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Simultaneous Measurement of Total Nitrogen and Phosphorus via Persulfate Digestion and Colorimetric Analysis

Introduction

Along with carbon, nitrogen and phosphorus containing (N/P) compounds are the most important nutrients found in fresh and natural waters, directly influencing algal and bacterial growths. Indirectly, the eutrophic effects of compounds containing these analytes can profoundly impact organisms within the ecosystems in which they are found [1]. Complete and accurate measurements of N/P–compounds as collectives are an increasingly important component of nutrient monitoring and management programs.

Methods widely used for the measurement of N and P are U.S. EPA methods 353.2, 350.1, and Standard Methods 4500 P E but only address one or two N and P species. U.S. EPA 365.1 analyzes total phosphorus (TP), hydrolyzable phosphorus, and orthophosphate, but the digestion does not quantitatively recover total nitrogen (TN). More vigorous digestion procedures found in U.S. EPA 351.2 (Total Kjeldahl Nitrogen [TKN]) and 365.4 (Total Kjeldahl Phosphorus [TKP]) broaden the scope of measurable compounds, but may use toxic mercury catalysts, or copper catalysts that contribute to high background coloration. The TKN/TKP digestions do not recover all compounds of interest and suffer from interferences that require speciation to be performed and results mathematically combined. Consolidating all of these processes into a single safe preparatory step combined with a single mutually expressive analysis would save valuable time, effort, and money, while reducing complexity, the potential for inaccuracy, and exposure to hazardous materials [2].

This application note presents a semi–automated continuous flow analysis method for the simultaneous detection of Total Nitrogen and Phosphorus (TN/TP). This methodology utilizes manual alkaline persulfate digestions for sample preparation, followed by Flow Injection Analysis (FIA) for sample detection. Calibration data, linear range measurements, method detection limits (MDLs), and representative sample analyses are reported.

Instrumentation

Instrumentation used for this study was an OI Analytical Flow Solution 3100 continuous flow analyzer consisting of an autosampler, a 16-channel peristaltic reagent pump, two valve modules equipped with heaters, two reaction mixing manifolds, two photometric detectors, and a data acquisition system (Figure 1).

Figure 1. OI Analytical Flow Solution 3100

Experimental

Prior to digestion and analysis, all glassware was thoroughly washed with 10% HCl in order to prevent contamination from any residual N/P–compounds commonly found in laboratory environments. In addition, exposure of all reagents to the laboratory atmosphere was severely limited, as ammonia (NH_3) is a prevalent gaseous contaminant. All reagents were prepared from stocks with low or ultra-low N and P contents.

The digestion procedure was derived from Standard Methods 4500-P J for concurrent digestion of samples for TN and TP [3] analysis. Modifications were made to the sample-to-reagent ratios in order to maximize recoveries on all compounds of interest. A standard COD-style digestion block, obtained from Pierce, was used in place of the suggested autoclave to improve the ease of operation and reduce capital equipment costs. Samples were digested in standard disposable glass 16 x 100 mm screw-cap vials. A study was conducted using N-containing compounds as an indicator of digestion efficiency in order to address variables such as reagent ratios, temperature settings, and reaction times under these conditions.

The measurement of TN and TP in water matrices was performed spectrophotometrically utilizing the reagents and mixtures described in Standard Methods 4500-NO$_3$ I and Standard Methods 4500-P G respectively. These reagents and procedures provided the foundations for a continuous flow methodology capable of simultaneous post-digestion analyses. Standards were prepared in the ranges of 0.02-2.0 mg/L P and 0.05-10 mg/L N. Samples consisted of varying N/P-containing compounds, and were digested as representative indicators of digestion efficiency. Optimizations were achieved by modifying experimental parameters such as reagent flow rates, reaction kinetics, pH, and reagent composition.

The P-dedicated valve module was equipped with a 200-μL injection loop, while the N-dedicated valve used a 100-μL loop, that supplied the sample to the mixing manifold. The mixing manifolds were composed of a polysulfone polymer for heat and acid resistance, utilized confluence mixing and radial dispersion, and 37 °C heat in the case of TP, to produce the colorimetric reaction. The photometric detectors consisted of a wide dynamic–range detector equipped with a 5-mm flowcell, and a 660- and 540-nm filter for TP and TN respectively. Refer to Figures 2 and 3.
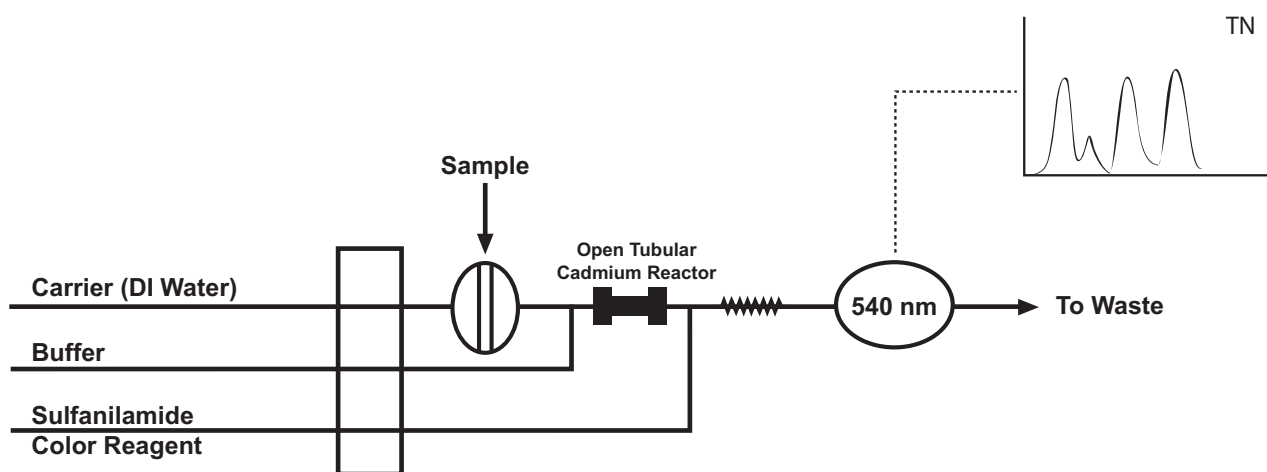

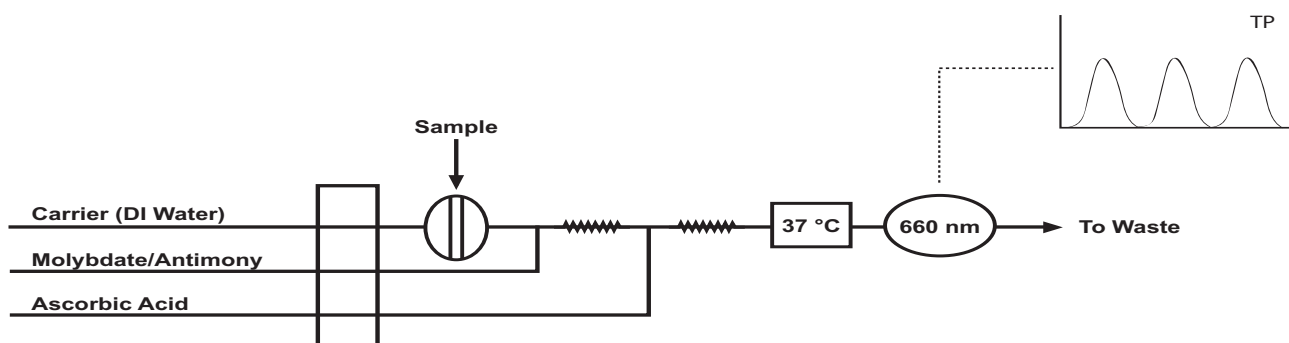Figure 2. Total Nitrogen (TN) Analytical Flow Diagram

Figure 3. Total Phosphorus (TP) Analytical Flow Diagram

Results and Discussion

Initially, the digestion conditions described by Standard Methods were found to be insufficient when performed using a bench-top digestion block. Method 4500-P J specifies the use of an autoclave at a temperature of 120 °C for 120 minutes for both TN and TP digestions. Digestions of urea and nicotinic acid, considered refractory N-containing compounds and excellent indicators of digestion efficiency, consistently yielded recoveries of 79-85% when comparing relative peak intensities of these compounds to a known standard (Figure 4)[6]. A systematic increase in temperature indicated operation at 150 °C was required for complete oxidation of these compounds, yielding recoveries of 97-102% respectively (Figure 5). A digestion time of 120 minutes was excessive and unnecessary, as each oxidation was complete after only 30 minutes.
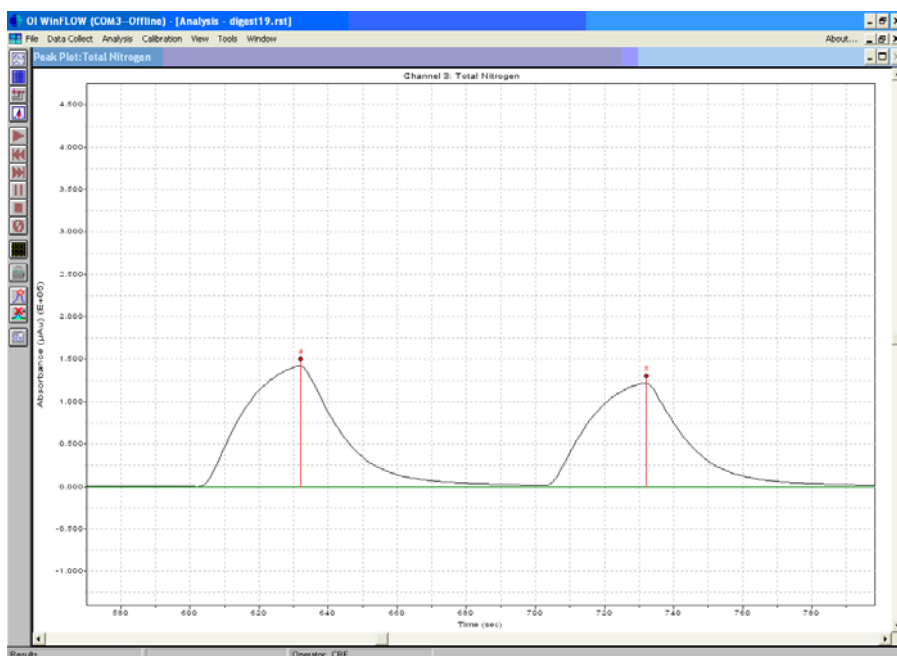

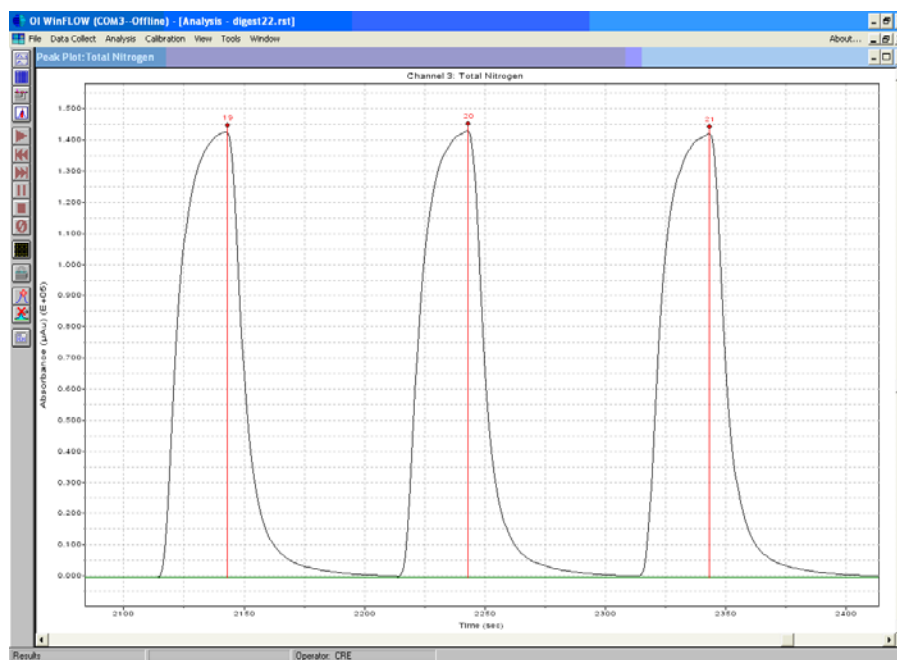Figure 4. Digestion of Nicotinic Acid relative to Nitrate at 85% Recovery

Figure 5. Digestion of Nicotinic Acid and Urea relative to Nitrate at 100% Recovery

When this procedure was extended to phosphorus, the results appeared equivalent to those for nitrogen when analyzing refractory P-containing compounds, such as trimethyl phosphate and phenyl phosphate (Figure 6). However, acid-hydrolyzable compounds such as sodium pyrophosphate and sodium tripolyphsosphate produced recoveries of 28-55% when digested under these conditions (Figure 7).
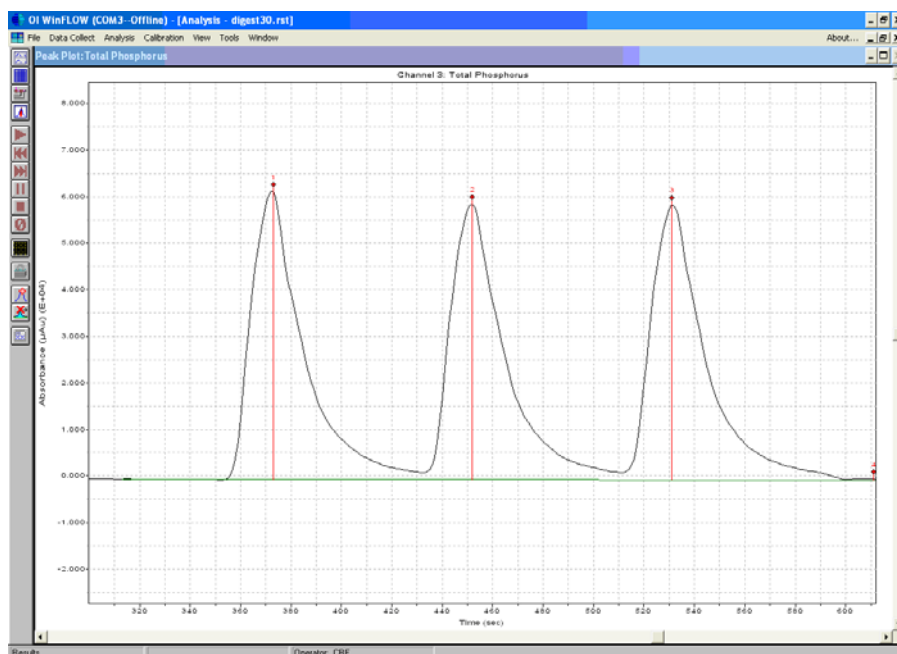

Figure 6. Digestion of Trimethyl- and Phenyl Phosphate Relative to
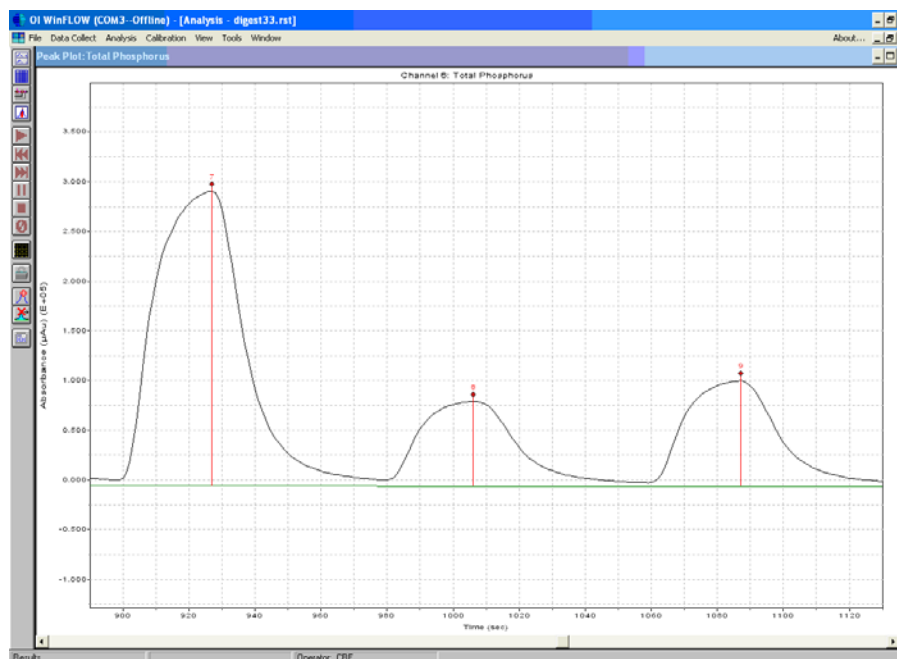Orthophosphate at 100% Recovery

Figure 7. Digestion of Tripoly- and Pyrophosphate Relative to Orthophosphate
at 28% and 26% Recovery

The low oxidation efficiency of these compounds was a direct result of the highly alkaline digestion conditions required by the Standard Methods procedure; pH measurements prior to digestion ranged from 12.5 to 12.8. The sample-to-digestion solution ratio was systematically increased from 4:8:1 (stated in Standard Methods), to a ratio of 8:1. This yielded 100% recoveries on pyrophosphate, without sacrificing efficiency on N-containing species; recoveries for tripolyphosphate were never improved beyond 65%, as this compound would likely require a preliminary acid-hydrolysis if it's presence is suspected (Figure 8).
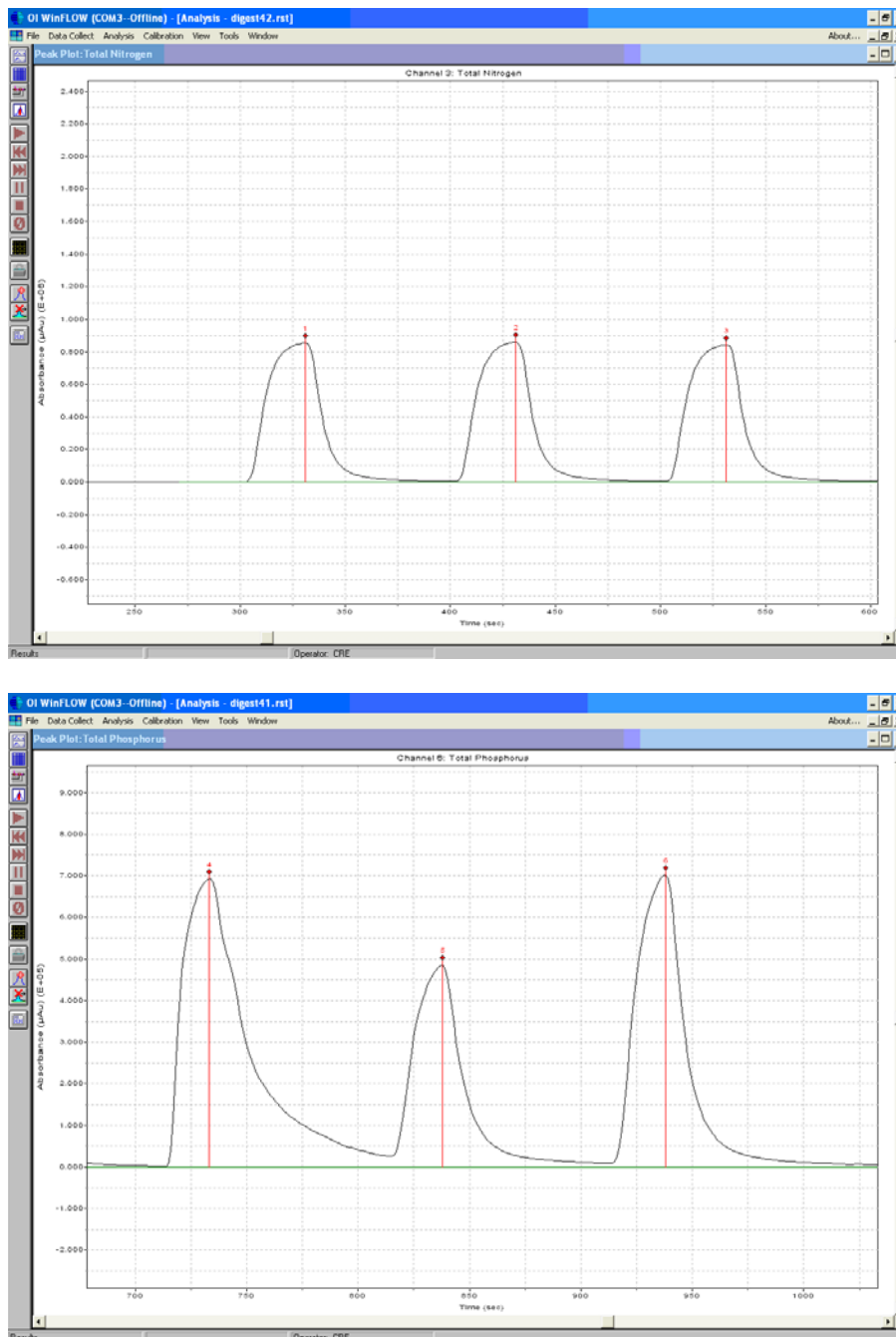

Figure 8. Digestion of Nicotinic Acid and Urea relative to Nitrate at 100% recovery, followed by Tripoly- and Pyrophosphate relative to Orthophosphate at 65% and 100% recovery.

Optimization of the analytical portion of the method identified two variables that needed to be reconciled in order to be functional. The flow rates described in Methods 4500-P G and $-NO_3$ I were excessively high and totaled 3.3 and 4.8 mL/min respectively. Operating at high flow rates reduced the incubation times of both reactions and limited the peak intensities. These rates were adjusted down by a factor of 2-3 in each case, ensuring the reactants had ample time to react.

Inherent nitrogen contamination was present in all forms of persulfate at concentrations as high as 2.0 mg/L (Figure 9). The result was a positive blank that limited the accuracy of the low end of the N analysis method. This positive interference is described in literature involving digestion procedures of this type, but not in the Standard Methods documentation on which it is based. Multiple recrystalizations of the persulfate oxidant as a means of purification reduced the background contribution enough to detect TN to a level of 0.05 mg/L; levels of TN below this concentration were obscured.

Recent studies conducted on thousands of environmental samples compared the efficacy of TP/TN measurements to TKN measurements and found the accuracy of these methods begins to break down below the 0.1-ppm level [7] (Figure 10).   Therefore, detection of N below the 0.05-ppm limit described by this method is meaningless and unnecessary. The inherent N background sets a lower limit on what is practically applicable for such analyses. With these facts in mind an accurate and precise method for simultaneous detection of TN and TP was successfully developed (Figures 11 and 12; Tables 1 and 2).
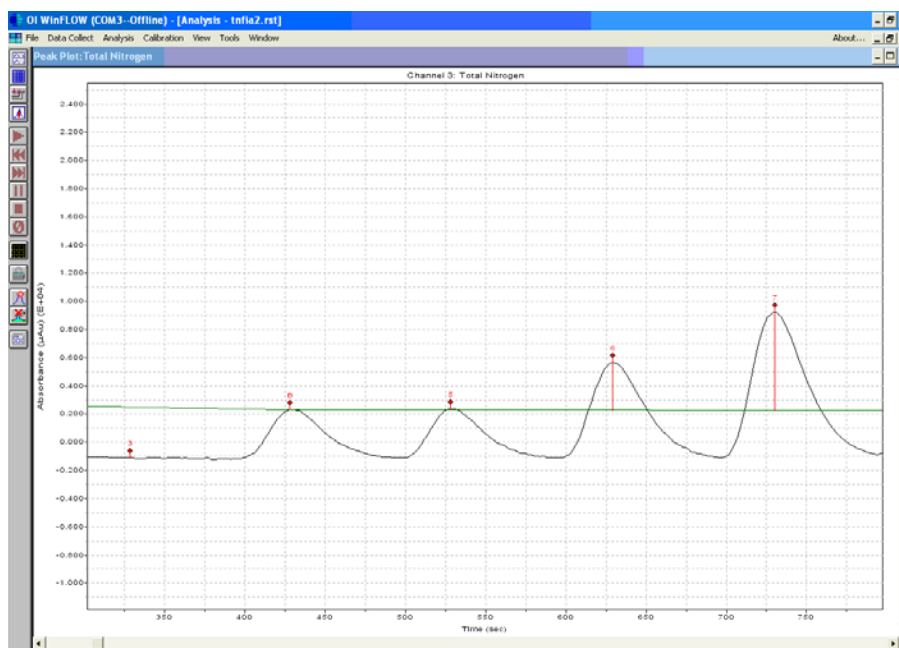

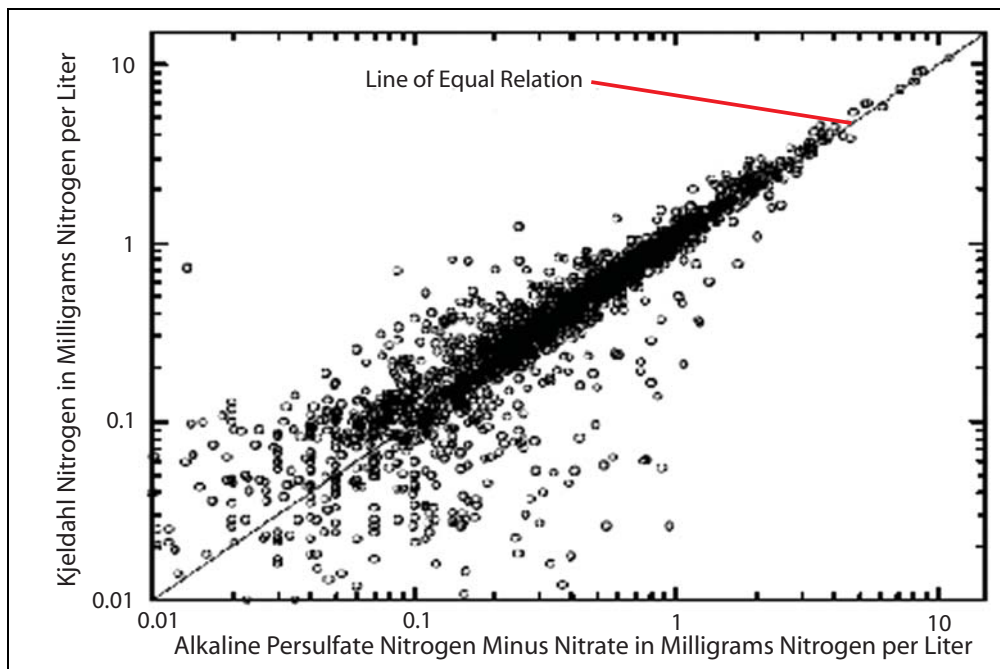Figure 9. Positive blank resulting from inherent nitrogen contamination.

Figure 10. Scatter plot relating efficacy of Total Persulfate Nitrogen
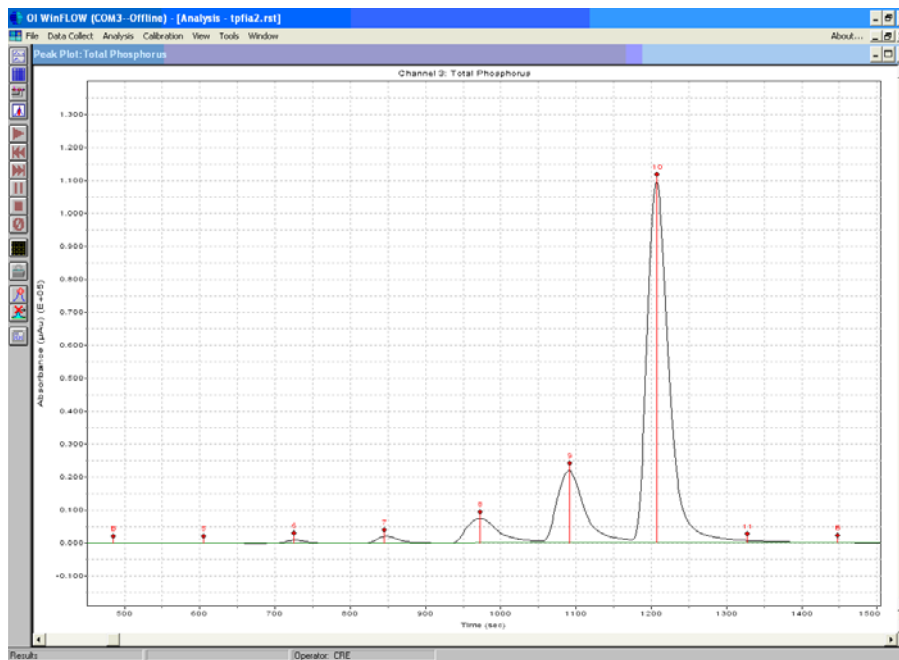Digests to traditional methods at various concentrations [7].
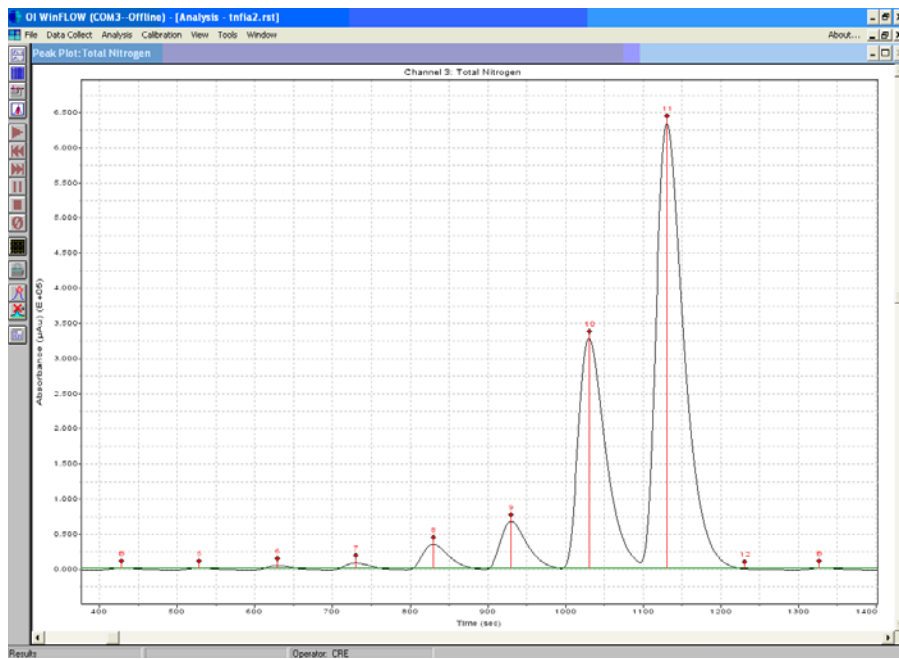
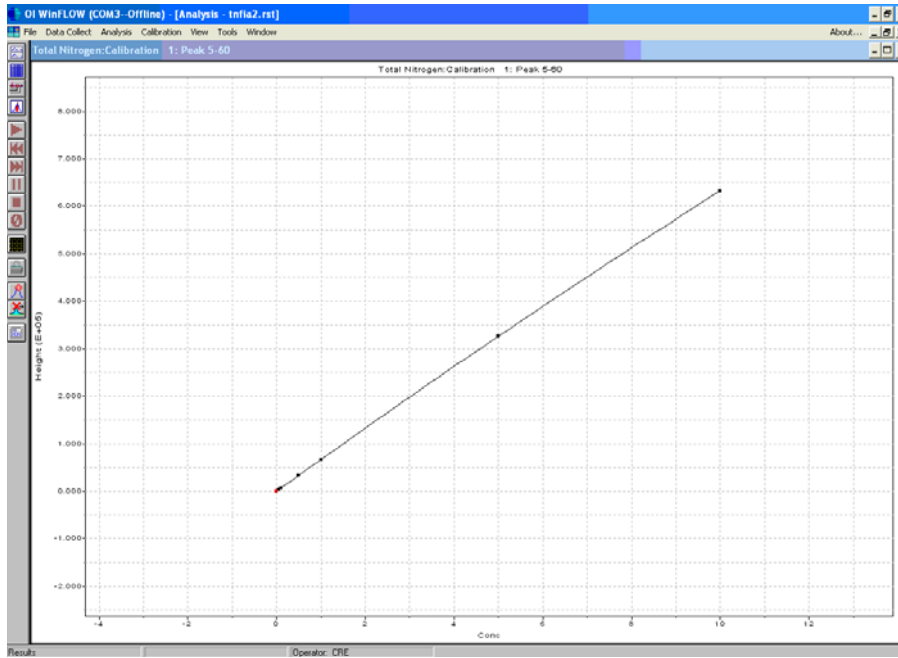Figure 11. Total Nitrogen and Total Phosphorus Calibrations
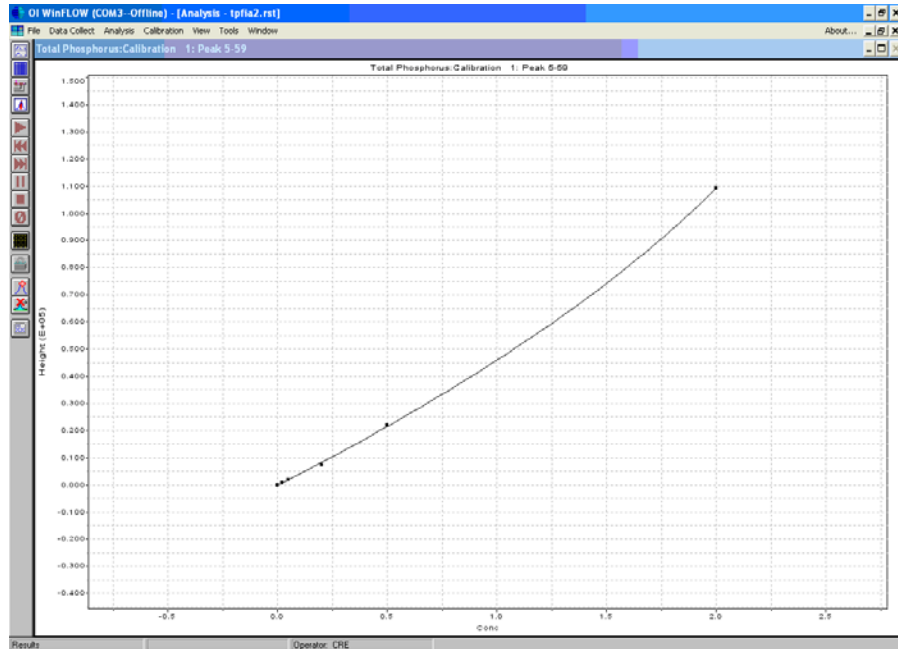
Figure 12. Total Nitrogen and Total Phosphorus Calibration Curves

Table 1. Total Nitrogen (TN) Calibration, MDL, and QC Data

Analyte	Actual Concentration (ppm N)	Calculated Concentration (ppm N)	%RSD	%Accuracy	Correlation (R-value)
Standard 1	0.00	0.00	—	—	0.999998
Standard 2	0.05	0.05	—	—	0.999998
Standard 3	0.10	0.10	—	—	0.999998
Standard 4	0.50	0.50	4.69%	—	0.999998
Standard 5	1.00	0.98	—	—	0.999998
Standard 6	5.00	5.00	1.36%	—	0.999998
Standard 7	10.00	10.00	—	—	0.999998
MDL	—	0.02	4.35%	—	—
QC	0.25	0.26	3.37%	104%	—

Table 2. Total Phosphorus (TP) Calibration, MDL, and QC Data

Analyte	Actual Concentration (ppm P)	Calculated Concentration (ppm P)	%RSD	%Accuracy	Correlation (R-value)
Standard 1	0.00	0.00	—	—	0.99992
Standard 2	0.02	0.02	—	—	0.99992
Standard 3	0.05	0.05	1.31%	—	0.99992
Standard 4	0.20	0.18	—	—	0.99992
Standard 5	0.50	0.51	—	—	0.99992
Standard 6	2.00	2.00	1.44%	—	0.99992
MDL	—	0.006	7.00%	—	—
QC	0.75	0.77	—	102.7%	—

Using the optimized digestion and detection procedures described within, representative samples were analyzed to ensure complete accuracy (Figure 13; Tables 3 and 4). Each sample produced significant peak intensities relative to the noise level at the expected 5 and 0.5 mg/L levels. Although one compound proved to be beyond the digestion conditions of the method, most inorganic and polyphosphates were effectively reduced to accurately–measurable orthophosphates. All organic and inorganic nitrogen species were completely converted to nitrate, which could then be subsequently reduced to measurable nitrite. The ammonium chloride buffer used by Standard Methods 4500-NO$_3$ I was exchanged for Imidazole, buffered at a pH of 7.5, to preserve the lifespan and efficiency of the cadmium reactor.
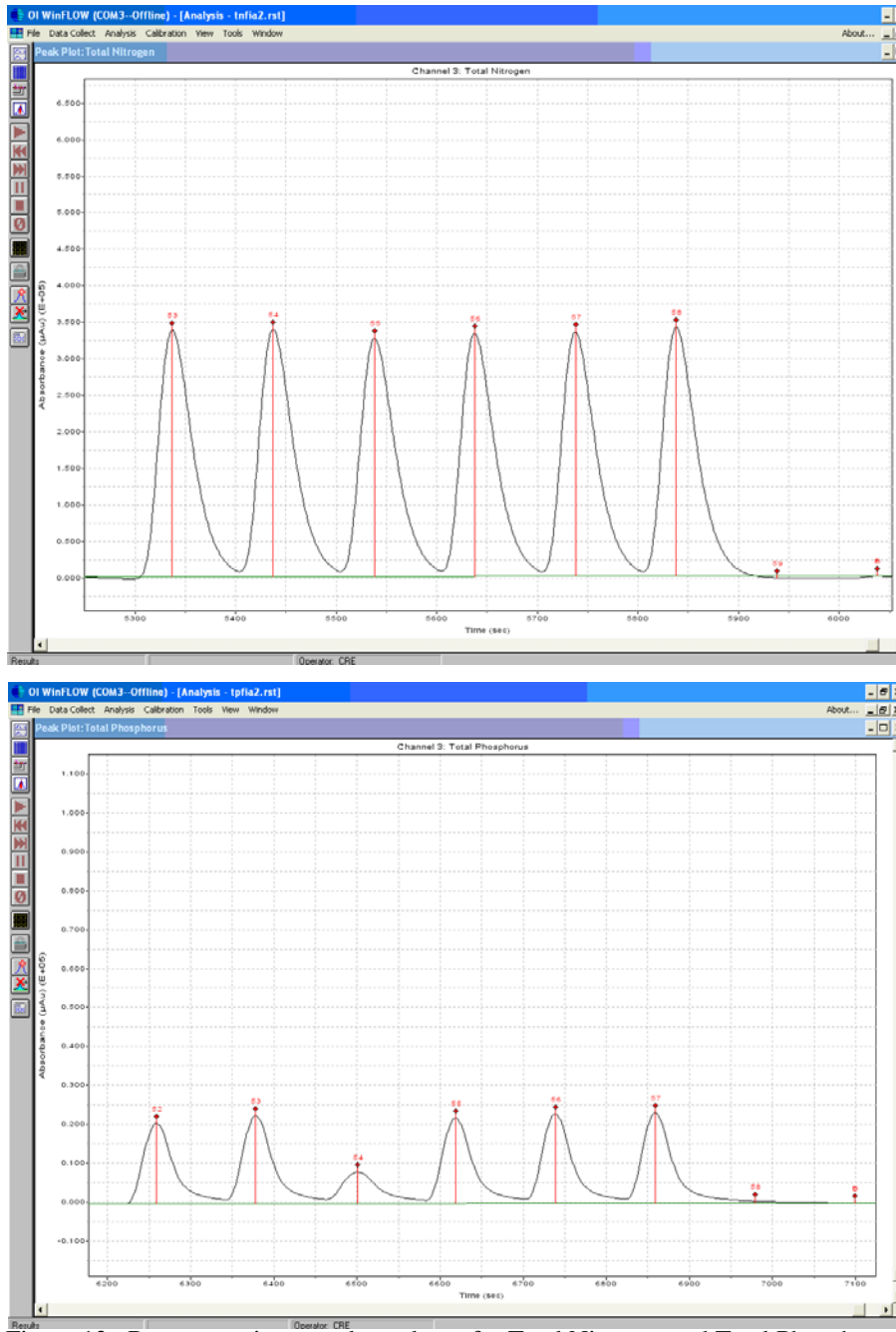
Figure 13. Representative sample analyses for Total Nitrogen and Total Phosphorus.

Table 3. Representative Sample Data for Total Nitrogen (TN)

Analyte	Actual Concentration (ppm N)	Calculated Concentration (ppm N)	%Accuracy
Urea	5.00	5.17	103.4%
EDTA	5.00	5.19	103.8%
Nicotinic Acid	5.00	4.99	99.8%
Ammonia	5.00	5.10	102%
Nitrite	5.00	5.12	102.4%
Glycine	5.00	5.23	104.6%

Table 4. Representative Sample Data for Total Phosphorus (TP)

Analyte	Actual Concentration (ppm P)	Calculated Concentration (ppm P)	%Accuracy
Trimethyl Phosphate	0.50	0.48	96
Sodium Pyrophosphate	0.50	0.52	104%
Sodium Tripolyphosphate	0.50	0.19	*38%
Phenyl Phosphate	0.50	0.51	102%
Sodium Hexametaphophate	0.50	0.53	106%
Sodium Phosphate	0.50	0.53	106%

Only one compound exhibited low recovery.

Summary and Conclusions

There is a need for simpler, more reliable methods of total nitrogen (TN) and total phosphorus (TP) analysis in environmental water samples to support nutrient pollution monitoring and control programs. The methodology for Simultaneous Detection of Total Nitrogen and Total Phosphorus following a Single Manual Persulfate Digestion described herein meets these requirements.

This method provides a means for determining the presence of nearly all N- and P-containing compounds with an average accuracy of 103% recovery. These analyses exhibited correlation values of 0.99992 or better on both analytes, and yielded percent relative standard deviations (%RSD) between 1 and 5%. In addition, the analytical ranges for each analyte are more than adequate to meet the detection criteria for "real world" samples.

The single persulfate digestion allows N/P–compounds to be digested into readily–measurable species without exposure to hazardous sulfuric acid vapors or toxic mercury catalysts specified in Kjeldahl digestions. The use of a COD-style block digester negates the need for cumbersome TKN and TP block digesters, and provides a complete digestion in 30 minutes, rather than the 1-2 hours required by methods such as U.S. EPA 365.1, 351.2, and Standard Methods 4500-P B. The two-channel simultaneous FIA system provides a reliable, accurate, and timesaving platform on which to analyze samples. This method can be a useful tool for collecting nutrient pollution data and monitoring water quality in natural water ecosystems.

References

1. Fuhrman, J.K., Zhang, H., Schroder, J.L., and Davis, R.L. Water-Soluble Phosphorus as Affected by Soil Extractant Ratios, Extraction Times, and Electrolyte. Communications in Soil Science and Plant Analysis; 2005, 36, pp 925-935.

2. Methods for Chemical Analysis of Water and Wastewater; EPA-600/4-79-020; U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1993.

3. Standard Methods for the Examination of Water and Wastewater, 21st ed.; American Public Health Association: Washington, D.C., 2005.

4. OI Analytical Method Abstract #3270, "FS3100 Automated Method for the Determination of Total Nitrogen in Manual Persulfate Digests".

5. OI Analytical Method Abstract #3271, "FS 3100 Automated Method for the Determination of Total Phosphorus in Manual Persulfate Digests".

6. Solorzano, Lucia, Sharp, Jonathan H. Determination of Total Dissolved Nitrogen in Natural Waters. Limnology and Oceanography; 1980, 25(4), pp 751-754.

7. Patton, Charles J., Kryskalla, Jennifer R. Evaluation of Alkaline Persulfate Digestion as an Alternative to Kjeldahl Digestion for Determination of Total Dissolved Nitrogen and Phosphorus in Water; Water Resources Investigations Report 03-4174; Methods of Analysis by the U.S. Geological Survey, National Water Quality Laboratory: Denver, CO, 2003.