Cooperation Centre for Scientific Research Relative to Tobacco

Tobacco and Tobacco Products Analytes Sub-Group

CORESTA Recommended Method No. 103

DETERMINATION OF NITRATE IN TOBACCO AND TOBACCO PRODUCTS BY ION CHROMATOGRAPHY

August 2023

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Status: Valid

Note: This document will be periodically reviewed by CORESTA

Document history:

Date of review	Information
August 2023	Version 1

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0. INTRODUCTION

In late 2021, the CORESTA Tobacco and Tobacco Products Analytes Sub-Group (TTPA) initiated a collaborative study for the determination of nitrate and nitrite in tobacco and tobacco products by ion chromatography [1]. A total of seven laboratories submitted results. The study included the analysis of four CORESTA reference smokeless tobacco products, 1R6F cigarette filler, two cigar fillers, and oriental tobacco. The results of the study demonstrate that the method is suitable for the determination of nitrate in tobacco and tobacco products. However, the results for nitrite were inconsistent and suggested either the laboratories needed further practice implementing the method or the method needed additional development for use as an international consensus standardized method. The nitrate results from this interlaboratory study are the basis for this CORESTA Recommended Method (CRM).

1. FIELD OF APPLICATION

This Recommended Method is used to quantitatively determine the concentration of nitrate in tobacco, cigarette filler, and a variety of smokeless tobaccos using ion chromatography. Results are reported in units of µg/g on an as-is basis. This method is applicable to samples with concentrations of nitrate in the range of 50 µg/g - 20000 µg/g.

2. NORMATIVE REFERENCES

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

3. PRINCIPLE

The nitrate in tobacco and tobacco products is determined by extracting a tobacco sample in Type 1 water. The extract is then analyzed by ion chromatography using suppressed conductivity detection. The results are reported as micrograms of analyte per gram of tobacco (µg/g).

4. EQUIPMENT AND APPARATUS

4.1 Ion Chromatograph (IC) consisting of an eluent generator, conductivity detector, conductivity suppressor, autosampler, and data collection system. An eluent degassing unit is recommended.

4.2 Weak anion exchange column of mid-capacity, (4 mm × 250 mm, nonmetallic), approximately 240 µeq per column with matching guard column[1].

4.3 Balance, 3-place, 0,001 g precision.

4.4 Orbital platform shaker.

4.5 Containers for sample extraction: 125-ml glass Erlenmeyer flask or similar flask of glass or polypropylene plastic for extracting samples.

4.6 1000 ml class A volumetric flask.

4.7 100 ml class A volumetric flask.

4.8 0,2 µm PVDF syringe filter, 25 mm.

5. REAGENTS

5.1 Sodium nitrate ($NaNO_3$) > 99 % purity

Note: A certified reference standards of 1000 µl/ml nitrate may also be used

5.2 Type 1 water

6. PREPARATION OF SOLUTIONS

6.1 Stock Solution:

Nitrate Stock Solution: Weigh 1,37 g of sodium nitrate and record the mass to the nearest 0,001 g. Add the reagent to a 1000 ml volumetric flask and dissolve in Type 1 water. This solution contains approximately 1000 µg/ml nitrate. Calculate the exact concentration. Store the prepared solution in a tightly sealed polypropylene bottle in the refrigerator at approximately 3 °C.

6.2 Calibration Standard Solutions:

Prepare a series of at least six calibration standard solutions using volumetric flasks whose concentrations cover the range expected to be found in the test portion. Dilute to volume using Type 1 water. An example calibration range is given in Table 1.

Table 1: Calibration Standards

Calibration Standards	Volume (ml)	Volume Nitrate Stock Solution (ml)	Nitrate Concentration (µg/ml)
Cal 1	100	0,100	1,00
Cal 2	100	0,500	5,00
Cal 3	100	2,00	20,0
Cal 4	100	10,0	100
Cal 5	100	25,0	250
Cal 6	100	40,0	400

[1] ThermoFisher IonPac® AS19 anion exchange analytical column is the trade name of a suitable product available commercially. Other column(s) may be suitable for use with this method; however, laboratories must verify suitable resolution in the test samples before use. This information is given for the convenience of the users of this Recommended Method and does not constitute endorsement of this product.

7. SAMPLE PREPARATION

7.1 Sample Requirements:

7.1.1 Sampling is conducted such that the laboratory test sample is representative of the sample received by the laboratory.

7.1.2 A homogeneous test portion shall be prepared for each test sample.

7.1.3 At least 2 grams of tobacco sample per replicate are required for this test method.

Note: Insufficient equilibration time for samples removed from the freezer has been identified as a source of variability. Samples removed from the freezer should be placed unopened in the refrigerator for a minimum of 24 hours to ensure water has sufficient time to fully equilibrate throughout the sample. At the time of analysis, samples should be allowed to equilibrate to room temperature before being opened for weighing.

7.2 Sample Grinding:

Tobacco and tobacco products shall be ground unless the samples are homogeneous and have a particle size <4 mm. It is important that the grinding procedure does not generate excessive heat or cause sample degradation. For further information, see CORESTA Guide No. 11.

7.2.1 Smokeless tobacco products supplied in the form of pouches shall be analyzed together with the pouch material and shall be cut into two halves and added directly into the extraction flask.

7.2.2 Cigarette filler does not need to be ground prior to analysis. Remove the filler from the cigarette paper and filter from a sufficient number of cigarettes to create a representative test sample.

7.2.3 Cigar filler typically needs to be ground prior to analysis. Testing may also involve the analysis of the entire cigar where the wrapper and filler are ground together. However, non-tobacco components such as filters must be removed prior to grinding. Grind a sufficient number of cigars to create a representative test sample.

7.3 Sample Extraction:

7.3.1 Mix the tobacco sample before aliquoting.

7.3.2 Weigh 2,0 g ± 0,3 g of tobacco sample with an analytical balance into a tared 125 ml Erlenmeyer flask (or similar). Record the exact weight of sample to the nearest 0,001 g.

7.3.3 When analyzing portioned smokeless tobacco products, select a unit number of pouches that comes closest to the target weight of 2,0 g. The pouch(es) shall be cut in half and the tobacco and pouch material shall be added to the extraction vessel. Record the exact weight of sample to the nearest 0,001 g.

7.3.4 Add 100,0 ml Type 1 water.

7.3.5 Cover the flask with a lid or equivalent.

7.3.6 Shake samples on an orbital platform shaker or equivalent device at 250 rpm for at least 30 ± 5 minutes. Ensure the shaking speed is sufficient to vigorously agitate the tobacco and for portioned smokeless products, the tobacco should become separated from the pouch material.

7.3.7 Filter approximately 1 ml of sample directly into 1,5 ml autosampler vial using a 0,2 µm PVDF syringe filter.

7.4 Sample Stability:

7.4.1 Sample extracts may be stored at room temperature or in a refrigerator.

Note 1: Stability should be determined by the laboratories using this Recommended Method under the conditions of use.

8. SAMPLE ANALYSIS

8.1 Instrument Setup:

The determination of nitrate is achieved using suppressed conductivity detection and a potassium hydroxide eluent generator in the recycled mode.

The instrument conditions specified below are recommendations and may be adjusted to obtain suitable chromatography. Set up the apparatus and operate the ion chromatograph in accordance with the manufacturer's instructions. Ensure that the analyte peaks and other peaks are well resolved.

Instrument operating conditions that have been found to be suitable for the specified column are as follows:

- Flow rate: 1,0 ml/min
- Column temperature: 30 °C
- Detector cell temperature: 35 °C
- Injection volume: 10 µl
- Suppressor current: 137 mA
- Flush volume: 500 µl
- Calibration type: linear with 1/X weighing and the y-intercept is not forced through zero
- Integration type: peak area
- Run time: 30 min

The gradient profile for IC is listed in the table below:

Table 2: IC Gradient Profile

Time (min)	Concentration of KOH (mM)	Eluent Generator Curve	Flow rate (ml/min)
0	10	5	1,0
12	10	5	1,0
25	55	5	1,0
26	10	5	1,0
30	10	5	1,0

Example chromatograms are provided in the Appendix.

8.2 Calibration:

Inject a 10 µl aliquot of each of the calibration solutions into the ion chromatograph. Generate a linear calibration curve with 1/X weighting without forcing regression through the origin.

Note: It is recommended to verify the calibration by injecting an aliquot of an intermediate concentration standard, which should be prepared from a separate stock, after calibration and after approximately every 20 test portions.

8.3 Determination of the concentration of nitrate in the test samples:

Inject a 10 µl aliquot of the Type 1 water used for sample extraction to evaluate for carryover or contamination. Inject 10 µl aliquots of each test portion into the ion chromatograph. Calculate the concentration of the analytes using the calibration curve.

8.4 Expression of results:

The concentration of nitrate (µg/ml) in the test portion is determined by the external standard calibration method using the linear regression equation derived from the calibration curve. Results are then converted and reported on an as-is basis using the formula below.

$$C_{nitrate}\ (\mu g/g) = \frac{c_{extract} \times v}{w}$$

Where:

$C_{nitrate}$ = the concentration of nitrate tobacco sample, on an as-is basis (µg/g)
$c_{extract}$ = the concentration of nitrate in the test portion, determined from the calibration curve (µg/ml)
v = the volume of the extraction solution, including any dilutions (ml)
w = the mass of the tobacco extracted (g)

9. REPEATABILITY AND REPRODUCIBILITY

In late 2021, the CORESTA TTPA Sub-Group initiated an international collaborative study involving 7 laboratories that used the specified test method [1]. The study included the analysis of four CORESTA reference smokeless tobacco products, 1R6F cigarette filler, two cigar fillers, and oriental tobacco. The repeatability (r) and reproducibility (R) values are shown in Table 3. The data were statistically evaluated in basic conformance with the recommendations of ISO 5725-5, using robust estimators of the within lab and between lab variability. The r & R results reflect both laboratory variability and product consistency.

Table 3: Repeatability (r) and Reproducibility (R) Results for Nitrate

Sample Name	Sample Description	N° of Labs[1]	Nitrate Mean (µg/g)	Repeatability[2]		Reproducibility[2]	
				r	% r	R	% R
CRP1.1	Swedish-style Snus	7	5894	298	5,1 %	719	12,2 %
CRP2.1	American-style loose moist snuff	7	17158	299	1,7 %	1411	8,2 %
CRP3.1	American-style dry snuff powder	7	38371	2733	7,1 %	2733	7,1 %
CRP4.1	American-style chopped loose-leaf chewing tobacco	7	7558	81,6	1,1 %	716	9,5 %
RT1	1R6F cigarette filler[3]	7	7046	146	2,1 %	255	3,6 %
RT3	Oriental tobacco[3]	7	483	30,8	6,4 %	61,5	12,7 %
RT6	Flavoured cigar filler[3]	7	18140	186	1,0 %	1119	6,2 %
RT8	Unflavoured cigar filler[3]	6	9021	138	1,5 %	497	5,5 %

1. The number of laboratory data sets included in the r & R calculations.
2. % r and % R reflect the corresponding variability value divided by the mean and expressed as a percent.
3. Samples were ground prior to distribution.

10. TEST REPORT

The test report shall state the amount of nitrate in micrograms per gram tobacco (wet weight) and shall include all conditions not specified in this Recommended Method which may affect the results. 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 No. 76: Determination of Moisture Content (Oven Volatiles) of Tobacco and Tobacco Products [2].

11. BIBLIOGRAPHY

[1] CORESTA Tobacco and Tobacco Products Analytes Sub-Group Technical Report: 2022 Collaborative Study for the Determination of Nitrate and Nitrite in Tobacco and Tobacco Products using Ion Chromatography – July 2023 [TTPA-319-1-CTR]

[2] CORESTA Guide No. 11: Technical Guideline for Sample Handling of Smokeless Tobacco and Smokeless Tobacco Products

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

APPENDIX – Example Chromatograms

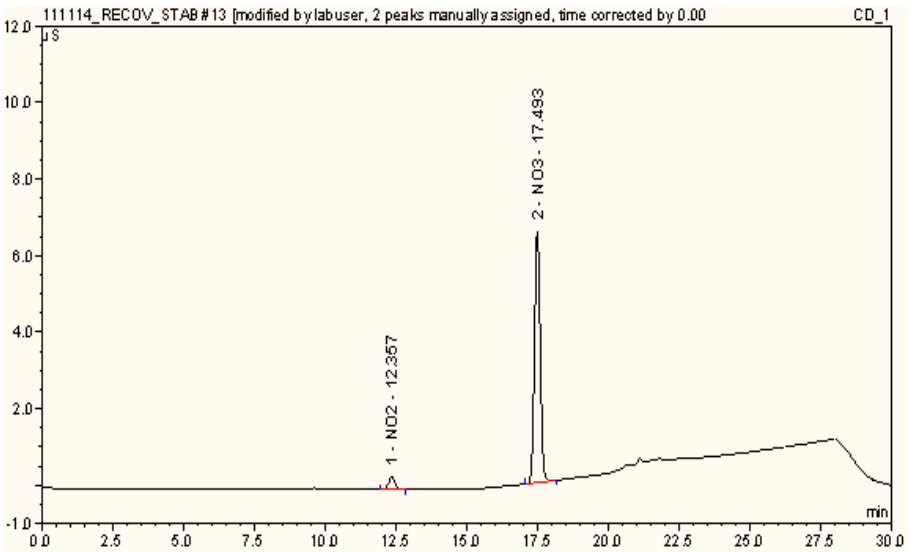

Figure 1: Chromatogram of a calibration standard

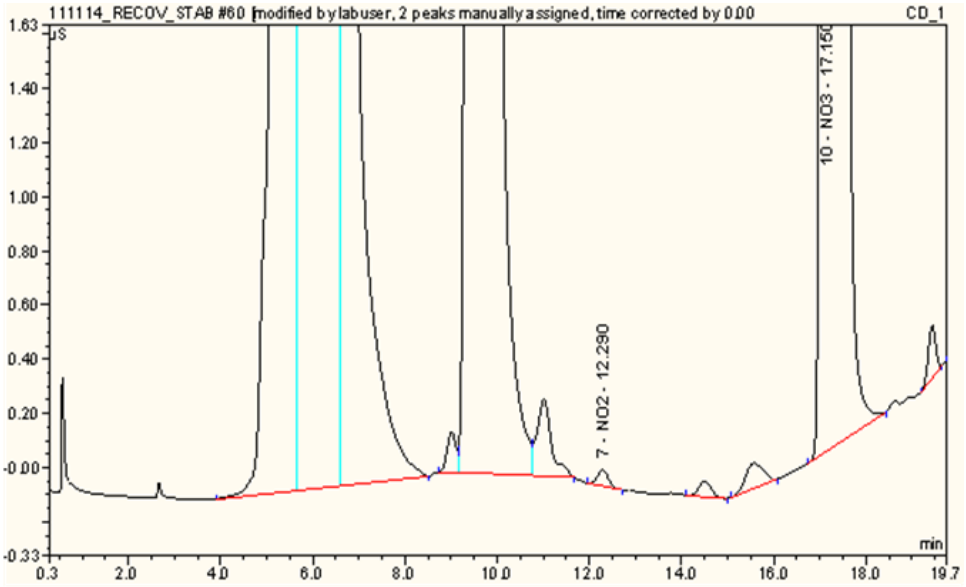

Figure 2: Chromatogram of a smokeless tobacco sample