

Foodstuffs — Determination of vitamin B6 (including its glycosylated forms) by HPLC

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National foreword

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English Version

Foodstuffs - Determination of vitamin B6 (including its glycosylated forms) by HPLC

Produits alimentaires - Dosage de la vitamine B6 (y compris ses formes glycosylées) par CLHP

Lebensmittel - Bestimmung von Vitamin B6 (einschließlich glucosidisch gebundener Verbindungen) mit HPLC

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Foreword

This document (EN 14663:2005) 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of an identical text or by endorsement, at the latest by June 2006, and conflicting national standards shall be withdrawn at the latest by June 2006.

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1 Scope

This document 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 as well as the β -glycosylated forms, calculated as pyridoxine.

This method has been successfully validated with semolina with milk (infant food), potato puree, vegetables with ham (convenient products) and a multi vitamin drink at levels from 0,034 mg/100 g to 1,21 mg/100 g.

2 Normative references

The following referenced documents are indispensable for the application of this document. 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 3696:1987)*.

3 Principle

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

The different derivatives of vitamin B₆ (pyridoxal, pyridoxamine and pyridoxine) are separated by HPLC and quantified by fluorometric detection [1], [2].

4 Reagents

4.1 General

During the analysis, unless otherwise stated, use only reagents of recognised analytical grade and water of at least grade 1 according to EN ISO 3696, or double distilled water.

4.2 Di-potassium hydrogen phosphate, mass fraction $w(\text{K}_2\text{HPO}_4 \cdot 3 \text{H}_2\text{O}) \geq 99,9 \%$

4.3 Sodium acetate, without crystal water, $w(\text{CH}_3\text{COONa}) \geq 99,0 \%$

4.4 Trichloroacetic acid (TCA), $w(\text{Cl}_3\text{CCOOH}) \geq 99,0 \%$

4.5 Sodium acetate solution, substance concentration $c(\text{CH}_3\text{COONa}) = 2,5 \text{ mol/l}$

Dissolve 205 g of sodium acetate (4.3) in 1 l of water.

4.6 Post-column reagent (optional), K_2HPO_4 solution $c(\text{K}_2\text{HPO}_4) = 0,15 \text{ mol/l}$

Dissolve 34,2 g of di-potassium hydrogen phosphate (4.2) in water, dilute to 1 000 ml, mix and degas.

4.7 Hydrochloric acid, $c(\text{HCl}) = 1 \text{ mol/l}$

4.8 Hydrochloric acid, $c(\text{HCl}) = 0,1 \text{ mol/l}$

4.9 Hydrochloric acid, $c(\text{HCl}) = 0,2 \text{ mol/l}$

4.10 Sulfuric acid, $c(\text{H}_2\text{SO}_4) = 1 \text{ mol/l}$

4.11 Light petroleum, boiling range of 40 °C to 60 °C

4.12 Acid phosphatase, from potatoes. Enzymatic activity approximately 5,3 U/mg¹⁾.

It is important that the enzyme used complies with the activity check 4.13.2, for further information see [2], [7].

4.13 Acid phosphatase solution

4.13.1 General

Dissolve/solubilise 60 mg of acidic phosphatase (4.12) in 10 ml of water in a beaker by stirring for 2 min. Prepare this solution on the day of analysis.

4.13.2 Activity check of Acid Phosphatase

Weigh 10 g of pork, 5 g of potato puree or 5 g of whole meal into a beaker, and extract with acid as described in 6.2.1. Add 1 ml of acid phosphatase solution (4.13.1) and optional 1 ml of β -glucosidase solution (4.15) to 12,5 ml of the extracted sample solution and mix. Incubate the solution at least 12 h or overnight at 37 °C with continuous stirring. Repeat this step with the double amount of acid phosphatase solution.

Determine the mass concentration of vitamins according to 6.6. The activity of the enzyme used is sufficient, if the resulting mass concentrations of vitamin B₆ compounds in both sample solutions are equivalent. The chromatogram shall not show a peak arising from pyridoxamin phosphate.

NOTE For the interlaboratory test, the acid phosphatase from Sigma Nr P 3752²⁾ has been used.

4.14 β -Glucosidase, from almonds. Enzymatic activity of approximately 3,2 U/mg.

It is important that the enzyme used complies with the activity check 4.15.2, for further information see [2], [7].

4.15 β -Glucosidase solution

4.15.1 General

Dissolve/solubilise 100 mg of β -glucosidase (4.14) in 10 ml of water in a beaker by stirring for 2 min. Prepare this solution on the day of analysis.

1) U, this unit (often called the International unit or standard unit) is defined as the amount of enzyme which catalyses the transformation of 1 μmol substrate per minute under standard conditions.

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

4.15.2 Activity check of β -glucosidase

Weigh 10 g of pork, 5 g of potato puree or 5 g of whole meal into a beaker, and extract with acid as described in 6.2.1. Add 1 ml of acid phosphatase solution (4.13.1) and 1 ml of β -glucosidase solution (4.15.1) to 12,5 ml of the extracted sample solution and mix. Incubate the solution at least 12 h or overnight at 37 °C with continuous stirring. Repeat this step with the double amount β -glucosidase solution.

Determine the mass concentration of vitamin B₆ compounds according to 6.6. The activity of the enzyme used is sufficient, if the resulting mass concentrations of vitamin B₆ compounds in both sample solutions are equivalent. The chromatogram shall not show a peak arising from pyridoxamin phosphate.

NOTE For the interlaboratory test, the β -glucosidase from Sigma Nr G-0395 ¹⁾ has been used.

4.16 Mobile phase for HPLC (Sulfuric acid, $c(\text{H}_2\text{SO}_4) = 0,015 \text{ mol/l}$ containing 0,005 mol/l TCA)

Dissolve 817 mg $\pm$ 5 mg of trichloroacetic acid (4.4) in 15 ml of 1 mol/l sulfuric acid (4.10), transfer into a 1 000 ml volumetric flask, dilute to the mark with water, mix and degas.

4.17 Silicon oil, for defoaming

4.18 Standard substances

4.18.1 General

Pyridoxamine (PM), Pyridoxal (PL) and pyridoxine (PN) can be obtained from various suppliers. The purity of the standards may vary, and it is therefore necessary to determine the concentration and purity (see 4.19.4 and 4.20.7).

4.18.2 Pyridoxamine (PM) dihydrochloride, $w(\text{C}_8\text{H}_{12}\text{N}_2\text{O}_2 \cdot 2\text{HCl}) \geq 98 \%$

4.18.3 Pyridoxal (PL) hydrochloride, $w(\text{C}_8\text{H}_9\text{NO}_3 \cdot \text{HCl}) \geq 98 \%$

4.18.4 Pyridoxine (PN) hydrochloride, $w(\text{C}_8\text{H}_{11}\text{NO}_3 \cdot \text{HCl}) \geq 98 \%$

4.19 Stock solutions

4.19.1 Pyridoxamine (PM) stock solution, mass concentration $\rho(\text{PM})$ approximately 500 $\mu\text{g/ml}$

Dissolve 71,7 mg of pyridoxamine dihydrochloride (4.18.2) in a 100 ml volumetric flask in 0,1 mol/l HCl (4.8) and dilute to the mark with 0,1 mol/l HCl. The solution can be stored without any losses for up to one week at 4 °C or up to two months at -18 °C.

4.19.2 Pyridoxal (PL) stock solution, $\rho(\text{PL})$ approximately 500 $\mu\text{g/ml}$

Dissolve 60,9 mg of pyridoxal hydrochloride (4.18.3) in a 100 ml volumetric flask in 0,1 mol/l HCl (4.8) and dilute to the mark with 0,1 mol/l HCl. The solution can be stored without any losses for up to one week at 4 °C or up to two months at -18 °C.

¹⁾ This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead to the same results.