
**Chillies and chilli oleoresins —
Determination of total capsaicinoid
content —**

Part 2: **STANDARD PREVIEW**

**Method using high-performance liquid
chromatography**

[ISO 7543-2:1993](#)

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[a145d68aa557/iso-7543-2-1993](#)
*Piments et leurs oléorésines — Détermination de la teneur en
capsaïcinoïdes totaux —*

*Partie 2: Méthode par chromatographie en phase liquide à haute
performance*



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 7543-2 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Sub-Committee SC 7, *Spices and condiments*.

ISO 7543-2:1993

ISO 7543 consists of the following parts, under the general title *Chillies and chilli oleoresins — Determination of total capsaicinoid content*:

- Part 1: *Spectrometric method*
- Part 2: *Method using high-performance liquid chromatography*

Annex A of this part of ISO 7543 is for information only.

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Chillies and chilli oleoresins — Determination of total capsaicinoid content —

Part 2:

Method using high-performance liquid chromatography

1 Scope

This part of ISO 7543 specifies a method for the determination, by high-performance liquid chromatography, of the total capsaicinoid content of whole or powdered chillies (usually *Capsicum frutescens* L.) and their extracts (oleoresins). This content is calculated from the total of capsaicin, nordihydrocapsaicin and dihydrocapsaicin, expressed as nonyl acid vanillylamide, which is the chosen reference substance.

This method enables the separation of capsaicin and nonyl acid vanillylamide.

NOTE 1 A spectrometric method is given in ISO 7543-1.¹⁾

2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this part of ISO 7543. At the time of publication, the edition indicated was valid. All standards are subject to revision, and parties to agreements based on this part of ISO 7543 are encouraged to investigate the possibility of applying the most recent edition of the standard 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*.

1) To be published.

3 Principle

3.1 Chillies in powder form

Extraction with tetrahydrofuran using a Soxhlet-type continuous extraction apparatus, then determination of the capsaicinoids by high-performance liquid chromatography (HPLC), in accordance with the procedure described in this part of ISO 7543.

3.2 Whole chillies

Preparation by grinding the sample, then extraction of the powder so obtained, followed by determination of the capsaicinoids by HPLC, in accordance with the procedure described in this part of ISO 7543.

3.3 Oleoresins of chillies

Dilution of the oleoresin in a tetrahydrofuran/methanol solution, then determination of the capsaicinoids by HPLC, in accordance with the procedure described in this part of ISO 7543.

4 Reagents

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

4.1 Reference substance

Nonyl acid vanilylamide (synthetic capsaicin) of at least 95 % purity, determined by chromatography under the test conditions given in this part of ISO 7543.

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

4.2 Solvents

4.2.1 Tetrahydrofuran

4.2.2 Methanol

4.2.3 Silver nitrate, aqueous solution, 0,1 mol/l.

WARNING — This product should be handled with care as skin contact will cause black spots.

4.2.4 Tetrahydrofuran/methanol solution, 50/50 (V/V).

4.3 Elution solvent

Mix 70 volumes of methanol (4.2.2) and 30 volumes of silver nitrate (4.2.3).

5 Apparatus

Usual laboratory equipment and, in particular, the following.

5.1 Pipettes, of 5 ml capacity.

5.2 Volumetric flasks, of 25 ml and 100 ml capacity.

5.3 Filtration diaphragm, made of cellulose acetate, of diameter 25 mm and pore size 0,45 μm .

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 280 nm.

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

5.6 Column

Material: stainless steel

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} , and 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 Continuous-extraction apparatus, Soxhlet type.

5.9 Test sieve, with aperture size 500 μm .

6 Calibration method

6.1 Reference solution

Prepare a stock solution of 1 g/l of nonyl acid vanilylamide (4.1) in the tetrahydrofuran/methanol solution (4.2.4).

6.2 Calibration curve

From the reference solution (6.1), prepare at least three standard solutions of nonyl acid vanilylamide 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 the nonyl acid vanilylamide 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 nonyl acid vanilylamide, in milligrams;

A is the area of the peak of nonyl acid vanilylamide;

P_r is the purity of the nonyl acid vanilylamide (4.1);

m' is the corrected mass of nonyl acid vanilylamide, 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 part of ISO 7543. A recommended sampling method is given in ISO 948²⁾.

8 Preparation of test sample

8.1 Chillies in powder form

8.1.1 Check that all of the test sample passes through the 500 μm sieve (5.9). If this is not the case, grind the powder in accordance with ISO 2825 until the required particle size is obtained.

8.1.2 From the test sample prepared as in 8.1.1, weigh, to the nearest 0,1 g, between 10 g and 15 g. Extract for 8 h in a continuous-extraction apparatus (5.8), using 100 ml of tetrahydrofuran (4.2.1).

Partially concentrate the solvent.

Quantitatively transfer the extract to a 100 ml one-mark volumetric flask (5.2). Make up to the mark with the tetrahydrofuran/methanol solution (4.2.4).

8.2 Whole chillies

8.2.1 Grind the test sample in accordance with ISO 2825 until the powder obtained passes entirely through the 500 μm sieve (5.9).

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

8.3 Oleoresins of chillies

Thoroughly homogenize the test sample of oleoresin.

9 Procedure

9.1 Test portion

9.1.1 Chillies, whole or in powder form

Take with the pipette (5.1) 5 ml of the extraction solution prepared as 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 tetrahydrofuran/methanol solution (4.2.4).

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

9.1.2 Oleoresins of chillies

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

Pipette 5 ml of this solution into a 25 ml one-mark volumetric flask and make up to the mark with the elution solvent (4.3).

NOTE 3 This dilution may be modified, if necessary, by the analyst depending upon the assumed total capsaicinoid content of the sample and the sensitivity of the detector.

9.2 Determination

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

On the chromatogram obtained, measure the area of the peaks of capsaicin (C_1), nordihydrocapsaicin (C_2) and dihydrocapsaicin (C_3) (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

Calculate the total capsaicinoid content, w_T , as a percentage by mass, using the following formula:

$$w_T = \frac{(A_{C_1} + A_{C_2} + A_{C_3})K}{m_x} \times 500 \times 100$$

where

- A_{C_1} is the area of the capsaicin peak;
- A_{C_2} is the area of the nordihydrocapsaicin peak;
- A_{C_3} is the area of the dihydrocapsaicin peak;
- m_x is the mass of the test portion, in milligrams;
- K is the response factor determined for the reference substance.

NOTE 4 The dilution factor of 500 in this formula may be adjusted by the analyst according to the capsaicinoid content of the sample to be tested.

2) ISO 948:1980, *Spices and condiments — Sampling*.

11 Repeatability

Take as the result for the response factor K and the content of the compound to be determined, 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 part of ISO 7543;

- 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.

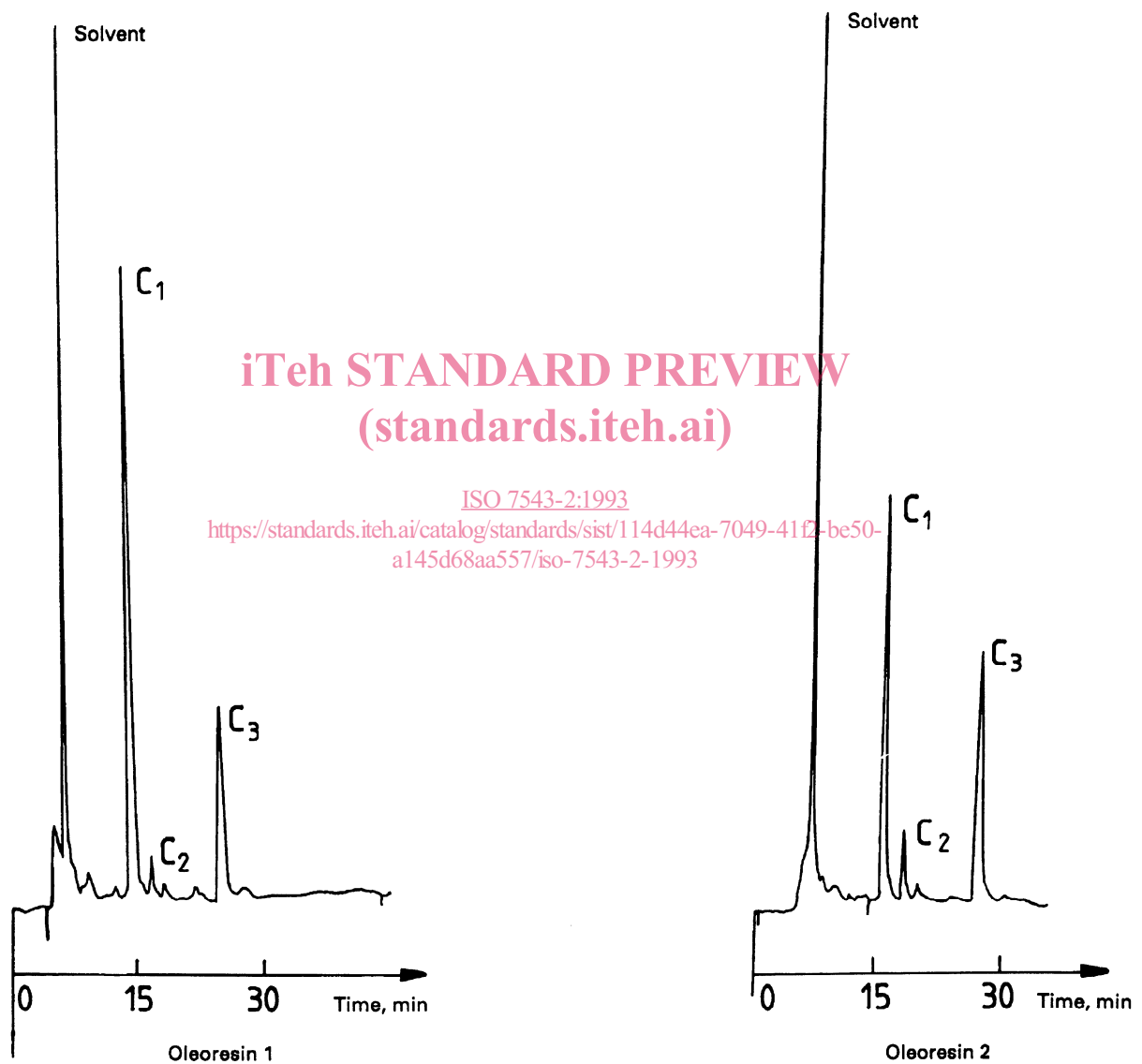
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Annex A (informative)

Typical chromatograms

Figure A.1 shows two examples of typical chromatograms taken under operating conditions given in this part of ISO 7543.



Identification of the peaks

- C₁ Capsaicin
- C₂ Nordihydrocapsaicin
- C₃ Dihydrocapsaicin

Figure A.1

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