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# SS-EN 12822:2014

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## Livsmedel – Bestämning av vitamin E med HPLC – Mätning av α-, ß-, γ- och δ-tokoferoler

## Foodstuffs – Determination of vitamin E by high performance liquid chromatography – Measurement of α-, ß-, γ- and δ-tocopherol

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Denna standard ersätter SS-EN 12822, utgåva 1.

The European Standard EN 12822:2014 has the status of a Swedish Standard. This document contains the official version of EN 12822:2014.

This standard supersedes the Swedish Standard SS-EN 12822, edition 1.



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Denna standard är framtagen av kommittén för Livsmedel och foder, SIS/TK 435.

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# EUROPEAN STANDARD
# NORME EUROPÉENNE
# EUROPÄISCHE NORM

**EN 12822**

June 2014

ICS 67.050

Supersedes EN 12822:2000

English Version

# Foodstuffs - Determination of vitamin E by high performance liquid chromatography - Measurement of α-, ß-, γ- and δ-tocopherol

Produits alimentaires - Détermination de la teneur en vitamine E par chromatographie liquide haute performance - Dosage des α-, ß-, γ- et δ-tocophérols

Lebensmittel - Bestimmung von Vitamin E mit Hochleistungs-Flüssigchromatographie - Bestimmung von α-, ß-, γ- und δ-Tocopherol

This European Standard was approved by CEN on 17 April 2014.

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

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



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# Foreword

This document (EN 12822:2014) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

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of an identical text or by endorsement, at the latest by December 2014 and conflicting national standards shall be withdrawn at the latest by December 2014.

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 12822:2000.

Annexes A, B and C are informative.

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**WARNING — The use of this standard can involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.**

# Introduction

This European Standard provides the base for the analytical methods. It is intended to serve as a frame in which the analyst can define his own analytical work in accordance to the standard procedure.

As the method in this European Standard deals with the measurement of the mass fraction of α-, β-, γ- and δ-tocopherol in food, reference is made to the literature for the calculation and expression of the vitamin E content in terms of biological activities. For further information see [1], [2], [3] and [4]. The differentiation of *RRR*-tocopherol and all racemic tocopherols is not possible with this method.

# 1 Scope

This European Standard specifies a method for the determination of vitamin E in foods by high performance liquid chromatography (HPLC). The determination of vitamin E content is carried out by measurement of α-, β-, γ- and δ-tocopherol. This method has been validated in two interlaboratory studies. The first study was for the analysis of α-tocopherol in margarine and milk powder ranging from 9,89 mg/100 g to 24,09 mg/100 g. The second study was for the analysis of α-, β-, γ- and δ-tocopherol in milk powder and of α-, and β-tocopherol in oat powder ranging from 0,057 mg/100 g (β-tocopherol) to 10,2 mg/100 g (α-tocopherol).

NOTE The vitamin E activity can be calculated from the tocopherol content assuming appropriate factors as given in [1], [2], [3] and [4].

# 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.

EN ISO 3696, *Water for analytical laboratory use - Specification and test methods (ISO 3696)*

# 3 Principle

α-, β-, γ- and δ-tocopherol are determined in a sample solution by HPLC separation and subsequent photometric (UV-range) or preferably fluorometric detection. In most cases a saponification of the test material followed by an extraction is necessary. Identification is carried out on the basis of retention times and quantitative determination by the external standard method using peak areas of peak heights. Internal standard methods can also be used if the corresponding recovery tests have proven the same behaviour of the internal standard during the analysis as the analyte itself, for more information see [4] to [14].

NOTE Using normal phase columns, the separation of tocopherols and tocotrienols is also feasible.

# 4 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and water of at least grade 1 according to EN ISO 3696.

**4.1 Methanol.**

**4.2 Ethanol absolute,** volume fraction $\varphi(C_2H_5OH)$ = 100 %.

**4.3 Ethanol,** $\varphi(C_2H_5OH)$ = 96 %.

**4.4 Sodium sulfate,** anhydrous.

**4.5 KOH solution,** for saponification, in suitable mass concentrations, for example $\rho$(KOH) = 50 g/100 ml or $\rho$(KOH) = 60 g/100 ml or alcoholic solutions, for example 28 g of KOH in 100 ml of a mixture of 9 parts per volume of ethanol and 1 part per volume of water.

**4.6 Antioxidants,** such as ascorbic acid (AA), sodium ascorbate, pyrogallol, sodium sulfide ($Na_2S$), hydroquinone or butylated hydroxytoluene (BHT).

**4.7 Solvents and extraction solvents,** such as diethyl ether (peroxide free), dicholormethane, light petroleum (boiling range of 40 °C to 60 °C), *n*-hexane, ethylacetate or appropriate mixtures thereof.

**4.8 HPLC mobile phase,** appropriate mixtures expressed as volume fractions of for example 3 % 1,4-dioxane or 0,5 % 2-propanol, 3 % tert-butyl methyl ether in *n*-hexane or *n*-heptane for normal phase chromatography (NP) or 1 % to 10 % water in methanol for reversed phase chromatography (RP).

For alternative HPLC systems, see Annex C.

**4.9 Standard substances**

**4.9.1 General**

β-, γ- and δ-tocopherol can be obtained from Calbiochem [1)] α-tocopherol can be obtained from various suppliers. The purity of the tocopherol standards can vary between 90 % and 100 %. It is therefore necessary to determine the concentration of the calibration solution by UV spectrometry (for purity tests, see 4.10.5).

**4.9.2 α-tocopherol,** $M(C_{29}H_{50}O_2)$ = 430,7 g/mol, with a known mass fraction of at least 95 %.

α-tocopherol acetate, $M(C_{31}H_{52}O_3)$ = 472,7 g/mol, may also be used as standard after saponification.

**4.9.3 β-tocopherol,** $M(C_{28}H_{48}O_2)$ = 416,7 g/mol, with a known mass fraction of at least 90 %.

**4.9.4 γ-tocopherol,** $M(C_{28}H_{48}O_2)$ = 416,7 g/mol, with a known mass fraction of at least 90 %.

**4.9.5 δ-tocopherol,** $M(C_{27}H_{46}O_2)$ = 402,6 g/mol, with a known mass fraction of at least 90 %.

**4.10 Stock solutions**

**4.10.1 α-tocopherol stock solution**

Weigh, to the nearest milligram, an amount of the α-tocopherol standard substance (4.9.2), e.g. approximately 10 mg, and dissolve it in a defined volume, e.g. 100 ml, of an appropriate solvent, e.g. *n*-hexane for a NP system or methanol for a RP system.

**4.10.2 β-tocopherol stock solution**

Weigh, to the nearest milligram, an amount of the β-tocopherol standard substance (4.9.3), e.g. approximately 10 mg, and dissolve it in a defined volume, e.g. 100 ml, of an appropriate solvent, e.g. *n*-hexane for a NP system or methanol for a RP system.

**4.10.3 γ-tocopherol stock solution**

Weigh, to the nearest milligram, an amount of the γ-tocopherol standard substance (4.9.4), e.g. approximately 10 mg, and dissolve it in a defined volume, e.g. 100 ml, of an appropriate solvent, e.g. *n*-hexane for a NP system or methanol for a RP system.

**4.10.4 δ-tocopherol stock solution**

Weigh, to the nearest milligram, an amount of the δ-tocopherol standard substance (4.9.5), e.g. approximately 10 mg, and dissolve it in a defined volume, e.g. 100 ml, of an appropriate solvent, e.g. *n*-hexane for a NP system or methanol for a RP system.

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1) This information is given for convenience of users of this European Standard and does not and does not constitute and endorsement by CEN. Equivalent products may be used if they can be shown to lead to the same results.

#### 4.10.5 Concentration and purity tests

Measure the absorbance of the stock solutions (4.10.1 to 4.10.4) at the appropriate wavelength using an UV spectrometer (5.1). If the solvent used is *n*-hexane, pipette 10 ml of the stock solution into an amber glass round bottomed flask and remove the solvent using a rotary evaporator (5.2) under reduced pressure at a temperature not higher than 50 °C. After restoring atmospheric pressure with nitrogen, remove the flask and dissolve the residue in 10 ml of methanol by swirling. Take this solution for the spectrometric measurement.

Calculate the mass concentration of vitamin E, $\rho$, of the respective of α-, β-, γ- and δ-tocopherol, in micrograms per millilitre by using Formula (1):

$$\rho = \frac{A \cdot M \cdot 1000}{\varepsilon} \tag{1}$$

where

$A$ is the absorption value of each tocopherol in the respective stock solution in methanol;

$\varepsilon$ is the molar absorption coefficient in methanol in l x $mol^{-1}$ x $cm^{-1}$ at the specific wavelength as given in Table 1;

$M$ is the molar mass, in grams per mol, of each tocopherol as given in Table 1.

**Table 1 — Examples for $E_{1cm}^{1\%}$ values and calculated $\varepsilon$**

| Substance | Wavelength (in methanol) | $E_{1cn}^{1\%}$ | Molar mass (in g · $mol^{-1}$) | $\varepsilon$ (in l · $mol^{-1}$ · $cm^{-1}$) | Reference |
|---|---|---|---|---|---|
| α-tocopherol | 292 nm | 76 | 430,7 | 3 273,3 | [12], [13], [15] |
| β-tocopherol | 296 nm | 89 | 416,7 | 3 708,6 | [12], [13], [15] |
| γ-tocopherol | 298 nm | 91 | 416,7 | 3 782 | [12], [13], [15] |
| δ-tocopherol | 298 nm | 87 | 402,6 | 3 502,6 | [12], [13], [15] |

In addition to the value for α-tocopherol obtained at a wavelength of 292 nm, the absorbance at 255 nm (minimum) should also be measured. The ratio at this wavelength should not exceed $E_{255}/E_{292}$ = 0,18. Otherwise the substance has degraded (for more information see [15]).

### 4.11 Standard solutions

#### 4.11.1 α-tocopherol standard solution

Pipette 10 ml of the α-tocopherol stock solution (4.10.1) into a one-mark 100 ml volumetric flask and dilute to the mark with the appropriate solvent (for NP e.g. *n*-hexane, for RP e.g. methanol). The standard solution should have a mass concentration of 1 µg/ml to 10 µg/ml of α-tocopherol. If an UV-detector is used to monitor the chromatography, a more concentrated solution shall be used.

The standard solution shall be stored protected from light and at a temperature below 4 °C and should be checked as described in 4.10.5.

#### 4.11.2 Standard solution of a mixture of α-, β-, γ- and δ-tocopherol

Pipette e.g. 10 ml of each of the stock solutions (4.10) into a one-mark 100 ml volumetric flask and dilute to the mark with the appropriate solvent (for NP e.g. *n*-hexane, for RP e.g. methanol). The standard solution should have a mass concentration of 1 µg/ml to 10 µg/ml of each of the tocopherols.