SLOVENSKI STANDARD
SIST EN 15890:2011

01-marec-2011

Živila - Določevanje patulina v sadnem soku in sadnih kašicah za dojenčke in majhne otroke - HPLC metoda z ekstrakcijo tekoče/tekoče, čiščenje z ekstrakcijo na trdni fazi in UV detekcijo

Foodstuffs - Determination of patulin in fruit juice and fruit based purée for infants and young children - HPLC method with liquid/liquid partition cleanup and solid phase extraction and UV detection

Lebensmittel - Bestimmung von Patulin in Fruchtsaft und Obstbrei für Säuglinge und Kleinkinder - HPLC-Verfahren mit Reinigung durch Flüssig/Flüssig-Verteilung, Festphasenextraktion und UV-Detektion

Denrées alimentaires - Dosage de la patuline dans le jus de fruits et la compote de fruits en alimentation infantile - Méthode par CLHP avec purification par partition liquide-liquide et extraction en phase solide et détection UV

Ta slovenski standard je istoveten z: EN 15890:2010

ICS:

67.080.10	Sadje in sadni proizvodi	Fruits and derived products
67.230	Predpakirana in pripravljena hrana	Prepackaged and prepared foods

SIST EN 15890:2011 **en,fr,de**

EUROPEAN STANDARD
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English Version

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Denrées alimentaires - Dosage de la patuline dans le jus de fruits et la compote de fruits en alimentation infantile - Méthode par CLHP avec purification par partition liquide-liquide et extraction en phase solide et détection UV

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Foreword

This document (EN 15890:2010) 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of an identical text or by endorsement, at the latest by March 2011, and conflicting national standards shall be withdrawn at the latest by March 2011.

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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.

1 Scope

This European Standard specifies a method for the determination of patulin in fruit juices and fruit-based purée, such as baby food purée, using high performance liquid chromatography with ultra-violet detection (HPLC-UV). Using naturally contaminated and spiked samples this method has been validated for the determination of patulin in apple juice, at levels ranging from 3,0 µg/kg to 15,5 µg/kg, and in fruit-based baby food purée, at levels ranging from 3,4 µg/kg to 17,9 µg/kg. Baby food fruit purée used in this study contained a mixture of the following ingredients which are commercially available on the European market: blueberry; apple; banana; lemon; wheat biscuits; wheat syrup; whole milk; and vegetable oil. A detailed listing, including the fractions, of each product used in this study is given in [1].

Further information on validation, see Clause 9 and Annex B.

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 3696:1995, *Water for analytical laboratory use — Specification and test methods (ISO 3696:1987)*

3 Principle

Patulin is extracted from apple juice, or fruit-based purée, with a mixture of ethyl-acetate and hexane in the presence of sodium sulfate and sodium hydrogen carbonate. An aliquot of the extract is purified by solid-phase extraction and evaporated. The residue is re-dissolved in water of pH = 4 and patulin is separated by reverse phase (RP)-HPLC and quantitatively determined by UV detection.

4 Reagents

4.1 General

Use only reagents of recognized analytical grade and water complying with grade 1 of EN ISO 3696:1995, unless otherwise specified. Solvents shall be of quality for HPLC analysis, unless otherwise specified. Commercially available solutions with equivalent properties to the reagents listed may be used.

4.2 Perchloric acid, the mass fraction $w(HClO_4) \geq 60$ % in water.

4.3 Sand, 50 mesh to 70 mesh particle size.

4.4 Silicagel solid phase extraction (SPE) cartridges (500 mg SiO_2).

4.5 Sodium sulfate anhydrous, Na_2SO_4.

4.6 Sodium hydrogen carbonate, $NaHCO_3$.

4.7 Glacial acetic acid, $w(CH_3COOH) \approx 98$ % in water.

4.8 Water of pH = 4.

Adjust water to pH = 4 with glacial acetic acid (4.7).

4.9 Absolute ethanol, $w(CH_3CH_2OH) \geq 99,7$ % in water.

4.10 Acetonitrile.

WARNING — Acetonitrile is hazardous and samples shall be blended using an explosion proof blender which is housed within a fume cupboard. After blending, samples shall be filtered inside a fume cupboard.

4.11 Ethyl acetate.

4.12 *n*-Hexane.

4.13 Extraction solvent.

Add 60 ml of ethyl acetate (4.11) to 40 ml of *n*-hexane (4.12).

4.14 Mixture of glacial acetic acid and ethyl acetate.

Add 3 ml of glacial acetic acid (4.7) to 97 ml of ethyl acetate (4.11).

4.15 HPLC mobile phase.

Mix 990 parts per volume of water with up to ten parts per volume of acetonitrile (4.10) and one part per volume of perchloric acid (4.2). The amount of acetonitrile will depend upon the type of samples analysed and their characteristic pattern of interferences after clean-up (see Annex A for typical chromatograms) and the HPLC column chosen for analysis. Degas this solution before use.

NOTE A mobile phase of 990 part of water with one part of perchloric acid has been found to give sufficient separation between patulin and other interfering substances (in particular 5-hydroxymethylfurfural when used in combination with a Synergy® [1)] column of 250 mm length and 4,6 mm diameter with a particle size of 4 µm and 8 nm porosity (see 5.13.4)).

4.16 Patulin.

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.

4.17 Patulin stock solution.

Dissolve 5 mg of patulin or the contents of one ampoule (if patulin has been obtained as a film) in ethyl acetate (4.11). Transfer the solution to a 25 ml volumetric flask and dilute to volume with ethyl acetate to produce a solution containing approximately 200 µg/ml of patulin.

1) Synergy® is a trade name of a suitable product available commercially. This information is given for the convenience of users of this European Standard 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.

Store this solution in a freezer at approximately - 18 °C. Confirm the mass concentration of the solution if it is older than six weeks. Ensure the solution is allowed to reach room temperature before use to avoid incorporation of water by condensation.

4.18 Patulin standard solution.

Evaporate 1 000 µl of the stock solution (4.17) to dryness under nitrogen and then immediately dissolve it in 20 ml of ethanol (4.9) to obtain a mass concentration of approximately 10 µg/ml of patulin.

To determine the exact mass 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:

$$\rho_{pat} = \frac{A_{max} \times M \times 100}{\varepsilon \times b} \quad (1)$$

where

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

M is the molar mass, in grams per mole, of patulin (M = 154 g/mol);

ε is the molar absorption coefficient, in square metres per mole, of patulin in ethanol (here: 1 460 m^2/mol, see [2]);

b is the optical path length, in centimetres, of the quartz cell.

Store this solution in a freezer at approximately - 18 °C. A solution stored in this way is stable for several months. Ensure that the standard solution is allowed to reach room temperature before use to avoid incorporation of water by condensation. Confirm the concentration of the solution if it is older than six weeks.

4.19 Spiking solutions.

For spiking experiments at levels of 10 ng/ml and 25 ng/ml patulin in the sample, prepare spiking solutions of patulin in water of pH = 4 (4.8) at mass concentrations of 200 ng/ml and 500 ng/ml, respectively.

These solutions can be obtained by evaporating exactly 100 µl and 250 µl respectively of the stock solution (4.17) to dryness under nitrogen in a 100 ml volumetric flask, followed by immediate dissolution in water of pH = 4 (4.8) to obtain a mass concentration of approximately 200 ng/ml and 500 ng/ml respectively of patulin, depending on the exact mass concentration of patulin in the stock solution. Make sure that the patulin is completely dissolved in the water of pH = 4 before the volumetric flask is filled up to the mark.

In case the patulin standard solution (4.18) has a different mass concentration than 10 µg/ml, adjust spiking solutions by calculating the correct aliquots in order to take account of the actual mass concentration of the standard solution determined in 4.18.

Store this solution in a refrigerator at 4 °C. A solution stored in this way is stable for at least eight weeks.

5 Apparatus

5.1 General

Usual laboratory apparatus and, in particular, the following.

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

5.3 Analytical balance, capable of weighing to 0,1 mg.

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

5.5 Quartz cells, with an optical path length of 1 cm.

5.6 Centrifuge, capable of operating at 400 *g*.

5.7 Centrifuge tubes, of 25 ml capacity with screw cap lids.

5.8 Mechanical shaker.

5.9 Evaporation block, capable of maintaining a temperature of 40 °C, with nitrogen supply.

5.10 Glass vial, of 6 ml capacity with screw cap.

5.11 Syringe, gas tight with a polytetrafluoroethylene (PTFE) plunger and with a volume of 3 ml to 5 ml.

5.12 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.

5.13 HPLC apparatus, comprising the following:

5.13.1 Injection system, a valve injection system with a 200 µl injection loop.

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

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

5.13.4 Analytical reverse-phase HPLC separating column, capable to run with 100 % water as mobile phase such as a polar end-capped or polar embedded alkyl phases (for example columns of the type Synergy® [2], Atlantis® [2] or Luna® [2] or similar).

The column dimensions may vary depending on the obtained peak separation of patulin from interfering peaks such as 5-hydroxymethylfurfural (5-HMF). 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.

Columns with a length of 250 mm, an inner diameter of 4,6 mm and a particle size of approximately 4 µm, with an 8 nm porosity have been shown to be suitable to meet these requirements when used in combination with the mobile phase proposed the Note under 4.15.

5.13.5 Data system.

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2) Synergy®, Atlantis® and Luna® are trade names of suitable products available commercially. This information is given for the convenience of users of this European Standard 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.