

**Foodstuffs - Determination of vitamin D  
by high performance liquid  
chromatography - Measurement of  
cholecalciferol (D3) and ergocalciferol  
(D2)**

Foodstuffs - Determination of vitamin D by high  
performance liquid chromatography - Measurement  
of cholecalciferol (D3) and ergocalciferol (D2)

## EESTI STANDARDI EESSÕNA

## NATIONAL FOREWORD

<p>Käesolev Eesti standard EVS-EN 12821:2000 sisaldab Euroopa standardi EN 12821:2000 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 12821:2000 consists of the English text of the European standard EN 12821:2000.</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>
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<p><b>Käsitlusala:</b> This draft European Standard specifies a method for the determination of vitamin D in foodstuffs by high performance chromatography (HPLC).</p>	<p><b>Scope:</b> This draft European Standard specifies a method for the determination of vitamin D in foodstuffs by high performance chromatography (HPLC).</p>
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ICS 67.040

Võtmesõnad:

**English version**

**Foodstuffs – Determination of vitamin D by high  
performance liquid chromatography**

Measurement of cholecalciferol (D<sub>3</sub>) and ergocalciferol (D<sub>2</sub>)

Produits alimentaires – Dosage de la  
vitamine D par chromatographie  
liquide haute performance – Dosage  
du cholécalfcérol (D<sub>3</sub>) et de  
l'ergocalciferol (D<sub>2</sub>)

Lebensmittel – Bestimmung von  
Vitamin D mit Hochleistungs-  
Flüssigchromatographie – Bestim-  
mung von Cholecalciferol (D<sub>3</sub>) und  
Ergocalciferol (D<sub>2</sub>)

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.

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**CEN**

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

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

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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 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.

## 1 Scope

This European Standard specifies a method for the determination of vitamin D in foodstuffs by high performance liquid chromatography (HPLC).

In the majority of foodstuffs vitamin D is naturally present as cholecalciferol, vitamin D<sub>3</sub>, and this is the form of the vitamin determined. Vitamin D<sub>2</sub>, ergocalciferol, is sometimes present in fortified foodstuffs and can also be determined using this European Standard. Some foods will contain both vitamin D<sub>3</sub> and D<sub>2</sub>. This method is not applicable to these samples.

## 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

Vitamin D<sub>3</sub> and D<sub>2</sub> are saponified in the foodstuffs using alcoholic potassium hydroxide solution and extracted by an appropriate solvent. The determination of vitamin D<sub>3</sub> or D<sub>2</sub> in an appropriate sample extract solution is carried out by semi-preparative normal phase HPLC followed by reversed-phase analytical HPLC.

If vitamin D<sub>3</sub> is to be determined, then vitamin D<sub>2</sub> is used as an internal standard. If vitamin D<sub>2</sub> is to be determined, then vitamin D<sub>3</sub> is used as an internal standard.

Vitamin D is detected by ultraviolet (UV) spectrometry and peaks are identified on the basis of retention times and additionally by UV spectral profile if diode-array detection is used. The determination is carried out by the internal standard procedure using peak areas or peak heights, see [1] to [9].

## 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**, volume fraction  $\varphi$  (C<sub>2</sub>H<sub>5</sub>OH) = 100%

**4.3 Ethanol**,  $\varphi$  (C<sub>2</sub>H<sub>5</sub>OH) = 96%

**4.4 Sodium sulfate**, anhydrous

**4.5 Potassium hydroxide (KOH) solutions**,

**4.5.1 KOH solutions for saponification**, in suitable 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 an ethanol/water mixture (9+1)(V+V).

**4.5.2 KOH solutions for extraction**, in suitable concentrations, e.g. 5 g/100 ml.

**4.6 Antioxidants**, such as ascorbic acid (AA), sodium ascorbate, pyrogallol, sodium sulfide (Na<sub>2</sub>S) 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.