



NSAI
Standards

Irish Standard
I.S. EN 14164:2014

Foodstuffs - Determination of vitamin B6 by high performance chromatography

I.S. EN 14164:2014

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English Version

Foodstuffs - Determination of vitamin B6 by high performance chromatography

Produits alimentaires - Détermination de la teneur en
vitamine B6 par chromatographie liquide haute performance

Lebensmittel - Bestimmung von Vitamin B6 mit
Hochleistungs-Flüssigchromatographie

This European Standard was approved by CEN on 17 April 2014.

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Foreword

This document (EN 14164:2014) 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of an identical text or by endorsement, at the latest by December 2014 and conflicting national standards shall be withdrawn at the latest by December 2014.

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This document supersedes EN 14164:2008.

The Annexes A, B, C and D are informative.

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EN 14164:2014 (E)

1 Scope

This European Standard specifies a method for the determination of vitamin B₆ in foodstuffs by high performance liquid chromatography (HPLC). Vitamin B₆ is the mass fraction of the sum of pyridoxine, pyridoxal, pyridoxamine including their phosphorylated derivatives determined as pyridoxine. The β -glycosylated forms are not taken into account. These can be determined with the method given in EN 14663 [1] by which the different vitamins of vitamin B₆ (pyridoxal, pyridoxamine and pyridoxine) are separated and individually quantified. A third European Standard, EN 14166 [2], determines the total vitamin B₆ by microbiological assay.

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

Pyridoxal, pyridoxamine and pyridoxine are extracted from food by acid hydrolysis and dephosphorylated enzymatically using acid phosphatase.

By reaction with glyoxylic acid in the presence of Fe²⁺ as a catalyst, pyridoxamine is transformed into pyridoxal, which is then reduced to pyridoxine by the action of sodium borohydride in alkaline medium. Pyridoxine is then quantified in the sample solution by HPLC with a fluorometric detection [3], [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, or double distilled water.

4.1 Acid phosphatase, (CAS 9001-77-8), from potatoes, enzyme activity is 33 nkat/mg¹⁾ with substrate p-nitrophenyl phosphate at pH = 4,8 and $T = 37\text{ }^{\circ}\text{C}$, for example from Boehringer or Sigma²⁾. 33 nkat/mg corresponds to 2 U/mg.

4.1.1 Acid phosphatase solution

Prepare a solution of 20 mg/ml acid phosphatase in sodium acetate solution (4.14).

It is necessary to use acid phosphatase rather than Taka-diestase to obtain a complete hydrolysis of phosphorylated forms of vitamin B₆. Where 300 mg of Taka-diestase is needed to obtain good dephosphorylation, only 0,5 mg of acid phosphatase is needed, see [5].

¹⁾ Katal (symbol "kat") is a derived SI unit of enzyme activity. One katal is that catalytic activity which will raise the rate of reaction by one mol/s in a specified assay system.

²⁾ This information is given for the convenience of users of this European standard and does not constitute an endorsement by CEN of the supplier. Equivalent products may be used if they can be shown to lead to the same results.

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