



Standard Test Method for Nitrite-Nitrate in Water by Nitrate Reductase¹

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1. Scope

1.1 This test method is applicable to the determination of nitrate plus nitrite (as nitrogen) in drinking water, surface, saline, wastewater, and ground waters. The applicable range of this test method is from 0.05 to 5 mg/L of nitrogen. The range may be extended upward by dilution of an appropriate aliquot. The 40 CFR Part 136 Method Detection Limit (MDL) is 0.02 mg /L.

1.2 It is the user's responsibility to ensure the validity of this test method for waters of untested matrices. The quality control criteria in Section 17 for method blanks, laboratory control samples, matrix spikes and matrix duplicates must be met.

1.3 The values stated in SI units are regarded as 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.*

2. Referenced Documents

2.1 *ASTM Standards:*²

D992

D1129 Terminology Relating to Water

D1141 Practice for the Preparation of Substitute Ocean Water

D1193 Specification for Reagent Water

D1254 Method of Test for Nitrite Ion in Water (Withdrawn 1980)³

D3867 Test Methods for Nitrite-Nitrate in Water

D5810 Guide for Spiking into Aqueous Samples

D6146 Guide for Monitoring Aqueous Nutrients in Watersheds

¹ This test method is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.05 on Inorganic Constituents in Water.

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² 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.

³ The last approved version of this historical standard is referenced on www.astm.org.

3. Terminology

3.1 *Definitions*—For definitions of terms used in these test methods, refer to Terminology D1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *bispecific NaR*, *n*—nitrate reductase that can use either NADH or NADPH as its electron donor (cofactor).

3.2.2 *discrete analyzer*, *n*—a programmable, computer-controlled instrument that automates wet-chemical analysis by using one or more robotic arms interfaced to high-precision volumetric dispensers to aspirate and dispense samples, standards, diluents, and reagents.

3.2.3 *Greiss reaction*, *n*—chemical formation of an azo dye by diazotization of nitrite ion with sulfanilamide and subsequent coupling with *N*-(1-naphthyl)ethylenediamine hydrochloride.

3.2.4 *NADH*, *n*—nicotinamide adenine dinucleotide, reduced form is a coenzyme found in all living cells.

3.2.5 *NADPH*, *n*—nicotinamide adenine dinucleotide phosphate, reduced form is a coenzyme found in all living cells; NADP⁺ is the oxidizing form and NADPH is the reducing form.

3.2.6 *nitrate reductase (NaR)*, *n*—NADH:NaR (EC 1.7.1.1 and CAS 9013-03-0) or bispecific NaR (EC 1.7.1.2 and CAS 9029-27-0) with 1 unit of enzyme activity defined as 1 micromol nitrite produced per minute at 30°C, at pH 7 with NADH (refer to 3.2.4 and 10.2) as an electron donor.

4. Summary of Test Method

4.1 *Nitrite-Nitrate Nitrogen*—The sample is mixed with a buffered solution containing NAD(P)H: nitrate reductase (EC 1.7.1.3) and NADH or NADPH to reduce nitrate ion to nitrite ion. The combined nitrite-nitrate (expressed as mg/L NO₃+NO₂-N) 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 at about 540 nm.

4.2 *Nitrite Nitrogen*—The nitrite ion (expressed as mg/L NO₂-N) originally present in the sample can be determined separately by carrying out the procedure and omitting the reduction step.