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EDB and DBCP in Drinking Water – EPA Method 504.1

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1. Scope and Application

1.1. Method 504.1 is used to determine the concentrations of EDB and DBCP from Drinking water. Water samples are extracted with hexane. The extract is injected into a temperature programmable gas chromatograph (GC) with and electron capture detector (ECD). Identifications are obtained by analyzing a standard curve under identical conditions used for samples and comparing resultant retention times. Analytes are quantitated using procedural standard calibration.

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 at a level equal to that of a Laboratory Control Sample (LCS) or that of a point on the calibration curve.

3. Interferences

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

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

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

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

3.5. Matrix interferences such as Di-bromo-chloromethane (DBCM) may be caused by containments that are co-eluted from the sample. DBCM may co-elute with the EDB.

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, screw cap

5.3. Microsyringes

5.4. Pipets: Pasteur, disposable glass

5.5. Autopipette Dispenser: Capable of dispensing 2ml ± 0.04ml of an organic solvent

5.6. Detergent: Steris Labklenz or equivalent

5.7. Balance: analytical, capable of weighing 0.0001 g

5.8. Gas Chromatograph capable of temperature and flow programming, with a linearized electron capture detector (ECD), and a capillary column split/splitless injector

5.8.1. Capillary columns:

5.8.1.1. Column #1: 30m, 0.53mm, ID DB-624 or equivalent

5.8.1.2. Column #2: 30m, 0.53mm, ID DB-1 or equivalent

6. Reagents and Standards

6.1. Sodium chloride (NaCl) – ACS grade or equivalent

6.1.1. Sodium chloride is baked at 400° C for 4 hours and stored into an amber glass jar

6.2. Reagent Water – Purified water which does not contain any measurable quantities of target analytes or any compounds which interfere with the targets analytes. (Deionized, HPLC, Milli-Q water or equivalent). Milli-Q water has a resistivity of 18 MΩ·cm or greater @ 25° C, and a TOC of 50µg/L or less.

6.3. Hexane – High purity, demonstrated to be free from analytes and interferences (HPLC grade or better)

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

6.5. Sodium Thiosulfate – Reagent grade or equivalent. 3 mg per 40 ml vial.

6.6. Stock Standard Solutions: **All standards that are made for the 504.1 analysis are to have a 4 week expiration date from the opening of the vendor stock ampule.**

6.7. Primary Stock #1 Solution: 200 µg/ml made up from vendor stock Mix of EDB and DBCP at 2000 µg/ml.

Table 6.7. 1 – 504.1 Primary Stock #1 Solution in Methanol (1st Dilution)

Compound	**Initial Concentration**	**Aliquot**	**Final Concentration**
EDB	2000 µg/ml	1.0 ml	200 µg/ml
DBCP	2000 µg/ml		200 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		10.0ml	

6.7.1. Primary Stock #2 Solution: 20 µg/ml is made up from Primary Stock #1 (Section 6.7.) at 200 µg/ml.

Table 6.7.1. 1 – 504.1 Primary Stock #2 Solution in Methanol (2nd Dilution)

Compound	**Initial Concentration**	**Aliquot**	**Final Concentration**
EDB	200 µg/ml	1.0 ml	20 µg/ml
DBCP	200 µg/ml		20 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	

6.7.2. Primary Stock # 3 Solution: 2.0 µg/ml is made up from Primary Stock #2 (Section 6.7.1.) at 20 µg/ml.

Table 6.7.2. 1 – 504.1 Primary Stock #3 Solution in Methanol (3rd Dilution)

Compound	**Initial Concentration**	**Aliquot**	**Final Concentration**
EDB	20 µg/ml	1.0 ml	2.00 µg/ml
DBCP	20 µg/ml		2.00 µg/ml
Total volume of Standard Aliquot		1.0 ml	
Addition of Methanol to Standard aliquots		9.0 ml	
Final Volume of Primary Stock #3		10.0 ml	

6.7.3. Spiking Solution #1: Must be used for spiking LCS/LCSD/MS/MSD and Curve levels 3-6 (see Tables 8.3.2. 1 and 8.4. 1 for concentrations). 0.25 µg/ml is made up from Primary Stock #3 (Section 6.7.2.) at 2.0 µg/ml.

Table 6.7.3. 1 – 504.1 Spiking Solution #1 Solution in Methanol (4th Dilution)

Compound	**Initial Concentration**	**Aliquot**	**Final Concentration**
EDB	2.00 µg/ml	1.25 ml	0.25 µg/ml
DBCP	2.00 µg/ml		0.25 µg/ml
Total volume of Standard Aliquot		1.25 ml	
Addition of Methanol to Standard aliquots		8.75 ml	
Final Volume of Spiking Solution #1		10.0 ml	

6.7.4. Spiking Solution #2: Must be used for spiking curve levels 1 and 2 (see Tables 8.3.2. 1 and 8.4. 1 for concentrations). 0.025 µg/ml is made from Spiking Solution #1 (Section 6.7.3.) at 0.25 µg/ml.

Table 6.7.4. 1 – 504.1 Spiking Solution #2 Solution in Methanol (5th Dilution)

Compound	**Initial Concentration**	**Aliquot**	**Final Concentration**
EDB	0.25 µg/ml	1.0 ml	0.025 µg/ml
DBCP	0.25 µg/ml		0.025 µg/ml
Total volume of Standard Aliquot		1.0 ml	
Addition of Methanol to Standard aliquots		9.0 ml	
Final Volume of Spiking Solution #2		10.0 ml	

6.7.5. MDL spiking solution: Must be made from Spiking Solution #1 per EPA method.

Table 6.7.5. 1 – 504.1 MDL Stock #1 Solution in Methanol (6th Dilution)

Compound	**Initial Concentration**	**Aliquot**	**Final Concentration**
EDB	0.25 µg/ml	2.0 ml	0.02 µg/ml
DBCP	0.25 µg/ml		0.02 µg/ml
Total volume of Standard Aliquot		2.0 ml	
Addition of Methanol to Standard aliquots		23.0 ml	
Final Volume of Primary Stock #1		25.0 ml	

6.7.6. ICV Stock Solution #1: Must be made the same way as the Spiking Solution #1 (Section 6.7.3.). 20 µg/ml is made up from vendor stock Mix of EDB and DBCP at 200 µg/ml.

Table 6.7.6. 1 – 504.1 ICV Stock Solution #1Solution in Methanol (1st Dilution)

Compound	**Initial Concentration**	**Aliquot**	**Final Concentration**
EDB	200 µg/ml	1.0 ml	20 µg/ml
DBCP	200 µg/ml		20 µg/ml

Table 6.7.6. 1 – 504.1 ICV Stock Solution #1Solution in Methanol (1st Dilution)

Compound	Initial Concentration	Aliquot	Final Concentration
Total volume of Standard Aliquot		1.0 ml	
Addition of Methanol to Standard aliquots		9.0 ml	
Final Volume of ICV Stock Solution #1		10.0 ml	

6.7.7. ICV Stock Solution #2: 2.0 µg/ml is made up from ICV Stock Solution #1 (Section 6.7.6.) at 20 µg/ml.

Table 6.7.7. 1 – 504.1 ICV Stock Solution #2 Solution in Methanol (2nd Dilution)

Compound	Initial Concentration	Aliquot	Final Concentration
EDB	20 µg/ml	1.0 ml	2.0 µg/ml
DBCP	20 µg/ml		2.0 µg/ml
Total volume of Standard Aliquot		1.0 ml	
Addition of Methanol to Standard aliquots		9.0 ml	
Final Volume of ICV Stock Solution #2		10.0 ml	

6.7.8. ICV Spiking Solution: 0.25 µg/ml is made up from ICV Stock Solution #2 (Section 6.7.7.) at 2.00 µg/ml.

Table 6.7.8. 1 – 504.1 ICV Spiking Solution, Solution in Methanol (3rd Dilution)

Compound	Initial Concentration	Aliquot	Final Concentration
EDB	2.00 µg/ml	1.25 ml	0.25 µg/ml
DBCP	2.00 µg/ml		0.25 µg/ml
Total volume of Standard Aliquot		1.25 ml	
Addition of Methanol to Standard aliquots		8.75 ml	
Final Volume of ICV Spiking Solution		10.0 ml	

6.7.9. Surrogate Spiking Solution: 5.00 µg/ml is made up from vendor stock at 200 µg/ml.

Table 6.7.9. 1 – 504.1 Surrogate Spiking Stock #1 Solution in Methanol (1st Dilution)

Compound	Initial Concentration	Aliquot	Final Concentration
SS: 1,2,3 Tri-chloropropane	200 µg/ml	0.25 ml	5.00 µg/ml
Total volume of Standard Aliquot		0.25 ml	
Addition of Methanol to Standard aliquots		9.75 ml	
Final Volume of Surrogate Spiking Stock #1		10.0 ml	

7. Sample Collection

7.1. Drinking water samples for EPA Method 504.1 are collected in a clear pre-certified 40 ml glass vials with Teflon lined screw caps, and preserved with 3 mg of Sodium thiosulfate. The 3 mg of Sodium thiosulfate is sufficient to neutralize up to 5 mg/L (ppm) residual chlorine.

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 the residual chlorine concentration determined by the collector. If the residual chlorine measured by the collector is less than 5 ppm, the 3 mg of Sodium thiosulfate in the vial 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 to 0-6ºC (not frozen) after sample collection. Three vials are to be collected for every sample. Samples must be extracted within 14 days and the sequence with the extracts is begun within 24 hours and run continuously until completion. Extract vials should remain stored at 0-6ºC (not frozen) for as long as possible, only adding enough vials to the autosampler to allow for 4-6 hours of runs. When sequences run overnight, only enough vials should be added to allow the instrument to run until the analyst can add more early in the next shift.

8. Calibration

8.1. This method utilizes a procedural curve, i.e. standards are extracted in the same manner as samples.

8.1.1. Standards are extracted in vials that are pre-preserved with Sodium Thiosulfate. See Section 7.1.

8.2. Calibration Curve

8.2.1. 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.

8.3. Calibration Standards

8.3.1. The calibration curve consists of the calibration standards at the following concentrations (µg/ml)

8.3.2. Calibration levels as actual concentrations of standard solutions:

Table 8.3.2. 1 - Calibration Curve for EDB and DBCP (µg/mL)

Name	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6
EDB	0.000350	0.00125	0.00250	0.00375	0.00500	0.00625
DBCP	0.000350	0.00125	0.00250	0.00375	0.00500	0.00625

8.4. Calibration levels as sample true value concentrations (See calculation 11.9.) (assumes exact 35 ml sample volume):

Table 8.4. 1 - Calibration Curve for EDB and DBCP (µg/L)

Name	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6
EDB	0.0200	0.0714	0.143	0.214	0.286	0.357
DBCP	0.0200	0.0714	0.143	0.214	0.286	0.357

8.5. The table below shows the amount of Spiking Stocks required to make an extracted 2 ml of each calibration standard: (**NOTE**: All of the separate levels of the calibration curve are spiked into individual vials at 35 ml of reagent water and then must follow through the extraction procedure to a final volume of 2 ml in Hexane).

Table 8.5. 1 - Aliquots of Spiking Stock #1 and #2 to make up all the levels in the above table. Aliquots correspond to each level directly above each column.

Name	**Level 1** **(Spiking Solution #2)**	**Level 2** **(Spiking Solution #2)**	**Level 3** **(Spiking Solution #1)**	**Level 4** **(Spiking Solution #1)**	**Level 5** **(Spiking Solution #1)**	**Level 6** **(Spiking Solution #1)**
EDB/DBCP Mix	0.028 ml (or 28 µl)	0.100 ml (or 100 µl)	0.020 ml (or 20 µl)	0.030 ml (or 30 µl)	0.040 ml (or 40 µl)	0.050 ml (or 50 µl)
SS: 1,2,3 Trichloropropane	0.030 ml (or 30 µl)	0.030 ml (or 30 µl)	0.030 ml (or 30 µl)	0.030 ml (or 30 µl)	0.030 ml (or 30 µl)	0.030 ml (or 30 µl)

8.6. Calibration Verification

8.6.1. 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.

8.6.2. A daily continuing calibration is performed every twelve-hour analysis period to monitor and validate the instrumentation, column and detector performance. See section 8.8. for details.

8.7. Record Keeping

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

8.8. Daily Calibration Verification and Continuing Calibration

8.8.1. A Continuing Calibration standard (CCC) ensures the instruments target compound retention times and quantitation parameters meet method performance criteria. For any 12-hour analysis period, prior to sample analysis, a one-point daily continuing calibration verification is performed. Continuing calibration standards, including the initial daily CCC, 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, then the instrument must be recalibrated. Two levels of calibration standards are alternated throughout the run. A CCC is required after running the standard curve and initial calibration verification.

8.8.2. A Method Detection Limit (MDL) 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 60-140%.

8.9. Daily Retention Time Update

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

8.10. Average Response Factor Calibration

8.10.1. To evaluate the linearity of the initial calibration, calculate the Average Response Factor ($\overline{RF}$), 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.

8.11. First Order Linear Calibration using Least Squares Regression

8.11.1. Linearity through the origin is not assumed in a least squares fit. The instrument responses versus the concentration of the standards for the minimum 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.

8.11.2. 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.

8.12. Retention Time Windows

8.12.1. 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 of CCCs established over the approximate time period needed for a 20 sample analytical batch sequence. The CCCs (all levels analyzed) for an actual 20 sample batch (or smaller batch with sufficient injections afterwards (solvent blanks and CCCs) to simulate a 20 sample batch) may be used. See calculation 11.6.

8.13. Verification of Linear Calibrations

8.13.1. Calibration verification for linear calibrations involves the calculations of % Drift of the instrument response between the initial calibration and each subsequent analysis of the verification standard (CCC). The % Drift may be no more than ± 30%. See calculation 11.7.

8.14. Sample Concentration

8.14.1. Sample results are expressed in μg/L.

8.14.2. If an analyte response is calibrated by Average Response Factor, $\overline{\text{RF}}$, the chromatographic software calculates the concentration of the extract per calculation 11.8.

8.14.3. 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. .

8.14.4. 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.

8.14.5. The sample concentration is calculated per calculation 11.9. Assuming a 35 ml initial sample volume and a 2 ml extract volume, equation 11.9. can be reduced to C_s multiplied by a factor of 57.1. The chromatographic report uses this factor to multiply the result from either paragraph 8.14.2. , 8.14.3 or 8.14.4 above and calculates the final result per calculation 11.10. .

8.14.6. If an initial volume of other than 35 ml is used or a dilution of the extract is analyzed, the final sample result is multiplied by the factor determined with per calculation 11.11. .

8.15. Autopipette Dispenser

8.15.1. Every day that samples are extracted, prior to initiation of extraction, the volume dispensed by the autopipette dispenser used for Hexane is calibrated.

8.15.1.1. 2 ml of Hexane are dispensed into a 5 or 10 ml graduated cylinder. The volume is observed and correction(s) to the amount dispensed are made as necessary.

8.15.2. Record in appropriate log book.

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 and whenever significant maintenance (maintenance that could affect response or sensitivity) is performed. See reference 13.6.

9.3. A retention time study is performed once per year and whenever significant maintenance (maintenance that could affect retention time windows) is performed. See section 8.12. .

9.4. At least once per quarter, an ICV must be extracted, even if no initial calibration is performed during that quarter. See section 8.6.1.

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

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

9.7. Control Limits

9.7.1. Default LCS/LCSD control limits for recovery are based on Section 9.3.2 of EPA Method 504.1. MS/MSD control limits for recovery are static per Section 9.5 of EPA Method (See SOP reference 13.1. The EPD Laboratory has set Surrogate recovery as static per Table 9.7.1. 1. Precision limit defaults are set by the EPD Laboratory and are static. In-house limits based on control charts may never exceed the default limits. These control limits are presented to assist in defining control limits established with control charts and are not used as batch acceptance criteria.

Table 9.7.1. 1 - Default Limits Criteria for Method 504.1

QC Type	Analyte	Accuracy (%R) LCL		Accuracy (%R) UCL	Precision (RPD)
LCS/LCSD	EDB	70	-	130	20%
	DBCP	70	-	130	20%

Table 9.7.1. 1 - Default Limits Criteria for Method 504.1

QC Type	Analyte	Accuracy (%R) LCL UCL	Precision (RPD)
Surrogate	SS: 1,2,3 Tri-chloropropane	70 - 130 (3.00-5.56 µg/L)	
MS/MSD	EDB	65 - 135	20%
	DBCP	65 - 135	20%

9.7.2. EPA Method 504.1 requires LCSs be analyzed at a frequency of 10% of all samples over time (see EPA Method 504.1 section 9.3 – SOP reference 13.1) or a minimum of one LCS (and one LCSD) per batch.

9.7.2.1. LCSDs are used to meet the 10% requirement for Laboratory Fortified Blanks in EPA Method 504.1 section 9.3.

9.7.2.2. For this analysis, a CCC and an LCS (or LCSD) are considered to be the same if both are spiked at the LCS concentration defined in section 10.4. below.

9.7.2.3. Because LCSD samples are used to satisfy the above criteria, LCSD recovery must meet the same recovery criteria as an LCS in order for a batch to meet EPD Laboratory validation criteria.

9.7.3. Matrix Spike (MS) samples must be analyzed at a frequency of 5% of all samples over time or a minimum of once per batch (if a suitable sample is available).

9.7.4. If sample is available, a Matrix Spike Duplicate (MSD) must be analyzed with each MS analyzed.

9.8. <u>Method Detection Limit Study (MDL):</u>

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

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

9.8.3. 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.

9.8.4. 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.

9.9. <u>Initial MDL study:</u>

9.9.1. 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.

9.9.2. 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.

9.10. Continuous MDL study:

9.11. 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 9.9.2 for requirements.)

9.12. 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.

9.12.1. The results of the MDLBlank will be entered into Labworks using the Method Blank test code, $B_504A. The MDLSpike result will be entered using the $ML504A. The MDL Spiked Amount will be entered into the test code $MA504A. The instrument used for the MDL and Blank analysis will be selected using the test code INSTR-504A.

9.12.2. 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. The analyst determines samples to be analyzed by checking the Labworks Backlog Report for the method prior to selecting samples for analysis.

10.1.1. During review of the Backlog Report, the analyst reviews the residual chlorine reported by the sample collector. See section 7.1. .

10.1.2. Samples preserved with insufficient Sodium thiosulfate must be recollected. The analyst should inform the supervisor or manager of the situation as soon as possible.

10.2. 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.

10.3. The extraction is carried out in the original sample vial in which the drinking water is collected and shipped (This is due to the volatility of EDB and DBCP).

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. The blank is defined as 35 ml of laboratory DI or Milli-Q water. The LCS/LCSD are 35 ml of DI or Milli-Q water spiked with 35 µL of a Spiking Solution #1 0.25 µg/ml EDB/DBCP standard. The Blank, LCS and LCSD are prepared in pre-preserved vials containing the same amount of Sodium thiosulfate as regular samples (see section 7.1). The MS/MSD are 35 ml aliquots of the designated batch QC sample spiked with 35 µl of a Spiking Solution #1 0.25 µg/ml EDB/DBCP standard.

10.5. For every batch that is extracted, make a "template" vial by measuring 35 ml of water into a vial, capping and marking the meniscus.

10.6. Added 6 g of NaCl to all QC and field samples. Recap and dissolve by shaking for about 20 seconds.

10.7. Spike 30 µl of surrogate to all QC and samples.

10.8. Add 2 ml of Hexane to all QC and samples.

10.8.1. Hexane is dispensed from an autopipette dispenser or a 2 ml volumetric pipet.

10.8.2. The autopipette dispenser must be calibrated each day of use. See section 8.15 for procedure.

10.9. Shake for 2 minutes.

10.10. Transfer only the hexane layer to an autosampler vial and cap.

10.11. Run on a GC equipped with an ECD.

10.12. Dilutions

10.12.1. Upon analysis of the extract, if a target compound response is greater than that of the highest standard of the calibration curve, the sample must be diluted with the final extraction solvent (Hexane) so that, upon analyzing the dilution (in a valid analysis sequence), the target response is between the lowest concentration standard (or the reporting limit, whichever is higher) and the highest concentration standard.

10.13. Sample extracts may be stored up to 24 hours if kept at 4° C or less. Keep the extracts in 2 ml glass autosampler vials with PTFE lined caps.

11. Calculations

11.1. Response Factor, RF, for a peak

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

11.1.1. Where:

RF = Response Factor
$\mathrm{Area}_{\mathrm{Analyte}}$ = 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, $\overline{\text{RF}}$

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

11.2.1. Where:
$\overline{\text{RF}}$ = Average 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
$\overline{\text{RF}}$ = 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 (µg/ml)

$$\mathrm{Y} = \mathrm{ax} + \mathrm{b}$$

This rearranges to:
$\mathrm{x} = \mathrm{Y} - \mathrm{b}/\mathrm{a}$

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, $\overline{\mathrm{RT}}$

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

11.6.1. Where:
$\overline{\mathrm{RT}}$ = Mean retention time for the target compound
RT = Retention time for the target compound
n = Number of values

11.7. Percent Drift, %Drift

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

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

11.8. Extract Concentration Calculation from Response Factors (µg/mL)

$$\mathrm{^{\mu g}/_{mL}} = \frac{(\mathrm{A_s})}{(\overline{\mathrm{RF}})}$$

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 35 ml and an extract volume of 2 ml, equation 11.9. reduces to:

$$\mu g/L = C_s * 57.1$$

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

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

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

11.12.1. Where:
R_{spike} =% recovery of spiked sample
R_{sample} =% recovery of sample
$R_{duplicate}$ =% recovery of duplicate sample

11.13. MDL Standard Calculations

11.13.1. An MDL standard is run at the beginning of each sample sequence prior to the analysis of samples to determine sensitivity, chromatographic and column performance. The MDL is equivalent to the second lowest standard on the curve.

11.13.2. Sensitivity

11.13.2.1. Instrument sensitivity is determined by comparing the MDL recovery of all analytes. The recovery of the analytes must be ±40% of the true MDL value.

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

11.14. Sample chromatograms generated from the processing software have calculation formulas already incorporated into the report format (see calculations 11.8., 11.9. or 11.10.). Manual adjustments are required for diluted samples, or samples of other than 35 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, online revision.

13. References

13.1. EPA/600/4-88-039 - EPA Method 504.1, Revision 1.1, 1995

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 Reporting Limits for EPA Method 504.1

Parameter/Method	Analyte	Matrix (Water)	
		RL	Unit
EPA 504.1	EDB	0.022	µg/L
	DBCP	0.044	µg/L

Table 14.1. 2 Summary of Calibration and QC procedures for Method 504.1

Method	Applicable Parameter	QC check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria
504.1	EDB/DBCP	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≥ 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	Correct problem then reanalyze all samples since the last retention time check	
		Retention time window update	Each analytical sequence.	First CCC of each sequence and first CCC of each 24-hour period.		
		Quality Control Sample (QCS)	Quarterly ICV with DBCM	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 504.1

Method	Applicable Parameter	QC check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria
504.1	EDB/DBCP	Calibration Verification	Beginning each analysis sequence prior to the analysis of the samples, after every 10 sample sand at the end of the analysis	All analytes within ±30% of expected values	Correct problem then repeat CCC and reanalyze all samples since the last calibration verification	If out of range high, report with corrective action. If out of range low, rerun CCC and affected samples. If rerun passes, use data, else correct the problem, recalibrate and reanalyze all samples analyzed since the last successful CCC.
		IDC - Demonstrate the ability to generate acceptable accuracy and precision using 4 replicate analyses of the QC check sample. Analyst must also produce a passing MDL study with 7 MDL spikes and 7 MDL blanks.	One per analyst	QC acceptance criteria **See Appendix A Table A.1. See section 9.8 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 or 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	

Table 14.1. 2 Summary of Calibration and QC procedures for Method 504.1

Method	Applicable Parameter	QC check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria
504.1	EDB/DBCP	MDL check	One per analytical batch	All analytes within 60% - 140% of expected values	Correct problem then repeat MDL.	If out of range high, report with corrective action. If out of range low, rerun MDL and affected samples. If rerun passes, use data, else correct the problem, reanalyze the MDL, and all samples analyzed since the last successful CCC.
		Method blank	One per analytical batch	No analytes detected >RL	Correct problem then re-prep 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	
		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.	
		Second column confirmation	100% for all positive results	Same as for primary column analysis	Sample as for primary column analysis if used for quantitation	

Table 14.1. 2 Summary of Calibration and QC procedures for Method 504.1

Method	Applicable Parameter	QC check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria
504.1	EDB/DBCP	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 with DBCM	Once per Quarter	All analytes within ± 30% of expected value and DBCM and EDB should be separated.	Correct problem then repeat initial calibration	
		Residual Chlorine check	Whenever needed. If collector does not check residual chlorine.	Must be checked for every sample.	Check residual chlorine levels and add information to extraction sheet.	

15. Associated Labworks Test Codes

15.1. Parent Test Code
15.1.1. $504A – Analysis results
15.2. Extraction Test Code
15.2.1. 504E – 40 mL vial – Liquid/ Liquid extraction
15.3. QC Test Codes
15.3.1. $B_504A – Extraction Blank Results
15.3.2. $LA504A – LCS/LCSD Spike Amount
15.3.3. $LS504A – LCS Results
15.3.4. $LD504A – LCSD Results
15.3.5. $LR504A – LCS Percent Recovery
15.3.6. $L2504A – LCSD Percent Recovery
15.3.7. $LP504A – LCS/LCSD Precision
15.3.8. $A_504A – MS/MSD Spike Amount
15.3.9. $S_504A – MS Results
15.3.10. $D_504A – MSD Results
15.3.11. $R_504A – MS Percent Recovery
15.3.12. $RD504A – MS Percent Recovery
15.3.13. $P_504A – MS/MSD Precision
15.3.14. $MA504A – MDL Spike Amount
15.3.15. $ML504A – MDL Results
15.3.16. INSTR-504A – Instrument associated with batch

Appendix A, Table A.1 – Quality Assurance Criteria for Method EPA 504.1

QC Type	Analyte	Accuracy (%R) LCL		Accuracy (%R) UCL	Precision (%RPD)
LCS/LCSD*					
	EDB	85	-	119	10
	DBCP	79	-	120	10
Surrogate**					
	SS: 1,2,3 Trichloropropane (1,2,3 TCP)	70	-	130	NA
	1,2,3 TCP (as ug/L)	3.00	-	5.56	NA
MS/MSD**					
	EDB	65	-	135	20
	DBCP	65	-	135	20

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

**Method 504.1 specifies 65 – 135% recovery limits for matrix spike and matrix spike duplicate analytes. The EPD Lab sets a static range of 0 – 20% for matrix spike/matrix spike duplicate precision. The method also sets static control limits of 70% - 130% for the surrogate.

Updates:

Updated to online revision.