

# INTERNATIONAL STANDARD

**ISO**  
**2881**

Third edition  
1992-09-01

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## **Tobacco and tobacco products — Determination of alkaloid content — Spectrometric method**

*Tabac et produits du tabac — Détermination de la teneur en  
alcaloïdes — Méthode spectrométrique*

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Reference number  
ISO 2881:1992(E)

## 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 2881 was prepared by Technical Committee ISO/TC 126, *Tobacco and tobacco products*.

This third edition cancels and replaces the second edition (ISO 2881:1977), which has been technically revised.

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International Organization for Standardization  
Case Postale 56 • CH-1211 Genève 20 • Switzerland

Printed in Switzerland

# Tobacco and tobacco products — Determination of alkaloid content — Spectrometric method

## 1 Scope

This International Standard specifies a reference method for the spectrometric determination of alkaloids, expressed as nicotine, in tobacco.

The method is applicable to unmanufactured tobacco, manufactured tobacco and tobacco products.

## 2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 565:1990, *Test sieves — Metal wire cloth, perforated metal plate and electroformed sheet — Nominal sizes of openings.*

ISO 3401:1991, *Cigarettes — Determination of alkaloid retention by the filters — Spectrometric method.*

ISO 4874:1981, *Tobacco — Sampling of batches of raw material — General principles.*

ISO 6488:1981, *Tobacco — Determination of water content (Reference method).*

ISO 8243:1991, *Cigarettes — Sampling.*

## 3 Principle

Submission of the ground tobacco sample to steam distillation under strongly alkaline conditions, spectrometric measurement of the absorption of the distillate and calculation of the alkaloid content, expressed as the percentage of nicotine.

## 4 Reagents

Use only reagents of recognized analytical grade and distilled water or water of at least equivalent purity.

### 4.1 Sodium chloride

4.2 Sodium hydroxide solution,  $c(\text{NaOH}) = 8 \text{ mol/l}$ .

4.3 Concentrated sulfuric acid,  $c(\text{H}_2\text{SO}_4) = 1 \text{ mol/l}$ .

4.4 Dilute sulfuric acid,  $c(\text{H}_2\text{SO}_4) = 0,025 \text{ mol/l}$ .

4.5 Nicotine, minimum purity 98 %.

## 5 Apparatus

Usual laboratory apparatus and the following items:

5.1 **Steam distillation apparatus**, as described in ISO 3401 or any other apparatus giving the same results.

Test the system according to the indicated procedure (see clause 7) with the pure nicotine (4.5) at the maximum expected level. Recovery shall be at least 98 % of the theoretical value. If not, optimize by modification of the distillation rate.

For routine tests it is possible to use nicotine hydrogen tartrate calibrated against the pure nicotine (4.5).

5.2 **Spectrometer**, covering a wavelength range from 230 nm to 290 nm.

5.3 **Matched quartz cells**, having an optical path length of 1 cm.

5.4 **Sieve**, aperture size 500  $\mu\text{m}$ , conforming to ISO 565.

**5.5 Grinder**, capable of grinding the tobacco sample without noticeable rise in temperature (see 7.1).

**5.6 Drying oven**, ventilated natural convection type.

**5.7 Volumetric flasks**, of capacity 250 ml, with ground glass stoppers.

**5.8 Volumetric flasks**, of capacity 100 ml, with ground glass stoppers.

**5.9 One-mark pipettes**, of capacities 5 ml, 10 ml and 25 ml.

**5.10 Glass funnels**, of diameter about 55 mm.

**5.11 Filter paper**, fast filtering grade.

## 6 Sampling

Carry out sampling in accordance with the methods specified in ISO 4874 for tobacco or ISO 8243 for cigarettes.

## 7 Procedure

### 7.1 Preparation of test sample

If required, randomly divide the laboratory sample obtained in accordance with clause 6 into two equal test samples.

If the determination of alkaloids will be combined with a determination of smoke condensate, prepare the test sample for the determination of alkaloid content from the laboratory sample after taking the test sample for the determination of smoke condensate.

In the case of cigarettes, ensure that the test sample is free from paper and filter material before grinding.

Protect the sample from atmospheric moisture prior to the determination.

Before grinding, dry the test sample in the drying oven (5.6) at a temperature not higher than 40 °C. Then grind the test sample to pass the sieve with aperture size 500 µm (5.4). Do not continue grinding for more than 2 min.

**NOTE 1** Excessive grinding may cause a rise in temperature of the sample with possible loss of alkaloids.

Retain a portion of the ground test sample for the determination of water content.

### 7.2 Determination

**NOTE 2** The quantities specified in this subclause refer to the type of apparatus used in ISO 3401. If other apparatus is used, these quantities may be modified provided that the results obtained are the same.

#### 7.2.1 Number of determinations

Carry out two determinations on the same test sample under identical conditions.

#### 7.2.2 Test portion

Weigh, to the nearest 0,001 g, 1 g to 2 g (depending on the expected alkaloid content) of the well-mixed ground test sample.

#### 7.2.3 Determination of water content

Determine the water content of the ground test sample in the portion retained for this purpose, in accordance with ISO 6488.

#### 7.2.4 Determination of alkaloid content

##### 7.2.4.1 Distillation

**WARNING** — Take care during the distillation while sodium hydroxide is being added.

Introduce the test portion (7.2.2) into the distillation chamber of the distillation apparatus (5.1) and wash down with 5 ml to 15 ml of water.

Add 20 g to 40 g of sodium chloride (4.1) and 5 ml of the sodium hydroxide solution (4.2) to the liquid contained in the distillation chamber, closing the inlet funnel as the last drops pass through.

**NOTE 3** The amount of sodium chloride should be sufficient to leave some undissolved salt at the end of the distillation.

Steam distil the mixture into a 250 ml volumetric flask containing 10 ml of concentrated sulfuric acid (4.3). The rate of distillate shall be at least 10 ml to 12 ml of distillate per minute. Do not allow the volume of liquid in the distillation chamber to change appreciably during distillation. Use auxiliary heating, if necessary. Collect 220 ml to 230 ml of distillate. Remove the flask whilst rinsing the delivery tube with a little water. Terminate the distillation and rinse the still. Ensuring that the flask is at room temperature, make up to the mark (volume  $V_1$ ) with water. Mix and use acidified distillate to determine spectrometrically the alkaloids in the tobacco. Filter the acidified distillate if it is not clear.

**NOTE 4** If filtration is necessary, either the first 150 ml of filtrate should be discarded or, before use, the filter paper should be washed with a sufficient amount of water and then dried.