BRITISH STANDARD

BS 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

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ICS 67.050

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National foreword

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The UK participation in its preparation was entrusted to Technical Committee AW/-/3, Food analysis - Horizontal methods, which has the responsibility to:

— aid enquirers to understand the text;

— present to the responsible European committee any enquiries on the interpretation, or proposals for change, and keep the UK interests informed;

— monitor related international and European developments and promulgate them in the UK.

A list of organizations represented on this committee can be obtained on request to its secretary.

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Summary of pages

This document comprises a front cover, an inside front cover, the EN title page, pages 2 to 13 and a back cover.

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This British Standard, having been prepared under the direction of the Consumer Products and Sector Committee, was published under the authority of the Standards Committee and comes into effect on 15 May 2000

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ISBN 0 580 34207 7

Amendments issued since publication

Amd. No.	Date	Comments

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Full document details: BS EN 12823-1:2000

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 12823-1

February 2000

ICS 67.040

English version

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

Produits alimentaires - Dosage de la vitamine A par chromatographie liquide haute performance - Partie 1: Dosage du tout-trans-rétinol et du 13-cis-rétinol

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

This European Standard was approved by CEN on 2 January 2000.

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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 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.

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1 Scope

This European Standard specifies a method for the determination of vitamin A in foodstuffs by high performance liquid chromatography (HPLC). The determination of vitamin A content is carried out by the measurement of all-trans-retinol, 13-cis-retinol and β-carotene. This part covers the measurement of all-trans-retinol and 13-cis-retinol.

The extract obtained after saponification in this method can be used for the determination of β-carotene, as described in EN 12823-2:1999, Measurement of β-carotene.

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).

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 ultra-violet (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 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 mass 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 diethyl ether (peroxide-free), *di*-isopropylether, light petroleum (boiling range of 40 °C to 60 °C), *n*-hexane, butanol or appropriate mixtures thereof.

4.8 HPLC Mobile phases

Examples of appropriate mixtures (expressed as volume parts) include:

n-hexane + 2-propanol (98 + 2);
iso-octane + 2-propanol (98,5 + 1,5);
iso-octane+ *iso*-butanol (98 + 2);
n-hexane + *n*-butanol (98 + 2);
and gradient with 2-propanol + *n*-heptane, (0,5 + 99,5) to (8,5 + 91,5) in 12 min.

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