CORESTA RECOMMENDED METHOD N° 20

DETERMINATION OF ALKALOIDS IN MANUFACTURED TOBACCO

(September 1968)

1. SCOPE

This method is applicable to manufactured tobacco and tobacco products.

2. PRINCIPLE OF THE METHOD

The ground tobacco sample is submitted to steam distillation under strongly alkaline conditions, the total alkaloid content of the distillate measured spectrophotometrically and calculated as nicotine.

3. SAMPLING

The sampling method used must be appropriate for the nature of the test and the product tested.

A detailed standard for sampling procedure is in preparation.

4. APPARATUS

4.1. Analytical balance, minimum accuracy ± 1.0 mg.

4.2. Sieve, mesh opening 0.63 mm, wire gauge 0.4 mm.

4.3. Mill, hammer or ball type, capable of grinding the tobacco sample without noticeable heat evolution.

4.4. Drying oven, ventilated natural convection type.

4.5. Volumetric flask, 250 ml, narrow-neck type with ground stopper.

4.6. Volumetric flask, 100 ml, narrow-neck type with ground stopper.

4.7. Pipettes, analytical, 25 ml.

4.8. Funnels, 55 mm diameter.

4.9. Filter paper.

4.10. Steam distillation apparatus as described by Griffith (Tobacco Science **1**, 130-137 (1957)), or by Seehofer and Borowski (Beiträge zur Tabakforschung **2**, 37-38 (1963)). The volume of the distillation flask shall not exceed 1000 ml. If the same apparatus is also to be used for the distillation of smoke alkaloids (CORESTA Recommended Method N° 12) from methanol solution the head space above the liquid in the flask must be large enough to prevent carry-over of liquid during the initial violent boiling when steam is first admitted into the methanol solution.

4.11. Spectrophotometer, covering the wavelength range 230-290 nm.

4.12. Quartz cells, path length 10 mm.

5. REAGENTS

Analytical grade reagents should be used.

5.1. 8 N Sodium hydroxide solution.

5.2. 2 N Sulphuric acid.

5.3. Sodium chloride.

5.4. 0.05 N Sulphuric acid.

6. SAMPLE PREPARATION

The laboratory sample supplied to the testing laboratory should satisfy the requirements of chapter 3 of this Recommended Method. If the test requires, the laboratory sample may be randomly divided into two test samples with equal, number of cigarettes.

If the alkaloid determination is to be combined with determination of the smoke condensate according to CORESTA Recommended Method N° 10, the test sample shall be drawn from the cigarettes remaining after the smoke condensate determination. The tobacco in the cigarettes shall be freed from paper and filtering material. The test sample is oven-dried below 40 °C, and when dry enough it is ground to pass a sieve with 0.63 mm mesh opening.

A portion of the ground sample is retained for determination of residual moisture.

7. PROCEDURE

The determination should be made in duplicate on different days.

The determination of residual moisture, b, in the ground sample should be carried out by the benzene co-distillation method. (Beiträge zur Tabakforschung **1**, 314 (1962)) or by any other suitable method which does not deviate by more than ± 0.5 per cent moisture from the benzene co-distillation method.

Weigh out 200-2000 mg (depending on the alkaloid content) of the well mixed test sample to the nearest (weight = t mg). Transfer to the distillation flask and wash down with 5-25 ml distilled water. Add 20-40 g sodium chloride* and 5 ml 8 N sodium hydroxide solution. Steam distill the mixture into a 250 ml volumetric flask containing 15 ml 2 N sulphuric acid**. Collect 220-230 ml of distillate and make up to the mark with distilled water (volume = V_D). Mix, and remove any cloudiness by filtration.

An aliquot, V_V (normally 25 ml), of the (filtered) distillate is pipetted into a volumetric flask V_M (normally 100 ml), diluted to the mark with 0.05 N sulphuric acid and mixed. The absorbance of this solution is measured at the wavelengths 236 nm (A_{236}), 259 nm (A_{259}) and 282 nm (A_{282}) with a solution of 15 ml 2 N sulphuric acid diluted to 250 ml with distilled water as reference.

If the absorbance at 259 nm exceeds 0.7 the dilution should be repeated with a smaller volume V_V.

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

** The volume of liquid in the distillation flask should not be allowed to increase during distillation. Keep constant by auxiliary if necessary.

8. CALCULATION

The following formulae apply :

Absorptivity : $a = \frac{A}{c \cdot d}$ $\qquad a_{259} = 34.3$ (1)

Corrected absorbance : $A = 1.059 \left(A_{259} - \frac{A_{236} + A_{282}}{2}\right)$ (2)

Nicotine content of diluted distillate, mg nicotine/ml solution

$$c = \frac{A}{a \cdot d} \qquad (3)$$

Total amount of tobacco alkaloids, expressed as mg nicotine, in the test sample

$$C = \frac{A \cdot V_D \cdot V_M}{a \cdot V_V \cdot d} \qquad (4)$$

Percentage of tobacco alkaloids (as nicotine) in the test sample (dry basis)

$$\% \text{ Nicotine} = \frac{100 \cdot A \cdot V_D \cdot V_M}{a \cdot V_V \cdot d \cdot t \left(\frac{100 - b}{100}\right)} \qquad (5)$$

where :

a = absorptivity (decadic extinction coefficient) of nicotine in 0.05 N sulphuric acid, *i.e.* 34.3 at the absorption maximum of 259 nm.

A = corrected absorbance (extinction).

V_V = aliquot of distillate taken for further dilution, ml.

c = nicotine concentration, g/1000 ml.

d = optical path length, cm.

V_M = volume of dilution flask, ml.

b = residual moisture content of the test sample, per cent.

t = weight of sample weighed in for distillation, mg.

V_D = volume of distillate, ml.

C = total amount of tobacco alkaloids.

For an explanation of the values for the absorptivity and the correction factor see Willits *et al.*, Analytical Chemistry **22**, 430-433 (1950).

Duplicate determination should agree within 0.05 per cent by weight of nicotine. If not, further determinations should be made until this requirement is fulfilled.

9. REPORT

The report on the results should include a reference to this Recommended Method, and :

9.1. description of the product tested, cf. CORESTA Recommended Method N° 10.

9.2. a) sampling method ;
b) number of samples ;
c) date and place of purchase.

9.3. test results :

a) mean residual moisture content, as percentage by weight to the nearest 0.5 per cent;
b) nicotine contents (results of all individual determinations as percentage of dry weight to the nearest 0.01 per cent ;
c) date of test.