Rapid Speciation Analysis of Cr(VI) and Cr(III) by Reversed-Phase High-Performance Liquid Chromatography with UV Detection

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Abstract

A simple and rapid method is developed for the simultaneous determination of Cr(VI) and Cr(III) based on the formation of their different complexes with ammonium pyrrolidine-dithiocarbamate (APDC). Separation is performed using reversed-phase high-performance liquid chromatography coupled with UV detection. The conditions for complex formation and speciation are determined, such as solution pH, amount of APDC, temperature, and type of mobile phase. In order to substantially reduce the analysis time, the separation is carried out without extraction of chromium-APDC complexes from the mother liquor. Under the optimum analysis conditions, the chromatograms obtained show good peak separation, and the absolute detection limits (3σ) are 2.2 µg/L for Cr(VI) and 4.5 µg/L for Cr(III). The calibration curves are linear from 3 to 5000 µg/L for Cr(VI) and 5 to 3000 µg/L for Cr(III). The relative standard deviations of peak areas in five measurements using a sample solution of 200 µg/L are less than 2% for Cr(VI) and 4% for Cr(III), indicating good reproducibility for this analytical method. Furthermore, simultaneous determination of Cr(VI) and Cr(III) is successful with the application of the proposed procedure in the synthetic wastewaters containing common heavy metal ions: Fe(III), Pb(II), Cd(II), Cu(II), and Zn(II).

Introduction

Speciation analysis of an element gives information about the individual concentrations of its various chemical forms. Speciation of chromium has attracted researchers' interest because of the high toxicity of its compounds and their widespread use in metallurgy, refractory, and chemical industries (1). Two common oxidation states of chromium are present in the environment, and their physicochemical properties are each drastically different from those of the other (1,2). Trivalent chromium, Cr(III), is essential for the maintenance of the normal glucose tolerance factor (3), whereas hexavalent chromium, Cr(VI), is a well-known carcinogen. The maximum permissible concentrations of chromium compounds in wastewaters are established as 0.5 mg/L for Cr(III) and 0.1 mg/L for Cr(VI) (USPS). Therefore, quantitative analysis of Cr(VI) and Cr(III) with low concentration levels is needed for environmental studies, as well as for industrial effluent controls.

Different analytical techniques for chromium speciation with atomic and molecular spectroscopy or voltammetry have been reported in the literature (1–11). In most methods, Cr(VI) can be determined directly, and then the Cr(III) concentration is calculated from the difference between Cr(VI) and the total chromium concentration. Because this calculation may induce some uncertainty (6), much attention in recent years has been focused on simultaneous determination methods of chromium compounds with different oxidation states, such as ion-chromatography (2,6,12), ion-pair chromatography (IPC) (2), and reversed-phase (RP) chromatography (4,11,13,14). For online detection, high sensitivity and better selectivity can be achieved by using RP high-performance liquid chromatography (HPLC) coupled with UV–spectrometry (4,13,14), atomic absorption spectrometry (AAS) (15), graphite furnace AAS (16), and inductively coupled plasma (ICP) mass spectrometry (MS) (1,17,18). The maximum calibration limits of AAS and ICP-MS are less than 0.1mg/L, and these methods require substantial dilution of sample solution, for example industrial effluents (Cr ≥ 10 mg/L).

Andrle et al. have proposed a novel simultaneous determination method of chromium compounds by RP-HPLC with UV detection (4). The method has long-range calibration limits but is a lengthy analysis. In the method, both Cr(VI) and Cr(III) react with ammonium pyrrolidine-dithiocarbamate (APDC) to form different types of complexes. The complexes are extracted with ethyl acetate, and the ethyl acetate is evaporated at reduced pressure. The separated complexes are dissolved in pure acetonitrile, and the solution is

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analyzed by using RP-HPLC–UV. This procedure consumes more than 3 h for one sample analysis.

The aim of the present study is to develop a simpler, time-saving procedure for the simultaneous determination of Cr(VI) and Cr(III) using RP-HPLC with UV detection, based on complexation with APDC.

Experimental

Cr-APDC complexes

The previous literature (4,8,19) showed that both Cr(VI) and Cr(III) react with APDC to form different types of complexes. Actually, Cr(III) reacts directly with APDC to give tris-[pyrrolidine-1-dithioato-S,S']-Cr(III) complex (denoted as Cr(III)–PDC). On the other hand, APDC reduces Cr(VI) to Cr(III) and leads to two different complexes: bis-[pyrrolidine-1-dithioato -S-S']-[pyrrolidine-1-peroxydithioato-O,S]-Cr(III) and tris-[pyrrolidine-1-dithioato-S,S]-Cr(III) [denoted as Cr(VI)-MP and Cr(VI)-BP, respectively] given as follows:

$$\text{Cr(III)} + \text{APDC} \rightarrow \text{Cr(III)-PDC complex (tris-[pyrrolidine-1-dithioato-S,S']-Cr(III)} \qquad \text{Eq. 1}$$

$$\text{Cr(VI)} + \text{APDC} \rightarrow \text{Cr(III)} \rightarrow \begin{cases} \text{Cr(VI)} - \text{MP (bis-[pyrrolidine-1-dithioato-S,S']-[pyrrolidine-1-peroxydithioato-O,S]-Cr(III)} \\ \text{Cr(VI)} - \text{BP (tris-[pyrrolidine-1-dithioato-S,S']-Cr(III))} \end{cases} \qquad \text{Eq. 2}$$

The known (4) structure of Cr(III)-PDC and Cr(VI)-MP complexes are shown in Figure 1. Andrle et al. have mentioned that the Cr(VI)-BP complex is similar to that of Cr(III)-PDC and is described in one name (4). However, the exact structure of Cr(VI)-BP complex is still unknown (19). The complexes Cr(III)-PDC and Cr(VI)-MP would have different types of polar attraction with organic solvents, but they have similar UV absorption characteristics (4). Therefore, Cr(VI)-MP, Cr(VI)-BP, and Cr(III)-PDC complexes can be separated from their mixtures with RP-HPLC (polarity of mobile phase > polarity of stationary phase) by using suitable mixed solvent as a mobile phase and detection using UV–spectrophotometry. Because the analysis of Cr(VI) and Cr(III) depends on the complex formation, as well as the separation, suitable complexation conditions were determined from the present investigation under various concentrations of APDC, solution pHs, and processing times.

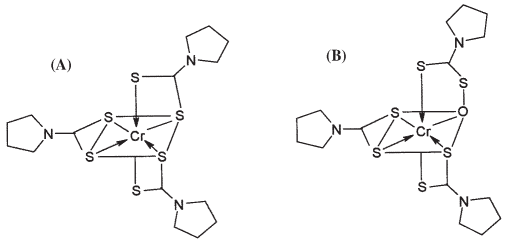

Figure 1. Complex structures of Cr(III) and Cr(VI) with APDC: (A) Cr(III)-PDC, (B) Cr(VI)-MP.

Standard solutions

Potassium dichromate and hexa-hydrate chromium trichloride (both from Wako Pure Chemical Industries, Osaka, Japan) were used as sources of Cr(VI) and Cr(III), respectively. Standard chromium solution with a concentration of 500 mg/L was prepared by using 18MΩ deionized water. A 1–5-mL volume of standard chromium solution was transferred to a 15-mL test tube, and 3 mL of an acetate buffer solution (pH = 4.66) was added. The acetate buffer was prepared from the mixture of acetic acid (Kanto Chemical Co., Tokyo, Japan) and sodium acetate trihydrate (Merck, Darmstadt, Germany). The APDC (Dojindo Corp., Kumamoto, Japan) solution was freshly prepared in deionized water. Because of long time storage, the APDC solution becomes turbid. To obtain the chromium–APDC complexes, 1 mL of 0.1% APDC solution was added to the chromium solution. The reaction mixture was held for 20 min at 55°C then diluted to 15 mL with deionized water. Different parameters in the procedure were optimized from various investigations.

Potassium permanganate (Wako), 1,5-diphenyl carbazide (Kanto), and sodium azide (Wako) were used for colorimetric analysis. Standard solutions of Fe(III), Cd(II), Pb(II), Zn(II), and Cu(II) (Ishizu Seiyaku, Osaka, Japan) were used to investigate interference of foreign ions with chromium analysis. Acetonitrile and methanol (Wako) were of HPLC grade, and other chemicals were analytical grade.

Instrumentation

The RP-HPLC–UV system (Tosoh Corp., Tokyo, Japan) consisted of a vacuum degasser (SD-8022), gradient pump (CCPS), column compartment oven (CO-8020), autosampler (AS-8020) with a variable injection valve, system controller (SC-8020), and UV–vis detector (UV-8020). The analytical column was prepacked with nonendcaped octadecyl silica gel (Figure 2) having a mean particle diameter of 5 µm (15 × 4.6 cm, TSK GEL ODS-120A) (Tosoh Corp.). The wavelength was set at 254 nm. A UV–vis recording spectrophotometer (UV-160A, Shimadzu, Kyoto,

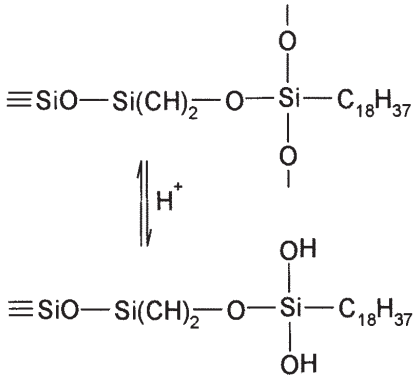

Figure 2. Equilibrium structure of nonendcaped octadecyl silica gel.

Japan) was used for colorimetric analysis of chromium [1,5-DPC-Cr(VI) complex] at a 540-nm wavelength.

Results and Discussion

Selection of mobile phase

Peak separation and intensity, as well as peak area of the chromatograms, were examined using different mobile phases. Figures 3A and 3B show chromatograms of Cr-APDC complexes for mixed solutions of Cr(VI) and Cr(III) using acetonitrile–water and methanol–water systems, respectively. Obviously, the peak separation in Figure 3B is better than that in Figure 3A. In Figure 3B, the initial peaks up to 5.22 min are for excess APDC, which were confirmed by blank experiments, and the other peak at 6.03 min is for Cr(VI)-MP, 7.18 min for Cr(III)-PDC, and 7.88 min for Cr(VI)-BP. Andrle et al. reported that the complexes of Cr(III)-PDC and Cr(VI)-BP have the chromatographic peaks at the same position and it makes some difficulties in determining Cr(III)-PDC complex (4). Such difficulties do not arise in our case because of the separate peak for Cr(VI)-BP (Figure 3B) and we considered only the complexes of Cr(III)-PDC and Cr(VI)-MP for determination of Cr(III) and Cr(VI), respectively, neglecting the existence of Cr(VI)-BP. The acetonitrile–water system at a volume ratio of 2:1 was chosen as a mobile phase for chromium analysis by RP-HPLC–UV.

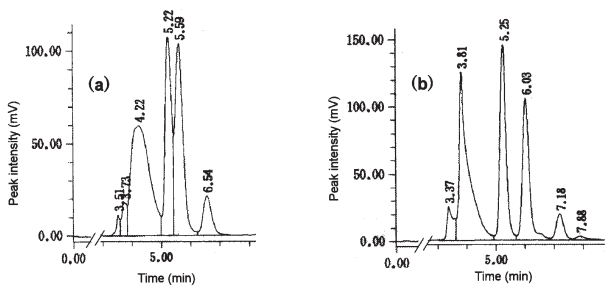

Figure 3. Chromatograms of Cr(VI, III)-APDC complexes of a mixed solution of 1.67 mg/L of Cr(VI) and 0.34 mg/L of Cr(III) with different mobile phases: (A) methanol–water (3:1, v:v) [peak at (t_R) 4.22 and 5.22; APDC, 5.59; Cr(VI)-MP, 6.54; Cr(III)-PDC] and (B) acetonitrile–water (2:1, v:v) [peak at (t_R) 3.81 and 5.25; APDC, 6.03; Cr(VI)-MP, 7.18; Cr(III)-PDC, 7.88; Cr(VI)-BP].

Determination of suitable complexation conditions

The suitable conditions for Cr-APDC complexation were optimized to enhance the analytical performance of the complexes. Parameters including solution pH, amount of APDC, and complexation time were determined by considering the chromatograms obtained.

Solution pH

The complexation reactions of Cr(VI) and Cr(III) with APDC are highly dependent on solution pH. The complexation reaction of Cr(VI) with APDC is more specific than Cr(III). It has been reported that the optimum pH for the reaction of APDC with Cr(VI) is 4.66, whereas a pH of less than 4.66 is suitable for Cr(III)

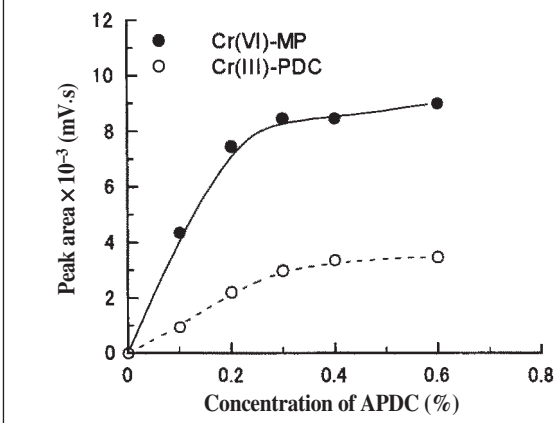

Figure 5. Effect of the APDC concentration for complexation on the peak area of Cr-APDC complexes: $C_{Cr(VI)}$ = 3.67 mg/L and $C_{Cr(III)}$ = 0.67 mg/L. Complexation conditions: pH = 4.66 and heating time at 55°C = 20 min.

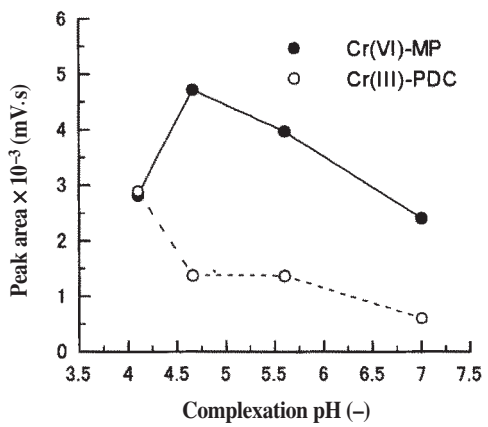

Figure 4. Effect of complexation pH on the peak area of Cr-APDC complexes: $C_{Cr(VI)}$ = 3.67 mg/L and $C_{Cr(III)}$ = 0.67 mg/L. Complexation conditions: APDC = 0.1%, and heating time at 55°C = 20 min.

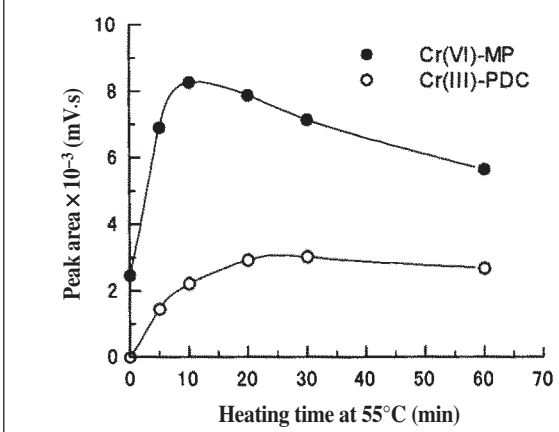

Figure 6. Effect of heating time for complexation on the peak area of Cr-APDC complexes: $C_{Cr(VI)}$ = 3.67 mg/L and $C_{Cr(III)}$ = 0.67 mg/L. Complexation conditions: pH = 4.66 and APDC concentration = 0.3% (1 mL in 15-mL solution).

(4). Figure 4 shows that the peak area of Cr(VI)-MP complex reaches a maximum value at a complexation pH of 4.66. On the other hand, the peak area of Cr(III)-PDC complex decreases with an increase in solution pH. Because of the presence of oxygen atom in Cr(VI)-MP complex, it becomes more protonated at a low pH of mother liquor, compared with Cr(III)-PDC complex and shows high peak area. Again, because the nature of used stationary phase also depends on the pH of solution (20) as shown in Figure 2; at a higher pH the stationary phase becomes electrically negative and less attractive to negative species. Thus, less Cr-APDC complexes attach with the stationary phase and show small peak areas. Therefore, the pH value of 4.66 was employed in subsequent experiments.

Amount of APDC

Figure 5 shows that both of the peak areas of Cr(VI)-MP and Cr(III)-PDC increase with an increasing APDC concentration, but there is no significant change of peak area in the concentration range above 0.3%. Thus, 1 mL of 0.3% APDC solution was used in subsequent Cr-APDC complexation.

Complexation time

Because chromium slowly reacts with APDC at ambient temperature, the complexation was carried out at 55°C. Figure 6 shows the relationship between peak areas of Cr-APDC complexes and heating time for complexation. The maximum peak area of Cr(VI)-MP can be found at 10 min, but the peak area of Cr(III)-PDC is almost constant with a heating time over 20 min. With heating more than 10 min, the agglomeration of the Cr(VI)-MP complex results the loss of complex in mother liquor and decrease in peak area. Thus the heating time at 55°C was specified as 10 min for Cr-APDC complexation.

Addition of acetonitrile

Under the previously mentioned specified conditions of pH, APDC concentration, and complexation time, calibration curves were constructed for both the Cr(VI) and Cr(III)-APDC complexes. Calibration limits for Cr(VI)-MP were found to be 5 to 1600 µg/L, and for Cr(III)-DPC they were 10 to 800 µg/L. Because the Cr-APDC complexes are less soluble in water, we added acetonitrile to the mixture of complexes before passage through the RP-HPLC–UV step to increase the solubility of the complexes and extend the maximum calibration limit. It should be mentioned that addition of acetonitrile does not significantly affect the maximum absorbance wavelength of Cr-APDC complexes in the UV spectrum. To optimize the amount of additional acetonitrile, various mixed solutions were passed through the HPLC, and their peak intensities and peak areas were investigated. Figure 7 shows the variation in peak area with the amount of acetonitrile added to the mixtures of complexes: 0.05 mg/L of Cr(VI) and 0.03 mg/L of Cr(III). The maximum peak areas for both Cr(VI)-MP and Cr(III)-PDC were observed at 33% of acetonitrile coexisting in the complex mixtures. Similar effects of acetonitrile addition were observed for the complexes of 2.67 mg/L of Cr(VI) and 2.00 mg/L of Cr(III).

Flow rate of mobil phase

The influence of the flow rate of the mobile phase on detection of the complexes was investigated in the range of 0.4 to 1.0

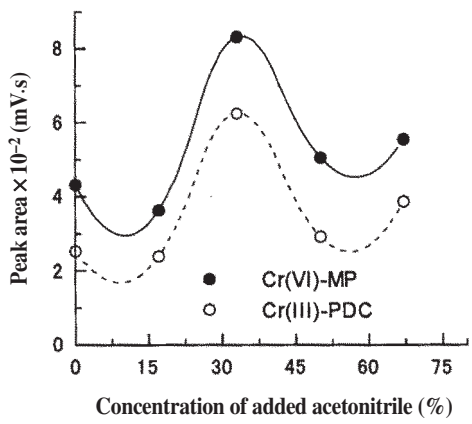

Figure 7. Effect of adding acetonitrile to the complexes on the peak area of Cr-APDC complexes: $C_{Cr(VI)}$ = 0.050 mg/L and $C_{Cr(III)}$ = 0.030 mg/L. Complexation conditions: pH = 4.66, APDC concentration = 0.3%, and heating time at 55°C = 10 min.

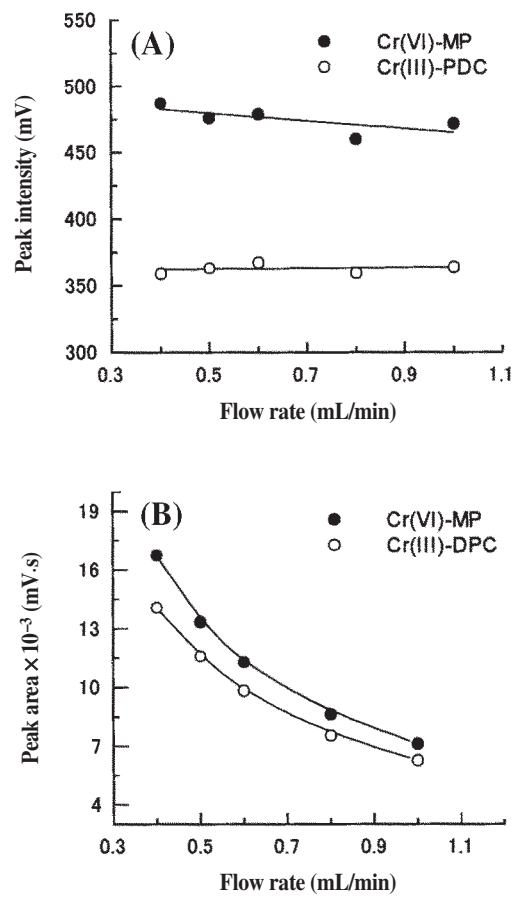

Figure 8. Effect of flow rate on the peak intensity and peak area of Cr-APDC complexes: $C_{Cr(VI)}$ = 2.67 mg/L and $C_{Cr(III)}$ = 2.00 mg/L. Complexation conditions: pH = 4.66, APDC concentration = 0.3%, heating time at 55°C = 10 min, and 33% acetonitrile in sample solutions.

mL/min. Generally, peak area decreases with increasing flow rate, but peak intensity increases. Figure 8A shows that for Cr(VI)-MP complex, the peak intensity slightly decreases with increasing flow rate; but for the Cr(III)-PDC complex, the peak intensity is nearly unchanged by an increase in flow rate. On the other hand, Figure 8B shows that the peak area decreases with increasing flow rate in both cases. Again, for low flow rate, the retention time becomes longer. Since the resolution of the Cr(VI)-MP complex increases towards lower flow rates, the flow rate of 0.6 mL/min was specified for both Cr(VI)-MP and Cr(III)-DPC complexes. The optimized conditions for complete analysis are shown in Table I.

Quantitation

Several important factors for quantitative analysis of chromium complexes, including reproducibility, detectable minimum concentration, and linearity, were examined under optimized conditions. Linear relationships between peak area and Cr-APDC concentration were obtained from 3 to 5000 µg/L for Cr(VI) and 5 to 3000 µg/L for Cr(III), with the correlation coefficients (R^2) of 0.999 and 0.996, respectively. These results are comparable with the previously reported values 5 to 5000 µg/L for both Cr(VI) and Cr(III) (4). Figure 9 shows the chromatogram of chromium complexes with APDC, including three peaks: APDC at 7.36 min, Cr(VI)-MP at 8.63 min, and Cr(III)-PDC at 10.53 min. Peak separation for the chromatogram in this figure has been improved when compared with that of Figure 3B. The present procedure is similar to that proposed by Andrle et al. (4). However, three samples can be analyzed within 1 h in this method, indicating that it is remarkably time-saving.

Table I. Determined Conditions for Complexation and HPLC Operation

Parameters	Optimized conditions
For complexation	
Acetate buffer pH	4.66
Concentration of APDC	0.3%
Heating time	10 min at 55°C
Acetonitrile in complex mixture	33%
For HPLC operation	
Mobile phase	Acetonitrile–water (2:1)
Flow rate	0.6 mL/min

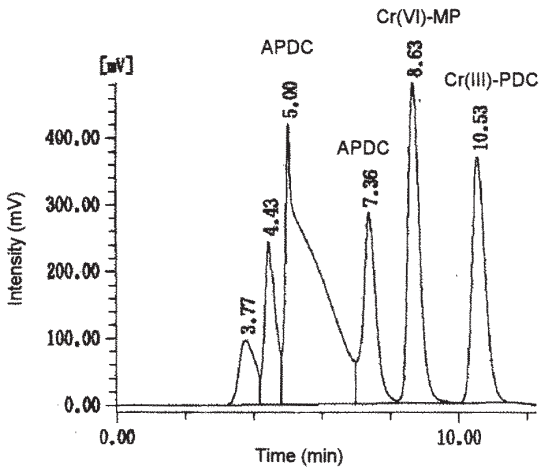

Figure 9. Chromatogram of Cr-APDC complexes with RP-HPLC–UV at optimized conditions. Solution pH = 4.66, concentration of APDC = 0.3%, complexation time = 10 min at 55°C, and acetonitrile in complex mixture = 33%.

The precision or reproducibility of measurements was examined by performing five consecutive analyses with a standard solution of 0.2 mg/L of each chromium species. The relative standard deviations of peak areas were found to be less than 2% for Cr(VI) and 4% for Cr(III)-PDC. These results indicated that the proposed method is reliable. Significance of the measurements was verified by student's *t*-test (compare the mean with true or standard values and significance of the comparison). The result showed that the *t* values were 7.2 and 9 for Cr(VI) and Cr(III), respectively. In these measurements, the mean values were 0.209 for Cr(VI) and 0.231 for Cr(III). From the *t*-table (21), for 4 degrees of freedom, $t = 2.13$ (for 10% probability), 2.78 (for 5% probability), and 3.75 (for 1% probability). Here the calculated values of *t* [± 7.19 for Cr(VI) and ± 9.0 for Cr(III)] were higher than the tabulated values, indicating that the measurements are highly significant.

The detectable minimum concentrations for both of Cr(VI) and Cr(III) were calculated as three times the standard deviation (3σ) of the blank analysis normalized by the slope of the calibration curves. The absolute detection limits were found to be 2.2 and 4.5 µg/L for Cr(VI) and Cr(III), respectively. These limits are nearly equal to the reported values: 2.1 µg/L for Cr(VI) and 2.4 µg/L for Cr(III) (RP-HPLC–UV) (4); 1.8 µg/L for Cr(VI) and 2.5 µg/L for Cr(III) (IC-UV) (12).

Interference

A synthetic wastewater sample containing approximately 0.2 mg/L each of Cr(VI) and Cr(III) was analyzed several times in the presence of common heavy metal ions such as Fe(III), Cd(II), Pb(II), Zn(II), and Cu(II). The chromatograms obtained showed that peak area slightly decreased approximately 1.9% for Cr(VI) and increased approximately 2.3% for Cr(III). Such

Table II. Comparison of the Measurement of a Standard Sample* by the RP-HPLC–UV Method (Present Method) and Colorimetric Method†

RP-HPLC–UV method				Colorimetric method			
Detected concentration (mg/L)		Coefficient of variation (%)		Detected concentration (mg/L)		Coefficient of variation (%)	
Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)
0.2123	0.2453			0.1931	0.2245		
0.2104	0.2354			0.1852	0.2286		
0.2066	0.2312	1.3	3.3	0.1879	0.2168	1.4	3.9
0.2061	0.2281			0.1813	0.2115		
0.2111	0.2147			0.1905	0.2102		

* 0.2 mg/L of both of Cr(VI) and Cr(III).
† F = 1.16 for Cr(VI) and F = 0.82 for Cr(III).

changes would be within the limit of repeatability of the measurement.

Comparison with colorimetric method

To demonstrate the accuracy and tprecision of the present procedure, synthetic wastewaters containing 0.2 mg/L of each chromium species were analyzed five times. One series of results was compared with those obtained with the conventional colorimetric method (22), as shown in Table II, in terms of variance ratio test (*F*-test). The calculated values of *F* were 1.16 for Cr(VI) and 0.82 for Cr(III), and they were less than the values tabulated in the literature (21). The present method, therefore, has significant precision comparable with the colorimetric method.

Conclusion

RP-HPLC–UV spectrometry was shown to be a rapid technique for the simultaneous determination of Cr(VI) and Cr(III). The linearity extended from 3 to 5000 µg/L for Cr(VI) and 5 to 3000 µg/L for Cr(III). The detection limits for both of Cr(VI) and Cr(III) were lower than the environmental safety levels and 10 times lower than the permissible limits for industrial wastewater, suggesting the suitability of this method for analysis of industrial waste samples, as well as its easy application to the research of remediation technologies.

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