O·I·Analytical **Methodology**

# Nitrate plus Nitrite Nitrogen and Nitrite Nitrogen, USEPA by Flow Injection Analysis (FIA)

**(Cartridge Part #A001559)**

## 1.0 Scope and Application

1.1 This method is used for the determination of nitrate ($NO_3^-$) plus nitrite ($NO_2^-$) or nitrite singly in drinking water, groundwater, surface water, saline water, and domestic and industrial waste according to USEPA Method 353.2 (Reference 15.7).

1.2 The Method Detection Limit (MDL) of this method is 0.001 mg/L nitrate plus nitrite nitrogen and nitrite nitrogen. The applicable range of the method is 0.01–10 mg/L. The range may be extended to analyze higher concentrations by sample dilution.

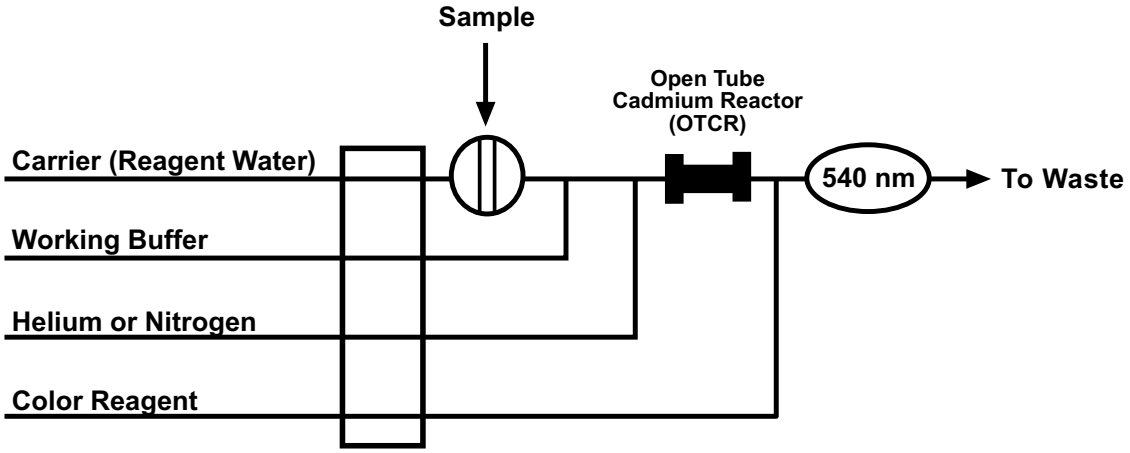
## 2.0 Summary of Method

2.1 Nitrate is reduced quantitatively to nitrite by cadmium metal. Nydahl (Reference 15.12) provides a good discussion of nitrate reduction by cadmium metal. The nitrite formed, in addition to any nitrite originally present in the sample, is diazotized with sulfanilamide and subsequently coupled with *N*-(1-naphthyl)ethylenediamine dihydrochloride. The resulting highly colored azo dye is colorimetrically detected at 540 nm (Reference 15.11). A calibration curve allows for accurate quantitation of the detected nitrite.

2.2 Nitrite singly may be measured by performing the same analysis as in Section 2.1 but without the cadmium reduction. Without the cadmium, nitrate is not reduced to nitrite. Nitrate is not detected since only nitrite forms the azo dye.

2.3 The quality of the analysis is assured through reproducible calibration and testing of the Flow Injection Analysis (FIA) system.

2.4 A general flow diagram of the FIA system is shown below (see Section 17.0 for a detailed flow diagram).




## 3.0 Definitions

Definitions for terms used in this method are provided in Section 16.0, "Glossary of Definitions and Purposes."

## 4.0 Interferences

4.1 Turbid samples may interfere with the photometric detector's ability to measure the true absorbance of the sample. Filter turbid samples prior to analysis.

4.2 Iron, copper, and other metals may interfere with the analysis by binding with the nitrate and/or nitrite in the sample, thus blocking the color formation reaction. Eliminate this interference by using ethylenediaminetetraacetic acid (EDTA) in the buffer solution.

4.3 Samples that are outside the functional pH range of the ammonium chloride buffer may affect the results obtained from this method. Adjust the pH of these samples to within a range of 5–9 using either concentrated hydrochloric acid (HCl) or ammonium hydroxide ($NH_4OH$).

4.4 Oil and grease will coat the cadmium surface, thus reducing its reduction efficiency. Extract samples containing large concentrations of oil and grease with an appropriate organic solvent (Reference 15.5).

4.5 Sulfide in the presence of cadmium will form cadmium sulfide (CdS), which will precipitate from solution. Samples containing sulfide cannot be determined by this method without first removing the sulfide by precipitation with cadmium salts (Reference 15.10).

4.6 Chlorine may reduce the reduction efficiency of the cadmium reactor. Samples that may contain residual chlorine should be tested for reduction efficiency through the analysis of Matrix Spike/ Matrix Spike Duplicate (MS/MSD) samples (Section 9.3). When necessary, dechlorinate samples with sodium thiosulfate ($Na_2S_2O_3$).

4.7 Method interferences may be caused by contaminants in the reagents, reagent water, glassware, etc., which may bias the results. Care should be taken to keep all such items free of contaminants.

4.8 Norwitz and Keliher (References 15.1 and 15.2) have compiled a comprehensive study of interferences in the spectrophotometric analysis of nitrite.

## 5.0 Safety

5.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been fully established. Each chemical should be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest possible level.

5.2 For reference purposes, a file of Material Safety Data Sheets (MSDS) for each chemical used in this method should be available to all personnel involved in this chemical analysis. The preparation of a formal safety plan is also advisable.

5.3 The following chemicals used in this method may be highly toxic or hazardous and should be handled with extreme caution at all times. Consult the appropriate MSDS before handling.

5.3.1 Ammonium Chloride, $NH_4Cl$ (FW 53.50)

5.3.2 Ammonium Hydroxide, $NH_4OH$ (FW 35.05)

5.3.3 Cadmium, Cd (FW 112.40)

5.3.4 Chloroform, $CHCl_3$ (FW 119.38)

5.3.5 Cupric Sulfate Pentahydrate, $CuSO_4 \cdot 5H_2O$ (FW 249.61)

5.3.6 Ethylenediaminetetraacetic Acid, Disodium Salt Dihydrate (EDTA), $C_{10}H_{16}N_2Na_2O_8 \cdot 2H_2O$ (FW 372.24)

5.3.7 Hydrochloric Acid, concentrated, HCl (FW 36.46)

5.3.8 *N*-(1-naphthyl)ethylenediamine Dihydrochloride, $C_{12}H_{14}N_2 \cdot 2HCl$ (FW 259.18)

5.3.9 Phosphoric Acid, concentrated, 85%, $H_3PO_4$ (FW 98.00)

5.3.10 Potassium Nitrate, $KNO_3$ (FW 101.11)

5.3.11 Potassium Nitrite, $KNO_2$ (FW 85.11)

5.3.12 Sulfanilamide, $C_6H_8N_2O_2S$ (FW 172.21)

5.4 Unknown samples may be potentially hazardous and should be handled with extreme caution at all times.

5.5 Proper personal protective equipment (PPE) should be used when handling or working in the presence of chemicals.

5.6 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method.

## 6.0 Apparatus, Equipment, and Supplies

6.1 Flow Injection Analysis (FIA) System (OI Analytical Flow Solution® 3000) consisting of the following:

6.1.1 120-Place Autosampler

6.1.2 Expanded Range (ER) Photometric Detector with 5-mm path length flowcell and 540-nm optical filter

6.1.3 Data Acquisition System (PC or Notebook PC) with WinFLOW™ software

6.1.4 Nitrate+Nitrite and Nitrite, USEPA Cartridge (Part #A001559)

6.2 Sampling equipment—Sample bottle, amber glass, with polytetrafluoroethylene (PTFE)-lined cap. Clean by washing with detergent and water, rinsing with two aliquots of reagent water, and drying by baking at 110°–150°C for a minimum of one hour.

6.3 Standard laboratory equipment including volumetric flasks, pipettes, syringes, etc. should all be cleaned, rinsed, and dried per bottle cleaning procedure in Section 6.2.

## 7.0 Reagents and Calibrants

7.1 Raw Materials

7.1.1 Ammonium Chloride, $NH_4Cl$ (FW 53.50)

7.1.2 Ammonium Hydroxide, $NH_4OH$ (FW 35.05)

7.1.3 Brij®-35, 30% w/v (Part #A21-0110-33)

7.1.4 Chloroform, $CHCl_3$ (FW 119.38)

7.1.5 Cupric Sulfate Pentahydrate, $CuSO_4 \cdot 5H_2O$ (FW 249.61)

7.1.6 Deionized Water (ASTM Type I or II)

7.1.7 Ethylenediaminetetraacetic Acid, Disodium Salt Dihydrate (EDTA), $C_{10}H_{16}N_2Na_2O_8 \cdot 2H_2O$ (FW 372.24)

7.1.8 Hydrochloric Acid, concentrated, HCl (FW 36.46)

7.1.9 *N*-(1-naphthyl)ethylenediamine Dihydrochloride, $C_{12}H_{14}N_2 \cdot 2HCl$ (FW 259.18)

7.1.10 Phosphoric Acid, concentrated, 85%, $H_3PO_4$ (FW 98.00)

7.1.11 Potassium Nitrate, $KNO_3$ (FW 101.11)

7.1.12 Potassium Nitrite, $KNO_2$ (FW 85.11)

7.1.13 Sulfanilamide, $C_6H_8N_2O_2S$ (FW 172.21)

7.2 Reagent Preparation

**Note**: For best results, filter and degas all reagents prior to use.

7.2.1 Reagent Water

7.2.1.1 Degassed and deionized reagent water can be prepared in one of the following manners:

7.2.1.1.1 Place distilled/deionized water under a strong vacuum for 15–20 minutes. Magnetic stirring or sonification will aid in the degassing process.

7.2.1.1.2 Purge distilled/deionized water with a stream of nitrogen gas (or other inert gas) through a glass frit for approximately 5 minutes.

7.2.1.1.3 Boil distilled/deionized water in an Erlenmeyer flask for 15–20 minutes. Remove the flask from the heat source, cover it with an inverted beaker, and allow it to cool to room temperature.

7.2.1.2 After preparing the degassed reagent water, store the reagent water in a tightly sealed container to protect it from reabsorption of atmospheric gases. For best results, store degassed reagent water under a slight vacuum when not in use.

7.2.2 Start-up Solution (1 L)

7.2.2.1 Add 2 mL of Brij-35 to approximately 800 mL of reagent water (Section 7.2.1) in a 1-L volumetric flask.

7.2.2.2 Dilute to 1,000 mL with reagent water and mix well.

7.2.3 Stock Ammonium Chloride/EDTA Buffer, pH 8.5 (1 L)

7.2.3.1 Dissolve 85 g of ammonium chloride and 0.1 g of EDTA in approximately 900 mL of reagent water in a 1-L beaker.

7.2.3.2 Adjust the pH to 8.5 with concentrated ammonium hydroxide.

7.2.3.3 Quantitatively transfer the solution to a 1-L volumetric flask and dilute to 1,000 mL with reagent water.

**Note**: For best results, filter this solution through a 0.45-µm filter before use.

7.2.4 Working Buffer (500 mL)

7.2.4.1 Add 2 mL of Brij-35 to 500 mL of stock ammonium chloride/EDTA buffer (Section 7.2.3) and mix gently.

7.2.5 Color Reagent (500 mL)

7.2.5.1 While stirring, carefully add 50 mL of concentrated phosphoric acid to approximately 400 mL of reagent water in a 500-mL volumetric flask.

7.2.5.2 Dissolve 20 g of sulfanilamide and 1 g of *N*-(1-naphthyl)ethylenediamine dihydrochloride in the phosphoric acid solution. Continue stirring until completely dissolved, which may take 2–3 hours.

7.2.5.3 Dilute to 500 mL with reagent water.

**Warning**: Mixing phosphoric acid and water produces a great amount of heat. Take appropriate precautions.

**Note**: For best results, filter this solution through a 0.45-µm filter before use. Store in an amber bottle and keep in the dark when not in use. If stored properly, this color reagent is typically stable for 2–3 months.

7.2.6 Carrier—Reagent Water

7.2.7 Reagents for Open Tubular Cadmium Reactor (OTCR) Activation

7.2.7.1 Stock Ammonium Chloride/EDTA Buffer (Section 7.2.3)

**Note**: Do not use buffer with Brij-35 for OTCR activation.

7.2.7.2 Reagent Water

7.2.7.3 2% Cupric Sulfate Solution (1 L)

7.2.7.3.1 Dissolve 20 g of cupric sulfate pentahydrate in approximately 900 mL of reagent water in a 1-L volumetric flask.

7.2.7.3.2 Dilute the solution to 1,000 mL with reagent water and mix well.

7.2.7.4 0.5 N Hydrochloric Acid Solution (100 mL)

7.2.7.4.1 Carefully add 4.15 mL of concentrated hydrochloric acid to approximately 70 mL of reagent water in a 100-mL volumetric flask.

7.2.7.4.2 Dilute to 100 mL with reagent water and mix well.

**Warning**: Mixing hydrochloric acid and water produces a great amount of heat. Take appropriate precautions.

7.3 Calibrant Preparation

7.3.1 Stock 1,000 mg/L Nitrate Nitrogen Solution (1 L)

7.3.1.1 Dissolve 7.218 g of potassium nitrate ($KNO_3$), dried at 110°C, in approximately 800 mL of reagent water in a 1-L volumetric flask.

7.3.1.2 Dilute this solution with reagent water to 1,000 mL and preserve it by adding 2 mL of chloroform (per liter).

**Note**: This solution is stable for approximately 4–6 weeks if stored at 4ºC.

7.3.2 Stock 1,000 mg/L Nitrite Nitrogen Solution (1 L)

7.3.2.1 Dissolve 6.076 g of potassium nitrite ($KNO_2$), dried at 110°C, in approximately 800 mL of reagent water in a 1-L volumetric flask.

7.3.2.2 Dilute this solution with reagent water to 1 L and preserve it by adding 2 mL of chloroform (per liter).

**Note**: This solution is stable for approximately 4–6 weeks if stored at 4ºC.

7.3.3 Intermediate 100 mg/L Nitrate Nitrogen or Nitrite Nitrogen Calibrant (100 mL)

7.3.3.1 Use a volumetric pipet to add 10 mL of stock nitrate nitrogen or nitrite nitrogen solution (Section 7.3.1 or 7.3.2) to approximately 80 mL of reagent water in a 100-mL volumetric flask.

7.3.3.2 Dilute to 100 mL with reagent water and mix well.

**Note**: Prepare intermediate calibrants fresh daily.

7.3.4 Working Calibrants (100 mL)

7.3.4.1 Add the designated volumes of stock or intermediate calibrant (see Equation 1) to the required number of 100-mL volumetric flasks that each contain approximately 80 mL of reagent water.

7.3.4.2 Dilute each solution to the mark with reagent water and mix well.

**Note**: Prepare working calibrants fresh daily.

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EQUATION 1

$$C_1V_1 = C_2V_2$$

*Where:*
$C_1$ = *Concentration (in mg/L) of stock solution (or calibrant)*
$V_1$ = *Volume (in L) of stock solution (or calibrant) to be used*
$C_2$ = *Desired concentration (in mg/L) of working calibrant to be prepared*
$V_2$ = *Final volume (in L) of working calibrant to be prepared*

By solving this equation for the volume of stock solution to be used ($V_1$), the following equation is obtained:

$$V_1 = \frac{C_2V_2}{C_1}$$

Since the desired concentration ($C_2$), the final volume ($V_2$), and the concentration of the stock solution ($C_1$) are all known for any given calibrant concentration in a defined volume, the volume of stock solution to be used ($V_1$) is easily calculated.

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7.3.4.3 Calibrants covering the entire range of this analysis can be prepared from the following tables.

| Final Concentration (mg/L) | Vol. of Inter. Cal. (µL) | Conc. of Inter. Cal. (mg/L) | Final Volume (mL) |
|---|---|---|---|
| 0.01 | 10 | 100 | 100 |
| 0.05 | 50 | 100 | 100 |
| 0.10 | 100 | 100 | 100 |
| 0.50 | 500 | 100 | 100 |
| 1.0 | 1,000 | 100 | 100 |

| Final Concentration (mg/L) | Vol. of Stock Cal. (µL) | Conc. of Stock Cal. (mg/L) | Final Volume (mL) |
|---|---|---|---|
| 5.0 | 500 | 1,000 | 100 |
| 10 | 1,000 | 1,000 | 100 |

## 8.0 Sample Collection, Preservation, and Storage

8.1 Samples should be collected in plastic or glass bottles that have been thoroughly cleaned and rinsed with reagent water (Section 7.2.1).

8.2 The volume of sample collected should be sufficient to ensure that a representative sample is obtained, replicate analysis is possible, and waste disposal is minimized.

8.3 Preserve samples by adding concentrated sulfuric acid ($H_2SO_4$) to a pH of less than 2 and refrigerating at 4°C from the time of collection. Do not preserve samples with mercuric chloride.

8.4 Sample analysis should be performed as soon as possible to eliminate loss of analyte. Should storage be required, preserved samples that have been refrigerated at 4°C may be held for a maximum of 28 days from the time of collection (Reference 15.8).

8.5 For analysis of nitrate or nitrite singly, samples should be refrigerated at 4°C and analyzed within 48 hours from the time of collection.

## 9.0 Quality Control

9.1 Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 15.4). The minimum requirements of this program consist of an initial demonstration of laboratory capability and the periodic analysis of Laboratory Control Samples (LCSs) and Matrix Spike/Matrix Spike Duplicates (MS/MSDs) as a continuing check on performance. Laboratory performance is compared to established performance criteria to determine if the results of the analyses meet the performance characteristics of the method.

9.1.1 The analyst shall make an initial demonstration of the ability to generate acceptable precision and accuracy with this method. This ability is established as described in Section 9.2.

9.1.2 In recognition of advances that are occurring in analytical technology and to allow the analyst to overcome sample matrix interferences, the analyst is permitted certain options to improve performance or lower the costs of measurements. Alternate determinative techniques, such as the substitution of spectroscopic or other techniques, and changes that degrade method performance are not allowed. If an analytical technique other than the techniques specified in this method is used, that technique must have a specificity equal to or better than the specificity of the techniques in this method for the analyte(s) of interest.

9.1.2.1 Each time a modification is made to this method, the analyst is required to repeat the procedure in Section 9.2. If the detection limit of the method will be affected by the change, the laboratory is required to demonstrate that the MDL is lower than one-third the regulatory compliance level or as low as or lower than that listed in Section 1.2. If calibration will be affected by the change, the analyst must recalibrate the instrument per Section 10.4.

9.1.2.2 The laboratory is required to maintain records of modifications made to this method. These records include the information in this subsection, at a minimum.

9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and of the quality control officer who witnessed and will verify the analyses and modification.

9.1.2.2.2 A narrative stating the reason(s) for the modification.

9.1.2.2.3 Results from all quality control (QC) tests comparing the modified method to this method including:

a) calibration (Section 10.4)

b) calibration verification (Section 9.5)

c) initial precision and recovery (Section 9.2.2)

d) analysis of blanks (Section 9.4)

e) ongoing precision and recovery (Section 9.6)

f) matrix spike and matrix spike duplicate (Section 9.3)

9.1.2.2.4 Data that will allow an independent reviewer to validate each determination by tracing the instrument output (peak height, area, or other signal) to the final result. These data are to include:

a) sample numbers and other identifiers

b) analysis dates and times

c) analysis sequence/run chronology

d) sample weight or volume

e) sample volume prior to each cleanup step, if applicable

f) sample volume after each cleanup step, if applicable

g) final sample volume prior to injection

h) injection volume

i) dilution data, differentiating between dilution of a sample or modified sample

j) instrument and operating conditions

k) other operating conditions

l) detector

m) printer tapes, disks, and other recording of raw data

n) quantitation reports, data system outputs, and other data necessary to link raw data to the results reported

9.1.3 Analyses of MS/MSD samples are required to demonstrate method accuracy and precision and to monitor matrix interferences (interferences caused by the sample matrix). The procedure and QC criteria for spiking are described in Section 9.3.

9.1.4 Analyses of laboratory reagent blanks (LRBs) are required to demonstrate freedom from contamination and that the compounds of interest and interfering compounds have not been carried over from a previous analysis. The procedures and criteria for analysis of an LRB are described in Section 9.4.

9.1.5 The laboratory shall, on an ongoing basis, demonstrate through the analysis of the LCS that the analytical system is in control. This procedure is described in Section 9.6.

9.1.6 The laboratory should maintain records to define the quality of data that is generated. Development of accuracy statements is described in Sections 9.3.8 and 9.6.3.

9.1.7 Accompanying QC for the determination of nitrate plus nitrite and nitrite is required per analytical batch. An analytical batch is a set of samples analyzed at the same time to a maximum of 10 samples. Each analytical batch of 10 or fewer samples must be accompanied by a laboratory reagent blank (LRB, Section 9.4), a laboratory control sample (LCS, Section 9.6), and a matrix spike and matrix spike duplicate (MS/MSD, Section 9.3), resulting in a minimum of five analyses (1 sample, 1 LRB, 1 LCS, 1 MS, and 1 MSD) and a maximum of 14 analyses (10 samples, 1 LRB, 1 LCS, 1 MS, and 1 MSD) in the batch. If more than 10 samples are analyzed at one time, the samples must be separated into analytical batches of 10 or fewer samples.

9.2 Initial Demonstration of Laboratory Capability

9.2.1 Method Detection Limit (MDL)—To establish the ability to detect nitrate plus nitrite and nitrite at low levels, the analyst shall determine the MDL per the procedure in 40 CFR 136, Appendix B (Reference 15.3) using the apparatus, reagents, and standards that will be used in the practice of this method. An MDL less than or equal to the MDL listed in Section 1.2 must be achieved prior to practice of this method.

9.2.2 Initial Precision and Recovery (IPR)—To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations:

9.2.2.1 Analyze four samples of the LCS (Section 9.6) according to the procedure beginning in Section 10.0.

9.2.2.2 Using the results of the set of the four analyses, compute the average percent recovery ($x$) and the standard deviation of the percent recovery ($s$) for nitrate plus nitrite and nitrite. Use Equation 2 for the calculation of the standard deviation of the percent recovery ($s$).

EQUATION 2

$$s = \sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n-1}}$$

*Where:*
*s = Standard deviation*
*n = Number of samples*
*x = Percent recovery in each sample*

9.2.2.3 Compare *s* and *x* with the precision and percent recovery acceptance criteria specified in Section 13.0. If the value of *s* exceeds the precision limit or the value of *x* falls outside the range for recovery, system performance is unacceptable and the problem must be found and corrected before the analysis may continue.

9.3 Matrix Spike/Matrix Spike Duplicate (MS/MSD)—The laboratory shall spike, in duplicate, a minimum of 10% of all samples (one sample in duplicate in each batch of 10 samples) from a given sampling site.

9.3.1 The concentration of the spike in the sample shall be determined as follows:

9.3.1.1 If, as in compliance monitoring, the concentration of nitrate plus nitrite and nitrite in the sample is being checked against a regulatory concentration limit, the spiking level shall be at that limit or at one to five times higher than the background concentration of the sample (determined in Section 9.3.2), whichever concentration is higher.

9.3.1.2 If the concentration of nitrate plus nitrite and nitrite in a sample is not being checked against a limit, the spike shall be at the concentration of the LCS or at least four times greater than the MDL.

9.3.2 Analyze one sample aliquot out of each set of 10 samples from each site or discharge according to the procedure beginning in Section 10.0 to determine the background concentration of nitrate plus nitrite and nitrite.

9.3.2.1 If necessary, prepare a stock solution appropriate to produce a concentration level in the sample at the regulatory compliance limit or at one to five times the background concentration of nitrate plus nitrite and nitrite (Section 9.3.1).

9.3.2.2 Spike two additional sample aliquots with the spiking solution (Section 9.3.2.1) and analyze these aliquots to determine the concentration after spiking.

9.3.3 Calculate the percent recovery of nitrate plus nitrite and nitrite in each aliquot using Equation 3.

EQUATION 3

$$P = \frac{A - B}{T} \times 100$$

*Where:*
*P = Percent recovery*
*A = Measured concentration of nitrate plus nitrite and nitrite after spiking (Section 9.3.2.2)*
*B = Measured background concentration of nitrate plus nitrite and nitrite (Section 9.3.2)*
*T = True concentration of the spike*

9.3.4 Compare the recovery to the QC acceptance criteria in Section 13.0. If percent recovery is outside of the acceptance criteria, and the recovery of the LCS in the ongoing precision and recovery test (Section 9.6) for the analytical batch is within the acceptance criteria, an interference is present. In this case, the result may not be reported for regulatory compliance purposes.

9.3.5 If the results of both the MS/MSD and the LCS test fail the acceptance criteria, the analytical system is judged to be out of control. In this case, the problem shall be identified and corrected, and the analytical batch must be reanalyzed.

9.3.6 Compute the relative percent difference (RPD) between the two spiked sample results (Section 9.3.2.2, not between the two percent recoveries) using Equation 4.

EQUATION 4

$$RPD = \frac{|D_1 - D_2|}{(D_1 + D_2) / 2} \times 100$$

*Where:*
*RPD = Relative percent difference*
*$D_1$ = Concentration of nitrate plus nitrite and nitrite in the spiked sample*
*$D_2$ = Concentration of nitrate plus nitrite and nitrite in the spiked duplicate sample*

9.3.7 If the RPD is greater than 10%, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected. The analytical batch must be reanalyzed.

9.3.8 As part of the QC program for the laboratory, method precision and accuracy for samples should be assessed and records should be maintained. After the analysis of five spiked samples in which the recovery passes the test in Section 9.3.4, compute the average percent recovery ($P_a$) and the standard deviation of the percent recovery ($s_p$). Express the accuracy assessment as a percent recovery interval from $P_a$–2$s_p$ to $P_a$+2$s_p$. For example, if $P_a$ = 90% and $s_p$ = 10% for five analyses, the accuracy interval is expressed as 70–110%. Update the accuracy assessment on a regular basis (e.g., after each 5–10 new accuracy measurements).

9.4 Laboratory Reagent Blanks (LRB)—Laboratory reagent blanks are analyzed to demonstrate freedom from contamination.

9.4.1 Analyze an LRB initially (i.e., with the tests in Section 9.2) and with each analytical batch. The LRB must be subjected to the exact same procedural steps as a sample.

9.4.2 If nitrate plus nitrite and nitrite is detected in the LRB at a concentration greater than the ML, analysis of samples is halted until the source of contamination is eliminated and consequent analysis of another LRB shows no evidence of contamination.

9.5 Calibration Verification—Verify calibration of the analytical equipment before and after each analytical batch of 14 or fewer measurements. (The 14 measurements will normally be 10 samples, 1 LRB, 1 LCS, 1 MS, and 1 MSD). This can be accomplished by analyzing the midrange calibration standard and verifying that it is within the QC acceptance criteria for recovery in Section 13.0. (The concentration of the calibration verification depends on the calibration range being used.) Failure to attain recoveries within the acceptance criteria requires recalibration of the analytical system (Section 10.4).

9.6 Laboratory Control Sample (LCS)—To demonstrate that the analytical system is in control and acceptable precision and accuracy is being maintained with each analytical batch, the analyst shall perform the following operations:

9.6.1 Analyze an LCS with each analytical batch according to the procedure in Section 10.0.

9.6.2 If the precision and recovery for the LCS are within the acceptance criteria specified in Section 13.0, analysis of the batch may continue. If, however, the concentration is not within this range, the analytical process is not in control. In this event, correct the problem, repeat the LCS test, and reanalyze the batch.

9.6.3 The laboratory should add results that pass the specification in Section 9.6.2 to IPR and previous LCS data and update QC charts to form a graphic representation of continued laboratory performance. The laboratory should also develop a statement of laboratory data quality for {chemical} by calculating the average percent recovery ($R$) and the standard deviation of the percent recovery ($s_r$). Express the accuracy as a recovery interval from $R$–2$s_r$ to $R$+2$s_r$. For example, if $R$ = 95% and $s_r$ = 5%, the accuracy is 85–105%.

9.7 Reference Sample—To demonstrate that the analytical system is in control, the laboratory may wish to periodically test an external reference sample, such as a Standard Reference Material (SRM) available from the National Institute of Standards and Technology (NIST). Corrective action should be taken if the measured concentration significantly differs from the stated concentration.

## 10.0 Configuration and Start-up

10.1 Instrument Configuration

10.1.1 Configure the OI Analytical Flow Solution 3000 Analyzer according to the Operator's Manual and verify that each module is properly powered on.

10.1.2 Verify that the Nitrate+Nitrite and Nitrite, USEPA Cartridge (Part #A001559) is configured as illustrated in the flow diagram shown in Section 17.0.

10.1.3 Connect the appropriate pump tubes to the cartridge and to their appropriate reagent containers according to the flow diagram.

10.2 Instrument Stabilization

10.2.1 Connect the reagent pump tubes to a reagent bottle containing the start-up solution (Section 7.2.2). Start the pump, allowing the start-up solution to flow through the entire system.

10.2.2 Verify that the flowcell of each detector is purged of all bubbles and that the flow is stable and free from surging before proceeding.

10.2.3 If nitrate plus nitrite nitrogen is to be determined, install the activated open tube cadmium reactor (OTCR) (Section 11.1) onto the cartridge manifold as shown in the flow diagram in Section 17.0. If nitrite nitrogen is to be determined singly, do not install the OTCR. If a two-channel system is operated, one cartridge may be configured for nitrate plus nitrite and one cartridge for nitrite only.

10.2.4 Stabilize the freshly activated OTCR before performing an analysis. Inject 10–20 replicates of a high level nitrate calibrant. When the peak height has stabilized, the OTCR is ready for analysis.

10.3 Baseline Verification

10.3.1 Create and save a Method in WinFLOW. Refer to the WinFLOW Operator's Manual (Reference 15.13) for help on creating a Method.

10.3.2 Create and save a Sample Table in WinFLOW that will be used to generate a calibration curve using at least three calibrants that cover the full range of expected concentrations in the samples to be analyzed. This Sample Table should also be used to analyze all necessary QC samples as well as the analytical batch of samples to be analyzed. For help on creating a Sample Table, refer to the WinFLOW Operator's Manual (Reference 15.13).

10.3.3 Select **Collect Data** in the WinFLOW main window, enter the user's identification, select the appropriate Method and Sample Table, and begin to collect baseline data. Very sharp fluctuations in the baseline and/or consistent drifting are typically signs of bubbles in the flowcell. The flowcell must be free of bubbles prior to beginning analysis.

10.4 Calibration and Standardization

10.4.1 Prepare a series of at least three working calibrants using the stock solutions (Section 7.3) according to Equation 1, covering the desired analysis range.

10.4.2 Place the calibrants in the autosampler in order of decreasing concentration. Each calibrant should be analyzed according to the analytical procedures in Section 11.0. A calibration curve will be calculated by the WinFLOW software.

10.4.3 Acceptance or control limits for the calibration results should be established using the difference between the measured value of each calibrant and the corresponding "true" concentration.

10.4.4 Each calibration curve should be verified by analysis of a Laboratory Control Sample (LCS, Section 9.5). Using WinFLOW software, calibration, verification, and sample analysis may be performed in one continuous analysis.

## 11.0 Procedure

11.1 Open Tube Cadmium Reactor (OTCR) Activation

**Note:** Read the entire procedure before continuing. Certain steps are time-sensitive.

**Note**: This procedure must be performed before being installed onto the cartridge.

**Note:** Do not introduce air into the OTCR during the activation process.

11.1.1 Using a 10-mL Luer-Lok™ syringe and a ¼"–28 female Luer-Lok fitting (Part #A000543), slowly flush the OTCR with 10 mL of reagent water. If any debris is seen exiting the OTCR, continue to flush with reagent water until all debris is removed.

11.1.2 Slowly flush the OTCR with 10 mL of 0.5 N hydrochloric acid solution (Section 7.2.7.4). Quickly proceed to the next step as the hydrochloric acid solution can cause damage to the cadmium surface if left in the OTCR for more than a few seconds.

11.1.3 Flush the OTCR with 10 mL of reagent water to remove the hydrochloric acid solution.

11.1.4 Slowly flush the OTCR with 10 mL of 2% cupric sulfate solution (Section 7.2.7.3). Leave this solution in the OTCR for approximately 5–10 minutes.

11.1.5 Forcefully flush the OTCR with 10 mL of stock ammonium chloride/EDTA buffer solution (Section 7.2.3) to remove any loose copper that may have formed within the reactor. Continue to flush until all debris is removed.

11.1.6 The OTCR should be stored filled with reagent water when not in use.

**Note**: Buffer containing Brij-35 should not be used when flushing or storing the OTCR.

11.2 Analysis

11.2.1 Begin pump flow with the start-up solution (Section 7.2.2). and verify a stable baseline (Section 10.3).

11.2.2 After the baseline has been verified, place all reagents on-line and allow to pump at least 10–15 minutes and verify there are no bubbles in the flowcell. Obtain a stable baseline at 540 nm and autozero the baseline before beginning the analysis.

11.2.3 Load the sampler tray with calibrants, blanks, samples, and QC samples.

**Note**: The matrix of the working standards, blanks, and QC samples should match that of the samples being analyzed.

11.2.4 Using the Method and Sample Table created for the analytical batch to be analyzed and with the baseline verified to be stable, begin the analysis by selecting the “Fast Forward” button on the left side of the Data Analysis window in WinFLOW. This will initiate the sequential analysis of samples as defined in the Sample Table.

11.2.5 When analysis is complete, pump start-up solution through the system for at least 10–15 minutes. Stop the pump, release the tension on all pump tubes, and power off the system.

11.3 Operating Notes

11.3.1 Reduction Efficiency of the OTCR

11.3.1.1 In the OTCR, nitrate is reduced to nitrite. However, under some conditions, reduction may proceed further with nitrite being reduced to hydroxylamine and ammonium ion. These reactions are pH dependent:

$$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O \qquad (1)$$
$$NO_2^- + 6H^+ + 6e^- \rightarrow H_3NOH^- + H_2O \qquad (2)$$
$$NO_2^- + 8H^+ + 6e^- \rightarrow NH_4^+ + 2H_2O \qquad (3)$$

At the buffered pH of this method, reaction (1) predominates. However, if the cadmium surface is overly active, reactions (2) and (3) will proceed sufficiently to give low results for nitrite.

11.3.1.2 If the cadmium surface is insufficiently active, there will be a low recovery of nitrate as nitrite (Reference 15.5). This condition is defined as poor reduction efficiency.

11.3.1.3 To determine the reduction efficiency, run a high-level nitrite calibrant followed by a nitrate calibrant of the same nominal concentration. The reduction efficiency is calculated as shown in Equation 5.

EQUATION 5

$$PR = \frac{|N3 - N2|}{N2} \times 100$$

*Where*:
*PR = Percent reduction efficiency*
*N3 = Nitrate peak height*
*N2 = Nitrite peak height*

11.3.1.4 If the response of the nitrite is as expected but the reduction efficiency is less than 90%, then the OTCR may need to be reactivated (Section 11.1).

11.3.1.5 If the response of the nitrite is lower than expected and/or the reduction efficiency is greater than 110%, the cadmium may need to be stabilized. To stabilize the reactor, analyze 15 replicates of a high-level concentration nitrate calibrant followed by three replicates of an equivalent nitrite calibrant. The high-level nitrate calibrant will allow the cadmium to stabilize by slightly reducing its activity to a more consistent level. The high-level nitrite calibrant should be compared to the nitrate calibrant to ensure that a reduction efficiency between 90% and 110% is being obtained.

## 12.0 Data Analysis and Calculations

12.1 The calibration curve allows for accurate quantitation of the concentration in each sample.

12.2 WinFLOW software reports the concentration of each sample relative to the calibration curve.

## 13.0 Method Performance

| | |
|---|---|
| Range: | 0.01–10 mg/L |
| Throughput: | 65 samples/hour |
| Precision: | |
| 0.05 mg/L | <1% RSD |
| Method Detection Limit (MDL): | 0.001 mg/L |

## 14.0 Pollution Prevention and Waste Management

14.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land-disposal restrictions. In addition, it is the laboratory's responsibility to protect air, water, and land resources by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations.

14.2 For further information on waste management, consult Section 13.6 of *Less is Better: Laboratory Chemical Management for Waste Reduction* (Reference 15.6).

## 15.0 References

15.1 Norwitz, G.; Keliher, P.N. Study of Interferences in the Spectrophotometric Determination of Nitrite Using Composite Diazotization-Coupling Reagents. *Analyst* **1985**, *110*, 689–694.

15.2 Norwitz, G.; Keliher, P.N. Study of Interferences in the Spectrophotometric Determination of Nitrite Using Composite Diazotization-Coupling Reagents. *Analyst* **1986**, *111*, 1033–1037.

15.3 *Code of Federal Regulations*, Part 136, Title 40, Appendix B, 1994.

15.4 *Handbook for Analytical Quality Control in Water and Wastewater Laboratories*; EPA-600/4-79-019; U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1979.

15.5 Fox, J.B. *J. Anal. Chem.* **1979**, 51, 1493.

15.6 *Less is Better: Laboratory Chemical Management for Waste Reduction.* Available from the American Chemical Society, Department of Government Regulations and Science Policy, 1155 16th Street, NW, Washington, DC, 20036.

15.7 Nitrogen, Nitrate-Nitrite (Colorimetric, Automated, Cadmium Reduction). *Methods for the Chemical Analysis of Water and Wastes*; EPA/600/R-79-020; U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1984; Method 353.2.

15.8 Sample Preservation. *Methods for Chemical Analysis of Water and Wastes*; EPA-600/4-79-020; U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1984; xvii.

15.9 Patton, C.J. Doctoral Dissertation, Michigan State University, 1982.

15.10 *Standard Methods for the Examination of Water and Wastewater*, 14th ed.; APHA-AWWA-WPCF: Washington, D.C., 1975, 365.

15.11 *Standard Methods for the Examination of Water and Wastewater*, 17th ed.; American Public Health Association: Washington, D.C., 1989; 4–178.

15.12Nydahl, F. *Talanta* **1976**, 23, 349–357.

15.13WinFLOW Software and Operator's Manual (Part #A002877). Available from OI Analytical, P.O. Box 9010, College Station, TX, 77842-9010.

## 16.0 Glossary of Definitions and Purposes

The definitions and purposes are specific to this method but have been conformed to common usage as much as possible.

16.1 Units of weights and measures and their abbreviations

16.1.1 Symbols

| | |
|---|---|
| °C | degrees Celsius |
| % | percent |
| ± | plus or minus |
| ≥ | greater than or equal to |
| ≤ | less than or equal to |

16.1.2 Alphabetical characters

| | |
|---|---|
| g | gram |
| L | liter |
| mg | milligram |
| mg/L | milligram per liter |
| µg | microgram |
| µg/L | microgram per liter |
| mL | milliliter |
| ppm | parts per million |
| ppb | parts per billion |
| M | molar solution |
| N | normal solution |

16.2 Definitions

16.2.1 Initial Precision and Recovery (IPR)—Four aliquots of the LRB spiked with the analytes of interest and used to establish the ability to generate acceptable precision and accuracy. An IPR is performed the first time this method is used and any time the method or instrumentation is modified.

16.2.2 Laboratory Control Sample (LCS)—An aliquot of LRB to which a quantity of the analyte of interest is added in the laboratory. The LCS is analyzed like a sample. Its purpose is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements.

16.2.3 Laboratory Reagent Blank (LRB)—An aliquot of reagent water and other blank matrix that is treated like a sample, including exposure to all glassware, equipment, and reagents that are used with other samples. The LRB is used to determine if the method analyte or other interferences are present in the laboratory environment, reagents, or apparatus.

16.2.4 Matrix Spike/Matrix Spike Duplicate (MS/MSD)—An aliquot of an environmental sample to which a quantity of the method analyte is added in the laboratory. The MS/MSD is analyzed like a sample. Its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentration of the analyte in the sample matrix must be determined in a separate aliquot, and the measured values in the MS/MSD must be corrected for the background concentration.

16.2.5 Method Detection Limit (MDL)—The minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero.

16.2.6 Minimum Level (ML)—The level at which the entire analytical system will give a recognizable signal and acceptable calibration point, taking into account method-specific sample and injection volumes.

16.2.7 Ongoing Precision and Recovery (OPR)—See Section 16.2.2, “Laboratory Control Sample.”

## 17.0 Figures

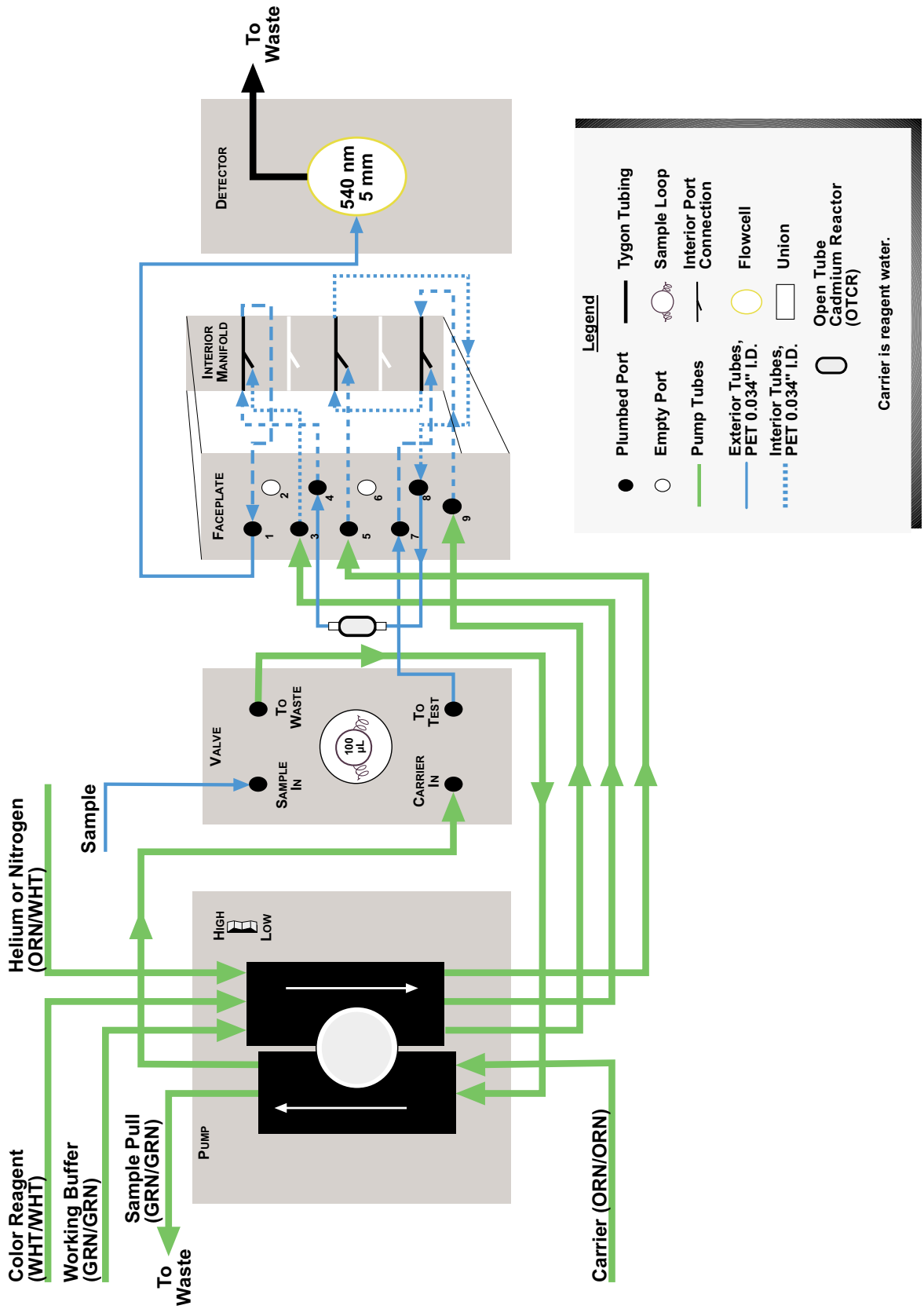


Figure 1. Detailed Flow Diagram for Nitrate+Nitrite and Nitrite, USEPA by FIA on a Flow Solution 3000, Cartridge Part #A001559

Results were obtained under optimal operating conditions. Actual results may vary depending on sample introduction, cleanliness of sample containers, reagent purity, operator skill, and maintenance of instruments.



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