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EPA Method 353.2- Nitrate/Nitrite Nitrogen in Water

Access to this SOP shall be available within the laboratory for reference purposes; the official copy of this SOP resides on the official Georgia EPD website at https://epd.georgia.gov/about-us/epd-laboratory-operations. Printed copies of this SOP will contain a watermark indicating the copy is an uncontrolled copy.

1. Scope and Application

1.1. The purpose of this method is to determine nitrate/nitrite in drinking water, surface waters, and industrial wastewaters. Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) is then determined by diazotizing with sulfanilamide followed by coupling with N- (1-naphthyl) ethylenediamine dihydrochloride. The resulting water-soluble dye has a magenta color, which is read at 520 nm. Nitrite alone also can be determined by isolating the cadmium column. The applicable range of 0.05 to 5.0 mg/L nitrate-nitrite nitrogen from the published methods has been modified to a range of 0.02 to 2.0 mg/L by the instrument manufacturer. The cadmium column used is prepacked. Tests covered by this SOP are NITNAT and NO3NO2 (as identified by the Labworks test codes). This procedure is modified using Lachat QuikChem Method 10-107-04-1-C. Volumes of standards and reagents may be changed, provided the quality control and performance requirements stated in this SOP are met.

1.2. Restricted Procedure:

1.2.1. This procedure is restricted to use by an analyst experienced in the operation of a Lachat Quikchem (FIA) System. Additionally, the analyst must complete the requirements of the GAEPD Initial Demonstration of Analyst Proficiency prior to the analysis of actual samples. Analysts are further warned that performance of this analysis involves the use of potentially hazardous chemicals; refer to the GAEPD Chemical Hygiene Plan (see reference 13.9.) for additional information regarding chemicals

required by this method.

2. Definitions

2.1. Refer to Chapter 3 of the Georgia EPD Laboratory Quality Assurance Plan (see reference 13.3.) 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 a 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 source calibration curve. The ICV is run at a level equal to that of a Laboratory Control Sample (LCS) or the midpoint on the calibration curve.

2.5. Continuing Calibration Check (CCC) or Continuing Calibration Verification (CCV) – A standard used to verify that the response of the instrument has not changed since initial calibration.

2.6. Calibration Blank (CB), Initial Calibration Verification Blank (ICB), Method Blank (MBLK) or Continuing Calibration Blank (CCB) – A volume of reagent water fortified with the same matrix as the calibration standards, but without the analytes.

2.7. MDLS (Method Detection Limit Spike) – MDLB spiked with analytes at the lowest calibration level to be used for the determination of MDL.

3. Interferences

3.1. Residual chlorine can produce a negative interference by limiting reduction efficiency. Before analysis, samples should be checked and if required, dechlorinated with sodium thiosulfate (See 7.2.).

3.2. Low results would be obtained for samples that contain high concentrations of iron, copper or other metals. In this method, EDTA is added to the buffer to reduce this interference.

3.3. Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. This interference may be eliminated by pre-extracting the sample with an organic solvent.

3.4. Sample turbidity may interfere. Turbidity can be removed by filtration through a 0.45 µm pore diameter membrane filter prior to analysis.(See 5.6.)

4. Safety

4.1. Refer to the EPD Laboratory Safety / Chemical Hygiene Plan & Fire Safety Plan, online revision. Reference 13.9.

5. Apparatus and Equipment

5.1. Sample Container: 250 ml Nalgene bottle

5.2. Plastic culture tubes, O.D. x L: 13mm x 100mm, Fisher Scientific part # 14-956-8E or equivalent or glass culture tubes 12mm x 75 mm VWR part # 60825-502 or equivalent.

5.3. Glassware – Class A volumetric flasks, graduated cylinders and pipettes

5.4. Cadmium-Copper Reduction Column (Lachat Part No. 50237), pre-packed

5.5. Lachat Quikchem flow injection analysis instrument

5.5.1. Lachat XYZ Autosampler

5.5.2. Auto-sampler racks (90 position)

5.5.3. Reagent pump

5.5.4. Reaction unit or manifold

5.5.5. Colorimetric detector with 520 nm interference filter

5.5.6. Computer with Microsoft Windows operating system with Lachat Omnion software or equivalent

5.6. Syringeless filter device containing 0.45 µm nylon membrane with glass microfiber prefilter (Whatman™ Autovial™ Syringeless Filters: 12mL Capacity, catalog number AV125UNAO or equivalent)

5.7. Air displacement pipettes of various volumes, auto- pipettor, pipette tips in various sizes. Air displacement pipettes and auto-pipettors may also be described as mechanical pipettes.

5.7.1. Each day of use, the volume dispensed by each mechanical pipette must be verified for the specific volume for which the pipette is being used.

5.7.1.1. Mechanical pipette volumes are verified by measuring the weight of a volume of water dispensed by the unit. At room temperature, 1 ml of water is equal to 1 g. Mechanical pipettes must be verified to be within ± 2.5% of the expected weight.

5.7.1.2. Auto-pipettors may be verified by measuring the volume dispensed with a graduated cylinder. The volume dispensed must be within ± 2.5 percent of the nominal volume.

5.7.1.3. Mechanical pipettes must be professionally calibrated every 6 months.

5.8. Balance: analytical, capable of accurately weighing to the nearest 0.0001g.

5.9. Flow cell: 10 mm path length, 80 µl, glass

5.10. Vacuum source for degassing mobile phases

5.11. Disposable transfer pipettes:

5.11.1. Plastic - VWR® Disposable Transfer Pipets PN # 16001-190 or Fisherbrand™ Standard Disposable Transfer Pipettes PN # 13-711-7 M

5.12. 50 ml Centrifuge Tubes: For standards. VWR Part Number 21008-240, or equivalent

5.13. Chlorine test strips:

5.13.1. HF Scientific – Catalog # 09941 (Fisher Catalog # 14-376-163) or equivalent

6. Reagents

6.1. Reagent Water:

6.1.1. Purified water which does not contain any measurable 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 18.2[MΩ·cm]@ 25ºC and a TOC of 50 ug/L or less).

6.2. Sulfanilamide Color Reagent:

6.2.1. Add 600 ml of reagent water (see 6.1.) to a 1 L volumetric flask. Add 100 ml of 85% Phosphoric acid (H_3PO_4), 40.0 g Sulfanilamide, and 1.0 g N-(1-naphthyl) Ethylenediamine dihydrochloride (NED). Shake to wet. Stir for 30 minutes to dissolve. Dilute to the mark, and invert to mix. Store the solution in a dark bottle. Degas before use. This solution is stable for one month. This solution is prepared using Lachat Methodology.

6.3. Sodium Hydroxide (15 N):

6.3.1. Only needed if not using commercially prepared buffer (see Ammonium Chloride Buffer, pH 8.5 section 6.4.). Store at room temperature.

6.3.2. Add 150 g NaOH very slowly to 250 ml of reagent water (see 6.1.). CAUTION: The solution will get very hot! Swirl until dissolved. Cool and store in plastic bottle. This solution is stable for one year.

6.4. Ammonium Chloride Buffer, pH 8.5:

6.4.1. In a 1 L volumetric flask, dissolve 85.0 g ammonium chloride (NH_4Cl) and 1.0 g disodium ethylenediamine-tetraacetic acid dihydrate ($Na_2EDTA{\cdot}2H_2O$) in about 800 ml reagent water (see 6.1.). Dilute to the mark. Invert to mix. Adjust pH to 8.5 with 15N NaOH solution (see 6.3.). Degas before use. Store solution at room temperature for up to one year. This solution is prepared using Lachat Methodology.

6.4.2. Commercially prepared buffer may also be purchased and used. This purchased buffer is stable until expiration date on bottle or within 1 year of opening date, whichever is sooner. Store solution at room temperature.

6.5. Carrier Solution/Diluent:

6.5.1. Fill a two-liter volumetric flask with reagent water to the mark. Add 20 ml of 10% Sulfuric acid (see 6.6). Prepare fresh daily.

6.6. 10% Sulfuric acid:

6.6.1. Purchased from VWR, Part # BDH 3358-4 or equivalent

6.7. Sodium Thiosulfate (0.375 N):

6.7.1. Purchased - Ricca Chemical Company – Catalog # 7925-32. This purchased chemical is stable until expiration date on bottle or within 1 year of opening date, whichever is sooner. Store at room temperature.

6.8. Sodium Nitrite ($NaNO_2$): Crystalline, ACS grade or equivalent

6.9. Nitrite Stock Solution, 100 mg/L:

6.9.1. Dissolve 0.493 g of $NaNO_2$ in 800 ml of reagent water (see 6.1.). Dilute to one liter with reagent water. Refrigerate at 6° C or less. This solution is stable for 3 - 5 days.

6.10. Nitrite Check Solution 1.00 (mg/L):

6.10.1. Pipette 1.0 ml of Nitrite Stock Solution (see 6.9.) into a 100 ml flask and dilute to volume with reagent water (6.1.). Prepare fresh daily.

6.11. Nitrate Stock Standard Solutions:

6.11.1. Potassium Nitrate (KNO_3): Crystalline, 99.0% minimum, ACS grade or equivalent.

6.11.2. Place approximately 8 grams in a partially covered glass dish and dry in a 103-105 ºC oven for 1 hour. Take out of oven and let cool in a desiccator for 2 hours before use in section 6.11.3.

6.11.3. Primary Source (PS) Nitrate Stock Solution (1000 mg/L):

6.11.3.1. 7.218 g of dried KNO_3 (see 6.11.1) is dissolved in and brought to volume in a 1L volumetric flask with reagent water (see 6.1.). Prepare every 6 months. Note: This standard can also be purchased from a commercially available source. This purchased standard is stable until expiration date on bottle or within 6 months of opening date, whichever is sooner. Refrigerate at 6° C or less.

6.11.4. *PS Nitrate Intermediate Stock Solution (100 mg/L) and Nitrate Spiking Solution:*

6.11.4.1. A 10 ml aliquot of PS Nitrate Standard Stock Solution (see 6.11.3.) is pipetted into a 100 ml volumetric flask and diluted to volume with reagent water (see 6.1.). Prepare every 3 to 5 days, as needed.

6.12. Calibration Standards

6.12.1. This method utilizes an 8-point calibration. The calibration includes a calibration blank.

Table 6.12.1. 1. – Nitrate Calibration Level Concentrations

Analyte	**(mg/L)**							
Nitrate	0	0.02	0.05	0.20	0.50	0.80	1.00	2.00

6.12.2. 8 levels of calibration standards are prepared by the addition of aliquots of

Table 6.12.2. 1. –Nitrate Calibration Level Spike Volumes into 100 ml of Reagent Water

	Nitrate Standard Concentrations							
	0 (ICB)	**0.02 mg/L**	**0.05 mg/L**	**0.20 mg/L**	**0.50 mg/L**	**0.80 mg/L**	**1.00 mg/L**	**2.00 mg/L**
ml needed of PS Nitrate Intermediate Stock Solution (100 mg/L)	NA				0.50	0.80	1.0	2.0
	OR							
ml needed of Nitrate Standard 2.00 mg N/L	NA	1	2.5	10				

PS Nitrate Intermediate Stock Solution (100 mg/L) (see 6.11.4.) or Standard 2.00 mg N/L to reagent water (see 6.1.) in a 100 ml volumetric flask and diluting to volume as follows:

6.12.3. After standards are brought to volume, add 1 ml of 10% Sulfuric acid (see 6.6.) for every 100 ml of final volume to each standard.

6.12.4. For convenience, the CB, ICB, CCB, MBLK and MDLB and the 1.00 mg/L (CCC or CCV) may be made by the addition of zero ml or 2 ml respectively of PS Nitrate Intermediate Stock Solution (100 mg/L) to final volumes of 200 ml. Adjust Sulfuric acid added in accordingly (2 ml for 200 ml of standard or reagent water).

6.12.5. Calibration standards must be made daily.

6.12.5.1 The auto-dilutor can be used to make up the calibration standards, the CCC and the Low level standard check if you use the 2.0 mg N/L standard to dilute from. Note: If the auto-dilutor makes the standards then they do not have to be logged into the standard log or assigned a number.

6.13. ICV Nitrate Stock Solution(see 2.4.) or Second Source(SS):

6.13.1. ICV Nitrate Stock Solution (1000 mg N/L):

6.13.2. The ICV stock standard is typically a standard intended as a "QC Sample" but used as a second source standard instead.

6.13.3. This stock standard must be from a different source than the stock standard used to make the calibration standards.

6.13.4. This standard is purchased from a commercially available source. The purchased standard is stable until expiration date on bottle or within 6 months of opening date, whichever is sooner.

6.14. *ICV Nitrate Solution (1.00 mg N/L):*

6.14.1. A 0.5ml aliquot of the ICV Nitrate Stock Solution (see 6.13.1.) is pipetted into a 500 ml volumetric flask and diluted to volume with reagent water (see 6.1.). After the ICV is brought to volume, add 5 ml of 10% Sulfuric Acid (see 6.6).

6.14.1.1. The ICV solution must be prepared fresh daily.

7. Sample Collection

7.1. Samples are collected in 250 ml plastic bottles.

7.1.1. Water Quality (WQ) – 2.5ml of 10% Sulfuric Acid is added to WQ sample bottles in the receiving lab, prior to sample collection in the field by sample collectors.

7.1.2. Drinking Water (DW) – Nitrate/Nitrite samples are preserved when received at the laboratory using 2.5 ml of 10% Sulfuric Acid to adjust to a pH of <2.

7.2. Sample preservation is checked in the receiving lab at the time of receipt.

7.2.1. Sample pH is checked with disposable transfer pipets. A clean disposable pipet (5.11.) is used to draw up a few drops of the sample. The drops are then placed on appropriate narrow range pH paper.

7.2.1.1. Never dip the test strip into the sample.

7.2.1.2. If the sample pH is not < 2 for Water Quality (WQ) samples, the collector is notified. The sample is then preserved in the receiving lab and a comment is placed on the NO3NO2 test code that the sample was preserved in the laboratory. The result is "J" flagged as estimated due to sample not preserved at time of collection

7.3. Drinking Water (DW) samples are checked for the presence of chlorine in the receiving lab prior to preservation.

7.3.1. Chlorine is checked with disposable transfer pipets. A clean disposable pipette (5.11.) is used to draw up a few drops of the sample. The drops are then placed on a chlorine test strip (5.13).

7.3.2. If the test strip indicates a chlorine level of 4 ppm or greater, add 10 drops of 0.375 Sodium thiosulfate (6.7.) to the drinking water sample, cap, mix, and retest the chlorine level with a new pipette and new test strip.

7.3.3. If the sample still has a high chlorine level, add 10 more drops of the Sodium thiosulfate solution. If the chlorine level is marginally above 4 ppm, only add 2 – 5 drops of Sodium thiosulfate solution. Cap, mix, and retest. Repeat as necessary, up to a maximum of 20 drops of Sodium thiosulfate.

7.3.4. If 20 drops of the Sodium thiosulfate solution are insufficient for neutralizing the available chlorine to less than 4 ppm, the Receiving Lab will notify the Inorganic Lab supervisor so that a new sample kit can be shipped to the customer for recollection. The original sample is voided in Labworks by adding the !VOID test code and commenting on the sample that the sample contains excess chlorine.

7.4. Holding time is 28 days.

7.5. Samples are stored at 0-6° C.

7.6. There is no temperature requirement on DW nitrate samples (NITNAT) received to the lab.

7.7. The temperature requirement for WQ Nitrate samples (NO3NO2) is 0-6° C (not frozen).

7.8. WQ Nitrate sample test code is NO3NO2.

7.9. DW Nitrate test code is NITNAT.

8. Calibration

8.1. Calibration Standards:

8.1.1. The calibration curve consists of the calibration standards and concentrations listed in Table 6.12.1. 1.

8.2. Calibration Curve:

8.2.1. The Lachat Quikchem is calibrated daily. Eight standards are used to construct the Nitrate/Nitrite calibration curve for water quality nitrates and drinking water nitrates. Minimum acceptable correlation coefficient is 0.995 using a linear regression.

8.2.2. Dilute all samples with a response greater the high standard 2.00 mg/L.

8.3. Calibration Verification:

8.3.1. An Initial Calibration Verification standard (ICV) (see 2.4. and 6.14), a Continuing Calibration Check (CCC) (see 2.5. and 6.12.4.) and an Initial Calibration Blank (ICB) (see 2.6. and 6.12.4.) must be analyzed immediately after the calibration standards.

8.3.1.1. The %Drift (see calculation 11.1.) of the ICV from the true value must be within ±10%. Repeat once if it fails. If it fails the second attempt,

determine the source of the problem, correct and recalibrate.

8.3.1.2. The ICB, CCB and MBLK values must be less than the method RL or the run will have to be repeated.

8.3.1.3. A CCC and a Continuing Calibration Blank (CCB) must be analyzed every 10 samples and at the end of the sample run and must meet the same criteria as the ICV and ICB respectively.

8.3.1.3.1. The CCC may be from the same source as the calibration standards.

8.3.1.4. If the CCC or CCB do not meet acceptance criteria, then all samples affected by the out of control CCC or CCB are to be rerun.

8.3.2. The Nitrite Check Solution at 1.0 mg/L (see 6.10.) is analyzed prior to sample analysis to verify that the Cadmium column is working correctly.

8.3.2.1. The %Drift (see calculation 11.1.) of the Nitrite Check Solution from the true value must be within ±10%. If the Nitrite Check Solution fails, the Cadmium column is not performing correctly. Service or replace the column and recalibrate the instrument.

8.4. A low-level calibration check standard at a concentration of 0.02 mg/L must be analyzed once per analytical run. Recovery of the standard must be ±50%.

8.5. A MDLS (low level spike) at the concentration of 0.02 mg/L must be analyzed with each batch to perform ongoing MDL study. All batch QC must be valid to report this result.

8.6. A MDLB (MDLB) must be analyzed once per analytical batch to perform an ongoing MDL study. All batch QC must be valid to report this result.

9. Quality Control

9.1. Refer to Table 14. 1. for Reporting Limits (RL's), Appendix A, Table A.1 for Quality Control Acceptance Criteria, and Table 14. 2. for Quality Control Procedures associated with this method.

9.2. See reference 13.6. for control charting procedures.

9.3. See reference 13.5. for training and certification procedures.

9.3.1. For Initial Demonstrations of Capability (IDC), Method 353.2 requires a recovery range of 90% - 110% (see calculation 11.8.).

9.3.1.1. The EPD Laboratory sets a 20% RSD requirement for IDC replicates (see calculation 11.4.).

9.3.2. The EPD Laboratory uses the most current accuracy and precision control ranges in use for samples for Continuing Demonstrations of Capability (CDC). If 4 replicates are performed (as opposed to two LCS/LCSD pairs) a 20% RSD is required (see calculation 11.5.).

9.4. Control Limits:

9.4.1. Method 353.2 requires control limits to be adjusted through the use of control charts.

9.4.2. Default control limits for recovery for LCS/LCSD pairs are based on Section 9.3.3 of EPA Method 353.2 (reference 13.1.) as noted in Table 9.4.7. 1. The default limits are 90% - 110% recovery.

9.4.3. The EPD Laboratory sets default LCS/LCSD precision control limits to 0-15% RPD.

9.4.4. Default control limits for recovery for MS/MSD pairs are based on Section 9.4.2 of EPA Method 353.2. The default limits are 90% - 110% recovery. These limits are static by EPA Method/EPD Lab default.

9.4.4.1. Method 353.2 section 9.4.1 requires that 10% of all routine samples must be spiked. See Section 9.5. for batching criteria.

9.4.5. MS/MSD default precision limits are set by the EPD lab as 0 – 15% RPD. These limits are static by EPA Method/EPD Lab default.

9.4.6. In-house limits based on control charts may never exceed the default limits.

9.4.7. See Administrative SOP for Control Charting and Control Limits, reference 13.6. for further details.

9.5. MDL Study:

9.5.1. MDL (method detection limit) is the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.

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

9.5.3. 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.

9.5.4. The Method Detection Limit Study for all analytes must be performed initially on a new instrument and performed after major instrument repairs or changes to procedures. There are two ways to perform the MDL. The first is with 7 samples and 7 blanks over 3 separate days. The second preferred way the MDL is run as a continuous format.

9.5.5. 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.

9.5.6. 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.

9.5.7. For NPW – NO3NO2 as N(NO3NO2), the results of the MDLBlank will be entered into Labworks using the Method Blank test code B_NO3NO2. The MDLSpike result will be entered using the MLNO3NO2. The MDL Spiked Amount will be entered into the test code MANO3NO2. The instrument used for the MDL and Blank analysis will be selected using the test code INSTR-NO3NO2. For DW - Nitrate/Nitrite as N(NITNAT) the results of the MDLBlank will be entered into Labworks using the Method Blank testcode B_NITNAT. The MDLSpike result will be entered using the MLNITNAT. The MDL Spiked Amount will be entered into the test code MANITNAT. The instrument used for the MDL and Blank analysis will be selected using the test code INSTR-NITNAT.

9.5.8. MDL study must be performed every six months and before the MDL for the instrument expires.

9.5.9. MDL data is pulled from a two year period.

Note: The default control limits are presented to assist in defining control limits established with control charts and are not used as batch acceptance criteria.

Table 9.4.7. 1. – Default Quality Assurance Criteria for Method EPA 353.2[1]

QC Type	Analyte	Accuracy(%R) LCL	Accuracy(%R) UCL	Precision (%RPD)
LCS/LCSD	Nitrate/Nitrite as N	90	110	0 – 15
MS/MSD	Nitrate/Nitrite as N	90	110	0 – 15

[1]*Default values apply to both drinking water and water quality.*

9.6. Batching:

9.6.1. Batch samples in groups of 20. For each batch, analyze a Matrix Spike (MS) and a Matrix Spike Duplicate (MSD) for a minimum of 10% of routine samples.

9.6.2. For batches of 1 to 10 routine samples, one MS/MSD pair must be spiked. For batches of 11 to 20 routine samples, two MS/MSD pairs must be spiked using different samples for each pair.

9.6.3. Each batch must have an LCS, LCSD and a Method Blank.

10. Procedure

10.1. Remove sample bottles, standards, and reagents from cold storage and

allow equilibration to room temperature prior to sample preparation or analysis.

10.1.1. If using an instrument with an auto-dilutor, refer to Appendix B for the procedure.

10.2. Air displacement and other mechanical pipetters must have the delivery volume verified each day of use for each specific volume for which the device is used on that day.

10.3. Prepare the color reagent, ammonia buffer, and Carrier Solution and make sure the buffer and color reagents have been degassed. (see 6.2., 6.4. and 6.2.5. respectively).

10.4. From Labworks, print a backlog of pending samples. Samples are batched in groups of 20. For each batch, select one or two QC samples to use as a matrix spike (MS) and matrix spike duplicate (MSD). See batching requirements Section 9.5.

10.5. Turn on the instrument computer, printer, sampler, pump, and colorimetric detector.

10.6. Log into the network and click on the Omnion 3.0 icon. Then log in to the Omnion software.

10.7. Click on the open button to open the folder containing all methods. Open the methods folder and click on either the "Water Quality Nitrates.omn" folder or the "Drinking Water Nitrates.omn" folder depending on which samples are being analyzed.

10.8. Input the sample list from the batch sheet into the appropriate template. If there are any dilutions make sure to type the dilution in the MDF column. Make sure the MDF box has a checkmark by it. MDF stands for manual dilution factor.

10.9. Select the run pull down menu and click on the Save As option.

10.10. Check pump tubes for wear. Replace if necessary. Secure lines to pump manifold. Make sure the cadmium column is offline. Insert waste lines into properly labeled waste container. If starting an empty waste container, be sure to update the label with the accumulation start date.

10.11. Press the manual start/stop button on the pump and make sure the pump speed is at 35. Pump DI water through all reagent lines and check for leaks and smooth flow. Let the water run for approximately 5 minutes.

10.12. Switch lines from DI water to reagents and allow system to equilibrate. Record maintenance checks and baseline readings in maintenance log as well as any other maintenance performed on instrument.

10.13. QC Samples and Standard Preparation:

10.13.1. *Initial Calibration Blank (ICB) / Continuing Calibration Blank (CCB/MDLB/MBLK)*:

10.13.1.1. Pour an aliquot of the 0 mg/L standard (also the CB, CCB, MDLB, MBLK

and ICB; see 6.12.4.) that was stored in a 250 ml sample collection bottle into a culture tube (see 5.2.) for each ICB, MBLK and CCB needed for the instrument sequence. Record the lot# of bottle used.

10.13.2. Pour an aliquot of the 1.00 mg/L standard (also the CCC; see 6.12.) into a culture tube for each CCC needed for the instrument sequence.

10.13.3. Prepare an LCS and LCSD by adding approximately 10 ml of the 0 mg/L standard or carrier that was stored in a 250 ml sample collection bottle (also the CB, CCB, MBLK, MDLB and ICB) to a 25 ml volumetric flask for each. Add a 250 µl aliquot of the Nitrate Intermediate Stock Solution 100 mg/L (see 6.11.4) to each flask and fill to volume with the 0 mg/L standard or carrier, stopper, and mix. Label each flask appropriately. LCS and LCSD concentrations are 1.00 mg/L N. Record the lot# of bottle used.

10.13.4. Prepare an MS and MSD by adding approximately 10 ml of the selected QC sample to a 25 ml volumetric flask for each. Add a 250 µl aliquot of the Nitrate Intermediate Stock Solution 100 mg/L (see 6.11.4) to each flask, fill to volume with the QC sample, cover and mix. Label each flask appropriately. MS and MSD spiked concentrations are 1.00 mg/L Nitrate over the Nitrate value of the QC Sample.

10.13.5. The MDLS (0.02 mg N/L standard) must be poured into a 250 ml sample collection bottle before it is poured into a culture tube for analysis. Record the lot# of bottle used.

10.14. Analysis:

10.14.1. Load samples/standards into the auto-sampler. If samples need to be filtered, the CCB/MBLK/ MDLB, LCS, LCSD in that batch must be filtered to ensure filtration does not negatively affect results. Initial calibration standards are loaded in order from high to low in the 50 ml centrifuge tubes in the standard rack.

10.14.2. Place the reagent line in the color reagent, the buffer line in the ammonia buffer and the carrier line in Carrier Solution.

10.14.3. Let the reagents run through the lines for approximately 5 minutes and then switch the cadmium column valve to the ON position by turning the blue knob located next to the cadmium column. Allow reagents to run through the column for a few minutes before starting the run.

10.14.4. Click the start button located above the run worksheet to start the run.

10.14.5. Verify that the cadmium column is working correctly by analyzing the Nitrite Check Solution (1.0 mg/L Nitrite). The %Drift (calculation 11.6) must not be greater than ±10% of the expected value. If this standard fails, the run needs to be stopped immediately and the column needs to be replaced.

10.14.6. After the run is finished, and before running reagent water through lines, turn the cadmium column valve to the offline position.

10.14.7. Select the tools pull down menu and click on custom report. Click on the custom report format and select layout. Once the report is created, the auto fields should contain the name of the individual that is logged in, the current date and the page numbers of the report.

10.14.8. Click Apply once, when all layout modifications have been made. If there are no layout modifications, click ok. Exit the custom report menu. Click on the printer button to print the report. The report should contain the calibration curve.

10.14.9. From the main menu, select the run button and click on export worksheet to print the run log.

10.14.10. *Dilutions*:

10.14.10.1. If the response of any sample or QC sample is greater than the high standard 2.0 mg/L N/L, those samples must be diluted and rerun in a valid sequence or at the end of the run, followed by an ending CCC and CCB. Dilution ratios should be determined, as nearly as possible, so that the response is near the mid-point of the calibration range.

10.14.10.2. To prepare dilutions, use the 0 mg/L standard or Carrier (see 6.12. and 6.5.) to dilute the samples.

10.14.10.3. If using the auto-dilutor to prepare dilutions, see Appendix B for instructions.

10.15. Volumes and amounts of reagents, chemicals, and standards may be altered as long as the final concentrations remain the same. Sample volumes and injection amounts may be altered as long as required detection limits can be met and sample/reagent ratios remain the same.

10.16. Shutdown Procedure:

10.16.1. After the cadmium column valve is in the offline position, place the color reagent and buffer lines into reagent water for 5 minutes and then place all lines in a dry beaker for 5 minutes.

10.16.2. Exit the Omnion program and shut down the computer, sampler, pump and colorimetric detector.

10.16.3. Be sure to loosen the platens on the pump tubes so that there isn't excessive wear.

10.16.4. Cap reagent waste container when not in use or neutralize waste as noted in the Laboratory Waste Management SOP, Reference 13.7.)

11. Calculations

11.1. The weighted external standard calibration is calculated by the instrument software and is documented in the instrument software.

11.2. Mean ($\overline{X}$):

$$\overline{X} = \frac{X_1 + X_2 + \cdots X_n}{n}$$

11.2.1. Where:

$X_1 + X_2 + \cdots X_n$ = The sum of a set of values X_i, i = 1 to n

n = The number of values in the set

11.3. Standard Deviation (n – 1) (σ_{n-1}):

$$\sigma_{n-1} = \sqrt{\sum_{i=1}^{n} \frac{(X_i - \overline{X})^2}{n-1}}$$

11.3.1. Where:

$\overline{X}$ = Mean of the values

X_i = Individual values 1 through i

n = Number of values

11.4. Percent Relative Standard Deviation (%RSD):

$$\%RSD = \frac{\sigma_{n-1}}{\overline{X}} * 100$$

11.4.1. Where:

σ_{n-1} = Sample Standard Deviation

$\overline{X}$ = Mean of the values

11.5. Relative Percent Difference (%RPD or RPD):

$$\%RPD = \frac{|X_1 - X_2|}{\frac{(X_1 + X_2)}{2}} * 100$$

11.5.1. Where:

$|X_1 - X_2|$ = Absolute difference between two values

$\frac{(X_1 + X_2)}{2}$ = Average of two values

11.6. Percent Drift, %Drift:

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

11.6.1. Where:

$Concentration_{Calculated}$ = Concentration calculated from result

$Concentration_{Expected}$ = Theoretical concentration of the standard

11.7. Extract Concentration:

11.7.1. The extract concentration is calculated relative to the calibration curve by the instrument software.

11.8. Percent Recovery:

11.8.1. *LCS/LCSD*:

$$\%\text{Recovery} = \frac{\text{Conc}_{\text{spiked}}}{\text{Conc}_{\text{expected}}} * 100$$

11.8.1.1. Where:

$\text{Conc}_{\text{spiked}}$ = Concentration found in the spiked sample

$\text{Conc}_{\text{expected}}$ = Expected concentration

11.8.2. *MS/MSD*:

$$\%\text{Recovery} = \frac{\text{Conc}_{\text{spiked}} - \text{Conc}_{\text{unspiked}}}{\text{Conc}_{\text{expected}}} * 100$$

11.8.2.1. Where:

$\text{Conc}_{\text{spiked}}$ = Concentration found in the spiked sample

$\text{Conc}_{\text{unspiked}}$ = Concentration found in unspiked sample

$\text{Conc}_{\text{expected}}$ = Expected concentration

11.9. Calculation of Dilution Factors

$$C \times D = F$$

11.9.1. Where:

C = concentration from instrument in mg N/L

D = dilution factor, if any

F = final concentration in mg N/L

12. Waste Management

12.1. See GA EPD Laboratory SOP-EPD Laboratory Waste Management Standard Operating procedures, reference 13.7.

13. References

13.1. EPA Method 353.2, Determination of Nitrate-Nitrite Nitrogen by Automated Colorimetry, Rev. 2.0, August 1993

13.2. Manual for the Certification of Laboratories Analyzing Drinking Water, EPA/815-R-05-004, January 2005.
13.3. EPD Laboratory Quality Assurance Plan, online revision.
13.4. Determination of Nitrate/Nitrite in Surface and Wastewaters by Flow Injection Analysis, Lachat QuikChem Method 10-107-04-1-C, August 28, 2000.
13.5. GA EPD Laboratory SOP's – Initial Demonstration of Capability SOP 6-001, online revision or Continuing Demonstration of Capability SOP 6-002, online revision.
13.6. GA EPD Laboratory SOP-EPD Laboratory Procedures for Control Charts and Control Limits SOP, SOP 6-025, online revision.
13.7. GA EPD Laboratory SOP-EPD Laboratory Waste Management SOP, SOP 6-015, online revision.
13.8. GA EPD Laboratory SOP – Determination of Method Detection Limit, Method Detection Limit SOP 6-007, online revision.
13.9. GA EPD Laboratory Safety Plan – EPD Laboratory Safety / Chemical Hygiene Plan & Fire Safety Plan, online revision.

14. Reporting Limits (RL's), Precision and Accuracy Criteria, and Quality Control Approach

Table 14. 1. – Reporting Limits for EPA 353.2

Parameter/Method	Analyte	Matrix (aqueous) Drinking Water (DW)		Matrix (aqueous) Water Quality Waters (WQ)	
		RL	Unit	RL	Unit
EPA 353.2	Nitrate/Nitrite	0.20	mg/L	0.02	mg/L

Table 14. 2. - **Summary of Calibration and QC Procedures for Method EPA 353.2**

Method	Applicable Parameter	QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria
EPA 353.2	Nitrate/Nitrite	Initial calibration for all analytes	Initial calibration prior to sample analysis	Correlation coefficient ≥ 0.995 linear regression	Correct problem then repeat initial calibration	
		Second source calibration verification (ICV)	Once per initial calibration or quarterly, whichever is sooner	Nitrate/Nitrite concentration within 10% of expected value	Correct problem then repeat initial calibration	

Table 14. 2. - **Summary of Calibration and QC Procedures for Method EPA 353.2**

Method	Applicable Parameter	QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria
EPA 353.2	Nitrate/Nitrite	Initial Calibration Blank (ICB)	Once per initial calibration	Nitrate/Nitrite value must be below reporting limit	Correct problem then repeat initial calibration	
		Nitrite (NO_2) Standard	Prior to sample analysis	NO_2 concentration within 10% of expected value	Correct problem then repeat initial calibration	
		Initial Demonstration: Demonstrate ability to generate acceptable accuracy and precision using four analysis of a QC check sample, a method blank, a blind sample, and an MDL study(NITNAT only). In addition, the analyst must prepare one standard.	Once per analyst	Default QC Criteria Table 9.4. and Initial Demonstration SOP	Recalculate results: locate and fix problem with system and then rerun demonstration for those analytes that did not meet criteria	
		Continuing Demonstration: Demonstrate ability to generate acceptable accuracy and precision using a variety of analysis options of a QC sample(s)	Every 6 months	QC Acceptance Criteria SOP 3-012 Appendix A and Continuing Demonstration SOP	Recalculate results: locate and fix problem with system and then rerun demonstration for those analytes that did not meet criteria	
		Method Blank (MBLK)	One per batch	Nitrate/Nitrite value must be below reporting limit	Correct problem then analyze method blank and all samples processed with the contaminated blank	If unable to reanalyze, flag with a “B”
		Laboratory Control Sample (LCS/ LCSD)	One LCS/LCSD per analytical batch	QC Acceptance Criteria Table SOP 3-012 Appendix A	Correct problem then re-analyze the LCS/LCSD and all samples in the affected batch	If unable to reanalyze, flag with a “J”
		Matrix Spike (MS/MSD)	10% of Samples	QC Acceptance Criteria Table SOP 3-012 Appendix A	Evaluate out of control event, reanalyze or flag data	
		Continuing Calibration Check (CCC)	Prior to sample analysis, after every 10 samples and at the end of a sample run	Nitrate/Nitrite concentration within 10% of expected value	Correct problem then reanalyze all samples associated with out of control CCC.	

Table 14. 2. - **Summary of Calibration and QC Procedures for Method EPA 353.2**

Method	Applicable Parameter	QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria
EPA 353.2	Nitrate/Nitrite	Continuing Calibration Blank (CCB)	After every 10 samples and at the end of a sample run	Nitrate/Nitrite concentration must be below reporting limit	Correct problem then reanalyze all samples associated with out of control CCB.	
		Auto-dilutor check	Prior to sample analysis only when using instrument with auto-dilutor	Nitrate/Nitrite concentration must within 10% of expected value.	Correct problem and repeat initial calibration	
		Low level standard (0.02 mg/L)	Once per analytical run	Value must be ± 50% of expected value	Evaluate recovery exceedances, reanalyze or recalibrate	
		MDL low level Spike (0.02 mg/L) (MDLS)	Once per analytical batch	All batch QC must be valid	Correct problem and reanalyze affected batch	
		MDL Blank (MDLB) *Can be combined with MBLK	Once per analytical batch	All batch QC must be valid	Correct problem and reanalyze affected batch	
		MDL study	Every six months 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	
		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	

Appendix A, Table A.1 – Quality Assurance Criteria for EPA Method 353.2- Nitrate/Nitrite Nitrogen in Water

Table A.1 – Current Control Limits – EPA 353.2 Drinking Water Analyses (DW) Labworks Test Code NITNAT			
QC Type	Analyte	Accuracy (%R) LCL – UCL	Precision (%RPD)
LCS/LCSD			
	DW - Nitrate/Nitrite as N	90 - 110	15
MS/MSD			
	DW - Nitrate/Nitrite as N	90* - 110*	15*
***MS/MSD Control limits are static by EPA Method/EPD Lab default.** ***Control Chart data generated from 01/01/2019 -01/01/2021**			

Table A.2 – Current Control Limits – EPA 353.2 Non-Potable Water Analyses (NPW) Labworks Test Code NO3NO2			
QC Type	Analyte	Accuracy (%R) LCL – UCL	Precision (%RPD)
LCS/LCSD			
	NPW – NO3NO2 as N	90 - 110	15
MS/MSD			
	NPW – NO3NO2 as N	90 - 110	15
Control charts are generated twice annually for informational purposes only **Control Chart data generated from 01/01/2018 -01/01/2020**			

Appendix B – Procedure for using Auto-Dilutor for EPA Method 353.2- Nitrate/Nitrite Nitrogen in Water

1. Procedure if using an auto-dilutor

1.1. Remove sample bottles, standards, and reagents from cold storage and allow equilibration to room temperature prior to sample preparation or analysis.

1.2. Air displacement and other mechanical pipetters must have the delivery volume verified each day of use for each specific volume for which the device is used on that day.

1.3. Prepare the color reagent, ammonia buffer, and Carrier Solution and make sure the buffer and color reagents have been degassed. (see 6.2., 6.4. and 6.5. respectively).

1.4 Prepare the 2.0 mg/L standard per Table 6.12.2.1 in main SOP and place in cup S1. The remaining standards will be made by the dilutor using the following dilution factors: 1.00 mg/L N(2.00 ADF), 0.80 mg/L N(2.50ADF), 0.50 mg/L N (4.00 ADF), 0.20 mg/L N (10.00 ADF), 0.05 mg/L N (40.00 ADF), 0.02 mg/L N
(100.00 ADF).

1.4.1 Make sure to type Cup No. S1 for all standards, except for the 0.00 mg/L standard which will be cup S9.

1.5. From Labworks, print a backlog of pending samples. Samples are batched in groups of 20. For each batch, select one or two QC samples to use as a matrix spike (MS) and matrix spike duplicate (MSD). See batching requirements Section 9.5.

1.6 Turn on the instrument computer, printer, sampler, auto-dilutor, pump, and colorimetric detector.

1.7 Log into the network and click on the Omnion 3.0 icon. Then log in to the Omnion software.

1.8 Click on the open button to open the folder containing all methods. Open the methods folder and click on either the "Water Quality Nitrates.omn" folder or the "Drinking Water Nitrates.omn" folder depending on which samples are being analyzed.

1.9 Input the sample list from the batch sheet into the appropriate template. If there are any manual dilutions, type the dilution factor in the MDF column.

1.10 Make sure the MDF box has a checkmark by it by all manually diluted samples.

1.11 If using the auto-dilutor for any straight samples that are known to be above the calibration curve, type the dilution factor into the ADF column to trigger the auto-dilutor.

1.12 For samples of unknown concentration, leave the ADF column blank and be sure the box under Trigger Off column is NOT checked.

1.13 Only standards and dilutions made by the auto-dilutor will have this box checked.

1.14 A check standard for the auto-dilutor is used to verify any dilutions made during the run.

1.14.1 The 2.00 mg/L check standard is analyzed after the initial calibration curve but before routine samples.

1.14.2 The auto-dilutor will make a 2x dilution of the 2.00 mg/l check standard.

1.14.3 The percent recovery of the check standard must be within 10% of the known diluted value of 1.00 mg/l.

1.15 Set the auto-dilutor trigger limit, go to the Run Properties window, click the Analytes tab and click NO3+NO2/NITNAT under Channel 1.

1.15.1 Ensure the "Auto Dilution Trigger" box is checked and that "% of Highest Standard" is set to 90.

1.15.2 To set auto-dilutor levels, click the Configuration drop-down menu from the top of the page and then click Autosamplers.

1.15.3 The three auto-dilutor levels should be set at 2, 5 and 10.

1.15.4 If a sample is above the auto-dilutor's trigger limit, then the auto-dilutor will append a row containing the most appropriate of these dilution factors as determined by the computer.

1.15.5 If a sample requires a higher dilution, manually append another row and type in the dilution factor.

1.15.6 A CCC and CCB are required after every ten dilutions and at the end of the sample run.

1.16 Dilutions are mixed by the auto-dilutor in the rightmost rack of the auto-sampler tray.

1.16.1 Always ensure there are enough culture tubes in this rack for the number of dilutions in the run and to prepare the standards for the curve.

1.16.2 Make sure to use glass culture tubes instead of plastic because they will hold more volume of sample which is needed for the dilutions.

1.17 Prime the auto-dilutor by selecting Configuration, Auto-sampler and then select Prime dilutor. Once it is done, close window.

1.18 Select the run pull down menu and click on the Save As option.

1.19 Check pump tubes for wear. Replace if necessary. Secure lines to pump manifold. Make sure the cadmium column is offline. Insert waste lines into properly labeled waste container. If starting an empty waste container, be sure to update the label with the accumulation start date.

1.20 Press the manual start/stop button on the pump and make sure the pump speed is at 35. Pump DI water through all reagent lines and check for leaks and smooth flow. Let the water run for approximately 5 minutes.

1.21 Switch lines from DI water to reagents and allow system to equilibrate. Place the auto-dilutor intake line into Carrier solution.

1.22 Record maintenance checks and baseline readings in maintenance log.

Updates to Previous Version:

Table A.1
Section 9
Table 14.2