Separation of Chromium (III) and Chromium (VI) by Ion Chromatography

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Introduction

Chromium in the environment exists primarily in two oxidation states: Cr(III) and Cr(VI). While the trivalent Cr(III) is only toxic at high concentrations, hexavalent Cr(VI), a strong oxidizer, is considered toxic to humans and the environment at µg/L concentrations. Because of this toxicity, many countries and states strongly regulate the concentration of Cr(VI) in drinking and wastewaters and require that its concentration is measured and reported. The state of California, for example, has established a public health goal (PHG) of 0.2 µg/L Cr(VI) in drinking water. Dissolved hexavalent chromium can be measured as the chromate ion following U.S. Environmental Protection Agency (EPA) Method 218.6. This method uses ion chromatography and the Thermo Scientific™ Dionex™ IonPac™ AS7 column with detection by postcolumn reaction to yield a compound measured by visible absorbance. In Dionex (now part of Thermo Scientific) Application Update (AU) 144 we updated Method 218.6 and Dionex (now part of Thermo Scientific) Application Note (AN) 80 (Determination of Dissolved Hexavalent Chromium in Drinking Water, Groundwater, and Industrial Wastewater Effluents by Ion Chromatography) to allow the method to be used to meet the California PHG of 0.2 µg/L Cr(VI).[1–2] Dionex (now part of Thermo Scientific) Technical Note (TN) 26 shows application of Method 218.6 for determination of Cr(VI) in wastewater and solid waste extracts.[3]

Reliable determination of Cr(III) in the presence of Cr(VI) is complicated by the aqueous chemistry of each oxidation state, as Cr(III) exists primarily as a cation in solution, and Cr(VI) exists primarily as an anion, although these states are dependent on solution pH. Approaching neutral pH, Cr(III) will form a hydroxo-Cr(III) species; at alkaline pH it forms a hydroxide precipitate. As pH increases, Cr(III) can oxidize to form Cr(VI) (chromate). Efforts to preserve Cr(III) (for example, lowering solution pH) can lead to the loss of Cr(VI). When designing sample preparation and analysis methods for determining the Cr(III) and Cr(VI) contents of soil, wastewater, or other samples, the biggest challenge is to ensure that the sample preparation and analysis procedures do not change the distribution of oxidation states in the sample.

In 2002, Ščancar, and Milacic designed a method for speciating airborne chromium.[4] They separated Cr(III) and Cr(VI) on a monolithic disc modified with weak anion-exchange groups. Cr(III) was not bound, and Cr(VI) (as chromate) was eluted using an ammonium nitrate solution buffered between pH 4 and 12 to match the pH of the sample. The authors noted that as pH increased, some Cr(III) was partially retained due to the formation of the hydroxo-Cr(III) species partially bound to the disc. Chromium was measured in the unretained and bound fractions using electrothermal atomic absorption spectrometry (EAAS). The authors then applied this method to determinations of Cr(VI) in soils and cement.[5–6]

European Union Directive 2003/53/EC restricts the use of cement and cement products to those that contain <2 mg/kg Cr(VI) when hydrated. For determination of Cr(VI) in cement, the authors also used the Dionex IonPac CS5A column with 350 mM ammonium nitrate eluent prior to EAAS, and noted that the Cr(III) remained bound to the column. One major theme pertaining to both of these studies was measurement of Cr(VI) in a variety of samples, while taking care not to convert Cr(III) to Cr(VI). Dionex (now part of Thermo Scientific) Technical Note (TN) 24 describes a method for simultaneous determination of Cr(III) and Cr(VI) (Method A).[7] TN24 shows separation of a mixed Cr(III)/(VI) standard using the Dionex IonPac CS5 column, and discusses the steps required for preparing a sample containing Cr(III) and Cr(VI) for chromatography. In this application update, the authors demonstrate separation of Cr(III) and Cr(VI) on the Dionex IonPac CS5A column, the replacement column for the CS5. A revised sample preparation method for stabilizing both Cr(III) and Cr(VI) during sample preparation, and discussion on the feasibility of using this method for soil and wastewater analyses are also provided.

Thermo SCIENTIFIC

Equipment

- Thermo Scientific Dionex ICS-3000 consisting of:
  - DP Dual-Gradient Pump*
  - DC Detector/Chromatography module with six-port injection valve
  - VWD Variable Wavelength Detector with PEEK™ flow cell, 11 µL, 10 mm (P/N 6074.0200)
- PC10 Postcolumn Delivery System
- Thermo Scientific Dionex Chromeleon™ 6.8 Chromatography Management Software*

This application can also be run using an Isocratic or Gradient Pump module.

Reagents and Standards

- Deionized water (DI), Type I reagent grade, 18 MΩ-cm resistivity.
- Pyridine-2,6-dicarboxylic acid (PDCA) (P/N 039671)
- Disodium hydrogen phosphate heptahydrate ($Na_2HPO_4 \bullet 7H_2O$)
- Sodium iodide (NaI)
- Ammonium acetate ($NH_4CH_3CO_2$)
- Lithium hydroxide monohydrate ($LiOH \bullet H_2O$)
- 1,5-diphenylcarbohydrazide (DPC)
- Nitric acid (HNO_3)
- Methanol (CH_3OH)
- Sulfuric acid (H_2SO_4) (96%; spectrophotometric grade)
- Chromium (III) nitrate ($Cr(NO_3)_3 \bullet 9H_2O$)
- Disodium chromate (Na_2CrO_4)

All compounds should be A.C.S. reagent grade or better, purchased from reliable sources.

Conditions	
Guard column:	Dionex IonPac CG5A, 4 × 50 mm (P/N 046100)
Column:	Dionex IonPac CS5A, 4 × 250 mm (P/N 046104)
Eluent:	2 mM PDCA, 2 mM Na_2HPO_4, 10 mM NaI, 50 mM $CH_3CO_2NH_4$, 2.8 mM LiOH
Flow Rate:	1.0 mL/min
Injection Volume:	50 µL
Expected System Pressure:	1500 psi
Detector Wavelength:	0.0–4.7 min, 335 nm; 4.7–8.0 min, 530 nm
Postcolumn Reagent:	2 mM DPC, 10% CH_3OH, 0.9 N H_2SO_4
Postcolumn Flow Rate:	0.5 mL/min*
Reaction Coil:	375 µL (Dionex P/N 043700)

*The flow rate of the eluent and postcolumn reagent are critical to this analysis. The combined flow rate should be 1.5 mL/min, as the eluent flow rate is 1.0 mL/min and the postcolumn reagent flow rate is 0.5 mL/min. After measuring the flow rate at the waste line, adjust the PC10 pressure regulator and measure to obtain the total flow rate of 1.5 mL/min.

Preparation of Solutions and Reagents

Eluent Stock: (10× concentrate)

To prepare 1 L, dissolve the following reagents in DI water: 3.34 g pyridine-2,6-dicarboxylic acid (PDCA), 5.36 g disodium hydrogen phosphate heptahydrate, 15 g sodium iodide, 38.5 g ammonium acetate, and 1.1 g lithium hydroxide monohydrate. PDCA is slow to dissolve. Heating the solution before adding the reagents increases the rate of dissolution.

Eluent

2 mM PDCA/2 mM Na_2HPO_4/10 mM NaI/ 50 mM $CH_3CO_2NH_4$/2.8 mM LiOH

To prepare 1 L, add 100 mL of the eluent stock to a 1 L volumetric flask and bring to volume with DI water. The pH of this solution should be between 6.70 and 6.80.

Postcolumn Reagent

2 mM DPC/10% CH_3OH/0.9 N H_2SO_4

To prepare 1L, dissolve 0.5 g 1,5-diphenylcarbohydrazide (DPC) in 100 mL HPLC grade methanol in a 1 L volumetric flask. Add approximately 500 mL DI water containing 25 mL of 96% sulfuric acid. Bring to volume with DI water.

Standard Solutions

Stock Standards

Cr (III) and Cr (VI) standards were prepared in 50 mM nitric acid.

50 mM Nitric Acid

To prepare 1 L, add approximately 100 mL DI water to a 1 L volumetric flask, weigh 3.15 g concentrated nitric acid into a 50 mL beaker and transfer to the volumetric flask. Bring to volume with DI water.

1000 mg/L Chromium Standards

To prepare 1000 mg/L Cr(III) and Cr(VI) stock standards, dissolve the weights of the appropriate chromium salt, listed below, in 100 mL of a 50 mM nitric acid solution.

Standard	Salt	Weight (g)
Cr(III)	$Cr(NO_3)_3 \bullet 9H_2O$	0.769
Cr(VI)	Na_2CrO_4	0.312

Standard Preparation

Standards are prepared for chromatography in the same manner as samples; preparation of a 0.75 mg/L Cr(VI) standard is shown below.

1. Add 1.5 mL 50 mg/L Cr(VI) in 50 mM nitric acid to a 100 mL Pyrex® beaker.
2. Add 10 mL eluent stock (10× concentration of the eluent) and 10 µL concentrated nitric acid.
3. Mix thoroughly, place the container (uncovered) in a boiling water bath for 30 min.
4. Cool the standard to room temperature; transfer to a 100 mL volumetric flask and bring to volume with DI water.

Cr(III) and mixed Cr(III)/Cr(VI) standards are prepared in the same manner.

Sample Preparation

Soil and wastewater samples were used to show the feasibility of determining Cr(III) and Cr(VI) using the chromatography method described in this application update. Sample preparation for analysis is shown below.

1. Add approximately 0.1 g soil sample or 5 mL wastewater sample to a 100 mL Pyrex beaker.
2. Add 5 mL 50 mM nitric acid, 10 mL eluent stock, and 10 µL concentrated nitric acid.
3. Mix thoroughly, place the container (uncovered) in a boiling water bath for 30 min.
4. Cool the sample to room temperature; transfer to a 100 mL volumetric flask, and bring to volume with DI water.
5. Filter sample with qualitative 2, 110 mm filter paper (Whatman Catalog No. 1002 110) and treat with a Thermo Scientific Dionex OnGuard™ II P sample pretreatment cartridge (P/N 057087)

For the feasibility demonstration, the authors used a simple qualitative filtration. To obtain the optimum column performance and prolong the life of the column, the authors recommend using a 0.45 µm filter.

Spike Sample Preparation

Standards of 1000 mg/L Cr(III) and 50 mg/L Cr(VI) were used to spike the soil and wastewater samples. An example of preparing a spiked sample with a final concentration of 10 mg/L Cr(III) and 0.5 mg/L Cr(VI) is presented below.

1. Add approximately 0.1 g soil sample or 5 mL wastewater sample to a 100 mL Pyrex beaker.
2. Add 1 mL 1000 mg/L Cr(III) and 1 mL 50 mg/L Cr(VI).
3. Add 5 mL 50 mM nitric acid, 10 mL eluent stock, and 10 µL concentrated nitric acid.
4. Mix thoroughly, place the container (uncovered) in a boiling water bath for 30 min.
5. Cool the sample to room temperature; transfer to a 100 mL volumetric flask, and bring to volume with DI water.
6. Filter sample, as above, and pretreat using a Dionex OnGuard II P sample pretreatment cartridge.

Results and Discussion

Discussion of the Chromatography Method

Cr(III) and Cr(VI) species are separated on the Dionex IonPac CS5A column, which is packed with resin containing both cation- and anion-exchange functional groups. The Dionex IonPac CS5A column was designed to replace the CS5 column for improved transition metal analysis using both PDCA and oxalic acid eluents. The resin in the Dionex IonPac CS5A column has lower capacity and a different ratio of anion-to-cation functionality than the resin used in the CS5 column (0.5:1 for the CS5, and to 2:1 for the CS5A). Both these changes affect the Cr(III)/Cr(VI) separation. Using the PDCA-based eluent system, trivalent chromium is separated as the $Cr(PDCA)^{2-}$ complex, while the hexavalent chromium is separated as the chromate ion (CrO_4^{2-}). Chromate does not form a complex with PDCA. Due to the slow kinetics of ligand exchange for Cr(III), a precolumn derivatization with PDCA is used to form the Cr(III)-PDCA complex in the sample.

After separation, the Cr(III)-PDCA complex is detected by absorbance at 335 nm. This value is 30 nm less than that used in TN24, but was found to be the wavelength maximum using a photodiode array detector. Chromate is subjected to postcolumn derivatization with DPC and detected at 530 nm as shown in AU144. The Cr(III)-PDCA complex does not react with DPC but can be detected at 530 nm. While not ideal, the product of the Cr(VI) and DPC reaction can also be detected at 335 nm. Figure 1 shows Cr(III) and Cr(VI) detected at both 335 and 530 nm. To maximize sensitivity for each species, wavelengths were set at 335 nm from 0.0 min to 4.7 min for Cr(III), and 530 nm from 4.7 min to 8.0 min for Cr(VI).

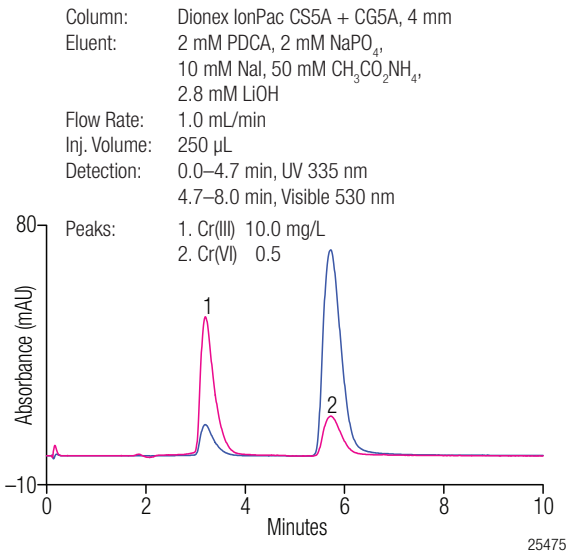

Figure 1. Overlay of chromatograms 10 mg/L Cr(III) and 0.5 mg/L Cr(VI) at 335 nm (pink) and 530 nm (blue).

Figure 2 shows an overlay of three concentrations of a mixed standard. The retention times of Cr(III) and Cr(VI) are 3.4 and 6.0 min, respectively. The baseline dip at 4.7 min is due to changing the detector wavelength. Note the sensitivity for the Cr(III)-PDCA complex at 335 nm is lower than for Cr(VI) at 530 nm after reaction with DPC. This method uses a 50 µL injection volume rather than a 250 µL injection volume loop used in TN24.

A 250 µL injection volume loop overloaded the Dionex IonPac CS5A column using the sample preparation method below. This may be a result of the reduced anion-exchange capacity of the Dionex IonPac CS5A column compared to the CS5, and/or the change in the sample preparation procedure. It may be possible to use injection volumes between 50 and 250 µL without overloading the column, but such volumes were not tested. When evaluating samples, use a Cr(VI) standard spiked into a prepared sample to evaluate recovery (that is, whether the sample is overloading the column).

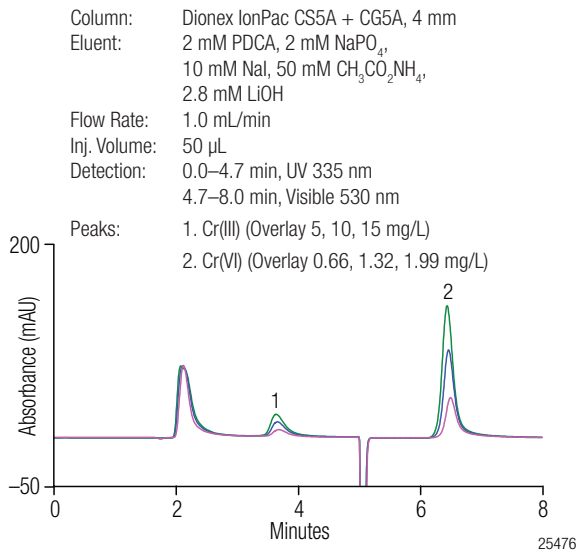

Figure 2. Overlay of chromatograms of mixed Cr(III) and Cr(VI) standards.

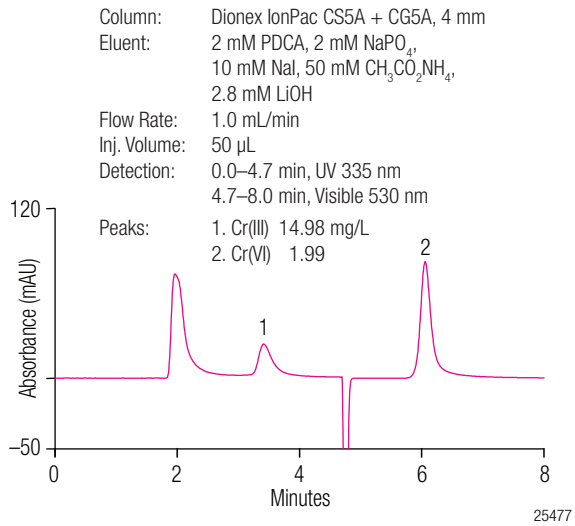

Figure 3. Chromatogram of a 15 mg/L Cr(III) standard.

Sample Preparation to Produce the Cr(III)-PDCA Complex

To produce the Cr(III)-PDCA complex, the sample must be treated with the PDCA eluent prior to injection. The PDCA eluent is buffered at pH 6.8. In a heated solution containing dissolved oxygen, Cr(III) can oxidize to Cr(VI) at neutral and higher pH values. The formation of the Cr(III)-PDCA complex is inhibited at pH values above 6, but as samples can contain both Cr(III) and Cr(VI), the sample preparation must also preserve Cr(VI). At pH values below 6, chromate can convert to dichromate, which can be harmful to the column and reduce the measurable Cr(VI). Dichromate can also oxidize the Cr(III). TN24 Figure 2 shows the effect of sample pH on the peak heights of Cr(III) and Cr(VI). The sample preparation procedure has been developed to maximize the formation of the Cr(III)-PDCA complex while minimizing the oxidation of Cr(III) to Cr(VI) and the conversion of chromate to dichromate.

A sample preparation method to maximize Cr(III) and Cr(VI) recovery in a sample was empirically determined using standards. Ten mL of the 10× PDCA and 10 µL concentrated nitric acid were added to 1.5 mL of standard prepared in 50 mM nitric acid and the sample was boiled. The addition of the nitric acid lowers the sample pH to approximately 5.9. Experimentation showed that the addition of nitric acid preserves Cr(III) while having no effect on the amount of Cr(VI). Figure 3 shows the analysis of a 15 mg/L Cr(III) standard. Under these conditions, 8–9% of the Cr(III) was converted to Cr(VI) irrespective of Cr(III) concentration in the range of 5 to 15 mg/L. Higher amounts of nitric acid (for example, 50 µL) resulted in greater preservation of Cr(III) but caused a loss of Cr(VI). Reducing the heating time from 30 min may reduce Cr(III) loss. A 1 min heating time was inadequate for Cr(III)-PDCA complex formation, but for this application, no other times <30 min were examined.

Prior to sample analysis, the system was calibrated to account for approximately 8–9% conversion of Cr(III) to Cr(VI). The system was first calibrated with Cr(VI) using concentrations of 0.25, 0.5, and 0.75 mg/L, which yielded a correlation coefficient of 0.9993. Figure 4 shows the chromatography of the Cr(VI) standards. This calibration was used to measure the amount of Cr(VI) formed from duplicate preparations of a 15 mg/L Cr(III) standard. As shown in Table 1, 1.24 mg/L Cr(VI) was produced from 15 mg/L Cr(III), which represents an 8.27% conversion of Cr(III). Three mixed standards were then prepared with the concentrations listed in Table 2; their chromatography is also shown in Figure 2. The third row of Table 2, (Cr(VI) Total), reflects the predicted amount of Cr(VI) from the amount added and the amount derived from Cr(III). Calibrations were linear for both oxidation states with correlation coefficients of 0.9999 and 0.9998, respectively. To ascertain the accuracy of this approach, a

Table 1. Determination of the amount of Cr(VI) in the 15 mg/L Cr(III) standard.

Number	Area mAU/min	Height mAU	Amount Cr(VI) (mg/L)
1	16.98	81.69	1.24
2	16.97	81.55	1.24
Average:	16.97	81.62	1.24

Table 2. Mixed standard concentrations.

Analyte	Concentration (mg/L)		
	Level 1	Level 2	Level 3
Cr(III)	5.0	10.0	15.0
Cr(VI)	0.25	0.5	0.75
Cr(VI) Total*	0.66	1.32	1.99

*This value reflects the amount of Cr(VI) added and the amount expected from Cr(III) oxidation.

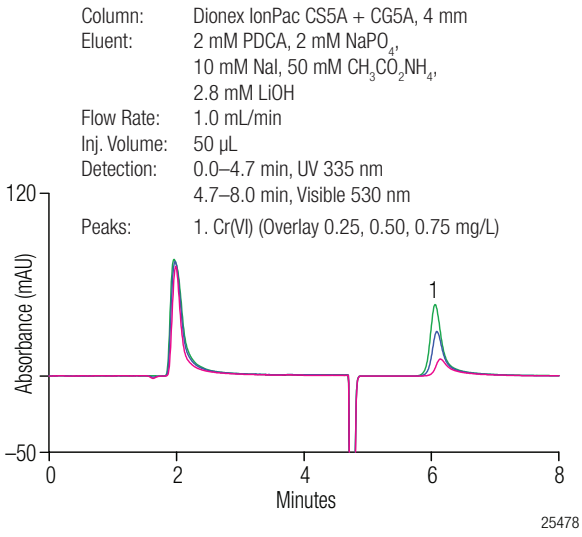

Figure 4. Overlay of chromatograms of Cr(VI) standards: 0.25, 0.5, and 0.75 mg/L, respectively.

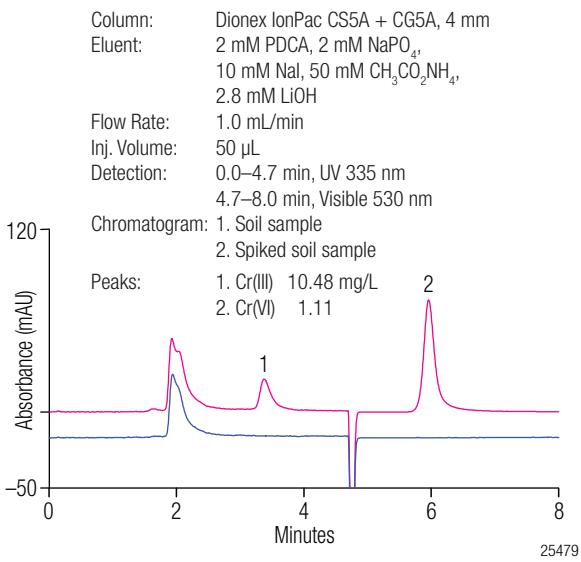

Figure 5. Overlay of the chromatograms of the soil sample and the same sample spiked with 10 mg/L Cr(III) and 0.5 mg/L Cr(VI).

mixed standard was analyzed with added concentrations of Cr(III) and Cr(VI) of 15 and 0.5 mg/L, respectively. Due to the expected conversion of some of the Cr(III) to yield an additional 1.24 mg/L, the authors expected a total of 1.99 mg/L Cr(VI). The measured values for Cr(III) and Cr(VI) were 15.0 mg/L and 1.99 mg/L.

Determination of Cr(III) and Cr(VI) in Soil and Wastewater

To determine the feasibility of this method for speciation of chromium in soil and wastewater samples, the authors spiked known quantities of a mixed standard into samples that contained no measurable amounts of chromium. Samples spiked with standards and unspiked samples were treated with 50 mM nitric acid to approximate the manner in which the standards were treated, and followed by immediate addition of the 10× eluent concentrate buffered at pH 6.8. In reference 5, the authors extracted soil using phosphate buffer at the pH of the suspended soil sample to efficiently extract Cr(VI) from the soil. In this method, the authors did not attempt this type of extraction, as pH values significantly different from 6.8 may interfere with production of the Cr(III)-PDCA complex.

After treatment with PDCA buffer and acid, the samples were filtered through a 0.45 µm filter, then through a Dionex OnGuard II P cartridge. This cartridge contains a polyvinylpyrrolidone (PVP) polymer with a high selectivity for phenolics, azo-containing compounds, aromatic carboxylic acids, and aromatic aldehydes. The Dionex OnGuard II P cartridge was used to remove the humic acids from soil and wastewater samples to avoid contaminating the Dionex IonPac CS5A column. Figures 5 and 6 each show an overlay of chromatograms of the soil or wastewater sample and the same sample spiked with a mixed Cr(III)/Cr(VI) standard. Both samples contain no measurable chromium. Tables 3 and 4 show recovery of Cr(III) and Cr(VI) from both samples. The recovery calculations assume an 8.27% loss of Cr(III) and con-

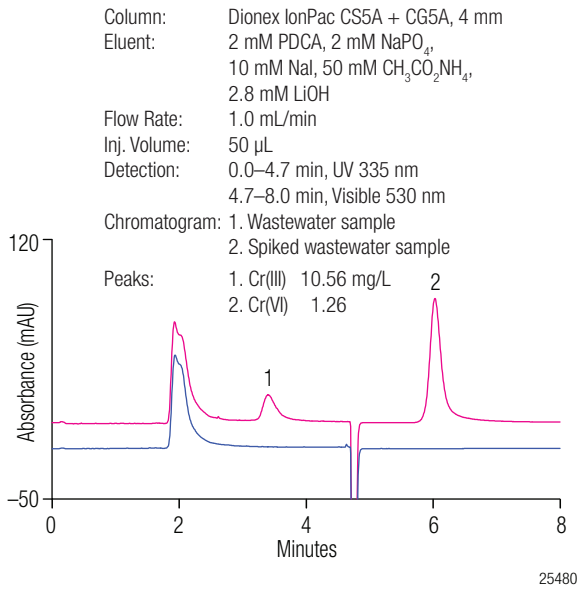

Figure 6. Overlay of the chromatograms of the wastewater sample and the sampled spiked with 10 mg/L Cr(III) and 0.5 mg/L Cr(VI).

comitant production of Cr(VI) during sample preparation, as measured in the experiments described earlier. This method allows an estimation of the Cr(III) and Cr(VI) amounts in each sample in a single injection. For an accurate measure of the sample concentration of Cr(VI), the authors recommend the method described in AU144. For a measure of total chromium, samples can be oxidized with ammonium persulfate then measured using the method in AU144. Cr(III) concentration can be derived from the difference of Cr(VI) and total chromium.

This application update demonstrates separation and detection of Cr(III) and Cr(VI) using a Dionex IonPac CS5A column and absorbance detection. This method also describes a sample preparation technique that can be used to estimate the concentrations of Cr(III) and Cr(VI) in soil and wastewater samples.

Table 3. Recovery of 10 mg/L Cr(III) and 0.5 mg/L Cr(VI) in a soil sample.

Sample No.	Amount (mg/L)	
	Cr(III)	Cr(VI)
1	10.49	1.11
2	10.46	1.11
3	10.49	1.11
Average	10.48	1.11
Spike	10	0.5
Total Cr*	10	1.33
% Recovery	104.8	83.5

*This value reflects the amount of Cr(VI) added and the amount expected from Cr(III) oxidation.

Table 4. Recovery of 10 mg/L Cr(III) and 0.5 mg/L Cr(VI) in a wastewater sample.

Sample No.	Amount (mg/L)	
	Cr(III)	Cr(VI)
1	10.70	1.26
2	10.40	1.26
3	10.58	1.26
Average	10.56	1.26
Spike	10	0.5
Total Cr*	10	1.33
% Recovery	105.6	94.7

*This value reflects the amount of Cr(VI) added and the amount expected from Cr(III) oxidation.

References

1. Dionex Application Update 144 .
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3. Dionex Technical Note 26.
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