INTERNATIONAL STANDARD

ISO 23906-1

First edition
2020-09

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

Part 1: Method using methanol 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 1: Méthode utilisant du méthanol comme solvant d'extraction

Reference number
ISO 23906-1:2020(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

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 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

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 total particulate matter (TPM) 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 conducted in 2012 involving 12 laboratories in 11 countries, provides a procedure for the determination of B[a]P in cigarette mainstream smoke.[4] 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 International Standards.

Cigarettes — Determination of benzo[a]pyrene in cigarette mainstream smoke with an intense smoking regime 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 (TPM) of cigarette mainstream smoke using gas chromatography/mass spectrometry (GC-MS) with methanol as extraction solvent.

This method is specified using ISO 20778 smoking parameters.

NOTE An alternative method for the determination of B[a]P is specified in ISO 23906-2 with a different clean-up using cyclohexane solvent and a shorter total analytical time.

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

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 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

— 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 TPM, collected on the appropriate glass-fibre filter pad, with methanol.

— Dilution of the methanol extract with water.

— Loading of the water/methanol solution onto 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 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 (5 %-phenyl)-methylpolysiloxane 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 or positive displacement pipettes, 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. Amber glassware may be required.

5.9 Shaker and filtration apparatus.

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.

6.7 **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

Certified B[a]P or B[a]P-d12 solutions can be used as reference material.

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 (see 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 (see 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 (see 7.4) and 10 µl to 2 000 µl of the secondary B[a]P stock solution (see 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 (see 7.2 to 7.6) are stable for up to six months if stored below –18 °C. Storage in amber glassware and away from the light is recommended.

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 onto a 92 mm diameter glass-fibre filter pad. Glass-fibre filter pads of 44 mm diameter are capable of retaining up to 150 mg of 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 on one glass-fibre filter pad to achieve a minimum TPM of 10 mg for a 44 mm diameter glass-fibre filter pad and 20 mg for a 92 mm diameter 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 two separate quarters of an unused conditioned glass-fibre filter pad. Refer to ISO 20779 for additional information.

8.3.2 Transfer the glass-fibre filter pad to a conical flask with stopper (100 ml for a 44 mm diameter glass-fibre filter pad; 200 ml for a 92 mm diameter glass-fibre filter pad).

8.3.3 For a 44 mm diameter glass-fibre filter pad, add 20 ml of methanol to the flask, then add 200 µl of the B[a]P-d12 spiking solution (see 7.5) with a suitable syringe or micropipette (5.7). Stopper the flask immediately.

For a 92 mm diameter glass-fibre filter pad, add 50 ml of methanol to the flask, then add 400 µl of the B[a]P-d12 spiking solution (see 7.5) with a suitable syringe or micropipette (5.7). Stopper the flask immediately.

8.3.4 Shake the flask vigorously (5.9) until the glass-fibre filter pad has disintegrated and filter the solution through a glass suction filter or using paper filtration.

8.3.5 Wash the glass-fibre filter remainder with approximately 15 ml of methanol for a 44 mm diameter glass-fibre filter pad or 25 ml of methanol for a 92 mm diameter glass-fibre filter pad. Add this washing solution to the filter extract and complete to a volume which is: ≥ 40 ml for a 44 mm filter pad or ≥ 80 ml for a 92 mm pad, with methanol. For convenience, bigger final volumes can be used, but without unnecessarily diluting the solution.

8.3.6 Transfer an aliquot of the solution obtained from 8.3.5 to a separating funnel. The volume of this aliquot shall not exceed 40 ml, which is convenient for this procedure. However, a smaller aliquot can be used in order to shorten the elution time during the clean-up step (see 8.4.2).

8.3.7 Add water to the funnel in order to obtain a solution containing 60 % water and 40 % methanol, then mix. For example, if an aliquot of 40 ml is used in 8.3.6, add 60 ml of water.

8.4 Sample clean-up

8.4.1 The CH SPE cartridge is pre-conditioned before use by passing 10 ml of methanol through it and then 10 ml of a mixture of water and methanol (60:40 volume fraction).

8.4.2 In the vacuum sample preparation unit, let the extraction solution (see 8.3.7) pass through the CH SPE cartridge under vacuum at a flow rate of approximately 2 ml/min (1 drop per second). Rinse the funnel with 10 ml of a mixture of water and methanol (60:40 volume fraction). Dry the cartridge with a stream of air for at least 30 min.

8.4.3 Elute the cartridge with 15 ml of cyclohexane (6.3).

8.4.4 Reduce the volume of the cyclohexane solution to about 0,5 ml using the rotary evaporator (5.4). Then add cyclohexane in order to obtain a volume of approximately 1 ml.

In spite of the drying procedure described in 8.4.2, the cyclohexane solution obtained in 8.4.3 can still contain a significant amount of water and a two-phase solution can be obtained after the volume reduction prescribed in 8.4.4. In this case, the cyclohexane phase shall be separated from the water phase before adjusting the final volume to 1 ml. Alternatively, the cyclohexane solution in 8.4.3 may be dried on a water adsorbent material before volume reduction.

Alternatively, the volume of cyclohexane solution may be reduced to dryness, then add 1 ml of cyclohexane, as long as it is proved that results are equivalent.

8.4.5 Transfer the obtained solution into a sample vial with a sealed cap and polytetrafluoroethylene (PTFE) faced septum.

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:	290 °C
— Mode:	Constant flow
— Flow rate:	0,9 ml/min
— Injection mode:	1 µl splitless
— Oven temperature programme:	80 °C for 3 min 5 °C/min to 290 °C hold at 290 °C for 20 min
— Carrier gas:	Helium
— Transfer line temperature:	270 °C
— MS source:	230 °C
— Ion traces:	B[a]P: *m/z* 252 (quantification) and 250 (confirmation) B[a]P-d12: *m/z* 264 (quantification) and 260 (confirmation)

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 (see 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. Inject one working standard solution (see 7.6) after 10 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).

NOTE During a normal analysis sequence, it has been observed by several laboratories that the absolute value of the B[a]P-d12 peak area can show significant variations. The reasons for this observed variability of the GC-MS response have not been investigated thoroughly. However, this phenomenon has no effect on the final result because the internal standard procedure used in this method compensates for these variations.

9.4 Calculation

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

$$m = \frac{C \times V \times V_e}{N_{cig} \times V_c} \quad (1)$$

where

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

V is the volume of the sample solution, expressed in millilitres (V = 1 ml);

V_e is the volume of the extraction solution (see 8.3.5) in millilitres;

N_{cig} is the number of cigarettes smoked;

V_c is the volume of the aliquot of the extraction solution used during the clean-up (see 8.3.6) in millilitres.

10 Repeatability and reproducibility

A major international collaborative study was conducted in 2012 involving 12 laboratories and 10 samples covering a wide range of blends and constructions.[4] This provided data on the determination of B[a]P in University of Kentucky reference products (3R4F/1R5F), the CORESTA Monitor 6 test piece (CM6), and seven commercial products smoked using Health Canada Method T-115 that uses the same smoking parameter as ISO 20778. The samples are shown in Table 1. 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 10 cigarette samples gave the estimates as summarized in Table 2.

Table 1 — 2012 Collaborative Study Sample Identification:

Sample ID	Product/Blend type
CM6	CORESTA Monitor 6 Test Piece
1R5F	Kentucky Reference 1R5F
3R4F	Kentucky Reference 3R4F
Sample 1	Dark air-cured
Sample 2	American blended
Sample 3	American blended
Sample 4	Virginia blended
Sample 5	Virginia blended
Sample 6	Virginia blended
Sample 7	Charcoal filtered

Table 2 — Repeatability (*r*) and reproducibility (*R*): TPM yield[a] and B[a]P[b]

Reference product	TPM yield	n^c	Mean value	*r*	*R*
3R4F	39,94	12	15,51	1,68	4,93
1R5F	26,72	12	7,13	1,25	2,28
Test piece sample	**TPM yield**	n^c	**Mean value**	*r*	*R*
CM6	41,99	11	27,86	4,65	8,45
Cigarette sample	**TPM yield**	n^c	**Mean value**	*r*	*R*
1	37,10	9	17,81	3,06	6,79
2	35,30	9	18,37	1,88	4,85
3	30,67	10	20,69	4,88	7,85
4	24,72	9	10,63	1,97	3,67
5	17,02	10	7,52	1,67	2,93
6	32,82	9	14,24	2,28	4,85
7	21,25	10	9,12	1,83	3,98

[a] mg/reference product, mg/test piece; mg/cigarette.

[b] ng/reference product, ng/test piece; ng/cigarette.

[c] Number of laboratories after removal of outliers.

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 the manufacturer, country of manufacture;

— product name;

— packet identifier (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 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

— Smoking regime 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.