



**Method for the quantitation of chromium (III) and (VI) in clean and effluent waters by ion-pair/chelation reversed phase HPLC-ICP-MS**

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**Analyst** John Entwisle  
**Our reference No.** AT20/13/1585-1612

Approved signatory

A handwritten signature in blue ink, appearing to read 'H Goenaga-Infante', is written over a horizontal line.

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## **Aim**

The objective of the study is to investigate the feasibility of a method based on ion pair/chelation reversed phase HPLC-ICP-MS for the reliable quantification of both Cr(VI) and Cr(III) species in water by external calibration. This study follows from a previous study - Chromium Speciation in LGC6177 Landfill Leachates Initial identification. <sup>1</sup> Unfortunately, BCR CRM-544 (lyophilised water) is no longer available therefore, a set of AQUACHECK proficiency testing scheme solutions (clean and waste waters) were analysed to evaluate the accuracy of the method. External calibration results were compared to those obtained using double spike IDMS calibration. An estimation of the limits of detection for the method was made and Cr (III) spike recovery experiments were also performed.

## **Method**

### **Chemicals and standards**

Ethylenediaminetetraacetic acid (EDTA, 99.995%), tetrapropyl ammonium bromide (TPABr, >99%), ammonium hydroxide solution (28-30% m/v) and chromium(III) chloride hexahydrate (98%) were obtained from Sigma-Aldrich (Poole, Dorset, UK). Potassium dichromate certified reference material (NIST SRM 136f), and chromium total solution (NIST SRM 3112a) were supplied by LGC Standards (Teddington, Middlesex, UK). Isotopically enriched <sup>53</sup>Cr and <sup>50</sup>Cr metal oxides were obtained from CK Gas Products Ltd (Hook, Hampshire, UK).

Preparation of diluent, double concentrated diluent and mobile phase :

The *diluent* (2.8 mM TPABr, 4 mM EDTA, pH 8.5) was prepared by dissolving TPABr (0.745 g) in 1 L deionised water, then adding EDTA (1.16 g) and sonicating the mixture. The pH was adjusted to 8.5 with aqueous ammonia solution. EDTA has a low solubility at pH less than 7 and therefore repeated sonication and pH adjustment was required to dissolve all the EDTA. The double concentrated *diluent x2* was prepared by doubling the quantity of TPABr and EDTA (1.49 g and 2.32 g respectively) and prepared as per the *diluent*. The *mobile phase* (0.18 mM TPABr, 1 mM EDTA, pH 8.5) is prepared in a similar manner as the *diluent* by dissolving TPABr (0.0478 g) and EDTA (0.29 g) in 1 L deionised water.

Preparation of natural Cr standard solutions :

Chromium (VI) and chromium (III) solutions with natural isotopic composition were prepared gravimetrically from potassium dichromate and chromium (III) chloride, respectively by dissolving  $\approx 0.1\text{g}$  in 150g of water in a plastic bottle. Aqueous potassium dichromate solutions have been found to be stable over 12 months or more. However, aqueous chromium chloride solutions are unstable. Therefore, dilutions from the stock aqueous chromium chloride solution were prepared immediately using *diluent* which stabilises Cr(III) in solution by chelation. Further dilutions of both species were prepared in *diluent*. Prior to injection, working standards were heated ( $85^\circ\text{C}$  for 60 minutes) in a similar manner to the samples to ensure entire complexation of Cr(III) with EDTA.

Preparation and characterisation of  $^{53}\text{Cr(VI)}$  and  $^{50}\text{Cr(III)}$  isotopically enriched solutions :

A stock solution of  $^{53}\text{Cr(VI)}$  was prepared by digesting an appropriate amount of  $^{53}\text{Cr}$  metal oxide and transforming it by oxidation to  $^{53}\text{Cr(VI)}$  using an oxidising agent. A stock solution of  $^{50}\text{Cr(III)}$  was prepared by gently heating an appropriate amount of  $^{50}\text{Cr}$  metal oxide with 6N hydrochloric acid. The concentrations of the isotopically enriched solutions were determined by ICP-OES analysis using single point calibration with NIST 3722a SRM. The isotopically enriched standard solutions were analysed for the possible presence of other Cr species using the chromatographic system coupled to ICP-MS. After characterisation, working standards of the two enriched solutions were gravimetrically prepared in *diluent* to provide a concentration of approximately 100 times the expected concentration of the species in the sample.

#### **Analysis of waste water round 443 by the double spike IDMS approach**

Procedure for the addition of isotopically enriched  $^{53}\text{Cr(IV)}$  and  $^{50}\text{Cr(III)}$  standards (Sample Blend preparation):

The sample solution was prepared as per the AQUACHECK scheme protocol and a 10g portion taken. 0.1g of the working spike standard solutions of  $^{53}\text{Cr(VI)}$  and  $^{50}\text{Cr(III)}$  were added gravimetrically. A portion of the solution was diluted 1:1 with *diluent x2* and heated ( $85^\circ\text{C}$  for 60 minutes). The solutions were analysed directly using the HPLC-ICP-MS system. Natural standards of Cr(VI) and Cr(III) were

injected periodically throughout the instrumental run for mass bias estimation correction.

The results were calculated according to a paper published by J. Meija, et al published in Journal of analytical atomic spectroscopy in 2006.<sup>3</sup> A Microsoft® Excel spreadsheet for the calculation is available as an appendix to J. Meija, et al paper.

### **Sample description and preparation**

On arrival, the AQUACHECK solutions were stored at 4°C and registered on the 4<sup>th</sup> June 2013 (internal reference AT20/13/1585-1612). Samples of rounds 443 and 441 clean water were supplied and 435, 439 and 443 waste water rounds. AQUACHECK is a long established UKAS accredited proficiency scheme run by LGC Standards, Proficiency Testing group.<sup>2</sup> Prior to analysis, the proficiency testing solutions were prepared according to the AQUACHECK protocol.<sup>2</sup>

Sample solutions were diluted 1:1 with double concentrated (*diluent x2*). The pH's of the solutions were measured to confirm they were in the range 8.0-9.0. No pH adjustment was needed. Prior to injection the samples were heated (85°C for 60 minutes) to ensure complete complexation of Cr(III) with EDTA. All dilutions were performed gravimetrically and with minimal delay to reduce species inter-conversion.

### **Instrumentation**

Ion-pairing reversed phase HPLC with online ICP-MS detection

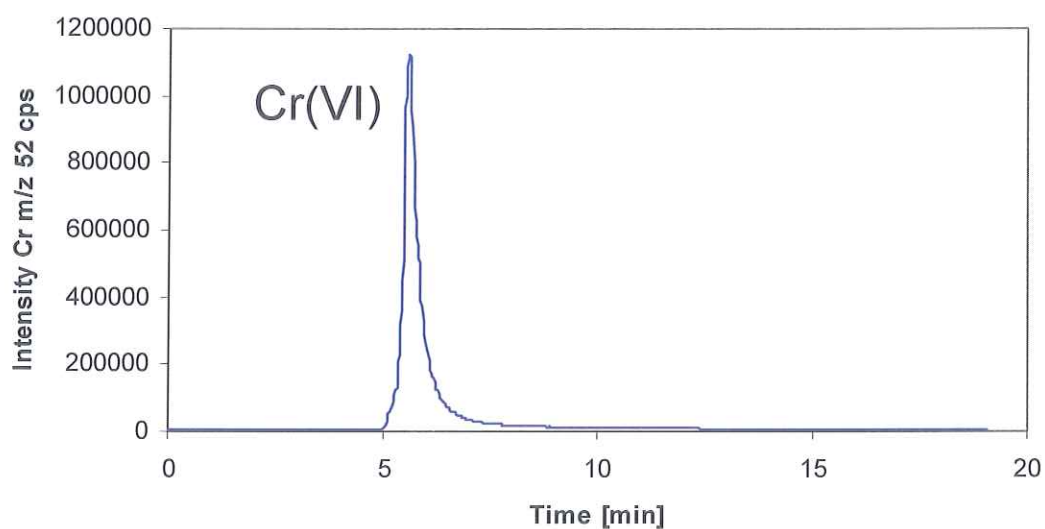
The chromatographic species separation was carried out isocratically using a 1200 Series HPLC (Agilent Technologies UK Ltd. Wokingham, Berkshire, UK). Ion pair/chelation reversed phase chromatography was performed using a PLRP-S 100Å in PEEK column (150 mm x 4.6 mm, 3 µm) (Agilent Technologies UK Ltd. Wokingham, Berkshire, UK). The column was kept at 40°C and an injection volume of 100µL was used with mobile phase flow rate of 0.8 mL min<sup>-1</sup>. The column eluent was introduced directly to an 8800 ICP-MS (Agilent Technologies UK Ltd. Wokingham, Berkshire, UK) via a microflow quartz concentric nebuliser coupled to a 2 °C cooled Scott type double pass spray chamber. The ICP-MS was operated in MS/MS mode with mixed ammonia 25% (mass flow controller flow) with 1ml/min helium reaction gas to minimise <sup>40</sup>Ar<sup>12</sup>C<sup>+</sup> interferences for element-specific detection of <sup>50</sup>Cr, <sup>52</sup>Cr and <sup>53</sup>Cr.

## Results and discussion

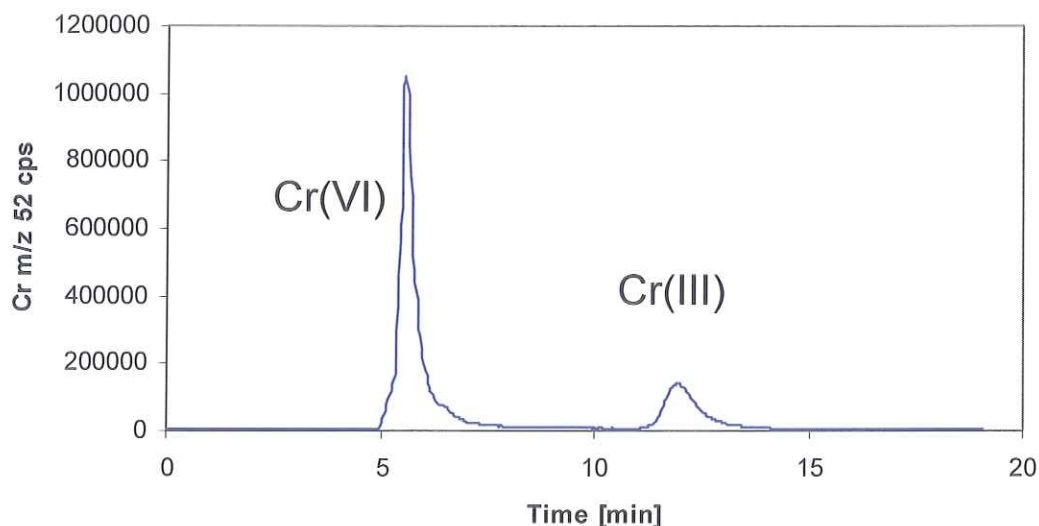
Cr concentration results obtained by monitoring the most abundant  $^{52}\text{Cr}$  isotope are presented in this report. The results obtained using the other isotopes compared well and are within the range of experimental error. The sample analysis throughput rate is 3 samples per hour. The results are compared to the assigned value, uncertainty and acceptable range, which are detailed in the participants AQUACKECK proficiency report for the individual rounds.

Figure 1 is a chromatogram of waste water round 435 and Figure 2 shows a typical chromatogram of waste water round 435 spiked with Cr(III). The figure demonstrates the separation of the species.

**Figure 1:** Chromatogram of waste water round 435. Cr (VI)  $85 \text{ ng g}^{-1}$  recovery %  $100.7 \pm 0.4$  ( $\pm 1\text{sd}$  from 3 replicated determinations)



**Figure 2:** Chromatogram of Cr(III) spiked waste water round 435. Cr (VI) 85 ng g<sup>-1</sup> recovery % 103.9±2.2, Cr (III) 25 ng g<sup>-1</sup> recovery % 98.8±3.3 (±1sd from 3 replicated determinations)



**Table 1: Limit of detection and limit of quantification**

	<sup>50</sup> Cr(IV)	<sup>52</sup> Cr(IV)	<sup>53</sup> Cr(IV)		<sup>50</sup> Cr(III)	<sup>52</sup> Cr(III)	<sup>53</sup> Cr(III)
Instrument LOD (ng g <sup>-1</sup> )	0.10	0.02	0.04		0.18	0.05	0.17
Method LOQ (µg L <sup>-1</sup> )	0.31	0.07	0.13		0.56	0.17	0.72

#### External calibration analysis

**Table 2:** Results AQUACHECK-clean water round 433 and 441 (mean ±1 standard deviations from 3 replicate determinations)

Identification Round	<sup>52</sup> Cr(VI) (µg L <sup>-1</sup> )	<sup>52</sup> Cr(III) (µg L <sup>-1</sup> )	Assigned Value, uncertainty and acceptable range Cr(VI) (µg L <sup>-1</sup> )	Recovery % Cr(VI)
433-1	4.46±0.01	<0.07	4.58±0.018 (3.58-5.58)	97.4±0.3
441-1	10.38±0.11	<0.07	10.37±0.045 (8.30-12.44)	100.1±1.1

The values are within the acceptable range and are in good agreement with the assigned values.

**Table 3:** Results AQUACHECK waste water round 435, 439 and 443 (mean  $\pm 1$  standard deviations from 3 replicate determinations)

Identification Round	$^{52}\text{Cr(VI)}$ ( $\mu\text{g L}^{-1}$ )	$^{52}\text{Cr(III)}$ ( $\mu\text{g L}^{-1}$ )	Assigned Value, uncertainty and acceptable range Cr(VI) ( $\mu\text{g L}^{-1}$ )	Recovery % Cr(VI)
435-1	85.58 $\pm$ 0.35	<0.07	85.0 $\pm$ 0.33 (68.0-102.0)	100.7 $\pm$ 0.4
439-1	116.16 $\pm$ 1.84	<0.07	112.3 $\pm$ 0.46 (89.8-134.8)	103.4 $\pm$ 1.6
443-1	78.29 $\pm$ 0.86	<0.07	76.5 $\pm$ 0.30 (61.2-91.8)	102.3 $\pm$ 1.1

The values are within the acceptable range and are in good agreement with the assigned values.

**Table 4:** Results for Cr (III) spiking experiment (25.5 ng g $^{-1}$ ) of AQUACHECK waste water round 435, 439 and 443 (mean  $\pm 1$  standard deviations from 3 replicate determinations)

Identification Round	Recovery % Cr(III)	$^{52}\text{Cr(VI)}$ ( $\mu\text{g L}^{-1}$ )	Assigned Value, uncertainty and acceptable range Cr(VI) ( $\mu\text{g L}^{-1}$ )	Recovery % Cr(VI)
435-spk	98.8 $\pm$ 3.3	88.2 $\pm$ 0.01	85.00 $\pm$ 0.33 (68.0-102.0)	103.9 $\pm$ 2.2
439-spk	100.1 $\pm$ 0.9	121.35 $\pm$ 1.2	112.30 $\pm$ 0.46 (89.8-134.8)	108.1 $\pm$ 1.6
443-spk	106.2 $\pm$ 0.1	85.08 $\pm$ 1.65	76.50 $\pm$ 0.30 (61.2-91.8)	111.2 $\pm$ 2.2

The recovery of Cr(III) is in good agreement with the expected level. The values for Cr(VI) are within the acceptable range and are in good agreement with the assigned values.

### Double spike species-specific IDMS analysis

**Table 5:** Results form double spike procedure AQUACHECK waste water 443 (mean  $\pm 1$  standard deviations from 3 replicate determinations)

Identification Round	$^{52}\text{Cr(VI)}$ ( $\mu\text{g L}^{-1}$ )*	$^{52}\text{Cr(III)}$ ( $\mu\text{g L}^{-1}$ )	Assigned Value, uncertainty and acceptable range Cr(VI) ( $\mu\text{g L}^{-1}$ )	Recovery % Cr(VI)
443-1	78.2 $\pm$ 0.4	<1.0	76.5 $\pm$ 0.30 (61.2-91.8)	102.3 $\pm$ 0.5

The values are within the acceptable range and are in good agreement with the assigned values.

**Table 6:** Results for Cr (III) spiking experiment (25.5 ng g $^{-1}$ ) of AQUACHECK waste water round 443 by double spike IDMS (mean  $\pm 1$  standard deviations from 3 replicate determinations)

Identification Round	Recovery % Cr(III)	$^{52}\text{Cr(VI)}$ ( $\mu\text{g L}^{-1}$ )	Assigned Value, uncertainty and acceptable range Cr(VI) ( $\mu\text{g L}^{-1}$ )	Recovery % Cr(VI)
443-spk-1	100.7 $\pm$ 0.8	79.9 $\pm$ 0.7	76.5 $\pm$ 0.30 (61.2-91.8)	104.5 $\pm$ 1.0

The recovery of Cr(III) is in good agreement with the expected level. The values for Cr(VI) are within the acceptable range and are in good agreement with the assigned values.

Cr concentration values obtained by external calibration and double spike IDMS are in good agreement. Round 443-1 (unit 1) contained 78.29 $\pm$ 0.86  $\mu\text{g L}^{-1}$  Cr(VI) (Table : 3) by external calibration and 79.7 $\pm$ 0.4  $\mu\text{g L}^{-1}$  (Table : 5) using the double spike IDMS approach.

### Conclusion

The feasibility of methodology for the quantitation of Cr(VI) and Cr(III) in clean and effluent water samples by external calibration with HPLC-ICP-MS has been demonstrated. The accuracy of the method was demonstrated by the analysis of proficiency testing scheme samples, spiking experiments and comparison to double spike species specific IDMS results.



Since SS-IDMS accounts for species inter-conversion, the fact that the external calibration results compared well with those obtained by SS-IDMS, makes routine external calibration procedure suitable for the reliable quantification of both Cr(VI) and Cr(III) species in clean and effluent water samples.

### **Suggestions**

The use of a metal free chromatography system may be advantageous as this would reduce the Cr background signal possibly enabling lower detection limits. As Cr species readily interconvert in the environment, the integrity of samples is a major concern. By immediately diluting samples taken in the field with *diluent x2* they can be stabilised during transit to the laboratory. Field blanks and quality control spikes and on-field isotope fortification would be advantageous for real water samples. This would provide a means of tracking and correcting for species inter-conversion during transit to the laboratory.

### **Acknowledgments**

The AQUACHECK solutions were kindly provided by Matthew Whetton and Helen Finch from LGC proficiency testing team.

### **References**

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