Application Note: 51862

# Analysis of Nitrate Nitrogen ($NO_3^-$) in Water by the EPA Approved Brucine Method

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**Key Words**

- Brucine Method
- Nitrate
- UV-Visible
- Water Analysis

## Introduction

In this experiment quantitative analysis of nitrate nitrogen ($NO_3^-$) was performed by the Brucine method. Data was acquired and processed using the Thermo Scientific Evolution Array UV-Visible spectrophotometer and Thermo Scientific VISION*collect* software.

## Background

The Brucine method for nitrate is approved by the United States EPA as Method No. 352.1.[1] When a water sample containing nitrate ion is treated with Brucine in sulfuric acid, a yellow solution results. The concentration of nitrate nitrogen may be calculated based upon the absorbance of the solution at 410 nm.

## Experimental

### Reagents and Apparatus

- Nitrate ion standard solution (0.001 mg $NO_3^-$ N/mL)
- Sodium chloride solution (30% w/v)
  - Dissolve 30 g NaCl in 100 mL deionized water
- Sulfuric acid solution (4+1)
  - 4:1 = Sulfuric acid ($H_2SO_4$):deionized water
  - *CAUTION: Wear appropriate protective clothing and observe proper protocols for diluting sulfuric acid*
- Brucine-Sulfanilic acid solution
  - Dissolve 1 g brucine dihydrate ($C_{23}H_{26}N_2O_4 \cdot 2H_2O$) and 0.1 g sulfanilic acid ($H_2NC_6H_4SO_3H \cdot H_2O$) in 3 mL conc. HCl. Dilute to 100 mL with deionized water
- Evolution™ Array™ UV-Vis spectrophotometer with VISION*collect*™ software
- Cuvette (10 mm pathlength)

### Standards and Measurement Procedure

1. Prepare a series of nitrate standards from the 0.001 mg/mL $NO_3^-$ standard. Each of the standards should be an accurately measured volume between 1 mL and 10 mL of stock diluted to a total volume of 10.0 mL. Also prepare a 0 ppm reagent blank. Between four and six standards are generally considered to be sufficient to establish a robust calibration curve.
2. Treat each standard according to the procedure described in the sample work-up procedure below
3. In Quantification Standard mode
   a. Measure the 0 ppm standard as the blank
   b. Measure the absorbance of the remaining standards
4. In Quantification Sample mode, measure the absorbance of the unknown sample(s) and calculate the concentration

### Sample Work-up Procedure

1. Measure a known, consistent volume (1–5 mL is a typical range) of sample into a calibrated tube or flask
2. Dilute with deionized water to a total volume of 10 mL
3. If samples are saline, add 2 mL sodium chloride solution
4. Add 10 mL sulfuric acid solution and mix well by shaking while cooling in flowing water. Do not stopper the tube/flask.

   ***CAUTION: This step is exothermic. The contents will heat up and pressure will result inside the container if it is stoppered.***
5. Add 0.5 mL brucine in sulfanilic acid solution and mix by shaking
6. Heat 25 minutes at 100 °C in a water bath
7. Cool with flowing water, dilute to 25 mL with deionized water, stopper and invert several times to mix thoroughly
8. Pour ca. 3 mL of solution into the 10 mm pathlength cuvette and record the absorbance using the spectrophotometer

## Instrument Parameters

Data collection was carried out with VISION*collect* software using the parameters displayed in Figure 1. The Curve Zero Offset and Curve Order settings specify a linear data fit that is not forced through the origin.

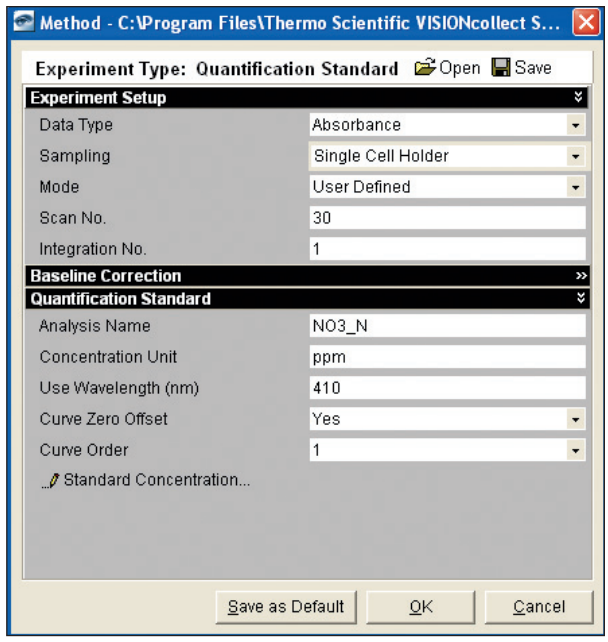


Figure 1: Experimental parameters for Nitrate assay

## Results

Figure 2 shows the software screen for the standard solution data collection. Full spectra of all standards are displayed in the Standard Spectrum window (upper left). Details of the standards used and their measured absorbance values at the analytical wavelength (410 nm) are shown at the bottom of the screen and a calibration curve is plotted (upper right). The equation of the line of best fit is calculated by the least squares method and displayed below the Standard Spectrum.

A sample containing an unknown amount of nitrate was prepared according to the procedure and measured in duplicate. The results were displayed as shown in Figure 3. The concentration of the sample was found to be 0.23 ppm.

## Conclusion

In this experiment quantitative analysis of nitrate nitrogen ($NO_3^-$) in water was successfully performed with the Evolution Array spectrophotometer and VISION*collect* software. Rapid acquisition of full-range spectra and excellent instrument sensitivity made the analysis quick, accurate and reproducible.

## References


1. Electronic: *http://www.epa.gov/waterscience/methods/method/files/352_1.pdf*. Date acquired 12-12-2009.


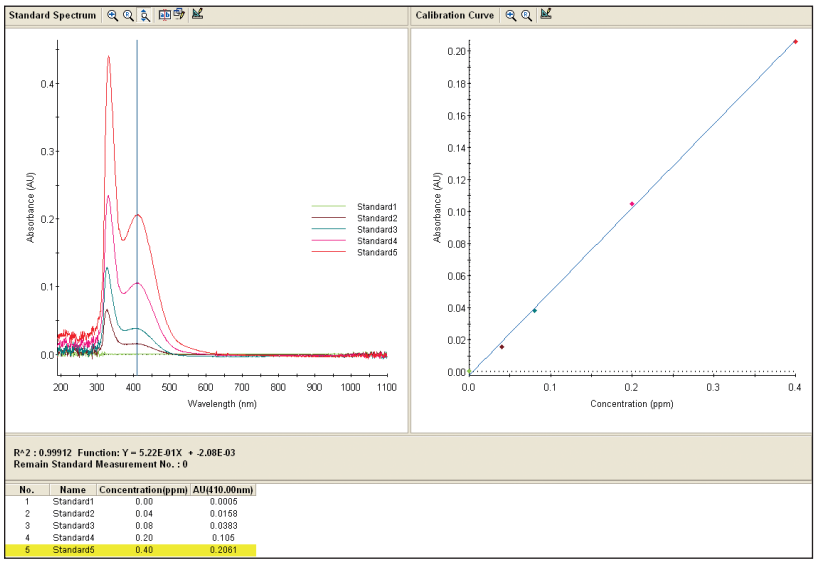


Figure 2: Standard solution data

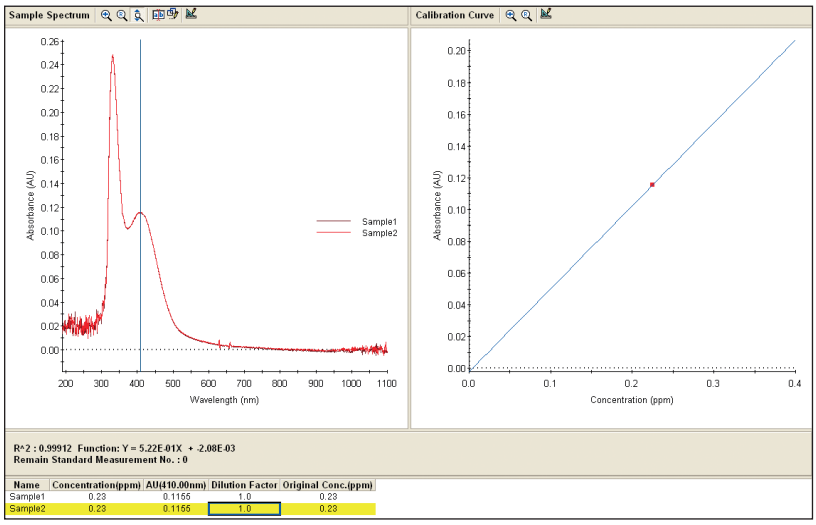


Figure 3: Sample solution data



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