

**Foodstuffs - Determination of vitamin A
by high performance liquid
chromatography - Part 2: Measurement
of beta-carotene**

Foodstuffs - Determination of vitamin A by high
performance liquid chromatography - Part 2:
Measurement of beta-carotene

EESTI STANDARDI EESSÕNA

NATIONAL FOREWORD

<p>Käesolev Eesti standard EVS-EN 12823-2:2000 sisaldab Euroopa standardi EN 12823-2:2000 ingliskeelset teksti.</p> <p>Käesolev dokument on jõustatud 19.07.2000 ja selle kohta on avaldatud teade Eesti standardiorganisatsiooni ametlikus väljaandes.</p> <p>Standard on kättesaadav Eesti standardiorganisatsioonist.</p>	<p>This Estonian standard EVS-EN 12823-2:2000 consists of the English text of the European standard EN 12823-2:2000.</p> <p>This document is endorsed on 19.07.2000 with the notification being published in the official publication of the Estonian national standardisation organisation.</p> <p>The standard is available from Estonian standardisation organisation.</p>
--	---

<p>Käsitlusala: This draft European Standard specifies a method for the determination of (all-E)-beta-carotene in foodstuffs by high performance liquid chromatography (HPLC).</p>	<p>Scope: This draft European Standard specifies a method for the determination of (all-E)-beta-carotene in foodstuffs by high performance liquid chromatography (HPLC).</p>
---	---

ICS 67.040

Võtmesõnad:

ICS 67.040

English version

Foodstuffs – Determination of vitamin A by high
performance liquid chromatography

Part 2: Measurement of β -carotene

Produits alimentaires – Dosage de la
vitamine A par chromatographie
liquide haute performance – Partie 2:
Dosage du β -carotène

Lebensmittel – Bestimmung von
Vitamin A mit Hochleistungs-Flüssig-
chromatographie – Teil 2:
Bestimmung von β -Carotin

This European Standard was approved by CEN on 2000-01-02.

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.

The European Standards exist 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, the Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, the Netherlands, Norway, Portugal, Spain, Sweden, Switzerland, and the United Kingdom.

CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

Central Secretariat: rue de Stassart 36, B-1050 Brussels

Contents

	Page
Foreword	2
Introduction	2
1 Scope	3
2 Normative references	3
3 Principle	3
4 Reagents	3
5 Apparatus	5
6 Sampling	5
7 Procedure	6
8 Calculation	7
9 Precision	7
10 Test report	8
Annex A (informative) Example of a HPLC chromatogramme	9
Annex B (informative) Precision data	10
Annex C (informative) Alternative HPLC-Systems	12
Bibliography	13

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 "Foodstuffs - Determination of vitamin A by high performance liquid chromatography" consists of two parts:

Part 1: Measurement of all-trans-retinol and 13-cis-retinol.

Part 2: Measurement of β -carotene.

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 draft European Standard deals with the measurement of total- β -carotene in foodstuffs, reference is made to the literature for the calculation and expression of β -carotene as vitamin A equivalents [1], [2].

Vitamin A activity can be calculated from the β -carotene data assuming appropriate factors.

1 Scope

This European Standard specifies a method for the determination of total- β -carotene in foodstuffs by high performance liquid chromatography (HPLC).

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 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)
- EN 12823-1 : 2000 Foodstuffs - Determination of vitamin A by high performance liquid chromatography - Part 1: Measurement of all-trans-retinol and 13-cis-retinol

3 Principle

Determination of the sum of β -carotene isomers in an appropriate sample solution by HPLC and spectrometric detection in the visible range. The extract obtained after saponification as described in EN 12823 -1 may be used for quantification. Identification on the basis of the retention times, and determination by the external standard method using peak areas or peak heights, see [3] to [7].

Internal standard methods may 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 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 φ (C_2H_5OH) = 100%
- 4.3 Ethanol**, φ (C_2H_5OH) = 96%
- 4.4 Sodium sulfate**, anhydrous
- 4.5 KOH solutions for saponification**, in suitable concentrations, e.g. ρ (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, sodium sulfide (Na_2S), butylated hydroxytoluene (BHT), pyrogallol or hydroquinone.
- 4.7 Solvents and extraction solvents** such as acetonitrile, diethyl ether (peroxide-free), *d*-isopropylether, light petroleum (boiling range of 40 °C to 60 °C), *n*-hexane, dichloromethane, tetrahydrofuran, toluene or appropriate mixtures thereof.
- 4.8 Methanolic ammonium acetate solution**, e.g. c ($CH_3CO_2NH_4$) = 0,05 mol/l
- 4.9 Triethylamine**
- 4.10 HPLC mobile phase**, for example acetonitrile (4.7) + methanolic ammonium acetate solution (4.8) + dichloromethane (4.7) (75+20+5) (volume parts) containing 0,1 percent by mass of butylated hydroxy toluene (4.6) and 0,05 percent by mass of triethylamine (4.9). For mobile phases of alternative HPLC-systems see Annex C.