

INTERNATIONAL STANDARD



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Revisé

Tobacco and tobacco products — Determination of alkaloids in tobacco — Spectrophotometric method

Tabac et produits du tabac — Détermination de la teneur en alcaloïdes dans le tabac — Méthode spectrophotométrique

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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 2881 was drawn up by Technical Committee ISO/TC 126, *Tobacco and tobacco products*, and circulated to the Member Bodies in July 1972.

It has been approved by the Member Bodies of the following countries :

Australia	India	South Africa, Rep. of
Austria	Ireland	Spain
Belgium	Israel	Switzerland
Czechoslovakia	Mexico	Thailand
Egypt, Arab Rep. of	Netherlands	Turkey
France	New Zealand	United Kingdom
Germany	Poland	U.S.S.R.
Hungary	Romania	

No Member Body expressed disapproval of the document.

Tobacco and tobacco products – Determination of alkaloids in tobacco – Spectrophotometric method

1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies a reference method for the spectrophotometric determination of alkaloids in tobacco.

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

2 REFERENCES

ISO/R 565, *Test sieves – Woven metal wire cloth and perforated plate – Nominal sizes of apertures.*

ISO 3401, *Tobacco and tobacco products – Determination of alkaloid retention by filters of cigarettes.*¹⁾

ISO . . ., *Tobacco and tobacco products – Sampling.*²⁾

3 PRINCIPLE

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

4 REAGENTS

The reagents used shall be of analytical reagent quality.

4.1 Sodium chloride.

4.2 Sodium hydroxide, 8 N solution.

4.3 Sulphuric acid, 2 N solution.

4.4 Sulphuric acid, 0,05 N solution.

5 APPARATUS

Usual laboratory apparatus not otherwise specified and the following items:

5.1 Analytical balance.

5.2 Sieve, aperture size 500 μm , conforming to ISO/R 565.

5.3 Grinder, capable of grinding the tobacco sample without noticeable rise in temperature.

5.4 Drying oven, ventilated natural convection type.

5.5 One-mark volumetric flasks, 250 ml capacity, with ground stoppers, complying with Class A of ISO/R 1042.

5.6 One-mark volumetric flasks, 100 ml capacity, with ground stoppers, complying with Class A of ISO/R 1042.

5.7 Pipettes.

5.8 Funnels, of glass, about 55 mm diameter.

5.9 Filter paper, fast filtering grade.

5.10 Steam distillation apparatus as described in ISO . . .²⁾ or any other apparatus giving the same results.

5.11 Spectrophotometer, covering a wavelength range from 230 to 290 nm.

5.12 Matched quartz cells, optical path length 1 cm.

NOTE — The absorbance of the cells must be equal before and after each measurement.

1) At present at the stage of draft.

2) In preparation.

6 SAMPLING

Carry out sampling by the method described in ISO ...¹⁾

7 PROCEDURE

7.1 Preparation of the sample

If required, randomly divide the laboratory sample obtained according to clause 6 into two equal test samples.

If the determination of alkaloids will be combined with a determination of smoke condensate, the test sample shall be prepared from the laboratory sample after the test sample for the determination of smoke condensate has been taken.

The sample shall be protected from atmospheric moisture prior to the determination.

Before grinding, dry the test sample in the drying oven (5.4) at a temperature not higher than 40 °C. Then grind the test sample to pass a sieve with aperture size 500 µm (5.2).

A portion of the ground test sample shall be retained for the determination of water content.

NOTE — In the case of cigarettes, the test sample shall be freed from paper and filter material before grinding.

7.2 Determination

For a complete analysis, carry out two independent determinations under identical conditions.

NOTE — The quantities specified in this sub-clause refer to the type of apparatus used. If other apparatus is used, these quantities can be modified provided that the results obtained are the same.

7.2.1 Test portion

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

7.2.2 Determination of water content

Determine the water content of the ground test sample in the portion retained for this purpose according to ISO ...¹⁾

7.2.3 Determination of alkaloid content

7.2.3.1 DISTILLATION

Transfer the test portion to the distillation flask of the steam distillation apparatus (5.10) and wash down with 5 to 25 ml of distilled water.

Add 20 to 40 g of sodium chloride (4.1) and 5 ml of 8 N sodium hydroxide solution (4.2) to the liquid in the distillation flask.

NOTE — The amount of sodium chloride shall be sufficient to leave some undissolved salt at the end of distillation.

Steam distil the mixture into a 250 ml volumetric flask containing 10 ml of 2 N sulphuric acid (4.3). Collect 220 to 250 ml of distillate and dilute to the mark (volume = *V*₁). If necessary filter through a dry filter paper (5.9) to remove cloudiness.

NOTE — The rate of distillation shall be at least 10 to 12 ml of distillate per minute. The volume of the liquid in the distillation flask shall not be allowed to change appreciably during distillation. Auxiliary heating shall be employed, if necessary.

7.2.3.2 DETERMINATION OF ALKALOIDS IN THE DISTILLATE

Pipette an aliquot (volume = *V*₂, normally 25 ml) of the solution from the volumetric flask into a second volumetric flask (capacity = *V*₃, normally 100 ml) and dilute to the mark with 0,05 N sulphuric acid (4.4).

Prepare a blank solution by diluting 10 ml of 2 N sulphuric acid (4.3) to 250 ml in a volumetric flask with distilled water and diluting further by the procedure described for the test solution.

Measure the absorbance of the test solution with the spectrophotometer (5.11), using the blank solution as reference at the wavelengths of 236 nm, 259 nm and 282 nm.

If the absorbance at 259 nm exceeds 0,7 repeat the measurement using a smaller volume *V*₂ in the dilution stage.

If the absorbance at 236 nm is greater than twice the absorbance at 282 nm the determination should be repeated because the background is too high and the distillation has been ineffective.

8 EXPRESSION OF RESULTS

8.1 Method of calculation and formulae

Calculate the corrected absorbance *A* (extinction) from the observed values of absorbance by means of the following formula :

$$A = 1,059 \left(A_{259} - \frac{A_{236} + A_{282}}{2} \right) \quad \dots (1)$$

where *A*₂₃₆, *A*₂₅₉ and *A*₂₈₂ are the observed absorbances at 236, 259 and 282 nm respectively.

1) In preparation.