Cooperation Centre for Scientific Research Relative to Tobacco

Cigar Smoking Methods Sub-Group

CORESTA Recommended Method No. 66

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

March 2020
CORESTA RECOMMENDED METHOD Nº 66

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

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<tr>
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CRM No. 66 – March 2020
CORESTA RECOMMENDED METHOD N° 66

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

(March 2020)

0. INTRODUCTION

This CORESTA Recommended Method (CRM) is part of a set of CRMs produced by the Cigar Smoking Methods Sub-Group which describes the machine smoking of cigars:

CRM N° 46 – Atmosphere for Conditioning and Testing Cigars of All Sizes and Shapes;
CRM N° 47 – Cigars – Sampling;
CRM N° 64 – Routine Analytical Cigar-Smoking Machine Specifications, Definitions and Standard Conditions;
CRM N° 65 – 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;
CRM N° 66 – Determination of Nicotine in the Mainstream Smoke of Cigars by Gas Chromatographic Analysis;
CRM N° 67 – Determination of Water in the Mainstream Smoke of Cigars by Gas Chromatographic Analysis;

This series of CRMs has been developed for use with machine smoking of cigar products. These CRMs are applicable to many commercially available products; however, deviations may be necessary to accommodate products not considered during the development of these CRMs.

1. FIELD OF APPLICATION

This method is applicable to the determination of nicotine in the mainstream smoke from cigars, generated and collected using a routine analytical smoking machine, by gas chromatography with flame ionization detection (GC-FID).

2. NORMATIVE REFERENCES

CORESTA Recommended Method N° 46, Atmosphere for Conditioning and Testing Cigars of All Sizes and Shapes
CORESTA Recommended Method N° 64, Routine Analytical Cigar-Smoking Machine Specifications, Definitions and Standard Conditions
CORESTA Recommended Method N° 65, 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
3. TERMS AND DEFINITIONS

For the purposes of this Recommended Method, the following definitions apply:

3.1 **Total Particulate Matter, Crude Smoke Condensate, TPM**
That portion of the mainstream smoke which is trapped on the glass fibre filter trap, expressed as milligrams per cigar.

3.2 **Dry Particulate Matter, Dry Smoke Condensate, DPM**
The total particulate matter after deduction of its water content, expressed as milligrams per cigar.

3.3 **Nicotine-free Dry Particulate Matter, Nicotine-free Dry Smoke Condensate, NFDPM**
The dry particulate matter after deduction of its water and nicotine content, expressed as milligrams per cigar.

4. PRINCIPLE

Cigars are conditioned according to CORESTA Recommended Method N° 46. Cigars are smoked using the procedure found in CORESTA Recommended Method N° 65 and the total particulate matter (TPM) of mainstream smoke is collected on a glass fibre filter trap. The TPM is dissolved in a solvent and the water content of this solution is determined by gas chromatography. Results are expressed as the weight of water delivered per cigar. 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 and equipped for smoking.

5.2 A gas chromatograph equipped with a flame ionisation detector, autosampler, and data handling software.

5.3 A packed or capillary column may be used for analysis. There are several suitable columns.

5.3.1 Packed column with a stationary phase consisting of 10 % poly(ethylene glycol) (PEG) 20 000 plus 2 % potassium hydroxide on an acid-washed silanized support material with a mesh of size of 100 (150 μm) to 80 (190 μm) and column dimensions consisting of an internal diameter between 2 mm and 4 mm and length of 1.5 m to 2 m have been shown to provide suitable results.

5.3.2 Non-polar poly siloxane capillary GC column[1]

5.4 The necessary general laboratory equipment for the preparation of samples, standards and reagents.

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[1] Agilent HP-5 GC column 30 m, 0.32 mm, 0.25 um 19091J-413 is the trade name of a suitable product available commercially. This information is given for the convenience of the users of this CORESTA Recommended Method and does not constitute endorsement of this product.
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 Gases: hydrogen, nitrogen or helium and compressed air necessary for operation of the gas chromatograph.

6.5 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 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 for a packed column 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: 2,0 µl

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

Condition the system by injecting smoke extract prior to use.

The instrumental conditions shown above are for the packed column. Alternative packed and capillary columns have been shown to provide suitable results for the determination of nicotine in TPM. If alternate columns are used, it is necessary to ensure that the nicotine and internal standard peaks are well resolved from the solvent peak and peaks due to other smoke components. Suitable operating conditions will depend on the type of column and injection system used.

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 5% 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 and CORESTA Recommended Method N° 65 set up the smoking machine, smoke the cigars and collect the TPM.

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 the average per cigar to the nearest 0.1 mg.

9. REPEATABILITY AND REPRODUCIBILITY

Collaborative Studies involving 9 laboratories conducted in 2018 found the following values for repeatability (r) and reproducibility (R) of this method.[2]

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.

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

<table>
<thead>
<tr>
<th>Product</th>
<th>weight (mg)</th>
<th>diameter (mm)</th>
<th>nicotine (mg/cigar)</th>
<th>r (mg/cigar)</th>
<th>R (mg/cigar)</th>
<th>r (%)</th>
<th>R (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2557</td>
<td>8.9</td>
<td>2.06</td>
<td>0.475</td>
<td>0.79</td>
<td>23.0%</td>
<td>38.3%</td>
</tr>
<tr>
<td>B</td>
<td>3287</td>
<td>9.6</td>
<td>2.11</td>
<td>0.778</td>
<td>1.44</td>
<td>36.8%</td>
<td>68.1%</td>
</tr>
<tr>
<td>C</td>
<td>4049</td>
<td>9.6</td>
<td>1.80</td>
<td>0.483</td>
<td>0.78</td>
<td>26.9%</td>
<td>43.2%</td>
</tr>
<tr>
<td>D</td>
<td>2754</td>
<td>10.4</td>
<td>2.36</td>
<td>0.754</td>
<td>1.19</td>
<td>32.0%</td>
<td>50.6%</td>
</tr>
<tr>
<td>E</td>
<td>2774</td>
<td>10.4</td>
<td>1.76</td>
<td>0.734</td>
<td>1.09</td>
<td>41.8%</td>
<td>62.0%</td>
</tr>
<tr>
<td>F</td>
<td>3026</td>
<td>~12</td>
<td>1.96</td>
<td>1.529</td>
<td>2.01</td>
<td>78.0%</td>
<td>102.5%</td>
</tr>
<tr>
<td>G</td>
<td>7721</td>
<td>14-15</td>
<td>2.01</td>
<td>0.831</td>
<td>1.5</td>
<td>41.4%</td>
<td>74.60%</td>
</tr>
<tr>
<td>H</td>
<td>8254</td>
<td>17</td>
<td>2.63</td>
<td>1.714</td>
<td>2.82</td>
<td>65.3%</td>
<td>107.4%</td>
</tr>
<tr>
<td>I</td>
<td>960</td>
<td>7.8</td>
<td>1.44</td>
<td>0.288</td>
<td>0.37</td>
<td>20.0%</td>
<td>25.7%</td>
</tr>
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</table>

These findings suggest that the product variability, inherent to cigar production, is reflected in the variability of the 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 yield obtained from smoking 1 cigar per filter pad for Products B through H. For Products A and I one “single” result was obtained from smoking 2 cigars per filter pad.

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

10. TEST REPORT

The test report shall give the water content from each cigar smoked and the method used, and shall include all conditions not defined in this Recommended Method that may affect the results (e.g. atmospheric conditions, cigar diameter, puff volume, etc.) as well as any deviations from this Recommended Method. The test report shall also give all details necessary for the identification of the cigars smoked.

11. BIBLIOGRAPHY

CORESTA Recommended Method N° 46, Atmosphere for conditioning and testing cigars of all shapes and sizes

CORESTA Recommended Method N° 47, Cigars - Sampling

CORESTA Recommended Method N° 64, Routine analytical cigar-smoking machine - specifications, definitions, and standard conditions

CORESTA Recommended Method N° 67, Determination of water in the mainstream smoke of cigars by gas chromatographic analysis

Cigar Smoking Methods Technical Report, 2018 Collaborative Study for CRM65 Update of Repeatability and Reproducibility [CSM-121-0-CTR]