AQ2 method

EPA-160-A Rev. 0

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Nitrate–N +Nitrite–N in Drinking and Surface Waters, Domestic and Industrial Wastes

1 SCOPE

This method covers the determination of Nitrate–N + Nitrite–N in sewage and effluents, ground and surface waters and industrial wastes.

This method is approved for the Clean Water Act, for use in wastewater compliance monitoring, under National Pollutant Discharge Elimination System (NPDES). This method has been listed in 40 CFR part 136.3, as an approved method, since at least 2012.

This method is not approved for use in drinking water compliance monitoring, under National Primary Drinking Water Regulations (NPDWR).

2 RANGE OF APPLICATION

Range	0.025 – 1.0 mg N/L

3 METHOD DETECTION LIMIT

By USEPA Procedure	MDL = 0.004 mg N/L

4 METHOD PRINCIPLE

Nitrate is reduced to nitrite by vanadium(III) chloride. Nitrite originally present, plus nitrite produced during vanadium(III) chloride reduction, reacts with sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride in dilute hydrochloric acid to form a reddish-purple azo dye that is measured photometrically at 520 nm.

5 REFERENCES

Timothy A. Doane, William R. Horwáth. Spectrophotometric Determination of Nitrate with a Single Reagent. *Analytical Letters* [Online] 2003, Vol. 36, Issue. 2.

6 REAGENTS: CHEMICALS REQUIRED AND SAFETY INFORMATION

Consult the safety data sheets (MSDS) for details on safe handling of chemicals.

Chemical	C.A.S. No.	Safety Information
Hydrochloric acid	7647-01-0	Causes severe burns to skin and eyes.
N-(1-napthyl)-ethylenediamine dihydrochloride	1465-25-4	Irritating to eyes, skin, and respiratory tract.
Sodium nitrate	7631-99-4	Irritating to eyes and skin. Harmful if inhaled or swallowed.
Sodium nitrite	7632-00-0	Irritating to eyes and skin. Harmful if inhaled or swallowed.
Sulfanilamide	63-74-1	Irritating to eyes, skin, and respiratory tract. Harmful if swallowed.
Vanadium(III) chloride	7718-98-1	Causes severe burns to skin and eyes. Harmful if swallowed. May cause severe respiratory tract irritation.

7 REAGENT SOLUTIONS

Use reagent-grade chemicals, certified for analytical or general laboratory use. Reagents of technical or commodity grade must be validated by the user. Use high-purity reagent water, distilled or deionized, and free from organic contamination. Grade 1 (ISO Standard 3696) or better than Type II (ASTM Standard D1193) are suitable.

7.1 HYDROCHLORIC ACID, 6 normal

Hydrochloric acid, concentrated	250 mL
DI water	dilute to 500 mL

Slowly add 250 mL concentrated hydrochloric acid, concentrated, to about 400 mL of deionized water. Caution, the container will become very warm. Cool to room temperature and dilute to 500 mL with deionized water. This reagent is available commercially.

7.2 COLOR REAGENT STOCK

Hydrochloric acid, 6 normal	10 mL
Sulfanilamide	6 g
N-(1-naphthyl)-ethylenediamine dihydrochloride	0.3 g
DI water	dilute to 200 mL

Carefully add 10 mL 6 normal hydrochloric acid to about 150 mL or deionized water and swirl to mix. Add 6.0 g sulfanilamide and 0.3 g N-(1-naphthyl)-ethylenediamine dihydrochloride and stir to dissolve. Dilute to 200 mL with deionized water. Filter this reagent, e.g., using filter paper. This reagent is stable for at least 6 weeks when stored in the dark at 4°C. As the reagent slowly turns pink, re-filter it as needed.

IN 100mL

HCl = 5mL

Sulfanilamide = 3g

N- ~~~ = 0.15g

DI = dilute to 100mL

7.3 VANADIUM(III) CHLORIDE STOCK, 16 g/L

Vanadium(III) chloride	1.6 g
Hydrochloric acid, 6 normal	16.7 mL
DI water	dilute to 100 mL

- Vanadium(III) chloride is highly toxic by inhalation, skin contact or ingestion. Contact with room air generates harmful brown fumes that form a brown film on nearby surfaces. Personal Protective Equipment includes eyeshields, faceshields, type P1 (EN143) respirator and gloves
- Do not allow vanadium(III) chloride powder to contact plastic or a balance pan. Wet the powder with dilute hydrochloric acid only, never with water.
- Store the vendor's bottle inside a wide-mouth plastic bottle, containing desiccant such as anhydrous calcium sulfate. Refrigeration is not recommended, because condensation may form inside the vendor's bottle.

Measure out 16.7 mL hydrochloric acid, 6 normal, for example, into a 25 mL graduated cylinder. Add additional deionized water to reach at least 25 mL total volume.

Use a light-weight dry glass vessel, only, to receive the vanadium(III) chloride. A glass beaker or dish (20 mL to 50 mL), with pouring spout, is ideal. Direct weighing is feasible, by using an open-pan balance and by covering the balance pan with protective paper. Weighing by difference is feasible by capping the vendor's bottle for each weighing. The indicated weight, 1.6 g, is a target value. Accept an actual weight between 1.4 g and 2.8 g, and note the exact weight to hundredths of a gram. Minimize exposure time prior to dilution, because potency diminishes, as the brown fumes evolve.

Immediately remove the weigh vessel from the balance and wet the powder cautiously, with 5 mL portions of dilute hydrochloric acid. Expect release of heat, evolution of gas and vigorous bubbling! The material dissolves readily. After cooling, transfer quantitatively to a 100 mL volumetric flask, using-up the diluted acid. Fill to the mark with deionized water and mix.

7.4 WORKING COLOR REAGENT, 12 g/L VCl_3, 1 normal HCl

Vanadium(III) chloride stock, 16 g/L	75 mL
Hydrochloric acid, 6 normal	3.7 mL
Color reagent stock	10 mL
DI water	dilute to 100 mL

To a 100 mL volumetric flask, combine 75 mL vanadium(III) chloride stock, 3.7 mL hydrochloric acid, 6 normal and 10 mL color reagent stock. Use small portions of DI water to wash-in each solution. Dilute to the mark with DI water and mix.

ALTERED RECIPE VOLUMES, if vanadium(III) chloride stock exceeds 16 g/L

U = actual concentration, g/L, of vanadium(III) chloride stock

M_1 = adjusted recipe volume, mL, for vanadium(III) chloride stock

M_2 = adjusted recipe volume, mL, for hydrochloric acid, 6 normal

Final volume of working color reagent is 100 mL

$$M_1 = 1200 \div U$$

$$M_2 = (97 - M_1) \div 6$$

8 STANDARD SOLUTIONS

8.1 NITRATE STOCK STANDARD SOLUTION (500 mg NO_3-N/L)

Sodium nitrate	3.034 g
Hydrochloric acid, 6 normal (above)	up to 10 mL
DI water	dilute to 1 L

Dissolve 3.034 g sodium nitrate (dried at 105°C) in about 800 mL deionized water in a 1 Liter volumetric flask. Up to 10 mL hydrochloric acid, 6 normal, may be added for preservation. Dilute to the mark with deionized water.

8.2 NITRITE STOCK STANDARD SOLUTION (500 mg NO_2-N/L)

Sodium nitrite	2.463 g
DI water	dilute to 1 L

Dissolve 2.463 g sodium nitrite (dried at least 4 hours in a dessicator) in about 800 mL deionized water in a 1 Liter volumetric flask. Dilute to the mark with deionized water. Do not add acid for preservation. Store up to 1 month in an amber bottle at 4°C.

8.3 NITRATE INTERMEDIATE STANDARD SOLUTION (50 mg NO_3-N/L)

NITRATE stock standard (500 mg N/L, above)	5.0 mL
DI water	dilute to 500 mL

To a 500 mL volumetric flask, pipet 5.0 mL NITRATE stock standard (500 mg NO_3-N/L) and dilute to the mark with deionized water. Prepare weekly.

8.4 NITRITE INTERMEDIATE STANDARD SOLUTION (50 mg NO_2-N/L)

NITRITE stock standard (500 mg N/L, above)	5.0 mL
DI water	dilute to 500 mL

To a 500 mL volumetric flask, pipet 5.0 mL NITRITE stock standard (500 mg NO_2-N/L) and dilute to the mark with deionized water. Prepare twice weekly.

8.5 NITRATE WORKING STANDARD SOLUTIONS (prepare weekly)

Calibrant concentrations shown are suggested for manual preparation of calibration standards.

Concentration, mg NO_3-N/L	0	0.05	0.1	0.25	0.5	0.75	1
Volume (mL) Intermediate nitrate standard solution diluted as indicated with deionized water	--	1	2	5	10	15	20
Final Volume, mL	100	100	100	100	100	100	100

8.6 NITRITE WORKING STANDARD SOLUTIONS

Concentration, mg NO_2-N/L	0.5	1.0
Volume (mL) Intermediate nitrite standard solution diluted to 100 mL with deionized water	10	20

Use nitrite-N working standards to test nitrate reduction efficiency. Prepare twice weekly.

9 SAMPLE PRESERVATION AND STORAGE

Samples may be collected into plastic or glass containers.

Sample preservation and holding time requirements are as follows, for samples to be reported for wastewater compliance monitoring under the Clean Water Act:

- For nitrate: Preserve the sample by cooling to 4°C and analyze the sample within 48 hours.
- For nitrite: Preserve the sample by cooling to 4°C and analyze within 48 hours.
- For nitrate+nitrite (combined): Preserve the sample by cooling to 4°C and acidifying to pH < 2 with sulfuric acid, concentrated, up to 2 mL sulfuric acid per liter. Analyze within 28 days.

10 PROCEDURE

Prepare standards and reagents as described in Sections 8 and 9. The laboratory must determine standard concentrations that are most suitable to their needs for calibration within the range of application for this method. The concentrations listed within this method are suggestions.

Pour calibration standards, quality control solutions and samples into sample cups and 45 mL reagent wedges, according to the user-defined worklist.

Before running the analysis batch, check the analyzer for sufficient reagent water, fresh reaction segments, correct location of reagent vessels and a waste collection bottle in place.

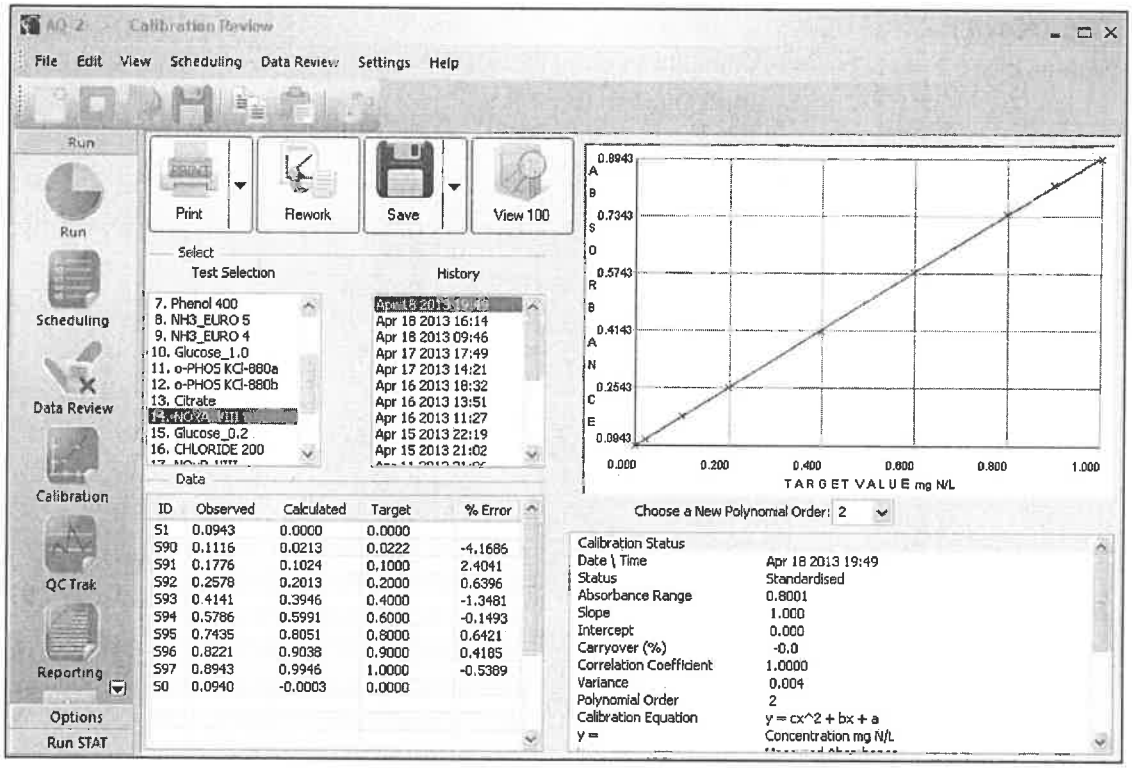
11 TEST PARAMETERS

PARAMETER	AQ2 SETTING
Test name	Nitrate VCl3
Units	mg N/L
Decimals	4
Test type	End Point
Sample volume (µL)	180
Water volume (µL)	0
Number of mixes	1
Cuvette primes	2
Cuvette washes	2
Baseline on Wash	Ticked
Reaction time (seconds)	1825
Wavelength (nm)	520
Polynomial order	2
Number of reagents	2
1. DI water (µL)	10
2. Working color reagent (µL)	310

DEFINITIONS:

Test Name:	Name which appears on the final report
Decimals:	Number of decimal places in the reported result
Units:	Concentration units appearing against the reported result
Number of mixes:	Number of mixes of the sample + reagent in the reaction well
Reaction time:	Time (in seconds) between addition of sample + reagent and measurement of the reaction mixture
Sample volume:	Volume (in microliters) of the sample used in the test
Number of reagents:	Number of reagents defined for the test
Reagent volume:	Volume (in microliters) of the reagent used in the test
Wavelength:	Interference filter wavelength to be used in the test
Cuvette primes:	Number of times the cuvette is flushed with reagent (no sample) prior to sampling
Cuvette wash:	Number of washes of the cuvette between aspirations of finished reaction liquid
Baseline on Wash:	Water baseline measured between aspirations to provide drift correction
Polynomial order:	Polynomial order to be used when fitting the standard points to the standard graph (1 = linear).

12 STANDARDIZATION



DEFINITIONS:

Abs. Range: Difference between the absorbance of the highest standard and the absorbance of the blank

Variance: Calculated as follows:

$$\text{Variance} = \frac{\sum (\text{Deviations, i.e., errors})^2 \times 100}{\sum (\text{Observed values})^2}$$

Carryover: Calculated as follows:

$$\text{Carryover} = \frac{(A - B)}{C}$$

Where, A = absorbance of carryover blank (S0)
B = absorbance of blank (S1)
C = calibration absorbance range

13 PERFORMANCE VALIDATION DATA

13.1 DETECTION LIMIT STUDY

Replicates of 0.02 mg N/L nitrate standard in reagent water***

	Concentration (mg N/L)			
	0.0206	0.0217	0.0226	0.0244
	0.0196	0.0210	0.0220	0.0217
	0.0169	0.0208	0.0222	0.0234
	0.0190	0.0217	0.0228	0.0201
	0.0212	0.0206	0.0221	0.0222
	0.0197	0.0194	0.0215	0.0254
	0.0158	0.0205	0.0228	0.0250
	0.0228	0.0177	0.0224	0.0212
	0.0185	0.0220	0.0212	0.0239
	0.0228	0.0227	0.0234	0.0258
	0.0196	0.0224	0.0212	0.0250
	0.0199	0.0206	0.0227	0.0251
	0.0169	0.0193	0.0232	0.0204
	0.0208	0.0201	0.0247	0.0232
	0.0206	0.0193	0.0255	0.0245
Statistics				
Mean	0.0196	0.0207	0.0227	0.0234
Std. Deviation	0.0020	0.0014	0.0012	0.0019
MDL*	0.0053	0.0036	0.0031	0.0049

13.2 PERFORMANCE STUDY

Replicates of 0.05 mg N/L nitrate standard in reagent water***

	Concentration (mg N/L)			
	0.0513	0.0498	0.0511	0.0547
	0.0521	0.0509	0.0511	0.0551
	0.0507	0.0506	0.0512	0.0486
	0.0532	0.0503	0.0512	0.0491
	0.0494	0.0485	0.0523	0.0530
	0.0531	0.0502	0.0538	0.0570
	0.0515	0.0526	0.0531	0.0548
	0.0491	0.0502	0.0480	0.0544
	0.0521	0.0494	0.0525	0.0541
	0.0543	0.0503	0.0515	0.0553
	0.0530	0.0503	0.0516	0.0550
	0.0539	0.0495	0.0517	0.0544
	0.0516	0.0514	0.0521	0.0556
	0.0525	0.0513	0.0532	0.0540
	0.0518	0.0509	0.0527	0.0562
Statistics				
Mean	0.0519	0.0504	0.0518	0.0542
Std. Deviation	0.0015	0.0010	0.0014	0.0023
% RSD**	2.9	1.9	2.6	4.2
% Recovery	103.8	100.8	103.6	108.3

* MDL is calculated by multiplying the Standard Deviation by the Student-*t* value for the number of replicates run.

** %RSD is calculated by dividing the Standard Deviation by the mean, then multiplying by 100.

*** To prepare these standards, refer to Section 9.

13.3 STUDIES FOR PRECISION AND ACCURACY

Seven replicates of 0.1 mg N/L nitrate standard in reagent water***

Concentration (mg N/L)

Statistics				
Mean	0.097	0.097	0.103	0.092
% Recovery	96.8	97.0	103.0	91.9
Std. Deviation	0.002	0.002	0.003	0.001
% RSD**	1.7	1.8	3.0	1.2
Maximum	0.100	0.100	0.107	0.094
Minimum	0.095	0.094	0.097	0.090

Seven replicates of 1 mg N/L nitrate standard in reagent water***

Concentration (mg N/L)

Statistics				
Mean	1.023	0.988	1.021	1.001
% Recovery	102.3	98.8	102.1	100.1
Std. Deviation	0.015	0.018	0.021	0.020
% RSD**	1.4	1.9	2.0	2.0
Maximum	1.052	1.017	1.044	1.023
Minimum	1.001	0.945	0.974	0.965

14 REVISIONS

May 6, 2013 Rev 0	Method document created
	Support data obtained