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International Standard



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**Tobacco and tobacco products — Determination of maleic hydrazide residues**

*Tabac et produits du tabac — Détermination des résidus d'hydrazide maléique*

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**Descriptors** : tobacco, chemical analysis, determination of content, residues, spectrophotometric analysis, test equipment.

## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards institutes (ISO member bodies). The work of developing International Standards is carried out through ISO technical committees. Every member body interested in a subject for which a technical committee has been set up 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.

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 4876 was developed by Technical Committee ISO/TC 126, *Tobacco and tobacco products*, and was circulated to the member bodies in November 1978.

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

Australia	Ireland	Sweden
Belgium	Italy	Switzerland
Brazil	Korea, Rep. of	Thailand
Bulgaria	Mexico	Turkey
Czechoslovakia	Netherlands	United Kingdom
Egypt, Arab Rep. of	Romania	USSR
Greece	South Africa, Rep. of	Yugoslavia
India	Spain	

The member body of the following country expressed disapproval of the document on technical grounds :

Germany, F. R.

# Tobacco and tobacco products — Determination of maleic hydrazide residues

## 1 Scope and field of application

This International Standard specifies a method for the determination of maleic hydrazide residues in tobacco and tobacco products.

The method is applicable to tobacco and tobacco products and to the residues from maleic hydrazide sucker control agents used on tobacco crops.

## 2 References

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

ISO 1770, *Solid-stem general purpose thermometers.*<sup>1)</sup>

ISO 4793, *Laboratory sintered (fritted) filters — Porosity grading, classification and designation.*<sup>2)</sup>

ISO 4874, *Tobacco and tobacco products — General conditions of sampling.*<sup>2)</sup>

## 3 Principle

Boiling of the test portion with sodium hydroxide solution to remove volatile basic compounds. Addition of granulated zinc and reduction by the nascent hydrogen of the maleic hydrazide to succinic hydrazide which is then hydrolyzed. Distillation of the liberated hydrazine and spectrophotometric determination of its yellow complex with 4-dimethylaminobenzaldehyde.

If necessary, an acid pre-digestion of the test portion and a carbon clean-up of the distillate may be included.

## 4 Reagents

During the analysis, use only reagents of recognized analytical grade and distilled water or water of equivalent purity.

**4.1 4-dimethylaminobenzaldehyde**, 20 g/l solution in 0,5 M sulphuric acid solution.

### 4.1.1 Purification of reagent

Dissolve 20 g of 4-dimethylaminobenzaldehyde in 150 ml of absolute ethanol. Add 5 g of powdered activated charcoal and stir mechanically for 5 min. Filter through a Buchner funnel and add, slowly and with constant stirring, 200 ml of water at 0 °C to the filtrate. Filter the white or pale yellow crystals on a Buchner funnel and wash the crystals with 50 ml of cold water. Dry in a vacuum desiccator over phosphorus(V) oxide and store in a dark bottle.

### 4.1.2 Preparation of reagent solution

Dissolve 2 g of the purified crystals in 100 ml of the 0,5 M sulphuric acid solution (4.6); filter through a sintered glass filter funnel, if necessary.

The reagent is stable for up to 1 month if stored in the dark in a refrigerator; otherwise fresh reagent should be prepared daily.

**4.2 Maleic hydrazide**, standard solution corresponding to 10 µg/ml.

Weigh, to the nearest 0,1 mg, 10 mg of pure maleic hydrazide, dissolve it in 100 ml of 0,1 M sodium hydroxide solution (4.5) and dilute to 1 000 ml with water.

**4.3 Granulated zinc**, of particle size 500 µm, having a bulk density of not more than 1,70 g/cm<sup>3</sup>.

It has been found that the grade of zinc used is of prime importance. It is recommended that the zinc to be used should be checked by comparing the colour produced by standard solutions of hydrazine sulphate and 4-dimethylaminobenzaldehyde with that from maleic hydrazide after reduction and distillation.

**4.4 Sodium hydroxide**, 12,5 M solution.

**4.5 Sodium hydroxide**, 0,1 M solution.

**4.6 Sulphuric acid**, 0,5 M solution.

1) At present at the stage of draft. (Revision of ISO/R 1770-1970).

2) At present at the stage of draft.

**4.7 Iron(II) chloride tetrahydrate** ( $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ ).

The reagent should be checked to ensure that no coloration is produced with 4-dimethylaminobenzaldehyde solution.

**4.8 Anti-foam agent** (paraffin wax or refined vegetable oil).

**4.9 Anti-bumping granules.**

**4.10 High boiling point oil**, for thermometer well.

**4.11 Tobacco**, known to be free from maleic hydrazide and similar in character to that under test (see the note to 7.4).

## 5 Apparatus

Usual laboratory apparatus and the following :

**5.1 One-mark volumetric flasks**, of capacity 25 ml, 100 ml and 1 000 ml, complying with the requirements of ISO 1042, class A.

**5.2 Measuring cylinder**, of capacity 100 ml.

**5.3 Beaker**, of capacity 250 ml.

**5.4 Sintered glass filter funnel** (Buchner funnel), porosity grade P 100, complying with the requirements of ISO 4793.

**5.5 Steam distillation apparatus** (see figure), comprising :

- a) steam generator;
- b) reaction/distillation flask;
- c) condenser;
- d) variable heat controlled hot plate.

The reaction/distillation flask shall be constructed from a 500 ml flat bottomed, thick-walled flask fitted with a spherical joint and a thermometer well containing a 0 to 360 °C thermometer complying with the requirements of ISO/R 1770, designation 9/75.

The steam generator and the reaction/distillation vessel shall be connected through a three way tap. The third outlet shall be used to pass steam to waste through a condenser when not required. This enables a steady boiling rate to be maintained in the generator while the reaction/distillation flask is being changed.

**Warning — The whole apparatus shall be surrounded by a safety screen.**

**5.6 Spectrophotometer**, suitable for measurements at wavelengths of 425, 455 and 485 nm and fitted with 10 mm cells.

## 6 Sampling

Carry out sampling in accordance with the procedures specified in ISO 4874.

## 7 Procedure

### 7.1 Preparation of the sample

Grind the sample in a laboratory grinding mill so that the whole of the ground material passes a 2 mm mesh screen. Mix the ground material thoroughly.

### 7.2 Test portion

Weigh, to the nearest 0,01 g, 1 g of the prepared sample.

### 7.3 Determination

If required, alkaline digestion may be preceded by acid pre-digestion (see annex A).

#### 7.3.1 Alkaline digestion

Transfer the test portion to the reaction/distillation flask. Add 50 ml of the sodium hydroxide solution (4.4), a small amount of the anti-foam agent (4.8) and a few anti-bumping granules (4.9). Insert a 0 to 360 °C thermometer into the thermometer well containing the high boiling point oil (4.10) and heat the flask carefully, swirling the contents occasionally. Reduce the contents of the flask until the thermometer indicates 165 °C; this should take about 10 to 15 min. Allow to cool for 5 min.

#### 7.3.2 Distillation (see annex B)

Start up the steam generator and place a 100 ml measuring cylinder (5.2), containing 10 ml of the sulphuric acid solution (4.6), so that the end of the condenser delivery tube is below the surface of the acid. Add 0,5 g of the iron(II) chloride (4.7) and 15 g of granulated zinc (4.3) to the contents of the reaction/distillation flask. Immediately connect the flask to the apparatus and start the distillation by admitting steam to the flask and heating the contents to  $200 \pm 10$  °C. During distillation maintain a rapid flow of cooling water to the condenser. Maintain the flask at this temperature during the distillation to ensure that 100 ml of distillate is collected in about 20 min. Rinse the end of the condenser into the distillate. Chill the distillate and filter through a sintered glass funnel (5.4) into a 250 ml beaker, washing the cylinder and funnel with about 5 ml of water. Add a few anti-bumping granules to the beaker and evaporate the contents on the hot plate to about, but not less than, 6 ml. Alternatively, the distillate may be concentrated in a rotary evaporator.

If required, the distillate may be cleaned before proceeding further (see annex A).

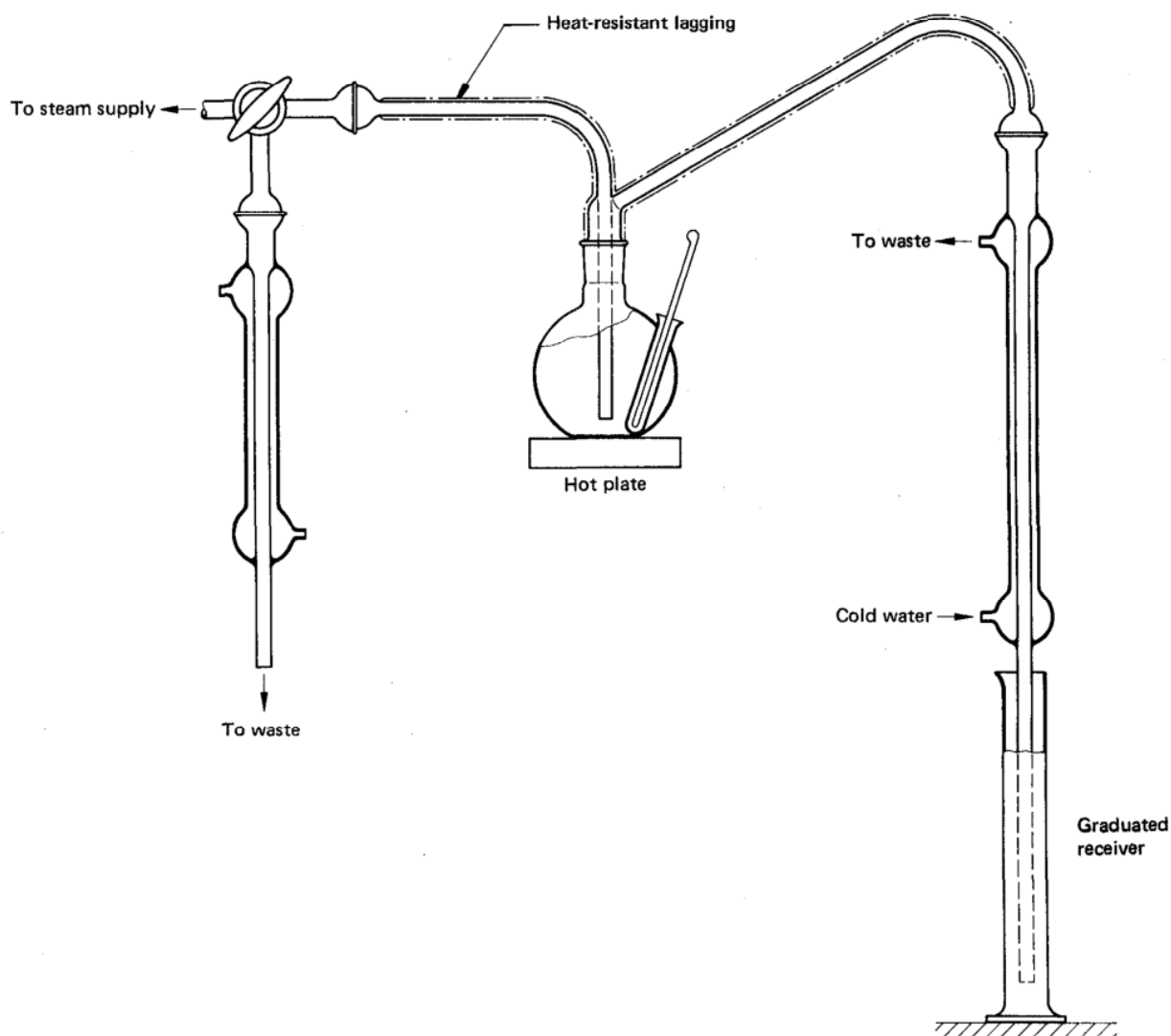


Figure — Apparatus for determination of maleic hydrazide in tobacco

### 7.3.3 Reaction with 4-dimethylaminobenzaldehyde

Cool the concentrated distillate obtained as described in 7.3.2, transfer it quantitatively to a 25 ml volumetric flask (5.1) and add 2 ml of the 4-dimethylaminobenzaldehyde solution (4.1) before making up to the mark with water. Stopper the flask, mix the contents well and stand it in the dark for about 30 min.

### 7.3.4 Spectrophotometric measurements

Fill a 10 mm cell with the test solution obtained as described in 7.3.3.

Add 10 ml of the sulphuric acid solution (4.6) to 2 ml of 4-dimethylaminobenzaldehyde solution (4.1), dilute to 25 ml with water in a volumetric flask and fill the reference cell with this solution.

Measure the absorbance of the test solution on the spectrophotometer (5.6) at wavelengths of 425, 455 and 485 nm.

Calculate the corrected absorbance,  $A_{\text{corr}}$ , of the test solution from the formula

$$A_{455} - \frac{A_{425} + A_{485}}{2}$$

where  $A_{425}$ ,  $A_{455}$  and  $A_{485}$  are respectively the absorbances of the test solution at 425, 455 and 485 nm.

If the absorbance at 455 nm exceeds 0,8, dilute the test solution further with a mixture of 2 ml of 4-dimethylaminobenzaldehyde solution (4.1) and 10 ml of the sulphuric acid solution (4.6), diluted to 25 ml with water.

Carry out two determinations on the same prepared sample.

## 7.4 Calibration curve

Add aliquots of 0, 1, 2, 5 and 8 ml of the standard maleic hydrazide solution (4.2), corresponding to 0 to 80 µg of maleic hydrazide, to 1 g portions of the tobacco (4.11) and treat as described in 7.3.1, 7.3.2 and 7.3.3 above.

Measure the absorbance of the standard test solutions thus prepared as indicated in 7.3.4. Prepare a calibration curve relating the absorbance to the maleic hydrazide content, in micrograms, of the standard test solutions.

NOTE — If no suitable tobacco is available for calibration, then calibration of the standard maleic hydrazide solution in the absence of tobacco is acceptable.

## 8 Expression of results

### 8.1 Method of calculation and formula

Read from the calibration curve the amount of maleic hydrazide present in the sample test solution.

The content of maleic hydrazide, expressed in micrograms per gram of tobacco, is given by the formula

$$\frac{m}{m_o} \times \frac{100}{100 - m_w}$$

where

$m$  is the mass of maleic hydrazide, in micrograms, present

in the sample test solution as read from the calibration curve;

$m_o$  is the mass, in grams, of tobacco taken as the test portion;

$m_w$  is the average water content of the sample.

A correction should be made for any further dilution (see 7.3.4).

Take as the result, the mean of the two values provided that the requirements for repeatability (8.2) are satisfied.

### 8.2 Repeatability

The difference between the results of duplicate determinations, carried out at the same time or rapidly one after another, by the same analyst, shall not exceed 5 % of their mean value, when the mean value is greater than 10 µg/g, or 1 µg/g when the mean value is less than 10 µg/g.

## 9 Test report

The test report shall show the method used and the results obtained. It shall also mention any operating conditions not specified in this International Standard or regarded as optional (for example a special procedure described in annex A), as well as any circumstances that may have influenced the result.

The test report shall include all details required for complete identification of the sample.

## Annex A

### Optional extra procedures

#### A.1 General

**A.1.1** Proteins present in tobacco can sometimes cause excessive foaming during the alkaline digestion and distillation stages. The inclusion of the acid pre-digestion stage described in A.2 breaks down the protein matter and reduces the foaming.

**A.1.2** Some tobacco, especially that containing a high proportion of bases, contains natural substances that interfere with the colour reaction and give a red colour with 4-dimethylaminobenzaldehyde. Treatment of the distillate with activated carbon as described in A.3 eliminates this interference.

**A.1.3** Neither of these procedures should be used unless the analyst regards it as necessary for the particular samples being tested.

#### A.2 Acid pre-digestion

##### A.2.1 Additional reagents required

**A.2.1.1** Hydrochloric acid, approximately 3 M solution.

Dilute 270 ml of hydrochloric acid,  $\rho$  approximately 1,18 g/ml, to 1 000 ml with water and mix.

**A.2.1.2** Sodium hydroxide, 700 g/l solution.

##### A.2.2 Procedure

Transfer the test portion to the reaction/distillation flask. Add 50 ml of the hydrochloric acid (A.2.1.1) and a small amount of paraffin wax. Heat the flask and boil the contents at a moderate rate until the volume has been reduced to between 20 and 25 ml. Wash down the walls of the vessel with about 25 ml of water and again boil until the volume has been reduced to between 20 and 25 ml. Allow to cool.

NOTE — The partially digested tobacco may be left overnight at this stage, but once sodium hydroxide has been added the method must be completed as soon as possible.

Add, slowly and with swirling, 50 ml of the sodium hydroxide solution (A.2.1.2) and proceed with the alkaline digestion as in 7.3.1.

#### A.3 Carbon clean-up

##### A.3.1 Additional reagent required

**A.3.1.1** Activated carbon.<sup>1)</sup>

##### A.3.2 Procedure

Shake the distillate obtained as described in 7.3.2 with 2 g of activated carbon (A.3.1.1) for 1 min and filter before concentrating to 6 ml as directed in 7.3.2.

1) Suitable material is available commercially. Details may be obtained from the Secretariat of ISO/TC 126 (DIN, Germany, F. R.) or from the ISO Central Secretariat.

## Annex B

### Notes on distillation procedure

**B.1** A safety screen shall be placed in position during distillation.

**B.2** After distillation, remove the hot flask from the apparatus using heat resistant gloves and wearing safety glasses. Remove the thermometer and seal the well with a small cork. Rinse the steam inlet tube free of sodium hydroxide. Pour the contents of the distillation flask into an iron sieve in the sink to trap the zinc granules and rinse the flask three times with water and twice with 10 % (V/V) hydrochloric acid solution to remove the encrusted caustic and zinc granules. Fill the flask with the hydrochloric acid solution until next used.

Before re-use rinse the flask three times with water. It is essential to remove all residual zinc as it would cause premature decomposition of maleic hydrazide in the pre-digest stage of the next determination.

**B.3** The distillation flasks shall be regularly inspected as they have a very limited life owing to the action of the concentrated sodium hydroxide solution.

**B.4** High vacuum silicone grease should be used on all spherical joints of the apparatus. When adding the iron(II) chloride and zinc granules to the contents of the reaction/distillation flask, care should be taken that no particles adhere to the grease and thus prevent proper closing of the joint.

**B.5** Care should be exercised in the disposal of residual zinc particles because of the danger of fire.