METHOD #: 352.1	Approved for NPDES and SDWA (Issued 1971)
TITLE:	Nitrogen, Nitrate (Colorimetric, Brucine)
ANALYTE:	CAS # N Nitrogen 7727-37-9 NO3 Nitrate
INSTRUMENTATION:	Spectrophotometer
STORET No.	Total 00620

1.0 Scope and Application

1.1 This method is applicable to the analysis of drinking, surface and saline waters, domestic and industrial wastes. Modification can be made to remove or correct for turbidity, color, salinity, or dissolved organic compounds in the sample.

1.2 The applicable range of concentrations is 0.1 to 2 mg NO_3-N/liter.

2.0 Summary of Method

2.1 This method is based upon the reaction of the nitrate ion with brucine sulfate in a 13 N H_2SO_4 solution at a temperature of 100°C. The color of the resulting complex is measured at 410 nm. Temperature control of the color reaction is extremely critical.

3.0 Sample Handling and Preservation

3.1 Analysis should be made as soon as possible. If analysis can be made within 24 hours, the sample should be preserved by refrigeration at 4°C. When samples must be stored for more than 24 hours, they should be preserved with sulfuric acid (2 mL conc. H_2SO_4 per liter) and refrigeration.

4.0 Interferences

4.1 Dissolved organic matter will cause an off color in 13 N H_2SO_4 and must be compensated for by additions of all reagents except the brucine-sulfanilic acid reagent. This also applies to natural color present not due to dissolved organics.

4.2 The effect of salinity is eliminated by addition of sodium chloride to the blanks, standards and samples.

4.3 All strong oxidizing or reducing agents interfere. The presence of oxidizing agents may be determined with a total residual chlorine test kit.

4.4 Residual chlorine interference is eliminated by the addition of sodium arsenite.

4.5 Ferrous and ferric iron and quadrivalent manganese give slight positive interferences, but in concentrations less than 1 mg/L these are negligible.

4.6 Uneven heating of the samples and standards during the reaction time will result in erratic values. The necessity for absolute control of temperature

during the critical color development period cannot be too strongly emphasized.

5.0 Apparatus

5.1 Spectrophotometer or filter photometer suitable for measuring absorbance at 410 nm.
5.2 Sufficient number of 40-50 mL glass sample tubes for reagent blanks, standards and samples.
5.3 Neoprene coated wire racks to hold sample tubes.
5.4 Water bath suitable for use at 100°C. This bath should contain a stirring mechanism so that all tubes are at the same temperature and should be of sufficient capacity to accept the required number of tubes without significant drop in temperature when the tubes are immersed.
5.5 Water bath suitable for use at 10-15°C.

6.0 Reagents

6.1 Distilled water free of nitrite and nitrate is to be used in preparation of all reagents and standards.
6.2 Sodium chloride solution (30%): Dissolve 300 g NaCl in distilled water and dilute to 1 liter.
6.3 Sulfuric acid solution: Carefully add 500 mL conc. H_2SO_4 to 125 ml distilled water. Cool and keep tightly stoppered to prevent absorption of atmospheric moisture.
6.4 Brucine-sulfanilic acid reagent: Dissolve 1 g brucine sulfate $[(C_{23}H_{26}N_2O_4)_2 \bullet H_2SO_4 \bullet 7H_2O]$ and 0.1 g sulfanilic acid ($NH_2C_6H_4SO_3H \bullet H_2O$) in 70 mL hot distilled water. Add 3 mL conc. HCl, cool, mix and dilute to 100 mL with distilled water. Store in a dark bottle at 5 °C. This solution is stable for several months; the pink color that develops slowly does not effect its usefulness. Mark bottle with warning: CAUTION: Brucine Sulfate is toxic; take care to avoid ingestion.
6.5 Potassium nitrate stock solution: 1.0 mL = 0.1 mg NO_3-N. Dissolve 0.7218 g anhydrous potassium nitrate (KNO_3) in distilled water and dilute to 1 liter in a volumetric flask. Preserve with 2 mL chloroform per liter. This solution is stable for at least 6 months.
6.6 Potassium nitrate standard solution: 1.0 mL = 0.001 mg NO_3-N. Dilute 10.0 mL of the stock solution (6.5) to 1 liter in a volumetric flask. This standard solution should be prepared fresh weekly.
6.7 Acetic acid (1 + 3): Dilute 1 volume glacial acetic acid (CH_3COOH) with 3 volumes of distilled water.
6.8 Sodium hydroxide (lN): Dissolve 40 g of NaOH in distilled water. Cool and dilute to 1 liter.

7.0 Procedure

7.1 Adjust the pH of the samples to approximately 7 with acetic acid (6.7) or sodium hydroxide (6.8). If necessary, filter to remove turbidity.
7.2 Set up the required number of sample tubes in the rack to handle reagent blank, standards and samples. Space tubes evenly throughout the rack to allow

for even flow of bath water between the tubes. This should assist in achieving uniform heating of all tubes.

7.3 If it is necessary to correct for color or dissolved organic matter which will cause color on heating, a set of duplicate samples must be run to which all reagents except the brucine-sulfanilic acid have been added.

7.4 Pipette 10.0 mL of standards and samples or an aliquot of the samples diluted to 10.0 mL - into the sample tubes.

7.5 If the samples are saline, add 2 mL of the 30% sodium chloride solution (6.2) to the reagent blank, standards and samples. For fresh water samples, sodium chloride solution may be omitted. Mix contents of tubes by swirling and place rack in cold water bath (0 - 10°C).

7.6 Pipette 10.0 mL of sulfuric acid solution (6.3) into each tube and mix by swirling. Allow tubes to come to thermal equilibrium in the cold bath. Be sure that temperatures have equilibrated in all tubes before continuing.

7.7 Add 0.5 mL brucine-sulfanilic acid reagent (6.4) to each tube (except the interference control tubes, 7.3) and carefully mix by swirling, then place the rack of tubes in the 100°C water bath for exactly 25 minutes.
CAUTION: Immersion of the tube rack into the bath should not decrease the temperature of the bath more than 1 to 2°C. In order to keep this temperature decrease to an absolute minimum, flow of bath water between the tubes should not be restricted by crowding too many tubes into the rack. If color development in the standards reveals discrepancies in the procedure, the operator should repeat the procedure after reviewing the temperature control steps.

7.8 Remove rack of tubes from the hot water bath and immerse in the cold water bath and allow to reach thermal equilibrium (20-25°C).

7.9 Read absorbance against the reagent blank at 410 nm using a 1 cm or longer cell.

8.0 Calculation

8.1 Obtain a standard curve by plotting the absorbance of standards run by the above procedure against mg NO_3-N/L. (The color reaction does not always follow Beer's law).

8.2 Subtract the absorbance of the sample without the brucine-sulfanilic reagent from the absorbance of the sample containing brucine-sulfanilic acid and determine mg NO_3-N/L. Multiply by an appropriate dilution factor if less than 10 mL of sample is taken.

9.0 Precision and Accuracy

9.1 Twenty-seven analysts in fifteen laboratories analyzed natural water samples containing exact increments of inorganic nitrate, with the following results:

Increment as Nitrogen, Nitrate mg N/liter	Precision as Standard Deviation mg N/liter	Accuracy as Bias, %	Bias, mg N/liter
0.16	0.92	-6.79	-0.01
0.19	0.083	+8.30	+0.02
1.08	0.245	+4.12	+0.04
1.24	0.214	+2.82	+0.04

(FWPCA Method Study 2, Nutrient Analyses).

Bibliography

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition, p 427, Method 419D (1975).
2. Annual Book of ASTM Standards, Part 31, "Water", Standard D 992-71, p 363 (1976).
3. Jenkins, D., and Medsken, L., "A Brucine Method for the Determination of Nitrate in Ocean, Estuarine, and Fresh Waters", Anal Chem., 36, p 610, (1964).