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N-Methyl Carbamates and Oxime Compounds – EPA Method 531.2

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1. Scope and Application

1.1. Method 531.2 is used to determine the concentrations of various n-methyl carbamate and oxime compounds in drinking water. Samples are hydrolyzed with sodium hydroxide to methyl amine which reacts with Thiofluor to form isoindole. The analysis occurs on a high pressure liquid chromatograph equipped with post-column derivitization and fluorescence detector. Identifications are obtained by analyzing a standard curve under identical condition used for samples and comparing resultant retention times. Concentrations of the identified components 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 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.

2.4.1. 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 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. 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 amber glass with Teflon-lined screw caps.

5.2. Vials: autosampler, screw cap

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 or equivalent

5.8. Perkin-Elmer Totalchrom chromatographic software or equivalent

5.9. Column #1: Carbamate Analysis Column, 5 micron, 20 A, 4.6 mm ID X250 mm long, ODS (C-18) or equivalent

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

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

5.12. Leurlock filters: Millipore 0.22 µm PVDF membrane, Cat. #: SLGVX13NL (pack of 100), SLGVX13NK (pack of 1000) or equivalent.

5.13. Balance: analytical, capable of weighing 0.0001 g

5.14. EMD colorpHast pH Paper 2.5 - 4.5 range or similar
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. Sodium Thiosulfate: Reagent grade or equivalent. 4 mg per 40 ml vial.
6.4. Sodium hydroxide-Hydrolysis Reagent (Pickering Labs CB130):0.05 N, pH = 13 degassed with Helium before use.
6.5. O-Pthaladehyde (OPA) Diluent (Pickering Labs CB910): 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. Potassium dihydrogen citrate. Added to the samples to make the pH ~ 3.8. Biocide
6.10. Preserved Reagent Water:
6.10.1. To a 1 L volumetric flask, add 9.2 g Potassium dihydrogen citrate and 0.320 g Sodium thiosulfate to 500 ml DI water, swirl to dissolve. Bring to volume with DI water.
6.11. Stock Standard Solutions: All standards that are made for the Method 531.2 analysis are to have a 6 month expiration date from the opening of the vendor stock ampule. (Note: All stock standards, including Primary stock #1, Primary Surrogate Stock, and Primary ICV stock must be stored at a temperature of -10°C or less.)
6.12. Primary Stock #1 Solution: 10 µg/ml made up from vendor stock Mix of Carbamate mix at 100 µg/ml:

Table 6.12. 1 – 531.2 Primary Stock #1 Solution in Methanol (1st Dilution)

Compound	Initial Concentration (µg/ml)	Aliquot (ml)	Final Conc. (µg/ml)
Aldicarb Sulfoxide	100	1.0	10
Aldicarb Sulfone	100		10
Oxamyl	100		10
Methomyl	100		10
Aldicarb	100		10
Carbofuran	100		10

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. Primary Surrogate Stock Solution: 10 µg/ml is made up from Vendor stock at 100 µg/ml:

Table 6.13. 1 – 531.2 Primary Surrogate Stock Solution in Methanol (1st Dilution)

Compound	Initial Concentration (µg/ml)	Aliquot (ml)	Final Conc. (µg/ml)
BDMC	1000	1.0	100

Total volume of Standard Aliquot	1.0 ml
Addition of Methanol to Standard aliquots	9.0 ml
Final Volume of Primary Surrogate Stock	10.0 ml

6.14. Primary ICV Stock Solution: 10 µg/ml made up from vendor stock Mix of Carbamate mix at 100 µg/ml:

Table 6.14. 1 – 531.2 Primary ICV Stock Solution in Methanol (1st Dilution)

Compound	Initial Concentration (µg/ml)	Aliquot (ml)	Final Conc. (µg/ml)
Aldicarb Sulfoxide	100	1.0	10
Aldicarb Sulfone	100		10
Oxamyl	100		10

Table 6.14. 1 – 531.2 Primary ICV Stock Solution in Methanol (1st Dilution)			
Compound	**Initial Concentration (µg/ml)**	**Aliquot (ml)**	**Final Conc. (µg/ml)**
Methomyl	100		10
Aldicarb	100		10
Carbofuran	100		10

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

7. Sample Collection

7.1. Drinking water samples for Method 531.2 are collected in a pre-certified 40 ml amber 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 preserved with 368 mg of Potassium dihydrogen citrate to buffer the pH to approximately 3.8

7.1.2.1. Prior to extraction, the analyst checks the pH with pH paper (see section 5.14.). Indicated pH should be with the 3.6 – 3.9 range of this paper.

7.1.2.2. If the pH of a sample is in this range, the analyst puts a check mark in the pH column for that sample on the extraction sheet.

7.1.2.3. If the sample fails the pH test, it must be recollected.

7.1.3. Samples are cooled to 6°C or less (not frozen) after sample collection. Three vials are to be collected for every sample. Samples must be extracted within 28 days and stored at 0-6º C (not frozen) until validated.

8. Calibration

8.1. Calibration Curve

8.1.1. A six-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. Standards are made up to 25 ml with preserved reagent water (see 6.10.) and stored at 6℃ or less (not frozen). Prior to use, standards are filtered through Leurlock filters (see 5.12.) into autosampler vials and capped. The calibration curve consists of the calibration standards at the following concentrations (µg/ml):

Table 8.2.1. 1: Calibration Curve for Carbamates in 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
Aldicarb Sulfoxide	0.0016	0.006	0.008	0.010	0.016	0.020
Aldicarb Sulfone	0.0016	0.006	0.008	0.010	0.016	0.020
Oxamyl	0.0016	0.006	0.008	0.010	0.016	0.020
Methomyl	0.0016	0.006	0.008	0.010	0.016	0.020
Aldicarb	0.0016	0.006	0.008	0.010	0.016	0.020
Carbofuran	0.0016	0.006	0.008	0.010	0.016	0.020
BDMC - SS	0.04	0.04	0.04	0.04	0.04	0.04

Table 8.2.1. 2: Calibration Curve for Carbamates in 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
Aldicarb Sulfoxide	1.6	6.0	8.0	10	16	20
Aldicarb Sulfone	1.6	6.0	8.0	10	16	20
Oxamyl	1.6	6.0	8.0	10	16	20
Methomyl	1.6	6.0	8.0	10	16	20
Aldicarb	1.6	6.0	8.0	10	16	20
Carbofuran	1.6	6.0	8.0	10	16	20
BDMC - SS	40	40	40	40	40	40

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
Aldicarb Sulfoxide	4 µl (or 0.004 ml)	15 µl (or 0.015 ml)	20 µl (or 0.010 ml)	25 µl (or 0.025 ml)	40 µl (or 0.040 ml)	50 µl (or 0.050 ml)
Aldicarb Sulfone						
Oxamyl						
Methomyl						
Aldicarb						
Carbofuran						
BDMC - SS	10 µl (or 0.010 ml)	10 µl (or 0.010 ml)	10 µl (or 0.010 ml)	10 µl (or 0.010 ml)	10 µl (or 0.010 ml)	10 µl (or 0.010 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. The calibration must be confirmed by reprocessing all of the initial calibration standards using the newly created calibration file. When quantitated using the new curve, each calibration point, except the lowest point, for each analyte should calculate to be 70-130% of its true value. The lowest calibration point should calculate to be 50-150% of its true value.

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

8.4. Record Keeping

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

8.5. Daily Calibration Verification and Continuing Calibration:

8.5.1. A continuing calibration standard (CCC) ensures the instruments target compound retention times and quantitation parameters meet method performance criteria. For any analysis period, prior to sample analysis, a one-point daily continuing calibration verification is performed. After initial calibration verification is performed a continuing calibration check (CCC) will be ran every tenth sample and should alternate between medium and high concentrations and must end the analysis batch.

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, rerun once. If it fails the second time, then the instrument must be recalibrated. Two levels of calibration standards are alternated throughout the run.

8.5.2. **Note: CCCs and all calibration and verification standards must be filtered. See 8.2.1.**

8.5.3. A Laboratory Performance Check (LPC) standard must be run at the beginning of every batch sequence. This standard must be at a concentration at or below the RL and must have a recovery of 50 – 150%.

8.5.4. A resolution check must be performed at the beginning of every batch sequence. A mid-level CCC is run and the resolution of the two closest eluting peaks, typically Baygon and Carbofuran is calculated. Calculation 11.14. must result in a value equal to or greater than 1.0.

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 6

points are evaluated using the instrument data analysis software. The regression will produce the slope and intercept terms for a linear equation. The regression calculation will regenerate a correlation, r, a measure of goodness of fit of the regression line to the data. A value of 1.0 is a perfect fit. An acceptable correlation of coefficient r should be ≥ 0.990 (or $r^2 \geq 0.980$). See calculation 11.4.

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

8.9. Retention Time Windows:

8.9.1. The width of the retention time window for each analyte, 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 in µg/ml per, calculation 11.2.

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

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

8.11.5. The sample concentration is calculated in µg/L per, calculation 11.9. Assuming a 25 ml initial sample volume and a 25 ml extract volume, calculation 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 paragraph 8.11.2., 8.11.3. or 8.11.4 above and calculates the final result per calculation 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 calculation 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. Method 531.2 does not establish criteria for LCS/LCSD recovery as LCS and LCSD samples are prepared exactly as are CCCs. Default LCS/LCSD control limits for recoveries are based on CCC limits found in Section 8.5.1. and are updated through the use of control charts. See reference 13.3. MS/MSD control limits for recovery are static per Section 9.8.3 of Method 531.2. See SOP reference 13.1. Method 531.2 sets static Surrogate recovery limits. See Method 531.2 Section 9.7.1. LCS/LCSD Precision limit defaults are set by the EPD Laboratory and are modified through the use of control charts. See reference 13.3. MS/MSD Precision limit defaults are set by EPA 531.2. See Method 531.2 Section 9.9.3. In-house limits based on control charts may never exceed the default limits. These default control limits 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. 1:

Table 9.5. 1 Default Limits Criteria for Method 531.2

QC Type	Analyte	Accuracy (%R) LCL	UCL	Precision (RPD) %
LCS/LCSD	Aldicarb Sulfoxide	70	130	30
	Aldicarb Sulfone	70	130	30
	Oxamyl	70	130	30
	Methomyl	70	130	30
LCS/LCSD	Aldicarb	70	130	30
	Carbofuran	70	130	30
Surrogate	BDMC	70 28	130 52 µg/L	N/A
MS/MSD	All analytes	70	130	30

9.6. EPA Method 531.2 requires LCS to be analyzed at a frequency rate of 5% of all samples or one per analytical batch (see EPA Method 531.2, Section 9.6). The LCSD is used to satisfy precision requirements. For this analysis, a CCC is considered the same as the LCS if spiked at the same level as the LCS.

9.7. Matrix Spike (MS/MSD) is to be analyzed at a frequency of 5% of all samples.

9.8. Performance Test (PT) Sample:

9.8.1. EPA requires that the Laboratory perform a PT sample every 12 months to maintain certification in EPA method 531.2. Those PT result must fall within acceptable control limits for the PT testing facility. If those results are not within acceptable control limits the Laboratory will have a second chance to pass the PT study within the same 12 months of the study. If the results did not fall within acceptable control limits for the study over the 12-month testing period, the laboratory will be downgraded for those compounds listed in this SOP. With the failure of this nature the laboratory must notify all drinking water facilities within 30 days of the failure after the 12-month period has passed. It is not until the laboratory passes a PT study will the laboratory be able to test for those compounds of interest again.

9.9. Method Detection Limit Study (MDL):

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

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

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

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

9.10. Initial MDL study:

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

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

analysis day between each of the 3 days. A total of 14 vials are prepared, 7 spiked and 7 blanks.

9.11. Continuous MDL study:

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

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

9.11.3. The results of the MDLBlank will be entered into Labworks using the Method Blank test code, $B_531C. The MDLSpike result will be entered using the $ML531C. The MDL Spiked Amount will be entered into the test code $MA531C. The instrument used for the MDL and Blank analysis will be selected using the test code INSTR-531C.

9.12. 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 extraction 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 blank is defined as 25 ml of laboratory preserved reagent water (see 6.10.). The LCS/LCSD are made up 25 ml of preserved reagent water spiked with 40 µl of a Primary Stock solution 10 µg/ml Carbamate standard. The MS/MSD are 25 ml aliquots of the designated batch QC sample spiked with 40 µl of a Primary Stock solution 10 µg/ml Carbamate standard

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

10.5. Samples are adjusted to 25 ml by removing an appropriate amount (segregating a small portion in the cap for final volume adjustments) and comparing the remaining volume to the "template." Adjust the final volume as needed to achieve 25 ml of sample in the sample vial.

10.6. Add 4 mg of Sodium Thiosulfate to LPC, CCC's, Blank, LCS, and the LCSD. Recap and dissolve by shaking for about 20 seconds.

10.7. Spike 10.0 µl of 100 µg/ml surrogate to all QC and samples.

10.8. Filter and collect 1.5 ml of all CCCs, ICVs, QC and field samples with a 0.22 µm Leurlock filter specified in section 5.12. Filter into a 2 ml autosampler vial and cap the vial

10.9. Run on a HPLC equipped with a fluorescence detector and post column derivitization unit. (see section 10.13-10.15 for HPLC and post column settings)

10.10. Dilutions:

10.10.1. 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 Preserved Reagent Water (see section 6.10.) and filtered as per sections 10.7. through 10.10. Dilutions must be made 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.

10.11. The sample extract may be stored up to 28 days in total from collection date if kept at 0-6ºC (not frozen). Keep the extracts in the original amber glass vials with PTFE lined caps.

10.12. PT Study:

10.12.1. 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.)

10.13. HPLC Parameters:

10.13.1. Pump Parameters: (PE series 200 is a quaternary pump, but for this method it will only use a two of the four micro pumps.)

Injection volume: 100.00 µL. All sample will proceed through the following instrument parameters.							
Step	Time	Flow: mL/min	Pump A: % H2O	Pump B: % MeOH	Pump C:	Pump D:	Curve:
0	5.00	0.70	90.0	10.0	0.00	0.00	0.0
1	50.0	0.70	30.0	70.0	0.00	0.00	1.0
2	2.00	0.70	30.0	70.0	0.00	0.00	0.0
3	3.00	0.70	10.0	90.0	0.00	0.00	0.0
4	12.0	0.70	10.0	90.0	0.00	0.00	0.0

(Note: Due to ever changing technologies, it may be necessary to change pump parameters to suit new instrumentation.)

10.14. Pickering Pinnacle PCX Parameters:

10.15. Column temp: 42℃. Reactor temp: 100℃. Reactor volume: 0.5 mL. Run time: 59 minutes. Equilibration time: 5 minutes.

10.15.1. Pump #1: Hydrolysis Solution:

10.15.1.1. Pump rate: 0.3 mL/min

10.15.2. Pump #2: OPA solution:

10.15.2.1. Pump rate: 0.3 mL/min

(Note: Due to ever changing technologies, it may be necessary to change pump parameters to suit new instrumentation.)

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 $_{Analyte}$ = 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. 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:

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

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

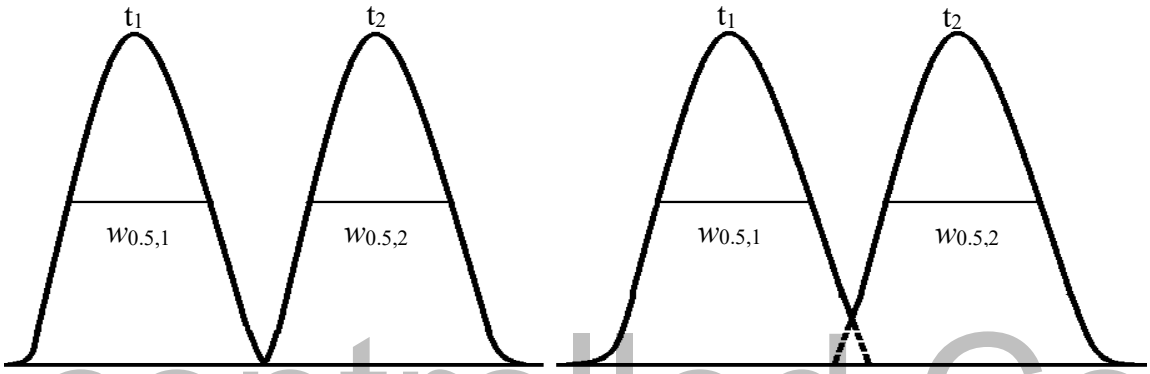
11.13. LPC Calculations:

11.13.1. An LPC standard is run at the beginning of each sample sequence prior to the analysis of samples to determine verify sensitivity. The LPC is equivalent to the low standard on the curve.

11.13.2. Instrument sensitivity is determined by checking the low level response of the compounds in the LPC. Recovery of all compounds must be 50% - 150% of the expected value.

11.14. Resolution Check (Column Performance):

11.14.1. Column performance is determined by calculating the resolution factor between the two closest eluting peaks, typically Baygon and Carbofuran, in the mid-level CCC.



Column Performance - Peak Resolution for Resolved Peaks **Column Performance - Peak Resolution for Overlapping Peaks**

11.14.2. Resolution between two peaks is determined by dividing the difference in the peak apex retention times by the average of the widths of the two peaks at the baseline. If the peaks overlap as in the second example above, estimate the peak widths as shown.

$$R_s = \frac{1.18(t_2 - t_1)}{W_{0.5,1} + W_{0.5,2}} \geq 1.0$$

11.14.3. Where:

R_s=Resolution between two peaks

t_2 = Retention time of Peak 2

t_1 = Retention time of peak 1

$W_{0.5,1}$ = Peak width of first peak at half the height of peak

$W_{0.5,2}$ = Peak width of second peak at half the height of the peak

11.14.4. R_s must be equal to or greater than 1.0.

11.15. Sample chromatograms generated from the processing software have calculation formulas already incorporated into the report format (see sections 11.9. and 11.10.). Manual adjustments are required for diluted

samples, or samples of other than 25 ml only (see section 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. Resolution checks must be measured with a ruler or other straight edge to mark time values on the chromatograms and calculated manually.

12. Waste Management

12.1. See GA EPD Laboratory SOP-EPD Laboratory Waste Management Standard Operating Procedures (reference 13.4.).

13. References

13.1. EPA/600/4-88-039 - EPA Method 531.2, Revision 1.0, 2001

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

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

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

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

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

13.7. GA EPD 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 531.2

Parameter/Method	Analyte	Matrix (Water)	
		RL	Units
EPA 531.2 N-Methyl Carbamate And Oxime Compounds	Aldicarb Sulfoxide	4.4	µg/L
	Aldicarb Sulfone	3.2	µg/L
	Oxamyl	2.4	µg/L
	Methomyl	2.9	µg/L
	Aldicarb	2.9	µg/L
	Carbofuran	1.9	µg/L

Table 14.1. 2 Summary of Calibration and QC procedures for Method 531.2

Method	Applicable Parameter	QC check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria
EPA 531.2	N-Methyl Carbamate and Oxime compounds	6 point initial calibration for all analytes	Initial calibration prior to sample analysis	Linear mean RSD for all analytes ≤ 30% 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	

Table 14.1. 2 Summary of Calibration and QC procedures for Method 531.2						
Method	**Applicable Parameter**	**QC check**	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**
EPA 531.2	N-Methyl Carbamate and Oxime compounds	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 update	Must be done every analytical sequence	First CCC of each sequence and the first CCC of subsequent 24-hour periods.		
		Calibration Verification (CCC)	CCC must be ran at the beginning of each analysis sequence prior to the analysis of the samples. After the initial CCC is ran a CCC must be ran after every 10 sample and one 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	Default acceptance criteria **(See Table 9.5. 1 for IDC) Plus,** in addition, the average recovery of the 4 replicates must be within ± 20% of the expected value for an IDC. The **Precision** (%RSD) for the 4 replicates must <20%. . **See section 9.9 for MDL requirements**	Recalculate results; locate and fix problem with system and then rerun demonstration for those analytes that did not meet criteria	

Table 14.1. 2 Summary of Calibration and QC procedures for Method 531.2

Method	Applicable Parameter	QC check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria
EPA 531.2	N-Methyl Carbamate and Oxime compounds	CDC – Continuing Demonstration of Capability	Required Every Six Months after IDC for each analyst	**See Appendix A, Table A.1** for acceptance criteria	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	
		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

Table 14.1. 2 Summary of Calibration and QC procedures for Method 531.2

Method	Applicable Parameter	QC check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria
EPA 531.2	N-Methyl Carbamate and Oxime compounds	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	
		Laboratory Performance Check (LPC)	One at the beginning of each analysis sequence prior to the analysis of samples	All analytes within ± 50% of the expected value	Correct the 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.	
		Resolution Check	Mid-level CCC run at the beginning of each analysis sequence prior to the analysis of samples	R_s in calculation 11.14. must be > 1.0 for the two closest eluting analytes	Correct the problem. Repeat initial calibration. Repeat retention time study if appropriate	

15. Associated Labworks Test Codes

15.1. Parent Test Code
15.1.1. $531C – Analysis results
15.2. Extraction Test Code
15.2.1. 531E - 40 mL amber glass vial
15.3. QC Test Codes
15.3.1. $B_531C – Extraction Blank Results
15.3.2. $LA531C – LCS/LCSD Spike Amount
15.3.3. $LS531C – LCS Results
15.3.4. $LD531C – LCSD Results
15.3.5. $LR531C – LCS Percent Recovery
15.3.6. $L2531C – LCSD Percent Recovery
15.3.7. $LP531C – LCS/LCSD Precision
15.3.8. $A_531C – MS/MSD Spike Amount
15.3.9. $S_531C – MS Results
15.3.10. $D_531C – MSD Results
15.3.11. $R_531C – MS Percent Recovery
15.3.12. $RD531C – MS Percent Recovery
15.3.13. $P_531C – MS/MSD Precision
15.3.14. $MA531C – MDL Spike Amount
15.3.15. $ML531C – MDL Results
15.3.16. INSTR-531C – Instrument associated with batch

Appendix A Table A.1 – Quality Assurance Criteria for Method EPA 531.2

QC Type	Analyte	Accuracy (%R) LCL	Accuracy (%R) UCL	Precision (%RPD)
LCS/LCSD*				
	Aldicarb Sulfoxide	77	124	15
	Aldicarb Sulfone	80	118	15
	Oxamyl	79	123	15
	Methomyl	79	120	15
	Aldicarb	74	126	15

	Carbofuran	70 - 130	17
Surrogate**			
	SS: BDMC	70 - 130	NA
	BDMC (as µg/L)	28 - 52	NA
MS/MSD**			
	All Analytes	70 - 130	30

*LCS/LCSD recovery and LCS/LCSD precision limits based on control charts of data collected from 12/31/2018 to 01/01/2021.
**Method 531.2 specifies 70 – 130% static recovery limits for surrogate and matrix spike and matrix spike duplicate analytes. The EPD Lab sets a static range of 0 – 30% RPD for matrix spike/matrix spike duplicate precision.

Updates:

Appendix A added.

Updated for online revision.