INTERNATIONAL STANDARD

ISO 22634-2

First edition
2017-11

Cigarettes — Determination of benzo[a]pyrene in cigarette mainstream smoke using GC/MS —

Part 2: Method using cyclohexane as extraction solvent

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

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

Reference number
ISO 22634-2:2017(E)

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

Contents

Page

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 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.*

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

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 brand;

— 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 2:
Method using cyclohexane 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 cyclohexane as extraction solvent.

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

This document provides an alternative method to that specified in ISO 22634-1, with a different clean-up, which is less time consuming and with a reduced analytical run allowing a potential increase of sample throughput in comparison with ISO 22634-1.

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:

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

— ISO Online browsing platform: available at http://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 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, 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 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 as long as 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.

6 Reagents

All reagents shall be of analytical grade quality.

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

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.

6.2 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.

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-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.

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 4387. Typically, five cigarettes should be smoked onto a 44 mm diameter Cambridge filter pad and 20 cigarettes on to a 92 mm Cambridge filter pad. Cambridge filter pads of 44 mm diameter are capable of retaining up to 150 mg of total particulate matter (TPM) and 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 pad and 20 mg for a 92 mm pad.

8.3 Filter pad extraction

8.3.1 Remove the filter pad from its holder, fold it twice (with the condensate inside) and wipe the inside of the holder with the pad. Refer to ISO 4387 for additional information.

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

8.3.3 For a 92 mm 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 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 giving equivalent results.

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®[1] 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 NH_2 SPE cartridge is pre-conditioned before use by passing 5 ml of hexane through it. Care has to 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 NH_2 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®[1] (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.

8.4.4 Transfer the obtained solution into two sample vials (vial inserts may be required) with a sealed cap and polytetrafluoroethylene (PTFE) faced septum.

NOTE The second vial is used in case a repetition of the GC/MS analysis is needed.

8.5 Blank solution

Proceed with 8.3 and 8.4 using a new glass fibre filter pad.

NOTE The programme described above can be adjusted depending on the SPE system used and SPE cartridge dimensions.

9 Determination

9.1 GC/MS operating conditions

The following operating conditions for a fused silica capillary column as specified in 5.3 have been found to be suitable for the determination. Conditions and column are regarded as an example.

—	Injector temperature:	300 °C
—	Mode:	Constant flow
—	Flow rate:	1 ml/min
—	Injection mode:	Pulsed splitless, 1 min
—	Pressure	200 kPa, 1 min
—	Injection:	1 µl splitless
—	Oven temperature programme:	100 °C for 1 min
		16 °C/min to 300 °C
		2 °C/min to 315 °C
		30 °C/min to 330 °C
		Hold at 330 °C for 20 min
—	Carrier gas:	Helium
—	Transfer line temperature:	330 °C
—	MS source:	230 °C
—	MS quad temperature:	150°C
—	Solvent delay:	17 min
—	Ion traces:	B[a]P: m/z 252 [quantification, Dwell (ms): 150] and 250 [confirmation, Dwell (ms): 100]
		B[a]P-d12: m/z 264 [quantification, Dwell (ms): 150] and 260 [confirmation, Dwell (ms): 100]

These chromatographic conditions shall be adapted in order to obtain a correct resolution of the B[a]P and B[a]P-d12 peaks. A typical chromatogram is given in Annex A.

9.2 Calibration

Successively inject each working standard solution (7.6) into the GC/MS system. Record the area of the B[a]P and the B[a]P-d12 peaks. A calibration curve for B[a]P is generated by calculating a linear regression equation as a function of the B[a]P to B[a]P-d12 concentration ratios. The intercept of this

regression line should be close to zero and the correlation coefficient shall be higher than 0,995. Use of a quality control of intermediate concentration after five sample analyses and if the measured concentration for this solution is different by more than 15 % of the nominal value, then repeat the calibration procedure.

9.3 Determination of B[a]P

Inject the sample, calculate the area ratio of B[a]P to B[a]P-d12 peaks and obtain the concentration of B[a]P in the solution by comparing this ratio with the B[a]P to B[a]P-d12 concentration ratio in the calibration curve.

If a sample does not show a concentration of B[a]P within the working standards range, a different number of cigarettes shall be smoked (see 8.2).

9.4 Calculation

The mass of B[a]P, m, expressed in nanograms per cigarette, is given by Formula (1):

$$m = (C - Cb) \times \frac{V_{B(a)P-d12}}{N_{cig}} \quad (1)$$

where

C is the mass concentration of B[a]P in the sample solution, expressed in nanograms per millilitre;

Cb is the mean B[a]P concentration in the blanks, expressed in nanograms per millilitre;

$V_{B(a)P\text{-}d12}$ is the volume of secondary spiking solution (7.3) added to the sample, expressed in millilitres;

N_{cig} is the number of cigarettes smoked.

10 Repeatability and reproducibility

A major international collaborative study involving 14 laboratories and six samples including the 3R4F (a reference cigarette produced by the University of Kentucky) and the CM7 (CORESTA Monitor) and covering a wide range of blends and constructions was conducted in 2014 and the values for repeatability limit, r, and reproducibility limit, R, given in Table 1, were obtained using this method. The statistical data analysis was done according to ISO 5725-2 and calculation of r and R according to ISO 5725-6:1994, 4.1.

The difference between two single results found on matched cigarette samples by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability limit, r, on average not more than once in 20 cases in the normal and correct operation of this method.

Single results on matched cigarette samples reported by two laboratories will differ by more than the reproducibility limit, R, on average not more than once in 20 cases in the normal and correct operation of the method.

Data analysis for the six cigarette samples gave the estimates as summarized in Table 1.

Table 1 — Repeatability (*r*) and reproducibility (*R*): B[a]P (ng/cigarette)

Cigarette sample	*n*[a]	Mean value	SD[b]	r	R
3R4F	12	6,453	0,770	0,622	2,214
CM7	13	14,427	1,420	1,316	4,119
A	12	1,328	0,276	0,469	0,863
B	13	4,491	0,466	0,791	1,455
D	13	6,382	0,748	0,931	2,228
E	13	11,369	1,182	1,504	3,529

[a] *n* = number of laboratories

[b] SD = standard deviation

11 Test report

11.1 General

The test report shall state the method used and the results obtained. It shall also mention any operating conditions not specified in this document or regarded as optional, as well as any circumstances that may have influenced the results.

The test report shall include all details required for complete identification of the sample. Where appropriate, record the information in 11.2 to 11.5.

11.2 Characteristic data about the cigarette

All details necessary for the identification of the cigarette smoked shall be given. In the case of a commercial cigarette, this may include:

— name of manufacturer, country of manufacture;

— product name;

— packet number (of that product sampled that day);

— marks on any tax stamp;

— printed mainstream smoke yields (if any);

— length of cigarette;

— length of filter;

— length of overwrap;

— diameter.

11.3 Data about sampling

— Type of sampling procedure

— Number of cigarettes in the laboratory sample

— Date and location of purchase

— Place of purchase or sampling

— Kind of sampling point

— Sampling point (e.g. address of retail outlet or machine number)

11.4 Description of the test

— Date of the test

— Type of smoking machine used

— Type of smoke trap used

— Number of cigarettes smoked into each smoke trap

— Butt length

— Room temperature (in degrees centigrade) during smoking operation

— Relative humidity (in percent) during smoking operation

— Atmospheric pressure (in kilopascals) during smoking operation

11.5 Test results

The expression of the laboratory data depends on the purpose for which the data are required and the level of laboratory precision. Confidence limits shall be calculated and expressed on the basis of the laboratory data before any rounding has taken place.

— Amount of B[a]P in the mainstream smoke of the cigarette (in nanograms per cigarette) to the nearest 0,1 ng.