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Cigarettes — Determination of selected carbonyls in the mainstream smoke of cigarettes with an intense smoking regime — Method using high performance liquid chromatography

Cigarettes — Dosage de carbonyles sélectionnés dans le courant principal de la fumée de cigarette avec un régime de fumage intense — Méthode par chromatographie liquide haute performance

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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).

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

At the outset of this work, discussions in the CORESTA (www.coresta.org) Special Analytes Sub-Group (since 2017 the Sub-Group changed its name to Smoke Analytes) determined that most laboratories used a method involving derivatization of carbonyls with 2,4-dinitrophenylhydrazine (DNPH) because they considered it the most suitable. This was chosen as the basis of the CORESTA Recommended Method (CRM). The CRM comprised smoke collection in impinger traps, derivatization of carbonyls with DNPH followed by their determination using reversed phase High Performance Liquid Chromatography with Ultra Violet or Diode Array Detection (HPLC-UV or HPLC-DAD).

This document was produced from a 2012 collaborative study involving 19 laboratories from 11 countries and included 10 samples with different tar yields[1][2]. This method includes recommendations about critical steps that should be controlled to provide data as robust and consistent as the repeatability and reproducibility data provided. Cigarettes were smoked using the intense smoking regime parameters given in Health Canada Official Method T-115. At the time the collaborative study was conducted, the study protocol stipulated the use of Health Canada Official Method (T-115) for intense conditions as there was not an International Standard that defined intense smoking conditions. ISO 20778 was published in 2018 and is equivalent to Health Canada Intense conditions. Statistical evaluations were carried out according to ISO 5725-1[3] and ISO 5725-2[4].

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 differences in 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 International Standards.

Cigarettes — Determination of selected carbonyls in the mainstream smoke of cigarettes with an intense smoking regime — Method using high performance liquid chromatography

WARNING — The use of this document can involve 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 determination of selected carbonyls (formaldehyde, acetaldehyde, acetone, acrolein, propionaldehyde, crotonaldehyde, 2-butanone and *n*-butyraldehyde) as their 2,4-dinitrophenylhydrazones by reversed phase HPLC-UV/DAD in mainstream smoke using an intense smoking regime.

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

ISO 8243, *Cigarettes — Sampling*

ISO 20778, *Cigarettes — Routine analytical cigarette smoking machine — Definitions and standard conditions with an intense smoking regime*

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

Cigarettes are smoked on a standard smoking machine as specified in ISO 20778 that has been fitted with impingers, but without a glass fibre filter pad as described in ISO 20778 [e.g. Cambridge filter pad[1)] (CFP), for example of equivalent product] and the filter pad holder, with the ISO 20778 smoking regime.

The carbonyls in mainstream tobacco smoke are trapped by passing each puff through an impinger device containing an acidified solution of 2,4-dinitrophenylhydrazine (DNPH) in 1:1 acetonitrile:water.

1) Cambridge filter pad (CFP) 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.

An aliquot of the smoke extract is then syringe-filtered and diluted with tris-(hydroxymethyl)-aminomethane dilution solution.

The samples are subjected to analysis using reversed phase HPLC-UV or HPLC-DAD.

5 Apparatus

The usual laboratory apparatus and equipment for use in preparation of samples and standards is needed in addition to the list provided below.

5.1 Equipment for conditioning of tobacco products.

5.2 Equipment for butt length marking.

5.3 Equipment for smoking of tobacco products, complying with ISO 20778.

5.4 Impingers for trapping mainstream smoke.

5.5 Polyvinylchloride (PVC) tubing, suitable for connection of the trapping system.

5.6 Analytical balance, capable of measuring to four decimal places.

5.7 Amber glass flasks, of capacities 10 ml, 25 ml, 200 ml, 1 l and 2 l.

5.8 Pipettes, of appropriate volumes.

5.9 Glass graduated measuring cylinders, of capacities 25 ml, 50 ml and 100 ml.

5.10 Dispenser, capable of delivering 35 ml.

5.11 Hot plate/stirrer.

5.12 Syringe filter, 0,45 µm PVDF or equivalent.

5.13 Disposable syringes, 5 ml.

5.14 Disposable glass Pasteur pipettes.

5.15 Amber Autosampler vials, caps and PTFE faced septa.

5.16 HPLC system, consisting of:

— tertiary gradient pump;

— auto-sampler with appropriate sampling loop;

— UV and/or DAD detector;

— data collection system;

— LC column: 250 mm × 4 mm, 100 Å, Reversed Phase (RP) C18 endcapped (5 µm), or equivalent;

— disposable guard column: 4 mm × 4 mm RP C18 endcapped (5 µm), or equivalent;

— vacuum filter;

— amber glass bottles 1 l and 4 l;

— desiccator.

6 Reagents

6.1 Acetonitrile, MeCN, HPLC grade.

6.2 Isopropanol, IPA, HPLC grade.

6.3 Ethyl acetate, HPLC grade.

6.4 Tetrahydrofuran, THF, HPLC grade.

6.5 Ethanol, HPLC grade.

6.6 Phosphoric acid, 85 %.

6.7 Deionized water, resistivity >18,0 MΩ.cm at 25 °C.

6.8 Formaldehyde-DNPH, min. 99 %.

6.9 Acetaldehyde-DNPH, min. 99 %.

6.10 Acetone-DNPH, min. 99 %.

6.11 Acrolein-DNPH, min. 99 %.

6.12 Propionaldehyde-DNPH, min. 98 %.

6.13 Crotonaldehyde-DNPH, min. 99 %.

6.14 2-Butanone-DNPH, min. 98 %; methyl ethyl ketone-DNPH derivative.

6.15 *n*-Butyraldehyde-DNPH, min. 99 %.

6.16 Tris-(hydroxymethyl)-aminomethane, ACS reagent grade[2)].

6.17 2,4-Dinitrophenylhydrazine (DNPH) (approximately 30 % water).

7 Preparation

7.1 Preparation of glassware

Glassware shall be cleaned and dried in such a manner as to ensure that contamination from glassware does not occur.

All possible sources of contamination shall be removed from the work area (e.g. acetone solvent wash bottles).

2) A reagent that meets the requirements of the American Chemical Society (ACS) Committee on Analytical Reagents.

7.2 Preparation of solutions

7.2.1 DNPH solution (using phosphoric acid)

Add approximately 150 ml deionized water to a 200 ml volumetric flask, then carefully add 28 ml of 85 % phosphoric acid (approximately 2,05 mol/l) and mix the solution.

Make up the solution to volume with deionized water.

Weigh approximately 6,8 g (24,0 mmol should be achieved) of DNPH (approximately 30 % water) into a 2 l volumetric flask and add 1 l of acetonitrile. Dissolve DNPH by alternately gently swirling the flask. Make sure there are no crystals remaining.

WARNING — Do not sonicate as a precipitation of DNPH may occur.

If using re-crystallized DNPH, weigh 4,8 g to achieve the same molarity (see Annex A).

After the DNPH is dissolved, add 58 ml of the diluted phosphoric acid solution while gently mixing. Dilute to volume with deionized water. The colour of the solution will become bright orange upon addition of the deionized water.

The addition of acid or water will cool the solution and may initiate the precipitation of the DNPH. Add the acid or water slowly. Gentle swirling may be required to maintain the solution at room temperature and to prevent the precipitation of DNPH. If crystals appear, do not sonicate.

Store the solution in an appropriately sized amber bottle at room temperature. This solution has been shown to be stable for one week. Stability shall be assessed by each laboratory.

7.2.2 Tris-(hydroxymethyl)-aminomethane dilution solution, 80:20 (volume fraction), MeCN: aqueous solution.

Dissolve 2,00 g of tris-(hydroxymethyl)-aminomethane in 200 ml of deionized water in a 1 l volumetric flask. Dilute to volume with acetonitrile.

Store in a 1 l amber bottle with PTFE-lined cap or equivalent at ambient temperature.

7.3 Preparation of standards

7.3.1 HPLC calibration standards and working solutions

The calibration should cover the concentration range of interest.

7.3.1.1 Primary carbonyl standards

Where available, certified reference solutions of the selected hydrazones can be used.

Weigh the hydrazones as described in Annex B into individual 25 ml volumetric flasks and dissolve in acetonitrile. Record the concentrations of the free aldehyde equivalents in µg/ml.

These solutions have been shown to be stable for up to one year when stored at approximately 4 °C. Stability and storage time should be checked by the laboratory.

7.3.1.2 Secondary carbonyl standards

Pipette predetermined volumes (Annex B) of each primary hydrazone standard into a 25 ml volumetric flask and dilute to the mark with acetonitrile.

Store at approximately 4 °C. Stability and storage time should be checked by the laboratory.

7.3.2 Carbonyl working standards

Take appropriate volumes (0,050 ml to 10 ml) of the secondary carbonyl standard (7.3.1.2) and dilute to 10 ml with acetonitrile to prepare calibration standards with approximate carbonyl concentrations (see Annex B).

Transfer to auto-sampler vials.

The calibration range described in Annex B has been shown to be suitable; however, it can be necessary to adjust the calibration range depending on factors such as the number of cigarettes smoked and the carbonyl yields of the test cigarettes. The user shall ensure the low calibration standard has a sufficient signal to noise ratio for accurate quantitation (≥10:1) and that the calibration curve is linear.

These solutions have been shown to be stable for 20 days when stored at approximately 4 °C. Stability and storage time should be checked by the laboratory.

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

Cigarettes are smoked in accordance with ISO 20778.

10.2 Smoking machine setup

An analytical cigarette smoking-machine complying with the requirements of ISO 20778 is required with the following modifications as detailed below.

No filter pad is required in the set up and therefore puff count information is the only means of monitoring whether the smoking process is controlled.

Add 35 ml of DNPH solution to each impinger. Assemble the carbonyl mainstream apparatus on the smoking machine without using the filter pads and filter holders (Figure 1).

A volume other than 35 ml of DNPH solution may need to be added to each impinger depending on the particular style of impinger used.

Check and adjust the puff volume drawn by the smoking machine at all channels at the cigarette end of the port as described in ISO 20778 with the impingers and DNPH in line.