

Improved Method for the Simultaneous Estimation of Trivalent Chromium and Hexavalent Chromium by Ionchromatography

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Abstract: Most of the health problems in chromium toxicity are caused by hexavalent chromium (Cr VI) and to lesser extent by trivalent chromium (Cr III). Therefore, simultaneous monitoring of both Cr (III) and Cr (VI) in environmental and biological samples is important. In this study, we describe an improved, simple and sensitive ionchromatographic method for the simultaneous estimation of Cr (III) and Cr (VI) that employed superior chromatographic performance of analytical column and better elution buffer system than those used earlier (Dionex TN, 24, AU 165, www.dionex.com). Analytical conditions were: guard column (Ion Pac AG7, 4x50 mm), analytical column (Ion Pac AS7, 4x250 mm), eluent buffer (NH₄)₂SO₄/NH₄OH, pH 7.2, reagent flow rate (1.5mL/min), post-column reagent (2mM DPC) in 10% CH₃OH, 0.9N H₂SO₄, flow rate (0.5 mL/min), detector PDA, wavelength (520 nm), sample loop (500 µl). The present method is simple, highly sensitive and reproducible and elutes both species of Cr within 7 min; the retention times (RTs) for Cr (III) and Cr (VI) were 2.408 and 5.675 min, respectively. Elution pattern Cr (III) and Cr (VI) was crucial to flow rate, buffer molarity, pH, and pre-complexation of Cr (III) with EDTA. The lowest molar concentration of the eluting buffer (35mM) rather eluted Cr (VI) at 12.13 min while highest molarity of the buffer (250 mM) reduced retention time to 3.03 min. These buffer systems also eluted Cr (III) as well, however, desirable separation of both species was achieved with 75mM eluting buffer, pH 7.2. Correlation Coefficients (r²) for Cr (III) and Cr (VI) were 0.995 and 0.998 with RSD 0.84% and 1.85%, respectively. The minimum detection limit (MDL) of Cr (III) was considerably improved to 20 µg/L compared to earlier MDL (100 µg/L) and to 0.5 µg/L for Cr (VI) against the previous reported value 1 µg/L (Dionne TN 24). Recovery of Cr (III) and Cr (VI) was more than 95% in spiked samples.

Keywords: Ionchromatography, Trivalent chromium [Cr (III)], Hexavalent chromium [Cr (VI)]

1. INTRODUCTION

Although, inductive coupled plasma mass spectrometry (ICP MS) coupled with high performance liquid chromatography (HPLC) is the current choice of estimation of chromium

species (trivalent chromium, Cr (III) and hexavalent chromium, Cr (VI) at ultra low level but this technique is cost-effective, and therefore cannot be routinely used by many laboratories. Ionchromatography (IC), a relatively less expensive technique is also a powerful tool for the estimation of ions when the very low detection limits of analytes are of less concern. Thus analysis of environmental samples like air, water and soil and effluents from factories and common effluents treatment plants (CETP) containing high levels of chromium can be conveniently done by ionchromatography. Dionex Company Pvt. Ltd. has a monography in ionchromatography and it described earlier a method for the simultaneous estimation of Cr (III) and Cr (VI) (Dionex TN 24, 1991). Later, this method was updated by the company (Dionex AU 144, 179). Though, these methods are highly sensitive for Cr (VI), it shows low sensitivity for Cr (III). Ion chromatographic method (Dionne (TN 24, 1991) described detection limit for Cr (III) and Cr (VI) 100 ppb and 1 ppb, respectively. Separate ionchromatographic method for the estimation of Cr (III) is still lacking.

It is clear from the literature that the sensitivity of the method and the resolution of peaks were much dependent on a number of factors including selection of analytical column, molarity of the buffer, flow rate, volume of the sample injected and the nature of eluting buffer. This was evidenced by the recent method (US EPA 2011) that determines Cr (VI) in drinking water at the proposed level of Public Health Goal (PHG) (0.02µg/L).

In this study we present a simple and improved ion chromatographic method for the simultaneous estimation of Cr (III) and Cr (VI) using Ion Pac AS7 analytical column (4x250 mm) and ammonium sulphate/ ammonium hydroxide (NH₄)₂SO₄/NH₄OH)-based eluting buffer system with some

modifications in experimental conditions than those used earlier by Dionex Company (Dionex TN 24, 1991, Dionex AU 165).

2. MATERIALS AND METHODS

All the chemicals used in the study were of high analytical purity grade and free of chromium. Standard solutions Cr (III) (1g/L) and Cr (VI) (999 mg/L \pm 4mg/L) were obtained from Fluke, NIST SRM (Switzerland). Deionized water (DI), type I reagent grade, 18 M Ω -cm resistivity was used throughout the studies. Prior to estimation, Cr (III) was complexed with EDTA solution (0.25 mM) by gently heating it at 60 $^{\circ}$ C for 10 min. Cr (VI) was prepared in DI water. Eluent stock buffer 250 mM $(\text{NH}_4)_2\text{SO}_4/\text{NH}_4\text{OH}$ was prepared and diluted suitably for further use. On-line post-column derivatization was done with 1, 5- diphenylcarbazine (DPC) with the help of PC-10 Pneumatic Controller equipped with instrument (Dionex IC- 2500). The chromatography was performed with PDA detector and Chromeleon 6.8 Chromatography Management Software system. Method described by Dionex (AU 165) was followed with some modifications. Conditions were: guard column (Ion Pac AG7, 4x50 mm), analytical column (Ion Pac AS7, 4x250 mm), eluent buffer $(\text{NH}_4)_2\text{SO}_4/\text{NH}_4\text{OH}$, pH 7.2, reagent flow rate (1.5 mL/min), post column DPC flow rate (0.5mL/min), sample loop (500 μ L). All samples were passed through acrodisc[®] syringe (0.45 μ m) filter and applied on to ionchromatograph.

3. RESULTS

Both species of Cr were efficiently separated by ion chromatography (Fig. 1).

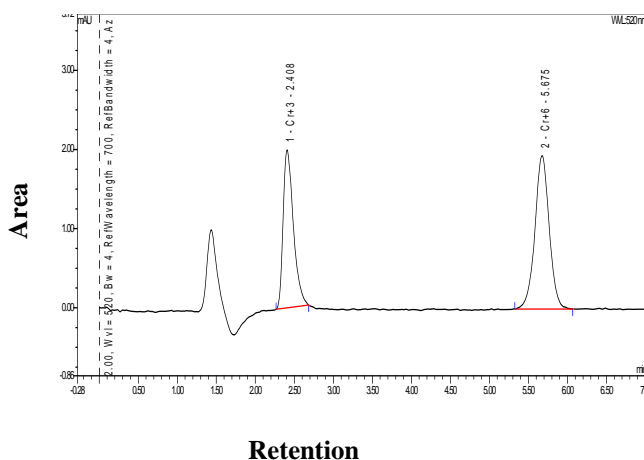


Fig. 1: Chromatogram of standard Cr (III) (500ppb) and Cr (VI) (4ppb)

Retention times (RTs) for Cr (III) and Cr (VI) were determined to be 2.408 and 5.675 min, respectively when 75mM of the eluting buffer was used. Cr (III) is eluted as Cr (III)-EDTA complex while Cr (VI) as chromate (CrO_4)²⁻. Calibration curve

of Cr (III) at a concentration of 200, 300, 400, 500 and 600 ppb ($\mu\text{g/L}$) and Cr (VI) using 1, 2, 3, 4 and 5 ppb was linear (Figs. 2 & 3). It yielded correlation coefficient (r^2) 0.995 and 0.998 for Cr (III) and Cr (VI), respectively with RSD 0.84% and 1.85%.

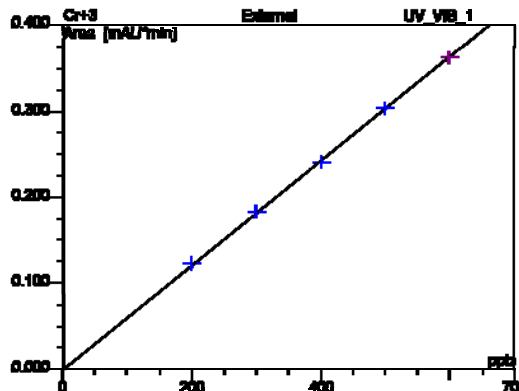


Fig. 2 Calibration curve of Cr (III)

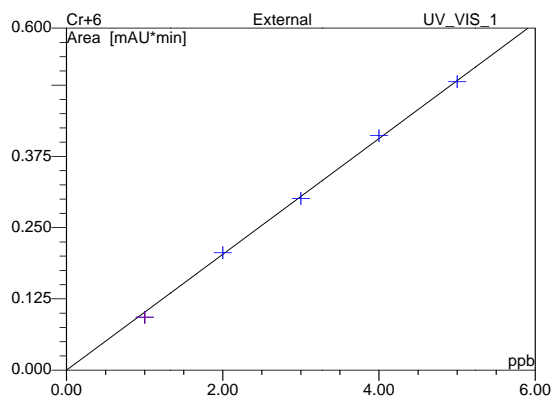


Fig. 3 Calibration curve of Cr (VI)

Elution buffer of various concentrations ranging from 35 to 250 mM were tried to elute both Cr (III) and Cr (VI) to get good resolution with suitable time within in a single run.

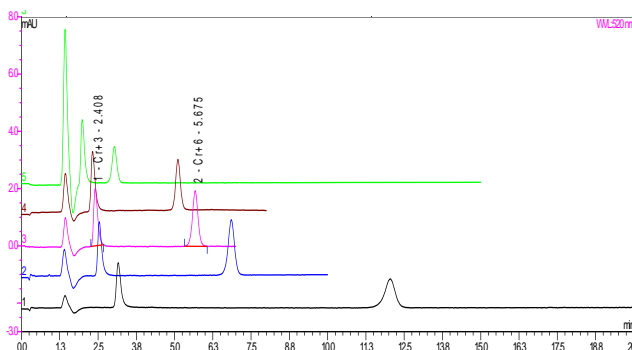


Fig. 4: Effects of molar concentrations of buffer on the elution profile of Cr (III) (500 ppb) and Cr

(VI) (4ppb) (Chromatograms 1, 2,3,4,5 eluted with 35, 65, 75,100, 250 mM of eluent buffer

Elution profile of Cr (III) and Cr (VI) as a function of buffer molarity is shown in Fig. 4.

As depicted in Fig. 4, elution pattern of Cr (III) and Cr (VI) with respect to retention time and resolution of peak was much dependent of the molar concentration of the buffer used, flow rate of the column and the pre-complexation of Cr (III) with EDTA. As shown in Fig 4, Cr (III) was eluted first as Cr (III)-EDTA-complex followed by Cr (VI) that required post column derivatization with DPC. RT of Cr (III) was changed narrowly as compared to Cr (VI) (Table 1).

Table 1: Chromatographic data of Fig. 4 showing retention time, height and area of Cr (III) and Cr (VI)

Sr. No	Elution buffer (NH4)2SO4/NH4 OH	Cr (III) (500 ppb)			Cr (VI) (4 ppb)		
		R.T	Height	Area	R.T.	Height	Area
	Molarity (mM)						
1.	35	3.17	1.564	0.274	12.13	0.937	0.379
2.	65	2.45	1.887	0.294	6.85	1.893	0.446
3.	75	2.40	1.997	0.303	5.67	1.939	0.411
4.	100	2.32	2.055	0.307	5.10	1.761	0.318
5.	250	1.98	2.236	0.323	3.03	1.270	0.231

Chromatogram 3 of Fig. 4, eluted with 75 mM eluent buffer, pH 7.2 shows that both species of Cr [Cr (III) and Cr (VI)] were suitably separated within 7 min in a single run. The lowest molarity of the eluting buffer (35 mM) eluted Cr (VI) at 12.13 min (Fig.4, chromatogram 1) while highest molarity of the buffer (250 mM) reduced retention time to 3.03 min (Fig.4, chromatogram 5). Suitable separation of both species of Cr was obtained at 75 mM concentration of eluting buffer showing reagent economy of the method used.

In our present study, minimum detection limits (MDLs) of Cr (III) and Cr (VI) were below to 20 ppb and 0.5 ppb, respectively. It was greatly improved and almost five times lower for Cr (III) when compared to the earlier MDL for Cr (III) as reported earlier by Dionex (TN 24, 1991). The sensitivity and validity of the present method was confirmed by analyzing Cr (III) and Cr (VI) in water un-spiked and spiked samples (Table 2 & 3).

Table 2 shows the determination of Cr (VI) and Cr (III) in tap water (background level) and spiked samples. Cr (VI) was spiked at two levels (10 and 30 ppb). More than 96% recovery was obtained. Cr (III) was not detected in tap water, therefore, it was fortified with external standard, Cr (III) and the

recovery was in the range from 94-102 %. Table 3 shows the simultaneous determination of Cr (III) and Cr (VI) in mixed-spiked samples in aqueous medium. Ultra pure water (DI) having no background level of Cr (III) and Cr (VI) were used and fortified with external standards of Cr (III) and Cr (VI). Ionchromatographic analysis of these samples is summarized in Table 3. Analysis of Cr (III) and Cr (VI) in mixed spiked samples appears satisfactory. The recovery of Cr (III) and Cr (VI) was in the range 94.6 -102.8% and 90-109%, respectively.

Table 2: Determination of Cr (III) and Cr (VI) in un-mixed tap and spiked water samples

Sr. No.	Sample (Water)	Cr (VI) (µg/L or ppb)			Cr (III) (ppb)		
		Spiked (ppb)	Amount of Cr (VI) obtained (ppb)	Recovery (%)	Spiked (ppb)	Amount of Cr (III) Obtained (ppb)	Recovery (%)
1.	Blank	-	Not detected	-	-	Not detected	-
2.	Background level	-	8.18	-	-	Not detected	94.8
3.	Spiked	10	17.58	96.69	110* (fortified)	109.35	99.4
4.	Spiked	30	37.04	97.01	230* (fortified)	236.2	102.6
5.	Standard Cr (VI) (25 ppb).	-	24.08	96.32	Standard Cr (III) (100 ppb)	96.54	96.5

(Samples were run in duplicate and the values represent the mean)

Table 3: Simultaneous determinations of Cr (III) and Cr (VI) in mixed-spiked samples

Parameters	Cr (III) (ppb)				Cr (VI) (ppb)			
	Expected	Obtained	Recovery (%)		Expected	Obtained	Recovery (%)	
Background (fortified)	200*	200	205.6	102.8	1	0.91	91	

Spiked Level 1	500	700	697.0	99.5	2	3	3.27	109
Level 2	150 0	1700	1609. 3	94.6	9	10	9.0	90

*Ultra pure water sample; * Samples were fortified with known concentrations of external standards

4. DISCUSSION

Ion chromatographic method reported in this study does not involve expensive instrument like ICP MS and it may be useful to analyse chromium species where very low detection limit of chromium is not an issue, for example, samples from tannery waste or sewage treatment plant. The Ion Pac AS7 column used in this study is a high capacity, high-efficiency hydrophobic anion-exchange column. It contains both anionic and cationic ion-exchange capacity. Superior chromatographic performance of AS7 column (Dionex data sheet, www.dionex.com) over the CS5 or CS5A (Thermo scientific 2002) is attributed to its unique polymer packing that may allow it for better analysis for a variety of anions including polyvalent anions even in high-ionic-strength matrices with great accuracy.

In our present method, the detection limit for Cr (III) and Cr (VI) was improved considerably. Larger sample loop (500 μ L) used in this study enabled us to improve the detection limit but at the same time it had not resulted in overloading of the column. Higher flow rate used in this study (1.5mL) prevented the broadening of the peak and it may be assigned to the high capacity of AS7 column (100 μ eq per column). The linearity of the calibration was evidenced by the correlation coefficient (r^2) value of each calibration curve of Cr (III) and Cr (VI) (Figs. 2 & 3). In ionchromatography, estimation of Cr (III) and Cr (VI) were reported to be highly pH-dependant (Lopez-Ruiz, 2000; Dionex TN 24 1991). These authors reported that alkaline pH favors Cr (VI) estimation while acidic pH promotes Cr (III) estimation. In our studies, a pH 7.2 that is near to neutral, estimation of both species of Cr [Cr (III) and Cr (VI)] was found suitable for the quantitative estimation of

each species (Table 2 and 3). In this study it was noted that molarity of the eluting buffer (75 mM) and pH (7.2) are crucial to elute both chromium species within 7 min of run (Fig. 4). Pre-complexation of Cr (III) with EDTA (0.25 mM) is essential and it is completed within 10 min at 60 $^{\circ}$ C. This incubation period of 10 min reduced the time to form complex Cr (III) with EDTA and it has significantly reduced the pre-complexation time of Cr (III) with EDTA over the previous reported method which stated 20 min at 80 $^{\circ}$ C (Choppala et al 2010). The improved method described in this report is simple, quick, highly sensitive and reproducible. It also shows reagent economy as both species of chromium (Cr III and Cr VI) are effectively eluted with relatively lower molarity of the eluting buffer (75 mM) against the early conventionally used concentration of eluting buffer (250 mM ammonium sulphate/ ammonium hydroxide buffer) used for the estimation of Cr (VI).

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