

CORESTA RECOMMENDED METHOD N° 12

DETERMINATION OF ALKALOIDS IN CIGARETTE SMOKE CONDENSATES

(September 1968)

1. SCOPE

This method is applicable to methanol solutions of cigarette smoke condensates.

2. PRINCIPLE OF THE METHOD

An aliquot of a methanol solution of a cigarette smoke condensate is steam distilled in two steps: the neutral and acid steam-volatile substances are removed by distillation after acidification of the solution with mineral acid and the pyridine alkaloids (nicotine) are subsequently distilled from the same solution made strongly alkaline. The absorbance of the distillate from the alkaline distillation is measured by ultraviolet spectrophotometry and the alkaloid content is calculated as nicotine.

3. APPARATUS

- 3.1. Steam distillation apparatus, cf. CORESTA Recommended Method N° 20, with auxiliary heating.
- 3.2. Spectrophotometer, covering the wavelength range 230-290 nm.
- 3.3. Quartz cells, path length 10 mm.
- 3.4. Volumetric flasks, 250 ml, narrow-neck type with ground stopper.
- 3.5. Pipettes, analytical.
- 3.6. Funnels, 55 mm diameter.
- 3.7. Filter paper.

4. REAGENTS

Analytical grade reagents should be used.

- 4.1. 8 N Sodium hydroxide solution.
- 4.2. 2 N Sulphuric acid.
- 4.3. 0.05 N Sulphuric acid.

5. SAMPLE PREPARATION

The cigarette smoke condensate is prepared according to CORESTA Standard Method N° 10 and dissolved in methanol as described in paragraph 8.6 of that method.

6. PROCEDURE

A single determination according to this method shall correspond to each single smoking run as described in CORESTA Standard Method N° 10.

Introduce an aliquot, V_A , of the crude smoke condensate (volume V_K), corresponding to 2-3 cigarettes, into the distillation flask. Add 5 ml 2 N sulphuric acid and about 25 ml distilled water and start the steam distillation. The volume of the contents in the distillation flask should not be allowed to increase. Keep constant by auxiliary heating if necessary. Stop the distillation when about 100 ml has been collected and discard the distillate.

Add slowly 10 ml 8 N sodium hydroxide solution and resume the distillation with a 250 ml volumetric flask containing 15 ml 2 N sulphuric acid as receiver. Collect 220-230 ml of distillate, make up to the mark with distilled water (volume V_D), mix and filter.

Measure the absorbance of the filtrate at 236 (A_{236}), 259 (A_{259}) and 282 (A_{282}) nm against a reference solution of 15 ml 2 N sulphuric acid diluted to 250 ml with distilled water. If the absorbance at 259 nm exceeds 0.7 dilute V_V ml of the filtered distillate further to V_M ml with 0.05 N sulphuric acid and measure the absorbance of this solution against a sulphuric acid reference diluted in the same way.

7. CALCULATION

Total amount of tobacco alkaloids, H_{nic} , per cigarette smoked :

$$H_{\text{nic}} = \frac{A \cdot V_K \cdot V_D \cdot V_M}{a \cdot d \cdot V_A \cdot V_V \cdot n} \quad A = 1.059 \left(A_{259} - \frac{A_{236} + A_{282}}{2} \right)$$

where:

- a = absorptivity (decadic extinction coefficient) of nicotine in 0.05 N sulphuric acid; *i.e.* 34.3 at 259 nm.
- A = corrected absorbance (extinction), cf. CORESTA Recommended Method N° 20.
- V_V = aliquot of distillate V_D taken for further dilution to V_M , ml.
- d = optical path length, cm.
- V_M = volume to which the aliquot V_V of the distillate was further diluted, ml.
- n = number of cigarettes smoked into the trap.
- V_K = volume of methanolic condensate solution, ml.
- V_A = aliquot of methanolic condensate solution taken for distillation, ml.
- V_D = volume of distillate from the alkaline distillation, ml.

The difference between determinations on two single smoking runs should agree within 6 per cent of the smallest value. If not, further determinations should be made until this requirement is fulfilled.

8. REPORT

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

- 8.1.** a description of the product tested, cf. CORESTA Standard Method N° 10, paragraph 10.1.
- 8.2.** a description of the sampling procedure, cf. CORESTA Standard Method N° 10, paragraph 10.2.
- 8.3.** test conditions, cf. CORESTA Standard Method N° 10, paragraph 10.3.
- 8.4.** test results:
 - a) nicotine content in mg per cigarette smoked to the nearest 0.01 mg for each single smoking run;
 - b) results as stated in CORESTA Standard Method N° 10, paragraph 10.4;
 - c) date of test.