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Tobacco and tobacco products — Determination of ammonia — Method using ion chromatographic analysis

Tabac et produits du tabac — Dosage de l'ammoniac — Méthode par chromatographie ionique

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 126, *Tobacco and tobacco products*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

In 2013, the CORESTA (Cooperation Centre for Scientific Research Relative to Tobacco) Smokeless Tobacco Sub-Group (STS) conducted a collaborative study for the determination of ammonia (as ammonium ion) in tobacco and smokeless tobacco products (STP). Eleven laboratories participated in the study. The method provides baseline resolution for ammonium and sodium, which is an important consideration for matrices with a high sodium content, such as moist smokeless tobacco. The method was shown to be appropriate for the determination of ammonia in tobacco, smokeless tobacco products, and cigarette filler. The repeatability and reproducibility values of this method were assessed in accordance with ISO 5725-2:1994 and ISO/TR 22971:2005.

Tobacco and tobacco products — Determination of ammonia — Method using ion chromatographic analysis

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1 Scope

This document specifies a method for the determination of ammonia in tobacco and tobacco products using ion chromatographic analysis.

It is applicable to tobacco, processed tobaccos, smokeless tobacco products and cigarette 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 1 000 µg/g ammonia in tobacco. Samples with higher levels of ammonia can be diluted prior to analysis to bring the samples within the calibration range.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at https://www.iso.org/obp
- IEC Electropedia: available at http://www.electropedia.org/

3.1
suppressor
device that reduces the background conductance of the eluent

Note 1 to entry: This can improve the signal-to-noise ratio.

3.2
portioned product
tobacco product that is pre-portioned into a serving size

EXAMPLE A snus pouch.

4 Principle

The ammonia content of tobacco or tobacco products, including smokeless tobacco products (STP) and cigarette filler, is determined by extraction 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 quantified against an external standard calibration. Results are reported as ammonia.

5 Apparatus

Usual laboratory apparatus and equipment for use in preparation of samples and standards, and in particular the following items. All labware shall be cleaned before use to avoid any contamination.

5.1 Analytical balance, capable of measuring to at least four decimal places (gram).

5.2 Syringe filter, 0,45 µm nylon, or equivalent.

5.3 Polypropylene volumetric flasks, of capacities 100 ml, 250 ml and 1 000 ml.

5.4 Mechanical pipettes with disposable plastic tips, 10 µl to 1 000 µl.

5.5 Laboratory shaker.

5.6 Polypropylene sample extraction vessels with caps, 100 ml approximate volume.

5.7 Weak cation exchange column of mid-capacity, (250 mm × 4 mm, non-metallic), approximately 2,8 milliequivalents per column.

EXAMPLE Thermo Scientific IonPac® CS12A[1], or equivalent.

Other column(s) might be suitable for use with this method; however, laboratories shall verify that sodium is sufficiently resolved from ammonium, in the test samples, before use.

5.8 Ion chromatograph (IC), consisting of a conductivity detector, suppressor and data collection system.

NOTE This method can be used without a suppressor; however, this will result in a reduced signal-to-noise ratio unless the eluent and chromatographic conditions are modified. As a consequence, it might not be possible to analyse samples with low levels of ammonia such as certain Virginia or Oriental tobaccos.

5.9 Eluent degassing unit, recommended.

6 Reagents

Use only reagents of recognized analytical grade.

6.1 Ammonium sulphate, [$(NH_4)_2SO_4$], >99 % purity (25,78 % ammonia); alternatively a certified solution of 1 000 µg/ml ammonia may be used.

6.2 Sulfuric acid, (H_2SO_4), >96 % purity.

6.3 Methanesulfonic acid (MSA), >99 % purity.

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[1] Thermo Scientific 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 document and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

7 Preparation

7.1 Preparation of labware

Labware shall be cleaned and dried in a manner which ensures that contamination does not occur.

It is important that all possible sources of contamination which might interfere with the analytical process are removed from the work area.

7.2 Preparation of solutions

7.2.1 Sulfuric acid, 0,012 5 mol/l (0,025 N), used for standards and extraction solution.

Carefully add 1,3 g of sulfuric acid to approximately 600 ml of deionised water in a 1 l polypropylene volumetric flask.

Mix and dilute to 1 l with deionised water.

7.2.2 Eluent A: 10 mM methanesulfonic acid (MSA).

Carefully add 650 µl of methanesulfonic acid (MSA) to approximately 600 ml of deionised water in a 1 l polypropylene volumetric flask.

Mix and dilute to 1 l with deionised water.

7.2.3 Eluent B: 40 mM methanesulfonic acid (MSA).

Carefully add 2,60 ml of methanesulfonic acid (MSA) to approximately 600 ml of deionised water in a 1 l polypropylene volumetric flask.

Mix and dilute to 1 l with deionised water.

7.3 Preparation of standards

7.3.1 Prepare a series of seven working calibration standard solutions as described in Table 1.

A minimum of seven standards are required because the calibration curve is quadratic.

NOTE 1 Quantitation is obtained from an external standard calibration using the peak area response of ammonium (NH_4^+) as ammonia (NH_3).

NOTE 2 All calculations are based on the ammonia molar mass.

NOTE 3 The stock and working standard solutions are stable for approximately 30 days when stored at 4 °C.

7.3.2 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 0,012 5 mol/l (0,025 N) sulfuric acid and mix thoroughly.

7.3.3 Working standards

Accurately pipette the specified volumes of ammonia stock solution according to Table 1 into 100 ml polypropylene volumetric flasks, containing approximately 50 ml of 0,012 5 mol/l (0,025 N) sulfuric acid.

Bring to a final volume with 0,012 5 mol/l (0,025 N) sulfuric acid.

Table 1 — Preparation of working calibration 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 Sampling procedure

8.1 Sample handling

Mill the tobacco sample to a mesh size <1 mm.

NOTE Samples that do not grind well due to a high moisture content can be cryoground using liquid nitrogen.

8.2 Preparation of the sample

Weigh approximately 0,50 g ± 0,05 g of the tobacco, cigarette filler or smokeless tobacco products into a suitable polypropylene extraction vessel and add 50,0 ml of the extraction solution.

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

It is recommended that a process control blank is prepared in the same fashion as the samples in order to assess laboratory and reagent contamination.

Place the extraction vessel on a laboratory shaker and shake at a moderate speed for 60 min at room temperature. Take an aliquot and filter through a 0,45 µm syringe filter and proceed to analysis by ion chromatography.

Samples may be centrifuged to remove particulate matter instead of using filtration.

Depending on the ammonia content of the tobacco sample, the extract might 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 0,012 5 mol/l (0,025 N) sulfuric acid. A dilution factor of 10 is sufficient for most samples. Alternatively, the volume of extraction solution used for the initial extraction can be increased if it is anticipated that the calibration range will be exceeded.

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 ± 2 °C.

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

8.3 Sample analysis

8.3.1 Example ion chromatography parameters

Set up the ion chromatograph according to the manufacturer's instructions.

Detection of ammonia, as ammonium, is achieved using suppressed conductivity detection. This method of detection reduces the background conductivity of the eluent, thus improving the signal-to-noise ratio.

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 = 0,257 8).

The operating conditions provided below are listed as an example. Ensure that the sodium and ammonium peaks are well resolved with the instrumental parameters utilized.

— A 25 µl injection loop is recommended and injection volume of all standards and samples is 25 µl.

— The gradient profile is stated in Table 2.

Table 2 — Example gradient profile

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

Other parameters (example):

— suppressor current = 88 mA;

— auto-sampler tray temperature = 4 °C ± 2 °C;

— column temperature = 30 °C;

— pressure range: 200 psi (min) and 3 000 psi (max);

— flush volume = 250 µl;

— data acquisition is throughout the period 0 min to 15 min.

Optimize 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.

8.3.2 Calibration of the ion chromatograph

Inject an aliquot of each ammonia standard into the IC.

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 the detector response. The response obtained for all test samples should fall within the working range of the calibration curve.

8.3.3 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, Kentucky Reference 3R4F cigarette filler, and CORESTA Reference Product 2 (CRP2) are shown in Annex A.

The amount of ammonia, NH_3, expressed in micrograms per gram, is calculated by Formula (1):

$$NH_3 = \frac{c \times v}{m} d \quad (1)$$

where

c is the concentration of ammonia, in micrograms per millilitre, obtained from the calibration curve;

v is the extraction volume, in millilitres;

m is the mass of the sample, in grams;

d is the dilution factor, e.g. 10 would be used if the sample was diluted tenfold.

The expression of the laboratory data depends on the purpose for which the data are required, and the level of laboratory precision. Any further statistical analyses should be calculated and expressed before any rounding has taken place. Moisture content may be determined on separate tobacco aliquots if it is necessary to present the final results on a dry-weight basis.

9 Repeatability and reproducibility

A major international study was conducted in 2013 involving 11 laboratories and 11 ground tobacco and smokeless tobacco products[4]. The following values for repeatability limit, *r*, and reproducibility limit, *R*, were obtained for this method.

The difference between two single results found on matched samples by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability limit, *r*, on average not more than once in 20 cases in the normal and correct operation of this method.

Single results on matched samples reported by two laboratories will differ by more than the reproducibility limit, *R*, on average not more than once in 20 cases in the normal and correct operation of the method. Data analysis for the 11 ground tobacco and smokeless tobacco product samples gave the estimates as summarised in Table 3.

The statistical evaluation was performed according to ISO 5725-2:1994 and ISO/TR 22971:2005.

Table 3 — Results overview

Product	Mean ammonia[a] µg/g	Number of labs[b]	*r*	*R*	*r* %	*R* %
CRP1 – Swedish style snus pouch	951	11	113	529	11,9	55,6
CRP2 – American-style loose moist snuff	2 581	10	103	1055	4,0	40,8
CRP3 – American-style loose dry snuff powder	4 786	10	149	870	3,1	18,2

[a] Results are presented on an as is basis, without correction for moisture.

[b] The number of laboratory data sets remaining after removal of outliers.

Table 3 *(continued)*

Product	Mean ammonia[a] µg/g	Number of labs[b]	*r*	*R*	*r* %	*R* %
CRP4 – American-style loose-leaf chewing tobacco	2 769	10	464	686	16,7	24,8
3R4F reference cigarette filler	1 081	9	100	373	9,3	34,5
Fine cut moist snuff	4 067	7	149	1227	3,7	30,2
Virginia tobacco	446	7	30	136	6,7	30,5
Burley tobacco	2 816	8	289	392	10,3	13,9
Oriental tobacco	362	7	43	52	11,8	14,4
Fire-cured tobacco	2 978	7	50	348	1,7	11,7
Dark air-cured tobacco	540	8	33	128	6,1	23,6

[a] Results are presented on an as is basis, without correction for moisture.

[b] The number of laboratory data sets remaining after removal of outliers.

10 Test report

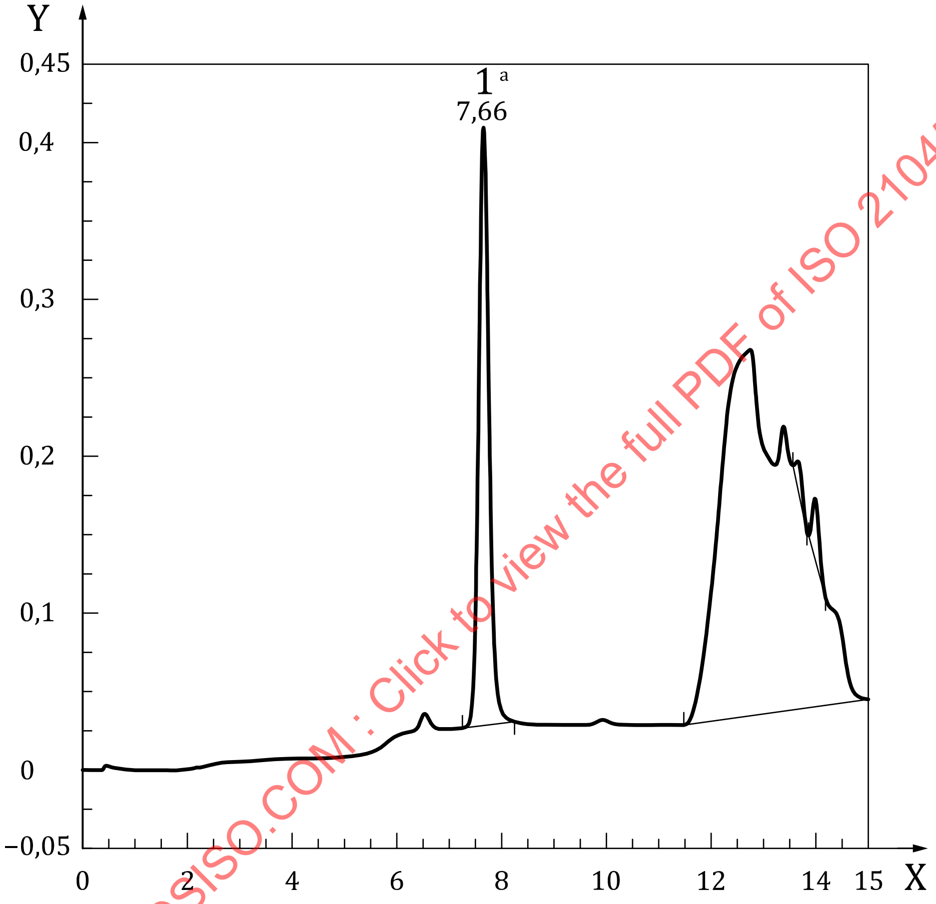
The test report shall state the method used and the results obtained. It shall mention any operating conditions not specified in this document, or regarded as optional, as well as any circumstances that might have influenced the results.

The test report shall include all details required for complete identification of the sample.

Annex A
(informative)

Example chromatograms

Example chromatograms are given in Figures A.1 to A.3.



Key

X time, min

Y conductivity, μS

1 ammonia – 7,66

a Detected as ammonium.

Figure A.1 — Example chromatogram for a 0,5 μg/ml ammonia calibration standard