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

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 manmade 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 ion chromatography (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 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

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 CRD 300 (4 mm) (P/N 064905)

Chromeleon® Chromatography Data System software
Version 6.80 SR7

*For analysis using Condition A (see below), any Dionex IC system can be used. Condition B requires the use of a Dionex RFIC system.

REAGENTS AND STANDARDS

Deionized water (DI), Type I reagent-grade, 18 MΩ-cm resistivity or better

Sodium carbonate (Na₂CO₃, Ajax)

Sodium hydrogen carbonate (NaHCO₃, Ajax)

Sodium fluoride (NaF, Fluka)

Sodium chloride (NaCl, Fluka)

Sodium nitrite (NaNO₂, Fluka)

Sodium bromide (NaBr, Fluka)

Sodium nitrate (NaNO₃, Fluka)

Sodium sulfate (Na₂SO₄, Fluka)

Potassium dihydrogen orthophosphate (KH₂PO₄, Fluka)

8 mol/L Potassium hydroxide solution,
8 N (KOH, KANTO)

400 g/L Sodium hydroxide solution (NaOH, KANTO)

OXONE®, monopersulfate compound, Sigma-Aldrich
(P/N 228036)

AS22 Sodium carbonate/bicarbonate concentrate
(Dionex, P/N 063965)

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent Solution

Condition A

Eluent (4.5 mM Na₂CO₃/1.4 mM NaHCO₃)

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

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 EluGen EGC II KOH cartridge and deionized water (18 MΩ-cm resistivity or better) supplied by the pump. The eluent concentration is controlled by Chromeleon software. The EluGen 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 *ICS-3000 Ion Chromatography System Operator's Manual* (Dionex Document No. 065031-04) 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 (Whatman, Catalog No. 1002 110).

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.

CHROMATOGRAPHIC CONDITIONS

Condition A

Column: IonPac® AS22 (4 × 250 mm) (P/N 064141)
Guard: IonPac AG22 (4 × 50 mm) (P/N 064139)
Eluent: 4.5 mM Na₂CO₃/
1.4 mM of NaHCO₃
Flow Rate: 1.2 mL/min
Inj. Volume: 20 µL
Column Oven: 35 °C
Pressure: ~1800 psi
Detection: Suppressed conductivity
ASRS® 300, 4 mm (P/N 064554),
recycle mode
CRD 300, 4 mm (P/N 064637),
External chemical mode
(200 mM NaOH)
Suppressor Current: 45 mA

Condition B

Column: IonPac AS18 (4 × 250 mm) (P/N 060549)
Guard: IonPac AG18 (4 × 50 mm) (P/N 060511)
Eluent Source: KOH produced by an EG equipped with a EGC II KOH cartridge (P/N 058900) and CR-ATC (P/N 060477)
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 ASRS 300, 4 mm (P/N 064554), 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.

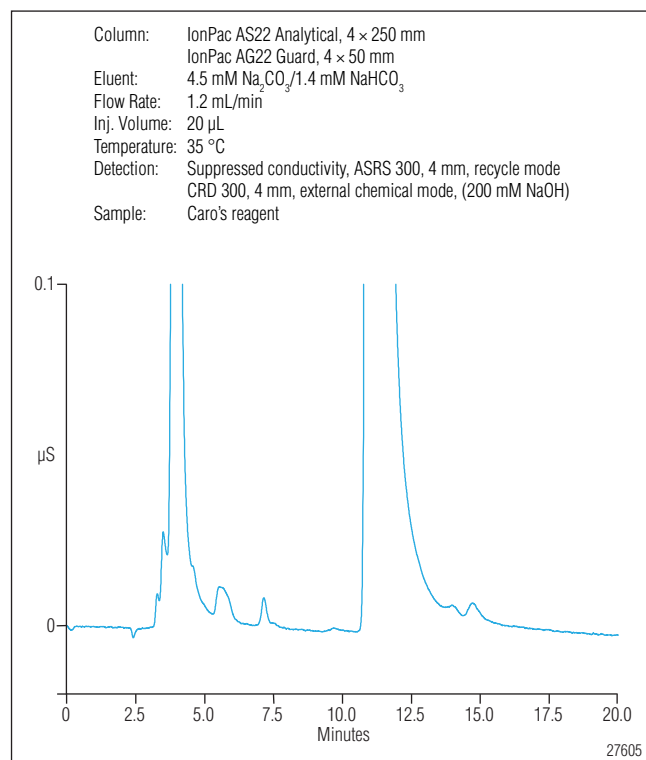


Figure 1. Chromatogram of Caro's reagent (Condition A).

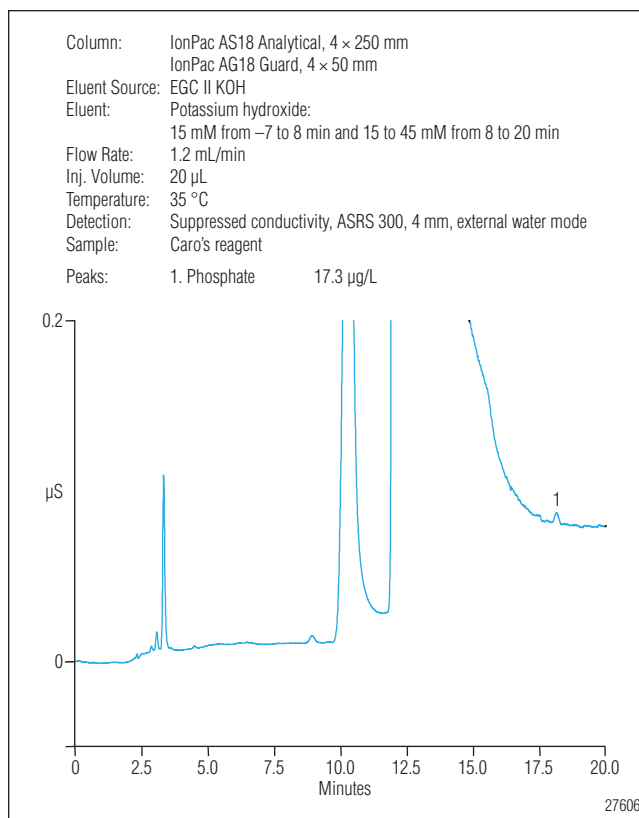


Figure 2. Chromatogram of Caro's reagent (Condition B).

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.

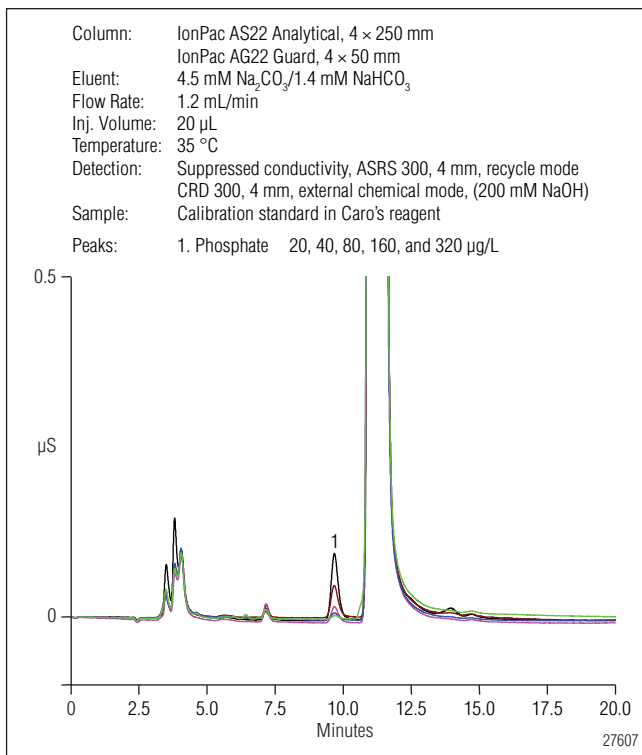


Figure 3. Overlay of chromatograms of five concentrations of phosphate standard for method calibration (Condition A).

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.

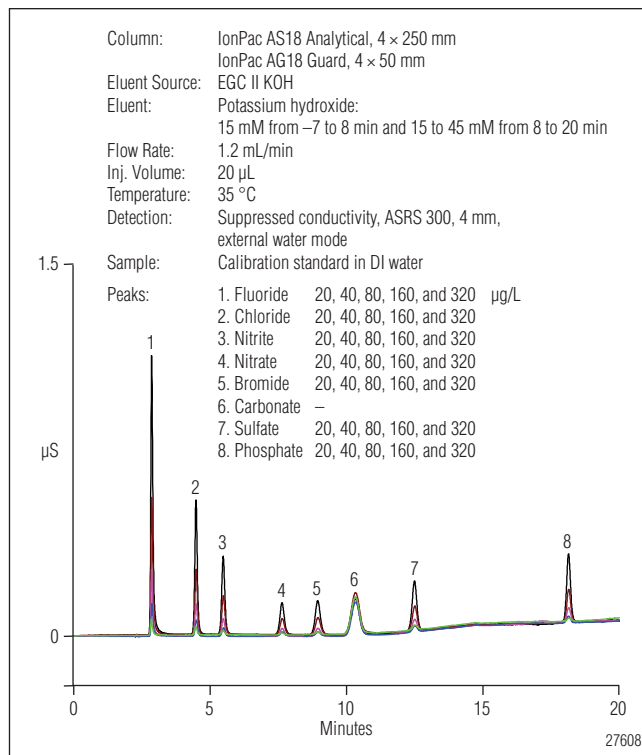


Figure 4. Overlay of chromatograms of five concentrations of the mixed anion standard, including phosphate, for method calibration (Condition B).

| 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 |

| 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 |

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.

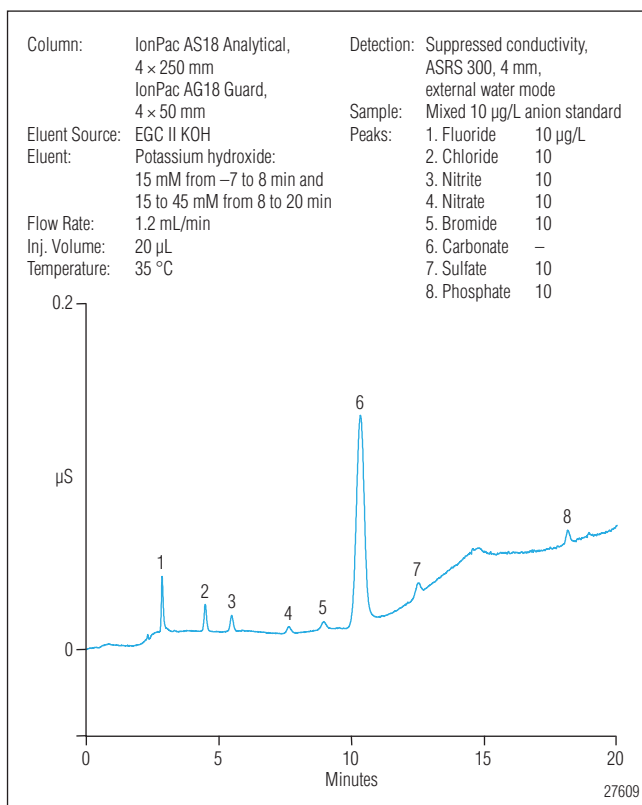


Figure 5. Chromatogram of a 10 µg/L mixed anion standard in DI water (Condition B).

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.

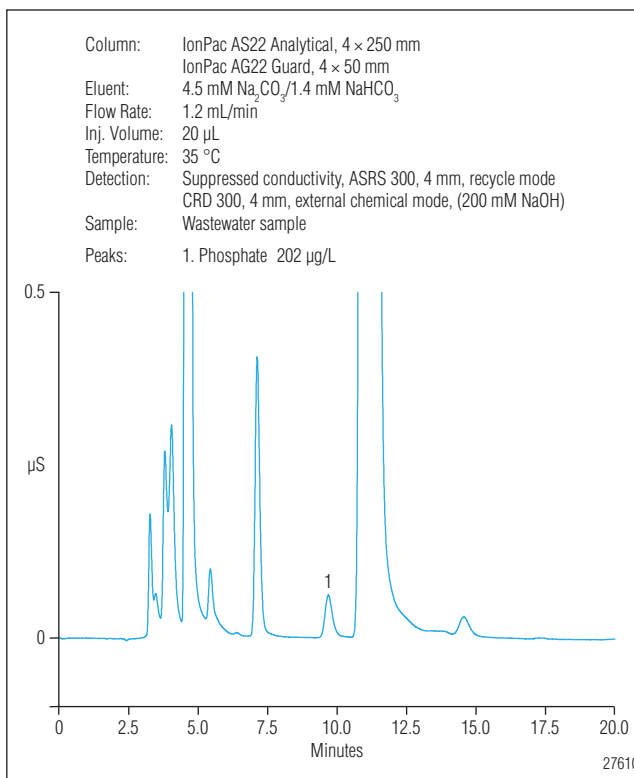


Figure 6. Chromatogram of wastewater sample (Condition A).

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 |

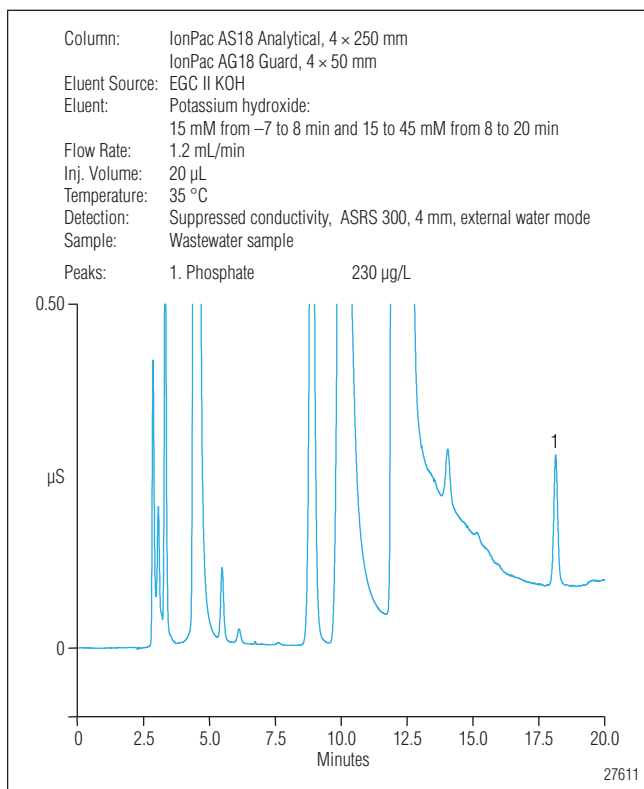


Figure 7. Chromatogram of wastewater sample (Condition B).

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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