INTERNATIONAL STANDARD

ISO 23919

First edition
2020-04

Cigarettes — Determination of ammonia in cigarette mainstream smoke using ion chromatography

Cigarettes — Dosage de l'ammoniac dans le courant principal de la fumée de cigarette par chromatographie par échange d'ions

Reference number
ISO 23919:2020(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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

The CORESTA Smoke Analytes Sub-Group[1)] conducted a survey among its members and determined that most laboratories used a method involving ion chromatography for the determination of ammonia in cigarette mainstream smoke. Two alternative trapping systems were used, either with a combination of glass fibre filter pad followed by impinger traps or with an impregnated glass fibre filter pad followed by a glass fibre filter pad.

A CORESTA recommended method (CRM) was written[1] on the basis of the results obtained in an interlaboratory study conducted in 2015 involving 17 laboratories from 8 countries using cigarettes manufactured from a range of blend styles[2]. The results demonstrated equivalency of the data obtained by using both trapping systems. It was observed that the method is not applicable to dark-air cured blended cigarettes.

This document is based upon the CRM 83 and includes statistical evaluations carried out according to ISO 5725-1 and ISO 5725-2.

No machine smoking regime can represent all human smoking behaviour.

— It is recommended that cigarettes also be tested under conditions of a different intensity of machine smoking than those specified in this document.

— Machine smoking testing is useful to characterize cigarette emissions for design and regulatory purposes, but communication of machine measurements to smokers can result in misunderstandings about exposure and risk across brands.

— Smoke emission data from machine measurements may be used as inputs for product hazard assessment, but they are not intended to be nor are they valid as measures of human exposure or risks. Communicating differences between products in machine measurements as differences in exposure or risk is a misuse of testing using ISO standards.

1) Until 2017, the sub-group has been previously known as CORESTA Special Analytes Sub-Group.

Cigarettes — Determination of ammonia in cigarette mainstream smoke using ion chromatography

WARNING — The use of this document involves hazardous materials, operations and equipment. This document does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this document to establish appropriate safety and health practices and determine the applicability of any other restrictions prior to use.

1 Scope

This document specifies a method for the quantification of ammonia by ion chromatography in mainstream smoke using ISO 3308 smoking parameters.

This method is applicable to cigarettes with ammonia yields between 1 µg/cigarette and 30 µg/cigarette. It is not applicable for the determination of ammonia in dark-air cured cigarettes.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3308, *Routine analytical cigarette-smoking machine — Definitions and standard conditions*

ISO 3402, *Tobacco and tobacco products — Atmosphere for conditioning and testing*

ISO 4387, *Cigarettes — Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine*

ISO 8243, *Cigarettes — Sampling*

3 Terms and definitions

No terms and definitions are listed in this document.

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/

4 Principle

Ammonia is collected by passing the mainstream smoke of cigarettes through either

a) a glass fibre filter pad as specified in ISO 3308 followed by impinger traps containing dilute sulphuric acid (trapping system 1 in the document), or

b) an impregnated glass fibre filter pad followed by an untreated glass fibre filter pad (trapping system 2 in the document).

The glass fibre filter pad is extracted either with the impinger solutions (trapping system 1) or with dilute hydrochloric acid (trapping system 2). The obtained solutions are analysed by ion chromatography using an external standard calibration.

5 Apparatus

The usual laboratory apparatus for use in preparation of samples, solutions and standards and, in particular, the following.

5.1 Routine analytical cigarette-smoking machine, complying with the requirements of ISO 3308.

5.2 Impinger trapping system, capable of being connected in series.

5.3 High performance liquid chromatography system, consisting of a conductivity detector and conductivity suppressor, an eluent degassing unit, a gradient pump, an autosampler with sampling loop and cooling unit, a data collection system.

5.4 Cation exchange analytical column, e.g. Dionex™ IonPac[2)] CS16 IC or equivalent.

5.5 Disposable guard column, e.g. Dionex™ IonPac CG16[2)] or equivalent.

5.6 Analytical balance, suitable for measuring to the nearest 0,1 mg.

5.7 Glassware, Erlenmeyer flasks of appropriate volumes with ground glass stoppers.

5.8 Polypropylene, tubing, volumetric flasks (25 ml, 50 ml, 100 ml and 1 l).

5.9 Mechanical pipettes with disposable plastic tips.

6 Reagents

All reagents shall be at least of analytical reagent grade.

6.1 Ammonium sulfate [$(NH_4)_2SO_4$] > 99 % purity.

6.2 Sulphuric acid (H_2SO_4) > 96 % purity (for trapping system 1).

6.3 Hydrochloric acid (HCl) > 36,5 % to 38 % purity (for trapping system 2).

6.4 Methanesulphonic acid (MSA) > 99 % purity.

6.5 Ethanol > 99 % purity.

6.6 Deionised water, with a resistivity > 18 MΩ·cm at 25 °C.

7 Preparation

7.1 General

Polypropylene and glass containers shall be cleaned and dried in such a manner which ensures that contamination does not occur.

2) Dionex Ion Pac is an example of a suitable product available commercially. This information is given for the convenience of 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.

7.2 Preparation of solutions

7.2.1 Sulphuric acid, 0,01 M — Impinger solution (trapping system 1)

Carefully add 1,022 g of H_2SO_4 (w = 96 %) to a minimum of 500 ml of deionised water in a 1 l volumetric flask. Mix and dilute to volume with deionised water. Store the solution in a bottle at ambient temperature.

7.2.2 Sulphuric acid, 0,1 M — Solution C (Ion chromatography eluent)

Carefully add 10,22 g of H_2SO_4 (w = 96 %) to a minimum of 500 ml of deionised water in a 1 l volumetric flask. Mix and dilute to volume with deionised water.

7.2.3 MSA 0,003 M — Solution A (Ion chromatography eluent)

Carefully add 0,288 g MSA to 900 ml of deionised water in a 1 l volumetric flask. Mix and dilute to volume with deionised water.

7.2.4 Sulphuric acid, 0,01 M — Ammonium standards preparation

Carefully add 1,022 g of H_2SO_4 (w = 96 %) to a minimum of 500 ml of deionised water in a 1 l volumetric flask. Mix and dilute to volume with deionised water. Store the solution in a bottle at ambient temperature.

7.2.5 Hydrochloric acid, 0,05 M — Solution for glass fibre filter pad impregnation (trapping system 2)

Carefully add 4,3 ml of HCl (w = 36,5 % to 38 %) to a minimum of 500 ml of ethanol in a 1 l volumetric flask. Mix and dilute to volume with deionised water. Store the solution in a bottle at ambient temperature.

7.2.6 Hydrochloric acid, 0,01 M — Extraction solution (trapping system 2)

Carefully add 0,9 ml HCl (w = 36,5 % to 38 %) to a minimum of 500 ml of deionised water in a 1 l volumetric flask. Mix and dilute to volume with deionised water. Store the solution in a bottle at ambient temperature.

7.3 Preparation of standards

7.3.1 Primary ammonium stock solution

Accurately weigh 0,10 g of $(NH_4)_2SO_4$ into a 25 ml volumetric flask. Note the exact weight in order to accurately calculate the standard concentrations. Dissolve in the 0,01 M sulphuric acid solution (see 7.2.4) and dilute to volume with the same solution.

NOTE 1 The solution is stable for about 30 days when stored in a refrigerator.

NOTE 2 It corresponds approximately to a 1 000 µg/ml ammonium stock solution.

NOTE 3 A certified reference material at 1 000 µg/ml is suitable as well.

7.3.2 Calibration standards

A range of calibration standard solutions is prepared with appropriate volumes (0,02 ml to 0,20 ml) of the primary ammonium stock solution (see 7.3.1) diluted to prescribed volumes with 0,01 M sulphuric acid (see 7.2.4), according to Table 1.

Table 1 — Preparation of calibration standards — Trapping system 1

Standard	Volume of primary stock solution (µl)	Final volume (ml)	Concentration (µg/ml)
0	0	25	0
1	20	100	0,200
2	50	100	0,500
3	50	25	2,000
4	100	25	4,000
5	175	25	7,000
6	250	25	10,00

NOTE It is assumed that the primary ammonium stock solution (see 7.3.1) has a concentration of exactly 1 000 µg/ml.

The calibration standards shall cover the concentration range of interest. The example provided in Table 1 can require an adjustment in case the trapping system 2 has been used.

8 Sampling

Carry out sampling in accordance with ISO 8243.

9 Tobacco product preparation

Condition the cigarettes in accordance with ISO 3402.

10 Sample generation — Smoking of cigarettes

10.1 General

The smoking parameters for which the method has been studied are defined in ISO 3308.

10.2 Smoking machine setup

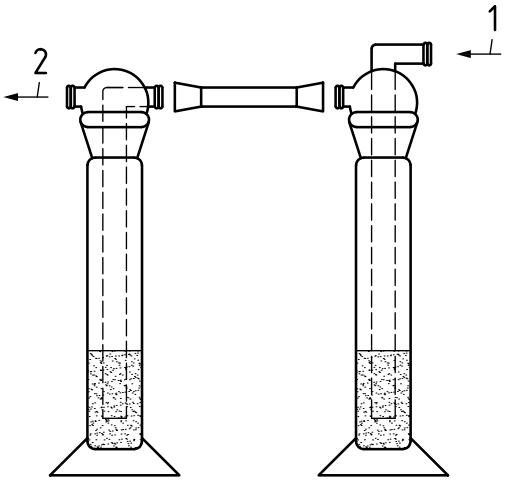
An analytical cigarette-smoking machine complying with the requirements of ISO 3308 is required.

Two trapping systems can be used. Trapping system 1 consists of a glass fibre filter pad combined with impinger traps. Trapping system 2 consists of an impregnated and an un-treated glass fibre filter pad.

10.2.1 Trapping system 1 — Glass fibre filter pad and impinger traps

Assemble the ammonia mainstream trapping system on the analytical smoking machine by connecting two impingers, each containing 15 ml of 0,01 M sulphuric acid (see 7.2.1), between the glass fibre filter pad and the puffing mechanism using polypropylene tubing (see Figure 1).

Check and adjust the puff volume drawn by the smoking machine at all channels, as described in ISO 4387.



Key

1 from collection pad

2 to puffing mechanism of smoking machine

Figure 1 — Example of trapping system 1

To determine whether a leak has occurred in the analytical smoking machine setup, use a leak tester. If the fluid column does not maintain its position but drops, there is a leak in the system.

It is recommended that tubing other than silicone tubing is used for connections between the analytical smoking machine and the impingers (i.e. polypropylene). Methyl silicone tubing is not recommended since adsorption of the analytes can occur. Tubing shall be as short as possible to minimize the potential for any adsorption.

It is recommended that the trapping efficiency is checked when validating this method. To check the trapping efficiency of the method, add an additional impinger and follow the method accordingly. Analyse each impinger individually for ammonia. If negligible amounts of ammonia are detected in the additional impinger, then only the prescribed number of impingers is required to trap the vapour phase ammonia effectively, otherwise an additional impinger is required. Poor trapping efficiency might be due to the impinger tip design.

If a carryover occurs, it is the responsibility of each laboratory to assess the carryover with respect to the specific trapping system design and decide how to manage it. Carryover should be repeatable, less than 5 % (ideally less than 1 %), should be reported or corrected in calculations.

10.2.2 Trapping system 2 — Impregnated glass fibre filter pad

10.2.2.1 Impregnation procedure

A glass fibre filter pad (44 mm for linear smoking machine and 92 mm for rotary smoking machine) is placed in a suitable container (e.g. culture vessel or a 100 ml beaker) and 2 000 µl or 6 000 µl (for 44 mm and 92 mm glass fibre filter pad respectively) of ethanol/HCl aqueous solution (see 7.2.5) is sprayed evenly drop by drop using a 100 µl to 1 000 µl pipette. Once saturated, the impregnated glass fibre filter pad is placed into a container with holes on the bottom. The container is placed in a laboratory incubator and conditioned according to the specifications provided in ISO 3402. To avoid contamination, the incubator shall be used only for this purpose.

The conditioning time shall be between 0,5 h and 8 h.

NOTE A longer conditioning time can lead to a significant decrease in the ammonia content in cigarettes mainstream smoke.

10.2.2.2 Assembly of trapping system 2

Place the impregnated glass fibre filter pad and the untreated glass fibre filter pad into the cigarette holder. The impregnated glass fibre filter pad shall be in the front facing the incoming smoke and the untreated glass fibre filter pad at the back. Assemble the cigarette holder in the analytical smoking machine and connect the rear section to the tubing leading to the smoking machine piston.

Check and adjust the puff volume drawn by the smoking machine at all channels, as described in ISO 4387.

10.3 Smoking

The cigarettes are smoked according to ISO 3308.

11 Sample analysis

11.1 Preparation of sample

11.1.1 Extraction of glass fibre filter pad — Trapping system 1

After all samples have been smoked following ISO 3308, remove the glass fibre filter pad from the smoking machine, fold into quarters and place into a 125 ml extraction flask. Add the content of the impingers. Rinse the impingers with equal volumes of water (e.g. 30 ml impingers rinsed with 30 ml water) and add to the extraction flask.

Close the flask, shake for 30 min on a wrist action shaker. Transfer the filtered extract to an autosampler vial.

NOTE Samples can be stored in the refrigerator for up to 48 h prior to analysis.

11.1.2 Extraction of glass fibre filter pad — Trapping system 2

After all samples have been smoked following ISO 3308, remove the two glass fibre filter pads from the smoking machine, fold into quarters and place into a 100 ml extraction flask containing 40 ml of diluted HCl solution (see 7.2.6)

NOTE 1 The volume of extraction was calculated for 44 mm glass fibre filter pad; adjust for 92 mm glass fibre filter pad.

Close the flask, shake for 30 min on a wrist action shaker. Transfer the filtered extract to an autosampler vial.

NOTE 2 Samples can be stored in the refrigerator for up to 48 h prior to analysis.

11.2 Determination

11.2.1 Ion chromatography operating conditions

Set up and operate the ion chromatography in accordance with the manufacturer's instruction.

The following parameters have been found to suitable for operation.

Chromatographic parameters:

Column temperature: 30 °C

Autosampler temperature: 4 °C (±2 °C)

Injection volume: 20 µl

Conductivity detector (e.g. Dionex™ suppressed conductivity)

Suppressor conductivity: 100 mA

Scale: 10 µS

Mobile phase

Solvent A: 0,003 M MSA (7.2.3)

Solvent B: Deionised water

Solvent C: 0,1 M sulphuric acid (7.2.2)

Flow: 1,5 ml/min

Gradient (see Table 2)

Table 2 — Example of gradient

Time (min)	Composition		
	% A	% B	% C
0:00	100	0	0
13:00	100	0	0
13:01	0	80	20
14:00	0	80	20
14:01	0	90	10
19:00	0	90	10
19:01	0	99	1
20:00	0	99	1
25:00	99	1	0
25:00	Method end action: Equilibrate (9 min)		

A typical chromatogram is provided in Annex A (see Figures A.1 and A.2).

11.2.2 Calibration

Analyse each calibration standard (see 7.2.3). Record the area. Generate a calibration curve using a quadratic function. The response obtained for tests samples shall fall within the working range of the calibration curve.

The concentration of ammonia in cigarette mainstream smoke samples is quantified by the external standard method. The identification of the ammonium peak in samples is determined by its comparison with the retention time of ammonium in standards.

11.2.3 Calculation

The yield of ammonia in the mainstream smoke of cigarettes, *m*, expressed in micrograms per cigarette, is given by Formula (1):

$$m = \frac{17}{18} \times \frac{A \times DF}{RF \times N_{cig}} \quad (1)$$

where

A is the peak area in the sample;

RF is the response factor;

DF is the dilution volume in ml;

N_{cig} is the number of cigarettes smoked.

NOTE The factor 17/18 corrects for the difference in molecular weight between ammonium and ammonia.

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 on the basis of the laboratory data before any rounding has taken place.

The yield of ammonia in the mainstream smoke of cigarettes is reported in micrograms per cigarette (µg/cigarette) to the nearest 0,1 µg.

12 Repeatability and reproducibility

12.1 General

An international collaborative study was conducted in 2015, involving 17 laboratories and 8 cigarette samples[2]. This provided data on the measurement of ammonia in replicate analyses of 8 samples (see Table 3) performed with ISO 3308 smoking regime. The values for repeatability, *r*, and reproducibility, *R*, given in Tables 4 and 5, were obtained using this method. The statistical evaluation was performed according to ISO 5725-2.

The difference between two single results found on matched cigarette samples by one operator using the same apparatus within the shortest feasible time interval exceeds 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 cigarette samples reported by two laboratories differs 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.

Table 3 — Cigarette test samples of 2015 collaborative study

Sample	Product characterization	NFDPM yield (mg/cigarette)
Sample 1	Dark-air cured product	9,5
Sample 2	American blended product	8,0
Sample 3	American blended product	6,4
Sample 6	Virginia blended product	9,7
Sample 7	Charcoal filtered/blended product	1,3
Ky 3R4F	Kentucky reference cigarette 3R4F/American blend	8,2
Ky 1R5F	Kentucky reference cigarette 1R5F/American blend	1,7
CM 7	CORESTA Monitor 7 test piece/Virginia blend	14,2