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WHO TobLabNet
Official Method
SOP 04

Standard Operating Procedure for Determination of Nicotine in Cigarette Tobacco Filler

Tobacco Free Initiative
Tobacco Laboratory Network (TobLabNet)

World Health Organization

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Standard operating procedure for determination of nicotine in cigarette tobacco filler

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Tobacco Laboratory Network

Standard operating procedure for method

Determination of nicotine in cigarette tobacco filler

Method:	**Determination of nicotine in cigarette tobacco filler**
Analytes:	**Nicotine(3-[(2S)-1-methylpyrrolidin-2-yl]pyridine) (CAS # 54-11-5)**
Matrix:	**Cigarette tobacco filler**
Last update:	**January 2014**

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The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by WHO in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

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.

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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 nicotine 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 (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
- 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, 1,3-butadiene) and one for carbon monoxide.

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

Smoking regimen	Puff volume (ml)	Puff frequency	Filter ventilation holes
ISO regimen: ISO 3308; *Routine analytical cigarette smoking machine—definitions and standard conditions*	35	Once every 60 s	No modifications
Intense regimen: Same as ISO 3308 but modified as indicated	55	Once every 30 s	All ventilation holes must be blocked 100% as described in WHO TobLabNet SOP 01.

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

1 SCOPE

This method is suitable for the quantitative determination of nicotine in cigarette tobacco filler by gas chromatography (GC).

2 REFERENCES

2.1 *ISO 3402: Tobacco and tobacco products—Atmosphere for conditioning and testing.*

2.2 *ISO 13276:Tobacco and tobacco products—Determination of nicotine purity—Gravimetric method using tungstosilicic acid.*

2.3 *ISO 8243: Cigarettes—Sampling*

2.4 United Nations Office on Drugs and Crime. *Guidelines on representative drug sampling*. Vienna, Laboratory and Scientific Section, 2009 (http://www.unodc.org/documents/scientific/Drug_Sampling.pdf).

2.5 World Health Organization. *Standard operating procedure for validation of analytical methods of tobacco product contents and emissions*. Geneva, Tobacco Laboratory Network, (WHO TobLabNet SOP 02 in preparation).

2.6 World Health Organization. *Method validation report of World Health Organization TobLabNet official method: Determination of tar, nicotine, and carbon monoxide in mainstream cigarette smoke under ISO and intense smoking conditions*. Geneva, Tobacco Laboratory Network, 2012, in preparation.

2.7 *ISO5725-1. Accuracy (trueness and precision) of measurement methods and results—Part 1: General principles and definitions.*

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

3 TERMS AND DEFINITIONS

3.1 *Nicotine content*: Total amount of nicotine in cigarette tobacco filler, expressed as milligrams per gram 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 entirely or partly made of leaf tobacco as the raw material that are manufactured to be used by 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 After conditioning, the cigarette tobacco filler is ground and mixed.

4.2 Nicotine is extracted from the cigarette tobacco filler with a mixture of *n*-hexane, sodium hydroxide solution and water.

4.3 The organic layer is analysed by GC with a flame ionization detector.

4.4 The ratio of nicotine peak area to internal standard is compared on a calibration curve created by analysis of standards with known concentrations of nicotine to determine the nicotine content of each test portion.

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 the 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 required to condition cigarette tobacco filler as specified in ISO 3402 [**2.1**].

6.2 Extraction flasks: Erlenmeyer flasks (100-ml) with stoppers, 100-ml Pyrex bottles with crimp seals and septa, 100-ml culture tubes with Teflon-lined caps or other suitable flasks

6.3 Linear shaker configured to hold the extraction flasks in position

6.4 Capillary GC equipped with a flame ionization detector

6.5 Capillary GC column capable of distinct separation of peaks for the solvent, the internal standard, nicotine and other tobacco components (e.g. Varian WCOT Fused Silica, 25 m × 0.25 mm ID; coating: CP-WAX 51)

6.6 Ultrasonic bath

7 REAGENTS AND SUPPLIES

All reagents shall be of 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 [7440-59-7] of high purity (> 99.999%)

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

7.3 *n*-Hexane [110-54-3], GC grade, with a maximum water content of 1.0 g/L

7.4 –(–)Nicotine [54-11-5] of known purity not less than 98% [**2.2**]. Nicotine salicylate [29790-52-1] of known purity not less than 98% may be used.

7.5 Sodium hydroxide [1310-73-2] pellets

7.6 Internal standard: *n*-Heptadecane (purity > 98% of mass fraction) [629-78-7]. Quinaldine [91-63-4], isoquinoline [119-65-3], quinoline [91-22-5] or other suitable alternatives may be used.

8 PREPARATION OF GLASSWARE

8.1 Clean and dry glassware in a manner to avoid contamination.

9 PREPARATION OF SOLUTIONS

9.1 Sodium hydroxide solution (2 mol/L)

9.1.1 Weigh approximately 80 g of sodium hydroxide.

9.1.2 Dissolve measured sodium hydroxide in water and dilute with water to 1 L.

9.2 Extraction solution (0.5 mg/ml)

9.2.1 Weigh approximately 0.5 g (to 0.001-g accuracy) of *n*-heptadecane or alternative internal standard.

9.2.2 Dissolve measured *n*-heptadecane or alternative internal standard in *n*-hexane, and dilute to 1 L with *n*-hexane.