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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 2881-1977, the second edition of this International Standard, has been drawn up by Technical Committee ISO/TC 126, *Tobacco and tobacco products*, and has been modified in accordance with the amendment circulated to the member bodies in August 1976.

This amendment has been approved by the member bodies of the following countries :

Australia	India	Spain
Belgium	Italy	Sweden
Canada	Mexico	Switzerland
Czechoslovakia	New Zealand	Thailand
Egypt, Arab Rep. of	Poland	Turkey
France	Romania	United Kingdom
Germany	South Africa, Rep. of	Yugoslavia

No member body expressed disapproval of the amendment.

This second edition cancels and replaces the first edition (i.e. ISO 2881-1974), which had 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 had 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 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.*

ISO . . . , *Tobacco and tobacco products – Sampling.*¹⁾

ISO . . . , *Tobacco and tobacco products – Determination of moisture content.*¹⁾

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 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, of capacity 250 ml, with ground stoppers, complying with class A of ISO 1042.**

5.6 **One-mark volumetric flasks, of capacity 100 ml, with ground stoppers, complying with class A of ISO 1042.**

5.7 **Pipettes, complying with ISO 648.**

5.8 **Funnels, of glass, of diameter about 55 mm.**

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

5.10 **Steam distillation apparatus, as described in ISO 3401, 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, of optical length 1 cm.**

NOTE – The absorbance of the cells must be equal before and after each measurement. If such is not the case, an appropriate correction shall be applied.

1) In preparation.

6 SAMPLING

Carry out sampling by the method specified in ISO...

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 the 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...

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 the sodium chloride (4.1) and 5 ml of the 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 the sulphuric acid solution (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 the sulphuric acid solution (4.4).

Prepare a blank solution by diluting 10 ml of the sulphuric acid solution (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.

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 formula

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

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

The test result [see also 9 g)] shall be calculated as the percentage by mass of tobacco alkaloids (as nicotine) in the test portion (referring to the dry mass) :

$$\% \text{ nicotine} = \frac{100 A V_1 V_3}{a V_2 l m \left(\frac{100 - b}{100} \right)} \dots (2)$$

NOTES — Other frequently used formulae are :

Nicotine content *c* of the diluted distillate, in milligrams of nicotine per millilitre of solution

$$c = \frac{A}{a l} \dots (3)$$

Absorptivity

$$a = \frac{A}{c l} \quad a_{259} = 34,3 \dots (4)$$