INTERNATIONAL STANDARD 5519

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION•МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ•ORGANISATION INTERNATIONALE DE NORMALISATION

Fruits, vegetables and derived products — Determination of sorbic acid content

Fruits, légumes et produits dérivés — Détermination de la teneur en acide sorbique

First edition — 1978-09-01

UDC 634.1/635 : 547.295.2 : 543.42

Ref. No. ISO 5519-1978 (E)

Descriptors : fruits, vegetables, fruit and vegetable products, chemical analysis, determination of content, sorbic acid, spectrophotometric analysis, colorimetric analysis.

Price based on 6 pages

FOREWORD

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Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council.

International Standard ISO 5519 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in March 1977.

It has been approved by the member bodies of the following countries :

Australia	Ghana	Poland
Austria	Hungary	Portugal
Bulgaria	India	Romania
Canada	Iran	South Africa, Rep. of
Czechoslovakia	Israel	Spain
Egypt, Arab Rep. of	Korea, Rep. of	Thailand
France	Mexico	Turkey
Germany	New Zealand	Yugoslavia

No member body expressed disapproval of the document.

Printed in Switzerland

Fruits, vegetables and derived products — Determination of sorbic acid content

0 INTRODUCTION

The determination of the sorbic acid content of fruits, vegetables and derived products has been studied in numerous projects during the acid's use as a fungicide, especially in wines. Because of its great volatility (very similar to that of acetic acid), the simplest extraction process is its entrainment by steam. This method has the advantage of producing an almost pure aqueous solution of sorbic acid.

Two techniques for the determination of the quantity of sorbic acid contained in this solution are described in this International Standard, namely :

Technique A : spectrophotometry in the ultra-violet range, carried out after oxidation of sulphur dioxide, which would interfere. The oxidation occurs spontaneously in a few minutes in air after the addition of a trace of a copper catalyst.

The natural essential oils of citrus fruits do not interfere with the determination provided that they are present in the small quantities normal in juice not enriched with essential oils. When the quantities of essential oils are significant, they may be eliminated beforehand by the same method as that applied in technique B.

Technique B : colorimetry, based on Schmidt's reaction, which requires the elimination of ethanol and essential oils by the evaporation of an aliquot portion of the distillate. This technique, not so rapid as technique A but giving comparable results, is provided for use when a spectrophotometer allowing measurements in the ultra-violet range is not available.

The interference caused by essential oils of garlic, onion or leek may be eliminated, when using either technique, by the evaporation of an aliquot portion of the distillate.

1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies a method for extracting sorbic acid present in fruits, vegetables and derived products, and two techniques for determining the sorbic acid extracted.

2 PRINCIPLE

Homogenization of the product, followed by quantitative entrainment, by steam, of the sorbic acid present in a test portion. Determination of this acid in the distillate obtained, either by spectrophotometry in the ultra-violet range (technique A), or by measuring by photocolorimetry or by spectrophotometry the pink colour obtained after oxidation by chromic acid and then treatment with thiobarbituric acid (technique B).

3 REAGENTS

All reagents shall be of recognized analytical quality, and the water used shall be distilled water or water of at least equivalent purity.

3.1 Tartaric acid, crystalline.

3.2 Sorbic acid, 0,010 g/l standard solution, prepared by one of the following methods (3.2.1 or 3.2.2).

3.2.1 Dissolve 0,100 g of sorbic acid in 10 to 12 ml of a 0,1 N sodium hydroxide solution. Transfer quantitatively into a 1 000 ml volumetric flask, and dilute to the mark with water.

Introduce 100 ml of the solution obtained into a second 1 000 ml volumetric flask, and dilute to the mark with water.

3.2.2 Dissolve 134 mg of potassium sorbate in water in a 1 000 ml volumetric flask, and dilute to the mark with water.

Introduce 100 ml of the solution obtained into a second 1 000 ml volumetric flask, and dilute to the mark with water.

3.3 Calcium hydroxide (if necessary), about 0,04 N solution.

For technique A :

3.4 Copper catalyst solution.

In a 1 000 ml volumetric flask, dissolve in a little water :

0,5 g of sodium hydrogen carbonate, and

0,001 g of pure copper(II) sulphate pentahydrate ($CuSO_4.5H_2O$).

Dilute to the mark with water.

For technique B :

3.5 Chromic-sulphuric acid solution.

Dissolve 0,050 g of potassium dichromate in approximately 90 ml of water. Transfer quantitatively into a 200 ml volumetric flask. Add 100 ml of a 0,3 N sulphuric acid solution. Dilute to the mark with water.

(1 litre of 0,3 N sulphuric acid solution contains 14,7 g of sulphuric acid, i.e. 8,4 ml of sulphuric acid ρ_{20} 1,84 g/ml.)

3.6 Thiobarbituric acid solution.

Dissolve 0,500 g of thiobarbituric acid in 50 ml of water to which has been added 10 ml of a 1 N sodium hydroxide solution. Transfer quantitatively into a 100 ml volumetric flask, and add 11 ml of a 1 N hydrochloric acid solution. Dilute to the mark with water.

This solution is not stable and must be used within the 5 h following its preparation.

4 APPARATUS

Usual laboratory equipment, and in particular :

4.1 Analytical balance (if necessary).

4.2 Homogenizer or mortar, as appropriate.

4.3 Boiling water bath (if necessary).

4.4 Steam distillation apparatus (see figure) comprising the items listed in 4.4.1 to 4.4.5.

4.4.1 Steam generator flask having a capacity of 1 000 to 1 500 ml.

4.4.2 Bubbler consisting of a cylindrical tube 30 mm in diameter and 270 mm in height, the lower part of which is closed and enlarged into a sphere having a diameter of 60 mm. The steam supply tube shall end 10 mm above the bottom of the bubbler. The spherical part, in which the product is placed, may be heated either electrically or by a flame; in the latter case, the burnt gases shall be deflected by a metal disc of 150 mm diameter, having a central orifice of approximately 40 mm diameter in which the bottom of the bubbler is engaged. This device avoids the pyrogenation of materials which may be extracted from the product. The auxiliary heating shall be controlled so that the volume of the product placed in the bubbler neither decreases nor increases by more than 5 ml during the distillation.

4.4.3 Fractionating column through which the vapour containing volatile acids passes. It may consist of

— a cylindrical tube of diameter 20 mm and height 500 mm, containing a corrugated helix of No. 100 stainless steel mesh, the helix having a pitch of 15 mm;

— or a column of diameter 20 mm and height 600 mm, having glass internal points;

— or any other device having the same fractionating efficiency.

NOTE — The fractionation of the vapour is indispensable to retain hydroxymethylfurfural when it is present. This substance and its hydrolysis products absorb ultra-violet radiations at 256 nm. The fractionating column may be reduced to 200 mm in height or be replaced by a Kjeldahl flask when the product is free of hydroxymethylfurfural.

4.4.4 Condenser of the West type, of effective length 400 mm and complying with ISO 4799, placed vertically to ensure condensation of the vapour and complete cooling of the distillate.

4.4.5 Receiver flask, as appropriate :

— *liquid products* : 200 ml flask with a graduation mark at 200 ml;

— *thick or solid products* : 500 ml flask.

4.4.6 *Checking of efficiency of distillation apparatus*

The distillation apparatus (4.4) shall allow 300 ml of distillate to be collected in 12 to 15 min, and shall also comply with the following minimal conditions :

a) in normal distilling conditions, 99,5 % of a known quantity of acetic acid added to the sample shall be found in the distillate, which shall be 200 ml. For this test, use 20 ml of a 0,1 N acetic acid solution;

b) in the same distilling conditions, not more than 5 parts per thousand of a known quantity of lactic acid added to the sample shall be found in the distillate, which shall be 200 ml. For this test, use 20 ml of a 1 N lactic acid solution.

4.5 Pipettes, capacities 10, 20 and 25 ml, complying with ISO 648.

4.6 Graduated pipettes, of appropriate capacities, complying with ISO/R 835.

For technique A :

4.7 Conical flasks, capacity 50 ml.

4.8 Spectrophotometer allowing measurements at a wavelength of 256 nm (ultra-violet), with **silica cells** of 10 mm optical path length.

For technique B :

4.9 Volumetric flasks, capacity 25 ml, complying with ISO 1042.

4.10 Photocolorimeter fitted with a green filter, or **spectrophotometer** allowing measurements at a wavelength of 532 nm.

5 PROCEDURE

5.1 Preparation of the test sample

5.1.1 *Liquid products* (juices, pulpy fluid products, syrups), *and thick products* (marmalades, jams)

Homogenize the laboratory sample after having carefully mixed it.

5.1.2 *Solid products* (fruits, vegetables)

Cut a part of the laboratory sample into small pieces, and remove seeds and carpellary cells, if necessary. Take approximately 40 g of the product and homogenize in a homogenizer or mortar (4.2).

Frozen or deep-frozen products shall first be thawed in a closed container and the liquid formed during thawing shall be added to the product before homogenization.

5.2 Test portion

5.2.1 *Liquid products*

Using a pipette (4.5), take 10 ml of the test sample (5.1) and introduce it into the bubbler (4.4.2).

NOTE — The test portion may also be taken by mass, by weighing, to the nearest 0,01 g, approximately 10 g of the test sample.

5.2.2 *Thick or solid products*

Weigh, to the nearest 0,01 g, approximately 10 g of the test sample (5.1) and introduce it into the bubbler (4.4.2) with the minimum of water necessary to entrain the whole of the test portion and to make the mixture sufficiently fluid.

NOTE — In certain cases it is necessary to leave the test portion to soak in the water for 1 to 2 h.

5.3 Distillation

Introduce 0,5 g of the tartaric acid (3.1) into the bubbler (4.4.2) containing the test portion (5.2). Connect the bubbler to the flask (4.4.1) and to the condenser (4.4.4) and simultaneously heat the flask and the bubbler; carry out the distillation, making sure that the volume of the contents of the bubbler remains constant to within 5 ml.

5.3.1 *In the case of liquid products* (5.2.1)

Collect the distillate in the 200 ml flask (4.4.5), stopping the distillation when the 200 ml mark is reached.

5.3.2 *In the case of thick or solid products* (5.2.2)

Collect in the 500 ml flask (4.4.5) a volume of distillate at least 20 times greater than the volume of the contents of the bubbler. Measure the volume (V) collected, using a graduated cylinder.

5.4 Technique A : Determination by spectrophotometry in the ultra-violet range

5.4.1 *Determination*

5.4.1.1 If the initial product contains essential oils of garlic, onion or leek, the presence of these essential oils causes significant absorbance, especially in the case of garlic. Complete evaporation[1] of the distillate, after its being made alkaline, allows the effect of this absorbance to be counteracted.

When these essential oils are present, therefore, take with a pipette (4.5) 25 ml of the distillate (5.3) and transfer it to a small dish; make it alkaline with 1,5 to 2 ml of the calcium hydroxide solution (3.3), evaporate it to dryness on the boiling water bath (4.3) and reconstitute it with water to re-establish the initial volume.

5.4.1.2 As appropriate, take with a pipette (4.5) 10 ml (see note) of the distillate (5.3) or of the reconstituted solution (5.4.1.1), place it in a 50 ml conical flask (4.7), and add 10 ml of the copper catalyst solution (3.4). Shake briefly and leave to stand in contact with air for several minutes.

NOTE — The volume of 10 ml is intended for products containing up to 200 mg of sorbic acid per litre or per kilogram. For higher contents, take only 5 or 2 ml and dilute to 10 ml with water.

Measure the absorbance of the solution using the spectrophotometer (4.8) at a wavelength of 256 nm.

Subtract from the value found the absorbance of the blank test solution (5.4.2).

5.4.2 *Blank test*

Carry out a blank test in parallel with the determination, replacing the 10 ml of distillate by 10 ml of water.

1) The evaporation to dryness does not destroy the sorbic acid if the conditions are sufficiently alkaline.

5.4.3 *Number of determinations*

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

5.4.4 *Preparation of calibration curve*

5.4.4.1 Into a series of six 50 ml conical flasks (4.7), introduce respectively, with a graduated pipette (4.6) :

0 – 1 – 2 – 3 – 5 – 10 ml of the standard sorbic acid solution (3.2); make up the volume to 10 ml by adding :

10 – 9 – 8 – 7 – 5 – 0 ml of water. The solutions obtained contain :

0 – 1 – 2 – 3 – 5 – 10 mg of sorbic acid per litre.

5.4.4.2 To each flask add 10 ml of the copper catalyst solution (3.4).

Measure the absorbances of the solutions using the spectrophotometer (4.8) at a wavelength of 256 nm.

Subtract from the values found the absorbance of the blank test solution (5.4.2).

5.4.4.3 Plot the calibration curve showing the absorbances of the solutions (5.4.4.2) as a function of the sorbic acid concentrations of the solutions obtained in 5.4.4.1, **i.e. before the addition of the copper catalyst solution (3.4)**, expressed in milligrams per litre.

5.5 Technique B : Determination by photocolorimetry or by spectrophotometry at 532 nm

5.5.1 *Determination*

5.5.1.1 If the initial product contains ethanol, remove it from the distillate by the following method :

Using a pipette (4.5) introduce 25 ml of the distillate (5.3) into a small dish, and make it alkaline with 1,5 to 2 ml of the calcium hydroxide solution (3.3); place the dish on the boiling water bath (4.3), and evaporate until the volume is reduced by about half, which normally takes about 30 min. Quantitatively transfer the residue into a 25 ml volumetric flask. Make up to the mark with rinsing water from the dish. Shake.

5.5.1.2 If the initial product contains essential oils (in the case of juice from citrus fruits), eliminate them from the distillate by the same method as described in 5.5.1.1, but prolong the evaporation so that a volume of 1 to 2 ml is attained.

5.5.1.3 If the initial product contains essential oils of garlic, onion or leek, proceed as indicated in 5.4.1.1.

NOTE – In the case of garlic, even when the distillate is evaporated to dryness, a very weak absorbance, corresponding to 1,5 mg of sorbic acid per kilogram, remains.

5.5.1.4 As appropriate, take with a pipette (4.5) 10 ml (see note below) of the distillate (5.3) or of the reconstituted solution obtained after treatment (5.5.1.1, 5.5.1.2, or 5.5.1.3), and introduce it into a 25 ml volumetric flask (4.9).

NOTE – The volume of 10 ml is intended for products containing up to 200 mg of sorbic acid per litre or per kilogram. For higher contents, take only 5 or 2 ml and dilute to 10 ml with water.

Add 4 ml of the chromic-sulphuric acid solution (3.5) and keep the flask for 10 min in the boiling water bath (4.3).

Add 4 ml of the thiobarbituric acid solution (3.6) and keep the flask in the boiling water bath for a further 20 min; a pink colour will develop. Cool in an iced water bath and dilute to the mark with water.

Within 30 min, measure the absorbance of the solution using the photocolorimeter or spectrophotometer (4.10) at a wavelength of 532 nm.

Subtract from the value found the absorbance of the blank test solution (5.5.2).

5.5.2 *Blank test*

Carry out a blank test in parallel with the determination, replacing the 10 ml of distillate by 10 ml of water.

5.5.3 *Number of determinations*

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

5.5.4 *Preparation of calibration curve*

5.5.4.1 Prepare a 2,0 mg/l standard sorbic acid solution by diluting 1 volume of the standard solution (3.2) with 4 volumes of water.

5.5.4.2 Into a series of six 25 ml volumetric flasks (4.9), introduce respectively, with a graduated pipette (4.6) :

0 – 2 – 4 – 6 – 8 – 10 ml of the diluted standard sorbic acid solution (5.5.4.1); make up the volume to 10 ml by adding :

10 – 8 – 6 – 4 – 2 – 0 ml of water. The solutions obtained contain :

0 – 0,4 – 0,8 – 1,2 – 1,6 – 2,0 mg of sorbic acid per litre.

5.5.4.3 To each flask add 4 ml of the chromic-sulphuric acid solution (3.5) and keep the flasks for 10 min in the boiling water bath (4.3).

Add 4 ml of the thiobarbituric acid solution (3.6) and keep the flasks in the boiling water bath for a further 20 min; a pink colour will develop. Cool in an iced water bath and dilute to the mark with water.

Within 30 min, measure the absorbances of the solutions using the photocolorimeter or spectrophotometer (4.10) at a wavelength of 532 nm.

Subtract from the values found the absorbance of the blank test solution (5.5.2).

5.5.4.4 Plot the calibration curve showing the absorbance of the solutions (5.5.4.3) as a function of the corresponding concentration of sorbic acid, expressed in milligrams per litre.

6 EXPRESSION OF RESULTS[1)]

6.1 Method of calculation and formulae

6.1.1 *Test portion measured by volume*

The sorbic acid content, expressed in milligrams per litre of product, is given by the formula

$$\frac{m_1 \times 200}{V_1}$$

where

m_1 is the mass of sorbic acid, expressed in milligrams, per litre of distillate (5.3.1), read on the calibration curve (see 5.4.4 or 5.5.4);

V_1 is the volume, in millilitres, taken in 5.4.1.2 or 5.5.1.4 (usually 10 ml, but may be reduced to 5 ml or 2 ml).

6.1.2 *Test portion measured by mass*

The sorbic acid content, expressed in milligrams per kilogram of product, is given by the formula

$$\frac{m_1 \times V \times 10}{m_0 \times V_1}$$

where

m_0 is the mass, in grams, of the test portion (5.2.2);

m_1 is the mass of sorbic acid, expressed in milligrams, per litre of distillate (5.3.2), read on the calibration curve (see 5.4.4 or 5.5.4);

V is the volume, in millilitres, of distillate collected (see 5.3.2);

V_1 is the volume, in millilitres, taken in 5.4.1.2 or 5.5.1.4 (usually 10 ml, but may be reduced to 5 ml or 2 ml).

6.2 Repeatability

The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst shall not exceed 5 % of the mean in relative value.

8 TEST REPORT

The test report shall show the method used and the result obtained. It shall also mention all operational details not specified in this International Standard, or regarded as optional, as well as any incidents likely to have affected the result.

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

1) Various vegetable products contain small quantities of volatile substances which may be extracted by organic solvents and which absorb 256 nm radiation or give the coloured reaction used in technique B (5.5). Therefore, results which are only slightly positive (less than 10 mg per litre or per kilogram) should be interpreted with caution and compared with results from the same products free from sorbic acid.

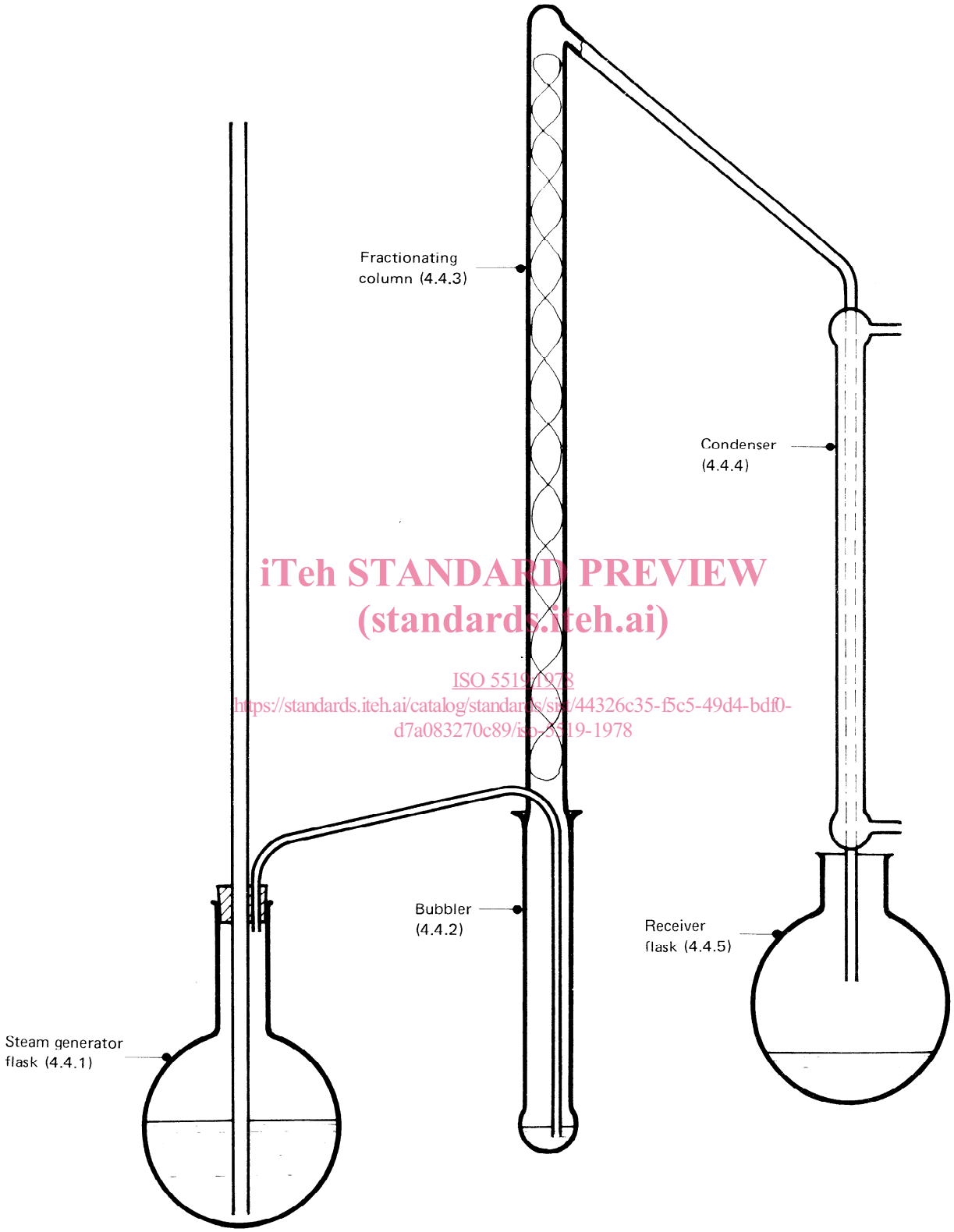

FIGURE — **Diagram of steam distillation apparatus (4.4)**