Cooperation Centre for Scientific Research Relative to Tobacco

Tobacco and Tobacco Products Analytes Sub-Group

CORESTA Recommended Method No. 62

DETERMINATION OF NICOTINE IN TOBACCO AND TOBACCO PRODUCTS BY GAS CHROMATOGRAPHIC ANALYSIS

December 2021

CORESTA RECOMMENDED METHOD Nº 62

Title:

DETERMINATION OF NICOTINE IN TOBACCO AND TOBACCO PRODUCTS BY GAS CHROMATOGRAPHIC ANALYSIS

Status: Valid

Note:

Document history:

Date of review	Information
February 2005	Version 1
April 2020	Version 2 – Extension of scope to include very low nicotine (VLN) content tobacco and tobacco products
December 2021	Version 3 – Extension of scope to include nicotine pouches

CORESTA RECOMMENDED METHOD N° 62

DETERMINATION OF NICOTINE IN TOBACCO AND TOBACCO PRODUCTS BY GAS CHROMATOGRAPHIC ANALYSIS

(December 2021**)**

0. INTRODUCTION

In 1999 to 2000, the Nicotine Sub-Committee of the Tobacco Science Research Conference Analytical Methods Committee (TSRC-AMC), the CORESTA Routine Analytical Chemistry (RAC) Sub-Group, and Deutsches Institut für Normung (DIN) coordinated an international collaborative study involving 37 laboratories to assess the repeatability and reproducibility of five methods for the determination of nicotine in 13 sample types including leaf, cigarette filler, smokeless tobacco products (STPs), cigar, and pipe tobacco. The intent of the study was to standardize a reference method for nicotine analysis in tobacco and tobacco products. Between 9 to 18 laboratories provided data using GC-FID based on n-hexane extraction using capillary column or packed-column, GC-FID based on methanol/ammonia extraction using capillary column, continuous flow analysis (CFA), and GC-FID based on MTBE extraction using capillary column that was a modified version of the CDC method published in the Federal Register [1].

The results for GC-FID MTBE and n-hexane extraction methods of this collaborative study were the basis for the original version of this CORESTA Recommended Method (CRM) [2]. During the development of this CRM, inter-laboratory tests were conducted on two different principles for the determination of the nicotine content of raw tobacco and tobacco taken from finished products:

- The gas-chromatographic procedure using MTBE as a solvent, and

- The gas-chromatographic procedure using n-hexane as a solvent.

In 2019, Tobacco and Tobacco Products Analytes (TTPA) Sub-Group conducted an inter-laboratory study for the determination of nicotine in a variety of traditional and experimental VLN tobacco products. These products included American blended cigarette fillers, a variety of smokeless tobacco products, and experimental very low nicotine (VLN) moist smokeless tobacco products, and VLN cigarette fillers. The intent of this study was to lower the calibration range for this Recommended Method. The collaborative study involved 25 laboratories; 16 labs provided data for CRM No. 62 MTBE and 11 labs provided data for CRM No. 62 hexane [3]. This Recommended Method has been shown to be fit for the analysis of the aforementioned matrices. The repeatability and reproducibility values of this method have been assessed in general accordance with ISO 5725-2:1994.

In 2021, the TTPA completed a collaborative study for the determination of nicotine in nicotine pouches. The intent of the study was to expand the scope of this Recommended Method to include nicotine pouches. Nicotine pouches do not contain tobacco leaf. Four commercial-like nicotine pouches were included, and fourteen laboratories participated in the study [4]. This Recommended Method has been shown to be fit for the analysis of nicotine pouches. The repeatability and reproducibility values of this method were assessed in general accordance with ISO 5725-2:1994.

1. FIELD OF APPLICATION

This Recommended Method is used to quantitatively determine the concentration of nicotine in traditional and very low nicotine (VLN) content tobacco and tobacco products using Gas Chromatography (GC) connected to a flame ionization detector (FID). The method is applicable to ground tobacco, cigarette filler, ground cigar filler, and smokeless tobacco products (e.g. snus, moist snuff, dry snuff, and chewing tobacco) and nicotine pouches. The calibration range specified in the method is from 0,0005 mg/ml to 0,8 mg/ml using MTBE as a solvent and 0,0006 mg/ml to 0,96 mg/ml using n-hexane as a solvent. This range corresponds to 0,025 mg/g to 40,0 mg/g using MTBE as a solvent and 0,024 mg/g to 38,4 mg/g using n-hexane as a solvent when 1 g of tobacco is extracted.

2. NORMATIVE REFERENCES

2.1 CORESTA Smokeless Tobacco Sub-Group - *Smokeless Tobacco Glossary*

2.2 CORESTA Guide No. 11 - *Technical Guideline for Sample Handling of Smokeless Tobacco and Smokeless Tobacco Products*

2.3 ISO 3696: *Water for analytical laboratory use – Specifications and test method*

3. PRINCIPLE

The nicotine content of a sample of tobacco or a tobacco product is determined by liquid/liquid extraction into an organic solvent containing an internal standard, followed by gas chromatographic (GC) analysis with flame ionization detection (FID).

4. APPARATUS

Normal laboratory apparatus and in particular, the following items:

4.1 Analytical balance, accurate to 0,0001 g

4.2 Volumetric pipettes of capacities 25 ml, 50 ml, and 100 ml

4.3 Volumetric dispensers of capacities 10 ml, 50 ml

4.4 Volumetric flasks, of capacities 50 ml, 100 ml, 250 ml and 2 l

4.5 Extraction vessels: Different styles may be utilized, including but not limited to: 100 ml Pyrex bottles (51,7 mm × 94,5 mm) with crimp seals and septa, 100 – 250 ml Erlenmeyer flasks with stoppers, and 25 mm × 200 mm culture tubes with teflon lined caps

4.6 Orbital shaker or wrist action shaker, or equivalent

4.7 GC column: A (5 % phenyl)-methylpolysiloxane (30 m × 0,25 mm I.D., 0,25 µm)[1] or a polar, base-deactivated, polyethylene glycol (PEG) column (30 m × 0,25 mm id × 0,25 µm df)[2]

[1] The following separation column has been found to provide acceptable performance: J&W HP-5, (30 m × 0.25 mm I.D., 0.25 µm film thickness (Catalog # 19091J-433, Agilent Technologies). This information is given for the convenience of users of this document and does not constitute an endorsement of this product.

[2] The following separation column has been found to provide acceptable performance: J&W DB-WAX, (30 m × 0.25 mm I.D., 0.25 µm film thickness (Catalog # 122-7032, Agilent Technologies). This information is given for the convenience of users of this document and does not constitute an endorsement of this product.

4.8 GC-FID system with data acquisition system and autosampler

4.9 Glass 4,0 mm I.D. deactivated split/splitless liner with glass wool[3]

4.10 Amber autosampler vials with PTFE lined septa

5. REAGENTS

All reagents must be of recognized analytical grade or better. Reagents specific to each analytical approach are identified as either *MTBE method* or *Hexane method*.

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

5.2 Nicotine salicylate ≥ 99 % purity, possible alternative to the use of Nicotine *(Hexane method)*

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

5.4 5N Sodium hydroxide solution**,** mass concentration (NaOH) = 200 g/L, *(MTBE method)*

5.5 8N Sodium hydroxide solution**,** mass concentration (NaOH) = 320 g/L, *(Hexane method)*

5.6 Methyl-t-butyl ether (MTBE), *(MTBE method)*

5.7 Quinoline [91-22-5] ≥ 98 % purity, internal standard *(MTBE method)*

5.8 n-hexane, with a maximum water content of 1,0 mg/ml, *(Hexane method)*

5.9 n-heptadecane or quinaldine (≥ 99 % purity), internal standard *(Hexane method)*

6. STANDARDS

All standards should be prepared in amber, or light protected glassware.

Prepare a series of at least five nicotine standard solutions whose concentrations cover the range expected to be found in the test portion, as in the example given in Tables 1-2. The standard solutions shall be made up fresh each time extraction solution is prepared. Transfer to autosampler vials and cap immediately.

Each laboratory shall establish the most suitable calibration range depending on the equipment used and the type of samples to be analysed. The standard preparation procedure is given as an example and is applicable for the range of the products described in the repeatability and reproducibility tables.

Solvents and solutions that are stored at low temperatures shall be allowed to equilibrate to room temperature before use. For each standard, calculate the exact concentration based on actual amount weighed.

6.1 MTBE method

6.1.1 Primary Internal Standard Stock Solution: Add 10 g of quinoline (5.7) to 0,01 g accuracy to a 250-ml volumetric flask. Dilute to volume with MTBE (5.6) and mix.

[3] The following liner has been found to provide acceptable performance: Topaz® Split/Splitless Straight Liner with glass wool (Catalog # 23300, Restek). This information is given for the convenience of users of this document and does not constitute an endorsement of this product

6.1.2 Extraction Solution (0,40 mg/ml): Transfer 20,0 ml of the Primary Internal Standard Stock Solution (6.1.1) to a 2-litre volumetric flask. Dilute to volume with MTBE (5.6) and mix.

Note: This solution will be used for preparation of the Primary Nicotine Stock Solution and Working Nicotine Standards.

6.1.3 Primary Nicotine Stock Solution: Purchase or prepare a 10 mg/ml nicotine stock solution in MTBE. Add 1 g of nicotine (5.1) to 0,0001 g accuracy to a 100-ml volumetric flask. Dilute to volume with Extraction Solution (6.1.2) and mix.

6.1.4 Working Nicotine Standards: Transfer the specified volumes of Primary Nicotine Stock Solution (6.1.3) according to the table below into 50-ml volumetric flasks. Bring to a final volume with Extraction Solution (6.1.2) and mix.

Table 1 - Preparation of Calibration Standards (MTBE Method)

Calibration Level	Volume of Primary Nicotine Stock (ml)	Final Conc. of Nicotine (mg/ml)	Final Conc. of Quinoline (mg/ml)
0[a]	0,0	0,000	0,4
1	0,0025	0,0005	0,4
2	0,005	0,001	0,4
3	0,025	0,005	0,4
4	0,25	0,05	0,4
5	0,5	0,1	0,4
6	1,0	0,2	0,4
7	2,0	0,4	0,4
8	3,0	0,6	0,4
9	4,0	0,8	0,4

[a] Use calibration level 0 as a solvent blank to evaluate carry over and system performance

6.1.5 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. Each laboratory should determine the shelf life of the standards and internal standards under their storage conditions. Solvents and solutions that are stored at low temperatures shall be allowed to equilibrate to room temperature before use.

6.2 Hexane method

6.2.1 Extraction Solution (0,50 mg/ml): Add 1 g of *n*-heptadecane or alternative internal standard (5.9) to 0,01 g accuracy to a 2-litre volumetric flask. Dilute to volume with n-hexane (5.8) and mix.

6.2.2 Primary Nicotine Stock Solution: Purchase or prepare a 2,4 mg/ml nicotine stock solution in n-hexane. Add 0,450 g of nicotine salicylate (5.2) or 0,240 g of nicotine (5.1) to 0,0001 g accuracy to a 200-ml Erlenmeyer flask. Add 50 ml of water (5.3) and mix to dissolve. Add 100 ml of Extraction Solution (6.2.1) and 25 ml of 8N sodium hydroxide solution (5.5). Shake the two-phase mixture vigorously for (60 ± 2) min using a shaker (4.6).

Note: Care should be taken to mix the phases well.

Transfer the supernatant (i.e. organic phase) to an amber bottle and store the Primary Nicotine Stock Solution at 4 °C.

6.2.3 Working Nicotine standards: Prepare at least five calibration standard concentrations. Transfer the specified volumes of Primary Nicotine Stock Solution (6.2.2) according to the table below into 20-ml volumetric flasks. Bring to a final volume with Extraction Solution (6.2.1).

Table 2 - Preparation of Calibration Standards (Hexane Method)

Calibration Level	Volume of Primary Nicotine Stock (ml)	Final Conc. of Nicotine (mg/ml)	Final Conc. of n-heptadecane (mg/ml)
0[a]	0,0	0,000	0,5
1	0,005	0,0006	0,5
2	0,010	0,0012	0,5
3	0,050	0,006	0,5
4	0,500	0,060	0,5
5	1,000	0,120	0,5
6	2,000	0,240	0,5
7	3,000	0,360	0,5
8	5,000	0,600	0,5
9	8,000	0,960	0,5

[a] Use calibration level 0 as a solvent blank to evaluate carry over and system performance

6.2.4 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. Each laboratory should determine the shelf life of the standards and internal standards under their storage conditions. Solvents and solutions that are stored at low temperatures shall be allowed to equilibrate to room temperature before use.

7. SAMPLE PROCEDURES

7.1 Sample Handling

Combine and mix sufficient tobacco to constitute at least 100 g for each test sub-sample. Refer to CORESTA Guide No. 11, *Technical Guideline for Sample Handling of Smokeless Tobacco and Smokeless Tobacco Products* for sample handling guidelines. Cut filler from cigarettes need not be reduced further in size.

7.2 Sample Preparation – MTBE Method

Note: Allow for adequate head space in the extraction vessel to increase extraction efficiency.

7.2.1 Loose tobacco: Weigh 1,000 g ± 0,020 g of the prepared tobacco sample into a suitable extraction vessel (4.5). Record the weight to the nearest 0,0001 g.

Portioned Products: 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. A sufficient number of portions should be used to come as close to the target weight as possible.

7.2.2 Add 7 ml of 5N NaOH solution (5.4) to each extraction vessel using a 10 ml volumetric dispenser (4.3), swirl to wet sample and allow to stand 15 minutes.

7.2.3 Add 50,0 ml of extraction solution (6.1.2) to the extraction vessel using a 50 ml volumetric dispenser (4.3).

7.2.4 Shake extraction vessels on a shaker (4.6) for two hours at a rate sufficient for vigorous mixing of the two phases.

7.2.5 Remove extraction vessels from shaker to allow the two phases to separate (approximately 15 min, maximum 24 h). Transfer an aliquot of the organic phase to one or more labelled amber autosampler vial(s) with PTFE lined cap.

7.2.6 If the sample needs to be stored, this is performed at 4°C to 8°C with exclusion of light.

7.3. Sample Preparation - Hexane Method

Note: Allow for adequate head space in the extraction vessel to increase extraction efficiency.

7.3.1 Loose tobacco: Depending on the expected nicotine content, weigh 1,000 to 2,000 g ± 0,050 g of the prepared tobacco sample into a suitable extraction vessel (4.5). Record the weight to the nearest 0,0001 g.

Portioned Products: 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. A sufficient number of portions should be used to come as close to the target weight as possible.

7.3.2 Add 20 ml of water (5.3), 40 ml of Extraction Solution (6.2.1), and 10 ml of 8N NaOH solution (5.5) to each extraction vessel.

7.3.3 Shake extraction vessels on a shaker (4.6) for one hour at a rate sufficient for vigorous mixing of the two phases.

7.3.4 Remove vessels from shaker to allow the two phases to separate (approximately 15 min, maximum 24 h). Transfer an aliquot of the organic phase to one or more labelled amber autosampler vial with PTFE lined cap. After phase separation transfer an aliquot of the supernatant organic phase to an autosampler vial for analysis.

7.3.5 If the sample needs to be stored, this is performed at 4 °C to 8 °C with exclusion of light.

8. SAMPLE ANALYSIS

8.1 Instrument Operating Conditions

Set up and operate the GC-FID system according to the manufacturer's instructions. Ensure that the peaks for solvent, internal standard, and nicotine are well resolved. Condition the system just prior to use by injecting two 1,0 µl aliquots of a sample solution or a nicotine standard as a primer followed by a solvent blank to evaluate carry over and system performance. The following conditions are suitable for analysis:

8.2 Gas Chromatography – MTBE Method

8.2.1 Injection Parameters

Mode: constant flow

Carrier Gas: Helium

Inlet Temp: 250 °C

Injection Mode:

- Splitless (recommended for very low nicotine content and traditional nicotine content tobacco)
- Split (20:1, ratio recommended for traditional nicotine content tobacco)

Injection volume: 1 µl injection

Flow rate: 1,7 ml/min

8.2.2 Oven Temperature

Initial 110 °C; hold for 0 min

Ramp 10 °C/min to 185 °C; hold for 0 min

Ramp 6 °C/min to 245 °C; hold for 10 min

Run time: 28,5 min

8.2.3 FID Parameters

Temp: 250 °C

Air and Hydrogen gas: See manufacturer's instructions

8.3 Gas Chromatography – Hexane Method

8.3.1 Injection Parameters

Mode: constant flow

Carrier Gas: Helium, Nitrogen, or Hydrogen

Inlet Temp: 270 °C

Injection Mode:

- Splitless (recommended for very low nicotine content tobacco and traditional nicotine content tobacco)
- Split (10:1, ratio recommended for traditional nicotine content tobacco)

Injection volume: 1 µl injection

Flow rate: 1,7 ml/min

8.3.2 Oven Temperature

Initial 170 °C

Run time: 10 min

8.3.3 FID Parameters

Temp: 270 °C

Air and Hydrogen gas: See manufacturer's instructions

8.4 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-FID system.

8.4.1 After installing a new column, condition the column by injecting a tobacco sample extract on the column, using the specified instrument conditions. Injections should be repeated until areas of IS and nicotine are reproducible. This will require approximately four injections.

8.4.2 Recondition the chromatographic column when the instrument has been used infrequently and after replacing the glass liner.

8.4.3 Hexane method Only: It is beneficial to purge high boiling point components from the column in between each large sample set. Typically, raising the temperature to 220 °C, for 30 minutes has been found to be sufficient.

8.4.4 When analyzing new tobacco products, extract product without IS to determine if any components co-elute with the IS. This interference could artificially lower the calculated values for nicotine.

8.5. Calibration of the gas chromatograph

8.5.1 Create an internal standard calibration method in the instrument operating software. A calibration curve is generated by calculating a linear regression model (y = mx + b) of the area ratios of nicotine to quinoline (y) as a function of the concentration ratios of nicotine to quinoline (x) (6.1.4 MTBE method or 6.2.3 Hexane method). Use both the slope (m) and the intercept (b) of the linear regression equation to process sample data. 1/x weighting is recommended.

Note: During the development of this method, a linear regression model using 1/x weighting was demonstrated to ensure that the low end of the calibration curve was not excessively biased by the high end of the calibration range. The user shall determine the level of weighting required in order to meet the acceptance criteria below.

8.5.2 Inspect the calibration model for the following acceptance criteria:

- The coefficient of determination (r^2) shall be greater than or equal to 0,99.
- Evaluate the difference between the measured and the true (expected) concentration of each calibration level used to create the linear regression model with the formula below:

$$\%\ Error = \frac{x_i - x'_i}{x_i} \times 100$$

where:

x'_i = Measured concentration of analyte at calibration level *i*

x_i = True (expected) concentration of analyte at calibration level *i*

- If the difference, % Error, for calibration level 1 is greater than 20 % or other calibration levels are greater than 10 % from the expected concentration (measured by linear regression model), the problem shall be investigated and corrected.
- The responses from the calibration levels used to create the linear regression model (calibration curve) shall bracket the responses from all test portions.

8.6 Determination of the nicotine concentration of samples

8.6.1 Inject an aliquot of each test portion and calculate the area (or height) ratio of the analyte to the internal standard response (y_t) for each sample.

8.6.2 Calculate the concentration of nicotine (C_t) for each test portion, expressed as milligrams per milliliter, using the (y_t) for each sample and coefficients of the linear regression equation in formula below:

$$C_t = (y_t - b)/m$$

where:

C_t = is the concentration of the test portion, in mg/ml from the calibration curve

y_t = is the peak area ratio: area of analyte per area of internal standard

b = is the regression y-intercept

m = is the regression slope

8.7 Expression of test sample results

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

$$\text{Nicotine (mg/g)} = \frac{C_t}{M} \times V$$

where:

C_t = is the concentration of the test portion, in milligrams per milliliter from the calibration curve

M = the mass of tobacco extracted (g)

V = the volume of extraction solvent added to the sample (50 ml MTBE, 40 ml Hexane)

9. REPEATABILITY AND REPRODUCIBILITY

In 1999, an international collaborative study was conducted including sample types of leaf, cigarette cut filler, pipe tobacco, loose leaf chewing tobacco, and moist snuff. Both capillary and packed columns were used in this study [2]. A statistical analysis of the results from 17 laboratories (MTBE method) and 14 laboratories (Hexane method) was conducted in accordance with ISO 5725 procedures to calculate repeatability and reproducibility values shown in Tables 3-4. The number of laboratories used to calculate the statistics after removal of outliers was not available. The values given in Tables 3 and 4 were originally presented on a dry weight basis both in Franke et al. (2001) and in CRM No. 62. For consistency with other CRMs, the dry weight values were converted to an as-is basis using the average of the two moistures shown in Table 1 of Franke et al. (2001).

Table 3 - Results of 1999 Collaborative Study for Nicotine (as-is basis) – MTBE

Product	Mean (mg/g)	Repeatability		Reproducibility	
		r	r (%)	R	R (%)
Loose leaf chewing tobacco	6,46	0,28	3,3 %	0,87	10,2 %
Oriental leaf	11,36	0,59	4,6 %	1,09	8,6 %
Cigar	11,51	0,31	2,3 %	1,29	9,7 %
Pipe tobacco	10,15	0,95	6,8 %	1,20	8,5 %
Menthol cigarette	15,97	0,36	2,0 %	1,43	7,7 %
Cigarette	16,31	0,56	3,0 %	1,57	8,3 %
1R4F cigarette	17,21	0,48	2,4 %	1,46	7,3 %
Moist snuff wintergreen	10,59	0,34	1,5 %	2,10	9,2 %
Moist snuff 1	11,70	0,67	2,6 %	2,35	9,1 %
Moist snuff long cut	13,16	0,36	1,3 %	2,55	9,0 %
Moist snuff 2	15,08	1,32	4,3 %	5,54	18,1 %
Bright leaf	31,07	0,84	2,4 %	2,41	6,8 %
Burley leaf	36,31	0,76	1,8 %	3,14	7,6 %

Table 4 - Results of 1999 Collaborative Study for Nicotine (as-is basis) – Hexane

Product	Mean (mg/g)	Repeatability		Reproducibility	
		r	r (%)	R	R (%)
Loose leaf chewing tobacco	6,69	0,62	7,0 %	1,29	14,6 %
Oriental leaf	11,36	1,43	11,2 %	2,13	16,8 %
Cigar	11,77	0,78	5,8 %	1,57	11,5 %
Pipe tobacco	10,22	1,18	8,3 %	1,79	12,6 %
Menthol cigarette	15,89	1,85	10,0 %	2,16	11,7 %
Cigarette	16,48	1,79	9,4 %	2,32	12,2 %
1R4F cigarette	17,38	1,04	5,1 %	2,60	12,9 %
Moist snuff wintergreen	10,69	1,12	4,8 %	2,94	12,7 %
Moist snuff 1	11,92	1,82	6,9 %	2,83	10,7 %
Moist snuff long cut	13,58	1,54	5,3 %	3,53	12,0 %
Moist snuff 2	15,48	1,60	5,1 %	4,06	12,9 %
Bright leaf	31,51	1,85	5,2 %	5,26	14,7 %
Burley leaf	36,39	2,21	5,4 %	6,38	15,5 %

The r and R were generated from the Hexane capillary method used in the 1999 collaborative study.

In 2019, the TTPA conducted a collaborative study involving 16 laboratories using the MTBE method and 11 laboratories using the n-hexane method in order to expand the scope of the Recommended Method beyond traditional nicotine content tobacco and tobacco products to include very low nicotine (VLN) content cigarette filler and moist smokeless tobacco [3]. In this study, the calibration ranges for MTBE and Hexane methods were expanded to bracket VLN and traditional tobacco nicotine concentrations in test portions. Generally, participants used a linear regression model with 1/x weighting to improve percent (%) Error at the low end of the calibration curve. Results were analyzed in basic conformance with ISO 5725-2:1994 and ISO/TR 22971:2005. The samples included in this study and the mean values, %r, and %R are shown in Tables 5-6.

Table 5 - Results of 2019 Collaborative Study for Nicotine (as-is basis) - MTBE

Product	Description	N*	Mean	Repeatability		Reproducibility	
			(mg/g)	r	r (%)	R	R (%)
NIST SRM 3222	VLNC Cigarette Tobacco Filler	14	0,152	0,029	18,8	0,057	37,7 %
VLNCF1	VLNC American blended cigarette filler - experimental prototype produced in limited quantities	13	0,391	0,034	8,8	0,083	21,1
VLNCMST	VLNC American-style loose moist snuff – experimental prototype produced in limited quantity	14	0,617	0,041	6,7	0,149	24,1
VLNCF2	VLNC American blended cigarette filler - experimental prototype produced in limited quantities	14	0,926	0,047	5,1	0,225	24,3
CRP1.1	Swedish-style Snus pouch	16	7,44	0,616	8,3	1,719	23,1
CRP4.1	American-style chopped loose-leaf chewing tobacco	16	8,72	0,446	5,1	1,361	15,6
CRP2.1	American-style loose moist snuff	15	10,47	0,599	5,7	1,608	15,4
RT6	Cigar filler, flavored, ground	14	10,91	0,366	3,4	1,125	10,3
RT8	Cigar filler, unflavored, ground	13	13,84	0,644	4,7	1,573	11,4
CRP3.1	American-style dry snuff powder	15	16,75	0,508	3.00	2,162	12,9
3R4F	3R4F Reference cigarette	15	17,92	0,666	3,7	2,607	14,5
RT1	1R6F Filler, American blended cigarette filler, ground	14	18,04	0,489	2,7	2,648	14,7

* The number of laboratory data sets after removal of outliers

Table 6 - Results of 2019 Collaborative Study for Nicotine (as-is basis) - Hexane

Product	Description	N*	Mean	Repeatability		Reproducibility	
			(mg/g)	r	r (%)	R	R (%)
NIST SRM 3222	VLNC Cigarette Tobacco Filler	8	0,156	0,03	19,4	0,075	47,8
VLNCF1	VLNC American blended cigarette filler - experimental prototype produced in limited quantities	10	0,396	0,036	9.0	0,083	21.0
VLNCMST	VLNC American-style loose moist snuff – experimental prototype produced in limited quantity	10	0,593	0,043	7,3	0,109	18,4
VLNCF2	VLNC American blended cigarette filler - experimental prototype produced in limited quantities	10	0,906	0,061	6,7	0,141	15,5
CRP1.1	Swedish-style Snus pouch	11	6,97	1,240	17,8	2,306	33,1
CRP4.1	American-style chopped loose-leaf chewing tobacco	11	8,71	0,212	2,4	1,289	14,8
CRP2.1	American-style loose moist snuff	11	10,09	0,361	3,6	2,684	26,6
RT6	Cigar filler, flavored, ground	9	10,84	0,371	3,4	2,041	18,8
RT8	Cigar filler, unflavored, ground	9	13,52	0,222	1,6	1,558	11,5
CRP3.1	American-style dry snuff powder	11	16,41	1,632	9,9	2,835	17,3
3R4F	3R4F Reference cigarette	10	17,37	1,890	10,9	2,826	16,3
RT1	1R6F Filler, American blended cigarette filler, ground	9	17,70	0,607	3,4	1,840	10,4

* The number of laboratory data sets after removal of outliers

In 2021, the TTPA completed a collaborative study in order to expand the scope of the Recommended Method to also include nicotine pouches [4]. The study included the analysis of four commercial-like nicotine pouches and fourteen laboratories participated using the MTBE method. Results were analyzed in basic conformance with ISO 5725-2:1994 and ISO/TR 22971:2005. The samples included in this study and the mean values, repeatability (r) and reproducibility (R) values are shown in Table 7.

Table 7 - Results of 2021 Collaborative Study for Nicotine (as-is basis) - MTBE

Product	Description	N*	Mean	Repeatability		Reproducibility	
			(mg/g)	r	r (%)	R	R (%)
NP1	Nicotine pouch (5 % moisture)	13	7,39	0,469	6,3 %	1,53	20,7 %
NP2	Nicotine pouch (40 % moisture)	13	13,24	0,759	5,7 %	2,59	19,5 %
NP3	Nicotine pouch (15 % moisture)	13	8,61	0,685	8,0 %	1,85	21,5 %
NP4	Nicotine pouch (32 % moisture)	13	6,60	0,441	6,7 %	2,02	30,6 %

* The number of laboratory data sets after removal of outliers

10. TEST REPORT

The test report shall state the amount of nicotine in mg per gram tobacco (wet weight). The test report shall also mention all operating conditions not specified in this Recommended Method, or conditions regarded as optional that may have affected the result. It shall also include all details required for the identification of the sample. Moisture content may be determined on separate tobacco aliquots if it is necessary to present the final results on a dry-weight basis. The determination of moisture is detailed in CORESTA Recommended Method N° 76: Determination of Moisture Content (Oven Volatiles) of Tobacco and Tobacco Products [5].

11. BIBLIOGRAPHY

[1] Federal Register, v.64, no. 55, March 23, 1999.

[2] Franke, J.E., Bennett, C.B., Davis, R.E., Thomsen, H.V., Johnston, K.S., and Shanmugan, S.M.: Determination of Nicotine in Tobacco: Collaborative Study; Beitr. Tabakforsch 19 (2001), 251-265.

[3] CORESTA Tobacco and Tobacco Products Analytes Sub-Group Technical Report: 2019 Collaborative Study for the Determination of Nicotine in Tobacco and Tobacco Products, April 2020.

[4] CORESTA Tobacco and Tobacco Products Analytes Sub-Group Technical Report: 2021 Nicotine Pouches Collaborative Study, October 2021

[5] CORESTA Recommended Method No. 76: Determination of Moisture Content (Oven Volatiles) of Tobacco and Tobacco Products.