
International Standard



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**Fruits, vegetables and derived products — Determination
of ascorbic acid —
Part 1: Reference method**

Fruits, légumes et produits dérivés — Détermination de la teneur en acide ascorbique — Partie 1: Méthode de référence

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Foreword

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International Standard ISO 6557/1 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*.

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Part 1: Reference method

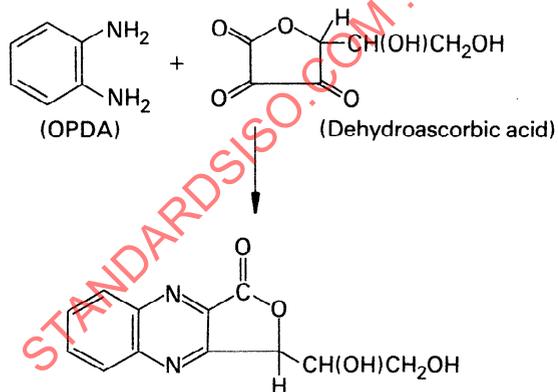
1 Scope and field of application

This part of ISO 6557 specifies the reference method, using molecular fluorescence spectrometry, for the determination of the combined ascorbic and dehydroascorbic acid content of fruits, vegetables and derived products.

2 Principle

Transformation of ascorbic acid into dehydroascorbic acid using activated charcoal.

Reaction of the dehydroascorbic acid obtained with *o*-phenylenediamine (OPDA) to give a fluorescent compound according to the reaction



(1-Oxo-2,4 *H*-3-(1,2-dihydroxyethyl) furo [3,4-*b*] quinoxaline)

In a control test in the presence of boric acid, formation of the complex H_3BO_3 -dehydroascorbic acid to prevent reaction with OPDA. Any fluorescent interference can thus be taken into account in the calculation.

NOTE — The initial dehydroascorbic acid content alone can be determined by omitting the step using activated charcoal. It is then possible, by subtraction, to determine the initial content of ascorbic acid alone.

3 Reagents and materials

All reagents shall be of recognized analytical grade and the water used shall be distilled or water of equivalent purity.

3.1 *o*-Phenylenediamine dihydrochloride ($C_6H_8N_2 \cdot 2HCl$), 0,2 g/l solution.

Prepare this solution just before use.

3.2 Sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$), 500 g/l solution.

3.3 Boric acid/sodium acetate solution.

Dissolve 3 g of boric acid (H_3BO_3) in 100 ml of the sodium acetate solution (3.2).

Prepare this solution just before use.

3.4 Ascorbic acid, 1 g/l standard solution.

Weigh, to the nearest 0,01 mg, 50 mg of ascorbic acid, previously dehydrated in a desiccator protected from light. Transfer quantitatively to a 50 ml one-mark volumetric flask and make up to the mark with the extraction solution (3.5), just before use.

3.5 Extraction solution.

3.5.1 Metaphosphoric acid/acetic acid.

Transfer 30 g of metaphosphoric acid (HPO_3) to a 1 000 ml beaker or conical flask containing 80 ml of glacial acetic acid (CH_3COOH) and about 500 ml of water. Warm and stir gently until complete dissolution.

Allow the solution to cool. Transfer it quantitatively to a 1 000 ml one-mark volumetric flask and make up to the mark with water.

or

3.5.2 Metaphosphoric acid/methanol.

Mix 3 volumes of a 4 % (*m/m*) metaphosphoric acid solution with 1 volume of methanol.

NOTE — Metaphosphoric acid is commercially available with an HPO_3 content of 40 to 44 %.

3.6 Activated charcoal.¹⁾

Weigh 200 g of activated charcoal and add 1 litre of 10 % (V/V) hydrochloric acid.

Heat to boiling, then filter through a sintered glass filter of porosity P 40 (16 to 40 µm). Transfer the "cake" of carbon into a beaker. Add 1 litre of water, shake and filter through a sintered glass filter. Repeat three times the operation of washing with water and filtering.

Place the residue in a drying oven maintained at 115 ± 5 °C and leave for 12 h (for example overnight).

4 Apparatus

Usual laboratory equipment and in particular

4.1 Mechanical grinder.

4.2 Centrifuge.

4.3 Stirrer, for conical flasks and test tubes.

4.4 Molecular fluorescence spectrometer. The optimum excitation and emission wavelengths for the test should be determined beforehand and depend on the instrument used. It shall be fitted with a lamp giving a continuous spectrum.

4.5 Conical flasks, of appropriate capacities.

4.6 Volumetric flasks, of capacity 100 ml.

4.7 Test tubes, of diameter 10 mm.

4.8 Pipettes, of appropriate capacities.

4.9 Filter paper.

5 Procedure

5.1 Preparation of the test sample

Mix the laboratory sample well. If necessary, first remove seeds and hard seed-cavity walls and pass the laboratory sample through a mechanical grinder (4.1). Allow frozen or deep-frozen products to thaw in a closed vessel and add the liquid formed during this process to the laboratory sample before mixing.

5.2 Test portion

Transfer a quantity, weighed to the nearest 0,1 mg, of the test sample (5.1) to a conical flask (4.5), so that after dilution with the extraction solution the ascorbic acid and dehydroascorbic acid concentration may be expected to be between 0 and 50 mg/l.

5.3 Preparation of the test solution

5.3.1 Add a known quantity of the extraction solution (3.5) to the test portion so that the ascorbic acid and dehydroascorbic acid content is between 0 and 50 mg/l. Agitate for 30 min, and centrifuge. Adjust the pH to 1,2 with a measured volume of the extraction solution (3.5).

Take 100 ml of this solution and add 1 g of activated charcoal (3.6). Mix well, then filter it through filter paper (4.9), discarding the first few millilitres of the filtrate.

5.3.2 Introduce, by means of a pipette (4.8), 5 ml of the sodium acetate solution (3.2) and 5 ml of the filtrate (5.3.1) into a 100 ml one-mark volumetric flask (4.6). Mix and make up to the mark with water.

5.4 Matching test

Introduce, by means of a pipette, 5 ml of boric acid/sodium acetate solution (3.3) and 5 ml of the filtrate (5.3.1) into a 100 ml one-mark volumetric flask. Allow to stand for 15 min, mixing occasionally, then make up to the mark with water.

5.5 Determination

Transfer 2 ml of the test solution (5.3.2) into a test tube (4.7) and 2 ml of the matching test solution (5.4) into another test tube.

Add 5 ml of the *o*-phenylenediamine dihydrochloride solution (3.1) to the contents of each tube, protected from light. Mix well using the stirrer (4.3), then allow the reaction to proceed for 30 min in the dark.

Carry out measurements on both tubes by means of the previously adjusted molecular fluorescence spectrometer (4.4) using minimum lamp power. Subtract the matching test solution reading from that of the test solution.

5.6 Calibration graph

5.6.1 Transfer, by means of a pipette, 2 and 5 ml of the standard solution (3.4) to two 100 ml one-mark volumetric flasks. Make up each to the mark with the extraction solution (3.5). These solutions contain 20 and 50 mg of ascorbic acid per litre.

Add 1 g of activated charcoal (3.6) to each of these solutions. Mix well, then filter through filter paper (4.9), discarding the first few millilitres of each filtrate.

¹⁾ A suitable product, available commercially, is Norite. This information is given for the convenience of the user of this part of ISO 6557 and does not constitute an endorsement of this product by ISO.

5.6.2 Repeat the operations 5.3.2, 5.4 and 5.5 with both calibration solutions (5.6.1), replacing the 5 ml of filtrate with 5 ml of each calibration solution.

Establish the calibration graph by plotting the spectrometer readings against concentration, expressed in milligrams per litre, of both calibration solutions.

Draw the calibration graph passing through the origin and the two points obtained.

5.7 Number of determinations

Carry out two determinations on the same test sample (5.1).

6 Expression of results

The ascorbic acid and dehydroascorbic acid content, expressed in milligrams per 100 g of product, is given by the formula

$$\frac{c V}{10 m_0}$$

where

m_0 is the mass, in grams, of the test portion;

V is the volume, in millilitres, of the extraction solution added;

c is the concentration, expressed in milligrams per litre, of ascorbic acid and dehydroascorbic acid in the test solution read from the calibration graph and corrected for the matching test solution.

7 Test report

The test report shall show the method used and the results obtained. It shall also mention any operating details not specified in this International Standard, or regarded as optional, together with details of any incidents likely to have influenced the results.

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

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