Foodstuffs - Determination of vitamin A by high performance liquid chromatography - Part 1: Measurement of all-E-retinol and 13-Z-retinol

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NATIONAL FOREWORD

See Eesti standard EVS-EN 12823-1:2014 sisaldab Euroopa standardi EN 12823-1:2014 inglisekeelset teksti.	This Estonian standard EVS-EN 12823-1:2014 consists of the English text of the European standard EN 12823-1:2014.
Standard on jõustunud sellekohase teate avaldamisega EVS Teatajas.	This standard has been endorsed with a notification published in the official bulletin of the Estonian Centre for Standardisation.
Euroopa standardimisorganisatsioonid on teinud Euroopa standardi rahvuslikele liikmetele kättesaadavaks 28.05.2014.	Date of Availability of the European standard is 28.05.2014.
Standard on kättesaadav Eesti Standardikeskusest.	The standard is available from the Estonian Centre for Standardisation.

Tagasisidet standardi sisu kohta on võimalik edastada, kasutades EVS-i veebilehel asuvat tagasiside vormi või saates e-kirja meiliaadressile standardiosakond@evs.ee.

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English Version

Foodstuffs - Determination of vitamin A by high performance liquid chromatography - Part 1: Measurement of all-E-retinol and 13-Z-retinol

Produits alimentaires - Détermination de la teneur en vitamine A par chromatographie liquide haute performance - Partie 1: Dosage du tout-E-rétinol et du 13-Z-rétinol

Lebensmittel - Bestimmung von Vitamin A mit Hochleistungs-Flüssigchromatographie - Teil 1: Bestimmung von all-E-Retinol und 13-Z-Retinol

This European Standard was approved by CEN on 24 April 2014.

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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.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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Foreword

This document (EN 12823-1:2014) 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 November 2014, and conflicting national standards shall be withdrawn at the latest by November 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 12823-1:2000.

This European Standard consists of two parts:

— *Part 1: Measurement of all-E-retinol and 13-Z-retinol*;

— *Part 2: Measurements of β-carotene*.

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.

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.

1 Scope

This European Standard specifies a method for the determination of vitamin A in foodstuffs by high performance liquid chromatography (HPLC). This method has been validated in an interlaboratory study with samples of margarine and milk powder with all-E-retinol levels ranging from 653 µg/100 g to 729 µg/100 g and with 13-Z-retinol levels ranging from 30 µg/100 g to 39 µg/100 g. The determination of vitamin A content is carried out by the measurement of all-E-retinol, 13-Z-retinol and β-carotene. This part covers the measurement of all-E-retinol and 13-Z-retinol.

The extract obtained after saponification in this method can be used for the determination of β-carotene, as described in EN 12823-2:2000, *Foodstuffs - Determination of vitamin A by high performance liquid chromatography - Part 2: Measurements of β-carotene*. In this case, the saponification temperature should preferably not exceed 80 °C in order to prevent isomerisation and oxidation of β-carotene.

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

Retinol is saponified by using methanolic or ethanolic potassium hydroxide solution and extracted by an appropriate solvent. The determination is carried out by high performance liquid chromatography (HPLC) with either fluorometric (F) or ultraviolet (UV) detection. The substances are identified on the basis of the retention times and determined by the external standard procedure using peak areas or heights, see [1] to [4].

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, e.g. $\rho(KOH) = 50$ g/100 ml or 60 g/100 ml, or alcoholic solutions, e.g. 28 g 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, sodium sulfide (Na_2S), butylated hydroxytoluene (BHT), pyrogallol or hydroquinone.

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