Application Note 1103

Determination of Total Nitrogen and Phosphorus in Wastewaters by Alkaline Persulfate Digestion Followed by IC

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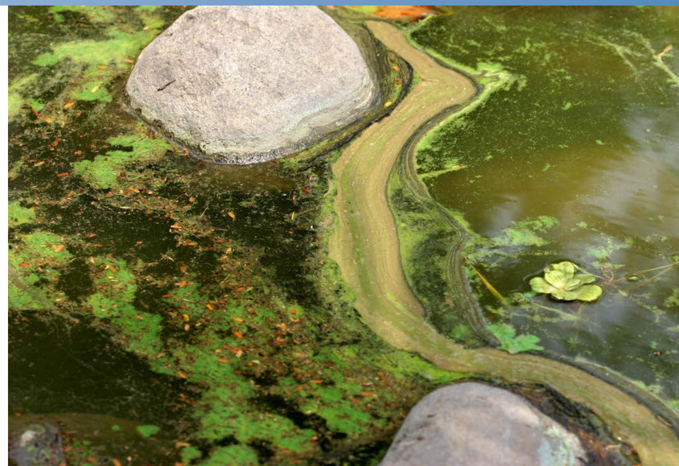
Key Words
Dionex IonPac AS19 Column, Suppressed Conductivity Detection, Eutrophication, Nutrient Pollution, Total Kjeldahl Nitrogen (TKN), Ion Chromatography (IC), Total Phosphorus



Introduction

Excess use of nutrients over the last several decades has caused significant water quality and health issues on a global scale. In the U.S., the impact of nutrient pollution has been identified as one of the most widespread, costly, and challenging environmental problems in the 21st century.[1] Nutrient pollution causes excess algae growth (i.e., algal blooms) over large bodies of water, which has a significant impact on the environment, human health, and the economy. Algal blooms consume significant amounts of oxygen and thus deprive fish, shellfish, and other aquatic organisms of the oxygen needed to survive. In addition, algae can have a negative impact on human health by emitting toxins that can cause stomach aches, rashes, and more serious health issues. It is estimated that the U.S. tourism industry loses approximately $1 billion annually because of algae-related decreases in fishing and recreational activities.[2]

There are about 14,000 nutrient-related impairment listings across 49 states that include 2.5 million acres of lakes and reservoirs and 80,000 miles of rivers and streams. Approximately 50% of the nation's streams contain medium to high levels of nitrogen and phosphorus. About 80% of the assessed U.S. coastal waterways are currently experiencing eutrophication.[3] Excess nutrients in bodies of water are primarily caused by fertilizer runoff, animal manure, sewage treatment plant discharges, storm water runoff, and the combustion of fossil fuels.[2] In the Mississippi River Basin, agriculture is the leading contributor of excess nutrients. About two-thirds of nitrogen loadings and about one-half of phosphorus loadings are contributed from crop agriculture, which is not regulated under the Clean Water Act.[3]

Although inorganic nitrate and phosphate can be determined directly in natural waters, this does not provide the wider environmental significance of the organic nitrogen and phosphorus fractions that contribute to the total nutrient loading in bodies of water. In water, nitrogen exists as inorganic and organic species. Inorganic nitrogen is present in the oxidized form (e.g., nitrite and nitrate) and reduced form (e.g., ammonia/ammonium and dinitrogen gas). Organic nitrogen is available in a variety of complex forms such as amino acids, proteins, humic acids, and urea. However, before being used as a nutrient, the organic nitrogen must first be converted to ammonia.[4] Total nitrogen (TN) is the sum of all forms of nitrogen in the water sample.

Phosphorus exists as inorganic orthophosphate, polyphosphate, and organic phosphate.[5] Particulate phosphorus—found in suspension or sediment—consists of plants, animals, phosphorus in minerals, and phosphate adsorbed on an iron oxyhydroxide mineral surface.[4] Total phosphorus (TP) is a measure of all forms of phosphorus found in water.

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The standard method for determining TN is based on the determination of total TKN plus the sum of nitrite and nitrate. The TKN procedure converts organic nitrogen—such as amino acids, proteins, and peptides—to ammonia.[6]

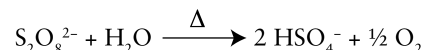
IC with suppressed conductivity detection, as described in U.S. Environmental Protection Agency (EPA) Methods 300.0 and 300.1, is one of the most common approaches used to determine nitrite and nitrate.[7,8] Although TKN is approved under the National Pollution Discharge Elimination System and Safe Drinking Water Act for compliance monitoring, the method has a number of drawbacks: 1) It poses environmental and safety hazards by using toxic reagents (e.g., mercury) at high temperatures. 2) Waters with high levels of nitrate are known to interfere with TKN, resulting in a negative bias. 3) Nitrate is not measured. 4) Because TKN does not provide a true TN value, it requires an additional analytical technique to achieve a true TN value.

An alternative method for determining TN and TP is the alkaline persulfate digestion technique. This is a well-established approach that provides an environmentally safer alternative to Kjeldahl digestion for the routine determination of nitrogen and phosphorus in water. The 22nd edition of the American Public Health Association's Standard Methods for the Examination of Water and Wastewater describes a protocol for determining TN by alkaline persulfate digestion, but the method excludes the determination of TP.[9] Over a 12 month period, the U.S. Geological Survey (USGS) conducted a large-scale and geographically diverse study for Kjeldahl nitrogen and Kjeldahl phosphorus in 2100 surface and groundwater samples. The samples were analyzed independently for TN and TP using alkaline persulfate digestion followed by colorimetric detection using continuous flow analysis.[10] The USGS concluded that the alkaline persulfate digestion technique is more sensitive, accurate, and uses less toxic reagents than the Kjeldahl digestion method.

IC is a well-established technique for the determination of inorganic anions in environmental samples and has been specified in a number of regulatory and standard methods.[7,8] The application of IC for the determinative step after alkaline persulfate digestion has focused primarily on TN only.[11–14] TP determination by IC after persulfate digestion is typically excluded because of the high sulfate concentration produced after decomposition of persulfate, which can interfere with phosphate determinations.[15] To overcome this challenge, some authors have used hydrogen peroxide as an alternative to persulfate for the oxidizing reagent or column-switching techniques after alkaline persulfate digestion.[16–18] However, significant advances in IC stationary phases have enabled the simultaneous and direct determination of TN and TP after alkaline persulfate digestion.[19]

Currently, there is no validated IC method for the simultaneous determination of TN and TP after alkaline persulfate digestion. IC offers several advantages for this type of analysis such as its simplicity, relatively low detection limits, and minimal interference from the digestion matrix when using higher-capacity anion-exchange columns. In addition, an electrolytically generated hydroxide eluent that only requires a source of deionized (DI) water can be used for this application, further simplifying the method and improving the quality of an interlaboratory method transfer.

This study demonstrates the simultaneous determination of TN and TP using a high-capacity hydroxide-selective Thermo Scientific™ Dionex™ IonPac™ AS19 Analytical Column with suppressed conductivity detection after alkaline persulfate digestion. The digestion procedure used here is adopted from the USGS, which uses equimolar concentrations of persulfate and hydroxide ions to yield samples with a pH >12 after a 1:2 dilution.[10] Under the initial alkaline conditions, nitrogen in the sample is oxidized to nitrate. As the digestion proceeds at high temperatures (i.e., 120 °C), bisulfate ions from the thermal decomposition of persulfate neutralize and then acidify the reaction mixture by the following chemical reaction:



$$S_2O_8^{2-} + H_2O \xrightarrow{\Delta} 2\,HSO_4^- + \tfrac{1}{2}\,O_2$$

After the persulfate is decomposed, the digest mixture approaches a pH of 2 and, under these conditions, the dissolved phosphorus hydrolyzes to orthophosphate.

Goal

To demonstrate the simultaneous IC determination of TN and TP in environmental waters after alkaline persulfate digestion as an alternative to TKN

Equipment and Consumables

- Thermo Scientific Dionex ICS-2100 Reagent-Free™ IC (RFIC™) System,* including:
  - Single Isocratic Pump
  - Degasser
  - Column Heater
  - Conductivity Detector
  - Eluent Generator
- Thermo Scientific Dionex AS-AP Autosampler
- Thermo Scientific Dionex EGC III Potassium Hydroxide (KOH) Eluent Generator Cartridge (P/N 074532)
- Thermo Scientific Dionex CR-ATC Continuously Regenerated Anion Trap Column (P/N 060477)
- Thermo Scientific™ Dionex™ AERS™ 500 Anion Electrolytically Regenerated Suppressor (P/N 082541)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, version 7.2
- Helium or Nitrogen, 4.5 grade (99.995%) or better (Praxair)

*Any Thermo Scientific Dionex IC system capable of eluent generation can be used for this application.

- Vial Kit, 10 mL, Polystyrene with Caps and Blue Septa (P/N 074228)
- Thermo Scientific™ Orion™ AQUAfast™ COD165 Thermoreactor (P/N COD165)
- FISHERBRAND™ Disposable Borosilicate Glass Tubes with Threaded End, round bottom, with marking spot, o.d. × L: 16 × 125 mm (Fisher Scientific P/N 14-959-35A)
- FISHERBRAND Screw Caps for Disposable Glass Tubes, Phenolic, 15 mm-415 thread (Fisher Scientific P/N 14-959-36A)

Reagents and Standards

- DI water, Type I reagent grade, 18 MΩ-cm resistance or better
- Sodium Hydroxide Solution (NaOH), 50% w/w (Fisher Scientific P/N SS254-1)
- Potassium Peroxodisulfate (Potassium Persulfate), ≥99% (Fluka P/N 60487, Sigma-Aldrich®)
- Sodium Nitrite, Certified ACS, ≥97% (Fisher Scientific P/N S347-250)
- Sodium Nitrate, Certified ACS, ≥99% (Fisher Scientific P/N S343-500)
- Potassium Phosphate Monobasic (KH_2PO_4), Certified ACS, ≥99% (Fisher Scientific P/N P285-500)
- Glycine ($C_2H_5NO_2$), 98.5 to 101% (Fisher Scientific P/N BP381)
- Glycerophosphate, Disodium Salt, Pentahydrate ($C_3H_9O_6Na_2 \cdot 5H_2O$, Fisher Scientific P/N ICN10291425)
- Urea (CH_4N_2O), Certified ACS, 99% (Fisher Scientific P/N AC42458)
- Nicotinic Acid (Niacin, $C_6H_5NO_2$), 99.5% (Fisher Scientific P/N AC12829)
- α-D-Glucose-1-Phosphate Dipotassium Salt Dihydrate ($C_6H_{11}O_9PK_2 \cdot 2H_2O$, Fisher Scientific P/N ICN19467325)
- Ammonium Chloride (NH_4Cl), Certified ACS, ≥99.5% (Fisher Scientific P/N A661)
- Phytic Acid, Dodecasodium Salt ($C_6H_{18}O_{24}P_6 \cdot 12Na$, Fisher Scientific P/N 50-121-7886)
- D-(+)-Glucose Monohydrate ($C_6H_{12}O_6 \cdot H_2O$), 99% (Fisher Scientific P/N AAA1109036)
- Adenosine 5'-Triphosphate (ATP), Disodium Salt Hydrate ($C_{10}H_{14}N_5Na_2O_{13}P_3 \cdot xH_2O$), 98% (Fisher Scientific P/N AC10280-0100)

Samples

Six wastewater samples (influent and effluent) were obtained from two treatment facilities in California's San Francisco Bay Area.

Conditions (Applicable to Figures 1–4)

Columns:	Dionex IonPac AG19 Guard, 2 × 50 mm (P/N 062888) Dionex IonPac AS19 Analytical, 2 × 250 mm (P/N 062886)
Eluent:	–6–10 min at 20 mM KOH, 10–12 min from 20 to 50 mM, 12–20 min at 50 mM
Eluent Source:	Dionex EGC III KOH cartridge with Dionex CR-ATC column
Flow Rate:	0.30 mL/min
Injection Volume:	5 µL (using a 5 µL sample loop in full-loop mode)
Detection:	Suppressed conductivity, Dionex AERS 500 suppressor (2 mm), recycle mode, 38 mA current
System Backpressure:	~2450 psi
Background Conductance:	~0.4 µS
Noise:	~0.5–1 nS/min, peak to peak
Run Time:	20 min

Preparation of Solutions and Reagents

Calibration Stock Standard Solutions

1000 mg/L nitrate-N stock solution

Prepare 1000 mg/L nitrate as nitrogen by dissolving 0.607 g sodium nitrate in 80 mL of DI water in a 100 mL volumetric flask. Fill to the mark with DI water, cap, and mix thoroughly by manual inversion. Transfer the solution to a polypropylene bottle, in which it will remain stable for 6 months at 4 °C.

1000 mg/L nitrite-N stock solution

Prepare 1000 mg/L nitrite as nitrogen by dissolving 0.492 g sodium nitrite in 80 mL of DI water in a 100 mL volumetric flask. Fill to the mark with DI water, cap, and mix thoroughly by manual inversion. Transfer the solution to a polypropylene bottle, in which it will remain stable for 1 month at 4 °C.

1000 mg/L phosphate-P stock solution

Prepare 1000 mg/L phosphate as phosphorus by dissolving 0.439 g potassium phosphate monobasic in 80 mL of DI water in a 100 mL volumetric flask. Fill to the mark with DI water, cap, and mix thoroughly by manual inversion. Transfer the solution to a polypropylene bottle, in which it will remain stable for 1 month at 4 °C.

Mixed calibration stock solution

Prepare a mixed calibration stock solution containing 1 mg/L each of nitrite-N, nitrate-N, and phosphate-P by adding 0.25 mL from each of the respective 1000 mg/L stock solutions to a 250 mL high-density polyethylene (HDPE) bottle. Dilute to 250 g with DI water. Prepare this solution fresh each time calibration solutions are prepared.

Digest Check Stock Solutions

1000 mg/L glycine-N stock solution

Prepare 1000 mg/L glycine as nitrogen by dissolving 0.536 g glycine in 80 mL of DI water in a 100 mL volumetric flask. Fill to the mark with DI water, cap, and mix thoroughly by manual inversion. Transfer the solution to a polypropylene bottle and store at 4 °C.

1000 mg/L urea-N stock solution

Prepare 1000 mg/L urea as nitrogen by dissolving 0.214 g urea in 80 mL of DI water in a 100 mL volumetric flask. Fill to the mark with DI water, cap, and mix thoroughly by manual inversion. Transfer the solution to a polypropylene bottle and store at 4 °C.

1000 mg/L nicotinic acid-N stock solution

Prepare 1000 mg/L nicotinic acid as nitrogen by dissolving 0.879 g nicotinic acid in 80 mL of DI water in a 100 mL volumetric flask. Fill to the mark with DI water, cap, and mix thoroughly by manual inversion. Transfer the solution to a polypropylene bottle and store at 4 °C.

1000 mg/L ammonium chloride-N stock solution

Prepare 1000 mg/L NH_4Cl as nitrogen by dissolving 0.382 g NH_4Cl in 80 mL of DI water in a 100 mL volumetric flask. Fill to the mark with DI water, cap, and mix thoroughly by manual inversion. Transfer the solution to a polypropylene bottle and store at 4 °C.

1000 mg/L ATP-P stock solution

Prepare 1000 mg/L ATP as phosphorus by dissolving 0.593 g ATP in 80 mL of DI water in a 100 mL volumetric flask. Fill to the mark with DI water, cap, and mix thoroughly by manual inversion. Adjust the final concentration according to the water content for the specific lot of ATP reagent. Transfer the solution to a polypropylene bottle and store at 4 °C.

1000 mg/L glucose-1-phosphate-P stock solution

Prepare 1000 mg/L glucose-1-phosphate (G1P) as phosphorus by dissolving 1.202 g G1P dipotassium salt in 80 mL of DI water in a 100 mL volumetric flask. Fill to the mark with DI water, cap, and mix thoroughly by manual inversion. Transfer the solution to a polypropylene bottle and store at 4 °C.

1000 mg/L phytic acid-P stock solution

Prepare 1000 mg/L phytic acid as phosphorus by dissolving 0.579 g phytic acid dodecasodium salt in 80 mL of DI water in a 100 mL volumetric flask. Fill to the mark with DI water, cap, and mix thoroughly by manual inversion. Adjust the final concentration according to the water content for the specific lot of phytic acid reagent. Transfer the solution to a polypropylene bottle and store at 4 °C.

1250 mg/L glucose stock solution

Weigh 1.564 g D-(+)-glucose monohydrate in a 500 mL volumetric flask. Fill to the mark with DI water, cap, and mix thoroughly by manual inversion. Transfer the solution to a polypropylene bottle, in which it will remain stable for 6 months at 4 °C.

Mixed digestion check solution (1.5 mg/L N, 1.6 mg/L P, 50 mg/L C)

To a 250 mL volumetric flask, add 0.375 mL glycine stock solution, 0.4 mL glycerophosphate stock solution, and 10 mL glucose stock solution. Fill to the mark with DI water, cap, and mix thoroughly by manual inversion. Transfer the solution to a polypropylene bottle, in which it will remain stable for 6 months at 4 °C. Use this solution as a continuous digest check standard and include it with each sample analysis batch.

Working Calibration Standard Solutions

Prepare calibration solutions at 2.5, 5, 10, 25, 50, 100, 200, and 300 µg/L for nitrite-N, nitrate-N, and phosphate-P by adding the appropriate volumes of the mixed calibration stock solution. Prepare concentrations ranging from 2.5 to 25 µg/L in a 250 mL HDPE bottle and concentrations ranging from 50 to 300 µg/L in a 100 mL HDPE bottle.

Digestion Reagents

1.5 M sodium hydroxide solution

In a 100 mL volumetric flask, add ~80 mL of filtered, degassed DI water. Add 7.7 mL (11.78 g) of 50% NaOH solution and swirl to mix. Fill to the mark with DI water, cap, and mix thoroughly by manual inversion. Transfer the solution to a polypropylene bottle and store at 4 °C.

Alkaline persulfate digestion reagent

In a 100 mL volumetric flask, add 80 mL of DI water. Add 10 mL of the 1.5 M stock NaOH solution followed by 4 g of potassium persulfate. Cap and sonicate for 10 min. Fill to the mark with DI water, cap, and mix thoroughly by manual inversion. Do not heat. Prepare fresh daily.

Sample Preparation

Prepare the alkaline persulfate digest solution at a 2:1 ratio by adding 2 mL of alkaline persulfate digestion reagent to 4 mL of sample in a glass digestion tube. Cap the digestion tube and place it in a heat block set at 120 °C for 60 min. After the digestion is complete, allow the tube to cool to room temperature. Dilute the cooled solution a minimum of 1:10 (total dilution 1:15), filter, then inject it onto the IC system. Some samples may require a total dilution of up to 150.

Every sample batch must include a water blank and the mixed digestion check solution. Prepare the blanks (4 mL DI water instead of 4 mL of sample) in the same manner to determine the amount of nitrate-N in the persulfate reagent. The mixed digestion check solution must be included to ensure the reagent is working properly.

Results and Discussion

Column Selection

Prior to IC analysis, alkaline persulfate digestion was used to convert all forms of nitrogen and phosphorus to nitrate and orthophosphate, respectively. During the digest reaction, bisulfate ions are produced from the thermal decomposition of persulfate. Therefore, the high concentration of sulfate that remains in the final sample can overload most anion-exchange columns. Although sample dilution can help minimize this effect, the low nitrogen and phosphorus concentrations present in most samples prohibit a dilution sufficient to remove potential interference from sulfate.

An alternative approach is to use a high-capacity anion-exchange column that can resolve nitrate-N and phosphate-P from high sulfate concentrations and other potential interferences in wastewater samples. In this study, the high-capacity Dionex IonPac AS19 column separated nitrate-N and phosphate-P in <20 min and resolved those components from potential interference, which was primarily sulfate in the alkaline persulfate digestions. Figure 1 shows a standard separation of nitrite-N, nitrate-N, and phosphate-P on the Dionex IonPac AS19 hydroxide-selective column using the optimized conditions described here.

Potential Interferences

Chloride, chlorate, carbonate, and sulfate can all potentially interfere with the determination of low concentrations of nitrate and phosphate by IC after alkaline persulfate digestion. Because most wastewater samples contain moderate to high chloride concentrations, potential interference can arise from the chlorate created by the oxidation of chloride toward the end of the reaction when the digestion pH becomes highly acidic.

In a previous study by Halstead et al.[14], the authors reported that the amount of chlorate produced during digestion is related to the initial ratio of persulfate to sodium hydroxide. At a persulfate to hydroxide ratio of 2:1, nearly 50% of the chloride present in the sample was oxidized to chlorate, compared to about 20% when the ratio was 1:1. In both scenarios, the final pH of the digestion reaction was 2, which is critical for converting inorganic and organic phosphorus fractions to phosphate. When the persulfate to hydroxide ratio was 1:2, however, very little to no oxidization of choride to chlorate was observed. Unfortunately, these initial conditions produced a final pH of 11, which prohibited the conversion of phosphorus to phosphate.

To overcome potential chlorate interference, a high-capacity anion-exchange column with good chlorate to nitrate resolution must be used. The Dionex IonPac AS19 column provides sufficiently high capacity, and gradient hydroxide conditions can be used to optimize the separation and achieve good resolution between chlorate and nitrate. Figure 2 demonstrates the separation of 10 µg/L each of nitrate-N and phosphate-P in a laboratory synthetic sample matrix containing 250 mg/L each of

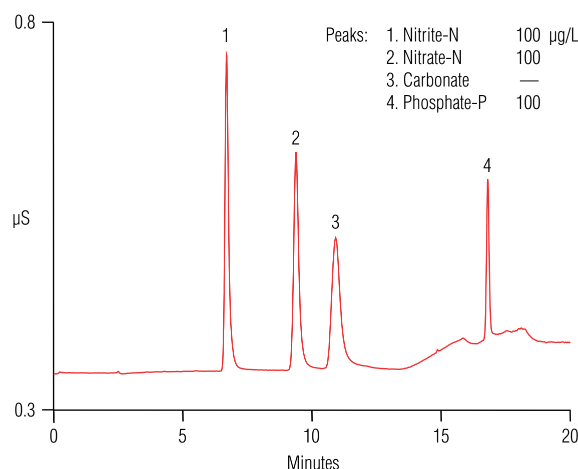


Figure 1. Separation of a mixed nitrite-N, nitrate-N, and phosphate-P standard.

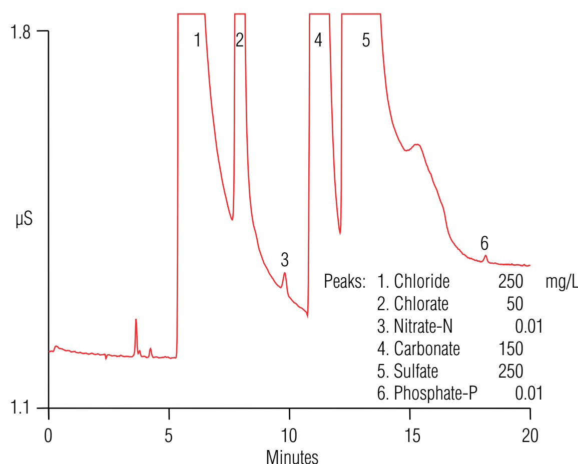


Figure 2. Separation of low concentrations of nitrate-N and phosphate-P in a laboratory synthetic sample matrix.

chloride and sulfate, 50 mg/L chlorate, and 150 mg/L carbonate. Although this matrix represents an extreme case relative to the samples encountered in this study, the separation demonstrates that nitrate and phosphate are resolved from these potential interferences.

In addition to potential chlorate interference, high chloride concentrations in samples can also be problematic for the determination of low concentrations of nitrate. To assess the influence of chloride on the determination of nitrate-N and phosphate-P, a standard consisting of known concentrations of glycine-N and glycerophosphate-P was prepared to obtain final chloride concentrations ranging from ~500 to 1500 mg/L after alkaline persulfate digestion.

Table 1. Effect of high chloride concentrations on the recovery of nitrate-N and phosphate-P.

Injected Chloride Concn (mg/L)	Test Compound	Nitrate-N or Phosphate-P Retention Time (min)[a]	Nominal Concn[b] (mg/L)	Recovery (%)
560	Glycine-N	9.1	1.49	105
	Glycerophosphate-P	16.8	1.63	96.2
1000	Glycine-N	8.9	1.49	101
	Glycerophosphate-P	16.7	1.63	94.0
1470	Glycine-N	8.7	1.49	87.5
	Glycerophosphate-P	16.7	1.63	88.9

[a] Retention times of nitrate and phosphate without chloride present were 9.3 and 16.7 min, respectively.
[b] Standards were diluted from 1:7 to 1:18.

Table 1 summarizes the results from this study. As shown, a chloride concentration of ≤1000 mg/L had no impact on the recovery of nitrate-N and phosphate-P from the standard. However, because increasing concentrations of chloride can have an eluting effect on the anions, there was some decrease in nitrate retention time. For chloride concentrations of 560 mg/L and 1000 mg/L, nitrate retention time decreased by ~2% and 4%, respectively. When the concentration reached nearly 1500 mg/L chloride, the nitrate-N and phosphate-P recovery decreased ~10–15%, while the retention time of nitrate decreased by 6.5%. The chloride concentration did not impact the phosphate retention time.

Due to the decomposition of persulfate during the reaction, sulfate is the primary anion in alkaline persulfate digestion solutions that causes interference. High sulfate concentrations can prohibit the determination of phosphate-P. This is likely the reason why many previous studies using IC excluded the determination of phosphate, avoided an alkaline persulfate digestion, or developed a more complex approach to minimize this interference.

Although the sulfate concentration was not determined in this study, a previous study estimated the concentration to be ~4500 mg/L after the digestion reaction.[18] Therefore, it is critical to use a high-capacity anion-exchange column that can provide good resolution between sulfate and phosphate. Despite using a high-capacity column in this method, however, the high sulfate concentration precludes a direct injection of the postdigest sample (1.5× dilution from the addition of the digest solution to the sample). The absolute minimum sample dilution determined for this method is 1:8, which still enables good resolution between nitrate and sulfate and between sulfate and phosphate. However, the minimum dilution implemented in this study was 1:15 to minimize other potential sample interferences.

Linearity, Limits of Detection, and Limits of Quantification

The initial performance of the method was assessed by determining the method linearity, limits of detection (LODs), and limits of quantification (LOQs). Because an electrolytically generated hydroxide eluent was used in this study, an exceptionally low suppressed background conductivity of ~0.35 µS was observed at 20 mM KOH (start of analysis) with only a slight increase to ~0.39 µS at 50 mM KOH (end of analysis). Therefore, a low background conductivity generated lower baseline noise (~0.5–1 nS), which enabled low detection limits.

In this study, nitrite-N, nitrate-N, and phosphate-P were calibrated from 2.5 to 300 µg/L using a 10-point calibration curve with each concentration injected in duplicate. The curve was generated by tabulating peak area vs concentration, which produced coefficient of determinations (r^2) ranging from 0.9998 to 0.9999. Nitrite-N was included as part of the calibration to determine the concentration of any nitrite-N present in the undigested samples. However, significant nitrite-N in the samples was not expected, based on the assumption that most would have already been oxidized to nitrate.

The system LODs and LOQs for the target analytes were determined based on 3× the signal-to-noise ratio (S/N) and 10× S/N, respectively. The calculated LODs for nitrite-N, nitrate-N, and phosphate-P were 0.76, 1, and 1.3 µg/L, respectively. The LOQs for nitrite-N, nitrate-N, and phosphate-P were 2.5, 3.4, and 4.2 µg/L, respectively. The sample LODs and LOQs for nitrate-N and phosphate-P were also estimated for postdigest samples.

For this study, it is critical to use a low-nitrogen persulfate salt because most reagents can have significant variations in their nitrogen content. For the potassium persulfate salt used in this study, the background nitrate-N concentration varied from 5 to 8 µg/L, whereas phosphate-P was not detected. Therefore, the estimated LOQ for nitrate-N was ~58 µg/L. For phosphate-P, the estimated LOD and LOQ in the sample were ~16 µg/L and 54 µg/L, respectively. Table 2 summarizes the calibration data.

Table 2. Linearity, LODs, and LOQs for nitrite-N, nitrate-N, and phosphate-P.

Analyte	Calibration Range (µg/L)	Linearity[a] (r^2)	System LOD[b] (µg/L)	System LOQ[c] (µg/L)
Nitrite-N	2.5–300	0.9999	0.76	2.5
Nitrate-N	2.5–300	0.9999	1.0	3.4
Phosphate-P	2.5–300	0.9998	1.3	4.2

[a] Ten calibration standards, each injected in duplicate
[b] LOD = 3 × S/N
[c] LOQ = 10 × S/N

Analytical Performance of the Quality Control Standards

Prior to analyzing the wastewater samples in this study, preliminary experiments were conducted to establish the performance of the alkaline persulfate digestion followed by IC with suppressed conductivity detection for nitrate-N and phosphate-P. Recoveries of nitrogen and phosphorus from individual nitrogen- and phosphorus-containing compounds prepared in DI water are shown in Tables 3 and 4, respectively.

Recoveries of nitrogen for the compounds tested ranged from 93 to 100% (Table 3), which indicates good conversion to nitrate-N by the alkaline persulfate digestion procedure. Recoveries obtained from the phosphorus compounds were somewhat lower (Table 4), which may be attributed to lower purity of the test compounds and variable water adsorption during long-term storage. The recoveries of phosphorus from adenosine triphosphate and phytic acid were comparable, though slightly lower when compared to the results presented by the USGS using the same alkaline persulfate digest procedure.[10] The recoveries of phosphorus from glucose-1-phosphate and glycerophosphate were significantly better than those from the other two phosphorus-containing compounds, with calculated recoveries at ~97 and 99%, respectively.

A solution of 1.5 mg/L glycine-N, 1.6 mg/L glycerophosphate-P, and 50 mg/L glucose-C was used as a continuous digest check solution. The addition of glucose to the check solution had no impact on the chromatography or recoveries of nitrogen and phosphorus. Figure 3 shows a chromatogram of the continuous digest check solution after a 1:15 dilution.

Analysis of Wastewater Samples

Wastewater treatment facilities in the U.S. process ~34 billion gallons of wastewater per day. This wastewater contains nitrogen and phosphorus from human waste, food, and detergents. After the water is treated according to state and federal regulations, it is typically released into local bodies of water where it can become a source of nitrogen and phosphorus pollution.[20] Phosphate concentrations have decreased because of the decline in use of phosphate detergents and the corresponding decrease in the amount of phosphate discharged from wastewater treatment facilities. Improved wastewater treatment, which converts ammonia to nitrate, has resulted in a decrease in ammonia concentrations but an increase in nitrate concentrations in streams. Therefore, TN from metropolitan areas has seen little change over the last 20 years.[21]

Table 3. Recovery of nitrogen (N) from quality control nitrogen-containing compounds.

Nitrogen Compound	Nominal Concn (mg N/L)	Expected Concn[a] (mg N/L)	Found Concn (mg N/L)	Recovery (%)
Nicotinic Acid	1.95	0.129	0.129	100.1
Urea	2.02	0.133	0.127	95.4
Glycine	1.49	0.098	0.094	95.6
Ammonium Chloride	2.07	0.137	0.127	93.1

[a] Standards were diluted 1:15.

Table 4. Recovery of phosphorus (P) from quality control phosphorus-containing compounds.

Phosphorus Compound	Nominal Concn (mg P/L)	Expected Concn (mg P/L)	Found Concn (mg P/L)	Recovery (%)
Glucose-1-Phosphate	1.93	0.129	0.125	97.1
Adenosine Triphosphate	1.76	0.116	0.099	85.2
Phytic Acid	1.86	0.123	0.105	85.4
Glycerophosphate	1.63	0.108	0.107	99.3

[a] Standards were diluted 1:15.

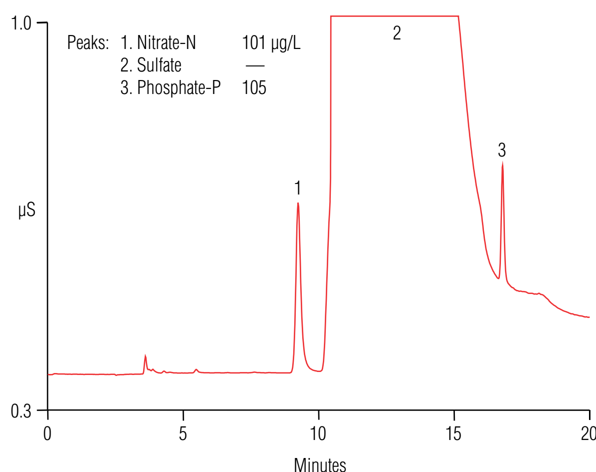


Figure 3. Separation of nitrate-N and phosphate-P after alkaline persulfate digestion of glycine-N, glycerophosphate-P, and glucose-C.

Table 5. Results for nitrogen and phosphorus determinations in untreated and digested wastewater samples.

Sample	Untreated (mg N/L)	Digested (mg N/L)	Untreated (mg P/L)	Digested (mg P/L)
City A Effluent T3	6.52	8.54	2.85	3.20
City A Primary Effluent	0.31	36.52	2.02	3.87
City B Filtered Effluent	11.0	13.0	0.23	0.43
City B Final Effluent	14.8	12.7	0.33	0.40
City B Effluent	11.7	13.4	0.25	0.39
City B Raw Sewage	0.55	39.9	2.19	4.50

In this study, six different wastewater samples were obtained from two wastewater facilities to evaluate the method performance of the alkaline persulfate digestion procedure and IC. From these samples, only one was collected from an influent stream (i.e., raw sewage), whereas the remaining samples were from effluent streams. Prior to processing through the alkaline persulfate digestion procedure, the samples were diluted, filtered, and injected directly into the IC system without further treatment. Therefore, the inorganic nitrite-N, nitrate-N, and phosphate-P present in the sample could be determined. However, the ammonia concentration could not be determined by this process. After the initial evaluation of the samples, the wastewaters were subjected to alkaline persulfate digestion to determine the TN and TP concentrations.

Table 5 summarizes the results from the analysis of the wastewater samples by IC before and after alkaline persulfate digestion. Nitrite-N was not detected in any of the samples tested because any residual nitrite-N was likely oxidized to nitrate-N. The nitrate-N concentrations in the undigested samples ranged from ~0.5 to ~15 mg/L nitrate-N. The concentrations of phosphate-P in the undigested samples were considerably less, with concentrations ranging between 0.2 and ~3 mg/L phosphate-P.

After alkaline persulfate digestions, no significant increase was observed for the measured phosphate-P concentrations. Overall, the TP concentrations (as phosphate-P) ranged from 0.4 to 4.5 mg/L. However, significant increases for TN concentrations for City A's primary effluent and City B's raw sewage samples were observed, which is likely indicative of high ammonia and/or organically bound nitrogen. Figure 4 compares the separation of nitrate-N and phosphate-P in undigested and digested raw sewage samples. As shown, a significant increase in the nitrate-N concentration was observed, whereas phosphate-P increased only slightly.

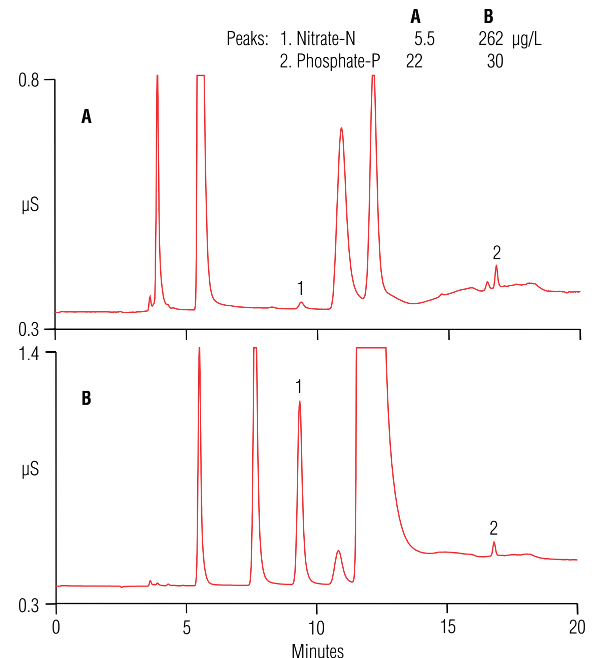


Figure 4. Comparison between (A) undigested and (B) digested raw sewage water.

To evaluate the day-to-day reproducibility of the alkaline persulfate digest, City A's primary effluent sample was digested over four consecutive days using digest solution freshly prepared each day. The average nitrate-N concentration over the four days was 36.4 ± 1.1 mg/L with an RSD of 3.1. For phosphate-P, the average concentration was 3.68 ± 0.14 mg/L with an RSD of 3.9. The retention time and peak area RSDs for nitrate-N varied from 0.03 to 0.04 and 0.11 to 0.13, respectively; for phosphate-P, the variation was from 0.01 to 0.05 and 0.58 to 1.14, respectively. To determine recoveries, the same sample was fortified with known concentrations of nitrate-N and phosphate-P prior to digestion. The average recoveries from this fortification were 84.5% and 89.5% for nitrate-N and phosphate-P, respectively.

Conclusion

The polymeric, high-capacity, hydroxide-selective Dionex IonPac AS19 column is effective for the simultaneous determination of TN and TP by IC with suppressed conductivity detection after alkaline persulfate digestion. A high-capacity column is essential for this application to minimize chromatographic interferences from chloride, chlorate (via oxidation of chloride), and sulfate. The electrolytically generated hydroxide eluent produces a significantly low suppressed background conductivity that enables single-digit µg/L detection limits of nitrate-N and phosphate-P. The recoveries of TN and TP from organic-containing nitrogen and phosphorus compounds are comparable to the colorimetric data reported by the USGS for similar or the same compounds. These results demonstrate that IC can be used as an alternative method without the inherent disadvantages typically associated with colorimetric methods.

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