Designation: D7771 – 17

Standard Test Method for Determination of Benzo-α-Pyrene (BaP) Content in Carbon Black[1]

This standard is issued under the fixed designation D7771; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

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 Polymers[4]

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 consists 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 $C_{20}H_{12}$, 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 16 h. The extract is concentrated and subsequently analyzed for BaP by gas chromatography with mass spectrometry (GC/MS). The BaP quantification is performed by the means of an internal standard.

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-cm^3 capacity.

6.2 *Extraction Thimbles,* glass or cellulose, approximately 50- to 70-cm^3 capacity. For instance, glass extraction thimble of 35-mm diameter by 90-mm height with course porosity (70 to 100 µm similar to Ace Glass Size C, Porosity B, Code-14).

[1] This test method is under the jurisdiction of ASTM Committee D24 on Carbon Black and is the direct responsibility of Subcommittee D24.66 on Environment, Health, and Safety.

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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.

TABLE 1 Native and Deuterated BaP Compounds

Native PAH	CAS#	Deuterated PAH	CAS#	C13 Labeled PAH	CAS#
Benzo-α-pyrene	50-32-8	d_{12}-Benzo-α-pyrene	63466-71-7	$13C_4$-Benzo-α-pyrene	Not Available

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 *Microliter 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 ± 10°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 ± 25°C, used to burn off organic contamination from glass surfaces.

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 native BaP standards and of the internal standard (deuterated or C13-labeled) can be purchased as premade solutions or prepared from solid materials (Table 1).

7.2.1 A concentration range of 80 to 100 µg/cm^3 for the native BaP analyte stock solution is recommended.

7.2.2 A similar concentration range of 80 to 100 µg/cm^3 of the deuterated (d_{12}-benzo-α-pyrene) or isotopically-labeled ($13C_4$-benzo-α-pyrene) is recommended for the internal standard (IS) stock solution.

7.2.3 All purchased BaP standard materials shall be 98 % pure or better and certified with respect to their purity and concentration by the manufacturer. Follow the manufacturer's recommendation on how best to store the standard solutions. Typically, the compounds are protected from light. They should be checked frequently for signs of degradation or evaporation. The BaP standard stock solutions shall be replaced/recertified on a yearly basis or sooner if comparisons with quality control (QC) samples indicate 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 gel columns for Solid Phase Extraction (SPE) removal of polar compounds.

[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.

7.7.1 *Silica SPE Cartridges,* single-use application, having a volume capacity of approximately 1 to 10 cm^3.[6]

7.7.1.1 *Preparation of Silica SPE Cartridge*—Follow the manufacturer's instruction for preparation and use. The typical method of preparation is to wet the cartridge with approximately 10 cm^3 of the elution solvent (toluene). Discard the wetting solvent.

7.7.2 *Alternative SPE Method: Silica Gel/13 % H_2O Gravity Column:*

7.7.2.1 *Preparation of a Silica Gel/13 % H_2O Adsorbent*—Pour 200 g silica gel (high purity grade, type 60, particle size 0.063 to 0.200 mm) into a screw cap glass bottle and add 30 g of deionized water in small portions (for example, 2 cm^3) using a suitable pipette. After addition of each portion of water, the silica gel bottle is shaken to evenly distribute the wetted silica gel. No aggregation should occur during this process. If so, shaking must be continued until a homogeneous material is obtained. Finally, the closed bottle is shaken for 8 hours by means of an overhead shaker. If properly closed and stored, the adsorbent should be viable for at least 6 months.

7.7.2.2 *Preparation of a Silica Gel/13 % H_2O Gravity Column*—Insert a glass wool plug into the bottom of a pipette tip (for example, 8 to 10 mm inner diameter and 5 cm^3 capacity). Place 1 g of the silica gel/13 % H_2O in the column and tap the column gently to settle the silica gel. Cover the adsorbent layer with a glass wool plug and pre-elute the column with 5 cm^3 of cyclohexane. Discard the cyclohexane eluate.

8. Hazards

8.1 This test involves hazardous materials, operations, and equipment. This procedure does not attempt to address the 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 using five solutions of the native BaP and internal standard (IS). The recommended BaP concentrations are to cover a range of 0.0125 to 1.0 µg/cm^3 (ppm). Other concentrations may be used as needed for the application. The IS concentration is kept constant within the calibration range. Preferably the IS concentration is in the middle of the selected calibration range (for example, 0.3 to 0.6 µg/cm^3). A lower BaP concentration range can be used for the case of high-purity carbon blacks. However, the IS concentration should maintain an S/N ratio of at least 15/1 for routine instrument performance. Subsections 9.2 – 9.4 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 below. Other concentrations may be used but the difference between any two concentration levels shall not exceed a factor of four. Before diluting to the final solution volume, spike each standard with the appropriate volume (for example, 100 µL IS solution described in 9.3) to give a final IS concentration of 0.500 µg/cm^3. Other aliquot volumes and final volumes may be used to obtain the desired concentrations. Cap the standard solutions securely, mix thoroughly, and label.

Native BaP Standard 5	1.00 µg/cm^3
Native BaP Standard 4	0.500 µg/cm^3
Native BaP Standard 3	0.200 µg/cm^3
Native BaP Standard 2	0.0500 µg/cm^3
Native BaP Standard 1	0.0125 µg/cm^3

9.3 *Preparation of Diluted IS Solution*—Using the IS stock solution described in 7.2, prepare a convenient volume (for example, 10 cm^3) of diluted internal standard solution that will be later added to samples and calibration standards. A concentration of 50.0 µg/cm^3 is suggested for this diluted IS solution in order to achieve a final concentration of 0.500 µg/cm^3 in the extract sample.

NOTE 1—Other concentrations within the calibration range can be used as well.

9.4 All standard solutions shall be stored in amber glassware and kept in a refrigerator (<6°C) when not in use. Care has to be taken not to exceed their shelf life. If any indication of degradation is perceived, then new standards have to be prepared.

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

[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.

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-cm^3 boiling flask and pour approximately 150 cm^3 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 2—Hamm et al[7] 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 centimeters 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 the Soxhlet assembly 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 Two extract preparation procedures (Part A and Part B) are given below. Either procedure may be used.

Part A

11.2 Once the set extraction time has elapsed, the Soxhlet assembly is cooled and the thimble is removed.

11.3 The raw toluene extract is transferred to a rotary evaporator and concentrated down to about 10 cm^3. The rotary evaporator shall be set to a maximum bath temperature of 40 ± 2°C and a vacuum of 5 ± 0.3 kPa. These parameters shall be monitored carefully during all evaporation steps as BaP losses may occur. Alternative evaporators or vacuum and temperature

[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.

settings or both may be used provided recovery studies are conducted and results of these studies are acceptable.

11.4 Quantitatively transfer the extract into a 20 cm^3 amber volumetric flask. Rinse the boiling flask at least 3 times with approximately 2 cm^3 of fresh toluene and pass them also into the volumetric flask. Bring the toluene extract to exactly 20 cm^3, cap securely, mix thoroughly, and label. If an aliquot of the concentrated extract is taken, then the initial extract volume (V_i) is 20 cm^3.

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

11.5 Depending on the expected level of BaP in the carbon black, the entire raw extract or a suitable aliquot is used for analysis. For low levels of BaP, 100 % or 50 % of the extract may be appropriate. If higher BaP levels are expected, aliquots of 10 % or 1 % may be used for BaP determination. The use of smaller aliquots for higher BaP level carbon blacks easily allows adjustment of the BaP concentration in the final measurement solution to the calibration range of the GC/MS, without further dilution steps.

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, 100 % or 50 % of the extract are taken in the case of colorless extracts. Only 10 % or 1 % of the extract is taken for the analysis of yellow or orange extracts.

11.6 Defined extract portions are typically taken up by means of volumetric pipettes or adjustable micropipettes. For SPE purification, the aliquot volume (V_A) selected for analysis is spiked with the diluted internal standard (from 9.3) and is brought to a volume of 1 cm^3. Concentration may be done either by means of a rotary evaporator or a nitrogen blow-down apparatus.

11.6.1 If the extract volume selected for analysis is larger than 1 cm^3, the aliquot is reduced to approximately 1 cm^3 prior to the SPE purification step. For these cases, add 10 µL of the diluted internal standard solution (from 9.3) into a pear-shaped rotary evaporator flask, add the extract aliquot selected for analysis, gently shake the solution and concentrate to approximately 1 cm^3 following the rotary evaporator temperature and vacuum guidelines mentioned (for example, 28 ± 2 kPa and 40 ± 2°C). If a nitrogen blow-down apparatus is used for the concentration step, 10 µL of the diluted internal standard solution and the extract aliquot selected for analysis are quantitatively transferred into a glass volumetric tube. The solution is mixed thoroughly and subsequently narrowed to the nearest of 1 cm^3 by applying suitable blow-down conditions. This final volume is recorded (V_f).

11.6.2 In the case of high BaP level carbon blacks, extract portions smaller than 1 cm^3 may be appropriate. Such small extract portions (recorded as aliquot volume, V_A) are transferred into a glass volumetric tube after addition of 10 µl of the diluted internal standard solution into the tube. The solution is brought to approximately 1 cm^3 by addition of fresh toluene and mixed thoroughly.

11.6.3 Record the volume of the extract portion taken for analysis (V_A) and the volume of the internal standard solution added to this extract aliquot.

11.7 Purification of the extract portion may be performed by means of commercially available Silica Solid Phase Extraction cartridges (SPE-cartridges, see 7.7.1). Follow the manufacturer's guidelines for removal of polar compounds from the toluene extract. Alternatively, self-prepared silica columns may also be used as described in 7.7.2. In both cases the elution behavior of the column with respect to BaP has to be validated by recovery studies for each method. Subsection 7.7.1 provides an example of the extract purification by means of a silica gel/13 % H_2O gravity column. Preparation of the silica gel/13 % H_2O solid phase is described in 7.7.2.1, whereas preparation of the column used for the example of 11.7.1 is specified in 7.7.2.2.

11.7.1 For extract purification using the silica gel/13 % H_2O gravity column described in 7.7.2.2, quantitatively transfer the concentrated or volume adjusted extract (approximately 1 cm^3) onto the top of the pre-eluted and still cyclohexane-wetted silica gel/13 % H_2O column. Rinse the pear-shaped rotary evaporator flask or the conical-bottom centrifuge glass 3 times with 0.5 cm^3 of cyclohexane and add the rinse solutions to the top of the SPE column. Subsequently, elute the PAH fraction from the SPE column by means of 9 cm^3 of cyclohexane. Collect the entire eluate in a new glass volumetric tube and narrow to 0.25 cm^3 by applying a nitrogen blow-down apparatus at appropriate conditions.

11.8 Completely transfer the concentrated eluate to a GC/MS amber vial by finally flushing the conical-bottom centrifuge glass three times with approximately 0.25 cm^3 toluene. The final volume of the measurement solution (V_f) is adjusted to 1 cm^3. Cap securely and label. The sample is now ready for injection.

11.9 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.

11.10 If these conditions are not met, start again from 11.3 using the retained extract volume in 11.5 and adjust the appropriate dilution or concentration factor.

Part B

11.11 Once the set extraction time has elapsed, the Soxhlet assembly is cooled and the thimble is removed.

11.12 Measure the volume of the extract to ±0.5 cm^3. Record the volume as V_i (the initial extract volume). If the entire extract is to be concentrated, the V_i and V_A terms in Eq 11 are equal and should be set to 1.

11.13 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 11.9.

11.13.1 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 can also act as a guide (see Note 5).

NOTE 5—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.

11.13.1.1 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 11.14.

11.13.1.2 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 11.15.

11.13.1.3 If the extract does not need concentrating or diluting, proceed also to 11.15.

11.14 Follow the procedure in this subsection if the extract from 11.12 needs to be concentrated.

11.14.1 Quantitatively transfer an aliquot of the extract to a glass jar with a PTFE rubber cap. Record the aliquot volume, V_A. For example, if a 50 cm^3 aliquot is transferred to the Erlenmeyer flask, the V_A = 50 cm^3. Add a known, accurately measured aliquot of the IS solution prepared in 9.3 to the glass jar (for example, 50 µL). Cap the jar and shake to thoroughly mix the IS solution and extract. If the entire extract is to be concentrated, the V_i and V_A terms in Eq 11 are equal and should be set to 1.

11.14.2 Pre-rinse the SPE with 10 cm^3 of toluene. This wetting toluene is discarded. Pass the contents of the glass jar through the SPE into the rotary evaporator flask. Rinse the glass jar three times with 10 cm^3 of toluene each and pass each rinse through the SPE into the rotary evaporator flask.

11.14.3 Concentrate the extract aliquot to approximately 2 cm^3. The rotary evaporator shall be set to a maximum bath temperature of 40 ± 2°C and a vacuum of 5 ± 0.3 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. Ensure that a volume of at least 1.0 cm^3 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.

11.14.4 Quantitatively transfer the concentrated extract to a 5 cm^3 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 cm^3. As in the calibration standard examples in 9.2, if 50 µL of dilute IS solution (of concentration 50.0 µg/cm^3) has been used, the 5 cm^3 amber volumetric flask should contain 0.500 µg/cm^3 of the IS (CIS = 0.500 µg/cm^3).

11.14.5 Proceed to 11.16.

11.15 Follow the procedure in this section if the extract from 11.12 needs to be diluted or if it does not need concentrating or diluting. This subsection will describe the method for mixing an aliquot of the extract with internal standard, passing it through the SPE, concentrating the extract, then quantitatively transferring it to a 5.0 cm^3 volumetric flask.

11.15.1 Pipette a known, accurately measured aliquot of the IS solution prepared in 9.3 (for example, 50 µL) into a 20 cm^3 glass vial (with PTFE-faced rubber cap).

11.15.2 An aliquot of the extract now needs to be added to the 20 cm^3 glass vial (that already contains the 50 µL of diluted IS solution). The volume of this aliquot needs to be chosen so that when diluted to a final volume of 5.0 cm^3, the final concentration of BaP falls within the range of the calibration curve. If no dilution is needed, then 5.0 cm^3 of extract is added to the 20 cm^3 vial. Should the extract prepared in 11.12 require dilution by a large factor, it is recommended to proceed via a two steps dilution. For instance, for a 100 times dilution, first dilute 1 cm^3 in 10 cm^3 then 0.5 cm^3 in 5 cm^3. Record the aliquot volume, V_A, as the effective volume of extract added to the 20 cm^3 vial. Once the extract aliquot has been added, cap the vial and shake to mix the extract aliquot with the internal standard. Three examples will illustrate how to determine V_A:

(1) If 5.0 cm^3 was added to the 20 cm^3 vial, then V_A = 5.0 cm^3.

(2) If 1.0 cm^3 of extract is added to the 20 cm^3 vial, V_A = 1.0 cm^3.

(3) If 1.0 cm^3 of extract is first diluted to 10.0 cm^3, then 0.5 cm^3 is added to the 20 cm^3 vial, then V_A = 0.05 cm^3.

11.15.3 Pre-rinse the SPE with 10 cm^3 of toluene. This wetting toluene is discarded. Pass the contents of the 20 cm^3 vial through the SPE into the rotary evaporator flask. Rinse the 20 cm^3 vial three times with 5 cm^3 of toluene each and pass each rinse through the SPE into the rotary evaporator flask.

11.15.4 Concentrate the extract aliquot to approximately 2 cm^3, following the rotary evaporator temperature and vacuum guidelines in 11.14. Ensure that a volume of at least 1.0 cm^3 of extract remains in the flask at the completion of this step.

11.15.5 Quantitatively transfer the concentrated extract to a 5 cm^3 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 cm^3. As in the calibration standard examples in 9.2, if 50 µL of dilute IS solution (of concentration 50.0 µg/cm^3) has been used, the 5 cm^3 amber volumetric flask contains 0.500 µg/cm^3 of the IS (C_{IS} = 0.500 µg/cm^3).

11.15.6 Proceed to 11.16.

11.16 Transfer at least 1 cm^3 of the final extract prepared in 11.14 or 11.15 to a GC/MS amber vial. Cap securely and label. The sample is now ready for injection.

11.17 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 13). 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.

11.18 If these conditions are not met, start again from 11.13 using the retained extract volume 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 instrument will have to be adjusted and tuned 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 9.2 and a carbon black extract as prepared in Section 10. 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 internal standard).

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 for deuterated BaP; 251.8, 251.9, 252.0, 252.1, 252.2 for native BaP

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 the chosen internal standard.

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

NOTE 6—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 9.2.

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/cm^3), and
C_{IS} = IS concentration in the GC vial (µg/cm^3).

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 0.98.

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 µg/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):

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

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

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

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

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 µg/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.

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

where:

V_f = final extract volume,
V_i = initial extract volume,
V_A = aliquot of extract volume, and
W_{CB} = carbon black weight (in grams) Soxhlet extracted, 10.8.

NOTE 7—If the entire extract was concentrated (that is, no aliquot was taken from the initial extract volume), then V_i and V_A are both equal to 1.

14.7 *Calculation Example:*

14.7.1 Using the calibration curve example from Fig. 3 and the sample example from Table 3:

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

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

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

W_{CB} = 10.024 g, V_A = 50 cm^3, V_i = 100 cm^3, and V_f = 5 cm^3 hence $C_{BaP,CB}$ = 485 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/cm^3 in the GC/MS vial.

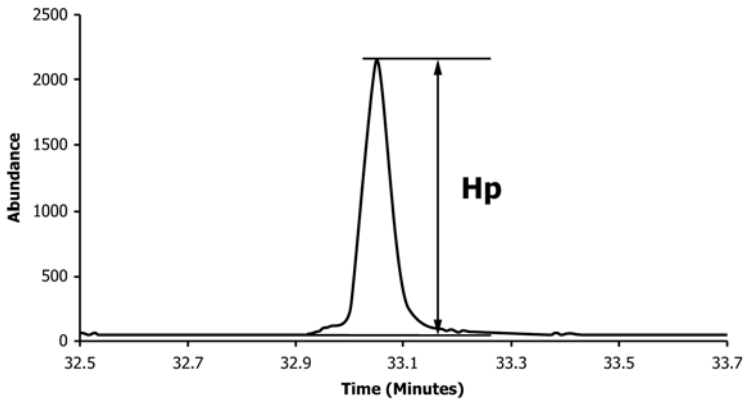

FIG. 1 Determination of Peak Signal

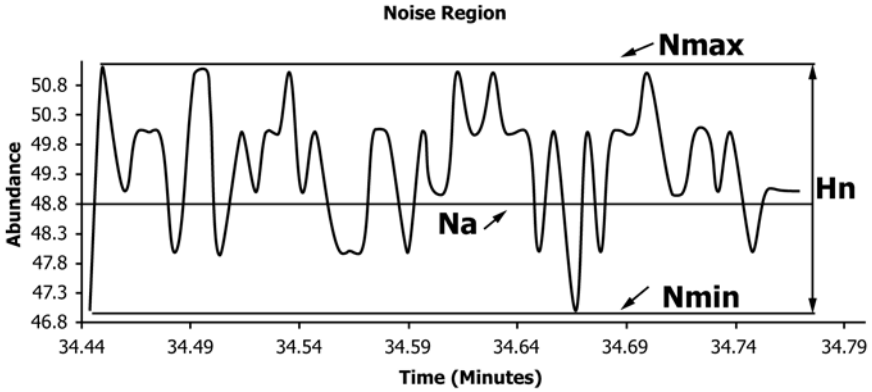

FIG. 2 Determination of Noise Parameters

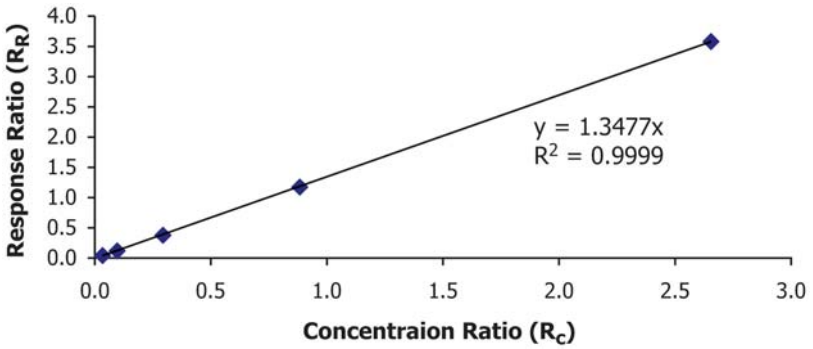

FIG. 3 Calibration Curve Example

TABLE 2 Quantities Used for Example Calculations

	Benzo(a)pyrene		d_{12}-Benzo(a)pyrene		Ratios[A]	
	C_{BaP} (µg/cm³)	A_{BaP}	C_{IS} (µg/cm³)	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/cm³)	A_{BaP}	C_{IS} (µg/cm³)	A_{IS}	R_C	R_R
Sample 1	unknown	362 524	0.330	182 506	unknown	1.986

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 h).

15.1.3 BaP content in the carbon black rounded to three significant figures in units of ppb (µg/kg).

16. Precision and Bias[8]

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.

[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**

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