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Cigarettes — Determination of selected carbonyls in the mainstream smoke of cigarettes — Method using high performance liquid chromatography

Cigarettes — Dosage de carbonyles sélectionnés dans le courant principal de la fumée de cigarette — 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 on 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 the following URL: 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).

Initial joint experiments and ongoing discussions addressed some methodological aspects that needed to be evaluated before the drafting of a CRM. This method was produced through a collaborative study undertaken in 2010 involving 15 laboratories from 11 countries using the ISO 3308 smoking regime[1]. Further data are provided for the same selected carbonyl compounds from 10 samples with different tar yields from the collaborative study in 2012, which involved 19 laboratories from 11 countries[2].

This method includes recommendations about some of the critical steps that should be controlled to provide data as robust and consistent as the repeatability and reproducibility data provided in the ISO document. Statistical evaluations were carried out according to ISO 5725-1 and ISO 5725-2[3],[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 ISO standards.

Cigarettes — Determination of selected carbonyls in the mainstream smoke of cigarettes — 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 in mainstream smoke using reversed phase HPLC-UV/DAD.

This method is applicable to cigarettes with nicotine-free dry particulate matter (NFDPM) yields between 1 mg/cigarette and 15 mg/cigarette using reversed phase HPLC-UV/DAD.

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

Cigarettes are smoked on a standard smoking machine as specified in ISO 3308 that has been fitted with impingers, but without a glass fibre filter pad as described in ISO 3308 (Cambridge filter pad; CFP, for example of equivalent product) and the filter pad holder, under the ISO 3308 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.

An aliquot of the smoke extract is then syringe-filtered and diluted with 1 % tris-(hydroxymethyl)-aminomethane in aqueous acetonitrile.

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

5 Apparatus

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

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 3308.

5.4 Impingers for trapping mainstream smoke.

5.5 Erlenmeyer flasks, of capacities 150 ml, with ground glass stoppers, (or equivalent for combining impinger solutions).

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

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

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

5.9 Glass micropipettes, of capacities 50 µl, 100 µl, 150 µl, 300 µl, 400 µl, 500 µl, 800 µl, 1 000 µl and 2 000 µl.

5.10 Volumetric pipettes, of capacities 1 ml, 2 ml, 5 ml, 6 ml, 7 ml, 8 ml and 20 ml.

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

5.12 Dispenser, capable of delivering 35 ml.

5.13 Hot plate/stirrer.

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

5.15 Disposable syringes, 5 ml.

5.16 Disposable glass Pasteur pipettes.

5.17 Rubber bulbs.

5.18 Autosampler vials, caps and PTFE faced septa.

5.19 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) 18e (5 µm), or equivalent;

— disposable guard column: 4 mm × 4 mm RP 18e (5 µm), or equivalent;

— vacuum filter;

— amber glass bottles 1 l and 4 l;

— desiccator;

— degasser (optional).

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[1)].

6.17 2,4-Dinitrophenylhydrazine (DNPH).

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

6.18 Helium, (UHP), if necessary for sparging of HPLC system mobile phase or equivalent degassing system.

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).

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 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 amber 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 a 4 l amber bottle at room temperature in the dark to prevent or significantly reduce the chances of DNPH precipitation. This solution, if properly sealed, will remain stable for one week.

7.2.2 Tris-(hydroxymethyl)-aminomethane dilution solution, 80:20 (volume fraction), MeCN: 1 % 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

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

The smoking parameters for which the method has been studied are defined in ISO 3308. See Table 1.

Table 1 — Smoking parameters for ISO smoking regime

Smoking regime	Puff volume (ml)	Puff frequency (s)	Puff duration (s)	Ventilation blocking (%)
ISO 3308	35	60	2	0

10.2 Smoking machine setup

An analytical cigarette smoking-machine complying with the requirements of ISO 3308 is required with the following modifications as detailed below.