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Cigarettes — Determination of benzo[a]pyrene in cigarette mainstream smoke using GC/MS — Part 1: Method using methanol as extraction solvent

Cigarettes — Dosage du benzo[a]pyrène dans le courant principal de la fumée de cigarette par CG/SM — Partie 1: Méthode utilisant du méthanol comme solvant d'extraction

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ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

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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.*

This first edition of ISO 22634-1 cancels and replaces ISO 22634:2008, of which it constitutes a minor revision.

The main changes compared to the previous edition are as follows:

— the number of ISO 22634 has been changed to ISO 22634-1 as a new part 2 of ISO 22634 has been elaborated;

— the titles of the two parts have been modified accordingly;

— the text has been editorially revised.

A list of all parts in the ISO 22634 series can be found on the ISO website.

Introduction

Between 1999 and 2003, a task force composed of Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) members studied the existing methodologies for the determination of benzo[a]pyrene (B[a]P) in the mainstream smoke of cigarettes. Several methods have been proposed for this determination, which are mainly based on two types of analytical methodology: high performance liquid chromatography (HPLC) with fluorescence detection and gas chromatography/mass spectrometry (GC/MS). In both cases, it is necessary to purify the smoke condensate extract before performing the chromatography in order to obtain a correct separation of the B[a]P peak.

The task force decided in the first instance to develop a method using HPLC with fluorescence detection. However, after several collaborative experiments, it appeared that achieving a significant reduction of the initially observed variability would be technically very difficult. The task force then decided to investigate a GC/MS method as an alternative and was able to demonstrate, through collaborative experiments, that a lower variability can be obtained with this methodology.

This document, produced through collaborative experiments involving many laboratories in many countries, provides an optimized procedure for the determination of B[a]P in cigarette mainstream smoke. The repeatability and reproducibility of this method have been assessed according to ISO recommendations and are included.

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.

Cigarettes — Determination of benzo[a]pyrene in cigarette mainstream smoke using GC/MS — Part 1: Method using methanol as extraction solvent

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 regulatory limitations 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 using gas chromatography/mass spectrometry (GC/MS) with methanol as extraction solvent.

This method was validated using ISO 3308 smoking parameters and is technically compatible with other smoking regimes.

An alternative method for the determination of B[a]P is specified in ISO 22634-2 with a different clean-up using cyclohexane solvent and a reduced analytical run.

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 3696, *Water for analytical laboratory use — Specification and test methods*

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:

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

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

4 Principle

— Sampling of the test cigarettes.

— Conditioning of the test cigarettes.

— Smoking of the test cigarettes according to the smoking procedure specified in ISO 4387.

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

— Dilution of the methanol extract with water.

— Elution of the water/methanol solution through a cyclohexyl solid phase extraction (CH SPE) cartridge, followed by the elution of B[a]P with cyclohexane.

— 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, complying with the requirements of ISO 3308 and equipped for smoking in accordance with ISO 4387.

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 5 % methylphenyl 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 Rotary evaporator or **equivalent equipment**.

5.5 Vacuum sample preparation unit or **equivalent equipment**.

5.6 Solid phase extraction cartridges, cyclohexyl bonded silica phase volume of 6 ml and packed with 1 g is suitable.

5.7 Gas tight syringes, of capacities 25 µl, 100 µl, 250 µl and 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.

6 Reagents

All reagents shall be of analytical grade quality.

6.1 Methanol, of known purity, not less than 99 %, CAS 67-56-1.

6.2 Water, complying with grade 2 of ISO 3696 or better.

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

6.4 Toluene, of known purity, not less than 99 %, CAS 108-88-3.

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

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

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.

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 stock solution

Dissolve 10 mg B[a]P, weighed to the nearest 0,01 mg, into a 10 ml volumetric flask and fill to the mark with toluene.

7.3 Secondary B[a]P stock solution

Dilute 1 ml of the primary B[a]P stock solution (7.2) into a 100 ml volumetric flask and fill to the mark with methanol.

7.4 B[a]P-d12 stock solution

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

7.5 B[a]P-d12 spiking solution

Using a gas syringe, transfer 100 µl of the B[a]P-d12 stock solution (7.4) into a 100 ml volumetric flask and fill to the mark with methanol. This solution has a mass concentration of approximately 1 µg/ml.

7.6 Working standard solutions

Prepare six working standard solutions that cover the concentration range of interest. For example, transfer 20 µl of the B[a]P-d12 stock solution (7.4) and 10 µl to 2 000 µl of the secondary B[a]P stock solution (7.3) into 100 ml volumetric flasks and fill to the mark with cyclohexane. These solutions have a mass concentration of approximately 0,2 µg/ml of B[a]P-d12 and mass concentrations from 1 ng/ml to 200 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 six months if stored below –18 °C.

8 Preparation of sample

8.1 Sampling

Sample the cigarettes in accordance with ISO 8243.