CORESTA RECOMMENDED METHOD N° 7

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

(August 1991)

1. FIELD OF APPLICATION

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

2. DEFINITION

Particulate matter is that part of mainstream smoke which is collected by a smoke trap conforming to CORESTA Recommended Method N° 22 or ISO 3308.

3. REFERENCES

CORESTA Recommended Method N° 5 : 1982
Determination of carbon monoxide in the mainstream smoke of cigarettes by non-dispersive infrared analysis.

CORESTA Recommended Method N° 8 : 1991
Determination of water in the mainstream smoke of cigarettes by Gas Chromatographic Analysis.

CORESTA Recommended Method N° 22 : 1991
Routine analytical cigarette-smoking machine - Specifications, definitions and standard conditions.

CORESTA Recommended Method N° 23 : 1991
Determination of total and nicotine-free dry particulate matter using a routine analytical cigarette-smoking machine - Determination of total particulate matter and preparation for water and nicotine measurements.

CORESTA Recommended Method N° 24 : 1991
Cigarettes - Sampling.

ISO 10362-1: 1991
Cigarettes - Determination of water in smoke condensates - Part 1: Gas chromatographic method.

ISO 3308:1991
Cigarettes - Routine analytical cigarette-smoking machine - Definitions and standard conditions.

ISO 4387:1991
Cigarettes - Determination of total and nicotine- free dry particulate matter using a routine analytical cigarette smoking-machine.

ISO 8243: 1991
Cigarettes - Sampling.

4. PRINCIPLE

Cigarettes 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 cigarette.

5. APPARATUS

5.1. A standard smoking machine complying with CORESTA Recommended Method N° 22 or ISO 3308 and equipped for smoking.

5.2. A gas chromatograph equipped with a flame ionisation detector together with a recorder or an integrator. 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.

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 (minimum purity 99%).

6.3. Solvent for samples and standards : propan-2-ol containing 0.5 gram per litre of a heptadecane as internal standard.

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 (minimum purity 98%) for the preparation of standard solutions. Store at 0 °C to +4 °C and exclude light.

7. STANDARDS

Dissolve nicotine 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 (usually 0.02 mg per cm^3 to 2.00 mg per cm^3). Store at 0 °C to +4 °C and exclude light.

8. PROCEDURES

8.1. *Gas Chromatography*

Set up and operate the gas chromatograph, recorder or integrator 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 cm^3 per minute
Injection volume :	2 µl

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

8.2. *Calibration of the gas chromatograph*

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

Calculate the ratio of the nicotine peak to the internal standard peak from the peak area (or height) 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. In addition, inject an aliquot of an intermediate standard after every 20 samples. If the value for this solution differs by more than 3% from the original calibration value, repeat the full calibration.

8.4. *Smoking and sample preparation*

8.4.1. Using CORESTA Recommended Methods N° 22 and N° 23 or ISO 3308 and 4387 set up the smoking machine, smoke the cigarettes and collect the particulate matter.

Extract using the solvent (20 cm^3 or 50 cm^3) described in section 6.3. The solutions should not be stored in daylight.

Note 1 - If measuring water, refer to CORESTA Recommended Method N° 8.

Note 2 - If measuring carbon monoxide, refer to CORESTA Recommended Method N° 5.

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

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

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

8.5.2. Using the calibration produced in section 8.2 determine the concentration of nicotine in the smoke solutions as mg per cm^3. Ensure that the values lie within the range of the standards prepared in section 7.

Deduce the amount in the cigarettes smoked. Express the test results in mg per cigarette for each channel to the nearest 0.01 mg and the average per cigarette to the nearest 0.1 mg.

9. REPEATABILITY AND REPRODUCIBILITY

A major international collaborative study involving 30 laboratories and 6 samples conducted in 1990 shows that when cigarettes are smoked according to CORESTA Recommended Method N° 23 and the resulting smoke solutions are analysed by this method the following values for repeatability (r) and reproducibility (R) are obtained.

The difference between two single results found on matched cigarette 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 cigarette 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.

Data analysis gave the estimates as summarised in the following table:

Mean Yield of Nicotine mg	Repeatability Conditions r	Reproducibility Conditions R
0.091	0.040	0.069
0.179	0.046	0.069
0.326	0.050	0.076
0.673	0.077	0.109
0.835	0.079	0.142
1.412	0.107	0.195

For the purposes of calculating r and R, one test result was defined as the mean yield obtained from smoking 20 cigarettes in a single run.

For further details of the interaction of r and R with other factors see CORESTA Report 91/1.

The subject of tolerances due to sampling is dealt with in CORESTA Recommended Method N° 24.

10. TEST REPORT

The test report shall give the yield of nicotine per cigarette smoked and the method used and include all conditions which may affect the result (*e.g.*, atmospheric pressure during smoking). It shall also give all details necessary for the identification of the cigarettes smoked.

ANNEX

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

1. The described method is a reference method which might be altered for practical purposes or special laboratory conditions in several aspects especially in sections:

5.2. column tubing material other than glass, such as deactivated stainless steel or nickel, may be used.

6.2. quinaldine may be used as the internal standard.

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° 8: 1991 - Determination of water in the mainstream smoke of cigarettes 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 which 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.