APPLICATION NOTE

LC-ICP-MS

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Chromium Speciation in Drinking Water by LC-ICP-MS

Introduction

With the growing concern of pollutants in the environment, more focus has been placed on identifying not only the total concentration of metals, but also the states in which they exist. Many elements can exist in various forms, either with different oxidation states or associated with various organic compounds or other elements. The toxicity and environmental impact of elements can vary depending in which form(s) they exist.

One element which has received considerable attention is chromium (Cr), which can exist in two different oxidation states: trivalent (Cr3) or hexavalent (Cr6). While trivalent chromium is an essential nutrient, hexavalent chromium is toxic. As a result, knowing the concentration of hexavalent chromium in environmental systems and samples which will be consumed is more important than knowing the total chromium concentration. This is especially true for drinking water.

Currently, the United States Environmental Protection Agency (U.S. EPA) only regulates the total chromium levels in drinking water and has set the limit at 100 ppb. However, the State of California set more aggressive limits for drinking water in 2014, both in regulating total chromium at 50 ppb and setting a MCL (maximum contaminant level) specific for hexavalent chromium at 10 ppb, with a public health goal of 20 ppt for Cr6.

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This work describes the use of LC-ICP-MS to measure both trivalent and hexavalent chromium in drinking waters, with the goal of presenting a suitable methodology for analyzing the range of concentrations encompassed by the new legislation.

Experimental

Two alternative methodologies are widely employed for the separation of Cr3 and Cr6: ion exchange and ion pairing chromatography. The ion pairing method described in a previous application note[1] is still widely used and has been thoroughly validated[2], is fast, robust, and suitable for multielemental speciation[3]. For this work, we opted for ion exchange on a short column with increased retention of species to maintain peak shape and separation at larger injection volumes utilized for lower detection limits.

Even though very acidic media can be employed to achieve separation of chromium species[4], the use of neutral media is preferred for equilibration-dependent methods for effective complexation of Cr3 by EDTA. Furthermore, the redox potential of the chromate/Cr3 redox couple is strongly pH dependent. Neutral pH has been demonstrated previously to provide suitable conditions for stability of chromium species when equilibration with EDTA was employed[1]. After initially testing the effect of pH on separation efficiency, we optimized the method utilizing neutral pH in order to build on the established complexation ability and species stability at this pH.

Samples and Reagents

Tap water samples were collected locally and internationally, and bottled water samples were purchased from a local grocery store. Bottled water does not strictly fall under the U.S. EPA or CDPH (California Department of Public Health) regulations (it is regulated by the Food and Drug Administration) but is included here to provide a wide range of drinking water samples to gauge the effectiveness of the methodology.

Chromium standards were made from stock solutions of trivalent and hexavalent chromium (PerkinElmer, Spex, respectively). The mobile phase was made from high purity nitric acid (GFS Chemicals), ammonium hydroxide (Fisher Scientific), and the dipotassium salt of ethylenediaminetetraacetic acid dihydrate (Sigma-Aldrich). Although the mobile phase can also be prepared from ammonium nitrate directly, it was found that using nitric acid and ammonium hydroxide produced lower chromium backgrounds. To prepare the mobile phase, 0.875 mL high-purity nitric acid and 202 mg of EDTA were added to 1L deionized water (18.2 MΩ-cm) and the pH was adjusted with 10% (v/v) ammonia hydroxide.

All quantitative measurements were made against external calibration curves with standards prepared in mobile phase. Samples were diluted 2-fold (i.e. 1+1) in mobile phase. To allow time for the Cr3 to complex with the EDTA, all standards and samples were allowed to equilibrate for a minimum of 3 hours at room temperature prior to analysis, although equilibration time can be substantially reduced by heating the solutions[5]. Standards were prepared daily, as they were found not to be consistently stable for more than 24 hours at room temperature.

Plastic vials were used uncapped in order to avoid the possibility of introducing contamination from the cap. Since samples are prepared just prior to analysis, there is no need to cap the vials.

Instrumental Conditions

All analyses were run on a PerkinElmer Altus™ UPLC System, fitted with a 250 µL stainless steel expansion loop, 30 µL stainless steel needle, and 250 µL syringe, coupled to a PerkinElmer NexION® 350D ICP-MS. The syringe draw rate for sample uptake was set to 500 µL/min. The instrumental parameters are shown in Tables 1 and 2. All analyses were performed in Reaction mode using ammonia as the cell gas to remove any carbon- and chlorine-based interference at $^{52}Cr^{+}$. Interference by $^{12}C^{40}Ar^{+}$ and $^{35}Cl^{16}OH^{+}$ can be effectively reduced by using ammonia as a reaction gas without loss of analyte sensitivity.[6] All data analysis and processing was done with Waters® Empower® 3 Software.

Table 1. Altus UPLC System Conditions.

Parameter	Value
Column	Hamilton PRP-x100, 4.1 x 50 mm, 5 µm
Mobile Phase	14 mM NH_4NO_3 (from HNO_3 + NH_4OH) + 0.5 mM EDTA
pH	7.0 (adjusted with 10% NH_4OH)
Flow Rate	1.2 mL/min
Separation Scheme	Isocratic
Column Temperature	30 °C
Injection Volume	200 µL
LC Vials	Plastic, 1.5 mL

Table 2. NexION 350D ICP-MS Conditions.

Parameter	Value
Nebulizer	Glass Concentric
Spray Chamber	Glass Cyclonic
RF Power	1600 W
Nebulizer Flow	Optimized for ≤ 2% oxides
Mode	Reaction
Cell Gas	NH_3 @ 0.5 mL/min
RPq	0.50
Isotope	Cr52
Dwell Time	1000 ms
Sampling Rate	1 point/second

Results and Discussion

Figure 1 shows the chromatogram of a mixed Cr standard. Both species are well separated and elute within five minutes, allowing for short run times. Additionally, the peaks elute well after the void volume (0.4 min).

Initial calibration curves were established with standards from 0.04 -50 ppb for Cr3 and from 0.008–10 ppb for Cr6, where the upper levels were chosen as the MCLs for Cr6 and total chromium (for the Cr3 curve). Under the selected instrumental conditions, the peaks are acquired with sufficient precision (30 pts per peak for the smallest peaks) for reproducible integration. All calibration curves gave R^2 values greater than 0.999, indicating the linearity of the method up to the levels of chromium set by the legislation.

Since the samples analyzed in this study read well below the MCLs, the calibration range was reduced to 10/2 ppb Cr3/6 to more accurately evaluate samples and spikes. Figure 2 shows the calibration curves used for the evaluation of samples, which gave $R^2 > 0.9999$ for both Cr3 (Figure 2a) and Cr6 (Figure 2b). Also shown are the concentrations calculated when the standards themselves are evaluated with the calibration curve (generally within 4% of stated value). The largest relative deviations are observed for the lowest concentrations, as expected, but in absolute terms these deviations are very small (4 ppt - lowest two Cr6 standards, Figure 2b). The slopes of the calibration curves for Cr3 and Cr6 (parameter B in Figures 2a and 2b) match very closely (1% difference), demonstrating that the species do not interconvert, and that complexation of Cr3 with EDTA is quantitatively complete.

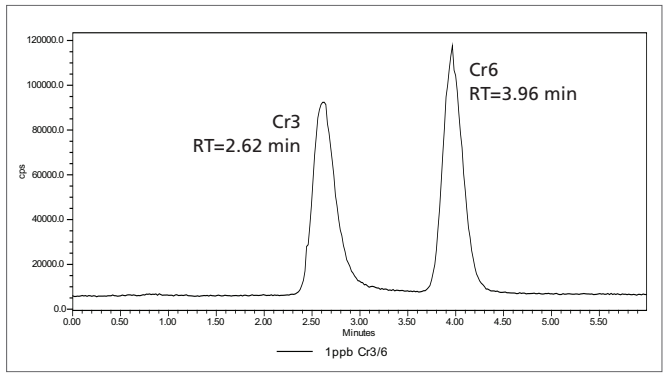
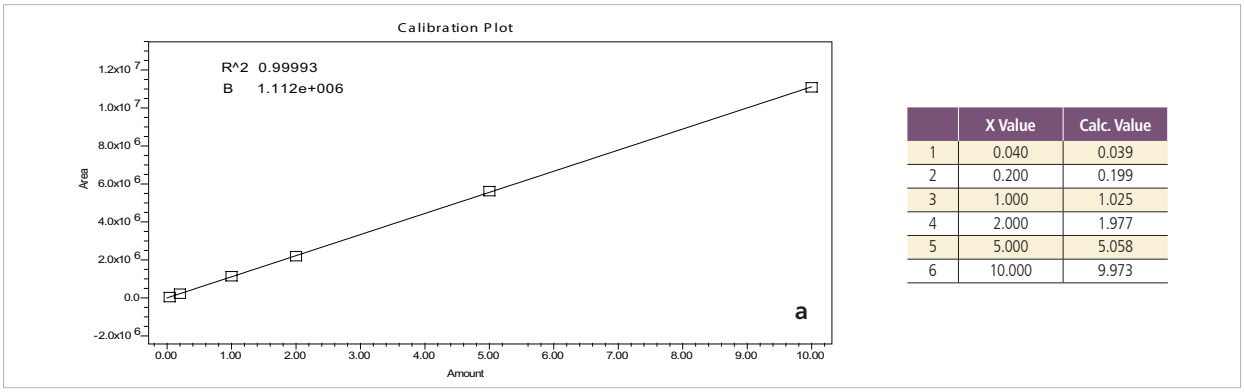
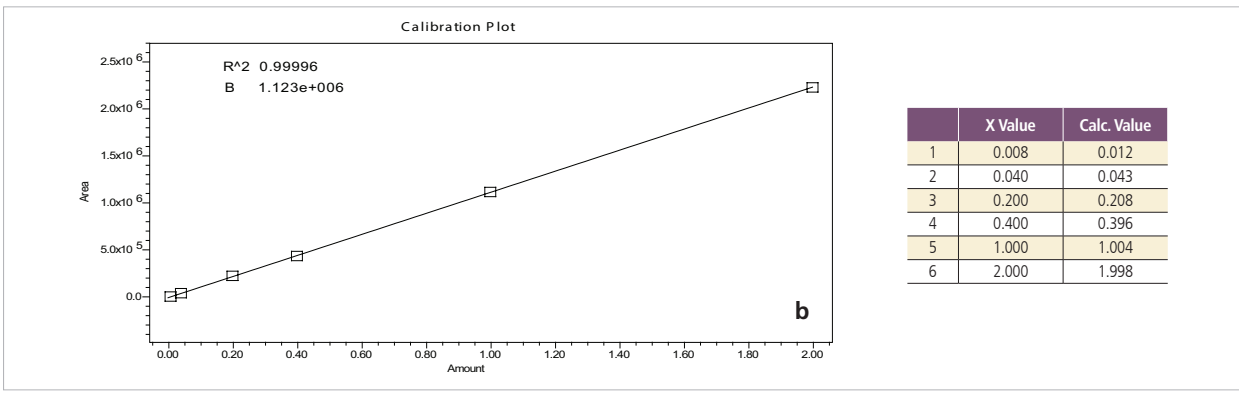


Figure 1. Chromatogram of 1 ppb Cr3/6 acquired with the instrumental and LC conditions stated in Tables 1 and 2.



	X Value	Calc. Value
1	0.040	0.039
2	0.200	0.199
3	1.000	1.025
4	2.000	1.977
5	5.000	5.058
6	10.000	9.973



	X Value	Calc. Value
1	0.008	0.012
2	0.040	0.043
3	0.200	0.208
4	0.400	0.396
5	1.000	1.004
6	2.000	1.998

Figure 2. Calibration curves and details for Cr3 (a) and Cr6 (b).

The LC vials are critical to preserving the species, as it has been found that vials of different material or from different suppliers assist in reducing Cr6 to Cr3[1]. The vials used in this work (listed in the Consumables Used table at the end) did not promote reduction of Cr6 to Cr3. These vials were used directly from the box and did not require any cleaning or pretreatment procedures.

The chromatograms of a 10 and 20 ppt mixed standard are shown in Figure 3. Both peaks are clearly visible above baseline and can be quantitated, indicating that under these conditions, detection limits are less than 10 ppt for each species. If lower levels are desired, larger injection volumes can be used, or the dilution ratio can be reduced by diluting samples with concentrated mobile phase. The injection volume and dilution ratio chosen here were found to be optimal for best detection while maintaining peak shape, peak separation, and ease of operation.

With the separation established, a variety of drinking water samples were analyzed, with the results appearing in Table 3. It is interesting to note that none of the samples contained Cr3, yet all had low levels of Cr6.

Table 3. Quantitative results for a variety of drinking water samples, corrected for dilution.

Sample	Cr3 (ppb)	Cr6 (ppb)
W1	---	0.084
W2	---	0.050
W3	---	0.068
W4	---	0.020
W5	---	0.046
W6	---	0.080
W7	---	0.076
W8	---	0.080

The chromatograms of several water samples are shown in Figure 4, demonstrating that the drinking water matrices do not affect the ability to measure low levels of Cr6. The Cr6 concentrations shown in Figure 4 are those read by the instrument and have not been corrected for the 2x dilution.

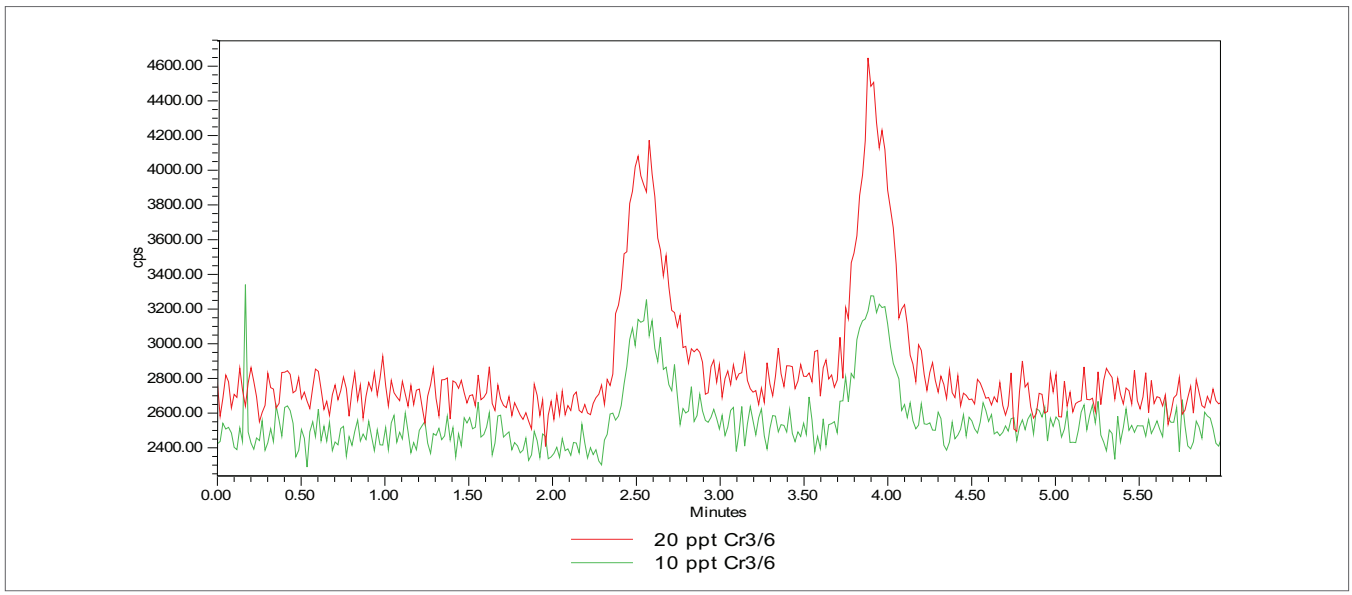


Figure 3. Chromatogram overlay of low level chromium solutions (0.010 and 0.020 ppb Cr3/6 mixed standards).

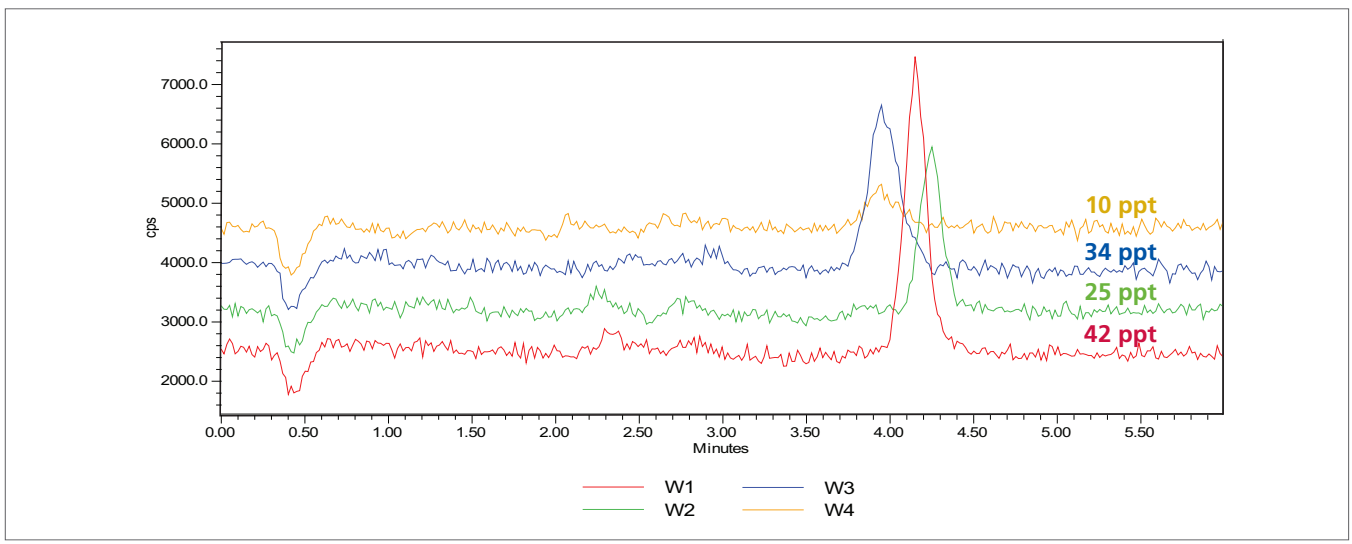


Figure 4. Chromatograms for several waters samples (with y-axes offset for clarity). All samples contain only detectable levels of Cr6. The raw analysis results (i.e., not corrected for dilution) for Cr6 are indicated on the chromatograms.

While for most samples shifts in retention time are small (see Figure 4), it was noted that in some samples, the Cr6 peak shifted to longer retention times by up to 30 seconds, compared to the standards. This peak shift is caused by the matrix composition of the water sample. To test the effect of salt concentration on retention time, solutions of varying concentrations of sodium chloride (up to 1000 mg/L), were diluted 2-fold with mobile phase and spiked with 1 ppb mixed Cr3/6. As seen in Figure 5, the retention time for both chromium species is only marginally affected by the sodium chloride content, indicating the robustness of the method towards elevated salt levels. Other matrix components are therefore causing the larger retention time shifts of Cr6 in some drinking water samples. However, these were not investigated further as they do not affect the ability to measure Cr6 in water. Any change in retention time can be accounted for in the Empower® 3 Software by expanding the peak search window or manually assigning the retention time when reviewing the data. Alternately, higher dilution factors may be employed to minimize retention time shifts. Results for samples with detectable Cr6 at 5-fold dilution agreed well with the data from 2-fold analysis, showing that higher dilutions can be used in cases where lowest method detection limits are not required. An associated benefit is lower sample loading on the column, which contributes to longer column lifetime.

To determine the accuracy of the results, all samples were spiked with 0.5 ppb of both chromium species. All spikes recovered within 10% of the target values (as shown in Figure 6), indicating the accuracy of the method.

With the separation, ruggedness, and quantitative accuracy of the method established, both short- and long-term stability were investigated. For short-term stability, one of the water samples (W7) was analyzed eight times within one hour. Figure 7 shows the resulting Cr6 concentrations, with all readings normalized to the first measurement. With concentrations varying by less than ± 4%, the short-term stability of the methodology is demonstrated.

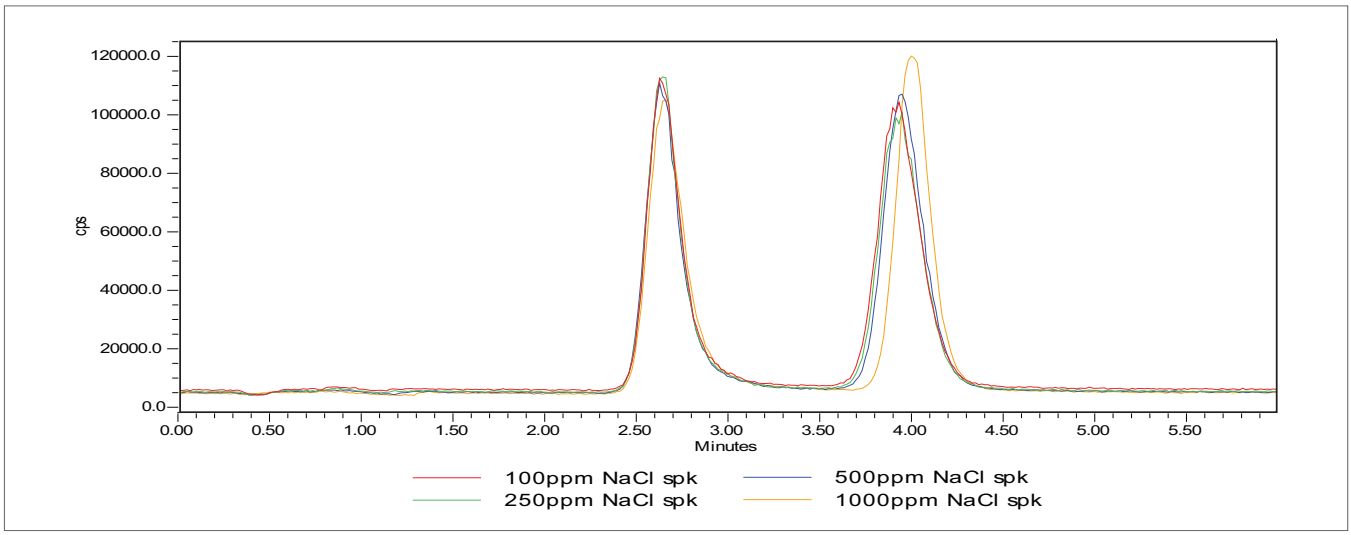


Figure 5. Method robustness: effect of salt concentration (100 – 1,000 ppm NaCl) on chromatography.

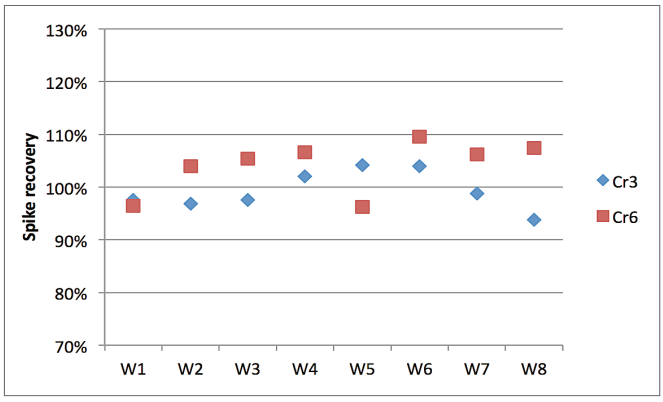


Figure 6. Recoveries for 0.5 ppb Cr3/6 spikes on all water samples.

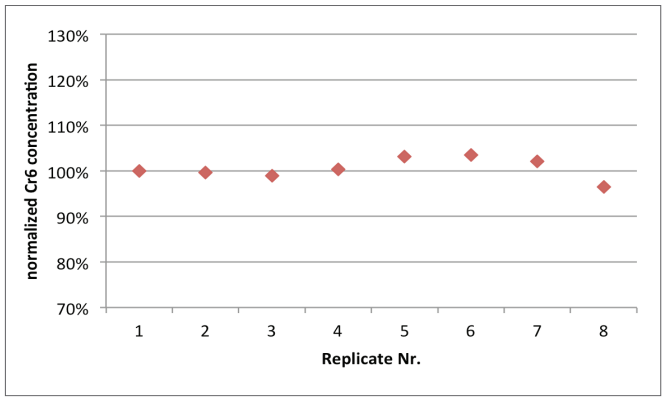


Figure 7. Repeated analysis of one sample (W8) over one hour.

Long-term stability of the analysis was explored by running all of the water samples continuously over 14 hours, with a check standard (Cr3=2 ppb and Cr6=0.4 ppb) run every 30 minutes. Figure 8 shows a plot of the check standard, with all results normalized to the initial reading. Variations of less than 6% for both species prove the stability of the standards, instrumentation and methodology.

Despite the long-term stability of standard solutions, another factor which must be considered is the stability of the species in the samples. Since water samples contain multiple components, it is possible that chromium interconversion may occur over time in the samples. Reanalyzing water samples 11 hours after their initial analysis (and 15 hours after sample preparation) showed that all samples were stable (raw results agreed within 5 ppt) for this time frame, except one sample (W5) where the Cr6 concentration decreased and Cr3 appeared. Spiking this sample also showed interconversion of the species over time. Since only this sample showed interconversion, it suggests that something in the sample is reducing the Cr6 to Cr3. Since the check standards did not show species interconversion (Figure 8), the possibility that the vial is causing the conversion is eliminated.

Although the methodology and instrumentation are stable over long runs, it is therefore recommended to prepare samples in smaller batches to minimize the possibility of species interconversion. Another option is to chill the autosampler tray (4-10°C) to slow the chemistry of interconversion.

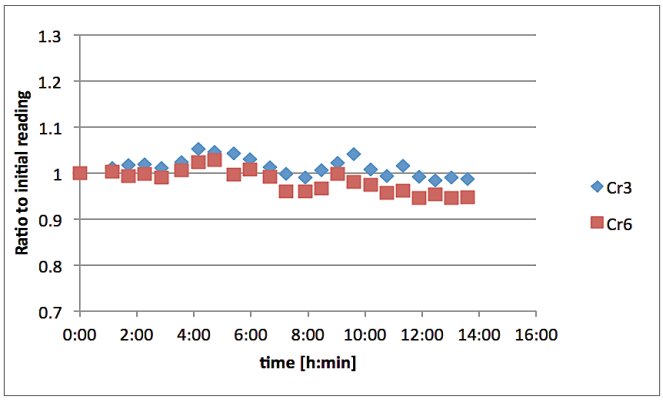


Figure 8. Data for check standard (2/0.4 ppb Cr3/6) run intermittently during drinking water analysis demonstrates long-term stability.

Conclusion

This work has shown that the Altus UPLC System together with the NexION 350D ICP-MS can be used successfully for chromium speciation at the levels relevant to recent, more stringent legislation. The presented method separates Cr3 and Cr6 within a 6-minute run, measures less than 10 ppt (20 ppt in the sample), and covers a wide linear range up to at least 50 ppb.

The optimum procedure uses a 2-fold sample dilution with mobile phase for sufficient complexation of Cr3 with EDTA, reduced retention time shifts caused by sample matrix, adequate peak shape and resolution, and the convenience of using identical solutions for sample dilution and the mobile phase.

The method is rugged with a relatively large salt tolerance (up to at least 1000 ppm NaCl) and is accurate for both chromium species as demonstrated by spike recoveries for a wide variety of drinking water samples. Both short-term (one hour) and long-term (14 hours) stability have been demonstrated, ensuring collection of high-quality data over long run times.

References

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Consumables Used

Component	Part Number
Autosampler Vials, clear, polypropylene, 1.5 mL (package of 100 with caps)	N9301736
PEEK Tubing, 0.007" ID x 1/16" OD (5 feet)	N9302678
PEEK Solvent Filter, 10 µm	N8122249
Nebulizer Connector from UPLC	WE024372
Connector for Peristaltic Pump Tubing to PEEK Tubing	N8122258
Finger Tight Connector for 1/16" OD PEEK Tubing	09920513

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