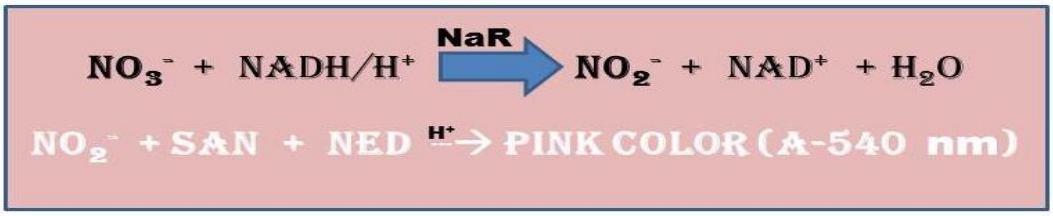
COMPARISON OF MANUAL AND DISCRETE ANALYZER METHODS FOR NITRATE ANALYSIS USING ENZYMATIC REDUCTION BASED ON EUKARYOTIC NITRATE REDUCTASE

WILBUR H. (Bill) CAMPBELL and ELLEN R. CAMPBELL

The Nitrate Elimination Company, Incorporated (NECi)
334 Hecla Street, Lake Linden, Michigan 49945 USA



The most widely used, certified nitrate analysis method for drinking water, wastewater, and seawater, employs cadmium metal reduction of the analyte to nitrite which is quantified after diazotization with specific dyes. This is a 20th century method which can now be replaced by enzymatic reduction, a Green Chemistry Method, and eliminate the use of hazardous and toxic cadmium. In addition, the cadmium reduction method for nitrate utilizes old technology such as the continuous flow analyzer, and cannot be done by modern equipment like the automated discrete analyzer (DA). The enzymatic reduction method for nitrate analysis is based on eukaryotic nitrate reductase (NaR; EC 1.7.1.1-3) is ideal for the DA system. In this new 21st century method, the enzyme nitrate reductase catalyzes the reduction of nitrate to nitrite, with the coenzyme nicotinamide adenine dinucleotide (NADH), driving the reduction which is an irreversible reaction. The nitrite is diazotized like in the old method, which yields a pinkish color absorbing at 540 nm. Certified nitrate calibrants are used for quantifying the results with a standard curve relating A-540 nm to Nitrate-N. We present here a comparison of the manual and DA method with a variety of drinking water, wastewater, and seawater matrices, which were analyzed in multiple laboratories on several different brands of instruments using robust quality control procedures. The results of the Inter-Laboratory Study demonstrate that the Enzymatic Nitrate Reduction Method using Nitrate Reductase is a valid method with high precision and low bias. For the Standard Range Nitrate analysis with calibrants from 0.05 to 5.00 mg nitrate-N per Liter for DA analysis, the mean Minimum Detection Limit (MDL) for 9 different DA is 0.02 mg nitrate-N per Liter (1.4 µM). Furthermore, the Enzymatic Nitrate Reduction Method using Nitrate Reductase is comparable to the cadmium reduction method when the same samples are analyzed. Thus, the Enzymatic Nitrate Reduction Method (see Appendix) is now certified as a replacement for the outdated cadmium reduction method by ASTM International (ASTM Method D7781 Nitrate Analysis in Water by Nitrate Reductase). Similar methods were recently approved by the U. S. Geological Survey - USGS Methods I-2547-11 and I-2548-11 for Standard- and Low-level Colorimetric Determination of Nitrate plus Nitrite in Water by Enzymatic Reduction, Automated Discrete Analyzer Methods.

Contact:
bill@nitrate.com

Key Words: Green Chemistry, Nitrate, Nitrite, Drinking Water, Wastewater, Seawater, Enzymes, Nitrate Reductase, Automated Discrete Analyzer, and Spectrophotometry.

Abbreviations:

AtNaR2	Recombinant *Arabidopsis thaliana* NADH Nitrate Reductase, purified
DA	Discrete Analyzer
ERA	Environmental Resource Associates
IMAC	Immobilized Metal ion Affinity Chromatography
IPR	Initial Performance and Recovery
MDL	Minimum Detection Limit
MS	Matrix Spike
MSD	Matrix Spike Duplicate
NADH	Reduced, Nicotinamide Adenine Dinucleotide
NADPH	Reduced, Nicotinamide Adenine Dinucleotide Phosphate
NaR	Nitrate Reductase
NECi	The Nitrate Elimination Company, Incorporated
NED	N-(1-naphthyl)ethylenediamine dihydrochloride
OPR	Ongoing Performance and Recovery
QC	Quality Control
SAN	Sulfanilamide
S	Spike Content
SR	Spike Recovery
SR%	Percent of Spike Recovery
SSC	Second Source Calibrant