

Foodstuffs — Determination of vitamin E by high performance liquid chromatography — Measurement of α -, β -, γ - and δ -tocopherols

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National foreword

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Contents

	Page
Foreword.....	2
Introduction	2
1 Scope	3
2 Normative references.....	3
3 Principle	3
4 Reagents.....	3
5 Apparatus.....	5
6 Sampling.....	6
7 Procedure	6
8 Calculation	9
9 Precision	9
10 Test report.....	10
Annex A (informative) Examples of HPLC chromatograms.....	11
Annex B (informative) Precision data	12
Annex C (informative) Alternative HPLC-systems.....	14
Bibliography	15

Foreword

This European Standard 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of an identical text or by endorsement, at the latest by August 2000, and conflicting national standards shall be withdrawn at the latest by August 2000.

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

This European Standard provide 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.

Introduction

As this European Standard method deals with the measurement of the mass fraction of α -, β -, γ - and δ -tocopherol in foodstuffs, reference is made to the literature for the calculation and expression of the vitamin E content in terms of biological activities [1], [2], [3].

1 Scope

This European Standard specifies a method for the determination of Vitamin E in foodstuffs by high performance liquid chromatography (HPLC). The determination of Vitamin E content is carried out by measurement of α -, β -, γ - and δ -tocopherol.

The vitamin E activity can be calculated from the tocopherol content assuming appropriate factors as given in the introduction.

2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this draft European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

EN ISO 3696 *Water for analytical laboratory use - Specification and test methods* (ISO 3696:1987).

EN ISO 5555 *Animal and vegetable fats and oils - Sampling* (ISO 5555:1991).

3 Principle

Determination of α -, β -, γ - and δ -tocopherol in an appropriate sample solution by high performance liquid chromatographic (HPLC) separation and subsequent photometric (UV-range) or preferably fluorometric detection. In most cases a saponification of the test material followed by an appropriate extraction is necessary. Identification is carried out on the basis of the retention times, and quantitative determination by the external standard method using peak areas or 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 [4] to [13].

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, abs., volume fraction $\varphi(\text{C}_2\text{H}_5\text{OH}) = 100 \%$.

4.3 Ethanol, $\varphi(\text{C}_2\text{H}_5\text{OH}) = 96 \%$.

4.4 Sodium sulfate, anhydrous.

4.5 KOH solutions for saponification, in suitable concentrations, e.g. (KOH) = 50 g/100 ml or 60 g/100 ml, or alcoholic solutions, e.g. 28 g KOH in 100 ml of an ethanol/water mixture (9+1)(V+V).

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), dichloromethane, 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 e.g. 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 Merck¹⁾; 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 (see purity tests [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 -tocopheryl 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

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

4.10.2 β -tocopherol stock solution

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

4.10.3 γ -tocopherol stock solution

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

4.10.4 δ -tocopherol stock solution

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

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.

¹⁾ This information is given for the convenience of users of this standard method and does not constitute an endorsement by CEN of the product named.