

## CORESTA RECOMMENDED METHOD N° 9

### DETERMINATION OF NICOTINE IN CIGARETTE FILTERS BY GAS CHROMATOGRAPHIC ANALYSIS

(April 2009)

#### 0. INTRODUCTION

In 2001 the CORESTA Routine Analytical Chemistry Sub-Group was charged by the Scientific Commission to review and update CORESTA Recommended Method N° 9 (CRM 9) originally published 1989 to reflect current technology and to include r and R statistics. In 2003 the scope of this work was expanded to investigate the method's application to the analysis of filters containing charcoal.

#### 1. FIELD OF APPLICATION

The method has been found to be applicable to the analysis of nicotine in machine-smoked cigarette filters made from paper or cellulose acetate plasticized with triacetin or triethyleneglycol-diacetate. It is not recommended for use with charcoal filters (see Appendix) or for the analysis of the nicotine content of human-smoked cigarette filters. An alternative approach, based on the analysis of a mouth-end section of the smoked filter and machine smoked calibration, has been used by the CORESTA Smoking Behaviour Sub-Group to estimate human smoked yields of nicotine (mouth level exposure or yield in-use).

#### 2. REFERENCES

*CORESTA Recommended Method N° 7*

Determination of Nicotine in the Mainstream Smoke of Cigarettes by Gas Chromatographic Analysis.

*CORESTA Recommended Method N° 21*

Atmosphere for Conditioning and Testing Tobacco and Tobacco Products.

*CORESTA Recommended Method N° 22*

Routine Analytical Cigarette-Smoking Machine: Specifications, Definitions and Standard Conditions.

*CORESTA Recommended Method N° 23*

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° 25*

Ambient Air-Flow Around Cigarettes in Routine Analytical Smoking Machines: Control and Monitoring.

*CORESTA Recommended Method N° 39*

Determination of the Purity of Nicotine and Nicotine Salts by Gravimetric Analysis – Tungstosilicic Acid Method.

*ISO 3308:2000*

Routine analytical cigarette-smoking machine – Definitions and standard conditions.

*ISO 3402:1999*

Tobacco and tobacco products – Atmosphere for conditioning and testing.

*ISO 4387:2000*

Cigarettes – Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine.

*ISO 10315:2000*

Determination of nicotine in smoke condensate of cigarettes (gas chromatographic method).

*ISO 13276:1997*

Tobacco and tobacco products – Determination of nicotine purity – Gravimetric method using tungstosilicic acid.

### **3. PRINCIPLE**

Cigarettes are machine-smoked according to ISO 4387. After smoking, the filters are removed from the remaining unburned tobacco and ash, slit open and extracted with methanol containing an internal standard. The nicotine content of this solution is determined by gas chromatography using flame ionization detection. Results are expressed as the weight of nicotine per cigarette filter.

### **4. APPARATUS**

- 4.1** An analytical cigarette-smoking machine.
- 4.2** Gas-chromatograph, equipped with a flame ionization detector, data recorder and integrator or other suitable data handling equipment.
- 4.3** Column, capillary or mega-bore capable of fully resolving the peaks of the solvent, internal standard, nicotine and other tobacco components. Typically, this would be a high polarity fused silica capillary column, *e.g.* 15 m x 0.53 mm I.D. x 1 µm with a polyethylene glycol stationary phase.
- 4.4** Shaker, orbital (capable of about 250 – 275 rpm) or wrist action.
- 4.5** The necessary general laboratory equipment for the preparation of samples, standards

and reagents.

## 5. REAGENTS

- 5.1** Methanol (analytical grade)
- 5.2** *n*-Heptadecane (minimum purity 99%)
- 5.3** Nicotine of known purity (minimum 98%) verified in accordance with ISO 13276 or CRM 39 for the preparation of standard solutions. Store in a tightly closed amber container in a refrigerator.
- Note:** Nicotine salicylate of known purity and verified in accordance with ISO 13276 or CRM 39 may also be used.
- 5.4** Extraction solvent for samples and standards
- Methanol (5.1) containing an appropriate concentration of internal standard (5.2); this is normally in the range of 0.2 mg/ml to 0.5 mg/ml.
- For example:
- Extraction Solvent – Stock Solution Preparation*  
Accurately weigh (to the nearest 0.1 mg) 5 g of *n*-heptadecane into a 50 ml volumetric flask and dilute to volume with methanol. Resulting concentration: 100 mg/ml.
- Extraction Solvent - Working Solution Preparation*  
Add 20 ml of the *n*-heptadecane stock solution to 2.5 l methanol in a 5 l volumetric flask with stirring, and dilute to volume with methanol.  
Resulting concentration: 0.4 mg/ml
- 5.5** Gases : hydrogen, nitrogen or helium and compressed air necessary for operating of the gas chromatograph

## 6. STANDARDS

Dissolve the nicotine in the extraction solvent working solution, described in section 5.4, 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/ml to 1.00 mg/ml). Store these solutions in a cool, dry place and protect from light.

## 7. PROCEDURE

- 7.1** Set up the apparatus and operate the gas chromatograph (4.2) in accordance with the manufacturer's instructions. Ensure that the peaks for solvent, internal standard, nicotine and other smoke component peaks are well resolved.

The following chromatographic conditions are suitable with the column type described in section 4.3:

Oven Program	
Column oven initial temperature	120 °C
Column oven initial time	1 minute
Column oven program rate	10 °C/minute
Column oven final temperature	230 °C
Column oven final time	5 minutes

- Injection temperature: 250 °C
- Detector temperature: 250 °C
- Carrier gas: nitrogen or helium at a flow rate of about 14 ml/minute
- Injection volume: 1 µl

Using the above conditions, the total analysis time is about 17 minutes.

## 7.2 Calibration of the gas chromatograph

Inject replicate aliquots of the calibration solutions (6) into the gas chromatograph. Record the peak areas of the nicotine and 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. Perform a linear least squares regression from these data. The graph should be linear and the regression line should theoretically pass through the origin.

Perform this full calibration procedure daily. In addition, inject an aliquot of an intermediate concentration standard after about every 20 samples. If the calculated concentration of this solution differs by more than 3 % from the target value, repeat the full calibration.

## 7.3 Smoking and sample preparation

Set up the smoking machine and smoke the cigarettes according to ISO 3308 and 4387. Immediately after smoking, remove the ash and all remaining tobacco from the filters; slit open the filters lengthwise and place them into an Erlenmeyer flask or other suitable container containing extraction solvent (5.4). Typically 20 ml per 5 cigarette filters is an adequate volume to cover the filters. Shake the flask on an orbital or wrist action shaker for 30 minutes. Allow the extraction mixture to stand overnight, and then shake again for 30 minutes to ensure complete extraction of nicotine from the filters.

**Note 1:** It is recommended that the filters are placed into the extraction solution within 30 minutes after completion of smoking. If filters must be stored prior to extraction it is recommended that they are stored at between 0 °C and 4 °C and protected from light. Experiments have shown that results decline slightly (~ 4 to 5 %) when the filters are stored for four weeks before extraction.

**Note 2:** Should the estimation of nicotine retention be required then nicotine in the mainstream pads should be determined as in ISO 10315 or CRM N° 7.

#### 7.4 Measurement and calculation of the nicotine content of samples

7.4.1 Inject replicate aliquots of the extracted test portion into the gas chromatograph using the conditions described in section 7.1 Calculate the mean value of the ratio of the peak area of nicotine to that of the internal standard for replicate injections.

7.4.2 Using the calibration curve produced in section 7.2, determine the concentration of nicotine in the test portions as mg/ml. Ensure that the values lie within the calibration range. Calculate the nicotine content in mg per cigarette filter and report to the nearest 0.01 mg.

### 8. CALCULATION OF NICOTINE RETENTION

If there is a requirement to determine the filter nicotine retention then the following formula should be used:

$$R = \frac{100 \times F}{MS + F}$$

Where:

R = nicotine retention (%)

F = nicotine retained by the Filter

MS = nicotine in the mainstream smoke

**Note:** for the calculation, figures for F and MS should be used with two decimal places.

### 9. TEST REPORT

The test report shall give the content of nicotine in mg/filter and shall include all conditions that may affect the result (smoking method, butt length, puff volume etc.). If the nicotine retention is reported the methods (*e.g.* ISO 4387 and ISO 10315) for the determination of nicotine in mainstream smoke has to be stated.

### 10. REPEATABILITY AND REPRODUCIBILITY

An international collaborative study involving 11 laboratories and 2 test samples (one test cigarette produced by Japan Tobacco Inc., using filters provided by Filtrona Technical Centre, Jarrow, U.K., and the CM4 test piece) was conducted by the CORESTA Routine Analytical Chemistry Sub-group in 2006. Cigarette samples were smoked according to ISO 3308, 3402 and 4387 in this collaborative study. Results from the 11 laboratories were analyzed according to ISO 5725-2 to calculate the mean values, repeatability ( $S_r$ ) and reproducibility ( $S_R$ ) standard deviations, repeatability ( $r$ ) and reproducibility limits ( $R$ ) of this method.

The sample information for the international collaborative study is shown in the Table 1:

Table 1: Sample Information

Sample Name	CM4	Sample A
Filter Type	Acetate filter	Acetate filter
NFPDM	~ 14.0 mg/cig	~ 4.0 mg/cig
Filter Ventilation	No	Yes
Sample Type	CORESTA Monitor Test Piece	Test Cigarette

Data analysis gave the estimates as summarized in Tables 2 and 3 below.:

Table 2 : Means & Standard Deviations ( $S_r$  &  $S_R$ )

	Number of Labs	Nicotine Mean Value mg/filter	Repeatability Standard Deviation $S_r$	Reproducibility Standard Deviation $S_R$
CM4	11	0.835	0.024	0.095
Sample A	11	0.400	0.021	0.055

Table 3: Repeatability and Reproducibility Limits

	Number of Labs	Repeatability Limit $r$ mg/filter	Reproducibility Limit $R$ mg/filter
CM4	11	0.067	0.266
Sample A	11	0.059	0.154

## APPENDIX

Experiments by the Routine Analytical Chemistry Sub-Group found this method to be inappropriate for charcoal filters. These studies indicated that the use of the liquid solvent extraction as described in CRM 9 may result in incomplete recovery of nicotine from charcoal filters, possibly due to adsorption of the nicotine. The degree to which the recovery is affected is dependent on filter age and charcoal loading. These studies also indicated that *n*-heptadecane is adsorbed onto the charcoal.

The applicability of this method to filters containing other adsorbents or chemical additives is unproven.

A technical report summarizing this work and findings will be available during 2009 from the CORESTA Secretariat. Any attempt to use the method for filters outside the fields of application should be prefixed by experiments to determine its validity.