

# Chromium Speciation in Water by HPLC/ICP-MS

## Introduction

Chromium exists primarily in two forms: trivalent and hexavalent. Trivalent chromium is present in the cationic form as  $\text{Cr}^{+3}$  and is an essential nutrient, but hexavalent chromium is toxic and exists as an anion, either as chromate ( $\text{CrO}_4^{-2}$ ) or dichromate ( $\text{Cr}_2\text{O}_7^{-2}$ ). Therefore, the analysis of total chromium does not always provide a true indication of the effects of chromium present in a sample. For example, a high total chromium concentration may not be harmful if the majority is  $\text{Cr}^{+3}$ . A more accurate assessment of the true effects of chromium can be gained by distinguishing trivalent and hexavalent species. As a result, chromium regulations have existed for some time for speciated analysis in water.

A common problem with chromium speciation is the well-known interconversion of  $\text{Cr}^{+3}$  and  $\text{Cr}^{+6}$ ; as a result, the determined  $\text{Cr}^{+3}/\text{Cr}^{+6}$  concentrations may not truly represent what exists in the sample. There has been much

work exploring the interconversion of these species and trying to limit this process<sup>1-9</sup>. Although some initial work is presented here, more in-depth discussions about chromium species preservation can be found in the literature. The focus of this work is to explore chromatographic and instrumental conditions and parameters necessary to distinguish  $\text{Cr}^{+3}$  from  $\text{Cr}^{+6}$  in water samples using HPLC/ICP-MS. The work here is an extension of earlier work.<sup>10</sup>

## Experimental

### Instrumental Parameters

Tables 1 and 2 show the chromatographic and ICP-MS operating conditions respectively. The HPLC separation of chromium species is accomplished isocratically by ion-pair chromatography using tetrabutylammonium hydroxide (TBAH) as the ion-pair reagent. Detection of the chromium species is accomplished with an ELAN® DRC (dynamic reaction cell) II ICP-MS.

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Table 1. HPLC Isocratic Method and Operating Parameters.

Parameter	Setting
Mobile Phase	1 mM Tetrabutylammonium Hydroxide (TBAH)+ 0.6 mM EDTA (potassium salt); pH 6.9 Then added 2% MeOH
Flow Rate	1.5 mL/min
Run Time	3 min
Column	3x3™ CR C8 (Part No. 0258-0191)
Column Temperature	Ambient
Autosampler Flush Solvent	5% Methanol / 95% HPLC-grade Water
Sample Injection Volume	50 µL
Sample Prep	Dilute with Mobile Phase
Detection	PerkinElmer SCIEX ELAN DRC II ICP-MS
Total Analysis Time	3 min

Table 2. DRC ICP-MS Operating Conditions and Parameters.

Parameter	Setting/Type
Nebulizer	Meinhard® Type A quartz (Part No. WE02-4371)
Spray Chamber	Quartz Cyclonic (Part No. WE02-5221)
RF Power	1100 w
Plasma Ar Flow	15 L/min
Nebulizer Ar Flow	0.96 L/min
Aux. Ar Flow	1.2 L/min
Injector	2.0 mm i.d. Quartz (Part No. WE02-3915)
Monitored Ion <i>m/z</i>	<sup>52</sup> Cr <sup>+</sup>
Dwell Time	1000 ms
Total Acquisition Time	180 sec
CeO <sup>+</sup> /Ce <sup>+</sup>	<2%
Reaction Gas	NH <sub>3</sub>
NH <sub>3</sub> Flow	0.50 mL/min
RPq	0.70

### Mobile Phase Preparation

The mobile phase was prepared by dissolving the appropriate amounts of tetrabutylammonium hydroxide (TBAH, Sigma-Aldrich, 1.0 M solution in water) and the dipotassium salt of ethylenediaminetetraacetic acid (EDTA, Sigma-Aldrich) in deionized water. The pH was then adjusted with nitric acid to 6.9, and methanol was added to make a 2% methanol solution.

### Conditioning the HPLC Column

The column used for this application is a high-speed, reversed-phase, C8 cartridge column. It is 3.3 cm long and packed with 3- $\mu$ m particles, which permit very rapid analysis times (typically 2-3 minutes) and reduced solvent consumption. The cartridge column consists of two pieces: the column holder (part number 0715-0028) and the cartridge column (part number 0258-0191, available only in a 5-pack). The main advantage of cartridge columns is their reduced cost compared to standard columns.

When the column is first received, it should be washed for 90 minutes with 100% methanol at a flow of 1.0 mL/min. This procedure ensures that the column is clean and does not need to be repeated.

Prior to using the column for the first time each day, mobile phase should be allowed to flow through the column for 30 minutes. This procedure conditions the column with the ion-pair reagent.

After use for the day, the column should be washed for 15 minutes with 5/95 v/v methanol/water to remove all salts from the system and to inhibit bacterial growth. For long-term storage, first rinse the column with 100% water and then 40/60 v/v methanol/water. After rinsing the column, cap the column to prevent it from drying out.

### Standards and Standard Preparation

Chromium standards were prepared from 1000 mg/L stock solutions of Cr<sup>+3</sup> (PerkinElmer, Cr(NO<sub>3</sub>)<sub>3</sub>·9 H<sub>2</sub>O, part number N930-0173) and Cr<sup>+6</sup> (Spex Certiprep, (NH<sub>4</sub>)<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>). Intermediate 2 mg/L solutions of each species were prepared in deionized water. Standards of different chromium concentrations were made by appropriate dilution of the intermediate standards with mobile phase.

## Results

Figure 1 shows a chromatogram of a mixture of Cr<sup>+3</sup> and Cr<sup>+6</sup> (10  $\mu$ g/L (ppb) each) acquired while monitoring <sup>52</sup>Cr<sup>+</sup>. The data was acquired in less than 3 minutes under the conditions stated above. Shorter analysis times can be accomplished using somewhat higher methanol concentrations. For this study, a 1mM TBAH concentration appeared to be optimal. Though 2mM TBAH has been used successfully with other 3x3 C8 columns, significantly increasing or decreasing the TBAH molarity may reduce peak resolution and may result in peak broadening.

The separation is accomplished by interaction of the chromium species with the different components of the mobile phase. The Cr<sup>+3</sup> forms a complex with the EDTA; it is this complex that is retained on the column. Cr<sup>+6</sup> exists in solution as dichromate. The net negative charge of the chromium-EDTA complex and the negative charge of the dichromate interact with the positive charge of the tetrabutylammonium group. In turn, the hydrocarbons of the tetrabutyl group interact with the C8 of the stationary phase, thus permitting the separation of a charged species on a reversed-phase column. The purpose of the methanol is to assist in the elution of these chromium species from the column.

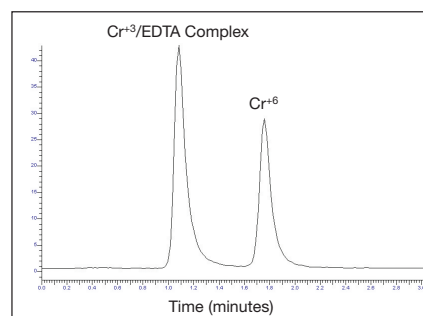


Figure 1. Optimal separation.

## Column Washing

The effect of washing the column with methanol is shown in Figure 2. Figure 2a displays the chromatogram of  $\text{Cr}^{+3}$  and  $\text{Cr}^{+6}$  (10  $\mu\text{g}/\text{L}$  each) on a new, unwashed C8 column. Clearly, there is no baseline resolution between the peaks. Figure 2b shows the same sample run on the same column after it has been washed for 90 minutes with 100% methanol at 1 mL/min. After the column wash, the individual peaks, resulting from the two different chromium species, are clearly distinguishable. The initial peak is an unidentified component that was not investigated further; it was only observed when polypropylene vials were used.

The reason that washing the column is important is due to the mechanism of ion pairing. With ion pairing, the organic side of the ion-pair reagent (TBAH in this work) interacts with the organic C8 stationary phase of the column, and the ionic side of the ion-pair reagent interacts with the analyte ( $\text{Cr}^{+3}$ ). With a new column, the original condition of the stationary phase prevents the TBAH from establishing a stable interaction equilibrium with the C8; therefore, at first, the chromium species are not well separated (Figure 2a). When the column is washed with methanol, the stationary phase is properly conditioned. Thus, indirectly, this allows the chromium species to be better retained and separated on the column (Figure 2b).

The use of a C18 column was also explored for this work. As with the C8 column, a before-and-after column wash experiment was performed on this column. As the results show (Figures 2c, 2d), there was no adequate peak separation achieved using the C18 column.

## Mobile Phase Concentrations

There are numerous parameters that may be optimized in a reversed-phase ion-pair separation. These parameters include:

- Type of ion-pair reagent
- Alkyl chain length of the ion-pair reagent

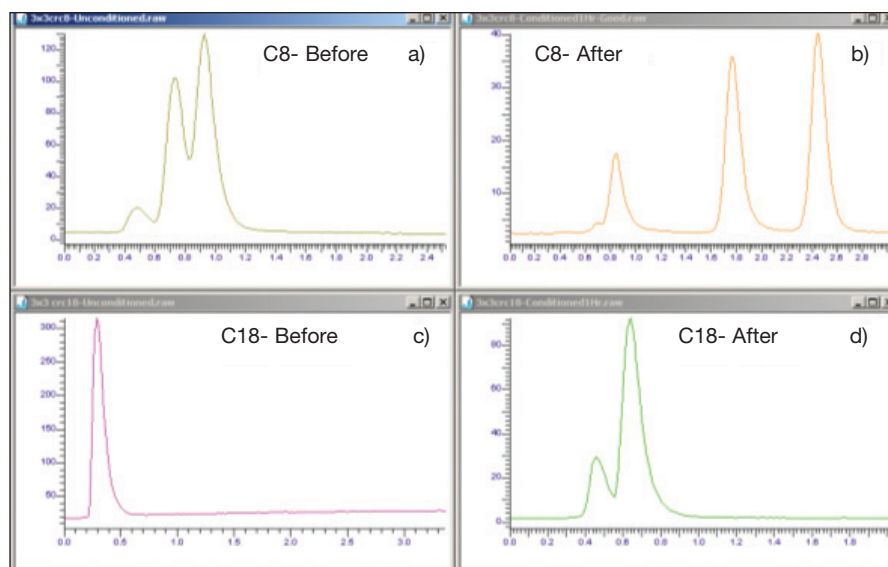


Figure 2. Effect of washing new columns with MeOH. Washing scheme: 90 minutes, 100% methanol at 1 mL/min. X-Axis: mV (millivolts), Y-Axis: Time (min).

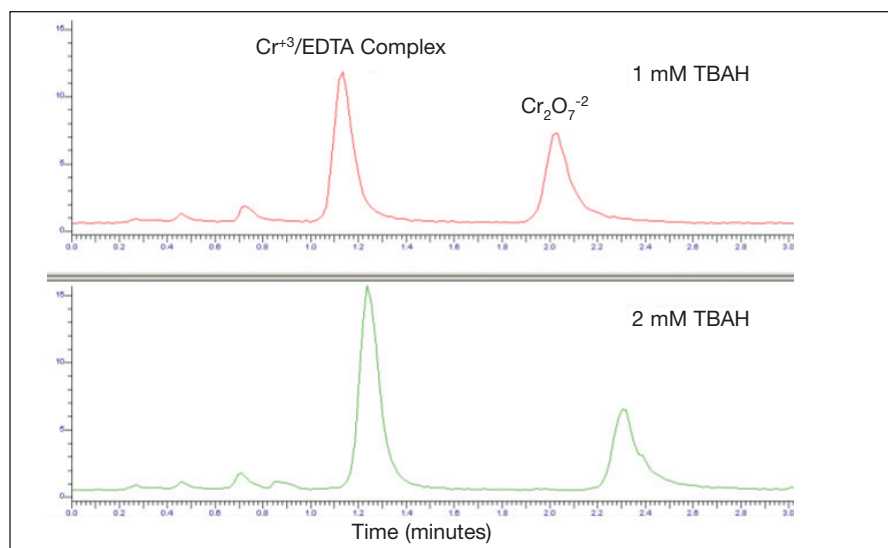


Figure 3. Effect of TBAH concentration on chromium separation.

- Concentration of the ion-pair reagent
- Percent organic modifier
- pH

In this work we investigated three of these parameters - the type of ion-pair reagent, the concentration of the ion-pair reagent and the percent organic modifier.

In the paper presented by Chang and Jiang<sup>10</sup> the ion-pair reagent used was tetrabutylammonium phosphate (TBAP). TBAP has two disadvantages. First, it is an expensive reagent. Second, TBAP is very hydroscopic, and hence, difficult to work with. Therefore, an alternative to TBAP was investigated.

Tetrabutylammonium hydroxide (TBAH), a very commonly used ion-pair reagent, was found to be an acceptable replacement.

Figure 3 shows the effect of TBAH concentration. If the TBAH concentration is increased, the retention times become longer and the peaks broaden. The reason for these phenomena is that, at higher concentrations, more TBAH can interact with the stationary phase, thus providing more ion pairing sites for the chromium species to be retained. Too much retention ultimately results in broader peak shapes.

The purpose of the methanol in the mobile phase is to reduce the interaction between the stationary phase and the ion-pair reagent. As a result, it is expected that higher methanol concentrations would result in shorter retention times and narrower peaks. The results shown in Figure 4 support this theory.

For the rest of the work presented here, a combination of 1 mM TBAH and 2% methanol was found to give adequate separation in a desired amount of time.

### Vial Material and Stability

An important aspect of an HPLC method is its reproducibility. To test this, consecutive injections of a 10 µg/L (ppb) mixed standard were made from a polypropylene HPLC autosampler vial. The chromatograms are displayed in Figure 5 and show that, over time, the Cr<sup>+6</sup> peak decreases while the Cr<sup>+3</sup> peak increases. These results suggest that something is causing the conversion of Cr<sup>+6</sup> to Cr<sup>+3</sup>.

Another test was performed where consecutive injections of a 10 µg/L Cr<sup>+3</sup> from a polypropylene vial were made over 1 hour. Figure 6 displays an overlay of six chromatograms taken at equal intervals throughout the hour. These chromatograms overlap very well, thus demonstrating the inherent stability of the method. This also suggests that the problems observed in Figure 5 resulted from a conversion of Cr<sup>+6</sup> to Cr<sup>+3</sup>, but not vice versa.

The above test was then repeated, except a glass autosampler vial was used instead of a polypropylene vial; the results are displayed in Figure 7. From this figure, it can be seen that in consecutive chromatograms, the intensities of both peaks remained quite constant. These results suggest that by using glass vials, as opposed to polypropylene vials, the unwanted conversion of Cr<sup>+6</sup> to Cr<sup>+3</sup> is significantly minimized. All further analyses were performed using glass HPLC autosampler vials.

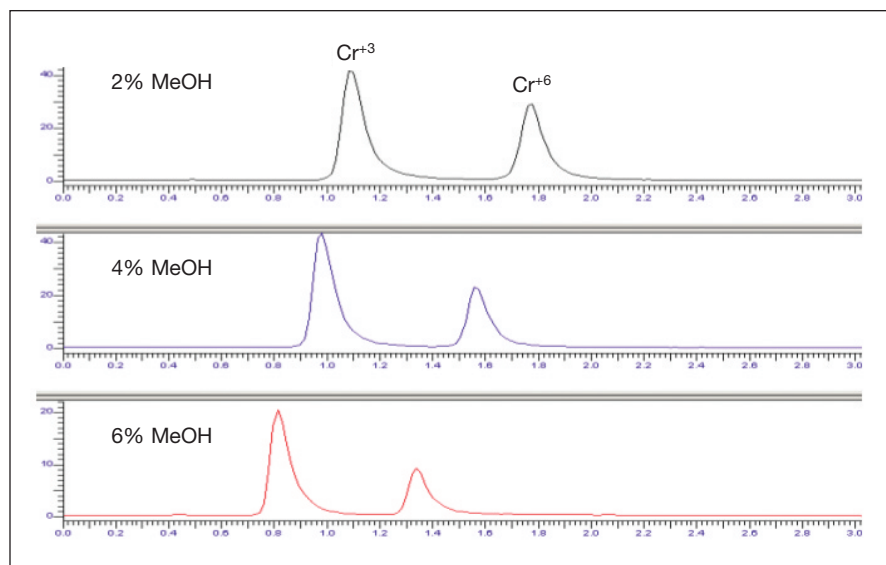


Figure 4. Effect of mobile phase MeOH on chromium separation.

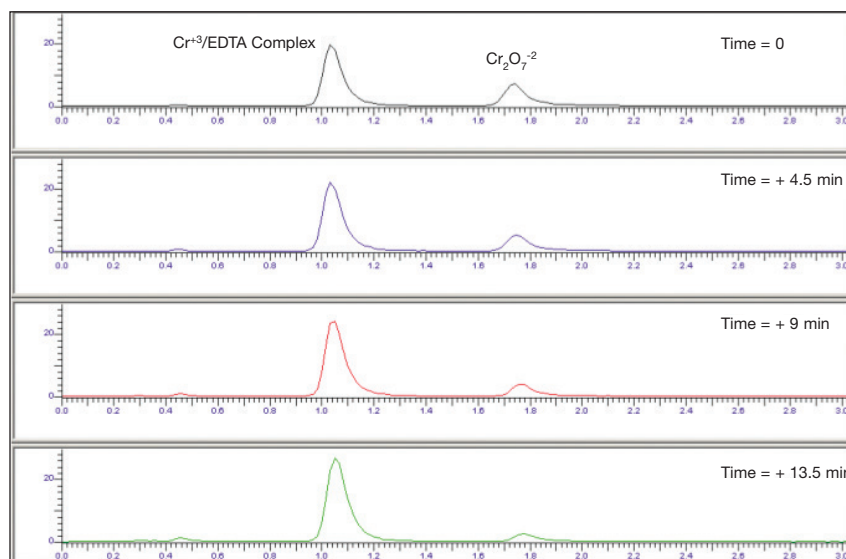


Figure 5. Cr<sup>+3</sup> / Cr<sup>+6</sup> reproducibility test using polypropylene vials.

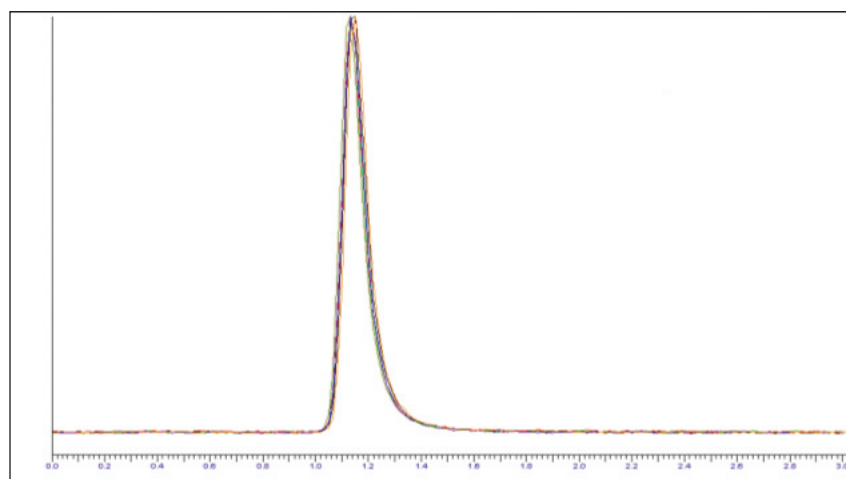


Figure 6. Cr<sup>+3</sup> reproducibility test using polypropylene vials: 10 µg/L Cr<sup>3+</sup> over 1 hour; 6 replicates.

## Interference Reduction

A potential interference for chromium determination by ICP-MS is  $\text{ArC}^+$  (argon carbide), which forms at  $m/z$  52, the same  $m/z$  as the major chromium isotope. Using 2% methanol in the mobile phase amplifies this interference, which would limit the ability to look at low levels of chromium. To solve this problem, ammonia was used as a reaction gas in the ELAN DRC II. Ammonia reacts with and eliminates  $\text{ArC}^+$ , thus leaving  $^{52}\text{Cr}^+$  interference-free. This effect can be seen in Figure 8, which displays two chromatograms of 1  $\mu\text{g/L}$   $\text{Cr}^{+3}$  and 1  $\mu\text{g/L}$   $\text{Cr}^{+6}$ , one acquired without a reaction gas (standard mode) and the other acquired with ammonia as a reaction gas (DRC mode).

The top chromatogram was obtained in the standard mode. The baseline is noisy and has an intensity of about 18,000 cps, the result of  $\text{ArC}^+$ . The amplitude of the baseline noise is about 1000 cps, and the peaks are about 3,000–4,000 cps above the baseline, thus leading to a signal to noise (S/N) of about 3–4. In addition, a broad, unidentified peak is seen in the top chromatogram.

In contrast, the bottom chromatogram was acquired in the DRC mode. The baseline is much less noisy and showed an intensity of only ~700 cps. The peaks are about 4,000–5,000 cps above the baseline, which fluctuates about 50 cps, yielding a S/N of about 80-100. Therefore, the quantitative sensitivity in DRC mode

is clearly superior to that in standard mode. In addition, in DRC mode, the unidentified peak disappeared, suggesting that this interference is a carbon species.

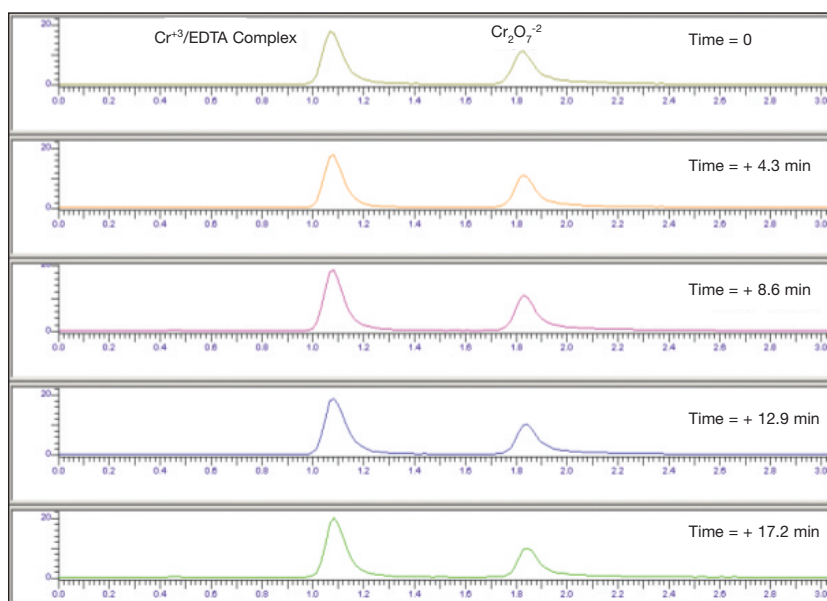


Figure 7.  $\text{Cr}^{+3}$  /  $\text{Cr}^{+6}$  reproducibility test using polypropylene vials.

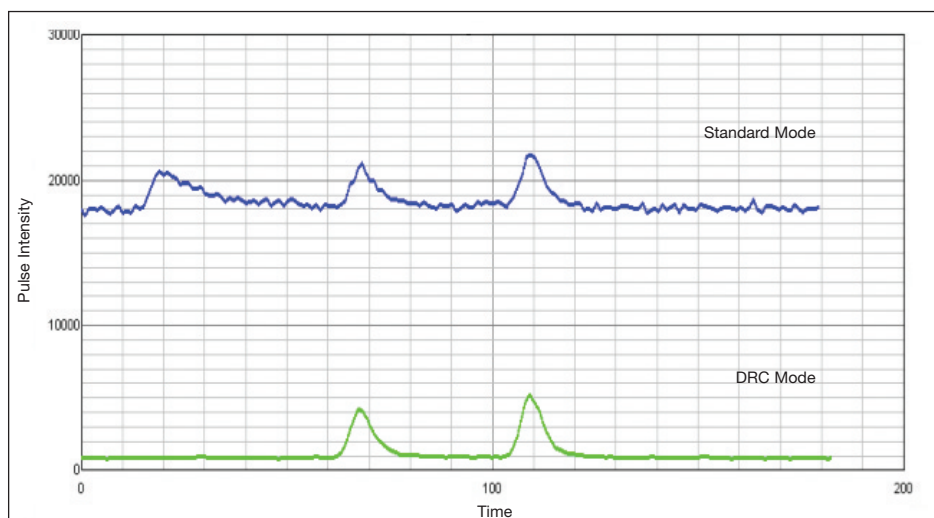


Figure 8. Comparison of DRC to Standard Mode (ELAN data): 1 ppb  $\text{Cr}^{+3}$  and  $\text{Cr}^{+6}$ .

## Detection Limits

To determine how low a Cr level can be detected in DRC mode, successive 50  $\mu\text{L}$  injections were made of varying chromium concentrations in mobile phase. Figure 9 shows chromatograms of 25, 50, and 100 ng/L chromium spikes (baselines artificially offset for clarity). From this figure, 50 ng/L (ppt) of each chromium species can be seen at about S/N - 3, thus demonstrating detection limits of about 50 ng/L for each chromium species.

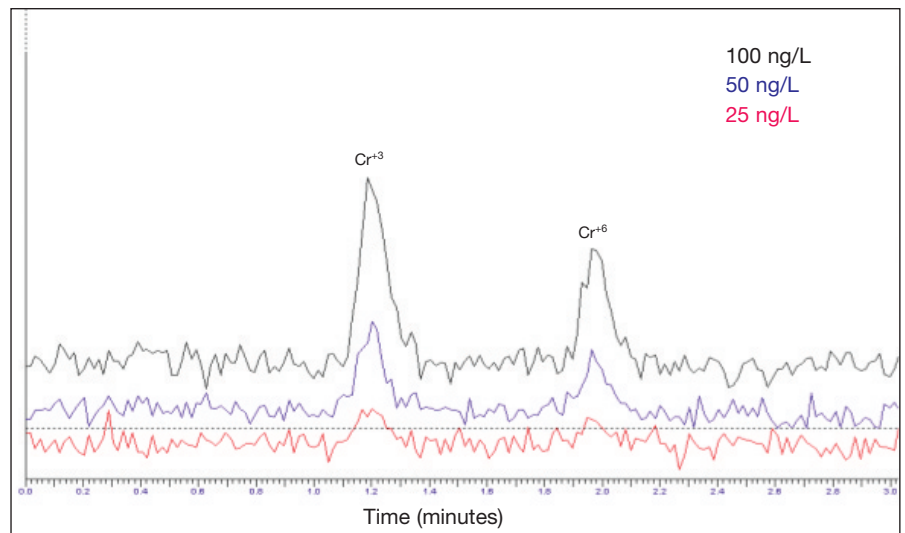


Figure 9. Cr<sup>3+</sup> / Cr<sup>6+</sup> detection limits.

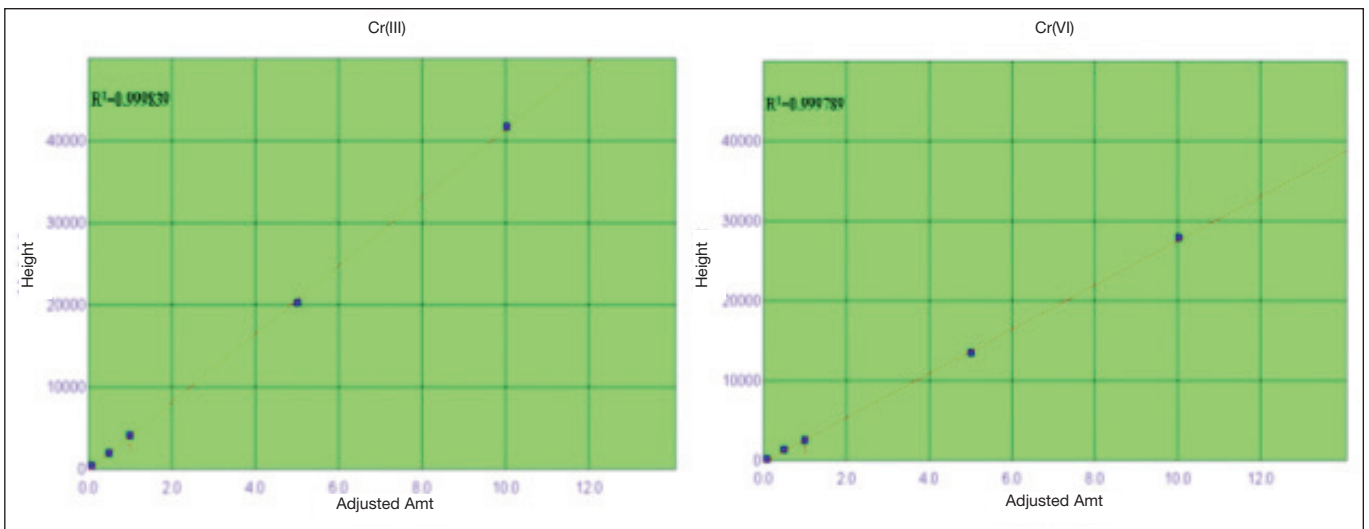


Figure 10. Chromium calibration curves.

## Quantification

The ability to quantitatively determine the amount of each species present was tested on a sample of tap water from our lab. External calibrations were made from 50  $\mu\text{L}$  injections of 0.1, 0.5, 1, 5, and 10  $\mu\text{g/L}$  of each chromium species in the mobile phase, and peak heights were measured. The resulting curves are shown in Figure 10. The regression for each species is 0.999, thus demonstrating the linearity and reproducibility of the technique.

The tap water sample was then analyzed, but the resulting chromatogram did not yield any peaks, thereby indicating the absence of or extremely low levels of chromium. (The same sample was later analyzed for total chromium, and the results

show < 75 ng/L of chromium present.) The tap water was then spiked with 0.5  $\mu\text{g/L}$  of each chromium species and run against the calibration curves. The results of this analysis show that Cr<sup>3+</sup>=0.62  $\mu\text{g/L}$  and Cr<sup>6+</sup>=0.36  $\mu\text{g/L}$ ; the chromatograms of the spiked sample and 0.5  $\mu\text{g/L}$  calibration standard appear in Figure 11. Subsequent analyses and sample preparations yielded the same results; therefore, it appears that the Cr<sup>6+</sup> is converted to Cr<sup>3+</sup> after it is spiked into the sample.

To attempt to minimize this conversion, the water sample was diluted five times with mobile phase prior to spiking the chromium species. Figure 12 shows the chromatograms of the diluted spiked sample and the 0.5  $\mu\text{g/L}$  calibration standard. These

chromatograms show that the Cr<sup>6+</sup> conversion in the spiked water sample is minimized since the peaks closely match those of the calibration standard. Quantitative analysis of the spiked sample shows that Cr<sup>3+</sup>=0.57  $\mu\text{g/L}$  and Cr<sup>6+</sup>=0.47  $\mu\text{g/L}$ , thus confirming that conversion of Cr<sup>6+</sup> to Cr<sup>3+</sup> is minimized. These results suggest that something present in the water sample catalyzes the chromium conversion; the addition of mobile phase to the sample either dilutes the catalyzing species or preferentially locks the chromium in its original state. Much work has been published about the interconversion of chromium species and ways to preserve the original oxidation state, but this remains a difficult and tricky part of the analysis.

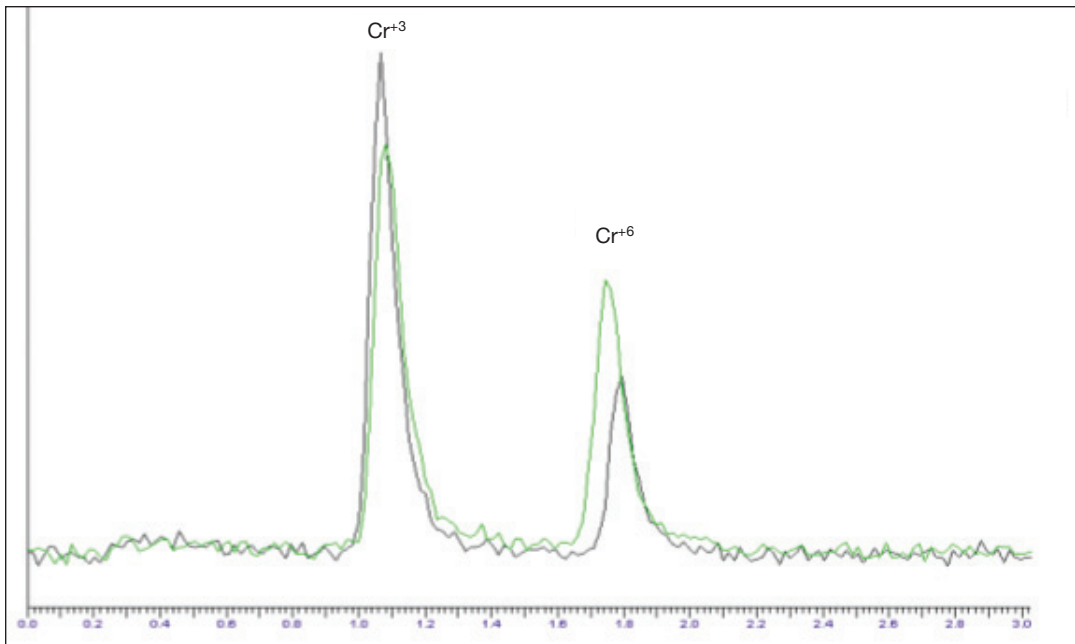


Figure 11. Chromatograms of 0.5 µg/L chromium spike in tap water (black) and 0.5 µg/L chromium calibration standard (green).

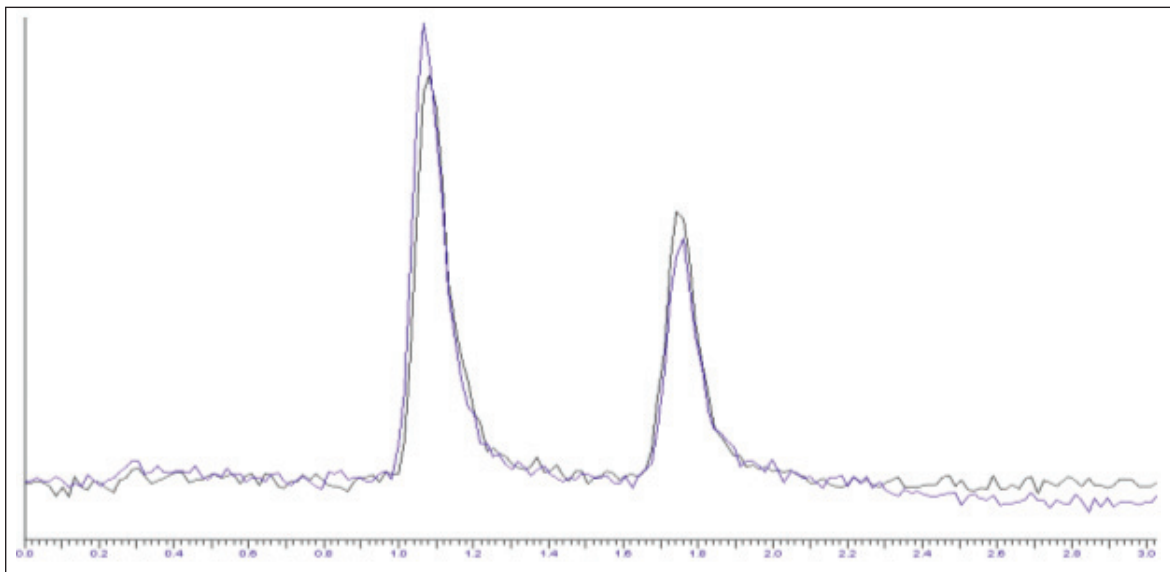


Figure 12. Chromatograms of 0.5 µg/L chromium spike in tap water diluted 5x (blue) and 0.5 µg/L chromium calibration standard (black).

## Conclusions

This work has shown that trivalent and hexavalent chromium can be separated using ion pair HPLC and detected with ICP-MS in less than 3 minutes. It was seen that many factors must be considered and optimized in order to achieve successful separation and reproducibility. These include column cleaning and pretreatment, ion-pair reagent concentration, organic modifier concentration

(methanol), vial material, and detection mode. Once these parameters are developed, low concentrations can be measured, with an estimated detection limit of 50 ng/L (ppt) for each species in DRC mode. The advantages of using a DRC ICP-MS are clearly evident in reducing interferences and minimizing baseline noise, making the method more rugged for the variety of matrices and concentrations that may be encountered.

## References

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