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Polynuclear Aromatic Hydrocarbons –EPA Method 550.1

Access to this SOP shall be available within the laboratory for reference purposes; the official copy of this SOP resides on the official Georgia EPD website at https://epd.georgia.gov/about-us/epd-laboratory-operations. Printed copies of this SOP will contain a watermark indicating the copy is an uncontrolled copy.

1 Scope and Application

1.1. Method 550.1 is used to determine the concentration of various polynuclear aromatic hydrocarbons in drinking water. Samples are extracted with a C_{18} extraction disk. The disk is solvent exchanged with Acetonitrile. The extract is injected into a high-pressure liquid chromatograph with an ultraviolet absorbance detector and fluorescence detector. Identification is obtained by analyzing a standard curve under identical conditions used for samples and comparing resultant retention times. Concentrations are measured by relating response produced for Benzo (a) pyrene to the standard curve response.

1.2. This method is restricted to analysts who have completed the requirements of the initial demonstration SOP. See SOP reference 13.2

2. Definitions

2.1. Refer to Section 3 and Section 4 of the Georgia EPD Laboratory Quality Assurance Manual for Quality Control definitions.

2.2. Primary Source (PS) – A standard that is used to make up the calibration points of a curve.

2.3. Second Source (SS) – A standard made from another manufacturer other than that of the primary source.

2.4. Initial Calibration Verification (ICV) – An ICV is a second source standard that is used to verify the correctness of the primary sources calibration curve. The ICV is run a level equal to that of a Laboratory Control Sample (LCS) or that of a point on the calibration curve.

3. Interferences

3.1. Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that may lead to discrete artifacts and/or elevated baselines in the chromatograms.

3.2. Glassware must be scrupulously cleaned with hot water and detergent followed by de-ionized water then rinsed with methanol followed by acetone.

3.3. The use of high purity reagents and solvents is absolutely necessary to minimize interference problems.

3.4. Interfering contamination may occur when a sample containing low concentrations of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes.

3.5. Matrix interferences may be caused by containments that are co-extracted from the sample.

3.6. All samples, standards, and extracts must be protected from light by using amber vials or wrapping clear sample bottles or vials with aluminum foil. A dark refrigerator is also appropriate.

4. Safety

4.1. Refer to Georgia EPD Laboratory Chemical Hygiene Plan.

5. Apparatus and Equipment

5.1. 1 L Amber glass bottle –Two for each sample location

5.2. Glass class A 1000 mL graduated cyclinders

5.3. 6 position manifold for Empore disks

5.4. C_{18} 47mm, liquid-solid exchange disk.

5.5. Culture tubes; glass 40 ml

5.6. Clear vials – 40 ml glass, disposable, with PTFE lined screw caps for extract and drying and derivation.

5.7. Pipets: Pasteur, disposable glass

5.8. Pipettes – Class A, 2.0 ml and 4.0 ml glass, or adjustable volume dispensers.

5.9. Volumetric flasks – Class A, suggested sizes 5 ml, 10 ml, and 100 ml.

5.10. Micro-syringes – Various sizes

5.11. Amber glass snap cap HPLC vials

5.12. Balance: analytical, capable of weighing 0.0001 g

5.13. Analytical concentrator: 6 or 8 position

5.14. High Performance Liquid Chromatograph (HPLC): HPLC system capable of injecting 1000 µl aliquots and performing linear gradients at a constant flow.

5.14.1. Variable UV/Vis detector set at 254 λ – Perkin Elmer Series 200 detector with a deuterium lamp.
5.14.2. Fluorescence Detector: Perkin Elmer LC series 200 fluorescence detector capable of an excitation at 330 nm and the detection of emission energies at 465 nm or equivalent. Set at 280λ.
5.14.3. Auto Sampler- Perkin Elmer: series 200 LC pump
5.14.4. Column: Hypercil Green PAH 250 mm x 4.6 mm analytical column or equivalent.
5.14.5. Perkin-Elmer Totalchrom or equivalent chromatography software
5.14.6. TurboVap concentrator
5.14.7. Detergent: Steris Labklenz or equivalent

6. Reagents and Standards

6.1. Reagent Water – Purified water which does not contain any measureable quantities of target analytes or interfering compounds for each compound of interest. (Deionized, HPLC, Milli-Q water or equivalent. Milli-Q water has a resistivity of 18 or greater [MΩ·cm] @ 25° C and a TOC of 50 µg/L or less)
6.2. Methanol: High purity, demonstrated to be free from analytes and interferences (HPLC grade or better).
6.3. Acetonitrile: High purity, demonstrated to be free from analytes and interferences (HPLC grade or better).
6.4. Methylene Chloride: High purity, demonstrated to be free from analytes and interferences (Pesticide grade or better).
6.5. Sodium Sulfate, $NaSO_4$ – Suitable for Pesticide Residue Analysis, granular, anhydrous. (Fisher Brand or equivalent):
6.5.1. Sodium sulfate is baked at 450° C for four hours and then returned to original amber glass jar after cooling.
6.6. Sodium Thiosulfate: Reagent grade 100 mg per 1 L amber glass bottle.
6.7. 1:1 Hydrochloric Acid Solution: Thermofisher 5 ml pre-mixed solution in sealed plastic vials or equivalent. One five ml vial is added to each sample in the field upon collection.
6.8. Standard Stock Solutions: All standards that are made for method 550.1 analysis are to have a 6 month expiration date from the opening of the vendor stock ampule.
6.8.1. Primary Stock # 1 Solution: 100 µg/ml made up from vendor stock at 1000 µg/ml.

Table 6.8.1.1 – 550.1 Primary Stock #1 Solution in Acetonitrile (1st Dilution)

Compound	Initial Concentration	Aliquot	Final Concentration
Benzo (a) pyrene	1000 µg/ml	1.0 ml	100 µg/ml

Total volume of Standard Aliquot	1.0 ml
Addition of Acetonitrile to Standard aliquots	9.0 ml
Final Volume of Primary Stock #1	10.0 ml

6.8.2. Primary Stock #2 Solution: 10.0 µg/ml made up from Primary Spiking stock #1 at 100 µg/ml.

Table 6.8.2.1 – 550.1 Primary Stock #2 Solution in Acetonitrile (2^{nd} Dilution)			
Compound	**Initial Concentration**	**Aliquot**	**Final Concentration**
Benzo (a) pyrene	100 µg/ml	1.0 ml	10.0 µg/ml
Total volume of Standard Aliquot		1.0 ml	
Addition of Acetonitrile to Standard aliquots		9.0 ml	
Final Volume of Primary Stock #2		10.0 ml	

6.8.3. Spiking Stock Solution: 1.00 µg/ml made up from Primary Spiking stock #2 at 10.0 µg/ml.

Table 6.8.3.1 – 550.1 Spiking Stock Solution in Acetonitrile (3^{rd} Dilution)			
Compound	**Initial Concentration**	**Aliquot**	**Final Concentration**
Benzo (a) pyrene	10.0 µg/ml	1.0 ml	1.00 µg/ml
Total volume of Standard Aliquot		1.0 ml	
Addition of Acetonitrile to Standard aliquots		9.0 ml	
Final Volume of Spiking Stock		10.0 ml	

6.8.4. ICV Stock #1 Solution: 100 µg/ml is made up from Vendor Stock at 1000 µg/ml.

Table 6.8.4.1 – 550.1 ICV Stock Solution #1 in Acetonitrile (1^{st} Dilution)			
Compound	**Initial Concentration**	**Aliquot**	**Final Concentration**
Benzo (a) pyrene	1000 µg/ml	1.0 ml	100 µg/ml
Total volume of Standard Aliquot		1.0 ml	
Addition of Acetonitrile to Standard aliquots		9.0 ml	
Final Volume of Primary Stock #2		10.0 ml	

6.8.5. ICV Stock #2 Solution: 10.0 µg/ml made up from Primary Spiking stock #1 at 100 µg/ml.

Table 6.8.5.1 – 550.1 ICV Stock #2 Solution in Acetonitrile (2^{nd} Dilution)			
Compound	**Initial Concentration**	**Aliquot**	**Final Concentration**
Benzo (a) pyrene	100 µg/ml	1.0 ml	10.0 µg/ml

Total volume of Standard Aliquot	1.0 ml
Addition of Acetonitrile to Standard aliquots	9.0 ml
Final Volume of Primary Stock #2	10.0 ml

6.8.6. ICV Spiking Stock Solution: 1.00 µg/ml made up from Primary Spiking stock #2 at 10.0 µg/ml.

Table 6.8.6.1 – 550.1 ICV Spiking Stock Solution in Acetonitrile (3rd Dilution)			
Compound	**Initial Concentration**	**Aliquot**	**Final Concentration**
Benzo (a) pyrene	100 µg/ml	1.0 ml	10.0 µg/ml
Total volume of Standard Aliquot		1.0 ml	
Addition of Acetonitrile to Standard aliquots		9.0 ml	
Final Volume of Spiking Stock		10.0 ml	

7. Sample Collection

7.1. Drinking water samples for EPA Method 550.1 are collected in pre-certified 1L amber glass bottles (to shield from light) with Teflon lined screw caps and preserved with 100 mg Sodium thiosulfate. The 100 mg of Sodium thiosulfate is sufficient to neutralize up to 5 mg/L (ppm) residual chlorine in a 1 L sample.

7.1.1. A residual chlorine check is done in the field by the collector. The collector writes down the numerical value for residual chlorine in ppm on the sampling form.

7.1.1.1. The collector also adds 5 ml of 1:1 HCl solution to the sample in the field to bring the pH below 2 to inhibit biological activity.

7.1.2. The shipping and receiving staff log in the samples and enter the information for residual chlorine in the DNR_LAB Labworks field. The analyst prints a backlog to determine samples to be analyzed.

7.1.3. The backlog report contains a field listing the residual chlorine determined by the collector. If the residual chlorine measured is less than 5 ppm, the Sodium thiosulfate preservative was sufficient to neutralize all of the residual chlorine in the sample.

7.1.3.1. If the collector reports 5 ppm or more residual chlorine, the sample must be recollected.

7.2. Samples are cooled and maintained at 0-6° C (not frozen) after sample collection. Two 1L amber glass bottles are to be collected for every sample. Additional bottles may be required for matrix spikes and matrix spike duplicates.

7.3. Samples must be extracted within 7 days of collection. Sample extracts should be stored in the dark at 0-6° C (not frozen) for a maximum of 40 days.

8. Calibration

8.1. Calibration Curve

8.1.1. A 7-point calibration is performed for all components. The calibration system uses traceable certified standards. The calibration is an external standard calibration with an average of response factor linear curve fit and should result in a percent relative standard deviation < 10% between calibration levels of each analyte. Alternatively, the calibration curve may be a least squares regression or quadratic fit.

8.2. Calibration Standards

8.2.1. The calibration curve consists of the calibration standards at the following concentrations made up to 10 ml with Acetonitrile (µg/ml):

Table 8.2.1. 1: Calibration Curve for 550.1 in µg/ml

Name	Level 1 µg/ml	Level 2 µg/ml	Level 3 µg/ml	Level 4 µg/ml	Level 5 µg/ml	Level 6 µg/ml	Level 7 µg/ml
Benzo (a) pyrene	0.00400	0.0100	0.0250	0.0500	0.100	0.150	0.200

Table 8.2.1. 2 Aliquots of Spiking Stock Solution to make up all the levels in the above table.

Name	Level 1 (use Spiking Stock)	Level 2 (use Spiking Stock)	Level 3 (use Spiking Stock)	Level 4 (use Spiking Stock)	Level 5 (use Primary Stock #2)	Level 6 (use Primary Stock #2)	Level 7 (use Primary Stock #2)
Benzo (a) pyrene	0.040 ml (40 µl)	0.100 ml (100 µl)	0.250 ml (250 µl)	0.500 ml (500 µl)	0.100 ml (100 µl)	0.150 ml (150 µl)	0.200 ml (200 µl)

8.3. Calibration Verification

8.3.1. Second source calibration verification (ICV) must be analyzed after initial calibration and at least once per quarter even if the system is not recalibrated. All analytes must be within ± 30% of the expected value.

8.3.2. A daily continuing calibration is performed every twelve-hour analysis period to monitor and validate the instrumentation, column, and detector performance.

8.4. Record Keeping

8.4.1. Documentation of an instrument calibration is reviewed for adherence to quality criteria and archived with project records.

8.5. Daily Calibration Verification and Continuing Calibration

8.5.1. A continuing calibration standard (CCC) ensures the instruments target compound retention times and quantitation parameters meet method

performance criteria. For any 12-hour analysis period, prior to sample analysis, a one-point daily continuing calibration verification is performed. Continuing calibration standards are analyzed during the analysis period to verify that instrument calibration accuracy does not exceed 20% of the initial calibration, i.e. %Drift ≤ 20% (see Calculations, Section 11.7.). If the continuing calibration does not meet method performance criteria, the CCC may be rerun once. If the CCC fails a second time, then the instrument must be recalibrated. Two levels of calibration standards are alternated throughout the run.

8.5.2. A laboratory performance check (LPC) standard must be run at the beginning of every batch sequence. This standard must be at or below the RL and will have a percent recovery of 50-150%.

8.6. Daily Retention Time Update

8.6.1. Retention Times (RT) are updated once per 24 hour period when analyses are performed. The first CCC is processed using chromatographic software (Totalchrom or an equivalent). The new RT's are saved in a copy of the chromatographic software method used for analyzing this batch of samples. To the existing chromatographic method an extension is added by using – Month-Day-Year. Then hard copies of the calibration parameters are added to the data package for that batch of samples. **NOTE**: If an analytical sequence is stopped for any reason longer than a typical work shift a new retention time update is necessary for the next sequence.

8.7. Average Response Factor Calibration:

8.7.1. To evaluate the linearity of the initial calibration, calculate the mean response factor (RF), the standard deviation (σ_{n-1}) and the relative standard deviation expressed as a percentage (%RSD). If the %RSD of the response factors is ≤ 10% over the calibration range, then linearity through the origin may be assumed, and the average calibration or response may be used to determine sample concentrations. See Calculations, Sections 11.1 – 11.3.

8.8. First Order Linear Calibration using Least Squares Regression:

8.8.1. Linearity through the origin is not assumed in a least squares fit. The instrument responses versus the concentration of the standards for the 7 points are evaluated using the instrument data analysis software. The regression will produce the slope and intercept terms for a linear equation. The regression calculation will regenerate a correlation, r, a measure of goodness of fit of the regression line to the data. A value of 1.0 is a perfect fit. An acceptable correlation of coefficient (r) should be ≥ 0.990 (or $r^2 \geq 0.980$). See Calculations, Section 11.4.

8.8.2. Alternatively, second order quadratic fit may be used with an acceptable correlation of coefficient of $r \geq 0.990$ (or $r^2 \geq 0.980$). Note: quadratic fit will be calculated by chromatographic software. See calculation 11.5.

8.9. Retention Time Windows

8.9.1. The width of the retention time window for each analyte is defined as ± 3 times the standard deviation of the mean absolute retention time established over an analytical batch sequence. See Section 11.6.

8.10. Verification of Linear Calibrations

8.10.1. Calibration verification for linear calibrations involves the calculations of % drift of the instrument response between the initial calibration and each subsequent analysis of the verification standard (CCC). The % drift may be no more than ± 20%. See Section 11.7.

8.11. Sample Concentration:

8.11.1. Sample results are expressed in µg/L.

8.11.2. If an analyte response is calibrated by Average Response Factor, $\overline{RF}$, the chromatographic software calculates the concentration of the extract per Calculations, Section 11.8. Results are in µg/ml.

8.11.3. If an analyte response is calibrated by linear regression, the chromatographic software calculates the concentration of the extract solving for x per Calculations, Section 11.4. Results are in µg/ml.

8.11.4. If an analyte response is calibrated by linear regression, the chromatographic software calculates the concentration of the extract solving for x per Calculations, Section 11.5. Results are in µg/ml.

8.11.5. The sample concentration is calculated per Calculations, Section 11.9 in µg/L. Assuming a 1000 ml initial sample volume and a 5 ml extract volume, equation 11.9 can be reduced to C_s multiplied by a factor of 5. The chromatographic report uses this factor to multiply the result from either paragraph 8.11.2. , 8.11.3. or 8.11.4. above and calculates the final result per Calculations, Section 11.9.2

8.11.6. If an initial volume of other than 1000 ml is used or a dilution of the extract is analyzed, the final sample result is multiplied by the factor determined with per Calculations, Section 11.10

9. Quality Control

9.1. Refer to Table 14.1.1 for the Reporting Limits (RL), Appendix A, Table A.1 for Quality Assurance Criteria and Table 14.1.2 for Quality Control (QC) procedures associated with this method.

9.2. A Method Detection Limit Study is performed once per year. See SOP reference 13.6

9.3. Refer to SOP reference 13.2 for training and certification procedures.

9.4. Refer to SOP reference 13.3 for control charting procedures.

9.5. Control Limits

9.5.1. EPA Method 550.1 section 10.3.2 (see SOP reference 13.3) sets the default LCS control limits as R (EPA Method 550.1 Table 2) ± 3 times the standard deviation, S_r (EPA Method 550.1 Table 2) or as R ± 30%, whichever produces the wider limits. For Benzo (a) pyrene, R ± 3* S_r produces the widest limits (see Table 9.6. 2).

9.5.1.1. The EPD Laboratory sets the LCSD recovery to the same limits as the LCS recovery.

9.5.1.2. The EPD Laboratory sets the default precision limits for the LCS/LCSD to be 0 – 30%

9.5.1.3. Method 550.1 sets the MS recovery limits to be the same as the LCS range.

9.5.1.4. The EPD Laboratory sets the MSD recovery limits to be the same as the LCS range.

9.5.1.5. The EPD Laboratory sets the MS/MSD precision limits to be the same as the LCS/LCSD limits.

9.5.2. When sufficient points have been collected control charts are used to establish in-house control limits. In-house control limits may never exceed the default limits.

9.6. The default control limits in Table 9.6. 1 are presented to assist in defining control limits established with control charts and are not used as batch acceptance criteria.

Note: Analysts must use the control limits presented in Appendix A, Table A.1. Those limits cannot exceed the default limits presented in Table 9.6. 1.

Table 9.6. 1 Default QC Limits for Method 550.1

QC Type	Analyte	Accuracy (%R) LCL	Accuracy (%R) UCL	Precision (%RPD)
LCS/LCSD* MS/MSD	Benzo (a) pyrene	46	107	30

*LCS/LCSD, and MS/MSD recovery ranges and LCS/LCSD, and MS/MSD precision ranges are determined by control charting per reference 13.3

Note: Analysts must use the data in Appendix A; Table 9.6. 2 is presented as information on how the default control limits in Table 9.6. 1 are established.

Table 9.6. 2 EPA 550.1 Defined Control Limits*

Compound	R** Recovery	30% R	σ (S_r)** Std. Dev.	3 * σ	R ± 30% R	R ± (3 * δ)
Benzo(a)pyrene	76.5	23.0	10.3	30.9	53.5 – 99.5	**45.6 - 107**

*Defined by EPA Method 550.1. R ± 3 * δ is greater than R ± 30% R and are therefore used as the basis for the control limits per the method.
**Defined in Table 2 of EPA Method 550.1

9.7. EPA Method 550.1 requires LCSs to be analyzed at a frequency rate of 5% of all samples (see EPA Method 550.1 Section 10.5).

9.8. Matrix Spike (MS/MSD) is to be analyzed at a frequency of 10% of all samples.

9.8.1. For batches of 1 – 10 samples, a minimum of one MS/MSD pair is required. For batches of 11 – 20 samples, a minimum of two MS/MSD pairs is required.

9.9. Method Detection Limit Study (MDL):

9.9.1. MDL is the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.

9.9.2. The actual MDL varies depending on instrument and matrix.

9.9.3. The MDL must be determined annually for each instrument prior to results being reported for that instrument. The MDL determined for each compound must be less than the reporting limit for that compound.

9.9.4. An MDL study may be done two different ways. The two different ways are considered and initial MDL study and a continuous MDL study. Both ways will be explained below.

9.10. Initial MDL study:

9.10.1. An initial MDL study may occur when a new instrument is brought online, changes to the method (which affect the compound of interest's peak area), and lastly major instrument repairs have been made.

9.10.2. An initial MDL study will consist of the following operating parameters, 7 MDL samples and 7 MDL blanks. The 7 MDL samples study is performed by preparing 7 spiked vials, MDLSpike, spiked at the lowest calibration point of the curve, and preparing 7 clean blank vials filled with DI water, MDLBlank. These 7 sets of spiked and blank vial "pairs" are analyzed over 3 separate days, there may or may not be a non-analysis day between each of the 3 days. A total of 14 vials are prepared, 7 spiked and 7 blanks.

9.11. Continuous MDL study:

9.11.1. A Continuous MDL study is preferred over the initial except in a few cases. For a continuous MDL study to be used on an instrument it must have a minimum of 7 MDL samples and 7 MDL blanks extracted over the course of multiple batches over a year. It is required that at a minimum 2 MDL samples and 2 MDL blanks must be ran per quarter per instrument. If this requirement is not met, then the initial MDL study must be performed for that instrument. (See section 9.10.2 for requirements.)

9.11.2. A continuous format MDL study is performed where one vial is spiked as an MDLSpike, at the lowest point of the calibration curve and analyzed with every batch of samples along with the method blank vial as an MDLBlank.

9.11.3. The results of the MDLBlank will be entered into Labworks using the Method Blank test code, $B_550B. The MDLSpike result will be entered using the $ML550B. The MDL Spiked Amount will be entered into the test code $MA550B. The instrument used for the MDL and Blank analysis will be selected using the test code INSTR-550B.

9.11.4. MDL studies must be pulled on a yearly basis or an initial MDL study must be performed before the current MDLs for the instrument expire.

10. Procedure

10.1. Remove the sample bottles, standards, and reagents from cold storage, and allow the samples to equilibrate to room temperature prior to sample preparation and/or analysis.

10.2. Sample Volume Adjustment:

10.2.1. For the Blank, Laboratory Control Sample (LCS), and Laboratory Control Sample Duplicate (LCSD) pour reagent water into clean 1000 mL amber glass bottles with 100 mg Sodium thiosulfate added to each. Reagent water should fill each bottle to the neck of bottle. To each QC bottle 5ml of 1:1HCL solution is added. Then the volume of Blank, Laboratory Control Sample (LCS), and Laboratory Control Sample Duplicate (LCSD) is adjusted by pouring the sample into a clean, glass, class A graduated cylinder up to the 1000 mL mark and then pour the reagent water back into the original sample bottle. A small amount of the excess is poured into the bottle cap for pH determination (see 10.3. below).

10.2.2. The volumes of each field sample is adjusted by pouring the sample into a clean, glass, class A graduated cylinder up to the 1000 mL mark and then poured back into the original sample container. A small amount of the excess is poured into the bottle cap for pH determination (see 10.3. below).

10.2.2.1. If there is less than 1 L of sample, perform the pH check with as little sample as possible, then measure, and record the volume of the sample in a clean 1 L graduated cylinder. Perform pH check (step 10.3.), then discard remaining

sample in the bottle and cap into an appropriately sized beaker, neutralize and dispose per the EPD Laboratory Waste Management SOP (SOP reference 13.4). Return the measured sample to the collection bottle.

10.3. pH Check:

10.3.1. During volume adjustment, pour a small amount of sample into the bottle cap. Test the pH of the fraction in the cap with a wide spectrum pH paper. The pH should be less than or equal to 2.

10.3.2. If the pH is not less than or equal to 2, the sample is not properly preserved and must be recollected.

10.3.3. Discard the sample fraction in the cap. Do not add this fraction back to the bottle.

10.4. Batch Creation:

10.4.1. Form a batch consisting of a Blank, Laboratory Control Sample (LCS), Laboratory Control Sample Duplicate (LCSD), Matrix Sample (MS), Matrix Sample Duplicate (MSD), and up to 20 samples. The blank is defined as 1000 ml of laboratory reagent water (see 6.1.). The LCS and LCSD are 1000 ml of reagent water spike with 500 µl of a 1.00 µg/ml Benzo (a) pyrene standard (Spiking Stock Solution – see 6.8.3). The MS and MSD are 1000 ml aliquots of the designated batch QC sample spiked with 500 µl of a 1.00 µg/ml Benzo (a) pyrene standard (Spiking Stock Solution – see 6.8.3).

10.4.2. Add a 5 ml aliquot of methanol to each 1L field and QC sample. Mix well.

10.5. Extraction:

10.5.1. Set up the manifold plastic ware using a C_{18} exchange disk.

10.5.2. *Disk Conditioning*:

10.5.2.1. Add 2.5 ml aliquot of methylene chloride to the disk and allow to soak for 1 minute. Add another 2.5 ml of methylene chloride then draw air through disk for one minute.

10.5.2.2. **Note**: do not allow the disk to go dry between the conditioning steps 10.5.2.3. - 10.5.2.4. If at any one of the following steps, 10.5.2.3. - 10.5.2.4. , it may be necessary to add an additional aliquot of solvent to keep the disk wet. It is acceptable to use an extra 5 ml per step.

10.5.2.3. Add a 5 ml aliquot of methanol to the disk and allow to soak for about 1 minute. Pull the methanol through the disk until approximately 1 mm remains on the disk.

10.5.2.4. Add a 5 ml aliquot of reagent water to the disk. Pull the water through the disk until approximately 1 mm remains on the disk.

10.5.3. Add sample to the manifold and pull the entire sample through the disk and allow disk to go dry.

10.5.4. *Analyte Elution*:

10.5.4.1. Position an appropriate size tube inside of the manifold to collect the eluent.

10.5.4.2. Add a 5 ml aliquot of the Acetonitrile (ACN) to the sample bottle. Rinse and then transfer the aliquot of ACN into the manifold. Pull approximately half of the ACN through the disk and then allow the ACN to soak for about 1 minute. Then pull the remaining ACN through the disk and allow the disk to go dry.

10.5.4.3. Add a 5 ml aliquot of the methylene chloride ($MeCl_2$) to the sample bottle. Rinse and then transfer the aliquot of $MeCl_2$ into the manifold. Pull approximately half of the $MeCl_2$ through the disk and then allow the $MeCl_2$ to soak for about 1 minute. Then pull the remaining $MeCl_2$ through the disk and allow the disk to go dry.

10.5.4.4. Repeat step 10.5.4.3.

10.5.4.5. Prepare a drying column by placing a small plug of glass wool at the bottom of a glass drying column and then add 10 - 25 g of Sodium sulfate (see 6.5.) to the column.

10.5.4.6. Rinse the drying column with approximately 10 ml of $MeCl_2$. Dispose of the eluted $MeCl_2$ per the EPD Laboratory Waste Management SOP (reference 13.4).

10.5.4.7. Capture eluent from the drying column in 50 ml conical tube.

10.5.4.8. Pour the eluent through the column to remove any residual water left from the extraction process.

10.5.4.9. Rinse with two 5 ml aliquots of $MeCl_2$.

10.5.5. *Solvent Exchange and Volume Adjustment*:

10.5.5.1. Concentrate sample down to 0.5 ml on TurboVap at 28°C with nitrogen pressure at 3-4 psi.

10.5.5.2. Once the sample has concentrated to 0.5 ml bring sample back up to a final volume of 5 ml with ACN. Transfer sample to a 7 ml vial. Cover sample vial with aluminum foil to block sample from degradation.

10.5.5.2.1. *The EPA allows changes in volumes from the methods to account for improvements in technologies, provided the changes do not affect the chemistry of the method. The EPD Laboratory is able to meet minimum reporting limits with a final volume of 5 ml instead of the 0.5 ml final volume prescribed by the method.*

10.5.5.3. Transfer 1 ml of sample to a 1.5 ml amber glass HPLC vial for analysis.

10.5.6. *Analysis*:

10.5.6.1. Analyze samples on an HPLC instrument equipped with an UV/Vis and Fluorescence detector.

10.6. Dilutions

10.6.1. Upon analysis of the extract, if a target compound response is greater than that of the highest standard of the calibration curve, the sample must be diluted with the final extraction solvent (Acetonitrile) so that, upon analyzing the dilution (in a valid analysis sequence), the target response is between the

lowest concentration standard (or the reporting limit, whichever is higher) and the highest concentration standard.

10.7. Extract Storage:

10.7.1. The sample extract may be stored up to 40 days if kept at 0-6º C (not frozen). Keep the extracts in amber glass vials or clear vials with PTFE lined caps. If clear vials are used, wrap aluminum foil around the racks used for storing the extracts.

11. Calculations

11.1 Response Factor, RF, for a peak:

$$RF = \frac{\text{Area}_{\text{Analyte}}}{\text{Concentration}_{\text{Analyte}}}$$

11.1.1. Where:
RF = Response Factor
Area $_{\text{Analyte}}$ = Area of the peak of the analyte of interest
Concentration $_{\text{Analyte}}$ = Concentration of the analyte of interest in µg/ml

11.2. Average Response Factor, $\overline{RF}$:

$$\overline{RF} = \sum \frac{RF_i}{n}$$

11.2.1. Where:
$\overline{RF}$ = Mean response factor
RF_i = Response factor of compound at each level *i*
n = Number of calibration standards

11.3. Sample Standard Deviation (n – 1) (σ_{n-1}) of response factors:

$$\sigma_{n-1} = \sqrt{\sum_{i=1}^{n} \frac{(RF_i - \overline{RF})^2}{n-1}}$$

11.3.1. Where:
σ_{n-1} = Sample Standard Deviation
$\overline{RF}$ = Mean response factor
RF_i = Response factor of compound at each level *i*
n = Number of calibration standards

11.4. Linear Regression Response Equation:

$$Y = ax + b$$

This rearranges to:

$$x = Y - b/a$$

11.4.1. Where:
Y = Instrument response
a = Slope of the line
b = Intercept
x = Concentration in the extract or standard

11.5. Second Order Quadratic Fit Equation

11.5.1. $Y = ax^2 + bx + c$

11.5.2. Where:
Y = Instrument response
a = Slope of the line
b = Intercept
c = constant
x = Concentration in the extract or standard

11.5.3. Subtract Y from c to get modified equation $0 = ax^2 + bx + c$

11.5.4. Solve for x using the quadratic formula:

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

11.5.5. A positive and negative value will be generated. Use positive value.

11.6. Average Retention Time, $\overline{RT}$:

$$\overline{RT} = \sum \frac{RT}{n}$$

11.6.1. Where:
$\overline{RT}$ = Mean retention time for the target compound

RT = Retention time for the target compound

n = Number of values

11.7. Percent Drift, %Drift:

$$\%\text{Drift} = \frac{(\text{Concentration}_{\text{Calculated}} - \text{Concentration}_{\text{Expected}})}{\text{Concentration}_{\text{Expected}}} * 100$$

11.7.1. Where:
Concentration $_{\text{Calculated}}$ = Concentration calculated from result
Concentration $_{\text{Expected}}$ = Theoretical concentration of the standard

11.8. Extract Concentration Calculation (µg/ml):

$$\mu g/ml = \frac{(A_s)}{(\overline{RF})}$$

11.8.1. Where:
A_s = Peak area of analyte
$\overline{RF}$ = Average Response Factor

11.9 Sample Concentration Calculation (µg/L):

$$\mu g/L = \frac{C_s * 1000 \frac{ml}{L} * V_t}{V_s}$$

11.9.1 Where:
C_s = Extract concentration in µg/ml
V_t = Extract volume in ml
V_s = Original sample volume in ml

11.9.2 Assuming an original sample volume of 1000 ml and an extract volume of 5 ml, equation 11.9.2reduces to:

$$\mu g/L = C_s * 200$$

11.10 Sample Concentration Adjustment for Varying Initial Volume and Dilutions:

$$\mu g/L_{Corrected} = \mu g/L_{Uncorrected} * \frac{(1000\ ml)(DF)}{V_s}$$

11.10.1 Where:
DF = Dilution Factor
V_s = Original sample volume in ml

11.11 Quality Control Calculations:

$$\text{LCS/LCSD/ICV \% Recovery} = \frac{R_{spike}}{\text{Expected Result}} X\,100$$

$$\text{\% RPD(precision)} = \frac{\left|R_{sample} - R_{duplicate}\right|}{\left(\frac{R_{sample} + R_{duplicate}}{2}\right)} X\,100$$

11.12 LPC Calculations

11.12.2An LPC standard is run at the beginning of each sample sequence prior to the analysis of samples to determine sensitivity. The LPC is a standard at or below the reporting limit.

11.12.3Sensitivity

11.12.3.1 Instrument sensitivity is determined by comparing the LPC recovery of all analytes. The recovery of the analytes must be ± 50% of the true LPC value.

$$\text{LPC \% Recovery} = \frac{R_{spike}}{\text{Expected Result}} X\,100$$

11.13 Sample chromatograms generated from the processing software have calculation formulas already incorporated into the report format (see Calculations, Sections 11.8 and 11.9.2). Manual adjustments are required for diluted samples, or samples of other than 1 L only (see Calculations, Section 11.10). The RPD calculations are not incorporated into report formats and must be calculated manually or by the use of an Excel spreadsheet. If Excel spreadsheets are used, RPD results may be manually written on LCSD and MSD reports.

12 Waste Management

12.1 See GA EPD Laboratory SOP-EPD Laboratory Waste Management Standard Operating procedures. See SOP reference 13.4

13 References

13.1 EPA/600/4-88-039 - EPA Method 550.1, 1990

13.2 GA EPD Laboratory SOP's- Initial Demonstration of Capability SOP 6-001, online revision and/or Continuing Demonstration of Capability SOP 6-002, online revision.

13.3 GA EPD Laboratory SOP- EPD Laboratory Procedures for Control Charting and Control and Control Limits SOP, SOP 6-025, online revision.

13.4 GA EPD Laboratory SOP- EPD Laboratory Waste Management SOP, SOP 6-015, online revision.

13.5 Manual for the Certification of Laboratories Analyzing Drinking Water, EPA/815-R-05-004, January 2005 or later.

13.6 GA EPD Laboratory SOP- Determination of Method Detection Limit, Method Detection Limit SOP 6-007, online revision.

13.7 GA EPD Laboratory Quality Assurance Plan, online revision.

13.8 GA EPD Laboratory Safety/Chemical Hygiene Plan & Fire Safety Plan, online revision.

14 Reporting Limits (RLs), Precision and Accuracy Criteria, and Quality Control Approach

14.1 Refer to Appendix A, Table A.1 for precision and accuracy criteria.

Table 14.1.1 RLs for EPA Method 550.1

Parameter/Method	Analyte	Matrix (Water)	
		RL	Unit
EPA 550.1	Benzo (a) pyrene	0.044	µg/L

Table 14.1.2 Summary of Calibration and QC procedures for Method 550.1

Method	Applicable Parameter	QC check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria
550.1	Benzo (a) pyrene	7 point initial calibration for all analytes	Initial calibration prior to sample analysis	Linear mean RSD for all analytes ≤ 10% with linear least squares regression correlation coefficient r ≥ 0.990, or r^2 ≥ 0.980	Correct problem then repeat initial calibration	
		Second source calibration verification (ICV)	Once per 7 point initial calibration	All analytes within ±30% of expected values	Correct problem then repeat initial calibration	
		Retention time window calculated for each analyte	Once per year or after major maintenance	± 3 times standard deviation for each analyte retention time for standard analytical batch sequence		
		Retention time window update	Must be done every analytical sequence	First CCC of each sequence and the first CCC of each 24 hour period.	Correct problem then reanalyze all samples since the last retention time check	

Table 14.1.2 Summary of Calibration and QC procedures for Method 550.1						
Method	**Applicable Parameter**	**QC check**	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**
550.1	Benzo (a) pyrene	Calibration Verification (CCC)	Beginning each analysis sequence prior to the analysis of the samples, after every 10 sample sand at the end of the analysis	All analytes within ±20% of expected values	Correct problem then repeat CCC and reanalyze all samples since the last calibration verification	If out of range high, high bias with no detects, generate a corrective action and use data. If low bias or with detects, rerun CCC and affected samples. If rerun passes, use data. If reruns do not pass, correct problem, repeat initial calibration verification and reanalyze all samples since last successful calibration verification
		IDC- Demonstrate the ability to generate acceptable accuracy and precision using 4 replicate analyses of the QC check sample, a Blind and a Blank Analyst must also produce a passing MDL study with 7 MDL spikes and 7 MDL blanks	One per analyst	See **Appendix A, Table A.1. See section 9.9 for MDL requirements**	Recalculate results; locate and fix problem with system and then rerun demonstration for those analytes that did not meet criteria	
		CDC- Continuing Demonstration of Capability	Required every Six Months after IDC for each analyst	See **Appendix A, Table A.1**	Recalculate results; locate and fix problem with system and then rerun demonstration for those analytes that did not meet criteria	
		Laboratory Performance Check	One at the beginning each analysis sequence prior to the analysis of the samples	All analytes within ± 50% of expected value	Correct problem then repeat initial calibration	If out of range high, high bias with no detects, generate a corrective action and use data. If low bias or with detects, rerun LPC and affected samples. If rerun passes, use data. If reruns do not pass, correct problem, repeat LPC and reanalyze all samples

Table 14.1.2 Summary of Calibration and QC procedures for Method 550.1						
Method	**Applicable Parameter**	**QC check**	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**
550.1	Benzo (a) pyrene	Method blank	One per analytical batch	No analytes detected >RL	Correct problem then reprep and analyze method blank and all samples processed with the contaminated blank	If unable to re-extract, flag samples with a "B"
		MS/MSD for all analytes	Minimum of one MS/MSD per batch or 10% of all samples analyzed over time (2 MS/MSD for batches of 11 to 20 samples)	QC acceptance criteria **Appendix A**, **Table A.1**	Flag report if recoveries are out of acceptable range	
		LCS/LCSD for all analytes	One LCS/LCSD per batch	QC acceptance criteria **Appendix A**, **Table A.1**	If an LCS/LCSD fail, it may be reran at least 24 hours from the original run or up to 12 hours from the end of the sequence. Then if the rerun of the LCS/LCSD result with a failure then all samples associated with the batch must be re-extracted.	Flag QC sample report if LCSD exceeds upper acceptable control limits with passing RPD when high bias with no detects
		Second column confirmation	100% for all positive results	Same as for primary column analysis	Sample as for primary column analysis if used for quantitation	
		MDL study	Once per year or after major maintenance of the instrument	All Spiked MDLs must have a value greater than 0. Minimum Detection Limits established shall be < the RLs in Table 14.1	Re-do MDL Study	None

Table 14.1.2 Summary of Calibration and QC procedures for Method 550.1						
Method	**Applicable Parameter**	**QC check**	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**
550.1	Benzo (a) pyrene	MDL analysis	Once per batch or as needed to acquire data points per SOP 6-007, online revision	All Spiked MDLs must have a value greater than 0. All other QC in the MDL blank and MDL sample (i.e. Surrogate Spike or Internal Standard, etc. if included) must meet established criteria	Correct problem and re-run the MDL sample or MDL blank once and initiate a corrective action. If the re-run fails a second time, do not use MDL data. Update corrective action, and use associated sample data	None
		Results reported between MDL and RL	None	None	None	
		Quarterly ICV	Once per Quarter	All analytes within ± 30% of expected value	Correct problem then repeat initial calibration	
		Residual Chlorine check	Whenever needed. If collector does not check residual chlorine.	Must be checked for every sample.	Check residual chlorine levels and add information to extraction sheet.	

15. Associated Labworks Test Codes

15.1. Parent Test Code
15.1.1. $555B – Analysis results
15.2. Extraction Test Code
15.2.1. 550E – 40 mL vial – Liquid/ Liquid extraction
15.3. QC Test Codes
15.3.1. $B_550B – Extraction Blank Results
15.3.2. $LA550B – LCS/LCSD Spike Amount
15.3.3. $LS550B – LCS Results
15.3.4. $LS550B – LCSD Results
15.3.5. $LR550B – LCS Percent Recovery
15.3.6. $L2550B – LCSD Percent Recovery
15.3.7. $LP550B – LCS/LCSD Precision
15.3.8. $A_550B – MS/MSD Spike Amount
15.3.9. $S_550B – MS Results
15.3.10. $D_550B – MSD Results
15.3.11. $R_550B – MS Percent Recovery
15.3.12. $RD550B – MS Percent Recovery
15.3.13. $P_550B – MS/MSD Precision
15.3.14. $MA550B – MDL Spike Amount
15.3.15. $ML550B – MDL Results
15.3.16. INSTR-550B – Instrument associated with batch

Appendix A – Quality Assurance Criteria for Method EPA 550.1

Table A.1: Default Limits Criteria for Method 550.1				
QC Type	**Analyte**	**Accuracy (%R)**		**Precision (RPD)**
		LCL	**UCL**	
LCS/LCSD* MS/MSD*	Benzo (a) pyrene	73 -	107	16%

*LCS/LCSD recovery and precision limits based on control charts of data collected from 12/31/2018 to 01/01/2021. EPA Method 550.1 requires MS recovery to be the same as that calculated for the LCS, the EPD Laboratory sets the recovery ranges for the LCSD and MSD to be the same as the range for the LCS and the MS/MSD precision to be the same as the LCS/LCSD precision.

Updates:
Appendix A added. Updated for online revision.