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Diquat Dibromide – EPA Method 549.2

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1. Scope and Application

1.1. Method 549.2 is used to determine the concentration of Diquat in drinking water. Samples are extracted with a 47 mm C_8 disk, which has been prepared for reverse-phase, ion-pair mode. The disk is eluted with an acidic aqueous solvent. The extract is injected into a high-pressure liquid chromatograph with an ultraviolet absorbance 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 Diquat 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. Plastic ware must be scrupulously cleaned with hot water and detergent followed by de-ionized water then rinsed with methanol.

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. This method has been shown to be susceptible to interferences from Ca^{+2} and Mg^{+2} ions which may be present in hard water samples. These divalent cations can cause low recovery of method analytes, by interfering with the ion exchange process.

4. Safety

4.1. Refer to Georgia EPD Laboratory Chemical Hygiene Plan.

5. Apparatus and Equipment

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5.1. 6 position manifold for Empore disks

5.2. C_8 47 mm, liquid-solid extraction disk.

5.3. 500 ml amber polyvinylchloride (PVC) bottles – Two per sampling point

5.4. Polypropylene 500 ml graduated cylinders

5.5. Culture tubes; polypropylene 15 ml

5.6. Plastic transfer pipets

5.7. Single channel pipettor:

5.7.1. 1 - pipettor capable of a measurement from 0.100 ml to 1.00 ml

5.7.2. 1 - pipettor capable of a measurement from 1.00 ml to 10.00 ml

5.8. Plastic snap cap HPLC vials

5.9. Balance: analytical, capable of weighing 0.0001 grams

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

5.10.1. Variable UV/Vis detector set at 308 λ – Perkin Elmer Series 200 detector with a deuterium lamp.

5.10.2. Auto Sampler- Perkin Elmer: series 200 LC pump

5.10.3. Column – Phenomenex PolymerX 5 µm RP-1 100A 150 x 4.1 mm or equivalent

5.11. Perkin-Elmer Totalchrom or equivalent chromatography software

5.12. 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 TOC of 50 µg/L or less).

6.2. Orthophosphoric acid, 85% (w/v) -- Reagent grade

6.3. Diethylamine-- Reagent grade

6.4. Concentrated sulfuric acid -- ACS reagent grade

6.5. Sodium Hydroxide -- Reagent grade

6.6. Concentrated Hydrochloric acid, 12 N -- Reagent grade

6.7. Cetyl trimethyl ammonium bromide, 95% -- Reagent grade

6.8. 1-Hexanesulfonic acid, sodium salt, 98%,

6.9. 1-Heptanesulfonic acid, sodium salt, 98%,

6.10. Ammonium Hydroxide, ACS, Concentrated

6.11. Methanol: – High purity, demonstrated to be free from analytes and interferences (HPLC grade or better).

6.12. Sodium Thiosulfate: pesticide grade 50mg per 500 ml amber polypropylene bottle. The sodium thiosulfate within this sample may handle up to 5 ppm of residual chlorine.

6.13. Conditioning Solution "A":

6.13.1. Dissolve 0.500 g of cetyl trimethyl ammonium bromide and 5 ml of concentrated ammonium hydroxide in 500 ml of reagent water and dilute to 1000 ml in a volumetric flask.

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6.14. Conditioning Solution "B":

6.14.1. Dissolve 10 g of 1-hexanesulfonic acid sodium salt and add 10 ml of concentrated ammonium hydroxide in 250 ml of reagent water and dilute to 500 ml in a volumetric flask.

6.15. Disk Eluting Solution:

6.15.1. Add 13.5 ml of orthophosphoric acid and add 10.3 ml of diethylamine to 500 ml of reagent water and dilute to 1000 ml in a volumetric flask.

6.16. Mobile Phase solution:

6.16.1. Add 13.5 ml of orthophosphoric acid and add 10.3 ml of diethylamine and then add 3.0 g of 1-hexansulfonic acid sodium salt to 500 ml of deionized water and dilute to 1000 ml in a volumetric flask.

6.17. 1-hexanesulfonic Acid Ion-pair solution:

6.17.1. Dissolve 3.75 g of 1-hexanesulfonic acid sodium salt in 15 ml of the disk or cartridge eluting solution and dilute to 25 ml in a volumetric flask with the disk eluting solution.

6.18. 1-heptanesulfonic Acid 2nd Ion-pair confirmation solution:

6.18.1. If necessary, a second ion-pair confirmation of 1-heptanesulfonic acid ion-pair will be used in the event of a confirmed hit.

6.18.2. Dissolve 3.75 g of 1-heptanesulfonic acid sodium salt in 15 ml of the disk or cartridge eluting solution and dilute to 25 ml in a volumetric flask with the disk eluting solution.

6.19. Standard Stock Solutions:

6.19.1. All standards that are made for the 549.2 analysis are to have a 6 month expiration date from the opening of the vendor stock ampule.

6.19.2. Primary Stock # 1 Solution: 100 µg/ml made up from vendor stock at 1000 µg/ml.

Table 6.19.2. 1: 549.2 Primary Stock #1 Solution in Reagent Water (1st Dilution)

Compound	Initial Concentration	Aliquot	Final Concentration
Diquat	1000 µg/ml	1.0 ml	100 µg/ml
Total volume of Standard Aliquot		1.0 ml	
Addition of Reagent Water to Standard aliquots		9.0 ml	
Final Volume of Primary Stock #1		10.0 ml	

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

Table 6.19.3. 1 549.2 Primary Stock #2 Solution in Reagent Water (2nd Dilution)

Compound	Initial Concentration	Aliquot	Final Concentration
Diquat	100 µg/ml	1.0 ml	10.0 µg/ml
Total volume of Standard Aliquot		1.0 ml	
Addition of Reagent Water to Standard aliquots		9.0 ml	
Final Volume of Primary Stock #2		10.0 ml	

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

Table 6.19.4. 1: 549.2 Spiking Stock Solution in Reagent Water (3rd Dilution)

Compound	Initial Concentration	Aliquot	Final Concentration
Diquat	10.0 µg/ml	1.0 ml	1.00 µg/ml
Total volume of Primary Stock #2 Aliquot		1.0 ml	

Table 6.19.4. 1: 549.2 Spiking Stock Solution in Reagent Water (3rd Dilution)			
Compound	**Initial Concentration**	**Aliquot**	**Final Concentration**
Addition of Reagent Water to Standard aliquots		9.0 ml	
Final Volume of Spiking Stock		10.0 ml	

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

Table 6.19.5. 1: 549.2 ICV Stock Solution in Reagent Water (1st Dilution)			
Compound	**Initial Concentration**	**Aliquot**	**Final Concentration**
Diquat	1000 µg/ml	1.0 ml	100 µg/ml
Total volume of Standard Aliquot		1.0 ml	
Addition of Reagent Water to Standard aliquots		9.0 ml	
Final Volume of Primary Stock #2		10.0 ml	

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

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Table 6.19.6. 1: 549.2 ICV Stock #2 Solution in Reagent Water (2nd Dilution)			
Compound	**Initial Concentration**	**Aliquot**	**Final Concentration**
Diquat	100 µg/ml	1.0 ml	10.0 µg/ml
Total volume of Standard Aliquot		1.0 ml	
Addition of Reagent Water to Standard aliquots		9.0 ml	
Final Volume of Primary Stock #2		10.0 ml	

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

Table 6.19.7. 1: 549.2 ICV Spiking Stock Solution in Reagent Water (3rd Dilution)			
Compound	**Initial Concentration**	**Aliquot**	**Final Concentration**
Diquat	10 µg/ml	1.0 ml	1.00 µg/ml
Total volume of Standard Aliquot		1.0 ml	
Addition of Reagent Water 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 549.2 are collected in pre-certifed 500 ml amber polyvinylchloride (PVC) bottles (to shield from light) with Teflon lined screw caps. Samples are preserved with 50 mg Sodium thiosulfate. 50 mg of Sodium thiosulfate is sufficient to neutralize up to 5 mg/L (ppm) of residual chlorine in a 500 ml 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.

7.1.2. 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. Stored samples must be protected from light exposure. Two sample bottles are collected for each sample. In addition, bottles are provided at a frequency to meet the method requirements for matrix spikes and duplicate analyses.

7.2.1. Samples must be extracted within 7 days of collection and the extracted samples must be analyzed within 21 days.

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8. Calibration

8.1. Calibration Curve:

8.1.1. A 6-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 < 20% 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 made up by closely matching the process of extracting the samples as follows:

8.2.1.1. Place a 47mm C8 disk on the extraction manifold and condition according to the steps outlined in section 10.7.

8.2.1.2. Pass through the disk 250 ml of reagent water and allow the disk to go dry.

8.2.1.3. Dry the disk by drawing 5 ml of Methanol through it and allowing the disk to dry.

8.2.1.4. Pass 4 ml of eluting solution through the disk and capture eluent in a PVC culture tube. Repeat with 4 more ml of eluting solution.

8.2.1.5. Add 200 µL of ionic pair solution to the eluent. Spike eluent with the appropriate volume (see Table 8.2. 3) of the Primary Stock #2 (see 6.19.3.) or the Spiking Stock Solution (see 6.19.4.) and then bring to a final volume of 10 ml.

8.2.2. Calibration standards are made up at the following concentrations (µg/ml):

Table 8.2. 1: Calibration Curve for Diquat in µg/ml						
Name	**Level 1**	**Level 2**	**Level 3**	**Level 4**	**Level 5**	**Level 6**
Diquat	0.010 µg/ml	0.020 µg/ml	0.060 µg/ml	0.10 µg/ml	0.20 µg/ml	0.40 µg/ml

Table 8.2. 2: Calibration Curve for Diquat in µg/L						
Name	**Level 1**	**Level 2**	**Level 3**	**Level 4**	**Level 5**	**Level 6**
Diquat	0.400 µg/L	0.800 µg/L	2.40 µg/L	4.00 µg/L	8.00 µg/L	16.0 µg/L

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Table 8.2. 3: Aliquots of Primary Spiking Stock Solutions to make up standard levels in the Tables 8.2. 1 or 8.2. 2						
Name	**Level 1 (use Spiking stock)**	**Level 2 (use Spiking stock)**	**Level 3 (use Spiking stock)**	**Level 4 (use Primary Stock #2)**	**Level 5 (use Primary Stock #2)**	**Level 6 (use Primary Stock #2)**
Diquat	0.100 ml (or 100 µl)	0.200 ml (or 200 µl)	0.600 ml (or 600 µl)	0.100 ml (or 100 µl)	0.200 ml (or 200 µl)	0.400 ml (or 400 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.

8.3.1.1. The ICV is made up in the same manner as the Level 5 calibration standard but spiking with the ICV Stock #2. Other levels can be made up using the appropriate spiking stock and volume.

8.3.2. A daily continuing calibration is performed every eight-hour analysis period (per EPA Method 549.2 section 10.4), or every 10 samples, whichever comes first, to monitor and validate the instrumentation, column, and detector performance.

8.3.2.1. CCCs are made up as needed in the same manner as other calibration standards using the appropriate volumes of the Primary Stock #2 or the Spiking Stock Solution as is appropriate.

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) (see section 8.3.2.1. for preparation of CCCs as needed) ensures the instrument target compound retention times and quantitation parameters meet method performance criteria. Prior to sample analysis, and for any 8-hour analysis period or every 10 samples, whichever comes first, 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 calculation 11.7). If the continuing calibration does not meet method performance criteria, re-analyze once. If the CCC continues to fail, determine the source of the problem, correct and if necessary, recalibrate. 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%.

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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 equivalent). The new RTs are saved in a copy of the chromatographic software method used for analyzing this batch of samples. To the existing chromatographic method file name and/or method title an extension is added by using – Month-Day-Year (mm-dd-yy format). Hard copies of the updated 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 ≤ 20% 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 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 6 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 calculation 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 calculation 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. The %Drift may be no more than ± 20%. See calculation 11.7.

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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 calculation 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 calculation 11.4. Results are in µg/ml.

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

8.11.5. The sample concentration is calculated per calculations 11.9 in µg/L. Assuming a 250 ml initial sample volume and a 10 ml extract volume, equation 11.9 can be reduced to C_s multiplied by a factor of 40. 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 calculation 11.9.

8.11.6. If an initial volume of other than 250 ml is used or a dilution of the extract is analyzed, the final sample result is multiplied by the factor determined per calculation 11.10.

9. Quality Control

9.1. Refer to Table 14.11 for the Reporting Limits (RL), Appendix A, Table A.1 for Quality assurance criteria and Table 14.12 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. See SOP reference 13.2 for training and certification procedures.

9.4. See SOP reference 13.3 for control charting procedures.

9.5. Default control limits for recovery are based on Section 9.3.2 of EPA Method 549.2. See SOP reference 13.1

9.5.1. The method defines the LCS default recovery range as 70% - 130% of the true value. The LCS control limits are updated through the use of control charts. See SOP reference 13.3

9.5.2. The EPD Laboratory sets the LCSD recovery control limits to be the same as the LCS limits.

9.5.3. LCS/LCSD precision limit defaults are set by the EPD Laboratory as 0% - 30% RPD. LCS/LCSD precision limits are updated through the use of control charts. See reference 13.3

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9.5.4. In-house limits based on control charts may never exceed the default limits shown in Table 9.6. 1.

9.5.5. The method sets the MS recovery control limits to be the same as those of the LCS. See EPA Method 549.2 section 9.6, reference 13.1.

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

9.5.7. Control limits are updated through the use of control charts.

9.6. The control limits below 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. Those limits cannot exceed the default limits presented in Table 9.6.

Table 9.6. 1: Default QC Limits for Method 549.2

QC Type	Analyte	Accuracy (%R) LCL	Accuracy (%R) UCL	Precision (%RPD)
LCS/LCSD* MS/MSD	Diquat	70	130	30

9.7. EPA Method 549.2 requires LCSs to be analyzed at a frequency rate of 5% of all samples (see EPA Method 549.2 Section 9.3, SOP reference 13.1.).

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

9.8.1. For batches (see section 10.4.) of 1 – 10 samples, one MS/MSD pair is extracted. For batches of 11 – 20 samples, two MS/MSD pairs are extracted.

9.9. Performance Test (PT) Sample:

9.9.1. EPA requires that the Laboratory perform a PT sample every 12 months to maintain certification in EPA method 549.2. Those PT result must fall within acceptable control limits for the PT testing facility. If those results are not within acceptable control limits the Laboratory will have a second chance to pass the PT study within the same 12 months of the study. If the results did not fall within acceptable control limits for the study over the 12-month testing period, the laboratory will be downgraded for those compounds listed in this SOP. With the failure of this nature the laboratory must notify all drinking water facilities within 30 days of the failure after the 12-month period has passed. It is not until the laboratory passes a PT study will the laboratory be able to test for those compounds of interest again.

9.10. Method Detection Limit Study (MDL):

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

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

9.10.3. 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.

9.10.4. 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.

9.11. Initial MDL study:

9.11.1. 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.

9.11.2. 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.

9.12. Continuous MDL study:

9.12.1. 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 9.11.2 for requirements.)

9.12.2. 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.

9.12.3. The results of the MDLBlank will be entered into Labworks using the Method Blank test code, $B_549. The MDLSpike result will be entered using the $ML549. The MDL Spiked Amount will be entered into the test code $MA549. The instrument used for the MDL and Blank analysis will be selected using the test code INSTR-549.

9.12.4. 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.

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10. Procedure

10.1. Pipettor check: Place an HPLC vial on a balance and tare. Pipette 1 ml of eluting solution to the vial. Record mass on extraction sheet.

10.2. Monthly Graduated cylinder check: (Note: 1gram of water equals 1 milliliter of water.) Measure out 250 grams of reagent water into your graduated cylinder. Record the mass and the volume in the graduated cylinder checklist in the back of the extraction notebook. Acceptable variance of 2% or ±5 mL.

10.3. 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.

10.4. 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. For the Blank, LCS and LCSD fill three 500 ml preserved sample collection bottles. For the Blank measure 250 ml from one of the filled 500 ml bottles into a graduated cylinder. For the LCS and LCSD measure 250 ml aliquots from each of the other two 500 ml bottles into graduated cylinders then spike with 1000 µl of a 1.00 µg/ml Diquat standard added to each. The MS and MSD are 250 ml

aliquots of the designated batch QC sample spiked with 1000 µl of a 1.00 µg/ml Diquat standard added to each.

10.4.1. *Note: If more CCC standards need to be prepared, the standard(s) may prepared during batch extraction per the steps outlined in sections 8.2. and 8.5.*

10.5. Adjust pH of the sample to a range of 7 - 9. If the pH is < 7 use the NaOH to adjust. If the pH is > 9 use the HCl to adjust.

10.6. Set up the manifold plastic ware using a C_8 extraction disk.

10.7. Disk Conditioning

10.7.1. The disk is conditioned by pulling the following reagents through the disk: ***Note****: do not allow the disk to go dry between the conditioning steps, if disk becomes dry the repeat steps 10.7.3. - 10.8.*

10.7.2. If at any point during steps 10.7.3. - 10.7.7. it becomes necessary, an additional aliquot of solvent may be added to keep the disk wet. Up to 5 ml of extra solvent may be used per step for this purpose.

10.7.3. Draw a 10 ml aliquot of methanol through disk until approximately 1 mm remains on the disk.

10.7.4. Draw 2 x 10 ml aliquot of reagent water through the disk until approximately 1 mm remains on the disk after each aliquot.

10.7.5. Add a 10 ml aliquot of conditioning solution "A" to the disk; Allow to soak the disk for about 1 minute. Draw this solution through the disk until approximately 1 mm remains on the disk.

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10.7.6. Draw 2 x 10 ml aliquot of reagent water to the disk until approximately 1 mm remains on the disk after each aliquot.

10.7.7. Add a 20 ml aliquot of conditioning solution "B" to the disk; Allow to soak the disk for about 1 minute. Draw this solution through the disk until approximately 1 mm remains on the disk.

10.8. Add sample to the manifold and draw the entire sample through the disk and allow disk to go dry.

10.9. Position polypropylene tube inside of the manifold to collect the eluent.

10.10. Draw a 1 ml aliquot of the methanol through the disk not allowing the disk to go dry.

10.11. Draw a 4 ml aliquot of Eluting solution through the disk two times not allowing the disk to go dry either time.

10.12. Remove the collection tube from the manifold. Spike with 200 µl of ion-pair concentrate, bring to a final volume of 10 ml with eluting solution, cap and mix.

10.13. Transfer sample to a 0.75 ml plastic HPLC vial.

10.14. Analyze samples on an HPLC instrument equipped with an UV/Vis detector set at 308 λ.

10.15. Dilutions

10.15.1. Any sample with a target analyte response greater than the highest level of the calibration curve must be diluted so that that analyte response is less than or equal to the highest calibration level and re-analyzed. Sample dilutions are made with the extraction solvent, the disk eluting solution (see section 6.15.) so that the analyte response is between the lowest standard (or the reporting limit, whichever is greater) and highest standard responses. Dilutions must be analyzed in a valid chromatographic sequence.

10.16. The sample extract may be stored up to 21 days if kept at 0-6ºC (not frozen). Keep the extracts in a Plastic tube with PTFE lined caps.

10.17. PT Study:

10.17.1. Once every 12-month period a PT study must be performed. An accredited testing facility will send the Laboratory an ampule for the compounds of interest listed in this SOP. The testing facility will send direction on how perform the dilutions necessary for the Analyst to spike into a sample. (Note: Please include a copy of instructions from the facility in the batch folder.)

10.18.

11. Calculations

11.1. Response Factor, RF, for a peak:

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

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

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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:

$$\%Drift = \frac{(Concentration_{Calculated} - Concentration_{Expected})}{Concentration_{Expected}} * 100$$

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11.7.1 Where:
$Concentration_{Calculated}$ = Concentration calculated from result
$Concentration_{Expected}$ = 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 100 ml and an extract volume of 1 ml, equation 11.8 reduces to:

$$\mu g/_L = C_s * 100$$

11.10 Sample Concentration Adjustment for Varying Initial Volume and Dilutions:

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

11.10.1 Where:

DF = Dilution Factor

V_s = Original sample volume in ml

11.11 Quality Control Calculations:

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

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

11.12 LPC Calculations:

11.12.1 An 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.

11.12.2 *Sensitivity*:

11.12.2.1 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 11.9 and 11.9.2). Manual adjustments are required for diluted samples, or samples of other than 1 ml only (see calculation 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 549.2, Revision 1.0, 1997

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 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

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

13.7 GA EPD Quality Assurance Plan, online revision.

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

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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 14.11: RLs for EPA Method 549.2

Parameter/Method	Analyte	Matrix (Water)	
		RL	Unit
EPA 549.2	Diquat	0.88	µg/L

Table 14.12: Summary of Calibration and QC procedures for Method 549.2						
Method	**Applicable Parameter**	**QC check**	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**
EPA Method 549.2	Diquat	6 point initial calibration for all analytes	Initial calibration prior to sample analysis	Linear mean RSD for all analytes ≤20% with linear least squares regression $r \geq 0.990$ or $r^2 \geq 0.980$	Correct problem then repeat initial calibration	
		Second source calibration verification (ICV)	Once per 6 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	Every 24-hours in which samples are analyzed.	First CCC of each sequence and the first CCC of subsequent 24-hour periods.	Correct problem then reanalyze all samples since the last retention time check	
		Calibration Verification (CCC)	Beginning each analysis sequence prior to the analysis of the samples, after every 10 samples or 8-hour period, whichever comes first, and 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

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Table 14.12: Summary of Calibration and QC procedures for Method 549.2						
Method	**Applicable Parameter**	**QC check**	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**
EPA Methods 549.2	Diquat	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 9.10 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	
		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	One MS/MSD per batch or 10% of all field samples analyzed over time	QC acceptance criteria **Appendix A**, **Table A.1**	Flag report if recoveries are out of acceptable range	

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Table 14.12: Summary of Calibration and QC procedures for Method 549.2						
Method	**Applicable Parameter**	**QC check**	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**
EPA Method 549.2	Diquat	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.	
		Second ion-pair confirmation	100% for all positive results	Same as for primary column analysis	Sample as for primary ion-pair 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
		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 ± 20% 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.	

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Table 14.12: Summary of Calibration and QC procedures for Method 549.2						
Method	**Applicable Parameter**	**QC check**	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging 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 LPC	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

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15 Associated Labworks Test Codes

15.1 Parent Test Code
15.1.1 $549 – Analysis results
15.2 Extraction Test Code
15.2.1 549E – 250 mL aliquot – Solid Phase Extraction (SPE)
15.3 QC Test Codes
15.3.1 $B_549 – Extraction Blank Results
15.3.2 $LA549 – LCS/LCSD Spike Amount
15.3.3 $LS549 – LCS Results
15.3.4 $LS549 – LCSD Results
15.3.5 $LR549 – LCS Percent Recovery
15.3.6 $L2549 – LCSD Percent Recovery
15.3.7 $LP549 – LCS/LCSD Precision
15.3.8 $A_549 – MS/MSD Spike Amount
15.3.9 $S_549 – MS Results
15.3.10 $D_549 – MSD Results
15.3.11 $R_549 – MS Percent Recovery
15.3.12 $RD549 – MS Percent Recovery
15.3.13 $P_549 – MS/MSD Precision
15.3.14 $MA549 – MDL Spike Amount
15.3.15 $ML549 – MDL Results
15.3.16 INSTR-549 – Instrument associated with batch

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Appendix A – Quality Assurance Criteria for EPA Method 549.2

Table A.1: QC Criteria for Method 549.2

QC Type	Analyte	Accuracy (%R) LCL	Accuracy (%R) UCL	Precision (RPD)
LCS/LCSD* MS/MSD*	Diquat dibromide	70	115	17%

*LCS/LCSD recovery and precision limits based on control charts of data collected from 12/31/2018 to 01/01/2021. EPA Method 549.2 requires MS recovery to be the same as that calculated for the LCS, 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.