Designation: D8143 – 17 (Reapproved 2022)

Standard Test Method for Determination of the EU-8 List of PAH Compounds in Carbon Black[1]

This standard is issued under the fixed designation D8143; 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 the EU-8 list of polycyclic aromatic hydrocarbons (PAH) on carbon black. The EU-8 list of PAH compounds is given in Table 1. The procedure involves Soxhlet extraction with toluene followed by extract analysis using 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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.4 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *Federal Standard:*[2]

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

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *polycyclic aromatic hydrocarbon, PAH, n*—organic compound(s) that consist of chemical structures of alternating single and double bonds of a ring like nature (for example, benzene), with no heteroatom or other substituents; frequently the rings are fused to make larger chemical structures.

3.1.1.1 *Discussion*—PAH naturally occur in oil, coal, and tar deposits; are produced by the incomplete combustion of hydrocarbons; and occur in many other products and processes.

3.1.2 *EU-8 PAH compound list, n*—a list of eight polycyclic aromatic hydrocarbons that are specified by the Commission Regulation (EU) 1272/2013 (December 6, 2013)[3] amending Annex XVII to Regulation (EC) 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards polycyclic aromatic hydrocarbons (2013) OJ L328/69.

3.1.2.1 *Discussion*—Regulation (EU) No. 1272/2013 sets PAH concentration limits on finished products specified by their application. The regulation does not refer directly to carbon black, though in many instances and for formulation purposes there is a need to know the quantitative, extractable PAH content present in carbon black that will become part of the finished product. In addition, no specific test method has been defined by the regulation for testing finished products or raw materials used in the finished products.

4. Summary of Test Method

4.1 A portion of carbon black is Soxhlet-extracted with toluene for 48 h. The extract is concentrated and subsequently analyzed for specific PAH compounds by gas chromatography with mass spectrometry (GC/MS). Each PAH compound is quantified individually using internal and external standards.

5. Significance and Use

5.1 This test procedure is used to determine the individual concentrations of the PAH compounds in the EU-8 list extracted from carbon black by the means of a Soxhlet extraction apparatus with toluene.

[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] Available from U.S. Government Printing Office, Superintendent of Documents, 732 N. Capitol St., NW, Washington, DC 20401-0001, http://www.access.gpo.gov.

[3] Commission Regulation (EU) No 1272/2013 of 6 December 2013. Available at http://eur-lex.europa.eu/eli/reg/2013/1272/oj.

TABLE 1 EU-8 PAH Compounds and Internal Standards

Native PAH	CAS#	Deuterated Internal Standard	CAS#
Benz[a]anthracene	56-55-3	Chrysene-d12	1719-03-5
Chrysene	218-01-9	Benzo[a]pyrene-d12	63466-71-7
Benzo[b]fluoranthene	205-99-2	1,12-Benzoperylene-d12	93951-66-7
Benzo[j]fluoranthene	205-82-3	Perylene-d12	1520-96-3
Benzo[k]fluoranthene	207-08-9		
Benzo[e]pyrene	192-97-2		
Benzo[a]pyrene	50-32-8		
Dibenz[a,h]anthracene	53-70-3		

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).

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 *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. Other evaporators may be used if they produce acceptable results.

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 *Mass Spectrometer (MS),* with electron impact capability and Selected Ion Monitoring (SIM) mode.

6.11.2 *GC Capillary Column,* usually a nonpolar GC column composed of 5 % diphenyl/95 % dimethyl polysiloxane coating is used for PAH analysis. Other similar column phases may be used if they provide acceptable peak resolutions.

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.

6.12 *Adjustable Micropipettes,* for example 1000, 200, and 20 µL, or similar analytical-grade volume measuring equipment.

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

6.14 *Volumetric Flasks,* for example 10 and 100 cm^3.

6.15 *Analytical Balance,* with an accuracy of 0.1 mg.

6.16 *Furnace,* capable of temperature regulation of 500 ± 25°C, used to burn off organic contamination from glass surfaces.

6.17 *Manometer,* capable of pressure readings in the range of 5 ± 0.3 kPa for controlling vacuum evaporators.

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.[4] 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 Stock solutions or mixtures of the native PAH standards and of the internal standard (deuterated PAH compounds) 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 each of the native PAH analytes is recommended for a stock solution mixture.

7.2.2 A similar concentration range of 80 to 100 µg/cm^3 for each of the deuterated internal standard compounds is recommended for the internal standard (IS) stock solution.

7.2.3 All purchased 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 to store the standard solutions and their recommended expiration date. Typically, the stock solutions are protected from light and stored at room temperature. They should be checked for signs of degradation or evaporation. The

[4] *ACS Reagent Chemicals, Specifications and Procedures for Reagents and Standard-Grade Reference Materials*, 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.

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 Cyclohexane, 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.

7.7.1 Silica SPE Cartridges, single-use application, having a volume capacity of approximately 1 to 10 cm^3.[5]

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. 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 h 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 PAH mixture in which internal standards (IS) have also been added. The recommended external standard concentrations are to cover a range of 0.0125 to 1.0 µg/cm^3. 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 PAH concentration range can be used for 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 PAH Standard Solutions for Calibration*—Using the native PAH standard stock solution described in 7.2, prepare at least 10 cm^3 of five toluene solutions at the concentrations suggested in the following list. 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 into 10 cm^3 volumetric flask 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. Store all standard solutions in the dark.

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

9.3 *Preparation of Diluted IS Solution*—Using the IS stock solution described in 7.2, prepare at least 10 cm^3 of diluted internal standard solution for adding to samples and calibration standards. A concentration of 50 µg/cm^3 is suggested for this solution but other concentrations can be used. For example, when adding 100 µL of a 50 µg/cm^3 IS solution to a 10 cm^3 final extract volume, the IS concentration will be 0.500 µg/cm^3.

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

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

10.3 For low PAH carbon blacks, a pre-extraction of the extraction unit for at least 4 h is recommended. If glass

[5] 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.

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.

10.4 Disposable devices such as cellulose thimbles can be 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, then certain parts of the glassware may also be baked for at least 6 h in a furnace at 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 without degradation or deformity.

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.

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 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 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 in 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 or fiberglass insulation. 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 48 h.

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.

10.14 After 5 to 20 min, the flasks should be on the verge of boiling. Gently agitate the apparatus; this should help initiate the toluene boiling. 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 During the 48 h extraction, the toluene level should be checked from time to time for potential losses through evaporation. To avoid degradation of the extracted PAH 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 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 GC/MS Analysis

11.1 Two extract preparation procedures (Procedure A and Procedure B) are given below. Either procedure may be used.

Procedure A

11.2 Once the 48 h extraction time has elapsed, the Soxhlet assembly is cooled and the thimble is removed.

11.3 In summary, the extract is concentrated, spiked with internal standards, and cleaned using solid phase extraction (SPE) prior to analysis. These steps are outlined below.

11.4 The purpose of the concentration step is take the PAH compounds in the toluene extract from sub part-per-billion concentrations to part-per-billion concentrations or higher for analysis by typical GC/MS instrumentation. The purpose of the internal standard spike is to improve quantitation by handling

variations in sampling and extract preparation. The purpose of the solid phase extraction is to remove polar compounds from the extract which may adversely affect gas chromatography analysis.

11.5 The concentration and SPE clean up steps may be removed or altered as long as the method change provides equivalent results to the method described below.

11.6 The toluene extract is quantitatively transferred to a rotary evaporator and concentrated down to around 2 cm^3. For rotary evaporators, the maximum bath temperature is 40 ± 2°C and vacuum is 5 ± 0.3 kPa. These parameters shall be monitored carefully during all evaporation steps as PAH losses may occur.

11.7 Alternative evaporators (concentrators) and vacuum/temperature settings may be used provided recovery studies are conducted and results of these studies are acceptable.

NOTE 1—Concentration tests with 200 cm^3 toluene solutions containing 50 ng of benzo-a-pyrene indicated that no measurable losses occurred when the volume reduced to 5 cm^3 (at 40°C water bath temperature and 5 kPa pressure).

11.8 Add 100 µL (other volumes can be used) of the diluted internal standard solution (from 9.3) into the evaporator flask with the extract and gently mix the solution. Take care that the final concentration of the internal standard spike is always the same for all samples and standards and defined appropriately in the chromatography quantitation application.

11.9 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 PAH compounds has to be validated by recovery studies for each method. Subsection 11.9.1 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 11.9.1 is specified in 7.7.2.2.

11.9.1 For extract purification using the silica gel/13% H_2O gravity column described in 7.7.2.2, quantitatively transfer the concentrated extract onto the top of the pre-eluted and still cyclohexane-wetted silica gel/13 % H_2O column. Rinse the evaporator flask 3 times with 0.5 cm^3 of cyclohexane and add the rinse solutions to the top of the SPE column.

11.10 Elute the PAH fraction from the SPE column by means of additional cyclohexane to collect the entire eluate in a new glass volumetric flask (for example, 10 cm^3) and dilute with cyclohexane to the mark. This final volume is recorded (V_f).

11.11 Transfer at least 1 cm^3 of the final extract to an amber GC/MS vial. Cap securely and label. The sample is now ready for injection into the GC/MS.

11.12 After the sample analysis and calculation (Section 14), both the raw peak areas (APAH), as well as the ratio of responses (RR = APAH/AIS) should be within the calibration range for each PAH compound (Section 13). In some cases, minor deviation from this guideline can be tolerated. On the low end of the curves, half of the lowest calibration value can be allowed; on the high end of the curves, twice the highest calibration value can be allowed.

11.13 If any of the individual PAH compound concentrations exceed the calibration range for that compound, then dilute an aliquot of the final volume by transferring an aliquot (for example, 1 cm^3) to a new volumetric flask (for example, 10 cm^3), adding appropriate amount of diluted internal standard solution (for example, 90 µL) to maintain the IS concentration, and diluting with toluene to a new final volume (10 cm^3). The example volumes given here will produce a 10× dilution of the extract for analysis.

11.14 A dilution multiplier in the chromatography software can be used to account for the dilution for final result calculations.

Procedure B

11.15 Once the 48 h extraction time has elapsed, the Soxhlet assembly is cooled and the thimble is removed.

11.16 In summary, the internal standard is added to an aliquot of the extract, optionally cleaned using solid phase extraction (SPE, described in 7.7.1), then either concentrated or diluted to a final volume. The purpose of the solid phase extraction is to remove polar compounds from the extract which may adversely affect gas chromatography analysis. The SPE clean-up steps may be removed or altered as long as the method change provides equivalent results to the method described below.

11.17 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 Equation 10 are equal and should be set to 1.

11.18 The extract may now need to be concentrated or diluted depending on the individual PAH level in the extract. The level of each individual PAH injected into the GC/MS shall fall within the range defined in 11.25. This may require more than one aliquot of the extract, although usually this will not be necessary for the EU-8 PAH compounds.

11.19 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 2).

NOTE 2—The color of the extract obtained might give an indication of the expected PAH level. Though difficult to assess and essentially based on experience, high-purity carbon blacks tend to exhibit colorless extracts or slightly yellow extracts, while higher PAH 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 or may need dilution.

11.20 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 glass jar, 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