1) Nitrite

a) Scope and Application

i) This method provides a procedure for the determination of low level nitrite concentrations normally found in estuarine and/or coastal waters.

ii) The method detection limit (MDL) is determined on a yearly basis. This MDL is defined as the student t times the standard deviation of at least seven replicates of a low level. The range is determined by the instrument used, its configuration, and the standard curve that is used.

iii) This method should be used by analysts experienced in the use of automated colorimetric analyses, matrix interferences and procedures for their correction. Analyst training and/or a demonstration of capability should be documented.

b) Summary of Method

i) This method is an automated colorimetric method for the analysis of low level nitrite concentrations. Filtered samples are analyzed by diazotizing with sulfanilamide and coupling with N-1-naphthylethylenediamine dihydrochloride to form a colored azo dye. The color produced is proportional to the nitrite concentration present in the sample.

c) Interferences

i) Metal ions may produce a positive error if present in sufficient concentrations.

ii) Sample turbidity should be removed by filtration prior to analysis.

iii) Refractive Index interferences should be corrected for when analyzing estuarine/coastal samples. Alternatively, match the salinity of the calibration standards to the salinity of the samples.

d) Apparatus and Materials

i) Continuous flow automated analytical system equipped with an autosampler, manifold, proportioning pump, colorimeter, phototube or recorder or computer based data system. Flow injection or discrete analysis may also be used.

ii) Nitrogen-free glassware: All glassware used in the determination must be low in residual nitrite to avoid sample/reagent contamination. Washing with 10% HCl and thoroughly rinsing with reagent water has been found to be effective. Some laboratories use critical cleaning liquid detergents instead of or before acid rinsing. A laboratory's glassware cleaning method will be considered sufficient if all quality control samples are within the expected ranges.

e) Reagents

i) Stock reagent solutions: The specific recipe for these reagents is generally instrument dependent, and may change due to the concentration of the samples being analyzed. In this SOP the chemical s needed for the reaction will be listed, but not the specific amounts. If continuous flow is being utilized an appropriate surfactant like Brij is added to one or more of the reagents.

(1) Ammonium Chloride Reagent: This reagent is used as a buffer solution. In may not be needed in discrete analysis. Ammonium chloride is used and the pH of the solution is adjusted to a basic solution using sodium hydroxide or ammonium hydroxide. Ethylenediamine tetra acetic acid disodium salt (EDTA) may be added to complex with metals to remove this source of interference.

NOTE: If analyzing ammonia at the same time as nitrite samples then the use of sodium hydroxide is recommended.

(2) Imidazole Buffer: This reagent can be used as a buffer solution in place of the Ammonium Chloride Reagent.
Imidazole and a small amount of hydrochloric acid is used for a buffer that has an approximate pH of 7.4.

(3) Color Reagent: Phosphoric acid, sulfanilamide and N-1 napthylethylenediamine dihydrochloride are combined. Another acid, such as hydrochloric acid, may be substituted depending on the instrument used.

(4) Refractive Reagent: Use if necessary. Should be made the same as the Color Reagent, omitting the sulfanilamide and N-1 napthylethylenediamine dihydrochloride

(5) Stock Nitrite Solution: A laboratory prepared or purchased stock standard can be used. If the stock standard is prepared in the laboratory, a purchased stock standard should be used as a calibration check standard.

ii) Reagent water: see Chapter VI, Section 4.2.

iii) Artificial Sea Water (ASW): see Chapter VI, Section 4.3. This can be used for the matrix at an appropriate salinity for the samples being analyzed. If precipitation occurs eliminate the magnesium sulfate.

iv) Prepare a series of standards by diluting suitable volumes with reagent or ASW water. Prepare these standards daily. When working with samples of known salinity it is recommended that the standard curve concentrations be prepared in substitute ocean water diluted to that salinity and that the sampler wash solution also be substitute ocean water diluted to that salinity. When analyzing samples of varying salinities, it is recommended that the standard curve be prepared in reagent water and Refractive Index corrections be made to the sample concentrations. Standards should bracket the expected concentration of the samples. In Chesapeake Bay Tidal Laboratories the range can be as low as 0.001to 0.040 mg N/L for samples near the Bay mouth, to as high as 0.03 – 0.30 mg N/L when very high nitrite samples are encountered.

v) Saline nitrite standards: When analyzing samples of varying salinities, it is also recommended that standards be prepared in a series of salinities in order to quantify the "salt error," the shift in the colorimetric response of nitrite due to the change in the ionic strength of the solution.

f) Sample Handling

i) Samples must be analyzed as quickly as possible. If the samples are to be analyzed within 48 hours of collection, then refrigeration at $4 \pm 2°C$ is acceptable.

ii) If samples will not be analyzed within 48 hours of collection, the sample must be stored at ≥ -20 for a maximum of 28 days.

g) Procedure

i) Calibration: Standard curve(s) bracket the concentration of expected samples should be analyzed.

ii) Sample analysis

(1) If samples have not been freshly collected and are frozen, thaw the samples to room temperature.

(2) Allow both colorimeter and recorder to warm up for 30 minutes or the specific instrument recommendation. Obtain a steady instrument state that is necessary for the instrument to be ready to collect data.

(3) Use a sampling rate which ensures reliable results.

(4) Analytical sequence: The samples and associated QC samples and standards should be run according to the following sequence.

(a) A calibration curve containing a minimum of four calibration standards with concentration within the linear range of the test and two zero standards.

(b) One LCS/QCS, one method blank and one initial calibration verification standard.

(c) Ten to twenty CBP samples.

(d) One duplicate sample, one matrix spike sample, one medium concentration calibration standard and one method blank.

(e) Steps c through d are repeated until samples are analyzed or QC samples indicate that the system is out of control and recalibration is necessary.

(5) If a low concentration sample peak follows a high concentration sample peak, a certain amount of carryover can be expected. It is recommended that if there is not a clearly defined low concentration peak, that the sample be reanalyzed at the end of the sample run.

iii) Calculations

(1) Nitrite concentrations are calculated from the linear regression obtained from the standard curve in which the concentrations of the standards are entered as the independent variable and their corresponding peak heights are the dependent variable.

(2) Refractive Index Correction For Estuarine/Coastal Systems can be performed if necessary for the instrument in use.

(3) Results should be reported in mg N/L.

h) Quality Control

i) Method detection limits (MDL): Method detection limits should be established using the procedures in Chapter VI, Section C.8.

ii) Calibration

(1) Linear calibration range: Calibration standards should bracket the range of CBP samples.

(2) Correlation coefficient: The correlation coefficient must be 0.995 or better for the calibration curve to be used.

iii) Method Blank: see Chapter VI, Section C.6.1.

iv) Matrix spike sample: see Chapter VI, Section C.6.4.

v) Laboratory duplicate: see Chapter VI, Section C.6.3.

vi) Reference materials: The laboratory must analyze a standard/certified reference material or proficiency testing sample at least once a year, as available.

Summary Table

QC INDICATOR	ACCEPTANCE/ ACTION LIMITS	ACTION	FREQUENCY (BATCH)
Correlation Coefficient	0.995	If < 0.995, evaluate data points of the calibration curve. If the lowest standard is an outlier, it can be rejected if no samples are reported below the lowest standard kept. If the highest standard is an outlier, it can be rejected if no samples are reported above the value of the highest standard kept.	1 per batch if acceptable.
LCS/QCS	± 10%	If QCS value is outside ± 10% of the target value, correct the problem and reanalyze LCS/QCS. If still outside the limit, recalibrate and reanalyze.	Beginning of run after calibration curve.
ICV	± 10%	Recalibrate if outside acceptance limits.	Beginning of run following standard curve.
CCV	± 10%	If outside 10%, correct the problem. Rerun all samples following the last in-control CCV.	After every 10 to 20 samples and at end of batch
Method Blank	Method Quantitation Limit	If the method blank exceeds the quantitation limit, results are suspect. Rerun the method blank. If the concentration still exceeds the quantitation limit, reject or qualify the data, or raise the quantitation limit.	One at the beginning after the calibration curve, than after every 10 to 20 samples and at the end of the run.
Method Quantitation Limit (MQL): The concentration of the lowest standard.		If not used in calibration curve and run as a calibration check: When the value is outside the predetermined limit and the ICV is acceptable, reanalyze the sample. If the reanalysis is unacceptable, increase the concentration and reanalyze. If this higher concentration meets the acceptance criteria, raise the reporting limit for the batch.	Beginning of run or in calibration curve.

Laboratory Fortified Sample Matrix Spike	80 to 120%	If the recovery of any analyte falls outside the designated acceptance limits and the QCS is in control, the recovery problem is judged matrix induced. Repeat the LFM and if the sample results are again outside the acceptable recovery range, the sample should be reported with a "matrix induced bias" qualifier.	After every 10 to 20 samples
Laboratory Duplicate	± 20% or ≤ method quantitation limit	If the RPD fails to meet the acceptance limits, the samples should be reanalyzed. If the RPD again fails to meet the acceptance limits, the sample must be reported with a qualifier identifying the sample analysis result as not having acceptable RPD for duplicate analysis.	After every 10 to 20 samples.

i) References

(a) EPA 1993. “Methods for the Determination of Inorganic Substances in Environmental Samples,’’ NERL–CI, EPA/600/R–93/100, August, 1993. Method 353.2, Rev. 2.0, Nitrite (as N) - Automated, spectrophotometric, “bypass” cadmium reduction.

(b) EPA 1997. Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Matrices - 2nd Edition: EPA EPA/600/R-97/072. Method 353.4 Determination of Nitrate and Nitrite in Estuarine and Coastal Waters by Gas Segmented Continuous Flow Colorimetric Analysis, Rev. 2

(c) Fishman, M.J., ed., 1993, Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory – Determination of inorganic and organic constituents in water and fluvial sediments: U.S. Geological Survey Open-File Report 93-125, 217 p. Method ID: I-2545-90

(d) MacDonald, R.W. and F.A. McLaughlin. 1982. The effect of storage by freezing on dissolved inorganic phosphate, nitrate, and reactive silicate for samples from coastal and estuarine waters. Water Research, 16:95-104.