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Endothall – EPA Method 548.1

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1. Scope and Application

1.1. Method 548.1 is used to determine the concentration of Endothall in drinking water. Samples are extracted with an anion exchange disk eluted with and acidic methanol and Methylene Chloride. The dimethyl ester of Endothall is formed by placing the extracted sample in a heating block at 50° C for one hour. After the addition of the salted reagent water, the ester partitions into methylene chloride. The extract is then to be analyzed by using a temperature programmable Gas Chromatographic (GC) instrument utilizing a Flame Ionization Detector (FID). Analyzing a standard curve under identical conditions used for sample extractions and comparing resultant retention times obtain an identification of the dimethyl ester of Endothall. Concentrations of the identified component are measured by relating the response produced for that compound to the standard curve response.

1.2. This method is restricted to analysts who have completed the requirements of the initial demonstration SOP. See 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. The glassware is rinsed again with extraction solvent, methylene chloride, immediately prior to use. The use of high purity reagents and solvents is absolutely necessary to minimize interference problems.

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

3.4. The major potential for interferences in the ion-exchange procedure are the naturally occurring ions in water, namely, dissolved calcium, magnesium, and sulfates. Sulfates can act as an effective counter ion and displaces Endothall from the column in high concentrations. Dissolved calcium and magnesium complex with the Endothall anion, resulting in Endothall no longer being available in the ionic form for the ion-exchange extraction. In the State of Georgia drinking water has shown very little concentrations of sulfates and magnesium. Therefore dissolved calcium tends to be the main ionic inference. The use of Ethylenediamine Tetraacetic Acid (EDTA) may be used to complex the cations to separate the effects of the ions on the recovery of Endothall.

Table 3.4. 1: Concentration of interfering ions and dilution factors for the use of EDTA

Interfering Ions in mg/L		Dilution Factor (initial to final volume ratio*)	Added EDTA ml (mg/100 ml final volume)
Sulfates	**Calcium/Magnesium**		
<250	<100	1:1	10 (186)
250-500	100-200	1:2	6.75 to 7.0 (126 to 130)
500-1250	200-400	1:5	4.0 (74.4)
>1250	>400	1:10	4.0 (74.4)

1:1 denotes undiluted sample.* ***Note**: Addition of 10 ml EDTA solution to 500 ml sample is insignificant in the final result.*

Note*: Dilutions may be necessary to obtain adequate results. Example: If a sample contained 800 mg/L of dissolved calcium, aliquot 10 ml of sample by graduated cylinder, add 4 ml EDTA solution (section 6.7.) and then bring to a volume of 100 ml with DI water (1:10 dilution factor).*

4. Safety

4.1. Refer to Georgia EPD Laboratory Chemical Hygiene Plan.

5. Apparatus and Equipment

5.1. Anion exchange, liquid-solid exchange disk.
5.2. Glass, Class A 250 mL graduated cylinders
5.3. Separatory funnels: 125 ml
5.3.1. Teflon stopcocks
5.3.2. Stoppers: Teflon or ground glass
5.4. Screw caps
5.5. Culture tubes; 25 ml
5.5.1. Culture tubes; 40 ml
5.6. Concentration tubes
5.7. TurboVap Concentrator
5.8. Pipets: Pasteur, disposable glass
5.9. Balance: analytical, capable of weighing 0.0001 g
5.10. Analytical concentrator; 6 or 8 position
5.11. Heating block
5.12. 2 ml amber autosampler vials and caps
5.13. Gas Chromatograph
5.13.1. Flame ionization detector
5.13.2. Column #1: 30 m, 0.53 mm, ID DB-1 or equivalent
5.13.3. Column #2: 30 m, 0.53 mm, ID DB-5 or equivalent
5.14. Perkin-Elmer Totalchrom or equivalent chromatography software
5.15. 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 [MΩ·cm] or greater @ 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. Methylene Chloride – High purity, demonstrated to be free from analytes and interferences (pesticide grade or better).
6.4. Sodium Sulfate, $NaSO_4$ – Suitable for Pesticide Residue Analysis, granular, anhydrous (Fisher brand or equivalent).
6.4.1. Sodium sulfate is baked at 450° C for four hours then returned to the original amber glass jar after cooling.
6.5. Sodium Thiosulfate - Reagent grade or equivalent: 40 mg per 500 ml amber glass bottle.
6.6. Disodium Ethylenediamine Tetraacetate (EDTA Disodium salt): 186 mg per sample
6.7. EDTA Solution:

6.7.1. Weigh 18.6 g into 1 L of reagent water (see section 6.1.). Adding 10 ml of EDTA solution is equal to 186 mg of EDTA per sample.

6.8. 10% Sulfuric acid in methanol:

6.8.1. Slowly and with stirring or careful swirling add 100 ml of concentrated sulfuric acid to 900 ml of HPLC or equivalent grade methanol to a 4 L bottle.

6.8.2. *The solution will become very hot and has a tendency to splash*.

6.9. Sodium hydroxide (NaOH) 1N:

6.9.1. Pour 90 ml of reagent water (see section 6.1.) into 10 ml of 10N NaOH to bring the final volume to 100 ml. This will make a 1N solution of NaOH.

6.9.1.1. Alternately, a commercially available 1N Sodium hydroxide solution may be used.

6.10. 10% sodium sulfate in water:

6.10.1. Dissolve 400 g of sodium sulfate in enough reagent water (see section 6.1.) to have a final volume of 4 L.

6.11. Standard Stock Solutions:

6.11.1. All standards that are made for the 548.1 analysis are to have a 6 month expiration date from the opening of the vendor stock ampule.

6.11.2. All Endothall standards are purchased in acid form and must be derivatized to dimethyl esters prior to analysis.

6.12. Primary Spiking Stock Solution:

6.12.1. 100 µg/ml made up from vendor stock at 1000 µg/ml.

Table 6.12.1. 1: 548.1 Primary Spiking Stock Solution in Methanol (1st Dilution)			
Compound	**Initial Concentration**	**Aliquot**	**Final Concentration**
Endothall	1000 µg/ml	1.0 ml	100 µg/ml
Total volume of Standard Aliquot		1.0 ml	
Addition of Methanol to Standard aliquots		9.0 ml	
Final Volume of Primary Stock #1		10.0 ml	

6.13. ICV Stock Solution:

6.13.1. 100 µg/ml is made up from Vendor Stock at 1000 µg/ml.

Table 6.13.1. 1: 548.1 ICV Stock Solution in Methanol (1st Dilution)			
Compound	**Initial Concentration**	**Aliquot**	**Final Concentration**
Endothall	1000 µg/ml	1.0 ml	100 µg/ml
Total volume of Standard Aliquot		1.0 ml	
Addition of Methanol to Standard aliquots		9.0 ml	
Final Volume of Primary Stock #2		10.0 ml	

7. Sample Collection

7.1. Drinking water samples for EPA Method 548.1 are collected in pre-certifed 500 ml amber glass bottles (to shield from light) with Teflon lined screw caps. Samples are preserved with 40 mg Sodium thiosulfate. 40 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. One 500 ml amber glass bottle is collected for every sample including matrix spikes and matrix spike duplicates.

7.2.1. Samples must be extracted within 7 days of collection and the extracted samples must be analyzed within 14 days.

7.2.2. Sample extracts must be stored in the dark at 0-6° C (not frozen) or less for a maximum of 14 days before analysis.

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 or quadratic 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.

8.2. Calibration Standards:

8.2.1. The Initial Calibration Verification standard (ICV) and the calibration curve points, which includes standards used for the Laboratory Performance Check (LPC), and Continuing Calibration Checks (CCCs) are prepared from the acid primary stocks (see 6.12. and 6.13.), then esterified.

8.2.1.1. Standards are derivitized to the methyl ester by the addition of primary stock aliquots of the volumes indicated in Table 8.2.2. 1. to a reaction mixture of 8 ml

of 10% H_2SO_4 in Methanol (see 6.8.) and 6 ml of Methylene chloride in screw cap culture tubes. The esterification is completed and the organic layer partitioned, collected and concentrated per sections 10.11. through 10.15. to a final volume of 1 ml.

8.2.2. The calibration standards have a final volume of 1 ml and are stored at 0-6° C (not frozen). The calibration standards have a usable shelf life of 14 days. The standards then should be discarded into the appropriate waste stream after the 14 day hold time. The calibration curve consists of calibration standards at the following concentrations (µg/ml):

Table 8.2.2. 1: Calibration Curve for Endothall in µg/mL or use µg/L when adjusted for the final extract concentration using calculation in section 11.9.1.

Name	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7
Endothall	1.50 µg/ml or 15.0 µg/L	3.00 µg/ml or 30.0 µg/L	4.00 µg/ml or 40.0 µg/L	5.00 µg/ml or 50.0 µg/L	8.00 µg/ml or 80.0 µg/L	10.0 µg/ml or 100 µg/L	15.0 µg/ml or 150 µg/L

Table 8.2.2. 2: Aliquots of Primary Spiking Stock Solution to make up all the levels in the above table.

Name	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7
Endothall	0.015 ml (or 15 µl)	0.030 ml (or 30 µl)	0.040 ml (or 40 µl)	0.050 ml (or 50 µl)	0.080 ml (or 80 µl)	0.100 ml (or 100 µl)	0.150 ml (or 150 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. The ICV is made up from the ICV Stock (Table 6.13. in the same manner and concentration as the Level 4 calibration standard.

8.3.2. A daily continuing calibration is performed every eight-hour analysis period (per EPA Method 548.1 section 10.4), or every 10 samples, whichever comes first, 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 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 30% of the initial calibration, i.e. %Drift ≤ 30% (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.

8.5.2. Two levels of calibration standards are alternated throughout the run. The first CCC is a mid-level CCC is made up of spiking 50 µL of the primary stock solution into a 14 mL's of eluent consisting of 8 mL of 10% sulfuric acid and methanol and 6 mL of methylene chloride.

8.5.3. The second CCC is another mid-level CCC is made up of spiking 80 µL of the primary stock solution into a 14 mL's of eluent consisting of 8 mL of 10% sulfuric acid and methanol and 6 mL of methylene chloride.

8.5.4. 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%. The LPC is made up of spiking 15 µL of the primary stock solution into a 14 mL's of eluent consisting of 8 mL of 10% sulfuric acid and methanol and 6 mL of methylene chloride

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 ≤ 30% 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 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 calculation 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 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 ± 30%. See calculation 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. 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 equation 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 equation 11.5 Results are in µg/ml.

8.11.5. The sample concentration is calculated per equation 11.9. in µg/L. Assuming a 100 ml initial sample volume and a 1 ml extract volume, equation 11.9. can be reduced to C_s multiplied by a factor of 100. 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 equation 11.10.

8.11.6. If an initial volume of other than 100 ml is used or a dilution of the extract 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), Table A.1, Appendix A 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 recovery are based on Section 9.3.2 of EPA Method 548.1. See SOP reference 13.1.

9.5.1.1. The method defines the LCS default range as R ± 20% (see EPA Method 548.1 section 9.6 and Table 2, reference 13.1.). R is concentration dependent. For 100 µg/L of Endothall in the LCS, R = 95. R ± 20% of R results gives a default range of 76% – 114% recovery. The LCS control limits are updated through the use of control charts. See SOP reference 13.3.

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

9.5.1.3. LCS/LCSD precision limit defaults are set by the EPD Laboratory as 0% - 40% RPD. LCS/LCSD precision limits are updated through the use of control charts. See reference 13.3.

9.5.1.4. In-house limits based on control charts may never exceed the default limits shown in Table 9.5.2. 1.

9.5.1.5. The method sets the MS recovery control limits to be the same as those of the LCS. See EPA Method 548.1 section 9.7, SOP reference 13.1.

9.5.1.6. 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.

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

9.5.2. 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, Table A.1. Those limits cannot exceed the default limits presented in Table 9.5.2. 1.

Table 9.5.2. 1: Default QC Limits for Method 548.1

QC Type	Analyte	Accuracy (%R) LCL	UCL	Precision (%RPD)
LCS/LCSD MS/MSD	Endothall	76 -	114	40

9.6. EPA Method 548.1 requires LCSs to be analyzed at a frequency rate of 5% of all samples (see EPA Method 548.1 Section 9.3).

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

9.8. For batches 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. EPA requires that the Laboratory perform a PT sample every 12 months to maintain certification in EPA method 548.1. 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):

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_548A. The MDLSpike result will be entered using the $ML548A. The MDL Spiked Amount will be entered into the test code $MA548A. The instrument used for the MDL and Blank analysis will be selected using the test code INSTR-548A.

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.

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. Form a batch consisting of a Blank, Laboratory Control Sample (LCS), Laboratory Control Sample Duplicate (LCSD), one or two Matrix Sample (MS) / Matrix Sample Duplicate (MSD) pairs (see section 9.7.), and up to 20 samples. For the Laboratory Blank, LCS and LCSD fill three 250 ml pre-preserved sample collection bottles with laboratory reagent water (see section 6.1.). From each sample collection bottle a 100 ml aliquot is measured into 250 ml glass, class A graduated cylinders. The LCS and LCSD are each spiked with 100 µl of a 100 µg/ml Endothall standard added to each aliquot. The MS and MSD are 100 ml aliquots for each using the designated batch QC sample spiked with 100 µl of a 100 µg/ml Endothall standard added to each aliquot that has been measured into 250 ml glass, class A graduated cylinders. For batches of 11 – 20 samples a second MS/MSD pair must also be prepared (see section 9.7.1.).

10.3. Take a 100 ml aliquot of each field sample measured into a 250 ml glass, class A graduated cylinder.

10.3.1. Most water samples tested in Georgia have very little hardness. Using 186 mg of EDTA treats samples with moderately high levels of hardness. This must be done prior to extraction by the addition of 10 ml of EDTA solution (section 6.7. to each field sample, the MS and the MSD. *Note*: *Blank, LCS/LCSD does not*

have to be treated with EDTA due to the lack of ionic interference from sulfates, dissolved calcium, and magnesium.

10.4. Set up the manifold glassware using an anion exchange disk.

10.5. 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 10.5.2. - 10.5.7. If the disk becomes dry while performing this series of steps, repeat steps 10.5.2. - 10.5.7.*

10.5.1. If, at any point during steps 10.5.2. - 10.5.7. it becomes necessary, an additional aliquot of solvent may be added to keep the disk wet. Up to 5 mls of extra solvent may be used per step for this purpose.

10.5.2. Add a 10 ml aliquot of methanol to the disk; pull methanol through disk until approximately 1 mm remains on the disk.

10.5.3. Add a 10 ml aliquot of reagent water (see section 6.1.) to the disk; pull water through the disk approximately until 1 mm remains on the disk.

10.5.4. Add a 10 ml aliquot of 10% sulfuric acid on methanol to the disk; pull acidic methanol through the disk until approximately 1 mm remains on the disk.

10.5.5. Add a 10 ml aliquot of reagent water to the disk; pull water through the disk approximately until 1 mm remains on the disk.

10.5.6. Add a 20 ml aliquot of 1N sodium hydroxide (NaOH) to the disk; pull NaOH through the disk approximately until 1 mm remains on the disk.

10.5.7. Add a 20 ml aliquot of reagent water to the disk; pull water through the disk approximately until 1 mm remains on the disk.

10.6. Add 100 ml sample (see 10.3.) to the manifold and pull the entire sample through the conditioned disk (section 10.5.) and allow disk to go dry.

10.7. Wash sample chamber with methanol so that no water is left on the side of the glass chamber and allow the disk to go dry for five minutes.

10.8. Position a collection tube or 40 mL vial inside of the manifold to collect the eluent.

10.9. Add an 8 ml aliquot of the 10% sulfuric acid and methanol. Allow the disk to go dry.

10.10. Add a 6 ml aliquot of methylene chloride. Allow the disk to go dry.

10.11. Remove the collection tube or 40 mL vial from the manifold and cap. Place the collection tube or 40 mL vial on a heating block for one hour at 50° C.

10.12. Remove the collection tubes or 40 mL vials from the heating block and allow to cool for 10 minutes.

10.13. Pour the contents of the culture tube or 40 mL vial into a 125 ml separatory funnel. Rinse the tube with two x 0.5 ml aliquots of methylene chloride and add the rinsing's to the separatory funnel. Add 20 ml of 10% sodium sulfate in reagent water to the separatory funnel. Shake the funnel three times vigorously, venting with the stopcock, and then shake vigorously for an additional 15 seconds. Swirl the separatory funnel a few times to get any remaining $MeCl_2$ off the upper sides of the separatory funnel.

10.13.1. After the phases have separated, drain the lower organic layer into a 25 ml screw cap culture vial.

10.13.2. Repeat the extraction procedure in section 10.13. with two additional 2 ml aliquots of methylene chloride, adding the organic phase to the culture tube each time.

10.14. Cap the collection vial that contains the extract.

10.14.1. Pour sample extracts into TurboVap tubes and concentrate to 1 ml at 45°C with nitrogen pressure at 3-4psi using the TurboVap concentrator.

10.15. GC vial: 1 ml final volume must be verified after concentration by comparison to a template vial. Prepare the template vial by measuring 1.0 ml of reagent water with a syringe and adding it to a clean vial. A new template vial must be made for each extracted batch.

10.16. Analyze samples on a GC instrument equipped with an FID.

10.17. Dilutions

10.18. 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, Methylene chloride (see section 6.3.) 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.

10.19. The sample extract may be stored up to 14 days if kept at 0-6º C (not frozen). Keep the extracts in amber glass autosampler vials with PTFE lined caps. Protect from light.

10.20. PT Study:

10.20.1. 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.)

11. Calculations

11.1. Response Factor, RF, for a peak:

$$RF = \frac{Area_{Analyte}}{Concentration_{Analyte}}$$

11.1.1. Where:
RF = Response Factor
Area $_{Analyte}$ = Area of the peak of the analyte of interest
Concentration $_{Analyte}$ = 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

11.10. Assuming an original sample volume of 100 ml and an extract volume of 1 ml, equation 11.9. reduces to:

$$\mu g/_{L} = C_s * 100$$

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 determine sensitivity. The LPC is a standard at or below the reporting limit. An LPC is not necessary for this method; however the EPD laboratory will run and process an LPC.

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 calculations 11.9. and 11.10.). Manual adjustments are required for diluted samples, or samples of other than 100 ml only (see calculation 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, SOP 6-015 (SOP reference 13.4.).

13. References

13.1. EPA/600/4-88-039 - EPA Method 548.1, Revision 1.0, 1992

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 Quality Assurance Plan, Rev. 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 548.1

Parameter/Method	Analyte	Matrix (Water)	
		RL	Unit
EPA 548.1	Endothall	19.8	µg/L

Table 14.1. 2 Summary of Calibration and QC procedures for Method 548.1

Method	Applicable Parameter	QC check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria
548.1	Endothall	7 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 7 point initial calibration	All analytes within ±30% of expected values	Correct problem then repeat initial calibration	
		Retention time window calculated for each analyte	Each initial calibration and calibration verifications	± 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 subsequent 24-hour periods.	Correct problem then reanalyze all samples since the last retention time check	

Table 14.1. 2 Summary of Calibration and QC procedures for Method 548.1						
Method	**Applicable Parameter**	**QC check**	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**
548.1	Endothall	Calibration Verification (CCC)	Beginning each analysis sequence prior to the analysis of the samples, after every 10 samples and at the end of the analysis	All analytes within ±30% 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	QC acceptance criteria **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	QC acceptance criteria **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	

Table 14.1. 2 Summary of Calibration and QC procedures for Method 548.1						
Method	**Applicable Parameter**	**QC check**	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**
548.1	Endothall	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 **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	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	

Table 14.1. 2 Summary of Calibration and QC procedures for Method 548.1						
Method	**Applicable Parameter**	**QC check**	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**
548.1	Endothall	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.	
		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

15. Associated Labworks Test Codes

15.1. Parent Test Code
15.1.1. $548A – Analysis results
15.2. Extraction Test Code
15.2.1. 548E – 100 mL aliquot – Solid Phase Extraction (SPE)
15.3. QC Test Codes
15.3.1. $B_548A – Extraction Blank Results
15.3.2. $LA548A – LCS/LCSD Spike Amount
15.3.3. $LS548A – LCS Results
15.3.4. $LD548A – LCSD Results
15.3.5. $LR548A – LCS Percent Recovery
15.3.6. $L2548A – LCSD Percent Recovery
15.3.7. $LP548A – LCS/LCSD Precision
15.3.8. $A_548A – MS/MSD Spike Amount
15.3.9. $S_548A – MS Results
15.3.10. $D_548A – MSD Results
15.3.11. $R_548A – MS Percent Recovery
15.3.12. $RD548A – MS Percent Recovery
15.3.13. $P_548A – MS/MSD Precision
15.3.14. $MA548A – MDL Spike Amount
15.3.15. $ML548A – MDL Results
15.3.16. INSTR-548A – Instrument associated with batch

Appendix A – Quality Assurance Criteria for Method EPA 548.1

Table A.1: Quality Assurance Criteria for Method 548.1

QC Type	Analyte	Accuracy (%R) LCL	Accuracy (%R) UCL	Precision (RPD)
LCS/LCSD* MS/MSD*	Endothall	76	114	27%

*LCS/LCSD recovery and precision limits based on control charts of data collected from 12/31/2018 to 01/01/2021. EPA Method 548.1 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.