SLOVENSKI STANDARD
SIST EN 14177:2004

01-maj-2004

Živila - Določevanje patulina v bistrem in motnem jabolčnem soku in jabolčni kaši - Metoda s HPLC s čiščenjem s porazdelitvijo tekočina-tekočina

Foodstuffs - Determination of patulin in clear and cloudy apple juice and puree - HPLC method with liquid/liquid partition clean-up

Lebensmittel - Bestimmung von Patulin in klarem und trübem Apfelsaft und Apfelpüree - HPLC-Verfahren mit Reinigung durch Flüssig/Flüssig-Verteilung

Produits alimentaires - Détermination de la teneur en patuline dans le jus de pommes clarifié et trouble et dans la compote de pommes - Méthode par CLHP avec purification par partage liquide/liquide

Ta slovenski standard je istoveten z: **EN 14177:2003**

ICS:

67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products
67.080.10	Sadje in sadni proizvodi	Fruits and derived products

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EUROPEAN STANDARD

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English version

Foodstuffs - Determination of patulin in clear and cloudy apple juice and puree - HPLC method with liquid/liquid partition clean-up

Produits alimentaires - Détermination de la teneur en patuline dans le jus de pommes et dans la compote de pommes, limpides et troubles - Méthode par chromatographie en phase liquide à haute performance avec purification par partage liquide/liquide

Lebensmittel - Bestimmung von Patulin in klarem und trübem Apfelsaft und Apfelpüree - HPLC-Verfahren mit Reinigung durch Flüssig/Flüssig-Verteilung

This European Standard was approved by CEN on 3 November 2003.

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 Management Centre or to any CEN member.

This European Standard exists 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 Management Centre has the same status as the official versions.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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Foreword

This document (EN 14177:2003) 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 2004, and conflicting national standards shall be withdrawn at the latest by June 2004.

Annex A is informative.

WARNING — The use of this standard can involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

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, Hungary, Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Slovakia, Spain, Sweden, Switzerland and the United Kingdom.

1 Scope

This European Standard specifies a method for the determination of patulin in apple juices and apple puree using high performance liquid chromatography (HPLC). The method has been validated for the determination of patulin via the analysis of naturally contaminated and spiked samples in clear apple juice at levels ranging from 26 µg/l up to 128 µg/l, in cloudy apple juice at levels ranging from 26 µg/l up to 106 µg/l and in apple puree at levels ranging from 23 µg/kg up to 121 µg/kg.

2 Normative references

This European Standard incorporates by dated or undated reference, provision 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 (including amendments).

EN ISO 3696, *Water for analytical laboratory use - Specification and test methods (ISO 3696:1987)*

3 Principle

Cloudy apple juice and apple puree are treated with a pectinase enzyme. Patulin is extracted from apple juice or enzyme treated puree with ethyl acetate. The solvent extract is cleaned up by liquid-liquid extraction with aqueous sodium carbonate solution. The ethyl acetate extract is dried with anhydrous sodium sulfate. After evaporation of ethyl acetate, patulin is quantitatively determined by high performance liquid chromatography (HPLC) with ultra violet (UV) detection.

4 Reagents

4.1 General

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and only distilled water or water of grade 1 as defined in EN ISO 3696. Solvents shall be of quality for HPLC analysis.

4.2 Sodium carbonate solution, mass concentration $\rho(Na_2CO_3) \approx 15$ g/l

Dissolve 1,5 g of sodium carbonate in 100 ml of water.

4.3 Acetic acid, volume concentration $\varphi(CH_3COOH) \approx 98$ %

4.4 Sodium sulfate anhydrous

4.5 pH 4 water

Adjust water to pH 4 with acetic acid.

4.6 Absolute ethanol, $\varphi(CH_3CH_2OH) \geq 99,7$ %

4.7 Ethyl acetate

4.8 Perchloric acid, $\varphi(HClO_4) = 60$ %

4.9 Acetonitrile

4.10 Endogalacturonase enzyme solution, typical activity 1 400 U/g

Unit definition - the amount of enzyme which catalyses the decrease in viscosity of a 1 % pectin solution by 20 % in 5 min at a pH 3,4 and at 25 °C.

NOTE Package volumes of 100 ml to 200 ml are practical.

4.11 HPLC mobile phase

Mix 95 parts per volume of water with 5 parts per volume of acetonitrile (4.9) and 0,095 parts per volume of perchloric acid (4.8), and degas.

4.12 Patulin stock solution

WARNING - Patulin is a suspect mutagen and has been reported to have immunotoxic and neurotoxic properties. Gloves and safety glasses should be worn at all times and all standard and sample preparation stages should be carried out in a fume cupboard.

Dissolve 5 mg of patulin or the contents of 1 ampoule (if patulin has been obtained as a film) in ethyl acetate (4.7). Transfer the solution to a 25 ml volumetric flask and dilute volume with ethyl acetate to produce a solution containing approximately 200 µg/ml of patulin. Store the stock solution in a freezer at less than 0 °C.

4.13 Patulin standard solution

Evaporate 1 000 µl of the stock solution (4.12) to dryness under nitrogen and then immediately dissolve it in 20 ml of ethanol (4.6) to obtain a mass concentration of approximately 10 µg/ml of patulin.

To determine the exact concentration, record the absorption curve between 250 nm and 350 nm in a 1 cm quartz cell with ethanol as reference. Identify the wavelength for maximum absorption. Calculate the mass concentration of patulin, ρ_{pat}, in micrograms per millilitre using equation 1:

$$\rho_{pat} = A_{\max} \times \frac{M \times 100}{\varepsilon \times \delta} \tag{1}$$

where:

A_{max} is the absorbance determined at the maximum of the absorption curve (here: at approximately 276 nm);

M is the relative molecular mass of patulin (M = 154,12 g/mol);

ε is the relative molar absorption coefficient of patulin in ethanol, (here: 1 460 m^2/mol, as given in AOAC Official Methods, 1995, Natural Toxins, Patulin, 49.6.01.C(d));

δ is the path length of the quartz cell in centimetres.

Store the standard solution in a freezer at less than 0 °C. Solutions stored this way are stable for 2 months. Ensure the standard solution is allowed to reach room temperature before use to avoid incorporation of water by condensation.

4.14 Patulin standard solution for calibration

Evaporate to dryness 500 µl of the standard solution (4.13) or an aliquot which is equivalent to an absolute amount of 5 µg of patulin, dissolve in 5 ml of pH 4 water (4.5) to obtain a mass concentration of 1 µg/ml of patulin.

This solution can be stored in a refrigerator at 4 °C. A solution stored in this way is stable for at least 8 weeks.

5 Apparatus

5.1 General

Usual laboratory equipment and, in particular, the following:

5.2 Displacement pipettes of 5 ml, 1 ml , 200 µl and 50 µl capacity with appropriate pipette tips

5.3 Analytical balance

5.4 UV spectrophotometer, double beam and recording suitable for measurement at 250 nm to 350 nm

5.5 Quartz cells, of optical path length 1 cm

5.6 Centrifuge, capable of operating at 4 500 *g*

5.7 Centrifuge tubes of 50 ml capacity with screw cap lids

5.8 Rotary evaporator, with water bath set at 40 °C

5.9 Filter paper, with pore size 20 µm to 25 µm

5.10 Disposable syringe filters, of 0,2 µm pore size (optional)

Test each batch before use to ensure that patulin is not adsorbed onto the filter.

5.11 HPLC apparatus, comprising the following

5.11.1 Injection system, a valve injection system with a 50 µl injection loop

5.11.2 Pump, isocratic, pulse free, capable of maintaining a volume flow rate of 1 ml/min

5.11.3 UV detector, fitted with an analytical flow cell and set at 276 nm

5.11.4 Analytical reverse phase HPLC column, for example C_{18} octyldecylsilane (ODS) (fully or super end capped with a carbon loading of 12 % to 17 % has been found suitable) which ensures resolution of patulin from all other peaks. The maximum height of overlapping peak shoulders shall be less than 10 % of the maximum peak height (it could be necessary to adjust the mobile phase for sufficient baseline resolution). A suitable pre-column should be used.