Cooperation Centre for Scientific Research Relative to Tobacco

Routine Analytical Chemistry
&
Tobacco and Tobacco Products Analytes Sub-Groups

CORESTA Recommended Method No. 87

DETERMINATION OF NICOTINE IN TOBACCO PRODUCTS BY GC–MS

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0. INTRODUCTION

In 2016, the CORESTA Smokeless Tobacco Sub-Group (STS), now named Tobacco and Tobacco Products Analytes Sub-Group (TTPA), and Routine Analytical Chemistry Sub-Group (RAC) conducted a collaborative study for the determination of nicotine in cigarette filler, cigar filler, ground cigars, and smokeless tobacco products (STP) using gas chromatography with mass spectrometric detection (GC-MS). Eighteen laboratories participated in the study. The method specified in this study was shown to be appropriate for the determination of nicotine in the aforementioned matrices. The repeatability and reproducibility values of this method have been assessed according to the recommendations in ISO 5725-2:1994 and are included.

1. FIELD OF APPLICATION

This Recommended Method is applicable to the determination of nicotine in cigarette filler, cigar filler, ground cigars (filler, wrapper, and binder), and smokeless tobacco products.

2. NORMATIVE REFERENCES

2.1 CORESTA Smokeless Tobacco Sub-Group (January 2010). *Smokeless Tobacco Glossary*

2.2 CORESTA Guide N° 11 - *Technical Guideline for Sample Handling of Smokeless Tobacco and Smokeless Tobacco Products*

2.3 ISO 3696, Water for analytical laboratory use – Specification and test methods

3. PRINCIPLE

The nicotine content of tobacco products is determined by extracting the tobacco with sodium hydroxide and methanol prior to gas chromatography/mass spectrometric (GC-MS) analysis in the selective ion monitoring (SIM) mode. The results are reported as milligrams of nicotine per gram of tobacco as is, wet weight.

4. APPARATUS

Normal laboratory apparatus is required, in particular, the following items:

4.1 Analytical balance, accurate to 0,0001 g.

4.2 Syringe (5 ml) and syringe filter, 0,45 µm nylon or equivalent.

4.3 Volumetric flasks of various capacities (25 ml and 50 ml are suitable)

Note: if standard solutions will be stored in the flasks, they should be amber or low-actinic glass.

4.4 Mechanical pipettes with disposable plastic tips 10 µl - 1000 µl.

4.5 GC column: A polar, base-deactivated, polyethylene glycol (PEG) column (30 m x 0,25 mm id x 0,25 µm df).

4.6 GC-MS system equipped with a computerized control and data acquisition and processing system. The system must be able to pilot the mass spectrometer in order to obtain chromatographic data under SIM data acquisition mode or equivalent. The GC must be configured to perform split injections on a capillary column. It is recommended to equip the GC with an auto-sampler for sample injection.

4.7 Glass 4,0 mm I.D. deactivated split liner with glass wool.

4.8 50-ml polypropylene centrifuge tube with screw-cap, or equivalent.

4.9 Orbital shaker or wrist action shaker.

4.10 Amber autosampler vials with PTFE-lined septa, or equivalent.

5. REAGENTS

All reagents must be of recognized analytical grade or better.

5.1 (-)-Nicotine [54-11-5] ≥ 99 % purity

5.2 Quinoline [91-22-5] ≥ 98 % purity

5.3 Water, complying with grade 2 of ISO 3696, or better

5.4 Methanol, HPLC grade or better

5.5 Sodium Hydroxide (NaOH), 2 mol/l solution or pellets (97 % purity)

WARNING — The use of this method can involve hazardous materials, operations and equipment. This method does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

6. PREPARATION OF SOLUTIONS

6.1 **2 mol/l Sodium Hydroxide:** If a 2 mol/l solution of sodium hydroxide must be prepared, the following is one example:

6.1.1 Add approximately 500 ml reagent water to a 1000 ml volumetric flask.

6.1.2 Carefully add 80 grams of solid sodium hydroxide pellets (≥ 97 % purity).

6.1.3 Mix carefully until dissolved.

6.1.4 Allow the solution to cool before bringing to volume with reagent water.

6.1.5 Close the flask and mix well.

7. STANDARDS

7.1 **Primary Nicotine Stock Solution:** Purchase or prepare a 50 mg/ml nicotine stock solution in methanol.

7.2 **Secondary Nicotine Stock Solution (1 mg/ml Nicotine)**: Transfer 1,0 ml of the primary analyte stock solution to a 50-ml volumetric flask. Dilute to volume with methanol and mix.

7.3 Internal Standard Stock Solution: Purchase or prepare a 50 mg/ml quinoline stock solution in methanol.

7.4 Working Internal Standard Solution (WISS, 4 mg/ml Quinoline): Transfer 2 ml of the Internal Standard Stock Solution to a 25-ml volumetric flask. Dilute to volume with methanol and mix.

7.5 Working Standards

Transfer the specified volumes of Secondary Nicotine Stock Solution (7.2) and Working Internal Standard Solution (7.4) according to the table below into 25-ml volumetric flasks. Bring to a final volume with methanol and mix.

7.6 Storage

All standard solutions should be stored in the refrigerator at approximately 4 ºC and have been shown to be stable for at least one month at these conditions.

Table 1 - Preparation of Working Calibration Standards

Calibration Standards	Volume of Secondary Nicotine Stock (ml)	Volume of WISS (ml)	Final Conc. of Nicotine (µg/ml)	Final Conc. of Quinoline (µg/ml)
0	0	0,250	0	40
1	0,100	0,250	4,0	40
2	0,200	0,250	8,0	40
3	0,500	0,250	20,0	40
4	1,00	0,250	40,0	40
5	3,00	0,250	120,0	40
6	5,00	0,250	200,0	40
7	10,00	0,250	400,0	40

Note: This calibration range is suitable for products ranging from 0,64 mg to 64 mg of nicotine per gram of tobacco (mg/g). Samples with lower or higher levels of nicotine may be analysed by extracting more or less tobacco to bring the samples within the calibration range.

8. SAMPLE PROCEDURE

8.1 Sample Handling

Due to the small sample aliquot size (0,25 g), samples must be ground and homogenized prior to removing aliquots for analysis. Refer to CORESTA Guide N° 11, *Technical Guideline for Sample Handling of Smokeless Tobacco and Smokeless Tobacco Products* for sample handling guidelines.

8.2 Sample Preparation

8.2.1 Weigh 0,25 g ± 0,05 g of the ground tobacco sample into a suitable extraction vessel (4.8). Record the weight to four decimal places. The recommended procedure for portioned products such as snus is to analyze the entire portion by cutting the pouch in half and adding the tobacco and pouch material to the extraction vessel.

8.2.2 Add 400 µl Working Internal Standard Solution (WISS, section 7.4) to each sample vial followed by 4 ml of 2 mol/l NaOH.

8.2.3 Let samples sit for approximately 30 minutes to allow the NaOH to completely wet the tobacco.

8.2.4 Add 40 ml methanol.

8.2.5 Shake samples on an orbital shaker (set to approximately 200 rpm) for approximately 30 minutes. Once sample extraction is complete, allow any solids to settle to the bottom of tubes (approximately 15 min.).

8.2.6 Decant the sample extract into a 5-ml disposable syringe fitted with a 0,45 µm syringe filter taking care not to add the tobacco to the syringe. Typically, 4 to 5 ml of sample extract is decanted. Filter the sample extracts into one or more labelled amber autosampler vials.

Note: For portioned products where grinding is not suitable or possible, the volume of extraction solution and internal standard may be increased proportionally or the sample may be diluted post-extraction using a dilution solution prepared by adding 400 µl of WISS to 40 ml of methanol and mixing well.

9. SAMPLE ANALYSIS

9.1 GC-MS Operating Conditions

Set up and operate the GC-MS system in accordance with the manufacturer's instructions. The following conditions are suitable for analysis:

9.1.1 Injection Parameters:

Mode: constant flow
Flow rate: 1,0 ml/min
Injection Mode: Split (60:1)
Inlet temp: 230 °C
Injection volume: 1 µl injection

9.1.2 Oven Temperature:

Initial 110 °C; hold for 1,0 min
Ramp 10 °C/min to 235 °C hold for 4,5 min
Run time: 18 min

9.1.3 Carrier Gas: Helium

9.1.4 Transfer Line Temperature: 230 ºC

9.1.5 MS Parameters:

MS Quad 150 ºC, MS Source 230 ºC
Solvent delay 5,00 min

Table 2 - MSD Quantitation/Qualifier Ions with Approximate Retention Times

Analyte	Retention time (min)	Quantitative Ion	Qualifier Ion
Quinoline	8,5	129	NA
Nicotine	7,8	84 or 162	162 or 84

9.2 System Suitability

The system performance must be evaluated for sensitivity, chromatographic performance, carry over and any other criteria necessary to ensure optimization of the GC-MS system.

9.3 Calibration of the GC-MS

Create an internal standard calibration method in the instrument operating software. A calibration curve is generated by calculating a linear regression of the area ratios of nicotine to quinoline (y) as a function of the concentration ratios of nicotine to quinoline (x). 1/x weighting is recommended.

9.4 Determination of the concentration of Nicotine

Inject each sample and calculate the area ratio of nicotine to quinoline for each sample and obtain the concentration ratio by comparing the area ratio with the calibration curve.

The amount of nicotine in the tobacco samples is quantified by the internal standard method. The concentration of nicotine in the samples is reported in µg/ml by the chromatography software. Examples of chromatograms are shown in Appendix 1.

9.5 Determination of the Nicotine Content of Samples

The concentration of nicotine expressed in milligrams per gram of tobacco is calculated with the formula below:

$$\text{Nicotine (mg/g)} = \frac{C}{M} \times \frac{Vol}{1000}$$

Where:

C = the concentration obtained from the calibration curve (µg/ml)
M = the mass of tobacco extracted (g)
Vol = the volume of methanol added to the sample (40 ml)
1000 = the conversion from µg to mg

Note: A correction factor does not need to be applied to account for the NaOH added; the internal standard corrects for this small dilution effect.

10. REPEATABILITY AND REPRODUCIBILITY

In 2016, an international collaborative study was conducted using cigarette filler, cigar filler, ground cigars (filler, wrapper, and binder) and smokeless tobacco products.[1] A statistical analysis of the results from 18 laboratories was conducted in accordance with ISO 5725-2:1994 and ISO/TR 22971:2005. After removal of outlying data, the final repeatability (r) and reproducibility (R) results were calculated. The r & R results are shown in Table 3.

[1] Routine Analytical Chemistry and Smokeless Tobacco Sub-Group Technical Report – 2016 Collaborative Study on Nicotine in Tobacco Products [RAC-STS-056-1-CTR] – published in February 2017.

Table 3 - Results of 2016 Collaborative Study for As-Is Nicotine (mg/g)

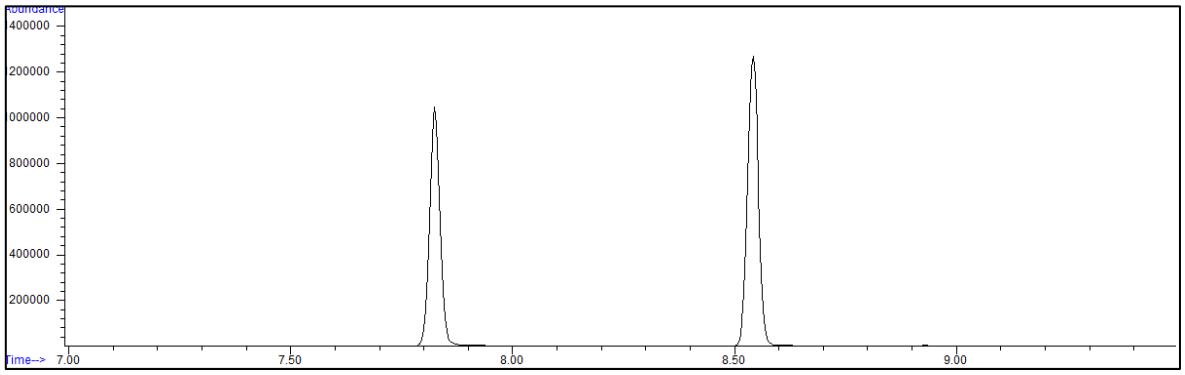
Product	No. of Labs *	Mean	Repeatability		Reproducibility	
			r	r (%)	R	R (%)
1R5F Ground Cigarette Filler	16	15,97	0,65	4,0 %	2,44	15,3 %
1R6F Ground Cigarette Filler	14	18,56	0,90	4,8 %	2,74	14,7 %
CM8 Ground Cigarette Filler	18	27,52	1,91	7,0 %	3,83	13,9 %
CRP1 Pouched Snus	16	10,36	1,40	13,5 %	3,18	30,7 %
CRP2 Loose Moist Snuff	15	12,98	0,93	7,2 %	2,09	16,1 %
CRP3 Loose Dry Snuff Powder	15	22,10	1,66	7,5 %	2,60	11,8 %
Flavoured Ground Cigar Filler	15	8,46	0,42	5,0 %	1,29	15,3 %
Dark Air-Cured Ground Cigar (Wrapper and Filler)	15	7,73	0,58	7,4 %	1,46	18,9 %
Mint MST	14	12,37	0,50	4,1 %	1,59	12,8 %

* The number of laboratory data sets after removal of outliers.

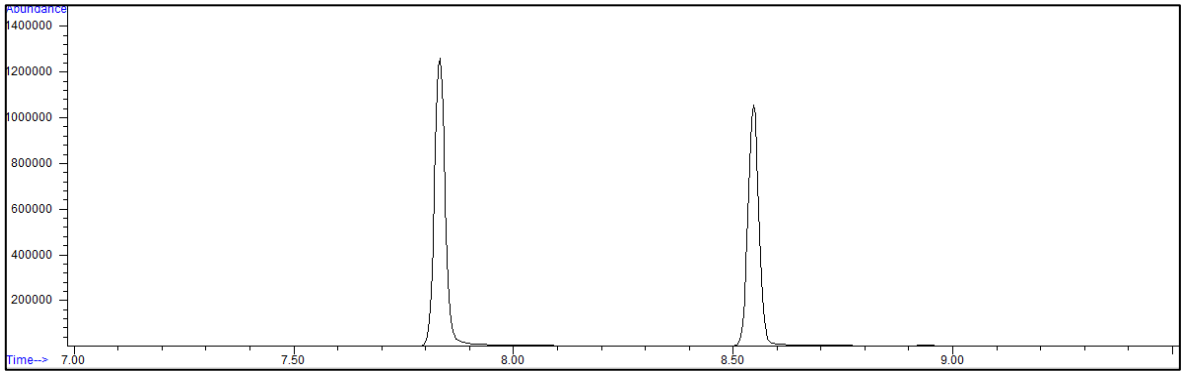
11. TEST REPORT

The test report shall state the amount of nicotine in mg per gram tobacco (wet weight) and shall include all conditions which may affect the result. The report shall also give all details necessary for the identification of each sample. As a reference basis for calculation (i.e. dry-weight basis) the following CORESTA Recommended Methods (CRMs) could potentially be used: CRM N° 56, 57 and 76.

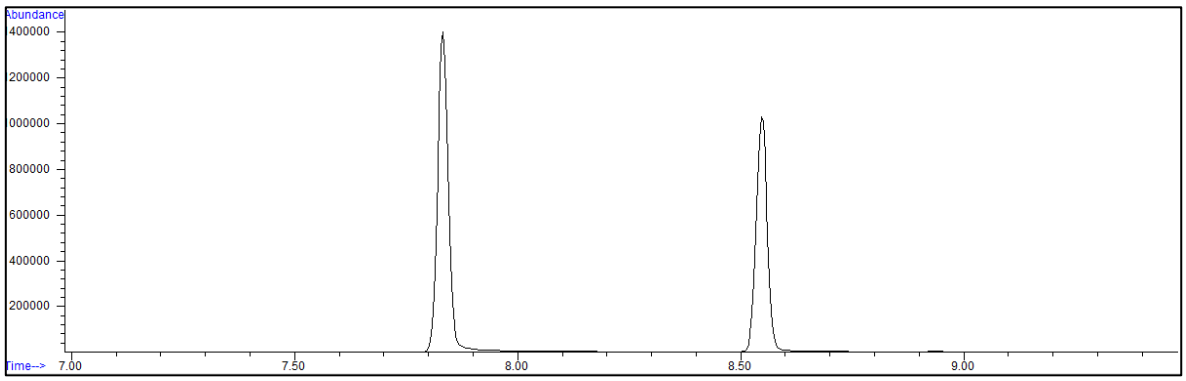
Appendix 1A - Example chromatogram of nicotine in calibration standard (100 µg/ml)



Appendix 1B - Example chromatogram of a Cigar sample extract



Appendix 1C - Example chromatogram of CRP1 sample extract



Appendix 1D - Example chromatogram of a US MST product (mint) extract