



Standard Test Method for Determination of Benzo- α -Pyrene (BaP) Content in Carbon Black¹

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1. Scope

1.1 This test method covers the qualitative and quantitative determination of only benzo- α -pyrene (BaP), a specific polycyclic aromatic hydrocarbon (PAH), on carbon black. The procedure involves Soxhlet extraction with toluene and analysis by gas chromatography with mass spectrometry (GC/MS). This method is not intended to test for U.S. Food and Drug Administration (FDA 21 CFR 178.3297) compliance of carbon blacks used for indirect food contact applications.

1.2 *Units*—The values stated in SI units are to be regarded as the standard. No other units of measurement are included in this standard.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 ASTM Standards:²

D4483 Practice for Evaluating Precision for Test Method Standards in the Rubber and Carbon Black Manufacturing Industries

2.2 EPA Standard:³

Method 8270D Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)

2.3 Federal Standard:

21 CFR 178.3297 Indirect Food Additives: Adjuvants, Productions Aids, and Sanitizers, Colorants for Polymers⁴

3. Terminology

3.1 Definitions of Terms Specific to This Standard:

3.1.1 *benzo- α -pyrene, BaP, n* —also known as 3,4-benzopyrene or benzo-a-pyrene is a specific polycyclic aromatic hydrocarbon (PAH) or polynuclear aromatic hydrocarbon that consist of fused aromatic rings with no heteroatom or substituent.

3.1.1.1 *Discussion*—PAHs naturally occur in oil, coal, and tar deposits; are produced by the incomplete combustion of hydrocarbons; and occur in many other products and processes. BaP is a pentacyclic PAH with the formula C₂₀H₁₂, related to pyrene by fusion of a phenylene group in the alpha position.

4. Summary of Test Method

4.1 A portion of carbon black is Soxhlet extracted with toluene for a specified period of time. The resultant extract is purified by solid-phase extraction (SPE), concentrated down to a known volume, and subsequently analyzed for BaP by gas chromatography with mass spectrometry (GC/MS). The BaP quantification is performed by the means of a deuterated internal standard (isotope dilution).

5. Significance and Use

5.1 This test procedure is used to determine the concentration of BaP extracted from carbon black by the means of a Soxhlet extraction apparatus with toluene.

6. Apparatus

6.1 *Soxhlet Extractor with Reflux Condenser*, 50- or 100-cm³ capacity.

6.2 *Extraction Thimbles*, glass or cellulose, approximately 50- to 70-cm³ capacity. For instance, glass extraction thimble

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² For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

³ Available from United States Environmental Protection Agency (EPA), Ariel Rios Bldg., 1200 Pennsylvania Ave., NW, Washington, DC 20004, <http://www.epa.gov/epaoswer/hazwaste/test/new-meth.htm#8270D>.

⁴ Available from the U.S. Government Printing Office, Superintendent of Documents, 732 N. Capital St., NW., Washington, DC 20402-0001.

of 35-mm diameter by 90-mm height with coarse porosity (70 to 100 μm similar to Ace Glass Size C, Porosity B, Code-14).

6.3 *Heating Mantle*, compatible with boiling flask described in 6.4.

6.4 *Boiling Flasks for Soxhlet*, for example, 250 cm^3 .

6.5 *Glass Beads*, approximately 180 to 250 μm (60/80 mesh) if glass thimbles are used. Need to be very clean or toluene extracted.

6.6 *PTFE or Glass Boiling Beads*, approximately 3 mm.

6.7 *Glass Wool*, need to be very clean or toluene extracted.

6.8 *Rotary Evaporator*, with temperature-controlled water bath, automatic pressure regulation, and solvent-proof membrane vacuum pump.

6.9 *Nitrogen Blow-Down Apparatus*, equipped with a controlled water bath and nitrogen pressure control.

6.10 *Pear-Shaped Flasks for Rotary Evaporator*, for example, 25, 50, and 100 cm^3 .

6.11 *GC/MS*, with autosampler.

6.11.1 MS with electron impact (EI) capability and single-ion monitoring (SIM) mode.

6.11.2 *GC Capillary Column*, usually a nonpolar GC column composed of 5 % phenyl-methyl silicone coating is used for PAH analysis.

6.11.3 *Deactivated Straight Borosilicate Liner with Small Piece of Deactivated Glass Wool*—This liner may be used as long as peak resolution is satisfactory.

6.11.4 Alternative liner is a split/splitless nondeactivated liner with glass wool (4-mm internal diameter, straight liner). This shall be deactivated with a silanizing agent before use. Another alternative is a split/splitless liner with fluorocarbon liner seals. Such a liner will already contain conditioned silanized glass wool. Other liners can be used if they produce acceptable results.

6.11.5 Gold-plated seal for GC injector port or similar nonreactive seal.

6.11.6 GC/MS amber autosampler vials with polytetrafluoroethylene (PTFE)-coated caps.

6.11.7 Crimping tool.

6.12 *Adjustable Micropipettes*, 1000, 200, and 20 μL .

6.13 *Microlitre Syringes of Different Volumes*, for example, 10 and 100 μL .

6.14 *Amber Glass Vials*, approximately 4 cm^3 with caps (rubber with PTFE back).

6.15 *Amber Volumetric Flasks*, 2, 3, 5, 10, 25, and 100 cm^3 .

6.16 *Analytical Balance*, with an accuracy of 0.01 mg.

6.17 *Drying Oven*, gravity convection type, capable of maintaining $40 \pm 10^\circ\text{C}$, used for slowing down the cooling when the glassware is taken out of the muffle furnace.

6.18 *Furnace*, capable of temperature regulation of $500 \pm 25^\circ\text{C}$, used to burn off organic contamination from glass surfaces.

TABLE 1 Native and Deuterated BaP Compounds

PAH	CAS#	Deuterated PAH	CAS#
Benzo(a)pyrene	50-32-8	d_{12} -Benzo(a)pyrene	63466-71-7

6.19 *Manometer*, capable of pressure readings in the range of 5 ± 0.3 kPa.

7. Reagents and Materials

7.1 *Purity of Reagents*—Reagent-grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society where such specifications are available.⁵ Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 Separate stock solutions of the deuterated internal standard (IS) and of the nondeuterated (native) BaP standards can be purchased prepared from solid materials or individual solutions (Table 1). A concentration range of 80 to 100 $\mu\text{g}/\text{cm}^3$ for both the native BaP and d_{12} -benzo(a)pyrene is recommended. All purchased BaP standard materials shall be 98 % pure or better and certified in respect of their purity, concentration level, and authenticity by the manufacturer.

7.2.1 Follow the manufacturer's recommendation on how best to store the standard stock solutions. It is generally accepted to keep them protected from light. They should be checked frequently for signs of degradation or evaporation, especially just before preparing calibration standards from them. The BaP standard stock solutions shall be replaced/recertified on a yearly basis or sooner if comparisons with quality control (QC) samples indicates a problem.

7.3 *Toluene*, suitable for high resolution gas chromatography analysis (99.99% pure).

7.4 *Acetone*, suitable for high resolution gas chromatography analysis (99.99% pure).

7.5 *Helium*, GC/MS purity grade.

7.6 *Nitrogen*, analytical purity grade.

7.7 *Silica Solid-Phase Extractor Cartridges*, single-use application, having a volume capacity of approximately 5 cm^3 or 2- to 3-cm length by 1-cm diameter.⁶

8. Hazards

8.1 This test involves hazardous materials, operations, and equipment. This procedure does not attempt to address the

⁵ *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial convention, Inc. (USPC), Rockville, MD.

⁶ The sole source of supply of the Sep-Pak cartridges known to the committee at this time is Waters, 34 Maple Street, Milford, MA 01757 (www.waters.com). If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,¹ which you may attend.

safety problems associated with this test. A hazards review shall be conducted by all personnel performing the test. It is the responsibility of the user to review all material safety data sheets (MSDS), manuals, and hazards procedures and establish the appropriate safety measures. Some PAH compounds have been shown to possess mutagenic as well as carcinogenic and teratogenic properties. As such, concentrated extracts of carbon blacks containing PAHs also may possess the same harmful properties. Solvents used are flammable. Appropriate personal protection equipment (PPE) shall be used.

9. Preparation of Standard Solutions

9.1 The GC/MS instrument is calibrated through five mixtures of the native BaP and deuterated IS. The recommended BaP concentrations are to cover a range of approximately 0.01 to 0.8 $\mu\text{g}/\text{cm}^3$ (ppm) while the IS concentration is kept constant at around 0.3 $\mu\text{g}/\text{cm}^3$. A lower BaP concentration range can be used for the case of high-purity carbon blacks, but the difference between any two concentration levels shall not exceed a factor of four. Furthermore, the IS concentration should maintain an S/N ratio of at least 15/1 for routine instrument performance. Subsections 9.2 – 9.5 describe the preparation of the various solutions required.

9.2 *Preparation of Native BaP Standard Solutions for Calibration*—Using the native BaP standard stock solution described in 7.2, prepare at least 10 cm^3 of five toluene solutions in amber glassware at the concentrations suggested in the following. Cap securely the standard solutions, mix thoroughly, and label.

Native BaP Standard 5	0.85 $\mu\text{g}/\text{cm}^3$
Native BaP Standard 4	0.28 $\mu\text{g}/\text{cm}^3$
Native BaP Standard 3	0.09 $\mu\text{g}/\text{cm}^3$
Native BaP Standard 2	0.03 $\mu\text{g}/\text{cm}^3$
Native BaP Standard 1	0.01 $\mu\text{g}/\text{cm}^3$

9.3 *Preparation of the Deuterated IS Solution*—Using the deuterated IS stock solution described in 7.1, prepare at least 50 cm^3 of a diluted toluene solution in amber glassware at the concentration suggested in the following. Cap securely the standard solutions, mix thoroughly, and label. This recommendation on the use of an internal standard is consistent with the practice described in EPA Method 8270D.

IS Standard	3.3 $\mu\text{g}/\text{cm}^3$
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9.4 *Preparation of the BaP Calibration Solutions*—Into five separate amber GC/MS vials, pipette 1.000 cm^3 of the native BaP standards prepared in 9.2 and 0.100 cm^3 of the IS standard solution prepared in 9.3. Cap securely the amber GC/MS vials, mix thoroughly, and label. The following is obtained:

Cal BaP 5	0.773 $\mu\text{g}/\text{cm}^3$ native BaP + 0.300 $\mu\text{g}/\text{cm}^3$ IS
Cal BaP 4	0.255 $\mu\text{g}/\text{cm}^3$ native BaP + 0.300 $\mu\text{g}/\text{cm}^3$ IS
Cal BaP 3	0.082 $\mu\text{g}/\text{cm}^3$ native BaP + 0.300 $\mu\text{g}/\text{cm}^3$ IS
Cal BaP 2	0.027 $\mu\text{g}/\text{cm}^3$ native BaP + 0.300 $\mu\text{g}/\text{cm}^3$ IS
Cal BaP 1	0.009 $\mu\text{g}/\text{cm}^3$ native BaP + 0.300 $\mu\text{g}/\text{cm}^3$ IS

9.5 All standard solutions shall be stored in amber glassware and kept in a refrigerator ($<6^\circ\text{C}$) when not in use. Care has to be taken not to exceed their shelf life. If any indication on degradation is perceived, the standard has to be prepared anew.

10. Carbon Black Sample Preparation and Extraction

10.1 All glassware parts coming into contact with the sample shall be BaP free on the basis of the limits of quantification. It is strongly recommended to use separate glassware and extraction units for high-purity carbon blacks and carbon blacks in which higher BaP levels are expected. Blanks should be run on a regular basis.

10.2 Glassware should be rinsed with toluene and acetone after use. The glassware is then dried at 150°C in a laboratory drying oven.

10.3 For low BaP carbon blacks, a pre-extraction of the extraction unit for at least 4 h is recommended. If glass thimbles are not baked in a furnace as described in 10.5, then the thimbles should be included in the 4 h pre-extraction. Cellulose thimbles should be included in the 4 h pre-extraction. In this case, the thimbles are further dried, for example, in a vacuum drying oven prior to use.

10.4 Disposable devices such as cellulose thimbles are rinsed with toluene and dried prior to use, for example, in a vacuum oven.

10.5 If repeated cleanings and extractions do not produce clean blanks, certain parts of the glassware may also be baked for at least 6 h in a furnace at, for example, 500°C . This is also valid for glass extraction thimbles if used. It shall be determined with the manufacturer if the glass parts can sustain such temperature—for example, it is unlikely the Soxhlet can sustain this.

10.6 If glass extraction thimbles are used, they should be checked for their drain rate at room temperature by pouring 50 cm^3 of toluene inside the thimble. The time for 40 cm^3 to drip out of the thimble should not exceed 95 s. Otherwise, the thimble is to be discarded. Dry the thimbles with clean nitrogen.

10.7 If glass extraction thimbles are used, pour the 60/80-mesh glass beads into the thimbles to a depth of 1 cm. Sizes other than 60/80 mesh may be used.

10.8 Weigh 10 ± 0.1 g to the nearest 1 mg of the beaded carbon black sample into the dried extraction thimble. Record the exact value as W_{CB} .

10.8.1 Fluffy or powder carbon black should be densified with toluene before extraction. This is accomplished by weighing 10 ± 0.1 g of carbon black into a beaker and adding toluene in approximately 1- cm^3 aliquots and mixing the toluene into the carbon black with a spatula after each aliquot is added. This densifies the carbon black and forms pellets. Add sufficient toluene to densify the entire sample into crude pellets. The beakers shall then be left in the hood overnight to evaporate the solvent. Once the solvent has evaporated, break up the larger of the carbon black pellets with a spatula. The entire amount of pelletized black is then transferred to the thimble.

10.9 Place a plug of glass wool or fold the top of the cellulose thimble above the black. The plug of wool should be placed such that the glass wool is in contact with the carbon

black. This facilitates drainage of solvent through the glass wool and into the carbon black bed.

10.10 Add a few glass boiling beads into the clean 250-cm³ boiling flask and pour approximately 150 cm³ of toluene. Assemble the extraction apparatus. To maintain temperature uniformity throughout the system, wrap the Soxhlet extractor and the boiling flask with aluminum foil. Record the Soxhlet position number, thimble identification (for glass thimbles), and extraction date.

10.11 *Extraction Settings and Conditions:*

10.11.1 Samples are extracted for 16 h.

NOTE 1—Hamm et al⁷ demonstrated that 16 h were sufficient to collect over 95 % of the BaP extractable in 48 h from a relatively high PAH content carbon black.

10.11.2 Turn on the water flow. It is recommended that a water-flow monitor, a solenoid valve to switch the water flow on or off, and a timer be assembled such that:

10.11.2.1 An interruption in the water flow triggers the timer to stop, the heating mantles to turn off, and the solenoid valve to turn off; and

10.11.2.2 A power outage stops the timer, switches off the solenoid valve, and prevents the heating mantles from turning back on once power is restored.

10.12 Nitrogen should continuously flow through a manifold connected to the condensers throughout the entire extraction period at a very low flow rate of approximately 10 to 15 sccm (standard cubic centimetres per minute). If the flow rate is too high, toluene may evaporate from the Soxhlet.

10.13 Turn the heating mantles on low.

10.14 After 5 to 20 min, the flasks should be on the verge of boiling. Gently agitate the apparatus; this should initiate the toluene boiling. Once this occurs, turn the heat to a higher setting. After about 30 min, check to ensure that the toluene is dripping from the condenser.

10.15 After at least 4 h of extraction, check and adjust the heater such that:

10.15.1 The cycle time of each Soxhlet is less than 6 min (approximately ten cycles per hour). This is the length of time for the Soxhlet to fill and the solvent to siphon out through the siphon arm. Note that this is not the drain time. Drain time is described in 10.15.2.

10.15.2 If glass extraction thimbles are used, the drain time of the thimbles should be less than 15 min. This is the time for the solvent to drain out of the thimble. This is measured by lowering the heat (to stop the solvent dripping from the condenser) and visually measuring the length of time for most of the solvent to drain through the thimble. If the drain time deviates from the above value, replace the thimble and restart the extraction.

10.16 Extract samples continuously for 16 h and the toluene level should be checked from time to time for potential losses

through evaporation. To avoid degradation of the extracted BaP by overheating of glass walls, a sufficient level of toluene shall always remain in the boiling flask especially at the point at which the Soxhlet is filled and the solvent is starting to siphon out.

10.17 If the remaining solvent volume becomes too small, the extraction has to be interrupted and fresh toluene be added to the extractor after cool down. The nitrogen flow should be checked and adjusted, if necessary. The extraction is subsequently continued to complete the required extraction time.

11. Extract Preparation before the GC/MS Analysis

11.1 Once the set extraction time has elapsed, the thimble is removed.

11.2 Depending upon the expected level of BaP in the carbon black sample, the raw toluene extract is processed differently.

11.3 For some extracts that contain very low levels of BaP, the IS solution prepared in 9.3 is added to the extract before the SPE and concentration steps. This is to correct for any potential loss of BaP during the SPE and rotary evaporation steps. In this case, add 200 µL of the IS solution prepared in 9.3 to the extract and swirl the boiling flask to mix in the IS solution. Then pass the entire extract through the silica SPE into the rotary evaporation flask. Rinse the boiling flask with a few cubic centimeters of fresh toluene through the SEP and into the rotary evaporation flask three times. Concentrate the extract under the conditions from 11.4 to about 0.5 cm³, then quantitatively transfer the concentrated extract into a 2-cm³ amber volumetric flask, rinsing twice with about 0.5 cm³ of fresh toluene. Bring the toluene level to exactly 2.0 cm³ with toluene, cap securely, mix thoroughly, and label. Record the final extract volume, V_f , as 2.0 cm³. Retain in the 2-cm³ amber volumetric flask or transfer to a 4-cm³ vial with PTFE/rubber cap (PTFE side faces the toluene). An aliquot of at least 0.6 cm³ can now be transferred to the amber GC/MS vial and analyzed by GC/MS as described in Section 12. For the IS standard example given in 9.3, the GC/MS vial allegedly contains 0.33 µg/cm³ of the IS ($CIS = 0.33 \mu\text{g}/\text{cm}^3$).

11.4 For all other cases, pass the entire extract through the silica SPE into the rotary evaporator flask. Rinse the boiling flask with fresh toluene and pass this through the SPE three times. For cases where it is known that the extract does not require concentration, it is acceptable to measure the volume of the extract to $\pm 0.5 \text{ cm}^3$, record the volume as V_i (the initial extract volume), and proceed to 11.6. Otherwise, concentrate the toluene extract on the rotary evaporator down to about 50 cm³ in the boiling flask. The rotary evaporator shall be set to a maximum bath temperature of $40 \pm 2 \text{ }^\circ\text{C}$ and a vacuum of $5 \pm 0.3 \text{ kPa}$. These parameters shall be monitored carefully during all evaporation steps as BaP losses may occur. Alternative evaporators or vacuum and temperature settings or both may be used provided recovery studies are conducted and results of these studies are acceptable.

NOTE 2—The purpose of the SPE is to remove some highly polar material that can contaminate the GC column and cause a problem with peak shape. The traditional way of performing SPE would be first to concentrate the extract to a much lower volume (2 to 3 cm³), then pour it

⁷ Hamm et al, "Investigations on the extraction and migration behavior of polycyclic aromatic hydrocarbons (PAHs) from cured rubber formulations containing carbon black as reinforcing agent," *Rubber Chemistry & Technology*, Vol 82, Issue 2, 2009.

on the top of the SPE bed, rinsing it with fresh toluene, and finally, go through a second concentration cycle. This traditional protocol would certainly minimize the amount of highly polar material in the final extract but would also increase the risk of BaP loss since a higher concentration of BaP is exposed to the SEP. This is especially critical when dealing with low BaP carbon black grades.

NOTE 3—Concentration tests with 200-cm³ toluene solutions containing 50 ng of BaP indicated that no measurable losses occurred when narrowing even to a volume of 5 cm³ (at 40°C water bath temperature and 5-kPa pressure).

11.5 Quantitatively transfer the extract into a 100-cm³ amber volumetric flask. Rinse at least three times with approximately 10 cm³ of fresh toluene each time. Bring the toluene extract to exactly 100 cm³, cap securely, mix thoroughly, and label. Record the initial extract volume, V_i , as 100 cm³.

11.6 The extract may now need to be concentrated or diluted depending on the BaP level in the extract. The level of BaP injected into the GC/MS shall fall within the range defined in 11.6.1.1 – 11.6.1.3.

11.6.1 One way to determine if the extract needs to be concentrated or diluted is from previous experience on the particular grade being tested. The color of the extract, many times, can act as a guide (see Note 4).

NOTE 4—The color of the extract obtained might give an indication of the expected BaP level. Though difficult to assess and essentially based on experience, high-purity carbon blacks tend to exhibit colorless extracts or with a slight tinge of yellow, while higher BaP level carbon blacks produce yellow to orange extracts. Typically, colorless extracts need to be concentrated. Typically, deeply yellow or orange extracts do not need concentration and may need dilution.

11.6.1.1 If the BaP level is lower than half the lowest point in the calibration curve, then the extract needs to be concentrated to bring the BaP level into the acceptable range. In this case, proceed to 11.7.

11.6.1.2 If the BaP level is more than twice the highest point in the calibration curve, then the extract needs to be diluted. In this case, proceed to 11.8.

11.6.1.3 If the extract does not need concentrating or diluting, proceed also to 11.8.

11.7 Follow the procedure in this section if the extract from 11.6 needs to be concentrated.

11.7.1 Quantitatively transfer an aliquot of the extract from the 100-cm³ volumetric flask to the rotary evaporator flask. Record the aliquot volume, V_A . For example, if a 50 cm³ aliquot is transferred to the rotary evaporator flask, $V_A = 50$ cm³. Add exactly 500 μ L of the IS solution prepared in 9.3 to the rotary evaporator flask.

11.7.2 Concentrate the extract aliquot to approximately 2 cm³, following the rotary evaporator temperature and vacuum guidelines in 11.4. Ensure that a volume of at least 1.0 cm³ of extract remains in the flask at the completion of this step. If a precipitate is observed on the rotary evaporator flask walls during the concentration, the concentration should be stopped and the extract brought to the most convenient known volume. A nitrogen blow-down apparatus may be used for this step instead of a rotary evaporator with the concentration being performed in a 40°C water bath at a suitable nitrogen flow rate.

11.7.3 Quantitatively transfer the concentrated extract to a 5-cm³ amber volumetric flask, rinsing the rotary evaporator

flask at least three times with fresh toluene. Bring the toluene extract to the set volume exactly, cap securely, mix thoroughly, and label. Record the final extract volume, $V_f = 5$ cm³. As in the calibration standard examples in 9.4, the 5-cm³ amber volumetric flask allegedly contains 0.33 μ g/cm³ of the IS ($C_{IS} = 0.33$ μ g/cm³).

11.7.4 Proceed to 11.9.

11.8 Follow the procedure in this section if the extract from 11.6 needs to be diluted or if it does not need concentrating or diluting.

11.8.1 Pipette exactly 500 μ L of the IS solution prepared in 9.3 into a 5-cm³ amber volumetric flask. As in the calibration standard examples in 9.4, the 5-cm³ amber volumetric flask allegedly contains 0.33 μ g/cm³ of the IS ($C_{IS} = 0.33$ μ g/cm³).

11.8.2 An aliquot of the extract prepared in 11.5 now needs to be added to the 5-cm³ amber volumetric flask that already contains 500 μ L of the IS. The volume of this aliquot needs to be chosen so that the final concentration of BaP falls within the range of the calibration curve (as defined in 11.10).

11.8.2.1 If no dilution is needed, then sufficient extract is added to the 5-cm³ amber volumetric flask to bring the final volume to the mark. In this case $V_A = 4.5$ cm³.

11.8.2.2 If the extract needs to be diluted, then dilute the extract appropriately with toluene into the 5-cm³ amber volumetric flask. Should the extract prepared in 11.5 require to be diluted by a large factor, it is recommended to proceed via a two steps dilution. For instance, for a 100 times dilution, first dilute 1 cm³ in 10 cm³ then 0.5 cm³ in 5 cm³. Record the aliquot volume, V_A , as the effective volume of extract added to the 5 cm³ volumetric flask. Three examples will illustrate how to determine V_A :

(1) If sufficient extract is added to the 5-cm³ amber volumetric flask to bring the volume to 5.0 cm³, $V_A = 4.5$ cm³.

(2) If 1.0 cm³ of extract is added to the 5-cm³ amber volumetric flask, $V_A = 1.0$ cm³.

(3) If 1.0 cm³ of extract is first diluted to 10.0 cm³, then 0.5 cm³ is added to the 5-cm³ amber volumetric flask, $V_A = 0.05$ cm³.

11.8.3 Bring the toluene extract to the set volume exactly, cap securely, mix thoroughly, and label. Record the final extract volume, $V_f = 5$ cm³.

11.8.4 Proceed to 11.9.

11.9 Transfer at least 1 cm³ of the final extract prepared in 11.5, 11.7, or 11.8 to a GC/MS amber vial. Cap securely and label. The sample is now ready for injection.

11.10 After the sample analysis and calculation (Section 14), both the raw peak area (A_{BaP}), as well as the ratio of responses ($R_R = A_{BaP}/A_{IS}$) should be within the calibration range (Section 9). In some cases, minor deviation from this guideline can be tolerated. On the low end of the curve, half of the lowest calibration value can be allowed; on the high end of the curve, twice the highest calibration value can be allowed.

11.11 If these conditions are not met, start again from 11.6 using the retained extract volume in 11.5 and adjust the appropriate dilution or concentration factor.

12. Procedure

12.1 GC/MS Instrument Operating Conditions:

12.1.1 Because of the different GC/MS systems available, it is impossible to specify detailed GC/MS parameters for this analysis. The following GC/MS conditions are presented as examples, but every user will have to adjust and tune his own device to obtain a suitable BaP peak separation and quantification.

12.1.2 *Example of Gas Chromatograph Operation Conditions:*

- 12.1.2.1 Carrier gas: Helium
- 12.1.2.2 Constant pressure: 70 kPa (corresponding to a linear velocity of approximately 40 cm/s)
- 12.1.2.3 Injector temperature: 300°C
- 12.1.2.4 Injector: Splitless with gold-plated seal, 1.5 µL
- 12.1.2.5 Initial oven temperature: 75°C
- 12.1.2.6 Initial hold time: 0.1 min
- 12.1.2.7 Ramp rate: 120°C/min to 180°C then 8°C/min to 240°C then 50°C/min to 300°C
- 12.1.2.8 Final temperature: 300°C
- 12.1.2.9 Final hold time: 5 min or until all organic compounds have eluted
- 12.1.2.10 Analytical time: Approximately 30 min

12.1.3 *PAH Identification and Retention Time Determination:*

12.1.3.1 Ensure the mass spectrometer is tuned for SCAN mode according to the manufacturer's instructions.

12.1.3.2 When starting a testing campaign or when the GC column has been changed, run in SCAN mode a calibration standard as prepared in 9.4 and a carbon black extract as prepared in 10.8. This SCAN protocol is not used for quantification purposes but for qualitative reasons and peak separation optimization. The various PAHs will be separated and identified through their full mass spectra confirming their corresponding retention times.

- (1) Transfer line temperature: 280°C
- (2) Source temperature: 230°C
- (3) Quadrupole temperature: 150°C (if available)
- (4) Electron energy: 70 V (nominal)
- (5) Ionization mode: EI
- (6) Mass range: 50 to 350 atomic mass units (amu), SCAN data acquisition
- (7) Scan time: at least five scans per peak not to exceed 0.5 s/scan
- (8) Solvent delay: 8 min (to protect mass detector)

12.1.3.3 The GC parameters (12.1.2) will need to be optimized to ensure an appropriate BaP peak resolution (both native and deuterated).

12.1.3.4 Once the peak resolution is satisfactory, write down the retention times of interest and determine the time window of interest.

12.1.4 *Dynamic Mass Calibration (DMC):*

12.1.4.1 As a way of further optimizing the MS signal, the DMC protocol is recommended for mass spectrometers capable of a resolution of at least 0.1 amu.

12.1.4.2 Tune the mass detector for SIM according to the manufacturer's instructions.

12.1.4.3 Run the DMC method on a calibration standard as prepared in 9.4 using the following parameters:

- (1) GC conditions: as optimized in SCAN mode (12.1.3.4)

(2) MS conditions: as in 12.1.3.2 apart for SIM data acquisition

(3) Ion masses: 263.8, 263.9, 264.0, 264.1, 264.2, 251.8, 251.9, 252.0, 252.1, 252.2

12.1.4.4 Observe the mass spectrum for every peak detected and write down the corresponding top three ion masses (with maximum signal) to the nearest 0.1 amu. Proceed then as per 12.1.5.

12.1.5 *SIM for BaP Quantification:*

12.1.5.1 This SIM protocol is based on the same GC and MS conditions as used for the DMC (12.1.4) apart from the MS SIM configuration in which only the top three ion masses (with maximum signal) are used. An example for SIM configuration from the DMC is shown in the following. If the DMC is not used, then only the nominal target ions should be used for the native BaP and its deuterated version.

- (1) Ion masses: 263.8, 263.9, 264.0, 251.8, 251.9, 252.0

NOTE 5—Ions 57, 92, and 182 are found in carbon black toluene extracts (bitolyl isomers and hydrocarbons). These ions can also be monitored as diagnostic aids if problems occur. The analyst is also encouraged to monitor characteristic fragment ions for each of the native PAHs. Presence of these ions and reasonable fragmentation ratio may help to confirm identity of the PAHs in case of interferences.

12.1.5.2 Once the DMC has been performed, do not tune the mass detector as this would reset the mass calibration.

12.1.6 *Peak Integration:*

12.1.6.1 Once BaP has eluted from the column, the chromatographic area for the molecular ion is integrated.

12.1.6.2 Some GC/MS instruments possess a built-in automatic integrator. The following parameters are presented as an example for the Agilent RTE integrator. This information is given for the convenience of users of this test method and does not constitute an endorsement of the product named. Equivalent products may be used if they can be shown to lead to the same results.

- (1) Detector point sampling: 2
- (2) Detection filter: 7
- (3) Start threshold: 0.060
- (4) Stop threshold: 0.03
- (5) Baseline reset: 0
- (6) Minimum area: 250 area counts
- (7) Peak location: Top

13. Preparation of Calibration Curves

13.1 Run the SIM protocol (12.1.5) on the five calibration solutions prepared in 9.4.

13.2 *Calibration Curve*—Plot the calculated ratio of response (R_R) versus the ratio of concentration (R_C) using each of the five calibration solutions.

$$R_R = A_{BaP}/A_{IS} \quad (1)$$

$$R_C = C_{BaP}/C_{IS} \quad (2)$$

where:

- A_{BaP} = integrated area of the BaP peak (peak area unit),
- A_{IS} = integrated area of the IS peak (peak area unit),
- C_{BaP} = BaP concentration in the GC vial (µg/cm³), and
- C_{IS} = IS concentration in the GC vial (µg/cm³).

13.3 *Calibration Curve Acceptance Criteria:*

13.3.1 Linear regression forced through the origin is used for the fit.

13.3.2 The correlation coefficient (R^2) shall be at least 0.95.

13.3.3 The signal-to-noise ratio (S/N, as defined in the following) for the lowest peak area of the least concentrated BaP standard (Cal BaP 1, 0.010 $\mu\text{g}/\text{cm}^3$) should be greater than 15:1. This is used as a check on instrument performance.

13.3.4 S/N is calculated as follows (see Figs. 1 and 2):

$$\text{Average Noise } (N_a) = \text{sum of noise/number of data points} \quad (3)$$

$$\text{Corrected Signal } (S_c) = \text{Height } (H_p) - N_a \quad (4)$$

$$\text{Noise } (H_n) = N_{\max} - N_{\min} \quad (5)$$

$$S/N = S_c/H_n \quad (6)$$

13.3.5 If the conditions in 13.3.2 and 13.3.3 are met, record the slope of the linear fit.

13.4 Calibration Curve Example:

13.4.1 In Table 2 is an example of the parameters used in constructing the calibration curve using hypothetical peak areas for BaP and d_{12} -BaP. The calibration curve should be a linear fit and forced through the origin.

13.4.2 The resulting BaP calibration curve is depicted in Fig. 3 with the resulting linear fit:

$$R_R = 1.348 R_C \quad (r^2 = 0.9999) \quad (7)$$

14. Sample Analysis and Calculation

14.1 Once the calibration system has been established, run the sample extract prepared in Section 11 using the SIM protocol described in 12.1.5.

14.2 Determine the peak areas for BaP (A_{BaP}) and the deuterated internal standard (A_{IS}).

14.3 Calculate the R_R :

$$R_R = A_{BaP}/A_{IS} \quad (8)$$

14.4 Calculate the R_C :

$$R_C = R_R/\text{slope} \quad (9)$$

14.5 Calculate the BaP concentration in the GC vial (in $\mu\text{g}/\text{cm}^3$):

$$C_{BaP} = R_C \times C_{IS} \quad (10)$$

14.6 Calculate $C_{BaP,CB}$, the BaP concentration in the carbon black sample, as shown in the following equation.

14.6.1 If the extract was prepared for analysis according to 11.3:

$$C_{BaP,CB} = (1000 \times V_f \times C_{BaP})/W_{CB} \quad (\text{in } \mu\text{g}/\text{kg or ppb}) \quad (11)$$

where:

V_f = final extract volume defined in 11.3, and

W_{CB} = carbon black weight (in grams) Soxhlet extracted, 10.8.

14.6.2 For the other cases (11.6):

$$C_{BaP,CB} = (1000 \times V_f \times V_i \times C_{BaP})/(V_A \times W_{CB}) \quad (\text{in } \mu\text{g}/\text{kg or ppb}) \quad (12)$$

where:

V_i = initial extract volume, 11.5,

V_A = aliquot volume, 11.7.1 or 11.8.2,

V_f = final extract volume, 11.7.3 or 11.8.3; and

W_{CB} = carbon black weight (in grams) Soxhlet extracted, 10.8.

14.7 Calculation Example:

14.7.1 Using the calibration curve example from Fig. 1 (Table 3):

$$R_R = A_{BaP}/A_{IS}: A_{BaP} = 362\,524 \text{ and } A_{IS} = 182\,506 \text{ hence } R_R = 1.986$$

$$R_C = R_R/\text{slope}: R_R = 1.986 \text{ and slope} = 1.348 \text{ hence } R_C = 1.473$$

$$C_{BaP} = R_C \times C_{IS}: R_C = 1.473 \text{ and } C_{IS} = 0.33 \mu\text{g}/\text{cm}^3 \text{ hence } C_{BaP} = 0.486 \mu\text{g}/\text{cm}^3$$

$$W_{CB} = 10.024 \text{ g and } V_A = 50 \text{ cm}^3 \text{ and } V_f = 5 \text{ cm}^3 \text{ and } V_i = 100 \text{ cm}^3 \text{ hence } C_{BaP,CB} = 485 \text{ ppb}$$

14.8 A blank run can be periodically performed to ensure the integrity of the results. Blank extracts are prepared as per Section 10 but with no carbon black. Trace quantities of certain PAHs could be found in the blanks as a result of many possible factors such as trace contamination of glassware and purity of the deuterated internal standards. The BaP level in the blanks should not exceed 5 ng/cm³ in the GC/MS vial.

15. Report

15.1 Report the following information:

15.1.1 Proper identification of the carbon black sample.

15.1.2 Time duration of Soxhlet extraction (16 hours).

15.1.3 BaP content in the carbon black to the nearest 1 ppb ($\mu\text{g}/\text{kg}$).

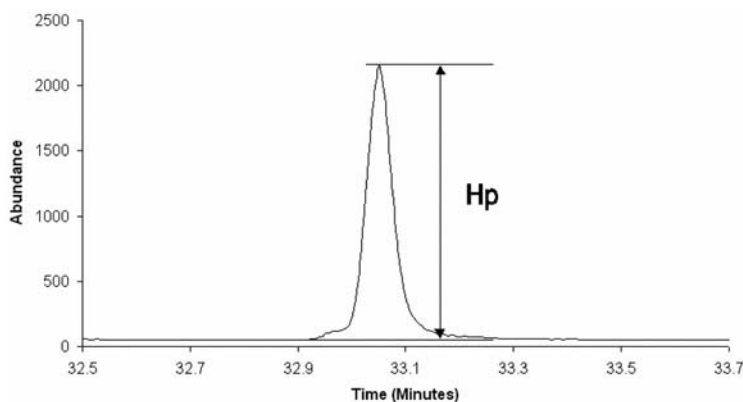


FIG. 1 Determination of Peak Signal

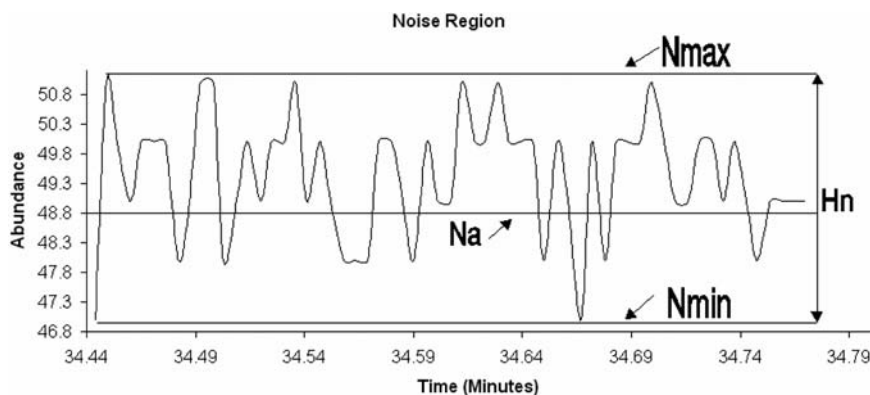


FIG. 2 Determination of Noise Parameters

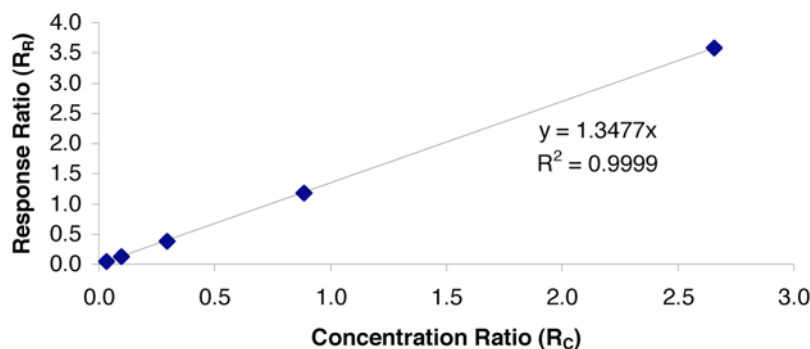


FIG. 3 Calibration Curve Example

TABLE 2 Quantities Used for Example Calculations

	Benzo(a)pyrene		d ₁₂ -Benzo(a)pyrene		Ratios ^A	
	C _{BaP} (μg/cm ³)	A _{BaP}	C _{IS} (μg/cm ³)	A _{IS}	R _C	R _R
Cal BaP 1	0.773	654 056	0.300	182 404	2.58	3.586
Cal BaP 2	0.255	170 169	0.300	144 156	0.85	1.180
Cal BaP 3	0.082	62 868	0.300	167 139	0.27	0.376
Cal BaP 4	0.027	20 264	0.300	157 126	0.09	0.129
Cal BaP 5	0.009	7 252	0.300	163 637	0.03	0.044

^A R_R = A_{BaP}/A_{IS}; R_C = C_{BaP}/C_{IS}

TABLE 3 Quantities Used for Example Calculations

	Benzo(a)pyrene		d ₁₂ -Benzo(a)pyrene		Ratios	
	C _{BaP} (μg/cm ³)	A _{BaP}	C _{IS} (μg/cm ³)	A _{IS}	R _C	R _R
Sample 1	unknown	362 524	0.330	182 506	unknown	1.986

16. Precision and Bias⁸

16.1 These precision statements have been prepared in accordance with Practice D4483. Refer to this practice for terminology and other statistical details.

16.2 The precision results in this precision and bias section give an estimate of the precision of this test method with the materials used in the particular interlaboratory program described below. The precision parameters should not be used for acceptance or rejection testing of any group of materials

without documentation that they are applicable to those particular materials and the specific testing protocols of the test method. Any appropriate value may be used from Table 4.

16.3 A type 1 inter-laboratory precision program was conducted. Both repeatability and reproducibility represent short-term (daily) testing conditions. The testing was performed using two operators in each laboratory performing the test once on each of two days (total of four tests). A test result is the value obtained from a single determination. Acceptable difference values were not measured. The between-operator component of variation is included in the calculated values for *r* and *R*.

16.4 The results of the precision calculations for this test are given in Table 4. The materials are arranged in ascending “mean level” order.

16.5 *Repeatability*—The pooled relative repeatability, (*r*), of this test has been established as 18.7 %. Any other value in Table 4 may be used as an estimate of repeatability, as appropriate. The difference between two single test results (or determinations) found on identical test material under the repeatability conditions prescribed for this test will exceed the repeatability on an average of not more than once in 20 cases in the normal and correct operation of the method. Two single test results that differ by more than the appropriate value from Table 4 must be suspected of being from different populations and some appropriate action taken.

NOTE 6—Appropriate action may be an investigation of the test method procedure or apparatus for faulty operation or the declaration of a

⁸ A research report is pending.

TABLE 4 Precision Parameters for Test Method D7771, Type 1 Precision, BaP Content of Carbon Black

Material	Number of Laboratories	Units ppb						
		Mean Level	Sr	r	(r)	SR	R	(R)
CB 1	5	6.9	2.2	6.3	91.9	2.4	6.8	100.0
CB 2	5	8220.8	374.2	1058.9	12.9	1904.3	5389.0	65.6
CB 3	5	18080.8	929.8	2631.2	14.6	3257.7	9219.2	51.0
Average		8769.5						
Pooled Values			578.6	1637.6	18.7	2178.6	6165.4	70.3

significant difference in the two materials, samples, etc., which generated the two test results.

16.6 Reproducibility—The pooled relative reproducibility, (R), of this test has been established as 70.3 %. Any other value in **Table 4** may be used as an estimate of reproducibility, as appropriate. The difference between two single and independent test results found by two operators working under the prescribed reproducibility conditions in different laboratories on identical test material will exceed the reproducibility on an average of not more than once in 20 cases in the normal and correct operation of the method. Two single test results produced in different laboratories that differ by more than the appropriate value from **Table 4** must be suspected of being from different populations and some appropriate investigative action taken.

16.7 Bias—In test method terminology, bias is the difference between an average test value and the reference (true) test property value. Reference values do not exist for this test method since the value or level of the test property is exclusively defined by the test method. Bias, therefore, cannot be determined.

17. Keywords

17.1 BaP; benzo-a-pyrene; carbon black; GC/MS; Soxhlet extraction; toluene extract

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