Laboratory Manager Approval: Mary K. Bowman / 08/19/2021

QA Manager Approval: Jeffrey Moore / 08/19/2021

Glyphosate – EPA Method 547

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 547 is used to determine the concentrations of Glyphosate in drinking water. The water sample is injected in to a high-pressure liquid chromatograph with separation achieved using an isocratic elution. The analyte is oxidized to glycine with sodium hypochlorite. Glycine is then coupled with Thiofluor to give a fluorophor detected by fluorescence detector. Identification is obtained by analyzing a standard curve under identical conditions used for the samples and comparing resultant retention times. Concentrations of identified compounds are measured by relating response produced for that compound to a 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 apparatus that lead to discrete artifacts or elevated baselines in chromatograms.

3.1.1. Glassware must be scrupulously cleaned with hot water and detergent followed by de-ionized water then rinsed with methanol followed by acetone. Glassware is pre-rinsed with reagent water (see 6.1.) prior to use.

3.1.2. The use of high purity reagents and solvents helps to minimize interference problems.

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

3.3. Matrix interferences may be caused by contaminants that are co-extracted from the sample.

4. Safety

4.1. Refer to Georgia EPD Laboratory Chemical Hygiene Plan.

5. Apparatus and Equipment

5.1. Sample containers: 40 ml glass with Teflon-lined screw caps.

5.2. Vials: autosampler, snap cap or equivalent

5.3. Leurlock Micro-syringes

5.4. Micro-syringes

5.5. Pipets: Pasteur, disposable glass

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

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

5.8. Column #1: Agilent Zorbax 300-scx or equivalent, 5 micron, 4.6 mm ID X 250 mm long, ODS (C-18) or equivalent

5.9. Pickering Labs Pinnacle PCX Post column reaction module or equivalent

5.10. Fluorescence Detector: Perkin Elmer LC series 200 fluorescence or equivalent detector capable of an excitation at 330 nm and the detection of emission energies at 465 nm or equivalent.

5.11. Perkin-Elmer Totalchrom or equivalent chromatography software

5.12. Leurlock filters at 0.45 µm

5.13. Balance: analytical, capable of weighing 0.0001 g

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

6.3. Sodium Thiosulfate: Reagent grade or equivalent. 4 mg per 40 ml vial

6.4. Hypochlorite Reagent (Pickering Labs GA116)

6.5. O-Pthaladehyde (OPA) Diluent (Pickering Labs GA104): pH = 9.1.

6.6. Thiofluor - N, N-Dimethyl-2-mecraptoethylamine hydrochloride, chromatographic grade.

6.7. O-Pthaladehyde (OPA) - chromatographic grade.

6.8. OPA Reaction Solution:

6.8.1. Dissolve 2.0 g of Thiofluor into approximately 25 ml of OPA diluent.

6.8.2. Dissolve 0.10 g of O-Pthaladehyde in about 10 ml of Methanol.

6.8.3. Combine these solutions into the OPA diluent and dilute to final volume of 950 ml.

6.8.4. Degas on instrument before use.

6.9. Sodium Hypochlorite: reagent grade, 4 – 6% hypochlorite solution

6.10. Hypochlorite Reaction Solution:

6.10.1. Remove the cap from Hypochlorite Reagent (GA116) and add 100 µL of Sodium Hypochlorite (4 - 6%) to the reagent. Recap and invert several times.

6.10.2. Degas on instrument before use.

6.11. Mobile Phase:

6.11.1. 0.005 M KH_2PO_4 (0.68 gm) in 960 mL reagent water, add 40 mL HPLC grade methanol, adjust pH of solution to 1.9 with concentrated phosphoric acid then filter with 0.22 µ filter and degas with helium before use.

6.12. Stock Standard Solutions: All standards that are made for the 547 analysis are to have a 6 month expiration date from the opening of the vendor stock ampule.

6.13. Primary Stock #1 Solution:

6.13.1. 10 µg/ml made up from vendor stock of Glyphosate mix at 100 µg/ml.

Table 6.13.1. 1 – 547 Primary Stock #1 Solution in Reagent Water (1st Dilution)

Compound	Initial Concentration	Aliquot	Final Concentration
Glyphosate	100 µg/ml	1.0 ml	10 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	

6.14. Primary ICV Stock Solution:

6.14.1. 10 µg/ml made up from vendor stock of Glyphosate at 100 µg/ml.

Table 6.14.1. 1 – 547 Primary ICV Stock Solution in Reagent Water (1st Dilution)			
Compound	**Initial Concentration**	**Aliquot**	**Final Concentration**
Glyphosate	100 µg/ml	1.0 ml	10 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	

7. Sample Collection

7.1. Drinking water samples for EPA Method 547 are collected in a pre-certified 40 ml glass vial with a Teflon lined screw cap.

7.1.1. Samples are preserved with 4 mg of Sodium Thiosulfate. 4 mg of Sodium thiosulfate is sufficient to de-chlorinate up to 5 parts per million (ppm) of residual chlorine in a 40 ml sample.

7.1.1.1. A residual chlorine check is performed in the field by the collector. The collector records the numerical value of the residual chlorine in ppm on the sampling form.

7.1.1.2. The laboratory shipping and receiving staff log in the samples and enter the residual chlorine value in the DNR_LAB field of the LIMS (Labworks).

7.1.1.3. The analyst prints a LIMS backlog showing the residual chlorine levels for every sample. The analyst verifies that the residual chlorine level is less than 5 ppm.

7.1.1.3.1. If a sample has more than 5 ppm of residual chlorine, recollection will be required.

7.1.2. Samples are cooled to 0-6º C (not frozen) after sample collection. Three vials are to be collected for every sample. Samples must be analyzed within 14 days.

8. Calibration

8.1. Calibration Curve:

8.1.1. A seven-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. Standards are made up to 25 ml with reagent water (see 6.1.) and stored at 0-6° C (not frozen). Prior to use, standards are filtered through Leurlock filters (see5.12.) into autosampler vials and capped. The calibration curve consists of the calibration standards made up in water at the following concentrations (µg/ml):

Table 8.2.1. 1: Calibration Curve for Glyphosate (µg/ml)							
Compound	**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**
Glyphosate	0.01	0.013	0.020	0.040	0.060	0.080	0.100

Table 8.2.1. 2: Calibration Curve for Glyphosate (µg/L)							
Compound	**Level 1 µg/L**	**Level 2 µg/L**	**Level 3 µg/L**	**Level 4 µg/L**	**Level 5 µg/L**	**Level 6 µg/L**	**Level 7 µg/L**
Glyphosate	10	13	20	40	60	80	100

Table 8.2.1. 3 Aliquots of Primary Stock Solution to make up all the levels in the above table. (Aliquots correspond to each level directly above each column.)							
Compound	**Level 1**	**Level 2**	**Level 3**	**Level 4**	**Level 5**	**Level 6**	**Level 7**
Glyphosate	25 µl (or 0.025 ml)	33 µl (or 0.033 ml)	50 µl (or 0.050 ml)	100 µl (or 0.100 ml)	150 µl (or 0.150 ml)	200 µl (or 0.200 ml)	250 µl (or 0.250 ml)

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. Prior to sample analysis, a one-point daily calibration verification is performed. For any 8-hour analysis period or after every 10 samples analyzed, whichever occurs first, another 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 equation 11.7.). If the continuing calibration does not meet method performance criteria, 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 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 ≤ 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 equations 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 equation 11.4.

8.8.2. Alternatively, second order quadratic fit may be used with an acceptable correlation of coefficient of r ≥ 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, surrogate and major constituent in multi-component analytes is defined as ± 3 times the standard

deviation of the mean absolute retention time established over an analytical batch sequence. See equation 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 CCC analysis. The %Drift may be no more than ± 20%. See equation 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 equation 11.8. 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 equation 11.4. 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 equation 11.5 in µg/ml.

8.11.5. The sample concentration is calculated per equation 11.9. in µg/L. Assuming a 25 ml initial sample volume and a 25 ml extract volume, equation 11.9. can be reduced to C_s multiplied by a factor of 1. The chromatographic report uses this factor to multiply the result from either 8.11.2. ,8.11.3. or 8.11.4. above and calculates the final result per equation 11.10.

8.11.6. If an initial volume of other than 25 ml is used or a dilution of the sample is analyzed, the final sample result is multiplied by the factor determined with per equation 11.11.

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. See SOP reference 13.2. for training and certification procedures.

9.4. See SOP reference 13.3. for control charting procedures.

9.5. Control Limits:

9.5.1. Default control limits for LCS recoveries are defined in sections 10.3.2 and 10.5.2 of the method (see reference 13.1.). The EPD laboratory sets LCSD limits to be the same as those for the LCS. Recovery limits are updated through the use of control charts (see SOP reference 13.3.).

9.5.2. The EPD Laboratory sets default precision control limits of 0 to 30% for LCS/LCSD precision. Precision limits are updated through the use of control charts (see SOP reference 13.3.).

9.5.3. Method 547 requires MS control limits to be the same the LCS limits. The EPD Laboratory sets the MSD recovery control limits to be the same as the LCS/MS limits.

9.5.4. The EPD Laboratory sets MS/MSD precision control limits to be equal to the LCS/LCSD precision limits.

9.6. **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 Limits Criteria for Method 547

QC Type	Analyte	Accuracy (%R) LCL	Accuracy (%R) UCL	Precision (RPD)
LCS/LCSD MS/MSD	Glyphosate	67	125	30%

9.7. EPA Method 547 requires an LCS to be analyzed at a frequency of 5% of all samples or a minimum of one per analytical batch. See EPA Method 547, Section 10.5.1. The LCSD is used to satisfy EPD Laboratory precision requirements.

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

9.8.1. For batches of 1 – 10 field samples, one MS/MSD pair must be analyzed.

9.9. For batches of 11 – 20 field samples a second MS/MSD pair must be analyzed. If possible, the sample selected for the second pair should be from the 11th through 20th samples, or, at least from a different sampling site/system from the first pair.

9.10. Performance Test (PT) Sample:

9.10.1. EPA requires that the Laboratory perform a PT sample every 12 months to maintain certification in EPA method 547. 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.

9.11. Method Detection Limit Study (MDL):

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

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

9.11.3. 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.

9.11.4. 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.

9.12. Initial MDL study:

9.12.1. 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.

9.12.2. 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.

9.13. Continuous MDL study:

9.13.1. 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 9.12.2 for requirements.)

9.13.2. 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.

9.13.3. The results of the MDLBlank will be entered into Labworks using the Method Blank test code, $B_547 The MDLSpike result will be entered using the $ML547. The MDL Spiked Amount will be entered into the test code $MA547. The instrument used for the MDL and Blank analysis will be selected using the test code INSTR-547.

9.13.4. 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 samples to equilibrate to room temperature prior to sample preparation and/or analysis.

10.2. The sample preparation is carried out in the original sample vial in which the drinking water is collected and shipped.

10.3. 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 laboratory blank is defined as 25 ml of laboratory reagent water (see 6.1.) added to a preserved sample collection vial, recap and shake for about 20 seconds. The LCS and LCSD are made up two 25 ml aliquots of reagent water added to two preserved sample collection vials. Before spiking the LCS and LCSD recap these vials and shake each for about 20 seconds. Then spike the LCS and LCSD with 150 µl of a Primary Stock solution 10 µg/ml Glyphosate standard. The MS/MSD are 25 ml aliquots of the designated batch QC sample spiked with 150 µl of a Primary Stock solution 10 µg/ml Glyphosate standard

10.4. For every batch prepared, make a “template” vial by measuring 25 ml of water into a vial, capping, and marking the meniscus.

10.5. The Laboratory Performance Check (LPC) is 25 µL of Primary stock solution into a 25 ml aliquot of laboratory reagent water (see 6.1) added to preserved sample collection vials. Recap and dissolve by shaking for about 20 seconds.

10.6. Two alternating Continuing Calibration Checks (CCC) consisting of 100 µL of Primary stock solution into a 25 ml aliquot of laboratory reagent water and the second CCC is 150 µL of the Primary stock solution into a 25 ml aliquot of laboratory reagent water (see 6.1.) added to preserved sample collection vials. Recap and dissolve by shaking for about 20 seconds.

10.7. Filter 1 ml of all QC and field samples with a 0.45 µm Leurlock filter into a 2 ml autosampler vial and cap.

10.8. Run on a HPLC equipped with a fluorescence detector and post column derivitization unit.

10.9. Dilutions:

10.9.1. 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 reagent water (see section 6.1.) and filtered prior to analysis (see 10.6.). Samples are diluted 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.

10.10. The samples may be stored up to 14 days in total from collection date if kept at 0-6º C (not frozen). Keep the samples in the original glass vials with PTFE lined caps.

10.11. PT Study:

10.11.1. 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.)

10.12.

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. First Order 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:

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

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

11.10. Assuming an original sample volume of 25 ml and an extract volume of 25 ml, equation 11.9. reduces to:

$$\mu g/_{L} = C_s * 1.0$$

11.11. Sample Concentration Adjustment for Varying Initial Volume and Dilutions:

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

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

11.12. Quality Control Calculations:

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

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

11.13. LPC Calculations:

11.13.1. An LPC standard is run at the beginning of each sample sequence prior to the analysis of samples to confirm sensitivity. The LPC is equivalent to a standard at or below the reporting limit. See the Drinking Water Certification Manual, section 7.2.12, SOP reference 13.5.

11.13.2. *Sensitivity*:

11.13.2.1. 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.

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

11.14. Sample chromatograms generated from the processing software have calculation formulas already incorporated into the report format (see equations 11.8, 11.9. and 11.10.). Manual adjustments are required for diluted samples, or samples of other than 25 ml only (see equation 11.11.). 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 547, 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
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 - RL's for Method 547

Parameter/Method	Analyte	Matrix (Water)	
		RL	Units
547	Glyphosate	13.0	µg/L

Table 14.1. 2 - Summary of Calibration and QC procedures for Method 547						
Method	**Applicable Parameter**	**QC check**	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**
EPA Method 547	Glyphosate	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 r ≥ 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 that would affect RTs	± 3 times standard deviation for each analyte retention time for standard analytical batch sequence	Correct problem then reanalyze all samples since the last retention time check	
		Retention time window	Must be done each analytical batch	First CCC of each sequence and the first CCC of subsequent 24-hour periods.		
		Calibration Verification (CCC)	Beginning each analysis sequence prior to the analysis of the samples, after every 10 samples or after 8 hours, whichever comes first, and at the end of the analysis	All analytes within ± 20% of expected values	Correct problem then repeat CCV 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

Table 14.1. 2 - Summary of Calibration and QC procedures for Method 547						
Method	**Applicable Parameter**	**QC check**	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**
EPA Method 547	Glyphosate	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	Default acceptance criteria (See **Appendix A, Table A.1).** . **See section 9.11 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	QC acceptance criteria **See Appendix A, Table A.1**	Flag report if recoveries are out of acceptable range	

Table 14.1. 2 - Summary of Calibration and QC procedures for Method 547						
Method	**Applicable Parameter**	**QC check**	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**
EPA Method 547	Glyphosate	LCS/LCSD for all analytes	One LCS/LCSD per batch	QC acceptance criteria **See 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	Same 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
		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	

Table 14.1. 2 - Summary of Calibration and QC procedures for Method 547						
Method	**Applicable Parameter**	**QC check**	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**
EPA Method 547	Glyphosate	Laboratory Performance Check	One standard at or below the reporting limit 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
		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. $547 – Analysis results

15.2. QC Test Codes

15.2.1. $B_547 – Extraction Blank Results

15.2.2. $LA547 – LCS/LCSD Spike Amount

15.2.3. $LS547 – LCS Results

15.2.4. $LD547 – LCSD Results

15.2.5. $LR547 – LCS Percent Recovery

15.2.6. $L2547 – LCSD Percent Recovery

15.2.7. $LP547 – LCS/LCSD Precision

15.2.8. $A_547 – MS/MSD Spike Amount

15.2.9. $S_547 – MS Results

15.2.10. $D_547 – MSD Results

15.2.11. $R_547 – MS Percent Recovery

15.2.12. $RD547 – MS Percent Recovery

15.2.13. $P_547 – MS/MSD Precision

15.2.14. $MA547 – MDL Spike Amount

15.2.15. $ML547 – MDL Results

15.2.16. INSTR-547 – Instrument associated with batch

Appendix A, Table A.1 – Quality Assurance Criteria for Method EPA 547

QC Type	Analyte	Accuracy (%R) LCL	UCL	Precision (RPD)
LCS/LCSD* MS/MSD*	Glyphosate	77	125	14%

*LCS/LCSD recovery and precision limits based on control charts of data collected from 12/31/2018 to 01/01/2021. EPA Method 547 requires MS recovery to be the same as that calculated for the LCS. The EPD Lab sets the recovery ranges for the LCSD and MSD to be the same as the range for the LCS. The EPD Lab sets the MS/MSD precision limits to be the same as the LCS/LCSD precision limits.

Updates:

Appendix A added. Updated for online revision.