

CORESTA RECOMMENDED METHOD N° 66

DETERMINATION OF NICOTINE IN THE MAINSTREAM SMOKE OF CIGARS BY GAS CHROMATOGRAPHIC ANALYSIS

(November 2005)

1. FIELD OF APPLICATION

This method is applicable to the particulate matter of mainstream cigar smoke.

2. DEFINITION

Particulate matter is that part of mainstream smoke which is collected by a smoke trap conforming to CORESTA Recommended Method N° 64: 2005.

3. REFERENCES

CORESTA Recommended Method N° 67: 2005

Determination of water in the mainstream smoke of cigars by Gas Chromatographic Analysis.

CORESTA Recommended Method N° 64: 2005

Routine analytical cigar-smoking machine - Specifications, definitions and standard conditions.

CORESTA Recommended Method N° 65: 2005

Determination of total and nicotine-free dry particulate matter using a routine analytical cigar-smoking machine - Determination of total particulate matter and preparation for water and nicotine measurements.

CORESTA Recommended Method N° 46: 1998

Atmosphere for conditioning and testing cigars of all shapes and sizes.

CORESTA Recommended Method N° 47: 2000

Cigars - Sampling.

CORESTA Recommended Method N° 39: 1994

Determination of the purity of nicotine.

CORESTA Recommended Method N° 12: 1968

Determination of alkaloids in cigarette smoke condensate.

4. PRINCIPLE

Cigars are smoked and the particulate matter of mainstream smoke is collected by a standard procedure. The particulate matter is dissolved in a solvent and the nicotine content of this solution is determined by gas chromatography. Results are expressed as the weight of nicotine delivered per cigar.

5. APPARATUS

- 5.1. A *standard smoking machine* complying with CORESTA Recommended Method N° 64: 2005 and equipped for smoking.
- 5.2. A *gas chromatograph* equipped with a flame ionisation detector together with a recorder and integrator or other suitable data handling instrument. Glass columns for the gas chromatograph should be 1,5 m to 2 m long and have an internal diameter of about 2 mm. A suitable syringe for sample injection or for automatic analysis an autosampler that is compatible with the gas chromatograph is required (see Annex).
- 5.3. The necessary general laboratory equipment for the preparation of samples, standards and reagents.

6. REAGENTS

- 6.1. Propan-2-ol (analytical grade).
- 6.2. n-Heptadecane or Quinaldine (minimum purity 99%) as internal standard.
- 6.3. Solvent for samples and standards: propan-2-ol containing an appropriate concentration of internal standard (see 6.2); this is normally in the range of 0,2 mg/ml to 0,5 mg/ml.
- 6.4. Column packing material: 10% Carbowax 20M plus 2% potassium hydroxide on an acid washed silanised support material, 80 - 100 mesh.
- 6.5. Gases: hydrogen, nitrogen or helium and compressed air necessary for operation of the gas chromatograph.
- 6.6. Nicotine or Nicotine salicylate or Nicotine tartrate of known purity (minimum purity 98%) for the preparation of standard solutions. Store at 0 °C to +4 °C and exclude light.

7. STANDARDS

Dissolve nicotine or a nicotine salt (6.6) in the solvent, described in Section 6.3, to produce a series of at least four calibration solutions whose concentrations cover the range expected to be found in the samples. Store at 0 °C to +4 °C and exclude light. In calculations, the weight of nicotine must be used (and not the weight of the nicotine salt).

8. PROCEDURES

8.1. *Gas Chromatography*

Set up and operate the gas chromatograph, recorder and integrator or other suitable data handling equipment, and autosampler (if one is used) according to the manufacturer's instructions.

Ensure that the peaks for solvent, internal standard, nicotine and other smoke component peaks, especially neophytadiene, are well resolved.

Suitable conditions are:

Column temperature: 170 °C (isothermal)
Injection temperature: 250 °C
Detector temperature: 250 °C
Carrier Gas: nitrogen or helium at a flow rate of about 30 cm³ per minute
Injection volume: 2,0 µl

The total analysis time is about 6 min to 8 min.

Condition the system by injecting smoke extract prior to use.

8.2. *Calibration of the gas chromatograph*

Inject single or replicate aliquots of the standard solutions into the gas chromatograph. Record the peak areas of nicotine and the internal standard.

Calculate the ratio of the nicotine peak to the internal standard peak from the peak area data for each of the calibration solutions. Plot the graph of the nicotine concentrations according to the area ratios or calculate a linear regression equation (concentration of nicotine according to the area ratios) from these data. The graph should be linear and the regression line should pass through the origin. Use the slope of the regression equation.

8.3. *Calibration check*

The full calibration procedure should be carried out daily, ensuring a correlation coefficient of at least 0,99 is obtained. In addition, inject an aliquot of an intermediate standard after at least every 20 samples. If the value for this solution differs by more than 3% from the original calibration value, repeat the full calibration. Check the gas chromatograph, and repeat the entire analysis.

8.4. *Smoking and sample preparation*

- 8.4.1.** Using CORESTA Recommended Method N° 64: 2005 and CORESTA Recommended Method N° 65: 2005 set up the smoking machine, smoke the cigars and collect the particulate matter.

Extract using the solvent (20 ml) described in Section 6.3. The solutions should not be stored in daylight.

The measurement can be done immediately after shaking, or after one night storing (without shaking).

Note:

If measuring water, refer to CORESTA Recommended Method N° 67.

8.5. *Measurement and calculation of the nicotine content of samples.*

- 8.5.1.** Inject single or replicate aliquots of the smoke solutions into the gas chromatograph using the conditions described in Section 8.1. Record the peak areas of nicotine and the internal standard.

Calculate the mean value of the ratio of the peak area of nicotine to that of the internal standard for the replicate injections.

Using the calibration produced in Section 8.2 determine the concentration of nicotine in the smoke solutions as mg per ml. Ensure that the values lie within the range of the standards prepared in Section 7.

Deduce the amount in the cigars smoked. Express the test results in mg per cigar for each channel to the nearest 0,01 mg and a range of two values expressed in mg per cigar to the nearest 0,1 mg/cigar where the minimum is the average minus two times the standard deviation and the maximum is the average plus two times the standard deviation.

Also you can express the test results in mg/g for each channel to the nearest 0,01 mg/g, dividing the nicotine (in mg per cigar) by the cigar mass (in g per cigar) of the conditioned cigars selected for the smoking in each particular channel and a range of two values expressed in mg per g to the nearest 0,1 mg/g where the minimum is the average minus two times the standard deviation and the maximum is the average plus two times the standard deviation.

9. REPEATABILITY AND REPRODUCIBILITY

Collaborative Studies involving between 10 and 15 laboratories conducted in 2003/2004 show the following values for repeatability (r) and reproducibility (R) of this method.

The difference between two single results found on matched cigar samples by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability value (r) on average not more than once in 20 cases in the normal and correct operation of the method.

Single results on matched cigar samples reported by two laboratories will differ by more than the reproducibility (R) on average not more than once in 20 cases in the normal and correct operation of the method.

Table 1: Data analysis gave the estimates (expressed in mg/cigar) as summarised in the following table.

Product	weight g	diameter mm	length mm	nicotine mg/cigar	r mg/cigar	R mg/cigar	r %	R %
O	0,95	8,5	75,0	1,25	0,16	0,40	13	32
P	1,36	9,0	87,7	2,48	0,24	0,82	10	33
Q	1,60	9,1	99,0	2,86	0,24	0,64	8	22
K	2,11	11,1	88,0	2,82	0,34	1,18	12	42
M	3,25	14,6	95,0	3,06	0,52	1,49	17	49
R	5,10	13,7	129,5	6,49	1,16	2,90	18	45
N	6,60	15,5	127,0	4,30	0,86	1,95	20	45
S	9,60	17,0	164,0	3,72	1,00	2,72	27	73
T	11,27	17,5	162,0	11,23	3,37	13,83	30	123
C1	14,28	18,3	174,0	4,92	1,54	3,99	31	81

Table 2: Data analysis gave the estimates (expressed in mg/g) as summarised in the following table.

Product	weight g	diameter mm	length mm	nicotine mg/g	r mg/g	R mg/g	r %	R %
O	0,95	8,5	75,0	1,34	0,17	0,45	13	33
P	1,36	9,0	87,7	1,90	0,21	0,74	11	39
Q	1,60	9,1	99,0	1,88	0,17	0,44	9	24
K	2,11	11,1	88,0	1,37	0,21	0,65	15	47
M	3,25	14,6	95,0	0,96	0,18	0,48	18	50
R	5,10	13,7	129,5	1,36	0,30	0,71	22	53
N	6,60	15,5	127,0	0,71	0,14	0,36	20	50
S	9,60	17,0	164,0	0,40	0,11	0,31	27	77
T	11,27	17,5	162,0	1,04	0,34	1,20	32	116
C1	14,28	18,3	174,0	0,39	0,15	0,35	38	90

These findings confirm that the product variability, inherent to cigar production, is reflected in a wide range of smoke yields when cigars are machine-smoked.

For the purposes of calculating r and R, one test result (or one “single” result) was defined as the mean yield obtained from smoking 8 cigars, i.e.: 2 cigars on 4 channels, at two cigars per filter pad for Product O, P and Q. For Products K, M, R, N, S, T and C1 one “single” result equally was obtained from smoking 8 cigars, but at 1 cigar per filter pad.

The subject of tolerances due to sampling is dealt with in CORESTA Recommended Method N° 47: 2000.

10. TEST REPORT

The test report shall give the yield of nicotine per cigar (and/or in mg/g) smoked and the method used and include all conditions that may affect the result (e.g., atmospheric pressure during smoking). It shall also give all details necessary for the identification of the cigars smoked.

ANNEX

(Informative, this Annex does not form an integral part of the Recommended Method)

1. The described method is a reference method that might be altered for practical purposes or special laboratory conditions in several aspects especially in sections:
 4. Nicotine in smoke condensate may also be determined by the spectrophotometric method. In this case reference to the method shall be made in the expression of results.
 - 5.2. Column tubing material other than glass, such as deactivated stainless steel or nickel, may be used.
Capillary columns may be used. If these are used, it is necessary to ensure that the peaks due to nicotine and the internal standard are well resolved from peaks due to other smoke components and the solvent.
 - 6.4. Other stationary phases such as 2% Versamid 900 plus 1% potassium hydroxide or 7% Carbowax 20M plus 3% Polyphenylether (6 ring) plus 2% potassium hydroxide or lower loadings of Carbowax 20M (with or without potassium hydroxide) may be used.
2. This method can be used in conjunction with CORESTA Recommended Method N° 67 - Determination of water in the mainstream smoke of cigars by gas chromatographic analysis. This may be done by:
 - (i) including the appropriate level of the internal standard required for the water determination in the solvent described in Section 6.3.
 - (ii) preferably using helium as a carrier gas.
 - (iii) injecting an aliquot of the smoke solution into a column for water analysis that is connected to a thermal conductivity detector as well as into the nicotine column and detector described in this method.

A simultaneous automated analysis of nicotine and water may be achieved by using a splitting system or an autosampler with two injection positions.