stopper and add 2 g of water (weighed to the nearest 0,01 g). Shake vigorously for 1 min and allow the silica gel to stand overnight in an airtight vessel.

**5.3 Petroleum ether**, boiling range 40 °C to 60 °C.

**5.4 Acetonitrile**, chromatography grade.

**5.5 *tert*-Butyl methyl ether**, chromatography grade.

**5.6 Isooctane**.

**5.7  $\Delta$ 3,5-Cholestadiene<sup>2)</sup>**, with a known purity of at least 95 g per 100 g.

Check the purity of the cholestadiene standard by gas chromatography using 5 $\alpha$ -cholestane as internal standard. For this test, see the conditions for the gas chromatographic method for the determination of sterols specified in ISO 12228. The response factor of the flame ionization detector should be 1,0. Take into consideration the obtained concentration during the determination of the steradienes.

**5.8 Stock solutions and standard solutions**

**5.8.1  $\Delta$ 3,5-Cholestadiene stock solution**, of concentration 1 mg/ml.

Weigh, to the nearest 0,1 mg, 50,0 mg of  $\Delta$ 3,5-cholestadiene into a 50 ml measuring flask. Dissolve and dilute to the mark with *tert*-butyl methyl ether (5.5).

**5.8.2  $\Delta$ 3,5-Cholestadiene standard solution for HPLC**

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1) For example, No. 7734 or 15101 from E. Merck, 64271 Darmstadt, Germany.

2) For example, No. C6012 from Sigma Chemie GmbH, Grünwalder Weg 30, 82041 Deisenhofen, Germany.

This information is given for the convenience of users of this part of ISO 15788 and does not constitute an endorsement by ISO of these products.