INTERNATIONAL STANDARD

ISO 5565-2

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Vanilla [*Vanilla fragrans* (Salisbury) Ames] —

Part 2: **Test methods**

Vanille [Vanilla fragrans *(Salisbury) Ames*] —
Partie 2: Méthodes d'essai

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

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.

Attention is drawn to the possibility that some of the elements of this part of ISO 5565 may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 5565-2 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Sub-committee SC 7, *Spices and condiments*.

This first edition of ISO 5565-2, together with ISO 5565-1, cancels and replaces ISO 5565:1982, which has been technically revised.

ISO 5565 consists of the following parts, under the general title *Vanilla [*Vanilla fragran*s (Salisbury) Ames]*:

— *Part 1: Specification*

— *Part 2: Test methods*

Vanilla [*Vanilla fragrans* (Salisbury) Ames] —

Part 2:
Test methods

1 Scope

This part of ISO 5565 specifies test methods for the analysis of vanilla belonging to the species *Vanilla fragrans* (Salisbury) Ames, syn. *Vanilla planifolia* Andrews.

This part of ISO 5565 is applicable to vanilla in pods, cut in bulk, and in the form of powder. It is not applicable to vanilla extracts.

Three test methods for the analysis of vanilla are described in this part of ISO 5565:

a) the determination of moisture content in vanilla pods and powder (4.1);

b) the determination of vanillin, vanillic acid, 4-hydroxybenzaldehyde and 4-hydroxybenzoic acid by high-performance liquid chromatography (4.2);

c) the determination of vanillin content by an ultraviolet spectrometric method (4.3).

NOTE Specifications for vanilla are given in ISO 5565-1.

2 Normative reference

The following normative document contains provisions which, through reference in this text, constitute provisions of this part of ISO 5565. For dated references, subsequent amendments to, or revisions of, this publication do not apply. However, parties to agreements based on this part of ISO 5565 are encouraged to investigate the possibility of applying the most recent edition of the normative document indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 1042, *Laboratory glassware — One-mark volumetric flasks*.

3 Terms and definitions

For the purposes of this part of ISO 5565, the following term and definition apply.

3.1
moisture content
quantity of water entrained and collected in accordance with the method specified in this part of ISO 5565

NOTE It is expressed as a mass fraction in percent [formerly given as % (m/m)].

4 Test methods

4.1 Determination of moisture content in vanilla pods and powder

NOTE The general method described in ISO 939 is not applicable to vanilla.

4.1.1 Principle

The amount of water entrained by azeotropic distillation is determined using a water-immiscible organic liquid. The water is collected in a graduated tube.

4.1.2 Reagent

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

4.1.2.1 Toluene

Saturate the toluene by shaking it with a small quantity of water and distil it. Use the distillate for the determination of the moisture.

4.1.3 Apparatus

Usual laboratory apparatus and, in particular, the following.

4.1.3.1 Distillation apparatus, consisting of a glass flask heated by a suitable means and provided with a reflux condenser discharging into a receiver connected to the flask.

The connections between the receiver, the condenser and the flask are interchangeable ground glass joints. The receiver serves to collect and measure the condensed water, and to return the solvent to the flask. The assembly of the apparatus is shown in Figure 1 and the various components are described below.

4.1.3.1.1 Flask, of capacity 500 ml, of the shape shown in Figure 1 and made of heat-resistant glass, well annealed and as free as possible from striae and similar defects.

4.1.3.1.2 Reflux condenser, water cooled, made of glass, having a jacket approximately 400 mm long and an inner tube of diameter 9,5 mm to 12,5 mm.

The tip of the condenser to be inserted in the receiver may be ground off at an angle of 30° from the vertical axis of the condenser. When inserted into the receiver, the tip of the condenser shall be 6 mm to 7 mm above the surface of the liquid in the receiver after distillation conditions have been established.

4.1.3.1.3 Receiver, of capacity 5 ml, made of heat-resistant glass, well annealed and as free as possible from striae and similar defects, provided with ground glass joints, with the shape, dimensions and tolerances given in Figure 1.

It consists essentially of an upper chamber, together with a tube and ground joint leading to a flask and graduated tube. The graduated portion shall have a capacity of 5 ml when filled to the highest graduation mark.

The scale shall cover the range of 0 ml to 5 ml and shall be graduated at intervals of 0,10 ml. The graduation marks corresponding to each millilitre shall be numbered and carried completely round the tube. The graduation marks midway between the numbered marks shall be carried three-quarters of the way round, and the remaining marks shall be carried half-way round the tube. The error at any indicated capacity shall not exceed 0,05 ml.

4.1.3.1.4 Heat source, either an **oil bath** or an **electric heater,** provided with a sliding rheostat or other means of heat control.

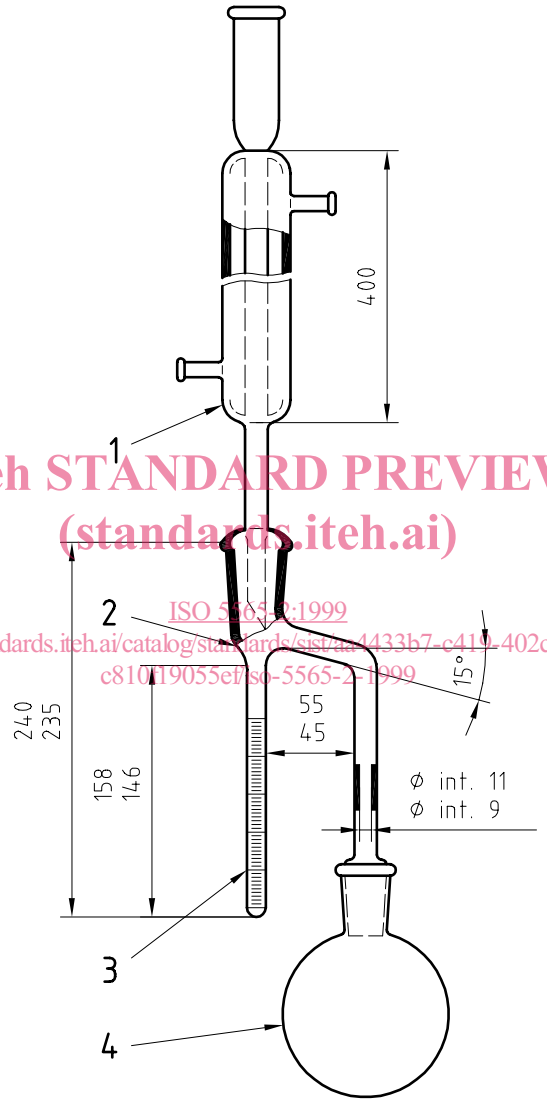
The temperature of the oil in the bath should not be very much higher than the boiling point of toluene.

4.1.3.1.5 **Copper wire**, long enough to extend through the condenser and with one end twisted into a spiral.

The diameter of the spiral shall be such that it fits snugly within the graduated portion of the receiver and yet can be moved up and down.

4.1.3.2 **Analytical balance**, accurate to 0,01 g.

Dimensions in millimetres



Key

1 Reflux condensor (4.1.3.1.2)
2 Receiver (4.1.3.1.3)
3 Graduated tube, of capacity 5 ml, graduated in 0,10 ml
4 Flask (4.1.3.1.1)

Figure 1 — Distillation apparatus

4.1.4 Preparation of test sample

4.1.4.1 Vanilla in pods, cut or in bulk

Prepare the test sample by cutting the vanilla pods into pieces of 5 mm maximum, taking care not to modify the moisture content.

4.1.4.2 Vanilla in powder

Thoroughly mix the laboratory sample.

4.1.5 Procedure

4.1.5.1 Preparation of apparatus

Clean the entire apparatus with a potassium dichromate/sulfuric acid cleaning solution to minimize the adherence of water droplets to the sides of the condenser and the receiver. Rinse thoroughly with water and dry completely before use.

4.1.5.2 Test portion

Weigh, to the nearest 0,1 g, about 10 g of the test sample (4.1.4) such that the quantity of water collected will not exceed 4,5 ml.

4.1.5.3 Determination

Transfer quantitatively the test portion (4.1.5.2) to the distillation flask (4.1.3.1.1) using toluene (4.1.2.1). Add sufficient toluene (about 75 ml in all) to cover the sample completely and swirl to mix. Assemble the apparatus and fill the receiver (4.1.3.1.3) with the toluene by pouring it through the condenser (4.1.3.1.2) until it begins to overflow into the distillation flask. If necessary, insert a loose cotton plug in the top of the condenser or attach to it a small calcium chloride tube to prevent condensation of atmospheric moisture within the condenser tube.

In order to control refluxing, wrap the flask and tube leading to the receiver with a cloth for insulation. Heat the flask so that the distillation rate is about 100 drops per minute. When the greater part of the water has distilled over, increase the distillation rate to about 200 drops per minute and continue until no more water is collected. Purge the reflux condenser occasionally during the distillation with 5 ml portions of the toluene to wash down any moisture adhering to the walls of the condenser.

The water in the receiver may be made to separate from the toluene by occasionally moving a spiral copper wire up and down in the condenser and receiver, thus causing the water to settle at the bottom of the receiver. Reflux until the water level in the receiver remains unchanged for 30 min and then shut off the source of heat. Flush the condenser with toluene as required, making use of the spiral copper wire to discharge any moisture droplets.

Immerse the receiver in water at room temperature for at least 15 min or until the toluene layer is clear; then read the volume of water.

4.1.6 Expression of results

The moisture content, w, expressed as a percentage by mass, is equal to:

$$w = \frac{100\,V}{m}$$

where

V is the volume, in millilitres, of water collected;

m is the mass, in grams, of the test portion.

It is assumed that the density of water is 1 g/ml exactly.

4.2 Determination of vanillin, vanillic acid, 4-hydroxybenzaldehyde and 4-hydroxybenzoic acid in vanilla in pods, bulk, cut or powder form, by high-performance liquid chromatography (Reference method)

4.2.1 Principle

A test portion is extracted and/or diluted (as necessary) then separated by high-performance liquid chromatography (HPLC), using an internal standard, then determined by ultraviolet spectrometry.

4.2.2 Reagents

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

4.2.2.1 **Ethanol**, 96 % (volume fraction).

4.2.2.2 **Methanol**

4.2.2.3 **Dilute phosphoric acid**, $c(H_3PO_4)$ = 0,01 mol/l.

4.2.2.4 **Mobile phase** (for guidance).

Mix 75 parts of the dilute phosphoric acid (4.2.2.3) with 25 parts of the methanol (4.2.2.2). Filter through a membrane (4.2.3.3). Degas.

4.2.2.5 **Reference standards**, with a minimum purity 99 %.

4.2.2.5.1 **Vanillin** (4-hydroxy-3-methoxybenzaldehyde).

4.2.2.5.2 **Vanillic acid** (4-hydroxy-3-methoxybenzoic acid).

4.2.2.5.3 **4-Hydroxybenzaldehyde**

4.2.2.5.4 **4-Hydroxybenzoic acid**

4.2.2.6 **Internal standard**: acetylsalicylic acid, with a minimum purity of 99 %.

4.2.2.7 **Stock standard solutions**, preferably dissolved in the mobile phase.

4.2.2.7.1 **Vanillin stock solution**, of concentration 1 g/l, weighed to the nearest 0,001 g.

4.2.2.7.2 **Vanillic acid stock solution**, of concentration 0,1 g/l, weighed to the nearest 0,001 g.

4.2.2.7.3 **4-Hydroxybenzaldehyde stock solution**, of concentration 0,1 g/l, weighed to the nearest 0,001 g.

4.2.2.8 **Working solution**

The working solution is obtained by dilution of the stock solutions (4.2.2.7) preferably in the mobile phase (4.2.2.4) in such a manner that the final concentration of the working solution should be as given in 4.2.2.8.1 to 4.2.2.8.4.

The solutions prepared in this manner can be preserved for some days in the dark, at a temperature of 4 °C.

4.2.2.8.1 **Vanillin working solution**, of concentration at 0,1 g/l.