STANDARD OPERATING PROCEDURE FOR DETERMINATION OF AMMONIA IN CIGARETTE TOBACCO FILLER

Tobacco Free Initiative
Tobacco Laboratory Network (TobLabNet)
Tobacco Free Initiative Tobacco Laboratory Network (TobLabNet)

No.: SOP 03
Date: June 2014

World Health Organization Tobacco Laboratory Network

Standard operating procedure for method

Determination of tobacco-specific nitrosamines in mainstream cigarette smoke under ISO and intense smoking conditions

Method: Determination of tobacco-specific nitrosamines in mainstream cigarette smoke under ISO and intense smoking conditions

Analytes: 3-(1-Nitrosopyrrolidin-2-yl)pyridine (CAS# 16543-55-8)
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (CAS# 64091-91-4)
N-Nitrosoanatabine (CAS# 71267-22-6)
N-Nitrosoanabasine (CAS# 37620-20-5)

Matrix: Tobacco cigarette mainstream smoke particulate matter

Last update: June 2014
Standard operating procedure for determination of ammonia in cigarette tobacco filler
Determination of tobacco-specific nitrosamines in mainstream cigarette smoke under ISO and intense smoking conditions.

Analytes: 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone

Matrix: Tobacco cigarette mainstream smoke particulate matter

Last update: June 2014

World Health Organization Tobacco Laboratory Network SOP 07

Tobacco Laboratory Network (TobLabNet)

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Tobacco Laboratory Network

Standard operating procedure for method

Determination of ammonia in cigarette tobacco filler

Method: Determination of ammonia in cigarette tobacco filler

Analytes: Ammonia (CAS # 7664-41-7)

Matrix: Cigarette tobacco filler

Last update: July 2016
No.: SOP 07  
Date: July 2016

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No machine smoking regimen can represent all human smoking behaviour: machine smoking testing is useful for characterizing cigarette emissions for design and regulatory purposes, but communication of machine measurements to smokers can result in misunderstanding about differences between brands in exposure and risk. Data on smoke emissions 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 risk. Representing differences in machine measurements as differences in exposure or risk is a misuse of the results of testing with WHO TobLabNet-recommended methods.
FOREWORD
This document was prepared by members of the World Health Organization (WHO) Tobacco Laboratory Network (TobLabNet) as an analytical method standard operating procedure (SOP) for measuring ammonia in cigarette tobacco filler.

INTRODUCTION
In order to establish comparable measurements for testing tobacco products globally, consensus methods are required for measuring specific contents and emissions of cigarettes. The Conference of the Parties to the WHO Framework Convention on Tobacco Control (WHO FCTC) at its third session, in Durban, South Africa, in November 2008, “recalling its decisions FCTC/COP1(15) and FCTC/COP2(14) on the elaboration of guidelines for implementation of Articles 9 (Regulation of the contents of tobacco products) and 10 (Regulation of tobacco product disclosures) of the WHO FCTC, noting the information contained in the report of the working group to the third session of the Conference of the Parties on the progress of its work ... requested the Convention Secretariat to invite WHO’s Tobacco Free Initiative to ... validate, within five years, the analytical chemical methods for testing and measuring cigarette contents and emissions” (FCTC/COP/3/REC/1).

Using the criteria for prioritization set at its third meeting in Ottawa, Canada, in October 2006, the working group on Articles 9 and 10 identified the following contents for which methods for testing and measuring (analytical chemistry) should be validated as a priority:

- nicotine
- ammonia
- propylene glycol (propane-1,2-diol)
- glycerol (propane-1,2,3-triol)
- triethylene glycol (2,2-ethylenedioxybis(ethanol)).

Measurement of these contents will require validation of three methods: one for nicotine, one for ammonia and one for humectants.

Using the criteria for prioritization set at the meeting in Ottawa mentioned above, the working group identified the following emissions in mainstream smoke for which methods for testing and measurement (analytical chemistry) should be validated as a priority:

- 4-(methylNitrosamino)-1-(3-pyridyl)-1-butanone (NNK)
- N-nitrosonornicotine (NNN)
• acetaldehyde
• acrylaldehyde (acrolein)
• benzene
• benzo[a]pyrene
• 1,3-butadiene
• carbon monoxide
• formaldehyde.

Measurement of these emissions with the two smoking regimens described below will require validation of five methods: one for tobacco-specific nitrosamines (NNK and NNN), one for benzo[a]pyrene, one for aldehydes (acetaldehyde, acrolein and formaldehyde), one for volatile organic compounds (benzene and 1,3-butadiene) and one for carbon monoxide.

The table below sets out the two smoking regimens for validation of the test methods referred to above.

<table>
<thead>
<tr>
<th>Smoking regimen</th>
<th>Puff volume (mL)</th>
<th>Puff frequency</th>
<th>Filter ventilation holes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISO regimen: ISO 3308: <em>Routine analytical cigarette smoking machine – definitions and standard conditions</em></td>
<td>35</td>
<td>Once every 60 s</td>
<td>No modification</td>
</tr>
<tr>
<td>Intense regimen: Same as ISO 3308, but modified as indicated</td>
<td>55</td>
<td>Once every 30 s</td>
<td>All ventilation holes must be blocked 100% as described in WHO TobLabNet SOP 01.</td>
</tr>
</tbody>
</table>

This SOP was prepared to describe the procedure for the determination of ammonia in cigarette tobacco filler.

1 **SCOPE**

This standard operating procedure is suitable for the quantitative determination of ammonia in cigarette tobacco filler by ion chromatography.

2 **REFERENCES**

2.1 *ISO8243: Cigarettes – Sampling.*

2.3 ISO5725-1: Accuracy (trueness and precision) of measurement methods and results – Part 1: General principles and definitions.

2.4 ISO5725-2: Accuracy (trueness and precision) or measurement methods and results - Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method.

2.5 ISO guide 34: General requirements for the competence of reference materials producers.


3 TERMS AND DEFINITIONS

3.1 Ammonia content: amount of ammonia in cigarette tobacco filler, expressed as milligrams per gram of cigarette tobacco filler

3.2 Cigarette tobacco filler: Tobacco-containing part of a cigarette, including reconstituted tobacco, stems, expanded tobacco and additives

3.3 Tobacco products: Products made entirely or partly of leaf tobacco as the raw material that are manufactured for smoking, sucking, chewing or snuffing (Article 1(f) of the WHO FCTC)

3.4 Laboratory sample: Sample intended for testing in a laboratory, consisting of a single type of product delivered to the laboratory at one time or within a specified period

3.5 Test sample: Product to be tested, taken at random from the laboratory sample

3.6 Test portion: Random portion from the test sample to be used for a single determination

4 METHOD SUMMARY

4.1 Ammonia is extracted from the cigarette tobacco filler with a dilute sulfuric acid extraction solution, followed by centrifugation (and filtration if necessary).

4.2 The extract is analysed by ion chromatography coupled with conductivity detection.

4.3 Quantification is achieved by calibration against an external standard, by comparing the conductivity response of the analytes in the samples with those of the standards.
5 SAFETY AND ENVIRONMENTAL PRECAUTIONS

5.1 Follow routine safety and environmental precautions, as in any chemical laboratory activity.

5.2 The testing and evaluation of certain products with this test method may require the use of materials or equipment that could be hazardous or harmful to the environment. This document does not address all the safety aspects associated with use of the method. All persons using this method are responsible for consulting the appropriate authorities and establishing health and safety practices as well as environmental precautions in conjunction with any existing applicable regulatory requirements prior to its use.

5.3 Special care should be taken to avoid inhalation or dermal exposure to harmful chemicals. Use a chemical fume hood, and wear an appropriate laboratory coat, gloves and safety goggles when preparing or handling undiluted materials, standard solutions, extraction solutions or collected samples.

6 APPARATUS AND EQUIPMENT

Usual laboratory apparatus, in particular:

6.1 Analytical balance capable of measurement to at least four decimal places

6.2 Erlenmeyer flasks, 250 mL, with stoppers, or equivalent

6.3 Beakers (various sizes)

6.4 Volumetric flasks (various sizes)

6.5 Volumetric pipettes for preparation of standard solutions (various sizes)

6.6 Autosampler

6.7 Autosampler vials, 2 mL, or equivalent

6.8 Mechanical wrist-action shaker or equivalent

6.9 Ion chromatograph equipped with a conductivity detector

6.10 Cation exchange column (250 × 4 mm)

6.11 Cation exchange guard column (50 × 4 mm)

6.12 Renewable cation suppressor (optional)

Note 1: The above is the typical instrument configuration for this analysis. Alternative configurations (for example, without a suppressor) are expected to yield similar results.

Note 2: Samples should be either filtered or centrifuged before analysis.
6.13 Filtration equipment
   6.13.1 Syringe, 10 mL, or equivalent
   6.13.2 Screen, 0.45 mm
   6.13.3 Water-phase membrane filters, 15 mm × 0.45 μm, or equivalent
   6.13.4 Ashless quantitative filter paper, for example Whatman No. 40, 8 μm, or equivalent

6.14 Centrifugation equipment
   6.14.1 Micro-17 centrifuge, or equivalent
   6.14.2 Microcentrifuge vials, or equivalent

7 REAGENTS AND SUPPLIES
All reagents shall be of at least analytical reagent grade unless otherwise noted. Reagents are identified by their Chemical Abstract Service [CAS] registry numbers when available.

7.1 Methanesulfonic acid [75-75-2], chromatographic purity
7.2 Concentrated sulfuric acid [7664-93-9], mass percentage: 95% to ~98%
7.3 Ammonium sulfate [7783-20-2] or, alternatively, a standard solution endorsed by ISO Guide 34: [2.5] of 1000 mg/L ammonia
7.4 Water, Type 1 [7732-18-5]

8 PREPARATION OF GLASSWARE
8.1 Clean and dry glassware in a manner to avoid contamination.
   Note: It is recommended that detergents not be used for cleaning, in order to minimize interference.

9 PREPARATION OF SOLUTIONS
The method for preparing extraction and mobile phase solutions described below is for reference purposes and can be adjusted if necessary.

9.1 Extraction solution (dilute sulfuric acid, approximately 0.0125 mol/L)
   9.1.1 To obtain 0.0125 mol/L sulfuric acid solution, use the equation \( \rho = \frac{m}{V} \), where \( \rho \) is the density of sulfuric acid (1.84 g/mL) and \( m \) is the mass of sulfuric acid.
Note: 1.29 g sulfuric acid added to 1 L of Type 1 water \[7.4\] gives a 0.0125 mol/L dilution of sulfuric acid.

9.1.2 Label as “dilute sulfuric acid extraction solution”, and store in a refrigerator.

9.2 Mobile phase (methanesulfonic acid in water, 0.02 mol/L)

Accurately transfer 1.32 mL of methanesulfonic acid \[7.1\] into a 1-L volumetric flask \[6.4\], and make up to volume with Type 1 water \[7.4\].

10 PREPARATION OF STANDARDS

The method for preparing standard solutions described below is for reference purposes and can be adjusted if necessary.

10.1 Ammonia standard solution (approximately 100 μg/mL)

10.1.1 Accurately weigh 0.092 g (± 0.002 g) of ammonium sulfate \[7.3\] into a 100-mL beaker, and dissolve it in extraction solution \[9.1\]. All solvents and solutions must be at room temperature.

10.1.2 Transfer the ammonium sulfate solution \[10.1.1\] into a 250-mL volumetric flask, and make up to volume with extraction solution \[9.1\].

10.2 Working standards

<table>
<thead>
<tr>
<th>Standard</th>
<th>Volume of standard solution [10.1] (mL)</th>
<th>Final volume (mL)</th>
<th>Approximate ammonia concentration in working standard solution (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.01</td>
<td>10</td>
<td>0.1</td>
</tr>
<tr>
<td>2</td>
<td>0.05</td>
<td>10</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>0.2</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Note 1: All standards are made in volumetric flasks at the dilutions described. Volumetric flasks are made up to volume with extraction solution \[9.1\].

Note 2: The range of the standard solutions may be adjusted, depending on the equipment used (with or without suppression equipment) and samples to be tested, keeping in mind a possible effect on the sensitivity of the method.

Note 3: The concentration of standard solutions can change significantly during storage, which will change the intercept of the calibration curve. They should not be stored for longer than 2 weeks.

Note 4: The purity of ammonia in the standard used must be considered in calculating the concentration.
11 SAMPLING

11.1 Sample cigarettes according to ISO 8243 [2.1]. Alternative approaches may be used to obtain a representative laboratory sample in accordance with individual laboratory practice or when required by specific regulation or the availability of samples.

11.2 Constitution of test sample

11.2.1 Divide the laboratory sample into separate units (e.g. packet, container), if applicable.

11.2.2 Take an equal amount of product for each test sample from at least √n [2.2] of the individual units (e.g. packet, container).

12 CIGARETTE PREPARATION

12.1 Remove all tobacco filler from all test cigarettes or quality control samples (when applicable) in a pack (e.g. containing 20 cigarettes). Alternatively, use at least 7 g of processed cigarette tobacco filler.

12.2 Combine and mix sufficient cigarette tobacco filler to constitute test portions of at least 7 g each. Prepare at least three replicates of test portions.

Note: Sample size may be adjusted if necessary.

13 PREPARATION OF THE SMOKING MACHINE

Not applicable.

14 SAMPLE GENERATION

Not applicable.

15 SAMPLE PREPARATION

15.1 For each test portion, take 0.7 g of well-mixed test portion, and weigh it to 0.0001 g into a 250-mL Erlenmeyer or other suitable flask.

15.2 Add 50 mL of the extraction solution [9.1] to the sample.

Note: Sample weight and dilution factor may be adjusted accordingly depending on the concentrations of ammonia or availability of sample.

15.3 Stopper the flasks, and place them on a wrist-action shaker or equivalent at a rate of at least 160 rpm for 30 min or at a rate suitable for the type of mechanical shaker used.
15.4 Remove the samples from the shaker, swirling each flask to disperse all the tobacco in the solvent.

15.5 Leave the samples for 30 min until the supernatant is clear.

15.6 Filter the extract through 8-µm ashless quantitative filter paper.

15.7 Syringe the extract [15.6] onto a 0.45-µm membrane filter.

15.8 Transfer the filtrate to an autosampler vial, and analyse it on the ion chromatograph.

Note: If the sample signal (peak area) does not fall within the working range of the calibration curve, the solutions should be adjusted accordingly, i.e. further diluted. All the final dilutions should be recorded in the sample report.

Alternatively, the following steps can be followed:

15.5 Centrifuge 1.5 mL of the supernatant at 13 000 rpm for 5 min.

15.6 Transfer the centrifuged extract into an autosampler vial, and analyse it on the ion chromatograph.

16 SAMPLE ANALYSIS

This method involves ion chromatography coupled with conductivity detection to quantify ammonia in cigarette tobacco filler. The analytes are resolved from potential interference on the column. Comparison of the area of the unknowns with the area of the known standard concentrations yields the concentrations of individual analytes.

16.1 Ion chromatography operating conditions

Mobile phase: Methanesulfonic acid in water, 0.02 mol/L [9.2]

Rate: 1.0 mL/min

Injection volume: 25 µL

Column temperature: 30 °C

These instrumental settings and other specifications (e.g. current setting of cation suppressor) shall be adapted for each equipment and column profile in order to obtain good resolution and chromatographic performance.

Note 1: For example, a more dilute solution of methanesulfonic acid in water is recommended for the mobile phase (up to 10-fold dilution) when working without suppression.

Note 2: The operating parameters may have to be adjusted to the instrument and column conditions and the resolution of chromatographic peaks.
16.2 Expected retention times

Note: Differences in, for example, the type and age of the column will alter retention times.

Under the above conditions, the expected total analysis time will be about 15 min.

16.3 Determination of ammonia

16.3.1 Condition the system before use, for example by injecting two 25-μL aliquots of sample solution as a primer. Other equilibrating procedures, as suggested by the manufacturer, can also be used.

Note: Depending on the stationary phase, a blank might have to be injected after the calibration standards. A blank should also be injected at the end of a batch, to reduce cross-contamination.

16.3.2 After conditioning, inject 25 μL of blank solution to check for any contamination in the system or the reagents.

16.3.3 Inject an aliquot of each ammonia standard solution [10.2] into the ion chromatograph.

16.3.4 Assess the retention times and responses (area counts) for the standards. The system is considered ready to perform the analysis when the retention times of the standards are similar (defined as ± 0.2 min) to the between-run retention times in the same system, the calculated resolution is ≥ 1.5, and responses are within 20% of the typical responses in previous runs.

16.3.5 Record the peak areas of ammonia.

16.3.6 Plot a graph of the areas in accordance with the concentration of ammonia.

16.3.7 The following alternatives should be used for calculating ammonia concentrations.

For ion chromatography with a suppressor:

Calculate a quadratic regression equation \( Y = ax^2 + bx + c \) from the data.

If the quadratic regression coefficient \( R^2 \) is < 0.99, the calibration should be repeated. An individual calibration point that differs by > 10% from the expected value (estimated by quadratic regression) should be omitted.

For ion chromatography without a suppressor:

Calculate a linear regression equation \( Y = a + bx \) from both the slope (b) and the intercept (a) of the linear regression. If the linear regression coefficient \( R^2 \) is < 0.99, the calibration should be repeated. An individual calibration point that differs by > 10% from the expected value (estimated by linear regression) should be omitted.
16.3.8 In both cases, the intercept should not be statistically significantly different from zero.

16.3.9 Inject the quality control samples and the test samples, and determine the peak areas with the instrument software.

16.3.10 The signal (peak area) obtained for all test portions must fall within the working range of the calibration curve.

Note: See Annex 1 for typical calibration lines and chromatograms.

17 DATA ANALYSIS AND CALCULATIONS

17.1 For each test portion, calculate the peak areas of ammonia.

17.2 Calculate the concentration of ammonium ion ($\text{NH}_4^+$) in mg/mL in each test portion from the coefficients of the quadratic or linear regression.

17.3 The amount of ammonia (in mg/g of tobacco) is determined from the following formula:

$$ M = \frac{C \times V \times 17.03}{m \times 18.04} $$

where:

$M$ is the concentration of ammonia ($\text{NH}_3$) in cigarette filler, expressed in mg/g.

$C$ is the concentration of $\text{NH}_4^+$ in the sample solution obtained from the calibration line, expressed in $\mu$g/mL.

$V$ is the volume of the sample solution, expressed in mL.

$m$ is the mass of tobacco filler, expressed in mg.

Note 1: The relative molecular mass of $\text{NH}_3$ is 17.03.

Note 2: The relative molecular mass of $\text{NH}_4^+$ is 18.04.

18 SPECIAL PRECAUTIONS

After installing a new column, condition it by injecting a tobacco sample extract under the specified instrument conditions. Injections should be repeated until the peak areas (or heights) of ammonia are reproducible according to the acceptance criteria of individual laboratories.
19 DATA REPORTING

19.1 Report individual measurements for each sample evaluated.

19.2 Report results as milligrams per gram of tobacco or as required.

19.3 Results may be reported as is or on a dry weight basis.

**Note:** Moisture can be measured with AOAC 966.02 [2.6] or a method of equivalent standard.

20 QUALITY CONTROL

20.1 Typical control parameters

If the control measurements are outside the tolerance limits of the expected values, appropriate investigation and action must be undertaken.

Additional quality assurance procedures should be used if necessary in order to comply with the policies of individual laboratories.

20.2 Laboratory reagent blank

To detect any contamination during sample preparation and analysis, include a laboratory reagent blank. The blank consists of all the reagents and materials used in analysing test samples and is analysed like a test sample. The result should be below the level of detection.

20.3 Quality control sample

To verify the consistency of the entire analytical process, analyse a reference cigarette, such as a University of Kentucky research cigarette (Lexington, Kentucky, USA) or CORESTA Monitor (Paris, France), with each analytical run.

21 METHOD PERFORMANCE

21.1 Limit of reporting

The limit of reporting is set to the lowest concentration of the calibration standards used, recalculated to approximately 0.1 μg/mL.

21.2 Laboratory-fortified matrix recovery

Recovery of analyte spiked onto the matrix is used as a surrogate measure of accuracy. It is determined by spiking known amounts of standards into tobacco and extracting the tobacco in the same way as for samples. Unspiked tobacco is also analysed. Recovery is calculated from the following formula:

\[
\text{Recovery} \% = 100 \times \left( \frac{\text{analytical amount} - \text{unspiked amount}}{\text{spiked amount}} \right)
\]
### Table 3. Sample recovery results for ammonia in tobacco filler

<table>
<thead>
<tr>
<th>Spiked amount (mg)*</th>
<th>Analytical amount (mg)</th>
<th>Unspiked amount (mg)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0799 (low)</td>
<td>0.1751</td>
<td>0.0960</td>
<td>98.90</td>
</tr>
<tr>
<td>0.0999 (medium)</td>
<td>0.1944</td>
<td>0.0956</td>
<td>98.88</td>
</tr>
<tr>
<td>0.1199 (high)</td>
<td>0.2141</td>
<td>0.0953</td>
<td>99.10</td>
</tr>
</tbody>
</table>

*“Low”, “medium” and “high” spiked amounts are about 80%, 100% and 120% of the unspiked amount.

#### 21.3 Limit of detection (LOD) and limit of quantification (LOQ)

The LOD can be determined as three times the standard deviation of results obtained by analysing the lowest standard a minimum of 10 times over several days. The LOQ can be determined as 10 times the standard deviation of results obtained by analysing the lowest standard a minimum of 10 times over several days.

The LOD of ammonia in tobacco filler is 0.0033 mg/g, and the LOQ is 0.011 mg/g.

Alternatively, the lowest standard used can be taken as the LOQ.

#### 22 REPEATABILITY AND REPRODUCIBILITY

An international collaborative study conducted in 2014-2015, involving testing of three reference cigarettes and two commercial brands by nine laboratories gave the following values for this method.

The difference between two single results found for matched cigarette tobacco filler samples by the same operator using the same apparatus within the shortest feasible time will exceed the repeatability, \( r \), on average no more than once in 20 cases with normal, correct application of the method.

Single results for matched cigarette tobacco filler samples reported by two laboratories will differ by no more than the reproducibility, \( R \), on average no more than once in 20 cases with normal, correct application of the method.

The test results were analysed statistically in accordance with ISO 5725-1 [2.3] and ISO 5725-2 [2.4] to give the precision data shown in Table 4. The ammonia concentrations in the commercial brands were within the range of those in the reference pieces.

To calculate \( r \) and \( R \), one test result was defined as the average of seven replicates.
Table 4. Precision limits for determination of ammonia in tobacco from reference test pieces

<table>
<thead>
<tr>
<th>Reference cigarette</th>
<th>n</th>
<th>m</th>
<th>Repeatability limit (mg/g), ( r )</th>
<th>Reproducibility limit (mg/g), ( R )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1R5F</td>
<td>9</td>
<td>1.214</td>
<td>0.002</td>
<td>0.153</td>
</tr>
<tr>
<td>3R4F</td>
<td>9</td>
<td>0.857</td>
<td>0.002</td>
<td>0.075</td>
</tr>
<tr>
<td>CM6</td>
<td>9</td>
<td>0.172</td>
<td>0.000</td>
<td>0.004</td>
</tr>
<tr>
<td>Commercial cigarette 1</td>
<td>9</td>
<td>0.383</td>
<td>0.001</td>
<td>0.010</td>
</tr>
<tr>
<td>Commercial cigarette 2</td>
<td>9</td>
<td>1.746</td>
<td>0.005</td>
<td>0.347</td>
</tr>
</tbody>
</table>

\( n \), number of laboratories that participated; \( m \), mean value of ammonia per cigarette

23 TEST REPORT

The following information shall be included in the test report:

(a) A reference to this method i.e. WHO TobLabNet SOP 07

(b) Date of receipt of the sample

(c) The results and its units
Annex 1. Typical calibration line and chromatograms obtained in the determination of ammonia in cigarette tobacco filler

Fig. 1a. Example of ammonia calibration line (without suppression)

![Ammonia Calibration Line (without suppression)](image)

Fig. 1b. Example of ammonia calibration line (with suppression)

![Ammonia Calibration Line (with suppression)](image)
Fig. 2. Representative chromatogram of a standard solution of ammonia (about 5 µg/mL), with suppression

Fig. 3. Representative chromatogram of a sample solution of tobacco filler, with ammonia peak, with suppression
This document was prepared by members of the World Health Organization (WHO) Tobacco Laboratory Network (TobLabNet) as an analytical method standard operating procedure (SOP) for determination of ammonia in cigarette tobacco filler under International Organization for Standardization (ISO) and intense smoking conditions.