भारतीय मानक

Indian Standard

IS 18625 (Part 2) : 2024
ISO 22634-2 : 2019

सिगरेट — सिगरेट मेनस्ट्रीम स्मोक में बेंजो[ए]पाइरीन का निर्धारण जीसी/एमएस द्वारा

भाग 2 निष्कर्षण विलायक के रूप में साइक्लोहेक्सेन उपयोग करने की विधि

Cigarettes — Determination of Benzo[a]Pyrene in Cigarette Mainstream Smoke Using GC/MS

Part 2 Method Using Cyclohexane as Extraction Solvent

ICS 65.160

भारतीय मानक ब्यूरो
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February 2024 **Price Group 7**

Tobacco and Tobacco Products Sectional Committee, FAD 04

NATIONAL FOREWORD

This Indian Standard (Part 2) which is identical to ISO 22634-2 : 2019 'Cigarettes — Determination of benzo[a]pyrene in cigarette mainstream smoke using GC/MS — Part 2: Method using cyclohexane as extraction solvent' issued by the International Organization for Standardization (ISO) was adopted by the Bureau of Indian Standard on recommendation of the Tobacco and Tobacco Products Sectional Committee and approval of the Food and Agriculture Division Council.

This standard is published in two parts. Other part in this series is:

Part 1 Method using methanol as extraction solvent

The text of ISO standard has been approved as suitable for publication as an Indian Standard without deviations. Certain conventions are, however, not identical to those used in Indian Standards. Attention is particularly drawn to the following:

a) Wherever the words 'International Standard' appear referring to this standard, they should be read as 'Indian Standard'; and

b) Comma (,) has been used as a decimal marker while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

In this adopted standard, reference appears to the following International Standards for which Indian Standards also exist. The corresponding Indian Standards which are to be substituted in their respective places, are listed below along with their degree of equivalence for the editions indicated:

International Standard	*Corresponding Indian Standard*	*Degree of Equivalence*
ISO 3308 Routine analytical cigarette-smoking machine — Definitions and standard conditions	IS 16022 : 2015/ISO 3308 : 2012 Routine analytical cigarette-smoking machine — Definitions and standard conditions (*first revision*)	Identical
ISO 3402 Tobacco and tobacco products — Atmosphere for conditioning and testing	IS 16121 : 2023/ISO 3402 : 2023 Tobacco and tobacco products — Atmosphere for conditioning and testing (*first revision*)	Identical
ISO 4387 Cigarettes — Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine	IS 16023 : 2021/ISO 4387 : 2019 Cigarettes — Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine (*first revision*)	Identical
ISO 8243 Cigarettes — Sampling	IS 12942 : 2018/ISO 8243 : 2013 Cigarettes — Sampling (*third revision*)	Identical

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 2022 'Rules for rounding off numerical values (*second revision*)'.

Contents

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Introduction

This document, produced through collaborative experiments involving many laboratories in many countries, provides a 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.

Indian Standard

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 (TPM) of cigarette mainstream smoke using gas chromatography/mass spectrometry (GC/MS) with cyclohexane as extraction solvent.

This method is specified using ISO 3308 smoking parameters. This document provides an alternative method to that specified in ISO 22634-1, with a different clean-up, and a shorter total 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:

— 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 4387.

— Extraction of the TPM, collected on the appropriate glass-fibre filter pad, with cyclohexane.

— Sample clean-up 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, e.g. sample vials (vial inserts may be required). All glassware shall be cleaned before use to avoid any contamination. Amber glassware may be required.

5.9 Ultrasonic bath.

5.10 Shaker, set to 200 r/min.

5.11 Vortex mixer.

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.

6.6 Nitrogen, of known purity, not less than 99,999 %, CAS 7727-37-9.

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-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 5,0 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. 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 4387. Typically, five cigarettes should be smoked onto a 44 mm diameter glass-fibre filter pad and 20 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 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 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 4387 for additional information.

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

8.3.3 For a 92 mm diameter 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 (5.7). Stopper the flask immediately.

For a 44 mm diameter glass-fibre filter pad, add 29 ml of cyclohexane and 1,0 ml of secondary B[a]P-d12 spiking solution. Stopper the flask immediately.

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.

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, then add 3 ml of cyclohexane.

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 Pre-condition the NH_2 SPE cartridge before use by passing 5 ml of hexane through it. Proceed to the next step without allowing the cartridge to become 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® (5.4) at 60 °C under nitrogen atmosphere. Then add 500 µl of cyclohexane.

Sonicate for 5 min and mix with a vortex mixer (5.11), repeat if necessary to achieve 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. Solution is stable for two weeks at 4 °C.

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 and consider reanalysing the five samples according to internal laboratory procedures.

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):

$$m=\left(C-C_{\mathrm{b}}\right)\times\frac{V_{B(a)P-d12}}{N_{\mathrm{cig}}} \quad (1)$$

where

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

C_{b} 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 interlaboratory 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/test piece; ng/reference product; ng/cigarette)

Test piece sample	*n*[a]	**Mean value**	*SD*[b]	*r*	*R*
CM7	13	14,43	1,420	1,316	4,119
Reference product	*n*[a]	**Mean value**	*SD*[b]	*r*	*R*
3R4F	12	6,45	0,770	0,622	2,214
Cigarette sample	*n*[a]	**Mean value**	*SD*[b]	*r*	*R*
A	12	1,33	0,276	0,469	0,863
B	13	4,49	0,466	0,791	1,455
D	13	6,38	0,748	0,931	2,228
E	13	11,37	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 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

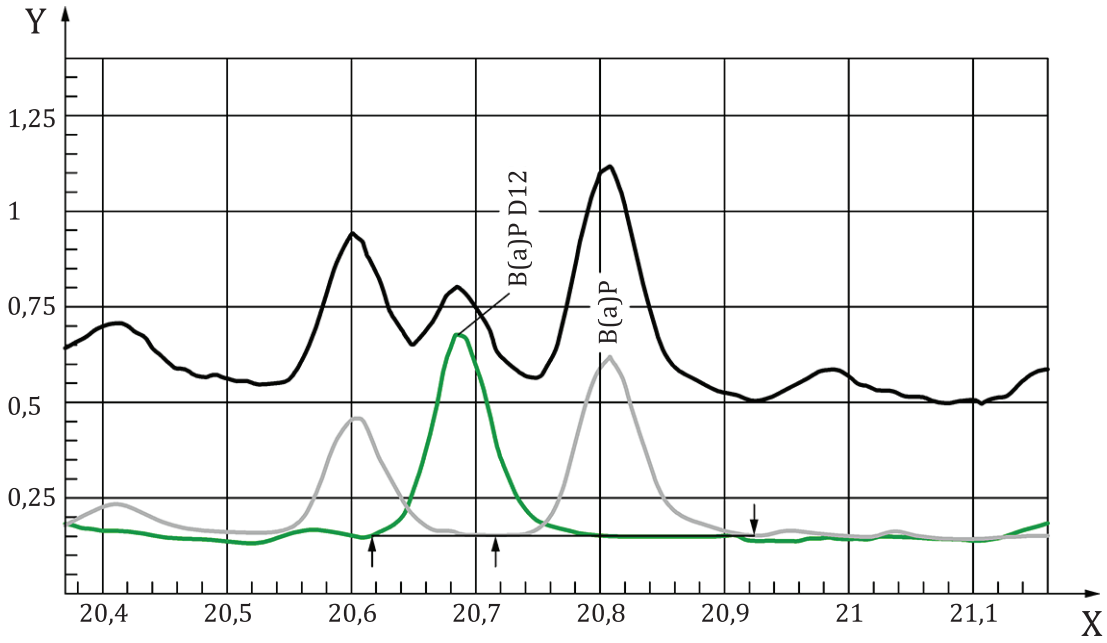
The expression of the laboratory data depends on the purpose for which the data are required and the level of laboratory precision. Confidence limits shall be calculated and expressed on the basis of the laboratory data before any rounding has taken place.

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

Annex A
(informative)

Example of a chromatogram of a cigarette smoke extract

The chromatogram in Figure A.1 has been obtained by using the column specified in 5.3 and the chromatographic set up in 9.1.



Key

X time in minutes

Y mass counts

NOTE Black — Total ion current (TIC) trace, Green — 264 ion trace, Grey — 252 ion trace.

Figure A.1 — Example of a chromatogram of a cigarette smoke extract

Bibliography

[1] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

[2] ISO 5725-6:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 6: Use in practice of accuracy values*

[3] ISO 22634-1, *Cigarettes — Determination of benzo[a]pyrene in cigarette mainstream smoke using GC/MS — Part 1: Method using methanol as extraction solvent*

[4] Collaborative study[2)]: "*Cigarettes — Determination of benzo[a]pyrene in cigarette mainstream smoke — Part 2: Method using cyclohexane as extraction solvent*", ISO/TC 126/WG 14, issued in June 2015

2) The collaborative study report can be made available upon request to ISO/TC 126 secretariat.

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Review of Indian Standards

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