



**Cooperation Centre for Scientific Research
Relative to Tobacco**

**Tobacco and Tobacco Products Analytes
Sub-Group**

**CORESTA Recommended Method
No. 79**

**DETERMINATION OF AMMONIA IN
TOBACCO
AND TOBACCO PRODUCTS BY ION
CHROMATOGRAPHIC ANALYSIS**

March 2018



CORESTA RECOMMENDED METHOD N° 79

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Status: Valid

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April 2015	Version 1
March 2018	Version 2 - Extension of the scope to include ground cigars

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DETERMINATION OF AMMONIA IN TOBACCO AND TOBACCO PRODUCTS BY ION CHROMATOGRAPHIC ANALYSIS

(March 2018)

0. INTRODUCTION

In 2013, the CORESTA Smokeless Tobacco Sub-Group (STS), now named Tobacco and Tobacco Products Analytes Sub-Group (TTPA), conducted a collaborative study for the determination of ammonia in tobacco, cigarette filler, and smokeless tobacco products (STP). Eleven laboratories participated in the study. This study was the basis for this CORESTA Recommended Method (CRM) as initially implemented.

In 2017, the TTPA (TTPA-150-1-STR 2017) conducted a collaborative study with the purpose of expanding the scope of the Recommended Method to include ground cigars. The CORESTA Reference Products (CRPs) produced in 2016 were also included in this study. This Recommended Method has been shown to be fit for purpose for the analysis of different tobacco varieties and tobacco products including a range of smokeless tobacco products, cigarette filler, and ground cigars. The repeatability and reproducibility values of this method have been assessed according to ISO 5725-2:1994.

1. FIELD OF APPLICATION

This Recommended Method is applicable to the determination of ammonia (as ammonium ion) in tobacco, cigarette filler, smokeless tobacco products (e.g. moist snuff, snus, chewing tobacco, and dry snuff), and ground cigars (wrapper, binder, and filler). The ammonia calibration range specified in the method is from 0,100 µg/ml to 10,0 µg/ml. This range correlates to 10 µg/g to 1000 µg/g ammonia in tobacco as is, wet weight. Samples with higher levels of ammonia may be analysed by dilution prior to analysis to bring the samples within the calibration range.

2. NORMATIVE REFERENCES

- 2.1 CORESTA Smokeless Tobacco Sub-Group. *Smokeless Tobacco Glossary*
- 2.2 CORESTA Guide N° 11 - *Technical Guideline for Sample Handling of Smokeless Tobacco and Smokeless Tobacco Products*

3. PRINCIPLE

The ammonia content of tobacco or tobacco products is determined by extraction of the tobacco sample into a sulfuric acid solution. Ion chromatographic analysis is used to separate ammonium ion from other cationic species. The response of ammonium ion is measured using a conductivity detector and is quantified against an external standard calibration. Results are reported as ammonia in micrograms per gram tobacco (wet weight).

4. APPARATUS

NOTE: Polypropylene volumetric flasks, sample flasks, and storage containers should be used to minimize sodium originating from borosilicate glassware.

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

- 4.1 Analytical balance (0,0001 g resolution)
- 4.2 Syringe filter, 0,45 µm nylon, or equivalent
- 4.3 Volumetric flasks of capacities 100 ml, 250 ml and 1000 ml
- 4.4 Mechanical pipettes with disposable plastic tips 10 µl - 1000 µl
- 4.5 Laboratory shaker
- 4.6 Polypropylene sample extraction vessels (with caps) of approximately 100 ml volume
- 4.7 Weak cation exchange column of mid-capacity, (250 mm × 4 mm, nonmetallic), approximately 2,8 milliequivalents per column with matching guard column
NOTE: Thermo Scientific IonPac® CS12A, or equivalent¹. Other column(s) may be suitable for use with this method; however, laboratories must verify that sodium is sufficiently resolved from ammonium in the test samples before use.
- 4.8 Ion Chromatograph (IC) consisting of a conductivity detector, conductivity suppressor and data collection system. An eluent degassing unit is recommended.

5. REAGENTS

Use only reagents of recognized analytical grade.

- 5.1 Ammonium Sulphate ((NH₄)₂SO₄) > 99 % purity (25,78 % ammonia)
(Alternatively a certified solution of 1000 µg/ml ammonia may be used)
- 5.2 Sulphuric Acid (H₂SO₄) > 96 % purity
- 5.3 Methanesulphonic acid (MSA) > 99 % purity
(Alternatively a Methanesulphonic Acid Eluent Generator Cartridge,)
- 5.4 Deionized water (resistivity ≥ 18,2 MΩ·cm)

6. PREPARATION OF SOLUTIONS

6.1 Sulfuric Acid, 0,025N (0,0125 mol/liter) (Standards and Extraction Solution)

Carefully add 1,277 g of H₂SO₄ to approximately 600 ml of deionised water in a 1000 ml polypropylene volumetric flask. Mix and dilute to 1000 ml with deionised water.

¹ ThermoFisher IonPac CS12A cation exchange analytical column 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. Other IC columns, such as the IonPac CS16A, may provide superior resolution between sodium and ammonia as compared to the ThermoFisher IonPac CS12A column.

6.2 Mobile Phase A: MSA 10 mM (Ion Chromatography Eluent A)

Carefully add 650 µl of methanesulphonic acid to approximately 600 ml of deionised water in a 1000 ml polypropylene volumetric flask. Mix and dilute to 1000 ml with deionised water.

6.3 Mobile Phase B: MSA 40 mM (Ion Chromatography Eluent B)

Carefully add 2,60 ml of methanesulphonic acid to approximately 600 ml of deionised water in a 1000 ml polypropylene volumetric flask. Mix and dilute to 1000 ml with deionised water.

Alternatively, the methanesulphonic acid can be prepared *in-situ* by an Ion Chromatograph fitted with an eluent generator.

7. STANDARDS

Prepare a series of seven ammonia standard solutions as described below. A minimum of seven standards should be used because the calibration curve is quadratic. Quantitation is obtained from an external standard calibration using the peak area response of ammonium sulphate as ammonia. All calculations are based on the ammonia molar mass. The stock and working standard solutions are stable for approximately 30 days when stored at 4 °C.

7.1 Ammonia Stock Solution = 100 µg/ml

Weigh approximately 0,097 g of ammonium sulphate into a 250 ml polypropylene volumetric flask. Record the exact weight in order to accurately calculate the actual concentration. Add 0,025 N H₂SO₄ and mix thoroughly.

7.2 Working Standards

Accurately pipette the specified volumes of Ammonia Stock Solution (7.1) according to the table below into 100 ml polypropylene volumetric flasks, containing approximately 50 ml of 0,025 N H₂SO₄. Bring to a final volume with 0,025 N H₂SO₄.

Table 1 - Preparation of Working Standards

Calibration Standard #	Volume to pipette (ml)	Working standard concentration (µg/ml)
1	0,100	0,100
2	0,250	0,250
3	0,500	0,500
4	1,00	1,00
5	2,50	2,50
6	5,00	5,00
7	10,0	10,0

8. SAMPLE PROCEDURE

8.1 Sample Handling

Mill the tobacco sample to a mesh size < 1 mm. Samples that do not grind well due to a high moisture content can be cryoground using liquid nitrogen.

8.2 Sample Preparation

8.2.1 Weigh approximately 0,500 g \pm 0,050 g of the tobacco sample into a suitable polypropylene extraction vessel and add 50,0 ml of the extraction solution (6.1.).

The recommended procedure for portioned products is to analyze unit pouches by cutting the pouch in half and adding the tobacco and pouch material to the extraction vessel. For portioned products it may be necessary to adjust the volume of extraction solution to keep the ratio of tobacco to extraction solution the same as loose tobacco products.

8.2.2 Place the extraction vessel on a laboratory shaker and shake at a moderate speed for 60 minutes.

8.2.3 Take an aliquot and filter through a 0,45 μ m syringe filter and proceed to analysis by ion chromatography. Samples may be centrifuged to remove particulate matter instead of using filtration.

NOTE: Depending on the ammonia content of the tobacco sample, the extract may require dilution in order to obtain a chromatographic response covered by the calibration range. If sample dilution is required, the sample should be diluted with 0,025 N Sulfuric Acid (6.1). A dilution factor of 10 is sufficient for most samples.

8.2.4 The extracts should be analyzed as soon as possible. Sample stability under storage and analysis conditions should be evaluated by each laboratory; however, samples have been shown to be stable for 72 h when stored at 4 °C \pm 2 °C.

NOTE: The use of a refrigerated auto-sampler has been shown to extend sample stability during analysis.

9. SAMPLE ANALYSIS

9.1 Example Ion Chromatography Parameters

Set up the ion chromatograph according to the manufacturer's instructions. Detection of ammonia, as ammonium, is achieved using a suppressed conductivity detector in either the external water mode or when using the MSA eluent generator cartridge in the recycled mode. This method of detection reduces the background conductivity of the mobile phase, thus increasing the sensitivity.

Quantitation is obtained from an external standard calibration using the peak area response of ammonium as ammonia. All calculations are based on the ammonia to ammonium sulphate molar mass ratio (molar mass ammonia/molar mass ammonium sulphate = 0,2578).

The operating conditions provided below are given as an example. Ensure that the sodium and ammonium peaks are well resolved with the instrumental parameters utilized or make adjustments accordingly:

- A 25 µl injection loop is recommended and injection volume of all samples is 25 µl.
- Gradient profiles are stated in Tables 2 and 3 for use with a manual system consisting of two eluents or an eluent generator, respectively:

Table 2 - Example Gradient Profile without an Eluent Generator

Time (min)	Eluent A 10 mM MSA (%)	Eluent B 40 mM MSA (%)	Gradient Profile	Flow Rate (ml/min)
0,0	100	0	linear	1,00
9,0	100	0	linear	1,00
9,5	0	100	linear	1,00
14,5	0	100	linear	1,00
15,0	100	0	linear	1,00

Table 3 - Example Gradient Profile when using Eluent Generator (Eluent A consists of Water)

Time (min)	Concentration of MSA (mM)	Gradient Profile	Flow Rate (ml/min)
0,0	10	linear	1,00
9,0	10	linear	1,00
9,5	40	linear	1,00
14,5	40	linear	1,00
15,0	10	linear	1,00

OTHER PARAMETERS (EXAMPLE)

Suppressor current = 88 mA

Auto-sampler tray temperature = 4 °C ± 2 °C

Column temperature = 30 °C

Pressure range: 200 psi (min) to 3000 psi (max)

Flush volume = 250 µl

Data Acquisition is throughout the period 0 min - 15 min

Optimise the IC conditions for analyte separation and sensitivity. Once these conditions are established, they should be used for the analysis of all standards and samples.

9.2 Calibration of the Ion Chromatograph

Inject an aliquot of each ammonia standard into the Ion Chromatograph. Record the analyte peak area. Plot a calibration curve of the peak area of ammonia versus the theoretical concentration in µg/ml.

The calibration curve is fitted by a quadratic function in keeping with weak base chemistry. The response obtained for all test samples should fall within the working range of the calibration curve.

9.3 Determination of the Ammonia Content of Samples

- Peak integration should be performed with a 'dropped baseline' instead of 'valley to valley' integration.
- Example chromatograms for a 0,5 µg/ml calibration standard, 3R4F cigarette filler and CRP2 are shown in Appendix 1.

9.4 The amount of ammonia is calculated in µg/g with the following equation:

$$\text{NH}_3(\mu\text{g} / \text{g}) = \frac{C \times V}{m} \times \text{dilution factor}$$

Where:

C = the concentration of ammonia, in µg/ml, obtained from the calibration curve

V = extraction volume (in ml)

m = mass of the sample (in g)

Dilution factor = factor as used (e.g.: 10 would be used if the sample was diluted 10-fold)

10. REPEATABILITY AND REPRODUCIBILITY

In 2013, an international collaborative study was conducted using ground tobacco, cigarette filler, and smokeless tobacco products². A statistical analysis of the results from eleven 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 4. All results are presented on an as is basis, without correction for moisture.

In June 2017 a study was conducted involving 14 laboratories to expand the scope of the recommended method to include ground cigars (wrapper, binder, and filler) and also to include r & R values for the CRPs produced in 2016.³ Results were analysed according to ISO 5752-2:1994 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 4. All results are presented on an as is basis, without correction for moisture. The value of 'N' is the number of the laboratories used to determine the statistics after the removal of outliers.

² Smokeless Tobacco Sub-Group Technical Report – Determination of Ammonia in Tobacco and Smokeless Tobacco Products, August 2014.

³ CORESTA Tobacco and Tobacco Products Sub-Group Technical Report – 2017 Collaborative Study on Ammonia and Benzo[a]pyrene in Tobacco Products, March 2018.

Table 4 - Results from 2014³ and 2017⁴ Collaborative Studies

Product	N ¹	Mean Ammonia (µg/g) ²	Repeatability		Reproducibility	
			r	r (%)	R	R (%)
CRP1 - Swedish- style snus pouch ³	11	951	113	11,9	529	55,6
CRP2 - American-style loose moist snuff ³	10	2581	103	4,0	1055	40,8
CRP3 - American-style loose dry snuff powder ³	10	4786	149	3,1	870	18,2
CRP4 - American-style loose-leaf chewing tobacco ³	10	2769	464	16,7	686	24,8
3R4F Reference Cigarette Filler ³	9	1081	100	9,3	373	34,5
Fine cut moist snuff ³	7	4067	149	3,7	1227	30,2
Virginia tobacco ³	7	446	30	6,7	136	30,5
Burley tobacco ³	8	2816	289	10,3	392	13,9
Oriental tobacco ³	7	362	43	11,8	52	14,4
Fire-cured tobacco ³	7	2978	50	1,7	348	11,7
Dark air-cured tobacco ³	8	540	33	6,1	128	23,6
CRP1.1 - Swedish- style snus pouch ⁴	12	982	63,6	6,5	687	69,9
CRP2.1 - American-style loose moist snuff ⁴	12	2400	161	6,7	1248	52,0
CRP3.1 - American-style loose dry snuff powder ⁴	12	7159	469	6,5	1647	23,0
CRP4.1 - American-style chopped loose-leaf chewing tobacco ⁴	12	2003	142	7,1	536	26,8
1R6F Ground Filler-RT1 - American blended cigarette filler ⁴	12	1005	85,1	8,5	260	25,8
Flavoured cigar (ground wrapper, binder and filler) ⁴	9	786	117	14,9	203	25,8
Traditional dark-air cured cigar (ground wrapper, binder and filler) ⁴	9	2813	262	9,3	1026	36,5

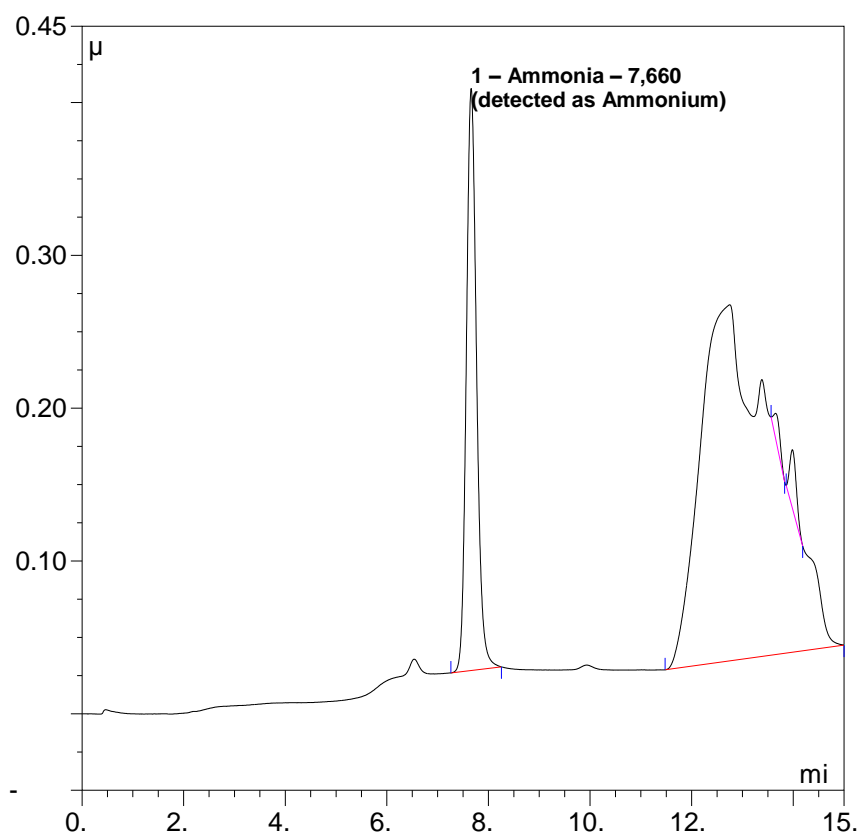
1. The number of laboratory data sets remaining after removal of outliers.
2. Results are presented on an as is basis, without correction for moisture.
3. Smokeless Tobacco Sub-Group Technical Report - Determination of Ammonia in Tobacco and Smokeless Tobacco Products, August 2014.
4. Tobacco and Tobacco Products Sub-Group Technical Report – 2017 Collaborative Study on Ammonia and Benzo[a]pyrene in Tobacco Products, March 2018.

11. TEST REPORT

The expression of the laboratory data depends on the purpose for which the data are required, and the level of laboratory precision. Any statistical analyses should be calculated and expressed before any rounding has taken place. The test report shall state the yield of ammonia in micrograms per gram tobacco (wet weight) and the method used shall include all conditions which may affect the result. The report shall also give all details necessary for the identification of each 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.

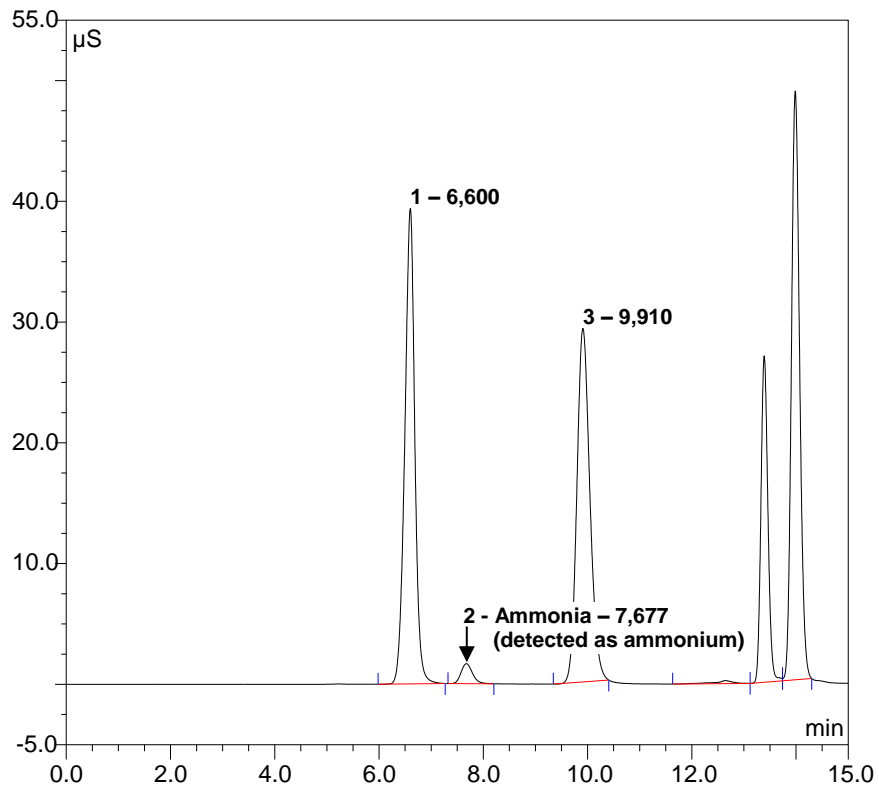
Appendix 1A

Example Chromatogram for a 0,5 $\mu\text{g/ml}$ Ammonia Calibration Standard



Appendix 1B

Example Chromatogram of 3R4F Cigarette Filler



Appendix 1C

Example Chromatogram of CORESTA Reference Product 2 (CRP2)

