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ORIGINAL PAPER

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Determination of Cr^{3+}, CrO_4^{2-}, and $Cr_2O_7^{2-}$ in environmental matrixes by high-performance liquid chromatography with diode-array detection (HPLC–DAD)

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Abstract A high-performance liquid chromatographic method with diode array detection (HPLC–DAD), based on chelation with ammonium pyrrolidinedithiocarbamate (APDC), has been developed for the determination of chromium species. Determination of Cr^{3+}, CrO_4^{2-}, and $Cr_2O_7^{2-}$ was performed for standards and synthetic environmental matrixes. This method is robust, rugged, and can be used for rapid routine determination of chromium species with high precision and reliability. Sample pretreatment is simple. The method is capable of discriminating not only between Cr(III) and Cr(VI) but also between the chemical forms of Cr(VI) – CrO_4^{2-} and $Cr_2O_7^{2-}$. By analysis of numerous samples the method has been shown to be selective, sensitive, and free from matrix interference, which is crucial for the determination of chromium species in difficult-to-analyze environmental matrixes. This method has been validated by means of an interlaboratory study. Although different speciation techniques were used during this study, there was good agreement between results from the two laboratories. The method detection limits were 7 and 4 mg L^{-1} for Cr^{3+} and $Cr_2O_7^{2-}$, respectively. Recoveries of the analytes from spiked samples were 98% and 100% for Cr^{3+} and $Cr_2O_7^{2-}$, respectively. Both were based on a 10-mL sample volume spiked with 0.4 mg L^{-1} chromium.

Keywords Chromium · Chelation · HPLC–DAD · Speciation · Environmental analysis

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Introduction

Chromium (Cr) occurs in several chemical forms with oxidation numbers ranging from zero (free metal) to six (chromate and dichromate). Only the trivalent (III) and hexavalent (VI) forms, however, are sufficiently stable to be found in the environment [1]. Because the physicochemical properties of Cr(III) and Cr(VI), e.g. their mobilities in the environment, and chemical and biochemical reactivity towards other chemical species, are very different, detailed information on each species, rather than total chromium, is crucial for evaluation of toxicological effects and for monitoring the performance of chromium remediation technologies [2, 3, 4]. The toxicity and environmental availability of the Cr species are also different [1].

A wide range of techniques has been established for quantification of chromium species, including atomic absorption spectrometry [5, 6], inductively coupled plasma–atomic emission spectrometry (ICP–AES) [7, 8], and liquid chromatography [9, 10, 11, 12, 13]. Liquid chromatography is currently regarded as one of the main analytical techniques for the determination of chromium species in environmental matrixes [14, 15, 16]; it can be easily interfaced with a variety of detection systems to enhance recoveries and detection limits. Despite this, there remain difficulties in determining traces of chromium species in environmental samples [1, 17]. The objectives of this work were to develop a routine liquid chromatographic method for routine speciation of Cr^{3+}, CrO_4^{2-}, and $Cr_2O_7^{2-}$, to validate the test method by means of an interlaboratory study, and to use it as a tool for evaluating a chromium remediation technology designed for treatment of Cr(VI)-contaminated soils, and to demonstrate its usefulness for the determination of chromium species in complex environmental matrixes.

Experimental

Reagents

All organic solvents were of highest quality available on the market. Methanol and acetonitrile were HPLC-grade from Caledon

Laboratories (Georgetown, Ontario, Canada) and were used without purification. Dichloromethane was distilled in glass, also from Caledon, used without purification. Deionized water was from a local water purification system (Millipore, Bedford, MA, USA). Chromium species and other metal salts were from Sigma–Aldrich (Oakville, Ontario, Canada), and were also of the highest quality. 1-Pyrrolidinecarbodithioic acid, ammonium salt (APDC) was from Sigma–Aldrich and was of high quality. Potassium chromate, potassium dichromate, and Cr(III) nitrate were from Sigma–Aldrich.

APDC reagent was prepared by dissolving 0.2 g in 100 mL ultra-pure water. Stock solutions of potassium chromate, potassium dichromate, and Cr(III) chloride were prepared by weighing appropriate amounts into small beakers, dissolution in water, quantitative transfer into 1000-mL volumetric flasks, then dilution to volume with additional ultra-pure water.

Preparation of interlaboratory samples

Two types of sample were used in the interlaboratory study. The first set of samples was a mixture of standard solutions containing Cr(VI), as dichromate, and Cr(III), as chloride, in 1% HCl, as preservative. The first set of solutions were prepared by mixing appropriate amounts of chromium atomic absorption standard solutions such that the final mixture contained different percentages of Cr(III) and Cr(VI). The second set of samples consisted of chromium-contaminated water, soil leachate, and solid samples, which were taken directly from a process stream. Both types of sample were split into two; one half was analyzed at this laboratory and the other half was sent to another accredited laboratory within the region.

Instrumentation

The system used for liquid chromatography comprised a solvent-delivery system, model 9012, an autosampler, model 9100, a diode-array detector, model 9065 Polychrom, and chromatography software, version 4.5, all from Varian Canada (Mississauga, Ontario, Canada). The software used has advanced application features that enable data acquisition, viewing, manipulation, and reporting. Separations were performed on a 250 mm×4.6 mm, 5-μm particle, C_{18} reversed phase carbamate column from Alltech Associates (Deerfield, IL, USA). The chromatographic conditions are given in Table 1.

Analysis of samples

Because the soil samples were soils spiked with Cr(VI) that were used in an experimental remediation process, aimed at cleaning chromium-contaminated soil, there was no need for digestion of the samples. Both soil and water samples were analyzed twice, once without addition of acid and the second time with acid, to differentiate between the chromate and dichromate in the matrix. The methods used for sample pretreatment and chelation with APDC were similar to those described elsewhere [9, 11]. Briefly, the soil sample (5 g) was weighed into a 15-mL test tube and APDC (0.2%, w/v; 1 mL) was added. After shaking vigorously the mixture was left to stand for 20 min, then extracted with dichloromethane (5 mL). The extract was then injected into HPLC–DAD for determination of dichromate. These steps were repeated, this time adding 10 μL conc. HCl to the soil sample before adding the APDC reagent, to ensure chromate was converted to dichromate. The extract was then injected into the HPLC for determination of total Cr(VI) originating from both chromate and dichromate. The difference between these two measurements was the concentration of the chromate. This procedure was repeated for analysis of water samples (10 mL). Because Cr(III) arises as a byproduct of the reaction between APDC and dichromate, and from the sample, the value had to be corrected for Cr(III) background before the final results were reported [9].

Table 1 Chromatographic conditions[a]

Time (min)	Amount of water (%)	Amount of methanol (%)
0	50	50
25	30	75
30	0	100

[a]Column: Alltech Carbamate 5 μm, length 250 mm, i.d. 4.6 mm; detection wavelength 254 nm; injection volume 10 μL

Results and discussion

Chelation and separation of the resulting Cr-APDC complexes

The chromium species, including Cr^{3+}, CrO_4^{2-}, and $Cr_2O_7^{2-}$, were reacted with 1-pyrrolidinecarbodithioic acid, ammonium salt (APDC) separately without pH or buffering adjustments and determined by high-performance liquid chromatography with diode-array detection (HPLC–DAD). Figure 1 shows the chromatograms obtained from the three chromium species. It is apparent there is good separation of Cr^{3+} and $Cr_2O_7^{2-}$; the HPLC–DAD did not respond to CrO_4^{2-} under these conditions. The solution of Cr^{3+}, except for the two chelant peaks APDC-Na (salt form) and APDC-H (acidic form), gave a single peak, which can be attributed to formation of the APDC-Cr(III) complex: tris[pyrrolidine-1-dithioato-S,S′]Cr(III) [9, 11]. The solution of $Cr_2O_7^{2-}$ yielded two peaks, one before the chelant peak (APDC-H) the second after it. The latter perfectly matched that of the Cr^{3+}signal. The two peaks of $Cr_2O_7^{2-}$ can be explained in terms of APDC-Cr(VI) complexes with different physicochemical properties. There is evidence in the literature [9, 11] that APDC reacts with $Cr_2O_7^{2-}$ to form two different species, one the main product (bis[pyrrolidine-1-dithioato-S,S′]Cr(III)) and the other a byproduct with a chemical structure and with physicochemical properties similar to those of the APDC-Cr(III) complex. When $Cr_2O_7^{2-}$ is present in the sample one must, therefore, take into account the Cr(III) background before reporting the Cr(III) result. It is of interest that the HPLC–DAD did not respond to CrO_4^{2-} under these experimental conditions, which meant that CrO_4^{2-} did not react with the chelant, or simply reacted but did not elute from the column. However, when the CrO_4^{2-} solution was acidified with HCl and re-analyzed with HPLC–DAD the CrO_4^{2-} was successfully detected in the sample.

This characteristic property of CrO_4^{2-} has been explored further to enable differentiation between the two chemical forms of hexavalent chromium. All that is necessary to obtain the concentrations of both chemical forms of Cr(VI) is to split the sample into halves and analyze one half under acidic conditions and the other in a non-acidic medium. Figure 2 shows the chromatograms obtained

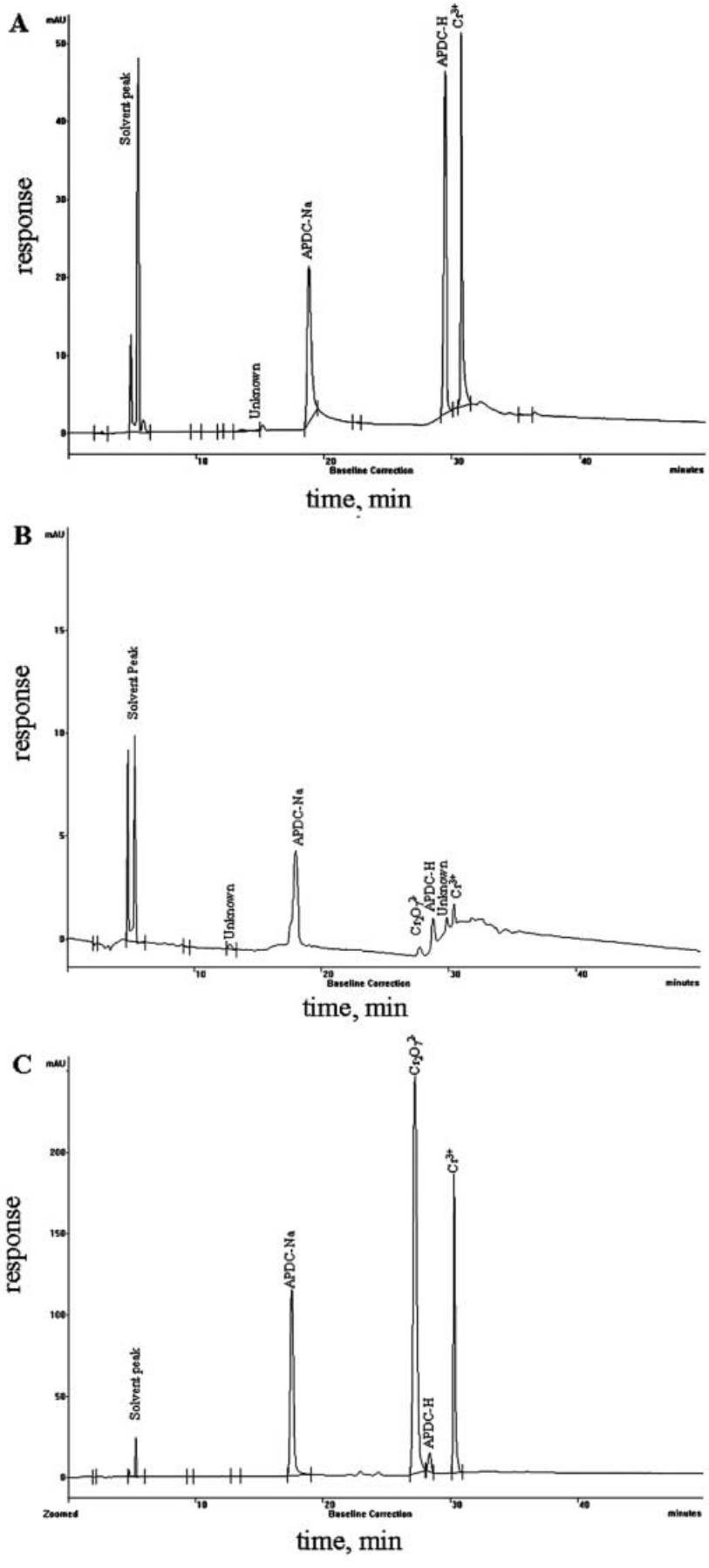

Fig. 1 Separation of Cr^{3+}, CrO_4^{2-}, and $Cr_2O_7^{2-}$ with methanol–water as mobile phase on the carbamate column; 10 μL of each species (20 mg L^{-1} Cr) was analyzed by HPLC–DAD. (**A**) Cr^{3+}, (**B**) CrO_4^{2-}, and (**C**) $Cr_2O_7^{2-}$

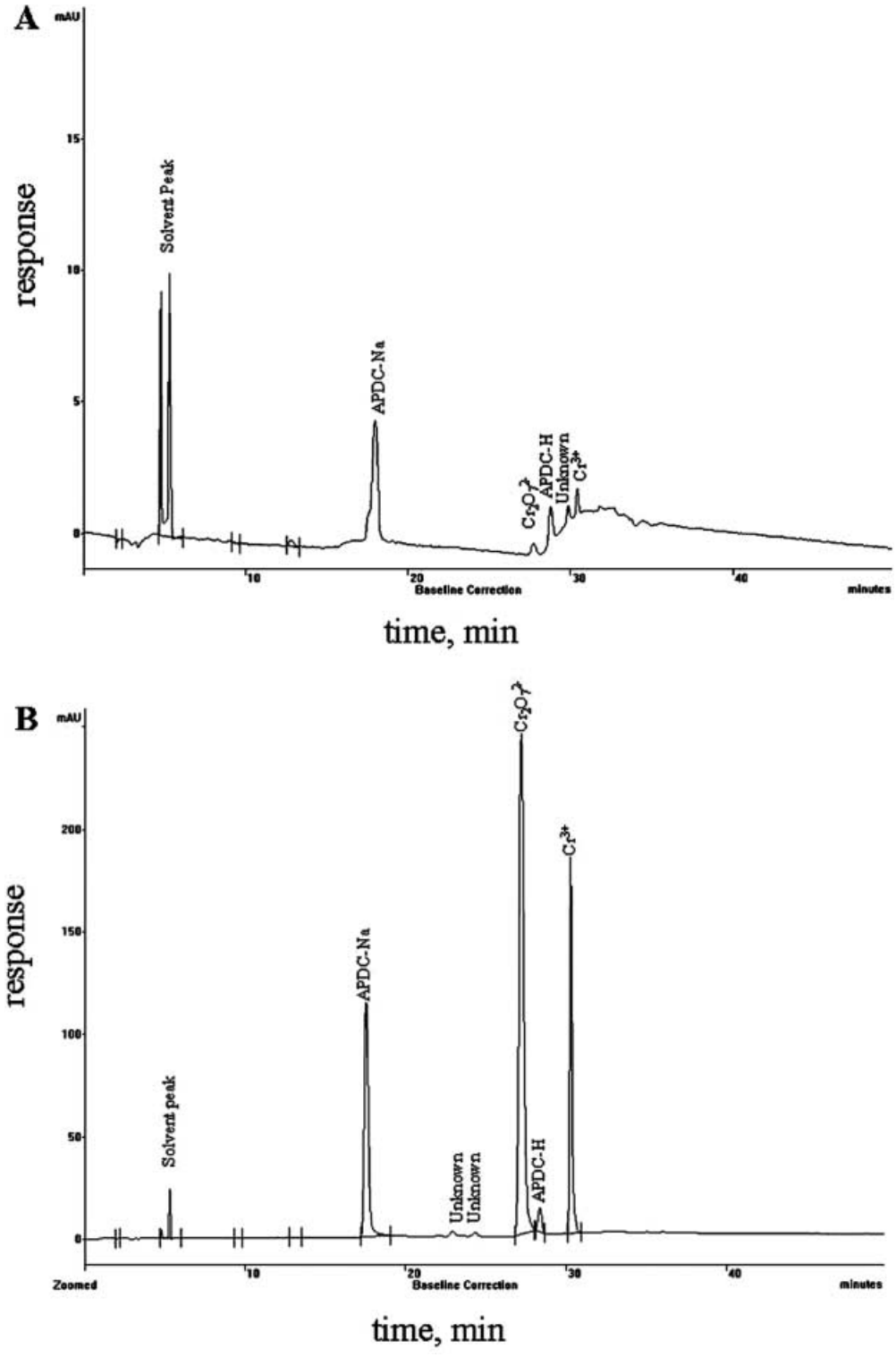

Fig. 2 Chromatographic separation of CrO_4^{2-} and $Cr_2O_7^{2-}$ after analysis (**A**) without acid and (**B**) with acid. The chromatographic conditions were same as those used for Fig. 1.

when a sample of CrO_4^{2-} is split and analyzed under these two different conditions. Chromatogram (A) was from a sample of CrO_4^{2-} analyzed without addition of HCl whereas chromatogram (B) was from the CrO_4^{2-} sample analyzed after addition of 10 μL conc. HCl. These experimental observations suggest that the chromium can be determined not only as Cr(VI) and Cr(III) but as the chemical forms CrO_4^{2-} and $Cr_2O_7^{2-}$and Cr^{3+}. There is also some evidence that the method is sensitive to the ionic form of Cr(III) but the result could not be confirmed.

Calibration and analysis of Cr^{3+} and CrO_4^{2-} and $Cr_2O_7^{2-}$

To establish the linearity of the calibration curve constructed by use of the HPLC–DAD results, a series of the standard solutions was prepared for each chromium species and analyzed under the optimum chromatographic conditions. The instrumental response (area under the curve) was plotted against the concentration of chromium species and found to be linear from 0.01 mg L^{-1} to 40 mg L^{-1} for Cr^{3+} and from 0.01 mg L^{-1} to 20 mg L^{-1} for $Cr_2O_7^{2-}$.

When determining Cr(III) as tris[pyrrolidine-1-dithioato-S,S′]Cr(III), the contribution to the Cr(III) response from the Cr(VI) chelation byproduct, which is also tris[pyrrolidine-1-dithioato-S,S′]Cr(III), must be corrected. This can be accomplished by determining the concentration ratio, r, of Cr(III) to Cr(VI) in the standard solution of Cr(VI) as follows:

$$r = \frac{Cr(III)_b}{Cr(III)_m} \quad (1)$$

where r is the concentration ratio, $Cr(III)_b$ (mg L^{-1}) is the contribution from Cr(III) in the standard solution of Cr(VI), and $Cr(III)_m$ (mg L^{-1}) is the concentration of the main product of the Cr(VI) reaction.

When both Cr(III) and Cr(VI) are present in the sample before chelation by treatment with APDC the first chromium peak detected by HPLC–DAD is that of the main product of reaction of Cr(VI) with APDC, bis[pyrrolidine-1-dithioato-S,S′]Cr(III), for short $Cr(III)_m$, which is well separated from Cr(III) and hence is used to quantify $Cr_2O_7^{2-}$. The second peak in the chromatogram, $Cr(III)_o$, corresponds to the sum of the background concentration arising from the APDC-Cr(VI) chelation byproduct, $Cr(III)_b$, and that of the Cr(III) originally present in the sample, $Cr(III)_s$. Hence, the value of $Cr(III)_s$ is given by the equation:

$$Cr(III)_S = Cr(III)_o - Cr(III)_b \quad (2)$$

where $Cr(III)_o$ is the sum of the background concentration arising from both $Cr_2O_7^{2-}$ and Cr(III) in the sample. Combination of Eqs. (1) and (2) gives:

$$Cr(III)_S = Cr(III)_o - r \times Cr(III)_m \quad (3)$$

To determine the value of r replicate measurements of $Cr_2O_7^{2-}$ samples were made at different levels of concentration. The value of r was found to be 0.30±0.05, over the entire dynamic range of the calibration plot under these chromatographic conditions.

Method detection limit

The method detection limit, which depends on many factors (reproducibility, confidence level, volume of sample extract, size of the sample, and recovery of the analyte from the sample matrix) can be expressed by the equation:

$$MDL = \frac{s \times t \times V_{extract}}{V_{sample} \times \%R} \quad (4)$$

where MDL (mg L^{-1}) is the method detection limit, s (mg L^{-1}) is the standard deviation of replicate measurements, t is a factor which depends on the number of measurements and the confidence level, $V_{extract}$ (mL) is the volume of sample extract, V_{sample} (mL or g) is the amount of sample, and %R is the percentage of recovery of analyte from the sample.

The method detection limit, MDL, was determined by analyzing five water samples spiked with Cr^{3+} and $Cr_2O_7^{2-}$ at 0.4 mg L^{-1} chromium and calculating the standard deviation and the percentage recoveries of the target analytes. Table 2 presents the results of these calculations; they were based on a 10-mL sample and a 1-mL sample extract. The MDL values were 0.0070 mg L^{-1} for Cr^{3+} and 0.0040 mg L^{-1} for $Cr_2O_7^{2-}$; these can be improved by preconcentrating a larger volume of sample before injection into the HPLC–DAD. The latter requires a very small sample, in this work only 10 μL. The reproducibility of these measurements ranged from ±0.0264 to ±0.0168 for Cr^{3+} and $Cr_2O_7^{2-}$, respectively, at the 0.4 mg L^{-1} level. The recoveries were 98%±7% and 100%±4% for Cr^{3+} and $Cr_2O_7^{2-}$, respectively. The estimated time for analysis of ten samples, excluding method quality-control samples but including the final report of the analysis, was approximately 2 h.

At the onset of the hexavalent chromium process it was decided that a validated chromium speciation test method would be beneficial for evaluating and interpreting process performance. A rapid, robust, and accurate speciation method was therefore required for determination of Cr^{3+}, CrO_4^{2-}, and $Cr_2O_7^2$ in process streams which might include water, soil leachate, and solid waste. Full descriptions of the process can be obtained on request from Environment Canada and SAIC Canada [18].

The process streams, including the method control samples (blank and standard solutions), and the chromium-contaminated soil leachates, were analyzed by this method. Each sample was analyzed twice, with and without addition of HCl, so that CrO_4^{2-} and $Cr_2O_7^2$ could be differentiated from one another. The total amount of chromium in the sample was determined at this laboratory by flame atomic absorption spectrometry (FAAS). The speciation test method was fully automated and used for intensive routine and non-routine determination of chromium

Table 2 Method detection limits for chromium species[b]

Species	Concentration (μg g^{-1})		Recovery (%)	MDL (mg L^{-1})
	Actual	Measured		
Cr^{3+}	0.4	0.3935±0.0264	98±7	0.0070±0.0005
$Cr_2O_7^{2-}$	0.4	0.3992±0.0168	100±4	0.0040±0.0002

[b]The sample extract volume was 1 mL, the volume of sample was 10 mL, and the number of replicate measurements was 5. Values are means±standard deviations

species in process samples and environmental matrixes. The method has been applied to soil spiked with Cr(VI). Because the chromium species in the spiked soil were water soluble, the sample preparation steps were minimal, which enabled rapid report generation and considerably aided assessment of the overall treatment process. The throughput of the HPLC–DAD system was found to be satisfactory, because of the high separating power of the column and the ability of the DAD to detect the analytes without significant interference.

Interlaboratory study

Although the technique is not very different from those reported in the literature [9, 11, 13], the proposed speciation methodology is, unlike others, capable of discriminating between CrO_4^{2-} and $Cr_2O_7^{2-}$. The method can also discriminate between complexed and free Cr^{3+} ions, as indicated by the lack of complete agreement between the total chromium determined using FAAS or ICP–AES and that obtained by use of HPLC–DAD, except for the standard samples. The initial intention was to use the method to evaluate the effectiveness of a chromium remediation technology, which required an accurate, reliable, and validated method. One way to ensure the test method is accurate and valid for testing a particular sample matrix is to analyze certified reference materials [14]. Another way of validating the method is to analyze conduct an interlaboratory study in which two different laboratories using their own methods split samples.

In this work the test method was validated by means of an interlaboratory study. The interlaboratory study samples were split in half and one half was analyzed at this laboratory for Cr^{3+}, CrO_4^{2-}, and $Cr_2O_7^{2-}$ whereas the other half was sent to another laboratory (Accutest Laboratories, Nepean, Ontario, Canada) for analysis of hexavalent and total chromium. The results revealed excellent agreement between the two laboratories on the level of Cr(VI) in the samples. When the total chromium concentration, Cr(tot), in soil leachates and solid matrixes was determined, however, results from the two laboratories deviated substantially. Accutest values, obtained by use of ICP–AES, were consistently higher than values obtained by use of HPLC–DAD. The discrepancy between the HPLC–DAD and ICP–AES measurements might be explained in terms of the techniques used to determine total chromium. Because ICP–AES detects total chromium, irrespective of the chemical form, whereas HPLC–DAD separates and detects only Cr^{3+}, CrO_4^{2-}, and $Cr_2O_7^{2-}$, other chromium species might have escaped detection by HPLC–DAD but not by ICP–AES. It is conceivable that the polymeric form of $Cr(OH)_n^{(3-n)+}$ and organochromium chemicals do not react with APDC, or react but do not absorb at 254 nm, or, for whatever reason, are not separated on the chromatographic column under these conditions, thereby resulting in low values for total chromium by HPLC–DAD.

Another experimental observation that might be relevant to this argument is that Cr(VI) and total chromium values for the standard solutions were both in good agreement whereas for soil leachates and solid matrixes HPLC–DAD values were lower than those obtained by ICP–AES. The Cr is still in the matrix but could not be detected by the speciation method, simply because it occurs in different chemical forms.

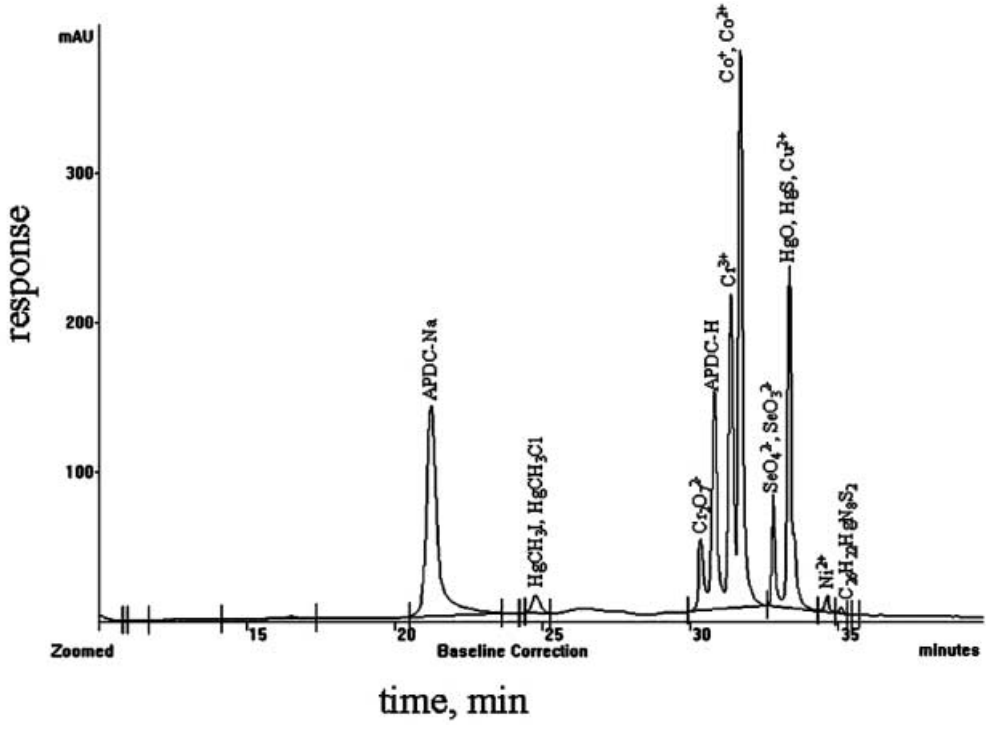

Fig. 3 Speciation of chromium in the presence of common elements in the different chemical forms found in soil and water

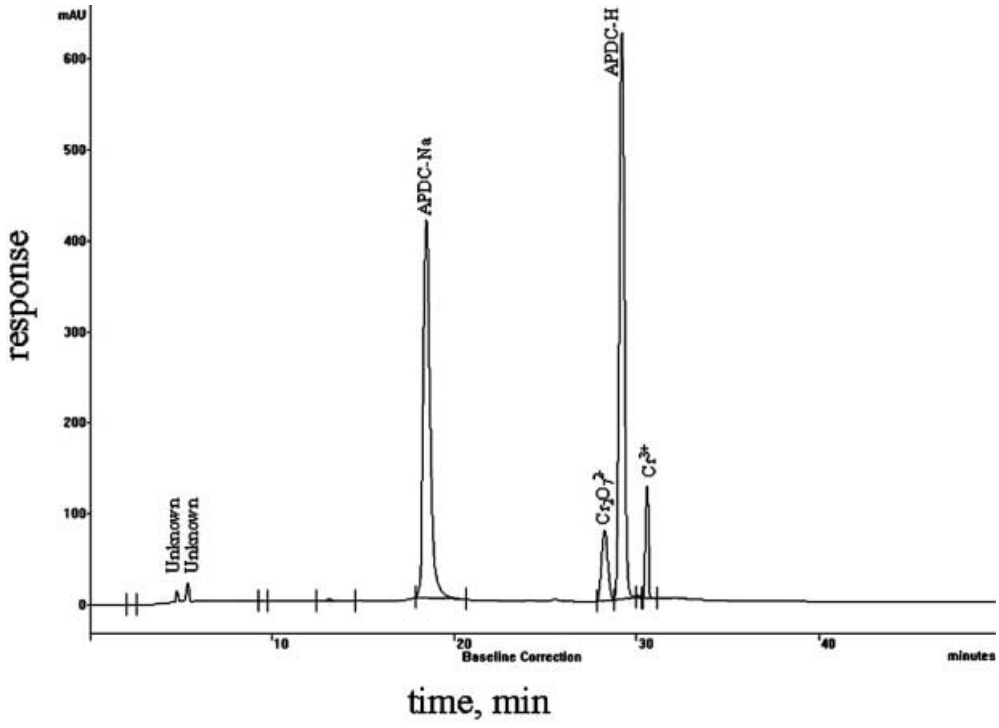

Fig. 4 Chromatogram obtained from a leachate of soil spiked with chromium

To conclude, on the basis of these experimental observations the proposed chromium speciation methodology is accurate and reliable, and can be suitable as a rapid, routine method for determination of chromium species in difficult to analyze matrixes.

Interference

Analysis of the chemical forms of chromium by reversed-phase liquid chromatography requires that the target analytes are separated from one another and that the sample matrix has no effect on the signal. It must also be demonstrated that common elements in all their chemical forms, organic and inorganic substances, do not interfere with the analysis of the chromium species. To investigate chromatographic and the matrix interference, common elements that were expected to interfere with the chromium species in the soil and water were determined by this method to demonstrate the separation and detection capabilities of the HPLC–DAD system. Most of these elemental chemical forms were not detected by HPLC–DAD; others were detected but did not interfere in the determination of Cr^{3+}, CrO_4^{2-}, and $Cr_2O_7^{2-}$. Figure 3 shows a typical chromatogram obtained from HPLC–DAD analysis of a mixture of chemical elements; it is readily apparent there is no discernable chromatographic interference under these experimental conditions. Figure 4 depicts a typical chromatogram obtained from a real sample of leachate from a soil contaminated with chromium. No matrix background or chromatographic interference was observed, which again demonstrates the ruggedness and accuracy of the proposed speciation method.

Conclusion

This chromium speciation method has enabled accurate and reproducible measurement of common chromium species in standard solutions, leachates, and solid samples. The method successfully discriminated not only between Cr(III) and Cr(VI), but also between the different chemical forms of chromium with the same oxidation states, CrO_4^{2-} and $Cr_2O_7^{2-}$. The method has been tested for the determination of chromium species in numerous samples and found to be sound, precise, and reliable. It has been validated by means of an interlaboratory study and found to be suitable for determination of Cr^{3+}, CrO_4^{2-}, and $Cr_2O_7^{2-}$ in difficult-to-analyze environmental matrixes. The estimated time required to process a batch of ten samples is approximately 2 h, including data processing and reporting.

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