

Determination of Total Phosphorus in Wastewater Using Caro's Reagent and Ion Chromatography

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Introduction

Phosphorus in the environment is beneficial for many biological processes, but too much phosphorus can create an imbalance in the ecosystem. Human activity can increase the concentration of phosphorus in the environment in many ways, particularly with the use of phosphorus-containing fertilizers and laundry detergents. Agricultural runoff is a major cause of increased phosphorus in both natural and man-made waterways. Phosphate stimulates the growth of plankton and aquatic plants, favoring some fast-growing species over others, which may have important roles in the ecosystem. Excess growth of fast-growing plants consumes large amounts of dissolved oxygen, which can suffocate fish. Excessive plant growth also blocks sunlight for bottom-dwelling species, compromising their health and prevalence, another unwanted change in the ecosystem. Monitoring the concentration of phosphorus in the environment is a good indicator because higher than normal concentrations lead to environmental problems.

Converting organic and inorganic phosphorus to orthophosphate allows phosphorus to be determined by ion chromatography (IC) with suppressed conductivity detection. Masson et al. demonstrated that soluble phosphate can be determined in soil water extracts in the $\mu\text{g/L}$ concentration range and with higher accuracy than by a colorimetric method.¹ In their sample analysis, Masson et al. used water to extract inorganic phosphate from the soil sample.

In the study presented here, the authors treat the wastewater sample with potassium peroxydisulfate (Caro's reagent) and heat to convert all the phosphorus to orthophosphate. The orthophosphate is determined by IC. The authors describe two IC methods to determine



phosphate after treating a wastewater sample with Caro's reagent. One IC method uses a classic carbonate/bicarbonate eluent system with a column designed to determine the common inorganic anions with a carbonate/bicarbonate eluent. The second method uses a hydroxide eluent prepared by an eluent generator with a column designed to determine the common inorganic anions with a hydroxide eluent. Further, the second method uses a Thermo Scientific™ Dionex™ Reagent-Free™ IC (RFIC™) system and delivers all its benefits, including excellent retention time reproducibility for accurate peak identification, and time and labor savings by eliminating eluent preparation and associated errors.

Here, two IC methods are presented for the determination of phosphorus content in wastewater. The sample's organic and inorganic phosphorus are converted to orthophosphate by treatment with Caro's reagent before analysis by IC.

Equipment

Thermo Scientific™ Dionex™ ICS-3000 system including*:

- DP Dual Pump
- DC Detector/Chromatography module with dual temperature zone equipped with 6-port injection valve
- EG Eluent Generator module
- AS Autosampler
- Peristaltic Pump with Thermo Scientific Dionex CRD 300 Carbonate Removal Device (4 mm)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software Version 6.80 SR7

For analysis using Condition A (see below), any Dionex IC system can be used. For Condition B, equivalent or improved results can be achieved using the Thermo Scientific Dionex ICS-5000 system.

Reagents and Standards

- Deionized water (DI), Type I reagent-grade, 18 MΩ-cm resistivity or better
- Sodium carbonate (Na₂CO₃)
- Sodium hydrogen carbonate (NaHCO₃)
- Sodium fluoride (NaF)
- Sodium chloride (NaCl)
- Sodium nitrite (NaNO₂)
- Sodium bromide (NaBr)
- Sodium nitrate (NaNO₃)
- Sodium sulfate (Na₂SO₄)
- Potassium dihydrogen orthophosphate (KH₂PO₄)
- 8 mol/L Potassium hydroxide solution, 8 N (KOH)
- 400 g/L Sodium hydroxide solution (NaOH)
- Monopersulfate compound
- Thermo Scientific™ Dionex™ AS22 Sodium carbonate/bicarbonate concentrate

Preparation of Solutions and Reagents

Eluent Solution

Condition A

Eluent (4.5 mM Na₂CO₃/1.4 mM NaHCO₃)

Dilute 10 mL Dionex AS22 sodium carbonate/bicarbonate concentrate to 1 L in a 1 L volumetric flask with DI water and mix.

Thermo Scientific Dionex CRD 300 Regenerant (200 mM NaOH)

Dilute 20 mL of 400 g/L sodium hydroxide solution to 1 L in a 1 L volumetric flask with DI water and mix.

Condition B

The eluent generator (EG) produces the eluent using the Thermo Scientific Dionex EGC II KOH cartridge and deionized water (18 MΩ-cm resistivity or better) supplied by the pump. The eluent concentration is controlled by Chromeleon CDS software. The eluent generation cartridge requires at least 14 MPa (2000 psi) of system backpressure, which ensures optimal removal of electrolysis gas from the eluent produced by the cartridge. See the *Dionex ICS-3000 Ion Chromatography System Operator's Manual* for instructions on adding backpressure.

Caro's Reagent

Dissolve 0.5 g potassium monopersulfate triple salt (2KHSO₄*KHSO₄*K₂SO₄) in a 100 mL volumetric flask with DI water and dilute to volume.

Standard Solutions

1000 mg/L Stock Standard Solution

Dissolve accurately weighed salts (Table 1) in separate 100 mL volumetric flasks with DI water and dilute to volume.

Table 1. Amounts of compounds used to prepare 100 mL of 1000 mg/L standards.

Ion	Compound	Weight (g)
Fluoride	Sodium fluoride (NaF)	0.221
Chloride	Sodium chloride (NaCl)	0.165
Nitrite	Sodium nitrite (NaNO ₂)	0.150
Bromide	Sodium bromide (NaBr)	0.129
Nitrate	Sodium nitrate (NaNO ₃)	0.137
Sulfate	Sodium sulfate (Na ₂ SO ₄)	0.148
Phosphate*	Potassium dihydrogen orthophosphate (KH ₂ PO ₄)	0.430

*The solution is 1000 mg/L phosphorus

10 mg/L Mixed Anion Stock Standard Solution

Pipette 1 mL each of 1000 mg/L fluoride, chloride, nitrite, bromide, nitrate, sulfate, and phosphorus standards into a 100 mL volumetric flask and bring to volume with DI water.

Working Standard Solutions for Condition A

The working standard solutions for analysis by Condition A are prepared as follows. Pipette the listed volume of 10 mg/L anion stock standard solution into 50 mL beakers (Table 2). Add 20 mL DI water to each beaker. Pipette 2 mL of Caro's reagent into each beaker. Boil the standard solutions using a hot plate for 30 min and then let them cool to room temperature. Transfer these standard solutions into 50 mL volumetric flasks and dilute to volume with Condition A eluent.

Working Standard Solutions for Condition B

The working standard solutions for analysis by condition B are prepared without adding Caro's reagent. Pipette the listed volumes of 10 mg/L anion stock standard solution into 50 mL volumetric flasks (Table 2). Dilute to volume with DI water.

Table 2. Volumes of 10 mg/L stock anion standard solution for Condition A and Condition B working standards.

Concentration of Phosphorus (µg/L)	Volume of 10 mg/L Mixed Anion Stock Standard Solution (mL)	Volume of Caro's Reagent (only for Condition A) (mL)	Final Volume (mL)
20	0.1	2	50
40	0.2	2	50
80	0.4	2	50
160	0.8	2	50
320	1.6	2	50

Sample Preparation

Filter the wastewater sample with qualitative 2, 110 mm filter paper.

Sample Preparation for Condition A

Accurately pipette 20 mL filtered wastewater sample into a 50 mL glass beaker. Add 2 mL Caro's reagent and mix thoroughly. Cover the beaker with a watch glass. Boil the sample by using a hot plate for 30 min and then let it cool to room temperature. Transfer this sample into a 50 mL volumetric flask and dilute to volume with Condition A eluent.

Sample Preparation for Condition B

Prepare as for Condition A but after transferring the sample to a 50 mL volumetric flask, add 5 µL 8 N potassium hydroxide and bring to volume with DI water.

Conditions

Condition A

Column:	Thermo Scientific™ Dionex™ IonPac™ AS22, 4 × 250 mm
Guard:	Dionex IonPac AG22, 4 × 50 mm
Eluent:	4.5 mM Na ₂ CO ₃ /1.4 mM of NaHCO ₃
Flow Rate:	1.2 mL/min
Inj. Volume:	20 µL
Column Oven:	35 °C
Pressure:	~1800 psi
Detection:	Suppressed conductivity Thermo Scientific™ Dionex™ ASRS™ 300 Anion Self-Regenerating suppressor, 4 mm, recycle mode; Dionex CRD 300 device, 4 mm, External chemical mode (200 mM NaOH)
Suppressor Current:	45 mA

Condition B

Column:	Dionex IonPac AS18, 4 × 250 mm
Guard:	Dionex IonPac AG18, 4 × 50 mm
Eluent Source:	KOH produced by an EG system equipped with a Dionex EGC II KOH cartridge and Thermo Scientific Dionex CR-ATC Continuously Regenerated Anion Trap column
Gradient Steps:	15 mM from –7 to 8 min and 15 to 45 mM from 8 to 20 min
Flow Rate:	1.2 mL/min
Inj. Volume:	20 µL
Column Oven:	5 °C
Pressure:	~2200 psi
Detection:	Suppressed conductivity Dionex ASRS 300 suppressor, 4 mm, external water mode
Suppressor Current:	170 mA

Results and Discussion

Chromatography

Two sets of chromatographic conditions were used in this application. The first set of conditions used a carbonate/bicarbonate eluent. The eluent was prepared manually. This first set of chromatographic conditions was referred to as Condition A. The second set used a hydroxide eluent. The eluent was automatically generated by the eluent generator module using deionized water and an eluent generator cartridge. This second set of chromatographic conditions was referred to as Condition B. This

application note presents a comparison of Conditions A and B. Condition B was a little more sensitive than Condition A, as shown by the Caro's reagent analysis. Caro's reagent was prepared and the analysis performed at the same time using both conditions (Figures 1 and 2). Condition B easily detected phosphate in Caro's reagent (Figure 2) while Condition A did not (Figure 1). Phosphate was observed by Condition A at about 10 min but it was difficult to integrate.

Column: Dionex IonPac AS22 Analytical, 4 × 250 mm
 Dionex IonPac AG22 Guard, 4 × 50 mm
 Eluent: 4.5 mM Na₂CO₃/1.4 mM NaHCO₃
 Flow Rate: 1.2 mL/min
 Inj. Volume: 20 µL
 Temperature: 35 °C
 Detection: Suppressed conductivity, Dionex ASRS 300 suppressor, 4 mm, recycle mode
 Dionex CRD 300 device, 4 mm, external chemical mode, (200 mM NaOH)
 Sample: Caro's reagent

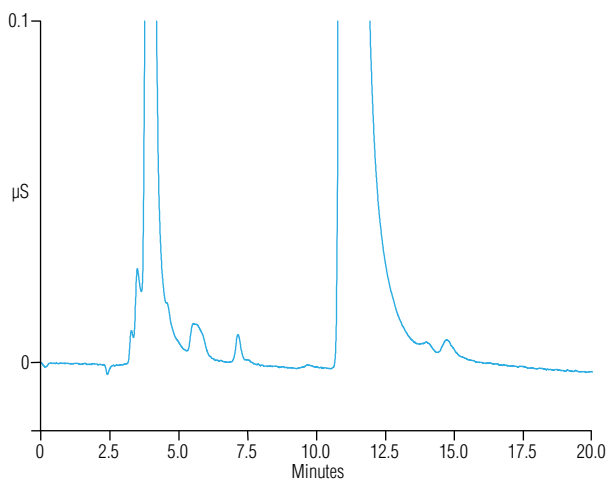


Figure 1. Chromatogram of Caro's reagent (Condition A).

Column: Dionex IonPac AS18 Analytical, 4 × 250 mm
 Dionex IonPac AG18 Guard, 4 × 50 mm
 Eluent Source: Dionex EGC II KOH cartridge
 Eluent: Potassium hydroxide:
 15 mM from -7 to 8 min and 15 to 45 mM from 8 to 20 min
 Flow Rate: 1.2 mL/min
 Inj. Volume: 20 µL
 Temperature: 35 °C
 Detection: Suppressed conductivity, Dionex ASRS 300 suppressor,
 4 mm, external water mode
 Sample: Caro's reagent
 Peaks: 1. Phosphate 17.3 µg/L

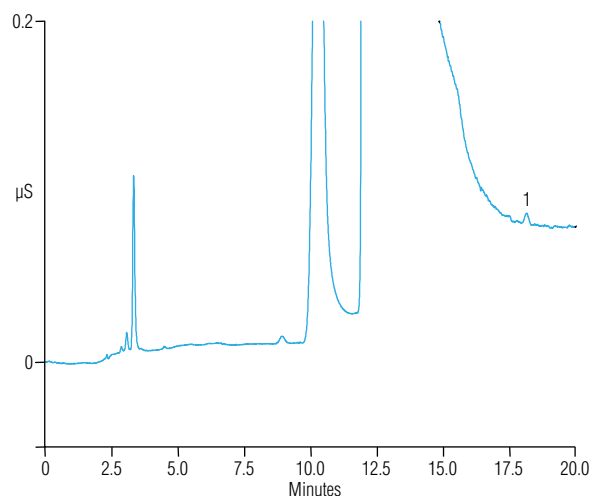


Figure 2. Chromatogram of Caro's reagent (Condition B).

Method Calibrations

Figures 3 and 4 show the chromatography of the calibration standards by Conditions A and B. Phosphate was present in Caro's reagent and difficult to detect by Condition A. To correct the measurement of phosphate by Condition A, the same amount of Caro's reagent was added to the calibration standard solutions. The calibration standards for Condition B were prepared in DI water and the

method blank, in which phosphate was detected, was subtracted from the result of sample analysis. The concentrations of the phosphorus standard solutions were the same for both sets of conditions. Table 2 shows the concentrations of the calibration standards for both sets of conditions. Table 3 shows that the calibration is linear in the range tested for both sets of conditions.

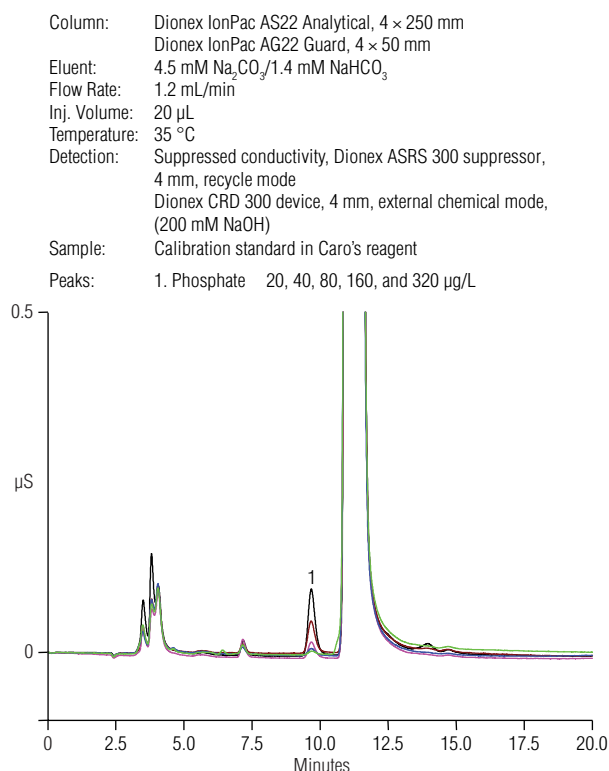


Figure 3. Overlay of chromatograms of five concentrations of phosphate standard for method calibration (Condition A).

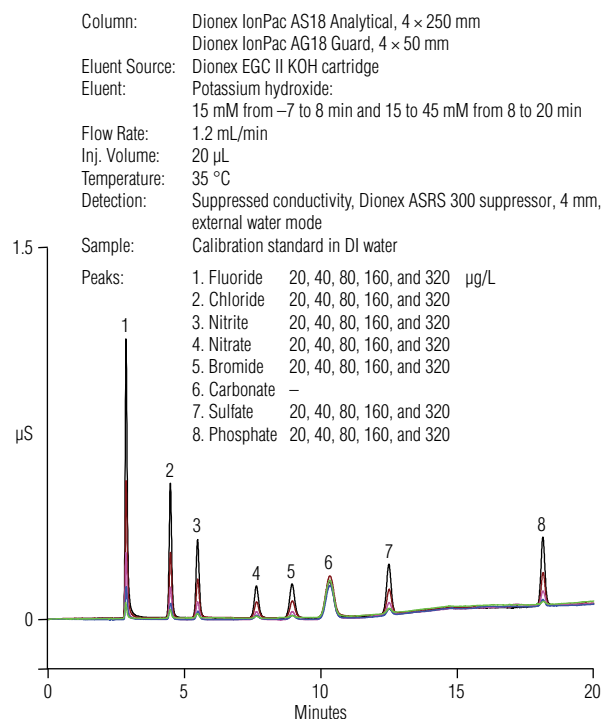


Figure 4. Overlay of chromatograms of five concentrations of the mixed anion standard, including phosphate, for method calibration (Condition B).

Table 3. Calibration results.

Chromatographic Condition	Name	% r ²	Offset (µS* min)	Slope (µS* min)/µg/L
A	Phosphorus	99.9659	-0.0001	0.0001
B	Phosphorus	99.8822	0.0008	0.0001

Table 4. Found concentration of phosphorus in Caro's reagent, sample, and spiked sample.

Injection No.	Found Concentration by Condition A (µg/L)			Found Concentration by Condition B (µg/L)		
	Caro's Reagent	Sample	Spiked Sample	Caro's Reagent	Sample	Spiked Sample
1	—	199	252	18.5	220	272
2	—	204	253	15.4	223	272
3	—	202	250	16.7	226	278
4	—	202	253	17.0	221	273
5	—	200	252	19.2	219	275
6	—	200	253	16.9	224	274
7	—	204	250	17.3	223	273
Average	—	202	252	17.3	222	274
%RSD	—	0.89	0.54	7.04	1.09	0.73

Method Detection Limits

As noted earlier, phosphorus was found in Caro's reagent, but it was not integrated when Condition A was used. The method detection limit (MDL) for Condition A was estimated from seven sample injections by using the standard deviation of found phosphorus and the Student's *t* value for the 99% confidence level (Table 4, column 3). This calculation estimated a value of 5.63 µg/L, which was lower than the amount of phosphorus in Caro's reagent determined by Condition B.

An analysis of Caro's reagent by Condition B found 17.3 µg/L phosphorus, which was not reliably determined by Condition A. The method detection limit for Condition B was estimated from seven injections of the 10 µg/L mixed anion standard that contained phosphate. Chromatography of the 10 µg/L of mixed anion standard is shown in Figure 5. This analysis estimated a MDL of 2.39 µg/L. For either set of chromatographic conditions, the amount of phosphorus in Caro's reagent set a practical limit on the MDL for this analysis. Samples submitted for this analysis were also likely to have phosphorus concentrations well above the MDL for either set of chromatographic conditions, thus making both suitable for this application.

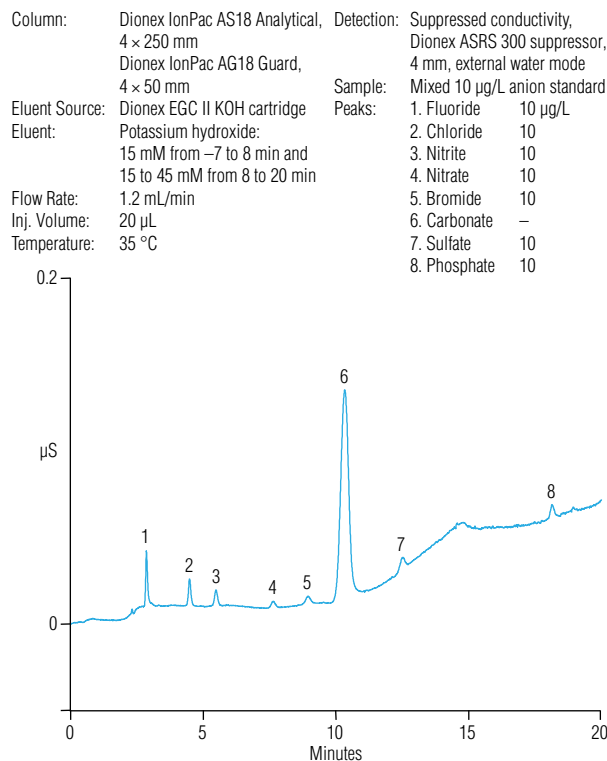


Figure 5. Chromatogram of a 10 µg/L mixed anion standard in DI water (Condition B).

Table 5. Recovery and corrected sample concentration results.

Analyte	Condition	Spiked Concentration	Average Found Concentration (µg/L)			Recovery (%)	Corrected Sample Concentration (µg/L)
			Caro's Reagent	Sample	Spiked Sample		
Phosphorus	A	50	—	202	252	100.05	202
	B	50	17.3	222	274	100.54	205

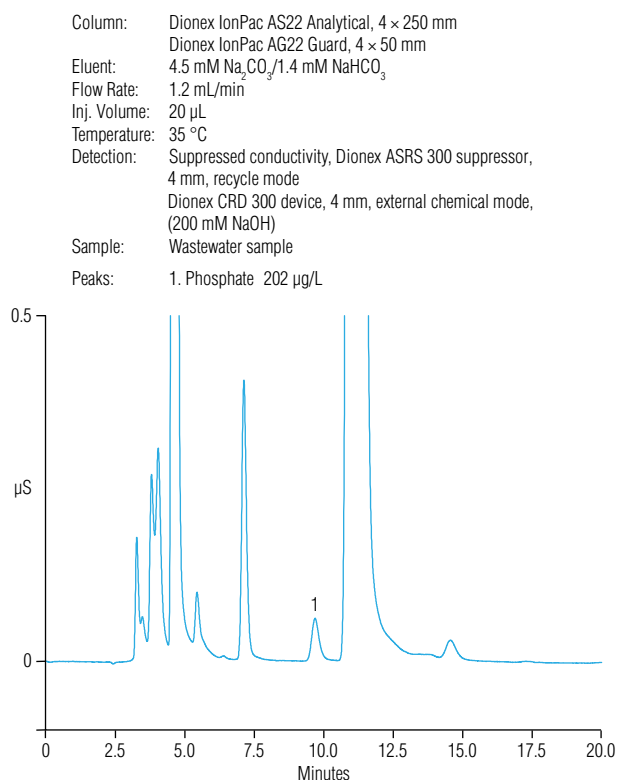


Figure 6. Chromatogram of wastewater sample (Condition A).

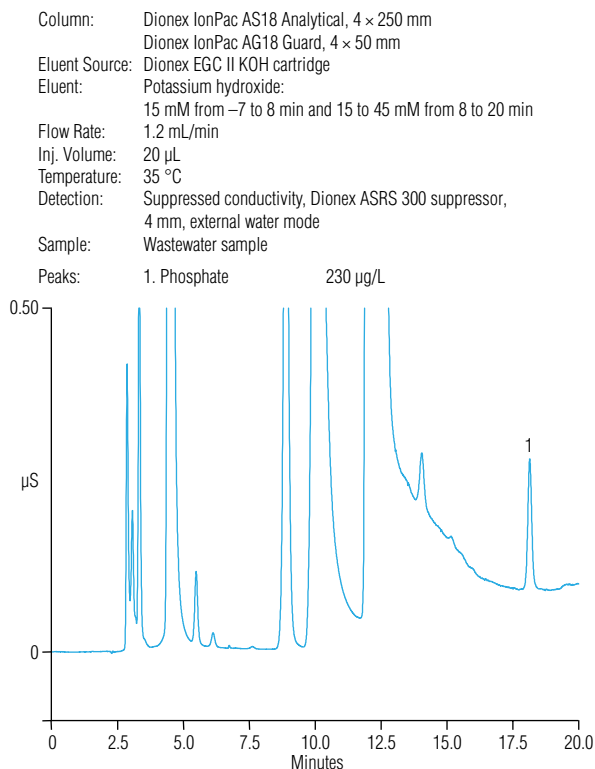


Figure 7. Chromatogram of wastewater sample (Condition B).

Sample Analysis

A wastewater sample was collected in Bangkok, Thailand. To compare the total phosphorus measurement using Conditions A and B, the same wastewater sample was prepared by following the sample preparations for Conditions A and B as described in the Sample Preparation section. Seven injections were made for both sample preparations and a method blank for Condition B. The average concentrations of phosphorus in the wastewater determined by Conditions A and B were 202 µg/L and 222 µg/L, respectively. The average amount of phosphorus in the method blank (Condition B) was 17.3 µg/L. Subtracting the method blank for Condition B yielded a value of 205 µg/L, which was very close to that determined

by Condition A. Table 4 shows the data from the phosphate determination of the wastewater sample using both analysis conditions; Figures 6 and 7 show the chromatography.

To evaluate the recovery of both conditions, the same wastewater sample was spiked with the 10 mg/L mixed anions stock standard solution before the sample preparation. This yielded 50 µg/L of spiked phosphorus after sample preparation. Both sets of conditions exhibited excellent recovery, with values of 100.05% and 100.54% for Conditions A and B, respectively. Tables 4 and 5 show the recovery data.

Summary

Both IC methods presented in this application note can be used to make an accurate measurement of the total phosphorus content of a wastewater sample after treatment with Caro's reagent. The analyst has a choice of a method with simple eluent preparation or a method that requires no eluent preparation and has the high reproducibility of a RFIC system.

Reference

1. Masson, P.; Morel, C.; Martin, E.; Oberson, A.; Friesen, D. *Commun. Soil Sci. Plant Anal.* 2001, 32, 2241–2253.

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