STANDARD OPERATING PROCEDURE FOR DETERMINATION OF NICOTINE AND CARBON MONOXIDE IN MAINSTREAM CIGARETTE SMOKE UNDER INTENSE SMOKING CONDITIONS

Tobacco Free Initiative
Tobacco Laboratory Network (TobLabNet)
World Health Organization Tobacco Laboratory Network SOP 03

Determination of tobacco-specific nitrosamines in mainstream tobacco smoke

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 nicotine and carbon monoxide in mainstream cigarette smoke under intense smoking conditions
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Tobacco Laboratory Network

Standard operating procedure for method

Determination of Nicotine and Carbon Monoxide in Mainstream Cigarette Smoke under Intense Smoking Conditions

Method:  Determination of Nicotine and Carbon Monoxide in Mainstream Cigarette Smoke under Intense Smoking Conditions

Analytes:  Nicotine (CAS# 54-11-5)
Carbon Monoxide (CAS# 630-08-0)

Matrix:  Mainstream Cigarette Smoke

Last update: 21 September 2016
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 risks. Representing differences in machine measurements as differences in exposure or risk is a misuse of testing with WHO TobLabNet standards.
FOREWORD
This document was prepared by members of the World Health Organization (WHO) Tobacco Laboratory Network (TobLabNet) as a standard operating procedure (SOP) for measuring nicotine and carbon monoxide in mainstream cigarette smoke under intense smoking conditions.

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 (COP) 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 measurement (analytical chemistry) should be validated as a priority:

- nicotine
- ammonia
- humectants (propane-1,2-diol, glycerol (propane-1,2,3-triol) and triethylene glycol (2,2-ethylendioxybis(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 four methods: one for tobacco-specific nitrosamines (NNK and NNN), one for benzo[a]pyrene, one for aldehydes (acetaldehyde, acrolein and formaldehyde) and one for volatile organic compounds (benzene, 1,3-butadiene and 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 modifications</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 method SOP was prepared to describe the procedure for the determination nicotine and carbon monoxide in mainstream cigarette smoke under intense smoking conditions.

1. **SCOPE**

This Standard Operating Procedure is suitable for the quantitative determination of nicotine and carbon monoxide in mainstream (MS) cigarette smoke. Nicotine will be analysed by gas chromatography and carbon monoxide by NDIR.

Note: Training in the use of the smoking machine and other analytical equipment is important for successful operation. For those not experienced in operating smoking machines or analysis using the analytical methods for measuring tobacco product emissions and contents, training should be obtained.
2. REFERENCES

2.1 ISO 3308: Routine analytical cigarette-smoking machine — Definitions and standard conditions

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

2.3 ISO 3402: Tobacco and tobacco products — Atmosphere for conditioning and testing

2.4 ISO 10315: Cigarettes — Determination of nicotine in smoke condensates — Gas chromatographic method

2.5 ISO 8454: Cigarettes — Determination of carbon monoxide in the vapour phase of cigarette smoke — NDIR method

2.6 ISO 8243: Cigarettes — Sampling

2.7 ISO 5725-1: Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions.

2.8 ISO 5725-2: Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method.

2.9 World Health Organization Tobacco Laboratory Network, Standard Operating Procedure for Intense Smoking (WHO TobLabNet SOP_01).

2.10 World Health Organization Tobacco Laboratory Network, Standard Operating Procedure for validation of analytical methods of tobacco product contents and emissions. (WHO TobLabNet SOP_02).


3. TERMS AND DEFINITIONS

3.1 \textit{TPM}: total particulate matter.

3.2 \textit{Smoke trap}: device for collecting such part of the smoke from a sample of cigarettes as is necessary for the determination of specified smoke components.
3.3 Vapour phase: portion of smoke which passes the particulate phase trap during smoking in accordance with ISO 4387 using a machine conforming to ISO 3308.

3.4 Clearing puff: any puff taken after a cigarette has been extinguished or removed from the cigarette holder.

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

3.6 Intense Regime: Parameters used to smoke tobacco products which include 55-mL puff volume, 30-second puff interval, and 100% blocking of the filter ventilation holes.

3.7 ISO Regime: Parameters used to smoke tobacco products which include 35-mL puff volume, 60-second puff interval and unblocked filter ventilation holes.

3.8 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.

3.9 Test sample: Product to be tested, taken at random from the laboratory sample. The number of products taken shall be representative of the laboratory sample.

3.10 Test portion: Random sample from the test sample to be used for a single determination. The number of products taken shall be representative of the test sample.

4. METHOD SUMMARY

4.1 All samples are conditioned and marked according to ISO standard procedures.

4.2 Ventilation holes are blocked 100%.

4.3 Cigarettes are smoked according to ISO standard procedures with the exception of puff volume and puff frequency.

4.4 The vapour phase of the cigarette smoke is collected and carbon monoxide is measured using a non-dispersive infrared (NDIR) analyser.

4.5 The particulate phase of the cigarette smoke is collected on a trap for further analyses.

4.6 After extraction from the trap the nicotine content is analysed using gas chromatography.
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 purport to address all the safety aspects associated with its use. All persons using this method have a responsibility to consult the appropriate authorities and to establish 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 Equipment needed to condition cigarettes as specified in ISO 3402 [2.3].

6.2 Equipment needed to mark butt length as specified in ISO 4387 [2.2].

6.3 Equipment needed to cover ventilation holes for the intense regimen as specified in WHO TobLabNet SOP 01 [2.10].

6.4 Equipment needed to perform smoking of cigarettes as specified in ISO 3308 [2.1].

6.5 Vapour phase collection system as specified in ISO 8454 [2.5].

6.6 Equipment needed to perform determination of carbon monoxide as specified in ISO 8454 [2.5].

6.7 Cambridge Filter Pad
Glass fibre filter pad as described in ISO 4387 (44 mm diameter for linear smoking machines, 92 mm diameter for rotary smoking machines) [2.2].

6.8 Extraction flasks: Erlenmeyer flasks (250 mL) or conical flasks (100 mL) with stoppers or other suitable flasks.

6.9 Wrist-action or circular shaker or equivalent device configured to hold extraction flasks in position.

6.10 GC equipped with flame ionization detector (FID) for nicotine determination.

6.11 GC columns capable of distinct separation of peaks for solvent, internal standard, nicotine and tobacco components (e.g. Varian WCOT Fused Silica, coating: CP-WAX 51, 25 m x 0.25 mm x 0.20 µm; DB-Wax, 30 m x 0.53 mm x 1 µm.).
6.12 Non-dispersive infrared (NDIR) analyser, selective and calibrated for the measurement of carbon monoxide in vapours and gases as specified in ISO 8454 [2.5].

6.13 Barometer, capable of measuring atmospheric pressures to the nearest 0.1 kPa ± 0.1 kPa.

6.14 Thermometer, capable of measuring temperature to the nearest 0.1 °C ± 0.1 °C.

7. REAGENTS AND SUPPLIES

All reagents shall be at least analytical reagent grade unless otherwise noted. When possible, reagents are identified by their Chemical Abstract Service (CAS) registry numbers.

7.1 Carrier gas: Helium [CAS Number: 7440-59-7] or nitrogen (CAS: 7727-37-9) of high purity (> 99.999%).

7.2 Auxiliary gases: Air and hydrogen [CAS Number: 1333-74-0] of high purity (> 99.999%) for the flame ionization detector.

7.3 Propan-2-ol [CAS Number: 67-63-0], with a maximum water content of 1.0 g/L.

7.4 (-) Nicotine [CAS Number: 54-11-5] of known purity not less than 98%. Nicotine salicylate [CAS Number: 29790-52-1] of known purity not less than 98% may also be used. The laboratory may verify nicotine purity if necessary.

7.5 Internal Standard for GC analyses:
Nicotine determination:
- n-heptadecane [CAS Number: 629-78-7] or quinaldine [CAS Number: 91-63-4] of purity not less than 99%.
- Eicosane [CAS Number: 112-95-8], Isoquinoline [CAS Number: 119-65-3] or Quinolone [CAS Number: 91-22-5] of purity not less than 98% of mass fraction or other suitable alternatives may also be used.

7.6 Standard Carbon Monoxide (CO) gas mixtures:
At least three standard gas mixtures of accurately known concentrations within a relative error of 2%, covering the expected range in such a way as to avoid extrapolation of the calibration curve.

The volume percentages of CO in nitrogen are typically 1%, 3% and 5%. For low levels of CO, extension of the calibration is recommended, for instance by 0.25%.

8. PREPARATION OF GLASSWARE

Clean and dry glassware so as to prevent contamination from residues.
9. PREPARATION OF SOLUTIONS

9.1 Extraction solvent

Propan-2-ol [7.3] containing an appropriate concentration of internal standard.

Weigh 0.10 g of n-heptadecane [7.5] into a 1-litre volumetric flask.
Mix thoroughly and transfer the solution into a storage container equipped with features to prevent contamination.

Note: The concentration and/or type of internal standard may be adjusted, keeping in mind the possible effect of internal standards on the sensitivity and selectivity, as well as the linear range of the method.

10. PREPARATION OF STANDARDS

10.1 Nicotine determination

10.1.1 Nicotine stock solution (10 mg/mL)
Weigh approximately 1000 mg of nicotine to 0.1 mg accuracy into a 100 mL volumetric flask and dilute to volume with the extraction solvent.
Mix thoroughly and store between 0 °C and 4 °C and exclude light.
Solvent and solutions stored at low temperatures shall be allowed to equilibrate to (22 ± 2) °C before use.

10.1.2 Nicotine working standard solutions
Pipette various volumes of the nicotine stock solution [10.1.1] into separate 100 mL volumetric flasks as indicated in Table 1 and dilute to volume with extraction solvent.
Store between 0 °C and 4 °C and exclude light. Solvent and solutions stored at low temperatures shall be allowed to equilibrate to (22 ± 2) °C before use.

Note: When, in addition to the nicotine determination, a water determination is performed simultaneously on a single GC, the working standard solutions of nicotine and water can be combined.

10.1.3 The final nicotine concentrations of standards (mg/L) are determined from:

\[
\text{Final concentration mg/L} = \frac{x \times y}{10} \times \text{purity of standard}
\]

where:

- \( x \) is the original weight (in mg) of nicotine as weighed in 10.1.1.
- \( y \) is the volume of stock standard solution as pipetted in 10.1.2.

The final nicotine concentrations in the standard solutions are shown in Table 1.
Table 1. Concentrations of nicotine in standard solutions

<table>
<thead>
<tr>
<th>Standard</th>
<th>Volume of nicotine stock solution (10 mg/mL)</th>
<th>Volume of internal standard solution (mL)</th>
<th>Total volume (mL)</th>
<th>Approximate nicotine concentration in final standard solution (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2</td>
<td>100</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>100</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>4</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>6</td>
<td>600</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>8</td>
<td>800</td>
<td></td>
</tr>
</tbody>
</table>

The range of the standard solutions may be adjusted, depending on the equipment used and the samples to be tested, keeping in mind the possible effect on the sensitivity of the method.

11. SAMPLING

Sample cigarettes according to ISO 8243 [2.6]. 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 [2.11] of the individual units (e.g. packet, container).

12. CIGARETTE PREPARATION

12.1 Condition all cigarettes to be smoked in accordance to ISO 3402 [2.3]

12.2 Mark cigarettes at a butt length in accordance with ISO 4387 [2.2] and World Health Organization Tobacco Laboratory Network, Standard Operating Procedure for Intense Smoking (WHO TobLabNet SOP_01) [2.9].

12.3 Prepare test samples to be smoked under intense smoking conditions as specified in World Health Organization Tobacco Laboratory Network, Standard Operating Procedure for Intense Smoking (WHO TobLabNet SOP_01) [2.9].

13. PREPARATION OF THE SMOKING MACHINE

13.1 Ambient conditions

The ambient conditions for smoking are specified in ISO 3308 [2.1].
13.2 **Smoking machine specifications**

Follow ISO 3308 [2.1] machine specifications, except for intense smoking as described in World Health Organization Tobacco Laboratory Network, Standard Operating Procedure for Intense Smoking (WHO TobLabNet SOP_01) [2.9].

14. **SAMPLE GENERATION**

Smoke a sufficient number of cigarettes on the specified smoking machine such that breakthrough does not occur and the concentrations of nicotine and carbon monoxide fall within the calibration range prepared for the analysis.

14.1 Smoke the cigarette test samples and collect the TPM, as specified in ISO 4387 [2.2] or in WHO TobLabNet SOP_01 [2.9].

14.2 Collect the vapour phase in a suitable collection system [6.5] as specified in ISO 8554 [2.5].

14.3 Include at least one reference test sample to be used for quality control, if applicable.

14.4 When testing sample types for the first time, breakthrough should be evaluated. The number of cigarettes might have to be adjusted to prevent breakthrough of the filter pad [6.7]. If the TPM exceeds 600 mg for a 92 mm filter pad or 150 mg for a 44 mm filter pad, the number of cigarettes smoked onto each pad must be decreased.

14.5 The number of cigarettes to be smoked per measurement for linear and rotary smoking machines at ISO and intense smoking regimens are shown in Table 2.

<table>
<thead>
<tr>
<th>Table 2. Number of cigarettes to be smoked for one measurement in linear and rotary smoking machines.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>No. of cigarettes per smoke trap</td>
</tr>
<tr>
<td>No. of smoke traps per result</td>
</tr>
</tbody>
</table>

14.6 After smoking an individual cigarette, remove the cigarette butt and take one clearing puff for each trap. After smoking the required number of test samples per trap, perform five clearing puffs for each trap and remove the smoke trap from the smoking machine.

14.7 Record the number of cigarettes and total puffs for each smoke trap, including clearing puffs.

14.8 Perform a blank measurement, at least once per day, by placing a Cambridge Filter Pad [6.7] in the smoking machine area during a smoke run.
15. **SAMPLE PREPARATION**

15.1 After collection of TPM, transfer each Cambridge filter (CF) pad [6.7] from the holder (for linear — three cigarettes smoked per 44 mm CF pad; for rotary — 10 cigarettes smoked per 92 mm CF pad) into a clean, dry, 100 mL conical flask for 44 mm disks and a 250 mL conical flask for 92 mm discs with glass stopper [6.8].

15.2 Wipe the front half of the holder twice using a ¼ clean 44 mm CF pad for linear smoking machines and twice using a clean 44 mm CF pad for rotary smoking machines and add these to the respective flask.

15.3 Add 20 mL extraction solvent [9.1] for 44 mm discs and 50 mL extraction solvent [9.1] for 92 mm discs into each flask. Ensure that the discs are fully covered.

15.4 Shake the flasks for about 30 minutes on the shaker [6.9] at about 200 rpm. Adjust the time or speed to prevent disintegration of the pads.

16. **SAMPLE ANALYSIS**

16.1 **TPM weight**

The method for determining TPM weight is described in ISO 4387 [2.2].

16.2 **Nicotine Content**

The method for determining nicotine in mainstream cigarette smoke is described in ISO 10315 [2.4].

Suitable GC–FID operating conditions: example

- Injector temperature: 250 °C
- Detector temperature: 250 °C
- Carrier gas: Helium, or nitrogen at a flow rate of 25 mL/min
- Injection: 1 μL or 2 μL
- Column temperature: 170 °C (Isothermal)

**Note:** The operating parameters may have to be adjusted to the instrument and column conditions and the resolution of the chromatographic peaks.

16.3 **Carbon Monoxide (CO) Content**

The method for determining carbon monoxide in the vapour phase of smoke is described in ISO 8454 [2.5].

17. **DATA ANALYSIS AND CALCULATIONS**

17.1 **Nicotine Content**

17.1.1 For each sample extract, calculate the ratio of the nicotine response to the internal standard response from the peak area.
17.1.2 Calculate the nicotine concentration in mg/L in each sample extract using the coefficients of the linear regression

\[ m_t = \frac{(y_i - a)}{b} \]

where;

- \( m_t \) is the concentration of nicotine in the sample extract in mg/L
- \( Y_i \) is the ratio of nicotine response to the internal standard response from the peak area
- \( a \) is the intercept of the linear regression obtained from the standard calibration curve
- \( b \) is the slope of the linear regression obtained from the standard calibration curve

17.1.3 The nicotine content for each sample extract, in mg per cigarette, is calculated as follows:

\[ \text{Nicotine content (mg/cigarette)} = \frac{m_t \times V}{1000 \times N} \]

where;

- \( m_t \) : Concentration of nicotine in the sample extract, in mg/L.
- \( N \) : Number of cigarettes smoked through each smoke trap (three for linear; 10 for rotary).
- \( V \) : Volume of extraction solvent in which the contents of the extract was dissolved (20 mL for linear; 50 mL for rotary).

17.2 Carbon Monoxide (CO) content

17.2.1 The average volume of carbon monoxide per cigarette is given by the following equation:

\[ V_{as} = \frac{C \times V \times N \times p \times T_o}{S \times 100 \times p_o \times (t + T_o)} \]

where;

- \( V_{as} \) is the average volume of carbon monoxide per cigarette, in mL
- \( C \) is the percentage by volume of carbon monoxide observed;
- \( V \) is the puff volume, in mL;
- \( N \) is the number of puffs in the measured sample (including clearing puffs);
17.2.2 The average mass of carbon monoxide per cigarette is given by the following equation

\[ m_{\text{CO}} = V_{\text{as}} \times \frac{M_{\text{CO}}}{V_{\text{m}}} \]

where;
- \( V_{\text{as}} \) is the average volume of carbon monoxide per cigarette, in mL
- \( m_{\text{CO}} \) is the average mass of carbon monoxide per cigarette, in mg
- \( M_{\text{CO}} \) is the molar mass of carbon monoxide, in g/mol;
- \( V_{\text{m}} \) is the molar volume of an ideal gas, in L/mol

18. SPECIAL PRECAUTIONS

18.1 After installing a new column, condition it as specified by the manufacturer by injecting a tobacco sample extract under the specified instrument conditions. Injections should be repeated until the peak areas (or heights) for the analyte of interest and internal standard are reproducible.

18.2 When the peak areas (or heights) for the internal standard are significantly higher than expected, it is recommended that the tobacco sample be extracted without internal standard in the extraction solution. This makes it possible to determine whether any component co-elutes with the internal standard, which would cause artificially lower values for nicotine.

19. DATA REPORTING

19.1 Report individual measurements for each of the samples evaluated.

19.2 Report results as specified by method specifications.

19.3 For more information, see World Health Organization Tobacco Laboratory Network, Standard Operating Procedures for validation of analytical methods of tobacco product contents and emissions. (WHO TobLabNet SOP_02). [2.10].
20. QUALITY CONTROL

20.1 Control Parameters

**Note**: If the control measurements are outside the tolerance limits of the expected values, appropriate investigation and action must be taken.

**Note**: Additional individual laboratory quality assurance procedures should be carried out if necessary in order to comply with the policies of individual laboratories.

20.2 Laboratory Reagent Blank (LRB)

To detect potential contamination during sample preparation and analysis, include a laboratory reagent blank. The blank consists of all reagents and materials used in analysing test samples and is analysed like a test sample. The blank should be assessed in accordance with the practices of individual laboratories.

20.3 Quality Control Sample

To verify consistency of the entire analytical process, analyse a reference cigarette (or an appropriate quality control sample) in accordance with the practices of the individual laboratories.

21. METHOD PERFORMANCE SPECIFICATIONS

21.1 Limit of Reporting (LOR)

The limit of reporting is set to the lowest concentration of the calibration standards used, recalculated to mg per cigarette. (e.g. for the linear smoking machine the lowest nicotine calibration standard of 20 mg/L corresponds to 0.1 mg per cigarette).

21.2 Analytical Specificity

For gas chromatographic determinations (nicotine), the retention time of the analyte of interest is used to verify the analytical specificity. An established range of ratios of the response of the component to that of the internal standard component of a quality control cigarette is used to verify the specificity of the results for an unknown sample.

21.2 Linearity

Nicotine Content: The nicotine calibration curves established are linear over the standard calibration range of 20–800 mg/L.

21.3 Possible interferences

The presence of eugenol can cause interference, as its retention time is similar to that of nicotine. This interference is most likely to occur with samples containing clove. The laboratory may need to resolve this by adjusting the analytical instrument parameters.
22. REPEATABILITY AND REPRODUCIBILITY

A global collaborative study conducted in 2007, involving 14 laboratories and three reference cigarettes (1R5F, 3R4F and CM6) and two commercial brands, was conducted to validate a method for assessing TNCO in mainstream cigarette smoke following ISO standards using an intense smoking regimen [2.12]. The precision limits for this method are indicated in Table 3 and Table 4.

The difference between two single results found for matched cigarette samples by the same operator using the same apparatus within the shortest feasible time will exceed the repeatability, r, on average not more than once in 20 cases in the normal, correct application of the method.

Single results for matched cigarette samples reported by two laboratories will differ by 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 [2.7] and ISO 5725-2 [2.8] to give the precision data.

Table 3. Precision limits for determination of Carbon monoxide (CO) (mg/cig) in cigarette

<table>
<thead>
<tr>
<th>Reference Cigarettes</th>
<th>N</th>
<th>m_{\text{mg}}</th>
<th>r_{\text{avg}}</th>
<th>R_{\text{avg}}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1R5F</td>
<td>13</td>
<td>27.39</td>
<td>2.53</td>
<td>4.62</td>
</tr>
<tr>
<td>3R4F</td>
<td>13</td>
<td>32.19</td>
<td>2.24</td>
<td>2.97</td>
</tr>
<tr>
<td>CM6</td>
<td>13</td>
<td>26.87</td>
<td>1.40</td>
<td>2.91</td>
</tr>
</tbody>
</table>

Table 4. Precision limits for determination of Nicotine (mg/cig) in cigarette

<table>
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<tr>
<th>Reference Cigarettes</th>
<th>N</th>
<th>m_{\text{mg}}</th>
<th>r_{\text{avg}}</th>
<th>R_{\text{avg}}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1R5F</td>
<td>13</td>
<td>1.02</td>
<td>0.08</td>
<td>0.17</td>
</tr>
<tr>
<td>3R4F</td>
<td>13</td>
<td>1.91</td>
<td>0.14</td>
<td>0.31</td>
</tr>
<tr>
<td>CM6</td>
<td>13</td>
<td>2.73</td>
<td>0.20</td>
<td>0.42</td>
</tr>
</tbody>
</table>
Appendix 1. Typical chromatograms obtained in the analysis of nicotine by GC method

Figure 1. Example of a chromatogram of a nicotine standard solution

Figure 2. Example of a chromatogram of a nicotine sample solution
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 nicotine and carbon monoxide in mainstream cigarette smoke under intense smoking conditions.