INTERNATIONAL STANDARD

ISO 11027

First edition
1993-07-15

Pepper and pepper oleoresins — Determination of piperine content — Method using high-performance liquid chromatography

Poivres, oléorésines de poivres — Détermination de la teneur en pipérine — Méthode par chromatographie en phase liquide à haute performance

Reference number
ISO 11027:1993(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 11027 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Sub-Committee SC 7, *Spices and condiments*.

Annex A of this International Standard is for information only.

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Case Postale 56 • CH-1211 Genève 20 • Switzerland

Printed in Switzerland

Pepper and pepper oleoresins — Determination of piperine content — Method using high-performance liquid chromatography

1 Scope

This International Standard specifies a method for the determination, by high-performance liquid chromatography, of the piperine content of peppers (*Piper nigrum* Linnaeus), whole or powdered, as well as their extracts (oleoresins).

This method enables the separation and, if necessary, the determination of the other alkaloids of pepper (isochavicine, isopiperine and piperittin).

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 2825:1981, *Spices and condiments — Preparation of a ground sample for analysis.*

ISO 5564:1982, *Black pepper and white pepper, whole or ground — Determination of piperine content — Spectrophotometric method.*

3 Principle

3.1 Ground pepper

Extraction with ethanol under reflux, then determination of piperine by high-performance liquid chromatography (HPLC), in accordance with the procedure described in this International Standard.

3.2 Whole pepper

Preparation by grinding the sample, then extraction of the powder obtained, followed by determination of piperine by HPLC, in accordance with the procedure described in this International Standard.

3.3 Oleoresins of pepper

Dilution of the oleoresin in ethanol, then determination of piperine by HPLC, in accordance with the procedure described in this International Standard.

4 Reagents

Use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

4.1 Reference substance

Piperine of at least 98 % purity, determined by the spectrometric method described in ISO 5564.

WARNING — This product should be handled with care as it is strongly irritating.

4.2 Solvents

4.2.1 Ethanol, 96 % (*V/V*).

4.2.2 Acetonitrile

WARNING — This product should be handled with care as it is lachrymatory.

4.2.3 Acetic acid, 1 % (*V/V*) aqueous solution.

4.3 Elution solvent

Mix 52 volumes of acetic acid solution (4.2.3) and 48 volumes of acetonitrile (4.2.2).

5 Apparatus

Usual laboratory equipment and, in particular, the following.

5.1 Pipettes, of 2 ml, 5 ml and 10 ml capacity.

5.2 Volumetric flasks, of 25 ml, 50 ml and 100 ml capacity (made of antiactinic glass or wrapped in aluminium foil).

5.3 Filtration diaphragms, non-adsorbing.

5.4 Separation system

5.4.1 Chromatograph, for high-performance liquid chromatography.

5.4.2 Pumping system, which enables a flow rate which is constant or programmed at high pressure to be obtained and maintained.

5.4.3 Degassing systems, for solvents.

5.4.4 Suitable detector system, adjustable to a wavelength of 343 nm.

5.5 Recorder or **integrator**, the performance of which is compatible with the apparatus as a whole.

5.6 Column

Material: stainless steel or glass

Length: 10 cm to 25 cm

Inside diameter: 0,4 cm to 0,5 cm

Stationary phase: grafted silica with derived octadecyl functional group, C_{18}, particle size 5 µm max.

5.7 Injection system

Injection valve with 10 µl loop or any other system giving the same injection accuracy.

5.8 Test sieve, of aperture size 500 µm.

6 Calibration method

WARNING — Throughout the operation, it is imperative that the solutions are not exposed to light (for instance wrap the flasks in aluminium foil) and are used as quickly as possible because of the instability of the piperine solutions.

6.1 Reference solution

Prepare for immediate use a 1 g/l stock solution of the piperine (4.1) in ethanol (4.2.1).

6.2 Calibration curve

From the reference solution (6.1), prepare at least three standard solutions of piperine with concentrations ranging from 0,05 g/l to 0,2 g/l. Inject each solution into the chromatograph (5.4.1). Repeat the determination at least once. Measure the peak areas and repeat the tests if the results deviate by more than 5 %.

Plot the calibration curve, i.e. the mass of piperine injected versus the peak area.

Define the mean slope of the curve.

6.3 Calculation of the response factor, K

Calculate the response factor K using the following formulae:

$$K = m'/A$$

$$m' = m \times P_r$$

where

m is the mass of piperine, in milligrams;

A is the area of the piperine peak, in integrator units;

P_r is the purity of the reference piperine (4.1);

m' is the corrected mass of piperine, in milligrams.

7 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.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 948[1].

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[1] ISO 948:1980, *Spices and condiments — Sampling*.

8 Preparation of test sample

WARNING — It is imperative to operate away from light because of the instability of the piperine solutions.

8.1 Peppers in powder form

8.1.1 Check that all of the test sample passes through the 500 µm sieve (5.8). If this is not the case, grind the powder in accordance with ISO 2825 until the required particle size is achieved.

8.1.2 From the test sample prepared as in 8.1.1, weigh, to the nearest 0,01 g, 0,5 g to 1 g, and transfer it to a 100 ml round-bottom flask (5.2) fitted with a reflux condenser. Add 50 ml of ethanol (4.2.1) and bring it to the boil. Maintain for 3 h under reflux.

Allow the solution to cool. Filter the solution and collect it in a 100 ml one-mark volumetric flask (5.2). Rinse the extraction flask and filter with successive portions of ethanol (4.2.1). Add the wash liquids to the 100 ml volumetric flask and make up to the mark with more ethanol.

8.2 Whole peppers

8.2.1 Grind the test sample in accordance with ISO 2825 until the powder obtained passes entirely through the 500 µm sieve (5.8).

8.2.2 Using the test sample prepared as in 8.2.1, proceed as described in 8.1.2.

8.3 Oleoresins of peppers

Thoroughly homogenize the test sample of oleoresin.

9 Procedure

9.1 Test portion

9.1.1 Peppers, whole or ground

Take with the pipette (5.1) 10 ml of the extraction solution, obtained as described in 8.1.2 or 8.2.2, and transfer it to a 25 ml one-mark volumetric flask (5.2). Make up to the mark with the elution solvent (4.3).

NOTE 1 This dilution may be modified, if necessary, by the analyst depending on the assumed piperine content of the sample and the sensitivity of the detector.

9.1.2 Oleoresins of pepper

Weigh into a 100 ml one-mark volumetric flask, to the nearest 0,1 mg, 0,2 g of oleoresin (see 8.3) and make up to the mark with ethanol (4.2.1).

Pipette 10 ml of this mixture into a 50 ml one-mark volumetric flask and make up to the mark with the elution solvent (4.3).

NOTE 2 This dilution may be modified, if necessary, by the analyst depending on the assumed piperine content of the sample and the sensitivity of the detector.

9.2 Determination

The final dilution shall be clear. If this is not the case, filter it on the filtration diaphragm (5.3). Inject the test solution into the chromatograph (5.4.1).

On the chromatogram obtained, measure the peak of the piperine (see typical chromatograms given in annex A).

Carry out two tests and repeat the determination if the results deviate by more than 5 %.

10 Calculation

10.1 Peppers, whole or ground

Calculate the piperine content, as a percentage by mass, using the following formula:

$$A \times K \times \frac{25}{10} \times \frac{100}{m_x} \times 100$$

where

A is the area of the piperine peak, in integrator units;

m_x is the mass of the sample, in milligrams;

K is the response factor determined for the reference substance.

NOTE 3 The ratio 25/10 is related to the dilution proposed in 9.1.1 and should be modified accordingly when another dilution is used.

10.2 Oleoresins of pepper

Calculate the piperine content, as a percentage by mass, using the following formula:

$$A \times K \times \frac{50}{10} \times \frac{100}{m_x} \times 100$$

where A, m_x and K have the same meaning as in 10.1.

NOTE 4 The ratio 50/10 is related to the dilution proposed in 9.1.2 and should be modified accordingly when another dilution is used.

11 Repeatability

Take as the result for the response factor K and the piperine content, the mean value of several (at least three) determinations carried out on the same test sample. These different values should not differ from their mean by more than 5 %.

12 Test report

The test report shall include the following information:

a) reference to this International Standard;

b) the apparatus system;

c) the characteristics of the column (material, dimensions, packing, stationary phase);

d) the characteristics of the detector (optional) and the operating conditions;

e) the characteristics of the mobile phase (flow rate and nature);

f) identification of the test sample (quantity injected and final dilution);

g) results obtained.

Annex A
(informative)

Typical chromatograms

Figure A.1 shows typical chromatograms, taken under the operating conditions given in this International Standard.

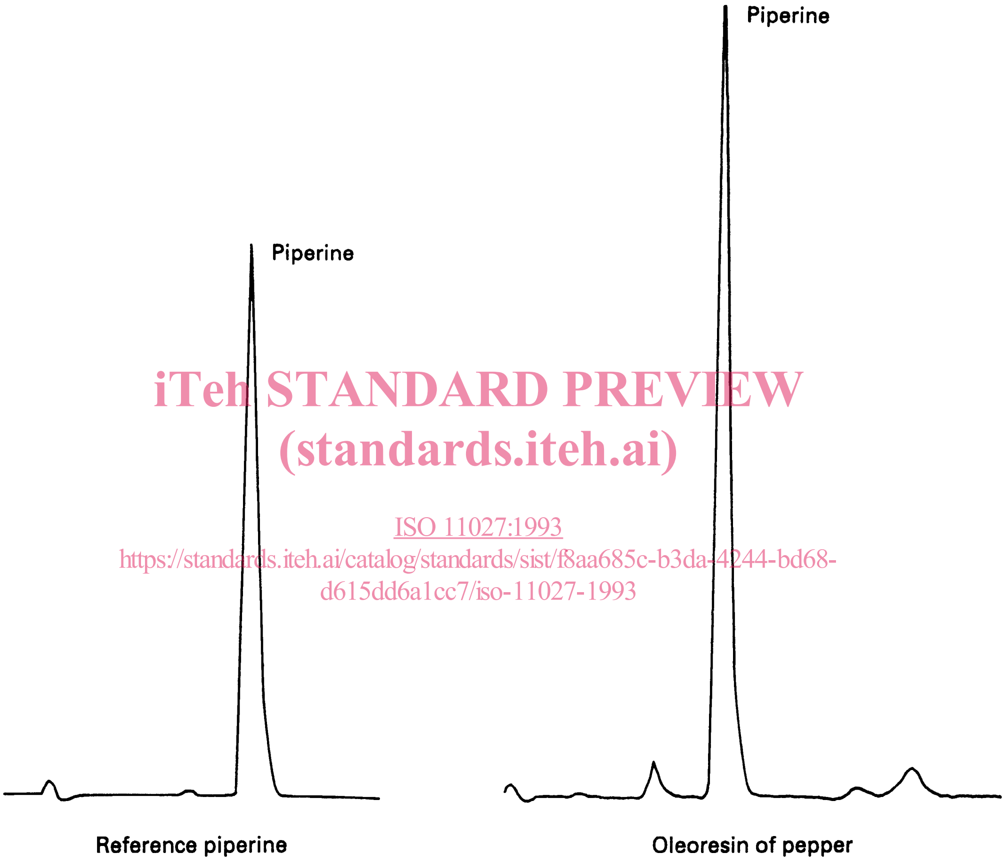

Figure A.1

UDC 633.841:543.544

Descriptors: agricultural products, food products, seasonings, spices, pepper, chemical analysis, determination of content, high performance liquid chromatography.

Price based on 5 pages
