Designation: D3867 – 16

Standard Test Methods for Nitrite-Nitrate in Water[1]

This standard is issued under the fixed designation D3867; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope*

1.1 These test methods cover the determination of nitrite nitrogen, nitrate nitrogen, and combined nitrite-nitrate nitrogen in water and wastewater in the range from 0.05 to 1.0 mg/L nitrogen. Two test methods[2] are given as follows:

	Sections
Test Method A—Automated Cadmium Reduction	9 to 18
Test Method B—Manual Cadmium Reduction	19 to 28

1.2 These test methods are applicable to surface, saline, waste, and ground waters. It is the user's responsibility to ensure the validity of these test methods for waters of untested matrices.

1.3 The values stated in either SI or inch-pound units are to be regarded as the standard. No other units of measurement are included in this standard.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* For specific hazard statements, see Notes 1 and 2.

2. Referenced Documents

2.1 *ASTM Standards:*[3]

D1129 Terminology Relating to Water

D1141 Practice for the Preparation of Substitute Ocean Water

D1193 Specification for Reagent Water

D2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water

D3370 Practices for Sampling Water from Closed Conduits

D5810 Guide for Spiking into Aqueous Samples

D5847 Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis

D7781 Test Method for Nitrite-Nitrate in Water by Nitrate Reductase

E60 Practice for Analysis of Metals, Ores, and Related Materials by Spectrophotometry

E275 Practice for Describing and Measuring Performance of Ultraviolet and Visible Spectrophotometers

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in these test standards, refer to Terminology D1129.

4. Summary of Test Methods

4.1 *Total Oxidized Nitrogen*—a filtered sample is passed through a column containing copper-coated cadmium granules to reduce nitrate ion to nitrite ion. The combined nitrite-nitrate nitrogen is determined by diazotizing the total nitrite ion with sulfanilamide and coupling with *N*-(1-naphthyl)ethylenediamine dihydrochloride to form a highly colored azo dye that is measured spectrophotometrically.

4.2 *Nitrite*—the nitrite ion originally present in the sample can be determined separately by carrying out the procedure and omitting the cadmium reduction step.

4.3 *Nitrate*—the nitrate ion can be calculated as the difference between the combined nitrite-nitrate nitrogen and the nitrite nitrogen.

5. Significance and Use

5.1 Both test methods use identical reagents and sample processing. The only difference between the two methods is that one test method is automated and the other is manual. The ranges and interferences are identical.

5.2 The automated test method is preferred when large numbers of samples are to be analyzed. The manual test method is used for fewer samples or when automated instrumentation is not available.

5.3 These test methods replace Test Methods D1254 (Nitrite) and D992 (Nitrate). The nitrite test method (Test Method D1254) used a reagent that is considered to be a potential carcinogen. The nitrate test method (Test Method D992) has

[1] These test methods are under the jurisdiction of ASTM Committee D19 on Water and are the responsibility of Subcommittee D19.05 on Inorganic Constituents in Water.

Current edition approved June 1, 2016. Published June 2016. Originally approved in 1979. Last previous edition approved in 2009 as D3867 – 09. DOI: 10.1520/D3867-16.

[2] Methods similar to these appear in *Methods of Chemical Analysis of Water and Wastes*, 2nd edition, U.S. Environmental Protection Agency.

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

***A Summary of Changes section appears at the end of this standard**

been shown to have relatively large errors when used in wastewaters and also has greater manipulative difficulties than the test method described herein.

5.4 Test Method D7781 uses a nitrate reductase enzyme for the reduction of nitrate to nitrite. Cadmium is considered a toxic metal. Also, the heterogeneous cadmium reductant creates greater difficulty than the reduction described in this test method.

6. Interferences

6.1 Turbid samples must be filtered prior to analysis to eliminate particulate interference. Furthermore, sample turbidity results in a buildup on the reduction column that restricts sample flow.

6.2 Sample color that absorbs at wavelengths between 520 and 540 nm interferes with the absorbance measurements. When color is suspect, analyze a sample blank, omitting the *N*-(1-naphthyl)ethylenediamine dihydrochloride from the color reagent.

6.3 Oil and grease in the sample coat the surface of the cadmium and prevent complete reduction of nitrate to nitrite. This interference is usually removed by filtration prior to analysis. If filtration is not adequate, the interference can be removed by extracting the sample with an *n*-hexane or a solid phase extraction (SPE) filter.

6.4 Certain metal ions, in concentrations above 35 mg/L, may cause interferences. For example, Hg (II) and Cu (II) may form colored complex ions having absorption bands in the region of color measurement. Iron and manganese are other reported examples of interference.

6.5 Excessive amounts of chlorine will deactivate the reducing column. Chlorine might be present in some Type II water. The use of chlorine-containing Type II water will lead to a negative interference because nitrite and chlorine do not normally coexist. This is of particular importance when preparing standards or spiked samples (Guide D5810).

6.6 In acid samples (pH less than 4.5) nitrate is not reduced in the cadmium column. To overcome this interference, the sample must be neutralized to a pH of between 6 and 8 prior to analysis.

7. Purity of Reagents

7.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, when such specifications are available.[4] Other grades may be used, provided it is first ascertained that the reagent is of sufficient high purity to permit its use without lessening the accuracy of the determination.

7.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Specification D1193, Type I. Other reagent water types may be used, provided it is first ascertained that the water is of sufficiently high purity to permit its use without adversely affecting the bias and precision of these test methods. Type II water was specified at the time of round-robin testing of these test methods.

8. Sampling and Sample Preservation

8.1 Collect the sample in accordance with Practices D3370.

8.2 When nitrite ion is to be determined separately, analyze as soon as possible after sampling. Even when sterile bottles are used, bacteria naturally present in the water may cause conversion of all or part of nitrite ion to other forms such as nitrate or ammonia. Ammonia and natural amines, which are frequently present in natural waters, may react with nitrites to form nitrogen. If samples are to be stored for 24 h or less, preserve the sample by refrigeration at 4°C. If the sample must be stored for more than 24 h, preserve it by the addition of 2 mL of chloroform per litre (11.7 and 11.9) in addition to refrigeration at 4°C.

NOTE 1—**WARNING**: Chloroform is toxic and is a suspected human carcinogen. Use with adequate ventilation or in a fume hood. Wear prescribed protective equipment. Use of chloroform is discouraged, since its use renders the solution a hazardous waste.

NOTE 2—**CAUTION**: The common prescribed use of sulfuric acid or mercury compounds as preservatives is discouraged. Sulfuric acid does not necessarily inhibit oxidation and mercury compounds should be avoided to prevent environmental pollution. Mercuric chloride is known to deactivate the column.

TEST METHOD A—AUTOMATED CADMIUM REDUCTION

9. Scope

9.1 The applicable range of this test method is from 0.05 to 1 mg/L of nitrite or nitrate nitrogen. The range may be extended upward by dilution of an appropriate aliquot. Many workers have found that this test method is reliable for nitrite and combined nitrite-nitrate levels to 0.01 mg N/L. However, the precision and bias data presented in this test method are insufficient to justify application of this test method in the 0.01 to 0.05 mg/L-N range.

9.2 This test method is applicable to surface, saline, waste, and ground waters. It is the user's responsibility to ensure the validity of this test method for waters of untested matrices.

10. Apparatus

10.1 *Automated Analysis System*[5] consisting of:

10.1.1 *Sampler.*

10.1.2 *Manifold or Analytical Cartridge.*

10.1.3 *Colorimeter* equipped with a 15- or 50-mm tubular flow cell and 540 ± 10-nm filters.

10.1.4 *Recorder or Electronic Data Acquisition Device.*

[4] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For Suggestions on the testing of reagents not listed by the American Chemical Society, see *Annual Standards for Laboratory Chemicals,* BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary,* U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

[5] The apparatus described is commercially available. ASTM does not undertake to ensure anyone utilizing an automated analysis system against liability of infringement of patent or assume such liability.

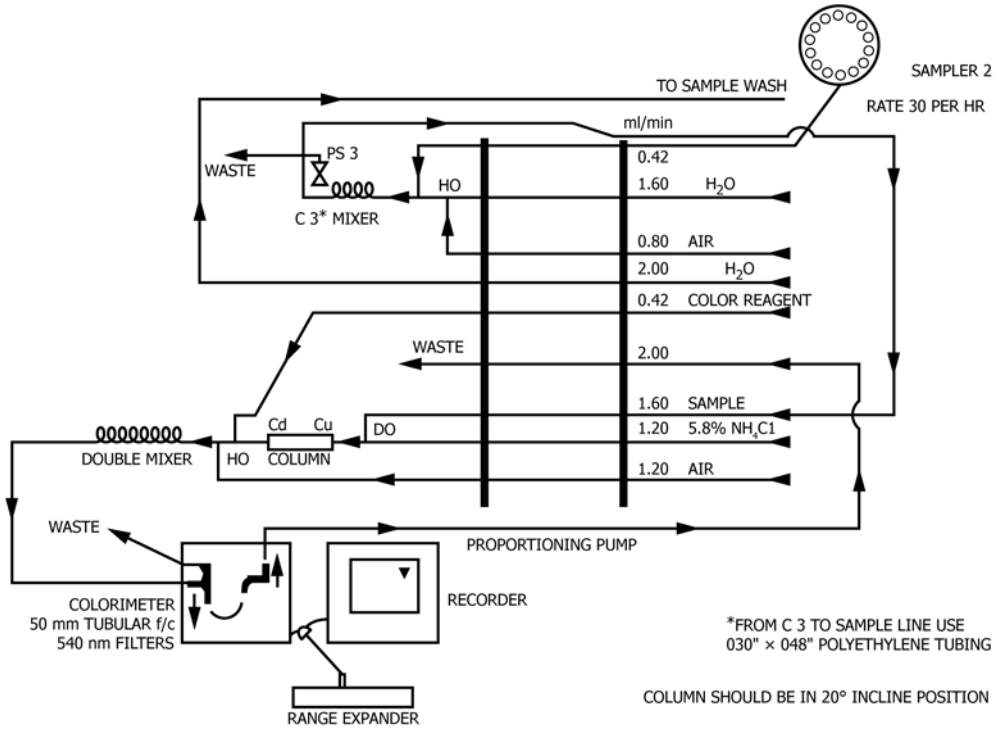

FIG. 1 Nitrite-Nitrate Manifold

10.1.5 *Digital Printer (Optional).*

10.1.6 *Continuous Filter (Optional).*

10.2 *Reduction Columns*—Choose the appropriate reduction column for the manifold system. A schematic drawing of the manifold system is shown in Fig. 1 and the cartridge system is shown in Fig. 2.

10.2.1 *Reduction Column,* a glass tube 8 by 50 mm with the ends reduced in diameter to permit insertion into the system (see Fig. 1).

10.2.2 *Reduction Column,* a U-shaped glass tubing, 350-mm length and 2-mm inside diameter.

NOTE 3—A pump tube with 0.081-in. (2.1-mm) inside diameter can be used in place of the 2-mm glass tube.

11. Reagents and Materials

11.1 *Ammonium Chloride Solution* (85 g/L)—Dissolve 85 g of ammonium chloride (NH_4Cl) in water and dilute to 1 L. Add 0.5 mL wetting agent.[6]

11.2 *Cadmium,* 40 to 60 mesh, granulated.[7]

11.3 *Color Reagent*—Add the following to 800 mL of water, while stirring constantly: 100 mL of concentrated phosphoric acid (H_3PO_4), 10 g of sulfanilamide, and 0.5 g of *N*-1-(naphthyl)ethylenediamine dihydrochloride. Stir until dissolved. Add 1 mL of wetting agent,[6] and dilute to 1 L with water. This solution is stable for about a month when stored in a brown bottle in a dark cool place.

11.4 *Copper Sulfate Solution* (20 g/L)—Dissolve 20 g of copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) in 500 mL of water. Dilute to 1 L.

11.5 *n-Hexane.*

11.6 *Hydrochloric Acid* (1 + 1)—Slowly add 50 mL of concentrated hydrochloric acid (HCl) to 40 to 45 mL of water and dilute to 100 mL.

11.7 *Nitrate Solution, Stock* (1.0 mL = 1.0 mg NO_3-N)—Dry potassium nitrate (KNO_3) in an oven at 105°C for 24 h. Dissolve 7.218 g in water in a 1-L volumetric flask. Dilute to the mark with water. This solution is stable for up to 1 month with refrigeration. If longer stability is required or refrigeration is not available, add 2 mL of chloroform as a preservative and store in a dark bottle. This solution is stable for 6 months. (See Note 1.) Alternatively, certified nitrate stock solutions are commercially available through chemical supply vendors and may be used.

11.8 *Nitrate Solution, Standard* (1.0 mL = 0.01 mg NO_3-N)—Dilute 10 mL of stock nitrate solution (11.7) to 1 L with water and store in a dark bottle. Prepare fresh as needed.

11.9 *Nitrite Solution, Stock* (1.0 mL = 1.0 mg NO_2-N)—Place about 7 g of potassium nitrite (KNO_2) in a tared 125-mL beaker and dry for about 24 h to a constant weight in a desiccator containing a suitable desiccant. Adjust the weight of the dry potassium nitrite to 6.072 g. Add 50 mL of water to the beaker, stir until dissolved, and transfer quantitatively to a

[6] A 30 % aqueous solution of Brij® 35, a polyoxyethylene compound with dodecyl alcohol (sp gr 1.18 to 1.22) has been found satisfactory for this purpose.

[7] Different sizes of granulated cadmium may be used. The analyst should ensure that adequate reduction occurs with the size chosen.

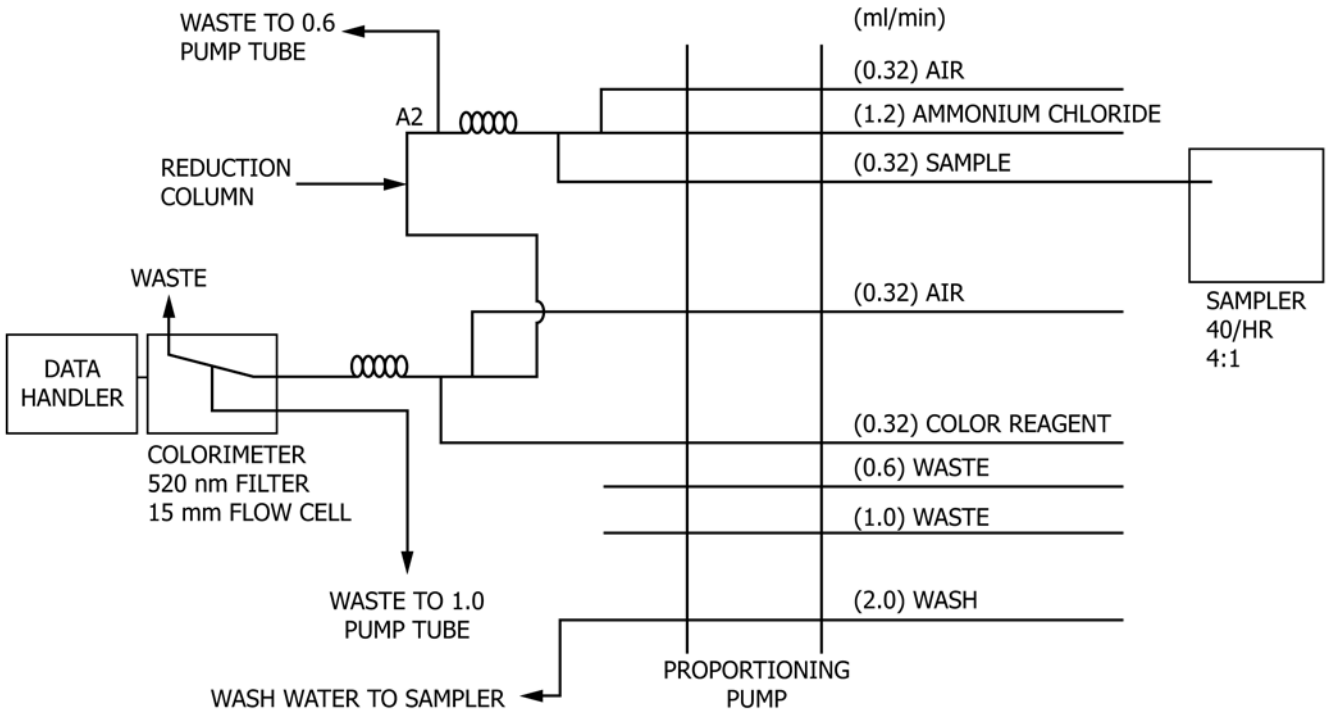

FIG. 2 Nitrite-Nitrate Cartridge

1000-mL volumetric flask. Dilute to the mark with water store in a sterilized bottle under refrigeration. Prepare fresh as needed. Alternatively, certified nitrite stock solutions are commercially available through chemical supply vendors and may be used.

NOTE 4—Potassium nitrite is easily oxidized, so use only fresh bottles of this reagent.

11.10 *Nitrite Solution, Standard* (1.0 mL = 0.01 mg NO_2-N)—Dilute 10 mL of stock nitrite solution (11.9) to 1 L with water. This solution is unstable; prepare fresh as needed.

11.11 *Filter Paper*—Purchase suitable filter paper. Typically the filter papers have a pore size of 0.45-µm membrane. Material such as fine-textured, acid-washed, ashless paper, or glass fiber paper are acceptable. The user must first ascertain that the filter paper is of sufficient purity to use without adversely affecting the bias and precision of the test method.

12. Preparation of Reduction Column

12.1 *Cadmium Granules Treatment*—Clean and copperize new or used cadmium granules in the following manner:

12.1.1 Clean about 10 g of cadmium granules (11.2) by washing with dilute HCl (11.6) and rinsing with water.

12.1.2 Swirl the clean cadmium in 100-mL portions of copper sulfate solution (11.4) in a beaker for 5 min or until the blue color partially fades. Decant and repeat with fresh copper sulfate until the first visible brown colloidal precipitate appears.

12.1.3 Wash the granules with water at least 10 times to remove all of the precipitated copper.

12.2 *Filling the Reduction Column:*

12.2.1 Insert a small plug of glass wool in one end of the column (10.2).

12.2.2 Fill the column with water to prevent the entrapment of air bubbles during the filling operation.

12.2.3 Fill the column with copper-cadmium granules, tap to pack the granules, and plug the open end with glass wool.

12.3 *Installation of Reduction Column*—Install the copper-cadmium reduction column in the automatic analyzer system. Purge the system with ammonium chloride solution (11.1) using water in the sample line. Observe the following precautions while installing the reduction column:

12.3.1 Place the column in the manifold system in an upflow 20° incline to minimize channeling (see Fig. 1).

12.3.2 Fill all pump tubes with reagents before inserting the column in the cartridge system to prevent the entrapment of air bubbles.

12.4 *Reduction Column Storage*—When it is not in use, put the sample line in water and purge the column with ammonium chloride solution (11.1) and water.

NOTE 5—Do not allow air to enter the column and do not let the cadmium granules become dry. If this occurs, refill the column with freshly treated cadmium granules.

13. Calibration

13.1 Using the standard nitrate solution (11.8) prepare calibration standards by pipetting specified volumes of the standard solution into 100-mL volumetric flasks and diluting to the mark with water. Table 1 specifies the millilitres of standard solution required.

13.2 Prepare at least one calibration standard from the standard nitrite solution (11.10) at the same concentration as

TABLE 1 Concentration of Calibration Standards, Automated Cadmium Reduction

NO_3-N or NO_2-N, mg/L	mL Standard Solution/100 mL
0.01	0.1
0.02	0.2
0.04	0.4
0.1	1.0
0.2	2.0
0.4	4.0
0.7	7.0
1.0	10.0

one of the nitrate standards to verify the efficiency of the reduction column. Repeat this when a suspected loss in NO_3-N reduction is observed.

NOTE 6—When the sample to be analyzed is a saline water, use substitute ocean water (SOW) to prepare the standards (Practice D1141). Run a reagent water blank in addition to a SOW blank because the reagents used to prepare SOW frequently contain nitrite or nitrate, or both. Adjust this curve for the contaminant level in SOW.

13.3 Develop the color and determine the absorbance of each standard as directed in the procedure (14.5).

13.4 Use a direct reading instrument or prepare a standard curve by plotting the peak heights of each processed calibration standard against its known concentrations.

14. Procedure

14.1 *Removal of Interferences*—Remove interferences (Section 6) by the following procedures:

14.1.1 For turbidity removal, when suspended solids are present, filter the sample through a glass-fiber filter or a 0.45-µm filter. Alternatively, use a continuous filter (11.11) as an integral part of the system to remove particulate matter. Centrifugation can be used as an option.

14.1.2 For oil and grease removal, if necessary after filtration, adjust the pH of the sample to 2 with concentrated HCl. Extract with two 25-mL portions of *n*-hexane (11.5) in a separatory funnel. Discard the *n*-hexane layer after each extraction. Alternatively, solid-phase extraction filters may be used.

14.1.3 For pH adjustment, determine the pH of the sample with a pH meter. Adjust the pH to within the range from 6 to 8 with concentrated HCl or concentrated NH_4OH, if needed.

14.1.4 For correction for color interferences, if there is a possibility that the color of the sample might absorb in the photometric range from 530 ± 10 nm, determine the background absorbance. Replace the color reagent (11.3) with a similar reagent where just the *N*-1-(naphthyl) ethylenediamine dihydrochloride is omitted and analyze the sample for background color absorbance as directed in the following procedure. Repeat the analysis using the complete color reagent.

14.2 Depending on the model of analysis system available, set up the manifold and complete the system as shown in Fig. 1 or Fig. 2.

NOTE 7—When determining nitrite alone, omit the reduction column from the manifold system.

14.3 Turn on the colorimeter and the recorder and allow them both to warm up for 30 min.

TABLE 2 Interlaboratory Precision for Nitrite Found in Selected Matrices

Water Matrix:				
Concentration (*x*),mg/L	0.05	0.09	0.42	0.80
S_T	0.024	0.006	0.033	0.049
S_O	0.012	0.005	0.029	0.043
Reagent Water:				
Concentration (*x*), mg/L	0.05	0.09	0.42	0.80
S_T	0.021	0.005	0.019	0.032
S_O	0.009	0.002	0.011	0.006

14.4 Obtain a stable baseline with all reagents (11.1 and 11.3), feeding water through the sample line.

14.5 Place the appropriate nitrate and nitrite calibration standards in the sampler in order of decreasing concentration of nitrogen. Fill the remainder of the sample tray with unknown samples.

14.6 For the manifold system, sample at a rate of 30/h, 1 + 1 cam. For the cartridge system, use a 40/h, 4 + 1 cam and a common wash.

14.7 Switch the sample line from water to sampler and begin the analysis, continuing until all unknowns have been analyzed.

15. Calculation

15.1 Determine the concentration of nitrate or nitrite nitrogen in the samples in milligrams per litre by comparing the peak heights of the samples with the standard curves (13.4) manually or by a computer-based data handler.

NOTE 8—If the background color absorbance has been measured (14.1.4), calculate the net absorbance by subtracting the background absorbance from the measured absorbance of the color developed sample. Use the net absorbance to determine the concentration of nitrogen in the sample.

15.2 Where separate values are required for nitrite-nitrogen and nitrate-nitrogen, calculate the nitrate-nitrogen by subtracting the nitrite-nitrogen from the total nitrate-nitrite nitrogen content.

16. Report

16.1 Report the following information:

16.1.1 Report the nitrogen content in milligrams per litre as:

16.1.1.1 Nitrite-Nitrogen (NO_2-N), mg/L,

16.1.1.2 Nitrate-Nitrogen (NO_3-N), mg/L, and

16.1.1.3 Combined Nitrate-Nitrite Nitrogen (NO_3, NO_2-N), mg/L.

17. Precision and Bias[8]

17.1 *Precision Statement:*

17.1.1 *Nitrite*—Based on the results of six operators in five laboratories, the overall and single-operator precision of this test method for nitrite within its designated range for reagent water and selected water matrices (including surface, saline, waste, and ground waters) varies with the quantity being tested in accordance with Table 2. No data were rejected as outliers

[8] Supporting data have been filed at ASTM International Headquarters and may be obtained by requesting Research Report RR:D19-1058.

TABLE 3 Reagent Water, Automated Cadmium Reduction

Radical	Amount Added, mg/L	Amount Found, mg/L	Bias, %	Statistically Significant 95 % Level
Nitrite-nitrogen	0.050	0.042	−16	no
	0.090	0.096	+ 6	yes
	0.420	0.416	−1	no
	0.800	0.798	0	no
Nitrate-nitrogen	0.050	0.044	−11	no
	0.090	0.092	+ 2	no
	0.420	0.404	−4	yes
	0.850	0.828	−3	no
Water Matrix				
Radical	Amount Added, mg/L	Amount Found, mg/L	Bias, %	Statistically Significant 95 % Level
Nitrite-nitrogen	0.050	0.060	+ 20	no
	0.090	0.097	+ 8	yes
	0.420	0.427	+ 2	no
	0.800	0.790	−1	no
Nitrate-nitrogen	0.050	0.053	+ 6	no
	0.090	0.081	−10	no
	0.420	0.396	−6	yes
	0.850	0.828	−3	no

for this statistical evaluation.

17.1.2 *Nitrate*—The precision of this test method for nitrate within its designated range for reagent water and selected water matrices may be expressed as follows (concentrations are given in mg/L):

Reagent Water	$S_T = 0.0400$
	$S_O = 0.0296$
Water Matrix	$S_T = 0.0437$
	$S_O = 0.0300$

17.2 Precision and bias for this test method conform to Practice D2777 – 77, which was in place at the time of collaborative testing. Under the allowances made in 1.4 of D2777 – 13, these precision and bias data do meet existing requirements for interlaboratory studies of Committee D19 test methods.

17.3 *Bias Statement*—Recoveries of known amounts of nitrites-nitrates from reagent water and selected water matrices are shown in Table 3.

17.4 It is the user's responsibility to ensure the validity of this test method for waters of untested matrices.

18. Quality Control

18.1 In order to be certain that analytical values obtained using these test methods are valid and accurate within the confidence limits of the test, the following QC procedures must be followed when analyzing nitrite-nitrate in water.

18.2 *Calibration and Calibration Verification*

18.2.1 Analyze at least four working standards containing concentrations of nitrite-nitrate in water that bracket the expected sample concentration prior to analysis of samples to calibrate the instrument.

18.2.2 Verify instrument calibration after standardization by analyzing a standard at the concentration of one of the calibration standards. The absorbance shall fall within 4 % of the absorbance from the calibration. Alternately, the concentration of a mid-range standard should fall within ±15 % of the known concentration. Analyze a calibration blank to verify system cleanliness.

18.2.3 If calibration cannot be verified, recalibrate the instrument.

18.2.4 It is recommended to analyze a continuing calibration blank (CCB) and continuing calibration verification (CCV) at a 10% frequency. The results should fall within the expected precision of the method or +15% of the known concentration.

18.3 *Initial Demonstration of Laboratory Capability*

18.3.1 If a laboratory has not performed the test before, or if there has been a major change in the measurement system, for example, new analyst, new instrument, and so forth, a precision and bias study must be performed to demonstrate laboratory capability.

18.3.2 Analyze seven replicates of a standard solution prepared from an Independent Reference Material containing a mid-range concentration of nitrite-nitrate in water. The matrix and chemistry of the solution should be equivalent to the solution used in the collaborative study. Each replicate must be taken through the complete analytical test method including any sample preservation and pretreatment steps.

18.3.3 Calculate the mean and standard deviation of the seven values and compare to the acceptable ranges of bias in Table 3. This study should be repeated until the recoveries are within the limits given in Table 2 and Table 3. If a concentration other than the recommended concentration is used, refer to Test Method D5847 for information on applying the F test and t test in evaluating the acceptability of the mean and standard deviation.

18.4 *Laboratory Control Sample (LCS)*

18.4.1 To ensure that the test method is in control, prepare and analyze a LCS containing a mid-range concentration of nitrite-nitrate in water with each batch (laboratory-defined or 10 samples). The laboratory control samples for a large batch should cover the analytical range when possible. It is recommended, but not required to use a second source, if possible and practical for the LCS. The LCS must be taken through all of the steps of the analytical method including sample preservation and pretreatment. The result obtained for the LCS shall fall within ±15 % of the known concentration.

18.4.2 If the result is not within these limits, analysis of samples is halted until the problem is corrected, and either all the samples in the batch must be reanalyzed, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

18.5 *Method Blank*

18.5.1 Analyze a reagent water test blank with each laboratory-defined batch. The concentration of nitrite-nitrate in water found in the blank should be less than 0.5 times the lowest calibration standard. If the concentration of nitrite-nitrate in water is found above this level, analysis of samples is halted until the contamination is eliminated, and a blank shows no contamination at or above this level, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

18.6 *Matrix Spike (MS)*

18.6.1 To check for interferences in the specific matrix being tested, perform a MS on at least one sample from each laboratory-defined batch by spiking an aliquot of the sample with a known concentration of nitrite-nitrate in water and taking it through the analytical method.

18.6.2 The spike concentration plus the background concentration of nitrite-nitrate in water must not exceed the high calibration standard. The spike must produce a concentration in the spiked sample that is 2 to 5 times the nitrite-nitrate in water concentration in the unspiked sample, or 10 to 50 times the detection limit of the test method, whichever is greater.

18.6.3 Calculate the percent recovery of the spike (P) using the following formula:

$$P = 100\left[A\left(V_s + V\right) - BV_s\right]/CV$$

where:

A = nitrite-nitrate in water concentration (mg/L) in spiked sample,
B = nitrite-nitrate in water concentration (mg/L) ini unspiked sample,
C = concentration (mg/L) of nitrite-nitrate in water in spiking solution,
V_s = volume (mL) of sample used, and
V = volume (mL) of spiking solution added.

18.6.4 The percent recovery of the spike shall fall within the limits, based on the nitrite-nitrate in water concentration, listed in Guide D5810, Table 1. If the percent recovery is not within these limits, a matrix interference may be present in the sample selected for spiking. Under these circumstances, one of the following remedies must be employed: the matrix interference must be removed, all samples in the batch must be analyzed by a test method not affected by the matrix interference, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

NOTE 9—Acceptable spike recoveries are dependent on the concentration of the component of interest. See Guide D5810 for additional information.

18.7 *Duplicate*

18.7.1 To check the precision of sample analyses, analyze a sample in duplicate with each laboratory-defined batch. If the concentration of the nitrite-nitrate in water is less than five times the detection limit for the nitrite-nitrate in water, a matrix spike duplicate (MSD) should be used.

18.7.2 Calculate the standard deviation of the duplicate values and compare to the precision in the collaborative study using an F test. Refer to 6.4.4 of Practice D5847 for information on applying the F test.

18.7.3 If the result exceeds the precision limit, the batch must be reanalyzed or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

18.8 *Independent Reference Material (IRM)*

18.8.1 In order to verify the quantitative value produced by the test method, analyze an Independent Reference Material (IRM) submitted as a regular sample (if practical) to the laboratory at least once per quarter. The concentration of the IRM should be in the concentration mid-range for the method chosen. The value obtained must fall within the control limits established by the laboratory.

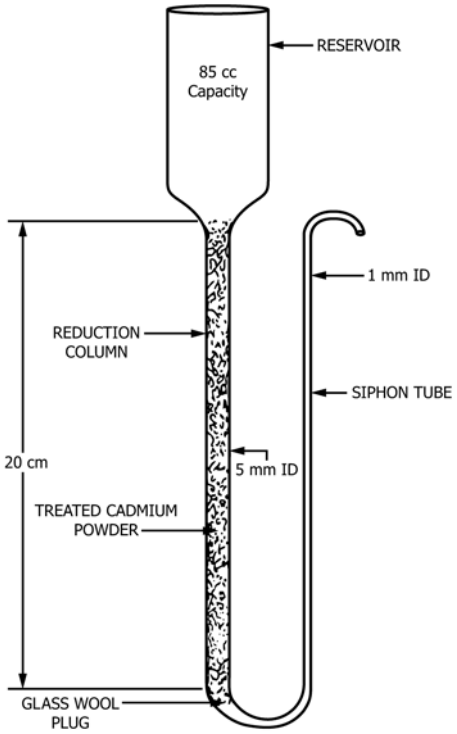

FIG. 3 Cadmium Reduction Column

TEST METHOD B—MANUAL CADMIUM REDUCTION

19. Scope

19.1 The applicable range of this test method is from 0.05 to 1 mg/L of nitrite or nitrate nitrogen. The range may be extended upward by dilution of an appropriate aliquot. Many workers have found that this test method is reliable for nitrite and combined nitrite-nitrate levels to 0.01 mg N/L. However, the precision and accuracy data presented in this test method are insufficient to justify application of this test method in the 0.01 to 0.05 mg/L-N range.

19.2 This test method is applicable to surface, saline, waste, and ground waters. It is the user's responsibility to ensure the validity of this test method for waters of untested matrices.

20. Apparatus

20.1 *Reduction Column* as shown in Fig. 3. The column shall be laboratory fabricated from the following:

20.1.1 *Pipet,* volumetric, 100-mL capacity, with 200-mm delivery stem. The top of the bulb shall be cut off before the constriction for the entry stem and the delivery tip curved to form a shallow hook.

20.1.2 *Tubing,* glass or vinyl, 1-mm inside diameter (1⁄16 in.) and about 350-mm (14 in.) in length.

NOTE 10—If glass tubing is used, it must be bent at the lower end to connect with the curved tip of the pipet and parallel to the pipet. Its upper end is then bent over to form an inverted U-siphon. This last bend should be level with the top of the lower stem of the pipet. With this arrangement,

liquid placed in the reservoir flows out of the system and stops when the level of the liquid just covers the cadmium powder packing.

20.2 *Cylinders,* graduated, 50-mL capacity.

20.3 *Test Tubes,* 15-mL capacity.

20.4 *Photometer*—A spectrophotometer or filter photometer suitable for use at 543 nm and equipped with absorption cells providing a light-path length of 1 cm. Spectrophotometers shall conform to Practice E275. Filter photometers and photometric practices prescribed in this method shall conform to Practice E60.

20.5 *Pipets,* serological, 1 and 10-mL capacity, calibrated to 0.1 mL, to deliver with ejection.

21. Reagents and Materials

21.1 *Ammonium Chloride Solution* (85 g/L)—Dissolve 85 g of ammonium chloride (NH_4Cl) in water and dilute to 1 L.

21.2 *Cadmium,* 40 to 60 mesh granulated.[7]

21.3 *Color Reagent*—Add the following to 800 mL of water, while stirring constantly: 100 mL of concentrated phosphoric acid (H_3PO_4), 10 g of sulfanilamide, and 0.5 g of *N*-(1-naphthyl)ethylenediamine dihydrochloride. Stir until dissolved. Dilute to 1 L with water. This solution is stable for about a month when stored in a brown bottle in a dark cool place.

21.4 *Copper Sulfate Solution* (20 g/L)—See 11.4.

21.5 *Trichloro-trifluoroethane.*

21.6 *Hydrochloric Acid* (1 + 1)—See 11.6.

21.7 *Nitrate Solution, Stock* (1.0 mL = 1.0 mg NO_3-N)—See 11.7.

21.8 *Nitrate Solution, Standard* (1.0 mL = 0.01 mg NO_3-N)—See 11.8.

21.9 *Nitrite Solution, Stock* (1.0 mL = 1.0 mg NO_2-N)—See 11.9.

NOTE 11—Potassium nitrite is easily oxidized, and only fresh bottles of this reagent are to be used.

21.10 *Nitrite Solution, Standard* (1.0 mL = 0.01 mg NO_2-N)—See 11.10.

21.11 *Filter Paper*—See 11.11.

22. Preparation of Reduction Column

22.1 *Cadmium Granules Treatment*—Clean and copperize (12.1) new or used cadmium granules (21.2) in accordance with 12.1.

22.2 *Filling the Reduction Column:*

22.2.1 Insert a small plug of glass wool in the tip of the pipet.

22.2.2 Fill the column with water to prevent the entrapment of air bubbles during the filling operations.

22.2.3 Pour sufficient cadmium powder into the apparatus to produce a column 300 mm in length; tap to pack the powder.

22.2.4 Wash the column thoroughly with ammonium chloride solution (21.1).

NOTE 12—Use a flow rate no greater than 8 mL/min. If the rate is too fast, slow it down by constricting the end of the siphon outlet or by raising the height of the siphon tube. Flow rates of less than 5 mL/min unnecessarily increase the time for analysis and may cause low results.

22.3 *Storing the Column*—When not in use, cover the cadmium in the column with ammonium chloride solution (21.2). Do not allow air to enter the packing, nor the packing to dry out. If this occurs, prepare another column.

TABLE 4 Concentration of Calibration Standards, Manual Cadmium Reduction

NO_3-N or NO_2-N, mg/L	mL Standard Solution/100 mL
0.04	0.4
0.1	1
0.2	2
0.4	4
0.7	7
1.0	10

23. Calibration

23.1 Using the nitrate standard solution (21.8) prepare calibration standards by pipetting specified volumes of the standard solution into 100-mL volumetric flasks and diluting to the mark with water. Table 4 specifies the millilitres of standard solution required.

23.2 Prepare at least one calibration standard from the nitrite standard solution (21.10) at the same concentration as one of the nitrate standards to verify the efficiency of the reduction column.

NOTE 13—When the sample to be analyzed is a saline water, use substitute ocean water (SOW) to prepare the standards (Specification D1141). A reagent water blank should be run in addition to a SOW blank because the reagents used to prepare SOW frequently contain nitrite or nitrate, or both. Adjust this curve for the contaminant level in SOW.

23.3 Treat the standards as directed in 24.2.

23.4 Develop the color and measure the absorbance of each standard as directed in 24.3 for nitrite standards or in 24.4 for nitrate standards.

23.5 Prepare a standard curve by plotting the absorbance of each processed calibration standard against its known concentration.

NOTE 14—The nitrite standard will fall on the nitrate standard curve if the reduction of nitrate was complete. If the nitrite standard is 5 % higher, prepare a fresh reduction column and repeat the analysis of the nitrate standards. Analyze a nitrate standard every 4 hours of continuous testing. The reduction column will usually last for several weeks of continuous analysis.

24. Procedure

24.1 *Removal of Interferences*—Remove interferences (Section 6) by the following procedures:

24.1.1 For turbidity removal, when suspended solids are present, filter the sample through a glass fiber filter or 0.45-µm filter (21.11).

24.1.2 For oil and grease removal, adjust the pH of the sample to 2 with concentrated HCl. Extract with two 25-mL portions of trichloro-trifluoroethane (21.5) in a separatory funnel. Discard the lower trichloro-trifluoroethane layer after each extraction.

24.1.3 For pH adjustment, determine the pH of the sample with a pH meter. If the pH is less than 6 or greater than 8, adjust the pH to within the range from 6 to 8 with concentrated HCl or concentrated NH_4OH.

24.1.4 For correction for color interferences, if there is a possibility that the sample might absorb at 543 nm, determine the background absorbance. Dilute the sample as directed in 24.2. Replace the color reagent (21.3) with a similar reagent where just the *N*-(1-naphthyl)-ethylenediamine dihydrochloride is omitted and follow the procedure as directed in 24.3. Using the complete color reagent (21.3), repeat the analysis as directed in 24.3 for nitrite determination or in 24.4 for combined nitrite-nitrate determination.

24.2 *Sample Treatment*—To a 20-mL sample add 80 mL of ammonium chloride solution (21.1). Mix well.

24.3 *Nitrite Determination:*

24.3.1 Pipet a 10-mL portion of the treated sample into a 15-mL test tube.

24.3.2 Pipet 3 mL of color reagent (21.3) into the test tube, mix, and let stand for 15 min.

24.3.3 Using water in the reference cell, determine the absorbance of the solution at 543 nm and record.

24.4 *Combined Nitrite-Nitrate Determination:*

24.4.1 Pour about 60 mL treated sample (24.2) into the reservoir of the reduction column on top of any liquid in the column.

NOTE 15—The cadmium in the reduction column will be covered by the ammonium chloride storage solution or by a previous sample because it should *never* be allowed to dry out.

24.4.2 Place a clean 50-mL graduate under the siphon outlet and allow approximately 30 mL of effluent to collect. Discard this initial 30 mL.

24.4.3 Place a clean 50-mL graduate under the siphon outlet and collect an additional 25 mL of effluent. Do not remove all the liquid from the column. Allow enough solution to remain in the column to completely cover the copper-cadmium granules.

24.4.4 Pipet a 10-mL portion of the collected effluent into a 15-mL test tube.

24.4.5 Pipet 3.0 mL of color reagent (21.3) into the test tube, mix, and let stand for 15 min.

24.4.6 Using water in the reference cell, determine the absorbance of the solution at 543 nm and record.

25. Calculation

25.1 From the standard curve (23.5) obtain the nitrogen content in mg/L that corresponds to the absorbance.

NOTE 16—If the background color absorbance has been measured (24.1.4), calculate the net absorbance by subtracting the background absorbance from the measured absorbance of the color-developed sample. Use the net absorbance to determine the concentration of nitrogen in the sample.

25.2 Where separate values are required for nitrite-nitrogen and nitrate-nitrogen, calculate the nitrate-nitrogen by subtracting the nitrite-nitrogen (24.3) from the combined nitrite-nitrate nitrogen content (24.4).

TABLE 5 Reagent Water, Manual Cadmium Reduction

Radical	Amount Added, mg/L	Amount Found, mg/L	Bias, %	Statistically Significant 95 % Level
Nitrite	0.05	0.05	0	no
	0.09	0.09	0	no
	0.42	0.42	0	no
	0.80	0.82	+ 2	yes
Nitrate	0.05	0.03	–40	yes
	0.09	0.08	–11	no
	0.42	0.43	+ 2	no
	0.85	0.81	–5	yes
Water Matrix				
Radical	Amount Added, mg/L	Amount Found, mg/L	Bias, %	Statistically Significant 95 % Level
Nitrite	0.05	0.04	–20	no
	0.09	0.06	–33	yes
	0.42	0.36	–10	no
	0.80	0.75	–6	yes
Nitrate	0.05	0.04	–20	no
	0.09	0.10	–11	no
	0.42	0.41	–2	no
	0.85	0.79	–7	yes

26. Report

26.1 Report the following information:

26.1.1 Report the nitrogen content in milligrams per litre as:

26.1.1.1 Nitrite-Nitrogen (NO_2-N), mg/L,

26.1.1.2 Nitrate-Nitrogen (NO_3-N), mg/L, and

26.1.1.3 Combined Nitrite-Nitrate Nitrogen (NO_2, NO_3-N), mg/L.

27. Precision and Bias[8]

27.1 Based on the results from eight operators from seven laboratories, the precision of this test method within its designated range for reagent water and water matrices selected (see 17.1.1) may be expressed as follows (concentrations are given in mg/L):

Reagent Water

Nitrite-Nitrogen: $S_T = 0.039\ X + 0.0108$; $S_O = 0.010$

Nitrate-Nitrogen: $S_T = 0.033$; $S_O = 0.014$

Water Matrix

Nitrite-Nitrogen: $S_T = 0.0901\ X + 0.035$; $S_O = 0.0841\ X + 0.019$

Nitrate-Nitrogen: $S_T = 0.057$; $S_O = 0.038$

27.2 *Bias Statement*—Recoveries of known amounts of nitrite and nitrate from reagent water and water matrices selected are shown in Table 5.

27.3 One set of nitrate results were rejected as outliers. The nitrite results from this laboratory were acceptable.

27.4 It is the user's responsibility to ensure the validity of this test method for waters of untested matrices.

27.5 Precision and bias for this test method conforms to Practice D2777 – 77, which was in place at the time of collaborative testing. Under the allowances made in 1.4 of D2777 – 13, these precision and bias data do meet existing requirements for interlaboratory studies of Committee D19 test methods.

28. Quality Control

28.1 In order to be certain that analytical values obtained using these test methods are valid and accurate within the confidence limits of the test, the following QC procedures must be followed when analyzing nitrite-nitrate in water.

28.2 *Calibration and Calibration Verification*

28.2.1 Analyze at least four working standards containing concentrations of nitrite-nitrate in water that bracket the expected sample concentration prior to analysis of samples to calibrate the instrument.

28.2.2 Verify instrument calibration after standardization by analyzing a standard at the concentration of one of the calibration standards. The absorbance shall fall within 4% of the absorbance from the calibration. Alternately, the concentration of a mid-range standard should fall within ±15 % of the known concentration. Analyze a calibration blank to verify system cleanliness.

28.2.3 If calibration cannot be verified, recalibrate the instrument.

28.2.4 It is recommended to analyze a continuing calibration blank (CCB) and continuing calibration verification (CCV) at a 10% frequency. The results should fall within the expected precision of the method or +15% of the known concentration.

28.3 *Initial Demonstration of Laboratory Capability*

28.3.1 If a laboratory has not performed the test before, or if there has been a major change in the measurement system, for example, new analyst, new instrument, and so forth, a precision and bias study must be performed to demonstrate laboratory capability.

28.3.2 Analyze seven replicates of a standard solution prepared from an Independent Reference Material containing a mid-range concentration of nitrite-nitrate in water. The matrix and chemistry of the solution should be equivalent to the solution used in the collaborative study. Each replicate must be taken through the complete analytical test method including any sample preservation and pretreatment steps.

28.3.3 Calculate the mean and standard deviation of the seven values and compare to the acceptable ranges of bias in sections 27.1 and 27.2. This study should be repeated until the recoveries are within the limits given in sections 27.1 and 27.2. If a concentration other than the recommended concentration is used, refer to Practice D5847 for information on applying the F test and t test in evaluating the acceptability of the mean and standard deviation.

28.4 *Laboratory Control Sample (LCS)*

28.4.1 To ensure that the test method is in control, analyze a LCS containing a mid-range concentration of nitrite-nitrate in water with each batch (laboratory-defined or 10 samples). The laboratory control samples for a large batch should cover the analytical range when possible. It is recommended, but not required to use a second source, if possible and practical for the LCS. The LCS must be taken through all of the steps of the analytical method including sample preservation and pretreatment. The result obtained for the LCS shall fall within ±15 % of the known concentration.

28.4.2 If the result is not within these limits, analysis of samples is halted until the problem is corrected, and either all the samples in the batch must be reanalyzed, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

28.5 *Method Blank*

28.5.1 Analyze a reagent water test blank with each laboratory-defined batch. The concentration of nitrite-nitrate in water found in the blank should be less than 0.5 times the lowest calibration standard. If the concentration of nitrite-nitrate in water is found above this level, analysis of samples is halted until the contamination is eliminated, and a blank shows no contamination at or above this level, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

28.6 *Matrix Spike (MS)*

28.6.1 To check for interferences in the specific matrix being tested, perform a MS on at least one sample from each laboratory-defined batch by spiking an aliquot of the sample with a known concentration of nitrite-nitrate in water and taking it through the analytical method.

28.6.2 The spike concentration plus the background concentration of nitrite-nitrate in water must not exceed the high calibration standard. The spike must produce a concentration in the spiked sample that is 2 to 5 times the nitrite-nitrate in water concentration in the unspiked sample, or 10 to 50 times the detection limit of the test method, whichever is greater.

28.6.3 Calculate the percent recovery of the spike (P) using the following formula:

$$P = 100\left[A(V_s + V) - BV_s\right]/CV$$

where:

A = nitrite-nitrate in water concentration (mg/L) in spiked sample,
B = nitrite-nitrate in water concentration (mg/L) in unspiked sample,
C = concentration (mg/L) of nitrite-nitrate in water in spiking solution,
V_s = volume (mL) of sample used, and
V = volume (mL) of spiking solution added.

28.6.4 The percent recovery of the spike shall fall within the limits, based on the nitrite-nitrate in water concentration, listed in Guide D5810, Table 1. If the percent recovery is not within these limits, a matrix interference may be present in the sample selected for spiking. Under these circumstances, one of the following remedies must be employed: the matrix interference must be removed, all samples in the batch must be analyzed by a test method not affected by the matrix interference, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

NOTE 17—Acceptable spike recoveries are dependent on the concentration of the component of interest. See Guide D5810 for additional information.

28.7 *Duplicate*

28.7.1 To check the precision of sample analyses, prepare and analyze a sample in duplicate with each laboratory-defined batch. If the concentration of the nitrite-nitrate in water is less

than five times the detection limit for the nitrite-nitrate in water, a matrix spike duplicate (MSD) should be used.

28.7.2 Calculate the standard deviation of the duplicate values and compare to the precision in the collaborative study using an F test. Refer to 6.4.4 of Practice D5847 for information on applying the F test.

28.7.3 If the result exceeds the precision limit, the batch must be reanalyzed or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

28.8 *Independent Reference Material (IRM)*

28.8.1 In order to verify the quantitative value produced by the test method, analyze an Independent Reference Material (IRM) submitted as a regular sample (if practical) to the laboratory at least once per quarter. The concentration of the IRM should be in the concentration mid-range for the method chosen. The value obtained must fall within the control limits established by the laboratory.

29. Keywords

29.1 cadmium reduction method; groundwater; nitrate; nitrite; saline water; waste water; water

SUMMARY OF CHANGES

Committee D19 has identified the location of selected changes to this standard since the last issue (insert designation and year date) that may impact the use of this standard. (Approved June 1, 2016.)

(1) The SI statement was update in Section 1.
(2) Test Method D7781 was added to Sections 2 and 5.
(3) Sections 11 and 21 were modified to include filter paper information.
(4) Section 13 was modified to allow for direct reading instruments.
(5) Subsections 18.2 , 18.4, 28.2, and 28.4 were modified.