



Standard Test Methods for Nitrite-Nitrate in Water¹

This standard is issued under the fixed designation D 3867; 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 approval. 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 methods² are given as follows:

	Sections
Test Method A—Automated Cadmium Reduction	9 to 16
Test Method B—Manual Cadmium Reduction	17 to 24

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 *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 Note 1 and Note 2.

2. Referenced Documents

2.1 ASTM Standards:

- D 992 Test Method for Nitrate Ion in Water³
- D 1129 Terminology Relating to Water⁴
- D 1141 Specification for Substitute Ocean Water⁵
- D 1192 Specification for Equipment for Sampling Water and Steam in Closed Conduits⁴
- D 1193 Specification for Reagent Water⁴
- D 1254 Test Method for Nitrite Ion in Water⁶
- D 2777 Practice for Determination of Precision and Bias of Applicable Methods of Committee D-19 on Water⁴
- D 3370 Practices for Sampling Water from Closed Conduits⁴

¹ These test methods are under the jurisdiction of ASTM Committee D-19 on Water and are the responsibility of Subcommittee D19.05 on Inorganic Constituents in Water.

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

³ Discontinued; see 1983 *Annual Book of ASTM Standards*, Vol 11.01.

⁴ *Annual Book of ASTM Standards*, Vol 11.01.

⁵ *Annual Book of ASTM Standards*, Vol 11.02.

⁶ Discontinued; see 1980 *Annual Book of ASTM Standards*, Part 31.

E 60 Practices for Photometric and Spectrometric Methods for Chemical Analysis of Metals⁷

E 275 Practice for Describing and Measuring Performance of Ultraviolet, Visible, and Near Infrared Spectrophotometers⁸

3. Terminology

3.1 *Definitions:* For definitions of terms used in these test methods, refer to Terminology D 1129.

4. Summary of Test Methods

4.1 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 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 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 D 1254 (Nitrite) and D 992 (Nitrate). The nitrite test method (Test Method D 1254) used a reagent which is considered to be a potential carcinogen. The nitrate test method (Test Method D 992) has been shown to have relatively large errors when used in

⁷ *Annual Book of ASTM Standards*, Vol 03.05.

⁸ *Annual Book of ASTM Standards*, Vol 03.06.

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

wastewaters and also has greater manipulative difficulties than the test method described herein.

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 preextracting 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 an interference. 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.

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.⁹ 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 D 1193, 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.

⁹ “Reagent Chemicals, American Chemical Society Specifications,” American Chemical Society, Washington, D.C. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar 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.

8. Sampling and Sample Preservation

8.1 Collect the sample in accordance with Specification D 1192 and Practices D 3370, as applicable.

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 (11.8 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*¹⁰ 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*.
- 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 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.

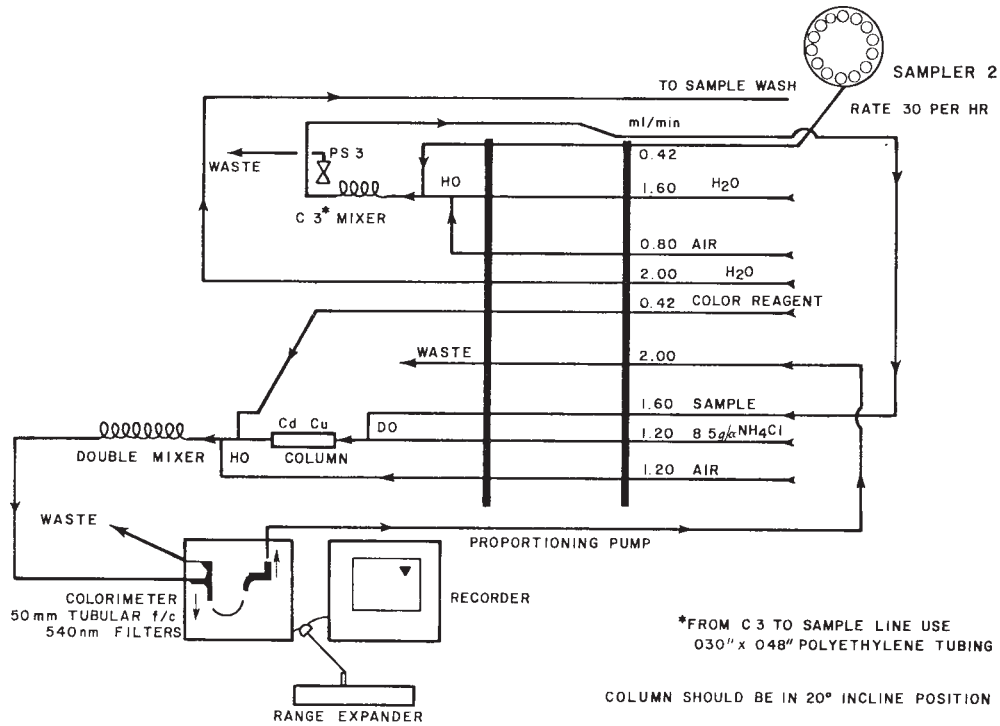


FIG. 1 Nitrite-Nitrate Manifold

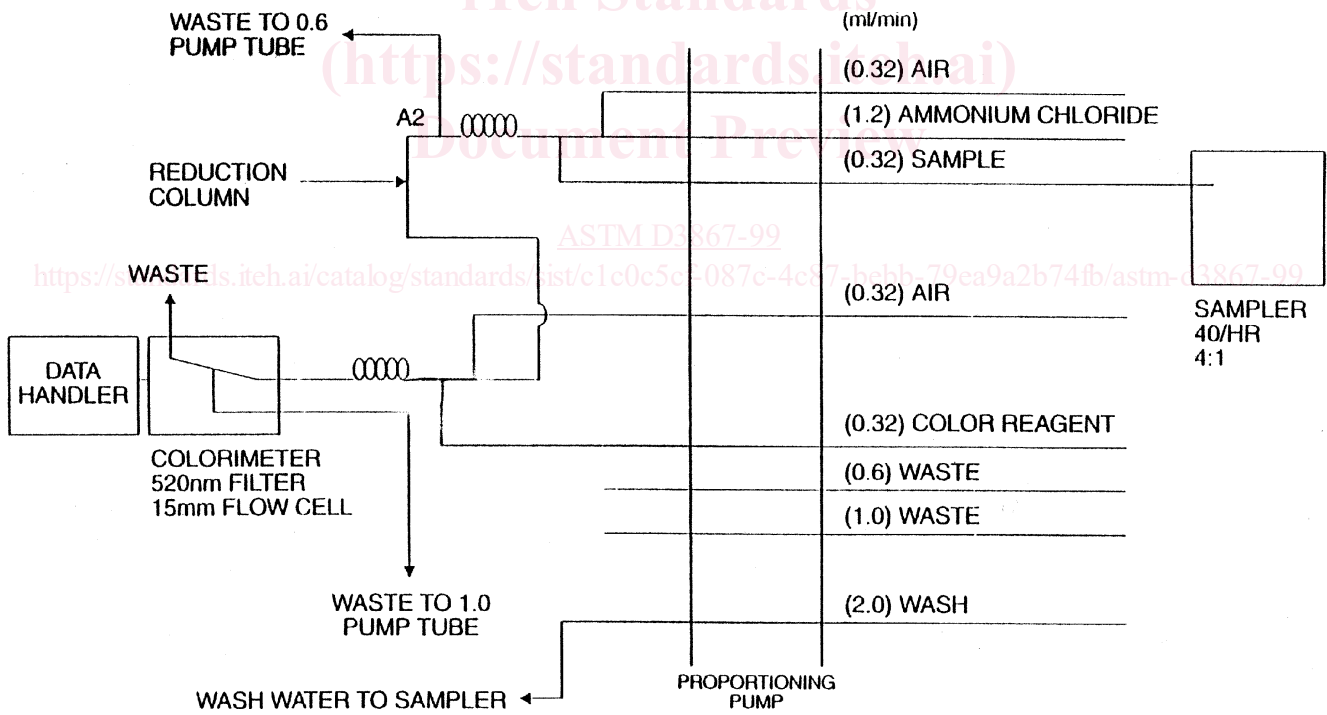


FIG. 2 Nitrite-Nitrate Cartridge

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

11.1 *Ammonium Chloride Solution* (85 g/L)—Dissolve 85 g of ammonium chloride (NH₄Cl) in water and dilute to 1 L. Add 0.5 mL wetting agent.¹¹

11.2 *Cadmium*, 40 to 60 mesh, granulated.¹²

11.3 *Color Reagent*—Add the following to 800 mL of water, while stirring constantly: 100 mL of concentrated phosphoric acid (H₃PO₄), 10 g of sulfanilamide, and 0.5 g of *N*-1-(naphthyl)ethylenediamine dihydrochloride. Stir until dissolved. Add 1 mL of wetting agent,¹¹ 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 (CuSO₄·5 H₂O) 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 NO₃-N)—Dry potassium nitrate (KNO₃) 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.)

11.8 *Nitrate Solution, Standard* (1.0 mL = 0.01 mg NO₃-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 NO₂-N)—Place about 7 g of potassium nitrite (KNO₂) 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 1000-mL volumetric flask. Dilute to the mark with water store in a sterilized bottle under refrigeration. Prepare fresh as needed.

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 NO₂-N)—Dilute 10 mL of stock nitrite solution (11.9) to 1 L with water. This solution is unstable; prepare fresh as needed.

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 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

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

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 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 at the same concentration as one of the nitrate standards to verify the efficiency of the reduction column. Repeat this when a suspected loss in NO₃-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 (Specification D 1141). 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).

¹¹ 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.

¹² Different sizes of granulated cadmium may be used. The analyst should ensure that adequate reduction occurs with the size chosen.