INTERNATIONAL STANDARD

ISO 8128-1

First edition
1993-07-01

Apple juice, apple juice concentrates and drinks containing apple juice — Determination of patulin content —

Part 1:
Method using high-performance liquid chromatography

Jus de pommes, concentrés de jus de pommes et boissons à base de jus de pommes — Détermination de la teneur en patuline —

Partie 1: Méthode par chromatographie en phase liquide à haute performance

Reference number
ISO 8128-1:1993(E)

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 8128-1 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Sub-Committee SC 3, *Fruit and vegetable products*.

ISO 8128 consists of the following parts, under the general title *Apple juice, apple juice concentrates and drinks containing apple juice — Determination of patulin content*:

— *Part 1: Method using high-performance liquid chromatography*

— *Part 2: Method using thin-layer chromatography*

Annex A of this part of ISO 8128 is for information only.

International Organization for Standardization
Case Postale 56 • CH-1211 Genève 20 • Switzerland
Printed in Switzerland

Apple juice, apple juice concentrates and drinks containing apple juice — Determination of patulin content —

Part 1:
Method using high-performance liquid chromatography

1 Scope

This part of ISO 8128 specifies a method using high-performance liquid chromatography for the determination of the patulin content of apple juice, apple juice concentrates and drinks containing apple juice.

The limit of detection of the method is 10 µg/l, based on 5 ml of ready-to-drink apple juice.

NOTE 1 ISO 8128-2 specifies a method using thin-layer chromatography.

2 Principle

Extraction of patulin from a test portion using ethyl acetate, followed by partitioning of the extract with aqueous sodium carbonate solution. Qualitative and quantitative determination of the patulin content by means of high-performance liquid chromatography (HPLC) using an ultraviolet (UV) detector.

3 Reagents

Use only reagents of recognized analytical grade, and water of HPLC grade.

3.1 Solvent, ethyl acetate.

3.2 Mobile phase, acetonitrile, 10 % (*V/V*) solution.

3.3 Extraction solution, 14 g/l aqueous solution of anhydrous sodium carbonate.

3.4 Acetate buffer, pH 4.

Mix 16,4 ml of dilute acetic acid [$c(CH_3COOH) = 0{,}2$ mol/l] with 3,6 ml of sodium acetate [$c(CH_3COONa) = 0{,}2$ mol/l].

3.5 Acetic acid, glacial.

3.6 Patulin standard solution ($C_7H_6O_4$).

3.6.1 Preparation

Weigh, to the nearest 0,1 mg, 10,0 mg of patulin in a 100 ml one-mark volumetric flask and dissolve it in the acetate buffer (3.4). Make up to the mark with the acetate buffer.

Pipette 10,0 ml of this solution into another 100 ml one-mark volumetric flask and make up to the mark with the acetate buffer.

The patulin content of this standard solution is 10 µg/ml approximately.

Measure the absorbance at 276 nm of this standard solution on an appropriate spectrometer using quartz cells of optical path length 10 mm.

NOTE 2 The preparation of the standard solution and the control of its purity are based on reference [3].

3.6.2 Calculation of the concentration

Calculate the concentration ρ_{ps}, expressed in micrograms per millilitre, of the patulin solution (3.6.1) using the formula

$$\rho_{ps} = \frac{A \times M_r \times 1\,000 \times C}{A_{276}}$$

where

A is the absorbance of the patulin standard solution;

A_{276} is the molecular absorbance of the patulin solution at the maximum (276 nm) of the absorption spectrum (see note 3);

M_r is the relative molecular mass of patulin;

C is the apparatus constant (usually 1).

NOTE 3 The molecular absorbance coefficient of patulin measured in ethanol at 276 nm is equal to 14 600.

4 Apparatus

Rinse the laboratory apparatus before use with a 10 g/l sodium hypochlorite solution.

Usual laboratory apparatus and, in particular, the following.

4.1 High-performance liquid chromatograph, equipped with a UV detector (capable of operating at 276 nm) and equipped with a recorder and/or integrator.

4.2 ODS[1] reverse-phase column, or any other equivalent column, with

— an efficiency of at least 35 000 theoretical plates per metre;

— length of 250 mm;

— inner diameter of 4,6 mm;

— a stationary phase[2] with particle size of 5 µm.

5 Sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

6 Procedure

6.1 Preparation of test solution

Dilute apple juice concentrates with water, using a 1:5 mixture of concentrate to water, by volume. Then proceed as for the other products, as follows.

6.1.1 Extract 5,0 ml of the laboratory sample (diluted with water if necessary) with 5,0 ml of ethyl acetate (3.1) for at least 1 min. Repeat the extraction twice more using 5,0 ml portions of ethyl acetate. Combine the three ethyl acetate phases and extract with 2,0 ml of sodium carbonate solution (3.3).

CAUTION — After combining the phases, carry out the extraction with sodium carbonate as quickly as possible, i.e. 1 min to 2 min, since patulin is not stable in alkaline media.

6.1.2 Extract the carbonate phase (6.1.1) with another 5,0 ml portion of ethyl acetate and combine with the preceding portions. Reject the carbonate phase. Add 5 drops of acetic acid (3.5), mix and evaporate using a vacuum evaporator until 1 ml to 2 ml remain.

6.1.3 Transfer quantitatively the solution thus obtained to a vial of about 5 ml capacity, using several portions (about 1 ml each) of ethyl acetate, and evaporate to dryness under a stream of nitrogen at about 40 °C. Dissolve the residue in 500 µl of either the mobile-phase solvent (3.2) or acetate buffer (3.4).

6.2 Preparation of the calibration curve

Using a pipette, transfer 1,0 ml, 2,0 ml, 3,0 ml, 5,0 ml and 7,5 ml of the patulin standard solution (3.6.1) to a series of five 10 ml one-mark volumetric flasks and make up to the mark with the acetate buffer (3.4).

Adjust the flow rate applied to the HPLC column to about 1 ml/min and adjust the sensitivity so that an absorbance of 0,01 gives a full-scale deflection.

Inject 10 µl to 30 µl of each of the calibration solutions into the chromatograph (4.1).

Prepare the calibration curve by plotting the peak heights (or areas) against the patulin concentration in micrograms per millilitre.

6.3 Determination

Inject 10 µl to 30 µl of the test solution (6.1.3) into the chromatograph using the same conditions as for the preparation of the calibration graph.

Identify the patulin peak of the test solution by comparison with the peaks of the calibration solutions. It is important to distinguish the patulin peak from the HMF peak.[3]

1) ODS = octadecylsilane

2) Bonded Octadecyl C_{18} is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

3) HMF = 5-hydroxymethylfurfural

7 Calculation

Determine the concentration of patulin in the test solution directly from the calibration curve (6.2). Calculate the patulin content of the sample, ρ_p, in micrograms per litre, as follows:

$$\rho_p = 100\rho_{pt}$$

where

ρ_{pt} is the concentration of patulin in the test solution, read from the calibration curve, expressed in micrograms per millilitre.

8 Precision

The precision of the method has been checked by collaborative studies[4].

Statistical parameters are expressed in accordance with ISO 5725[1].

8.1 Repeatability

$r = 41{,}9$; $s_r = 14{,}9$

where

r is the repeatability limit;

s_r is the standard deviation of repeatability.

8.2 Reproducibility

$R = 47{,}5$; $s_R = 22{,}6$

where

R is the reproducibility limit;

s_R is the standard deviation of reproducibility.

9 Test report

The test report shall specify

— the method used,

— the test result obtained, and

— if the repeatability has been checked, the final quoted result obtained.

It shall also mention all operating details not specified in this part of ISO 8128, or regarded as optional, together with details of any incidents which may have influenced the test result.

The test report shall include all information necessary for the complete identification of the sample.

Annex A
(informative)

Bibliography

[1] ISO 5725:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.*

[2] ISO 8128-2:1993, *Apple juice, apple juice concentrates and drinks containing apple juice — Determination of patulin content — Part 2: Method using thin-layer chromatography.*

[3] *Official Methods of Analysis,* Vol. 12, Washington DC: AOAC, 1975, p. 463.

[4] KUBACKI, S.J. and GOSZCZ, H. IUPAC Collaborative Study of HPLC methods for the determination of patulin in apple juice. *J. Pure Appl. Chem.*, **60** (6): 871-876 (1988).

[5] TANNER, H. and ZANIER, C. Ueber eine neue Patulin Bestimmung in Fruchtsäften und Konzentraten. *Schweiz. Z. Obst Weinbau,* **112:** 656-662 (1976).

[6] TANNER, H. and ZANIER, C. Amtliche Methoden zur Bestimmung von Patulin. *Mitt. Gebiete Labsm. Hyg.*, **75:** 506-513 (1984).

[7] POHLAND, A.E. *et al.* Physicochemical data for some selected mycotoxins. *J. Pure Appl. Chem.*, **54:** 2219-2284 (1982).