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Cigarettes — Determination of benzo[a]pyrene in cigarette mainstream smoke with an intense smoking regime using GC/MS —

Part 2:
Method using cyclohexane as extraction solvent

Cigarettes — Dosage par CG/SM du benzo[a]pyrène dans le courant principal de la fumée de cigarette avec un régime de fumage intense —

Partie 2: Méthode utilisant du cyclohexane comme solvant d'extraction

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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*.

A list of all parts in the ISO 23906 series can be found on the ISO website.

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

No machine smoking regime can represent all human smoking behaviours.

— 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 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.

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Part 2:
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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 benzo[a]pyrene (B[a]P) in the total particulate matter of cigarette mainstream smoke with an intense smoking regime using gas chromatography/mass spectrometry (GC/MS) with cyclohexane as extraction solvent.

This method is specified using ISO 20778 smoking parameters. An alternative method for the determination of B[a]P is specified in ISO 23906-1, with a different clean-up using methanol solvent.

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*

ISO 20779, *Cigarettes — Generation and collection of total particulate matter using a routine analytical smoking machine with an intense smoking regime*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

4 Principles

— Sampling of the test cigarettes according to the sampling procedure specified in ISO 8243.

— Conditioning of the test cigarettes according to the conditioning procedure specified in ISO 3402.

— Smoking of the test cigarettes according to the smoking procedure specified in ISO 20779.

— Extraction of the total particulate matter, collected on the glass-fibre filter pad, with cyclohexane.

— Clean-up procedure using solid phase extraction (SPE).

— Analytical determination of B[a]P by gas chromatography/mass spectrometry.

5 Apparatus

The usual laboratory apparatus and equipment and, in particular, the following.

5.1 Routine analytical cigarette-smoking machine, in accordance with the requirements of ISO 20778, and equipped for smoking in accordance with ISO 20779.

5.2 Gas chromatograph with a mass selective detector, equipped with its computerized control and data acquisition and processing system. This system shall be able to pilot the mass spectrometer in order to obtain chromatographic data under single ion monitoring (SIM) detection mode. The gas chromatograph shall be configured to perform splitless injections on a capillary column. It is recommended to equip the gas chromatograph with an autosampler for sample injection.

5.3 Fused silica capillary column, for example a 50 % phenyl-, 50 % methyl-polysiloxane stationary phase and a 30 m length, 0,25 mm internal diameter column with a 0,25 µm film thickness are suitable for this analysis.

NOTE Other columns can be used, provided that appropriate peak separation is obtained.

5.4 TurboVap®[1)] evaporator or equivalent equipment.

5.5 Vacuum sample preparation unit or equivalent equipment.

5.6 Solid phase extraction cartridges, NH_2 bonded silica phase volume of 3 ml and packed with 500 mg is suitable.

NOTE Other cartridges with the same phase but different dimensions can be used if it is proved that results are equivalent.

5.7 Positive displacement pipettes, suitable for a volume range of 10 µl to 1 000 µl.

5.8 General laboratory equipment, for the preparation of samples, standards and reagents. All glassware shall be cleaned before use to avoid any contamination.

5.9 Ultrasonic bath.

5.10 Shaker, set to 200 r/min.

5.11 Glassware, conical flasks with ground glass stoppers (50 ml, 100 ml), volumetric flasks (20 ml, 100 ml, 2 000 ml), test tubes (e.g. 16 mm diameter, 150 mm length).

1) TurboVap® 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.

6 Reagents

WARNING — Benzo[a]pyrene and benzo[a]pyrene-d12 are carcinogens. Appropriate safety precautions shall be taken when manipulating these compounds or any solution containing these compounds.

All reagents shall be of analytical grade quality.

6.1 Hexane, of known purity, not less than 99 %, CAS 110-54-3.

6.2 Cyclohexane, of known purity, not less than 99 %, CAS 110-82-7.

6.3 Benzo[a]pyrene, of known purity, not less than 98 %, CAS 50-32-8.

6.4 Benzo[a]pyrene-d12, of known purity, not less than 98 %, CAS 63466-71-7.

6.5 Helium, carrier gas of known purity, not less than 99,999 %, CAS 7440-59-7.

7 Standards

7.1 General

The use of certified B[a]P and B[a]P-d12 solutions as reference material is possible.

7.2 Primary B[a]P-d12 stock solution: 100 µg/ml

Dissolve 10 mg B[a]P-d12, weighed to the nearest 0,01 mg, into a 100 ml volumetric flask and fill to the mark with cyclohexane. Sonicate to ensure dissolution.

7.3 Secondary B[a]P-d12 spiking solution: 40 ng/ml

Transfer 800 µl of the primary B[a]P-d12 stock solution (7.2) into a 2 000 ml volumetric flask and fill to the mark with cyclohexane.

7.4 Primary B[a]P stock solution: 100 µg/ml

Dissolve 10 mg B[a]P, weighed to the nearest 0,01 mg, into a 100 ml volumetric flask and fill to the mark with secondary B[a]P-d12 spiking solution (7.3).

7.5 Secondary B[a]P stock solution: 1 000 ng/ml

Dilute 1 ml of the primary B[a]P stock solution (7.4) into a 100 ml volumetric flask and fill to the mark with secondary B[a]P-d12 spiking solution (7.3).

7.6 Working standard solutions

Prepare six working standard solutions that cover the concentration range of interest. For example, transfer 100 µl of the secondary B[a]P stock solution (7.5) into a 20 ml volumetric flask and then fill to the mark with secondary B[a]P-d12 spiking solution (7.3). These solutions have a mass concentration of approximately 40 ng/ml of B[a]P-d12 and mass concentrations from 2,5 ng/ml to 250 ng/ml of B[a]P.

7.7 Storage of standard solutions

The standard solutions (7.2 to 7.6) are stable for up to four months if stored in the refrigerator at maximum 4 °C. Laboratories must determine stability under their storage and use conditions.

8 Preparation of sample

8.1 Sampling

Sample the cigarettes in accordance with ISO 8243.

8.2 Smoking

Condition the samples according to ISO 3402 and smoke the cigarettes according to ISO 20779. Typically, three cigarettes should be smoked onto a 44 mm diameter glass-fibre filter pad and five cigarettes on to a 92 mm glass-fibre filter pad. Glass-fibre filter pads of 44 mm diameter are capable of retaining up to 150 mg of total particulate matter (TPM) and glass-fibre filter pads of 92 mm diameter up to 600 mg. If this mass is exceeded, the number of cigarettes shall be reduced. For low tar products, a greater number of cigarettes may be smoked to achieve a minimum TPM of 10 mg for a 44 mm glass-fibre filter pad and 20 mg for a 92 mm glass-fibre filter pad.

8.3 Glass-fibre filter pad extraction

8.3.1 Remove the glass-fibre filter pad from its holder, fold it twice (with the TPM inside) and wipe the inside of the holder with the glass-fibre filter pad. Refer to ISO 20779 for additional information.

8.3.2 Transfer the glass-fibre filter pad to a conical flask (100 ml for a 92 mm glass-fibre filter pad, 50 ml for 44 mm glass-fibre filter pad).

8.3.3 For a 92 mm glass-fibre filter pad, add 58 ml of cyclohexane to the flask, then add 2,0 ml of secondary B[a]P-d12 spiking solution (7.3) with a suitable syringe. For a 44 mm glass-fibre filter pad, add 29 ml of cyclohexane and 1,0 ml of secondary B[a]P-d12 spiking solution.

8.3.4 Shake the flask for at least 20 min on the shaker at approximately 200 r/min.

NOTE Shaking up to 60 min has been tested to give equivalent results.

Laboratories may change the time of shaking based on a result of extraction efficiency.

8.3.5 Transfer 15,0 ml of solution to a test tube, for example, a 16 mm × 150 mm test tube.

Concentrate the sample by evaporation in a TurboVap® at 60 °C under nitrogen atmosphere and down to approximately 3 ml. Adjust the volume to 3 ml with cyclohexane if necessary.

The volume of the sample can be adjusted depending on the cartridge dimension and/or the use of an automatic system. An automatic system can improve the efficiency and repeatability of the clean-up process and its use is recommended.

8.4 Sample clean-up

8.4.1 The NH2 SPE cartridge is pre-conditioned before use by passing 5 ml of hexane through it. Care shall be taken that the cartridge does not run dry.

8.4.2 In the vacuum sample preparation unit, load the 3 ml of sample and collect in a test tube. Let the extract pass through the NH2 SPE cartridge under vacuum at a flow rate of approximately 2 ml/min (1 drop per second). Load 5,5 ml of hexane and collect in the same test tube.

8.4.3 Evaporate to dryness using the TurboVap® (5.4) at 60 °C under nitrogen atmosphere. Then add 500 µl of cyclohexane.

Sonicate for 5 min and vortex. Repeat, if necessary, for achieving a homogenous solution.