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Cigarettes — Determination of benzo[a]pyrene in cigarette mainstream smoke — Method using gas chromatography/mass spectrometry

Cigarettes — Dosage du benzo[a]pyrène dans le courant principal de la fumée de cigarettes — Méthode par couplage de chromatographie en phase gazeuse/spectrométrie de masse

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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 22634 was prepared by Technical Committee ISO/TC 126, *Tobacco and tobacco products*.

Introduction

Between 1999 and 2003, a task force composed of CORESTA [1)] members has 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 mainly are based on two types of analytical methodology: HPLC [2)] with fluorescence detection and GC-MS [3)]. 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 International Standard, produced through collaborative experiments involving many laboratories in many countries, provides an optimized procedure for the determination of benzo[a]pyrene 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 International Standard;

— 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.

1) CORESTA: Cooperation Centre for Scientific Research Relative to Tobacco.

2) High performance liquid chromatography.

3) Gas chromatography-mass spectrometry.

Cigarettes — Determination of benzo[a]pyrene in cigarette mainstream smoke — Method using gas chromatography/mass spectrometry

1 Scope

This International Standard specifies a method for the determination of benzo[a]pyrene (B[a]P) in the total particulate matter of cigarette mainstream smoke.

This method is specified using ISO 3308 smoking parameters, but is technically compatible with other smoking regimes.

2 Normative references

The following referenced documents are indispensable for the application 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 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 4387 + ISO 4387:2000/Amd.1:2008, *Cigarettes — Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine*

ISO 8243, *Cigarettes — Sampling*

3 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.

4 Apparatus

Usual laboratory apparatus and equipment and in particular the following items.

4.1 Routine analytical cigarette-smoking machine, complying with the requirements of ISO 3308 and equipped for smoking in accordance with ISO 4387.

4.2 Gas chromatography — Mass spectrometry system, 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.

4.3 Fused silica capillary column, with a methylphenyl (5 %) polysiloxane stationary phase. For example, a 30 m, 0,25 mm internal diameter with a 0,25 µm film thickness column is suitable for this analysis.

4.4 Rotary evaporator or **equivalent equipment**.

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

4.6 Cyclohexyl Solid Phase Extraction (CH SPE) cartridges, phase: cyclohexyl bonded silica. A 6 ml, 1 g packing cartridge is suitable.

4.7 Gas tight syringes, of capacities 25 µl, 100 µl, 250 µl and 1 000 µl.

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

5 Reagents

All reagents shall be of analytical grade quality.

5.1 Methanol.

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

5.3 Cyclohexane.

5.4 Toluene.

5.5 Benzo[a]pyrene.

5.6 Benzo[a]pyrene-d12.

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

6 Standards

6.1 Primary B[a]P stock solution

Dissolve approximately 10 mg B[a]P, weighed to the nearest 0,01 mg, in 10 ml of toluene.

6.2 Secondary B[a]P stock solution

Dilute 1 ml of the primary B[a]P stock solution (6.1) to 100 ml with methanol.

6.3 B[a]P-d12 stock solution

Dissolve approximately 10 mg B[a]P-d12, weighed to the nearest 0,01 mg, in 10 ml of toluene.

6.4 B[a]P-d12 spiking solution

Using a gas syringe, transfer 100 µl of the B[a]P-d12 stock solution (6.3) into a 100 ml volumetric flask and fill to the mark with methanol. This solution has a mass concentration of approximately 1 µg/ml.

6.5 Working standard solutions

Prepare 6 working standard solutions that cover the concentration range of interest. For example, transfer 20 µl of the B[a]P-d12 stock solution (6.3) and 10 µl to 2 000 µl of the secondary B[a]P stock solution (6.2) into 100 ml volumetric flasks and make up 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.

6.6 Storage

The standard solutions (6.1 to 6.5) are stable for up to six months if stored below −18 °C.

7 Preparation of sample

7.1 Sampling

Sample the cigarettes in accordance with ISO 8243.

7.2 Smoking

Smoke the cigarettes according to ISO 4387. Typically 10 cigarettes should be smoked on to 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 nominal TPM of 10 mg for a 44 mm pad and 20 mg for a 92 mm pad.

7.3 Filter pads extraction

7.3.1 Remove the filter pad from its holder, fold it twice (with the condensate inside) and wipe the inside of the holder with the pad.

7.3.2 Transfer the filter pad to a volumetric flask (100 ml for a 44 mm pad; 200 ml for a 92 mm pad).

7.3.3 For a 44 mm pad, add 20 ml of methanol to the flask, then add 200 µl of the B[a]P-d12 spiking solution (6.4) using a suitable syringe. For a 92 mm pad, add 50 ml of methanol to the flask, then add 400 µl of the B[a]P-d12 spiking solution (6.4) with a suitable syringe.

7.3.4 Shake the flask vigorously until the filter pad has disintegrated and filter the solution through a glass suction filter or using paper filtration.

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

7.3.6 Transfer an aliquot of the solution obtained from 7.3.5 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 7.4.2).

7.3.7 Add water to the funnel in order to obtain a solution containing 60 % of water and 40 % of methanol, then mix. For example, if an aliquot of 40 ml is used in 7.3.6, add 60 ml of water.

7.4 Sample clean-up

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

7.4.2 In the vacuum sample preparation unit, let the extraction solution 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.

7.4.3 Elute the cartridge with 15 ml of cyclohexane (5.3).

7.4.4 Reduce the volume of the cyclohexane solution to about 0,5 ml using the rotary evaporator (4.4). Then add cyclohexane in order to obtain a volume of approximately 1 ml.

NOTE In spite of the drying procedure described in 7.4.2, the cyclohexane solution obtained in 7.4.3 might still contain a significant amount of water and a two-phase solution can be obtained after the volume reduction prescribed in 7.4.4. 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 7.4.3 may be dried on a water adsorbent material before volume reduction.

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

8 Determination

8.1 Suitable GC-MS operating conditions

— Injector temperature:	290 °C
— Mode:	constant flow
— Initial flow:	0,9 ml/min
— Injection:	1 µl splitless
— Column temperature:	80 °C for 3 min 5 °C/min to 290 °C hold at 290 °C for 20 min
— Transfer line temp:	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.

8.2 Calibration

Inject successively each working standard solution (6.5) 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 equation regression of the area ratios of B[a]P to B[a]P-d12 peaks as a function of the B[a]P concentrations. The intercept of this regression line should be close to zero. Inject one working standard solution (6.5) after 10 sample analysis and if the measured concentration for this solution is different by more than 15 % of the nominal value then repeat the calibration procedure.

8.3 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 calibration line.

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.

8.4 Calculation

The mass of B[a]P, m, expressed in nanograms per cigarette, is given by the equation:

$$m = \frac{C \times V \times V_e}{n \times V_c}$$

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 (7.3.5);

n is the number of cigarettes smoked;

V_c is the volume of the aliquot of the extraction solution used during the clean-up (7.3.6).

9 Repeatability and reproducibility

A major international study involving 13 laboratories and seven cigarette samples including the 2R4F (a reference cigarette produced by the University of Kentucky) and covering a wide range of blends and constructions was conducted in 2003 and the following values for repeatability limit, r, and reproducibility limit, R, were obtained for this method.

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 seven cigarette samples gave the estimates as summarized in Table 1.

Table 1 — B[a]P (ng/cigarette)

Values in nanograms per cigarette

Cigarette sample	Mean value	r	R
2R4F	7,28	1,27	2,52
A	1,81	0,49	1,01
B	5,27	1,06	2,52
C	6,54	1,11	2,21
D	7,76	1,47	2,88
E	8,71	1,39	2,72
F	14,07	2,26	5,94

10 Test report

10.1 General

The test report shall show the method used and the results obtained. It shall also mention any operating conditions not specified in this International Standard, 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 10.2 to 10.5.

10.2 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.

10.3 Data about sampling

— type of sampling procedure;

— number of cigarettes in 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).

10.4 Description of test

— date of 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.

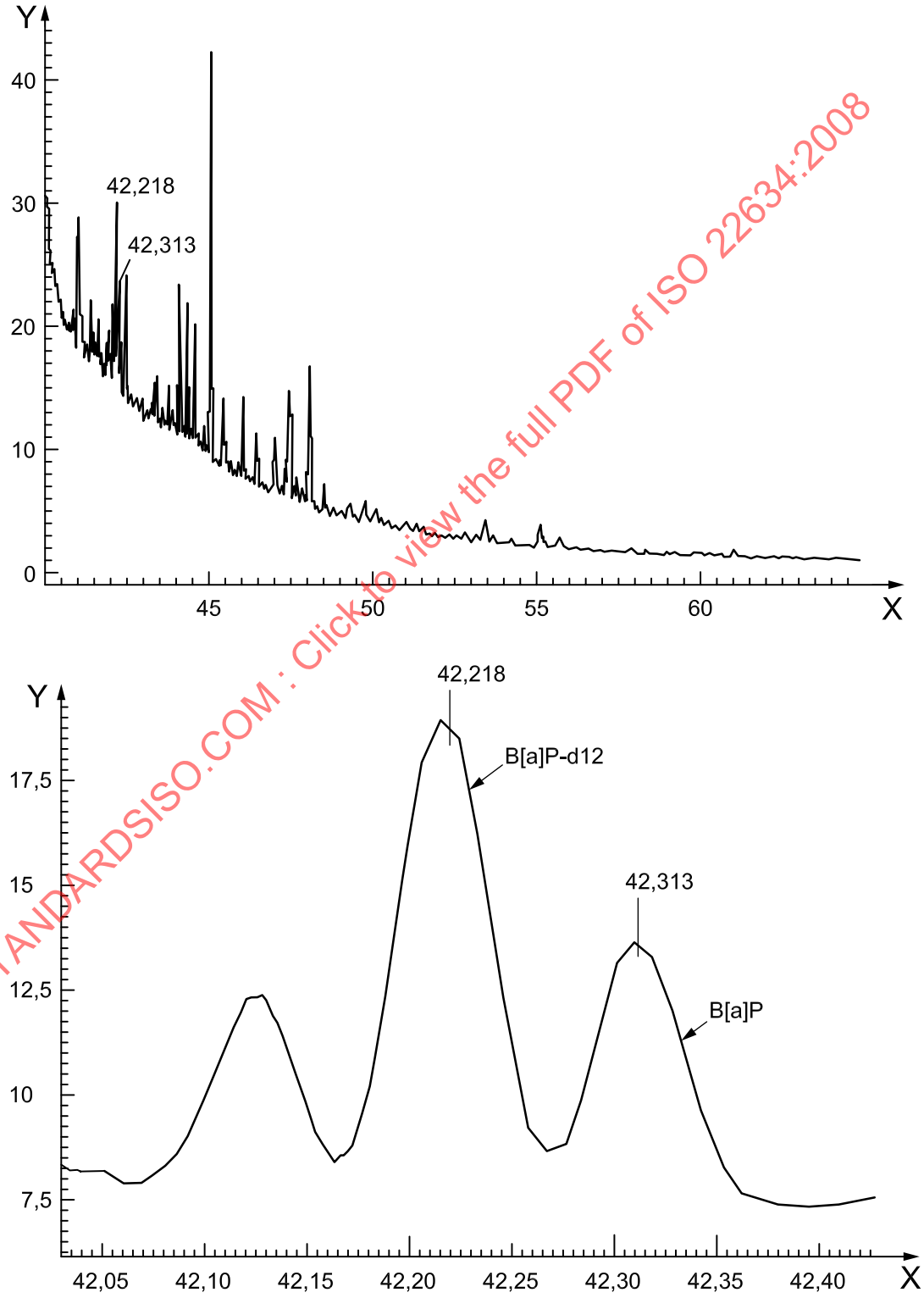
10.5 Test results

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 cigarette (in nanograms per cigarette) to the nearest 0,1 ng.

Annex A
(informative)

Example of a chromatogram of a cigarette smoke extract



Key
X time in minutes
Y mass counts

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