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Journal of Chromatography A, 1073 (2005) 291–295
JOURNAL OF CHROMATOGRAPHY A
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Simultaneous determination of Cr(III)–EDTA and Cr(VI) by ion interaction chromatography using a C_{18} column

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Abstract

The simultaneous determination of Cr(III) and Cr(VI) by ion interaction chromatography has been investigated. The mobile phase consisted of a 5 mM octylammonium orthophosphate at pH 4.0 with 35% (v/v) MeOH. The Nucleosil-100, C_{18} (5 μm, 250 × 4.6 mm) was used as the separating column and the component was detected at 200 nm. The separation of Cr(III) and Cr(VI) was based on anionic interaction. Since the Cr(III) did not exist as an anionic form like the Cr(VI) ($Cr_2O_7^{2-}$) presented at the optimum condition, Cr(III) was firstly reacted with EDTA (1:40 mole ratio) to form the anionic complex prior to injecting into the chromatographic system. The characteristics of the method for separation of Cr(III)–EDTA and Cr(VI) were satisfactory. The wide linear range (0.3–50.0 mg l^{-1}) was achieved. The repeatabilities (%R.S.D.) calculated from peak areas were 0.49% and 0.14%, detection limit (signal to noise ratio of 3) of 0.02 mg l^{-1} and 0.3 mg l^{-1} were obtained and the average of percent recoveries were found to be 98.5% and 99.6% for Cr(III) and Cr(VI), respectively.

Keywords: Ion interaction chromatography; Cr(III)–EDTA; Cr(VI)

1. Introduction

Chromium is a "paradox" since it is classified as both biologically important and a toxic industrial hazard depending upon its oxidation state [1]. Cr(III) is known to be an essential trace nutrient involved in the mechanism of the action of the pancreatic hormone insulin and/or glucose metabolism [2]. No minimum daily requirement of chromium has yet been established, but 50–200 μg per day is considered adequate. Cr(III) is found in fruits, vegetables, meats, cereals and various other foods. A deficiency of the nutrient may lead to glucose intolerance [3]. Conversely, Cr(VI) is known to be carcinogenic and mutagenic. Unlike Cr(III), Cr(VI) may cross cellular membranes by way of non-specific anion carriers [4], causing skin ulceration, nasal perforations, and lung cancer [5]. The subsequent reduction of Cr(VI) to intermediates such as Cr(IV) and Cr(V) is thought to play a role in its toxicity [4].

It is obvious that speciation is necessary to obtain an adequate toxicological sample assessment for chromium. As a result, several methods for inorganic chromium speciation have been described. Non-chromatographic methods are generally labor and time-intensive. A summary of some of these methods with UV, flame atomic absorption spectroscopy (FAAS), electrothermal atomic absorption spectroscopy (ETAAS), direct current plasma (DCP)-AES, and ICP-AES determination may be found in Ref. [6]. Often, Cr(III) is calculated by subtraction of Cr(VI) from total chromium. This type of calculation may involve some uncertainty. Simultaneous determination would not only be less time consuming, but would pose less risk of calculation errors.

Liquid chromatography (LC) is a convenient method for separating and determining metal ions simultaneously [7–11]. Some reports have shown the practical use of LC for the determination of chromium species with conventional spectrophotometric detection, or with coupling to techniques such as atomic absorption spectrometry and inductively couple and direct current plasma atomic emission spectrometry [12–18].

Ion interaction chromatography is a powerful technique, which permits the separation of anions on commercial reversed stationary phases and conventional high performance liquid chromatography (HPLC) instrumentation [19]. Litera-

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doi:10.1016/j.chroma.2004.09.053

ture methods for the determination of inorganic anions based on both ion chromatography and ion interaction chromatography published before 1984 were exhaustively reviewed by Haddad and Heckenberg [20]; and a review by Marina et al., published in 1989 was devoted to HPLC applications in the analysis of inorganic anion species [21].

In this work, a simultaneous determination of Cr(III) and Cr(VI) by ion interaction chromatography with UV detection was investigated. The separation of Cr(III) and Cr(VI) was based on anionic interactions. Since the Cr(III) did not exist in an anionic form like the Cr(VI) ($Cr_2O_7^{2-}$) presented at the optimum condition, Cr(III) was chelated with EDTA before injecting into a C_{18} column which had been dynamically coated with octylammonium. The optimum condition for complexation and elution were also studied.

2. Experimental

2.1. Instrumentation

The chromatographic analysis was carried out in a high performance liquid chromatograph (Hewlett-Packard, HP1100) equipped with a G 1311 A pump and a 7125 Rheodyne Injector with a 20 μl loop. The chromatographic separation was achieved with a Nucleosil-100, C_{18} (5 μm, 250 × 4.6 mm) chromatographic column. A Denver Instrument pH meter was used throughout for pH measurements.

2.2. Reagents

Distilled deionized water was used to prepare all solutions and the eluent. Stock standard solutions of 1000 μg ml^{-1} Cr(III) and Cr(VI) were prepared from chromium(III) chloride and potassium dichromate (RDH, Aktiengesellshaft, Germany), respectively. Fresh working standard solutions of Cr(III) and Cr(VI) (single or mixed) were prepared by appropriate dilution of the stock solutions with water. Ethylenediaminetetraacetate disodium salt (Na_2-EDTA) was obtained from Merck (Darmstadt, Germany). The LC eluents were prepared from LC-grade methanol (Mallinckrodt, St. Louis, MO, USA), water and octylamine (Fluka, Switzerland) and were adjusted to the desired pH with concentrated H_3PO_4. All eluents were filtered through a 0.45 μm cellulose acetate membrane filter (Millipore, Bedford, MA, USA) and degassed ultrasonically before being used.

2.3. Chromatography

The chromatographic system was conditioned by passing the eluent through the column until a stable signal was obtained. Usually, about 2 hrs as necessary. Then, 20 μl of a mixed standard of Cr(III)–EDTA and Cr(VI) was injected into the chromatographic system with flow rate of 1.0 ml min^{-1} and the analytes were detected at 200 nm. External standards were used in the calibration curves and the data presented at the present study were the average of triplicate injections of samples or standards.

2.4. Preparation of standard sample solution

A mixed standard sample solutions containing 5 ppm each of Cr(III) and Cr(VI) was prepared. Before injecting into the HPLC system, an appropriate amount of EDTA was added to the sample solution and the desired pH of the solution was adjusted with 0.1 M H_2SO_4 or 0.1 M NaOH.

3. Results and discussion

To establish the optimum conditions for the simultaneous determination of Cr(III) and Cr(VI), factors affecting the formation of chelates and retention behavior, such as the pH of the sample medium and of the eluate, concentrations of EDTA, ion interaction reagent (octylamine), pH and organic modifier (methanol) were studied in detail.

3.1. Cr(III) chelate formation

Conversion of both chromium ions into species with similar charge is necessary for them to be retained simultaneously in a C_{18} column which had been coated by octylammonium. Cr(III) exists in the form of $Cr(H_2O)_6^{3+}$ in aqueous solution, hence a complexation reagent with higher chelating ability than water is required to complex Cr(III). EDTA is known to complex with metal ions in a 1:1 ratio, and the chelates are always negatively charged whatever the charge on the cations [22]. Being chelated with EDTA, as CrY^- (where Y^{4-} represents the completely deprotonated EDTA species), Cr(III) will possibly be retained simultaneously with Cr(VI), dichromate, on an anion-exchange C_{18} column.

As Cr(III) was precolumn chelated with EDTA, the enrichment would depend on the extent of chelate. Chelation between metal ions and EDTA has been discussed elsewhere [23]. Both solution pH and EDTA concentration are important factors in the chelation.

The effect of pH of sample on chelation of Cr(III)–EDTA and stability of Cr(VI) was studied by preparing the mixed solution of 5 mg l^{-1} Cr(III), 5 mg l^{-1} Cr(VI) and 200 mg l^{-1} EDTA. The pH of sample (2.0, 4.0, 6.0, 8.0 and 10.0) was adjusted by using 0.1 M NaOH (high pH) and 0.1 M H_2SO_4 (low pH), then the samples were left for 30 min at room temperature before injecting into the HPLC system. The optimal chromatographic conditions were 5 mM octylammonium orthophosphate at pH 4.0, 1.0 ml min^{-1} flow rate, and 200 nm detection wavelength. The result obtained is shown in Fig. 1, it was found that Cr(III)–EDTA chelation offered the highest formation ability at pH 4.0, and Cr(VI) was relatively stable throughout the pH range studied. Equilibra among Cr(VI) species also depend on the pH of the solution. Although Cr(VI) species are still in anionic form at very low pH, Cr(III) chelation is difficult owing to the lack of Y^{-4}.

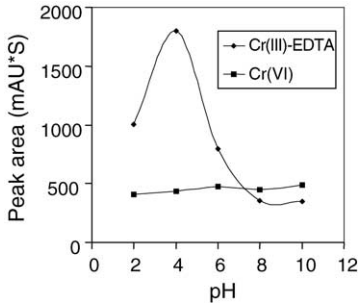

Fig. 1. Effect of pH of sample on the formation ability of Cr(III)–EDTA and stability of Cr(VI) during the sample preparation step.

Therefore, controlling the pH of sample solution to pH 4.0 gave the best result than other pH values. The chromatogram obtained of the standard mixture of Cr(III) and Cr(VI) after chelation with EDTA at pH 4.0 and at room temperature is shown in Fig. 2.

The effect of EDTA concentration on chelation of Cr(III)–EDTA was studied by preparing the mixed solution of 5 mg l^{-1} Cr(III) and varied concentration of EDTA. The pH of the sample was adjusted to 4.0, then the samples were left for 30 min at room temperature. Absorbance was measured spectrophotometrically at 200 nm. The chelation ability can be implied by the increase of the absorbance. The result obtained is shown in Fig. 3. As the mole ratio of EDTA:Cr(III) increased to 40:1, it took 30 min to complete the chelation at pH 4.0 at room temperature. As verified in this work, it was advantageous to chelate at high temperature (if the sample was heated to 40 °C at pH 4.0, only 10 min were required for chelation). It was unnecessary to consider the oxidation ability of chromate at the controlled pH. Thus, the ratio of the Cr(III) to Cr(VI) was not changed during the sample chelation. In order to make more flexibility and convenience to the researcher, 40:1 concentration ratio along with 30 min and at room temperature were selected.

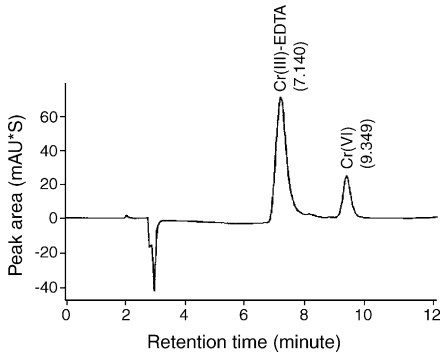

Fig. 2. Chromatogram of Cr(III)–EDTA and Cr(VI). Chromatographic conditions used were Nucleosil-100, C_{18} (5 μm, 250 × 4.6 mm); 5 mM octylammonium orthophosphate at pH 4.0 with 35% (v/v) MeOH; flow rate of 1.0 ml min^{-1}; detection at 200 nm.

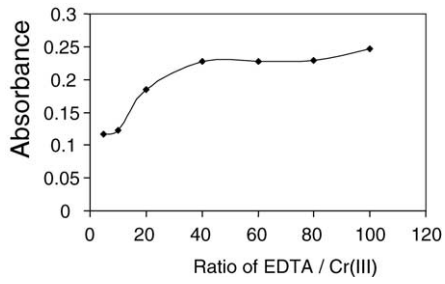

Fig. 3. Effect of EDTA concentration on the formation ability of Cr(III)–EDTA.

3.2. Retention behavior

In order to optimize the chromatographic conditions for the simultaneous determination of Cr(III)–EDTA and Cr(VI), the chromatographic parameters, such as the concentration of octylamine, pH and % organic modifiers, were studied. The effect of octylamine concentration on the retention of Cr(III)–EDTA and Cr(VI) was studied by injecting 5 mg l^{-1} of mixed standard of Cr(III)–EDTA and Cr(VI) into the HPLC system using the same chromatographic conditions as showed in Fig. 2, except varied the concentration of octylamine. The retention behavior as a function of the ion interaction reagent concentration (octylamine) is shown in Fig. 4. The left portion of the curve shows the increase in the retention time which can be easily explained in such a way that the stationary phase surface is becoming more ionogenic due to the adsorption of octylammonium ion onto the surface and overall energy increases due to ion–ion interactions. The plateau shape of the curve has been attributed to the formation of micells in the mobile phase and to the competition of adsorption reactions when the concentration of the interaction reagent exceeds the critical micelle concentration [25].

Fig. 5 indicates an increasing of the mobile phase pH resulted in an increase of the retention of both analytes. This result could be described by caused of a decrease of the amount of eluting anionic species (phosphate ion) leads to the increasing in retention. In addition, it was also found that the variation of mobile phase pH influenced the peak area of Cr(III) but did not have any significant effect on the peak area of Cr(VI), which agrees with other reported results [24]. At mobile phase pH higher than 5.0, Cr(III) ion forms $Cr(OH)_3$ precipitation. This event could affect column

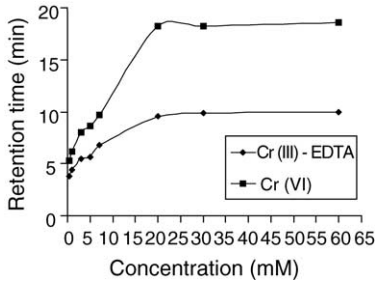

Fig. 4. Effect of ion interaction reagent concentration on retention of Cr(III)–EDTA and Cr(VI). The chromatographic conditions were the same as those in Fig. 2.

Table 1
Characteristics of the method for the simultaneous determination of Cr(III) and Cr(VI)

Metal	Regression equation[a]	r[b]	Range (mg l^{-1})	Detection limit (3σ) (mg l^{-1})	%R.S.D.[c]
Cr(III)–EDTA	$Y = 337.8X + 140.14$	0.9999	0.3–50.0	0.02	0.49
Cr(VI)	$Y = 109.7X + 70.08$	0.9997	0.3–50.0	0.30	0.14

[a] Y, peak area (mAU*S); X, metal concentration (mg l^{-1}).
[b] Correlation coefficient.
[c] Average of five individual determinations (calculated from peak area).

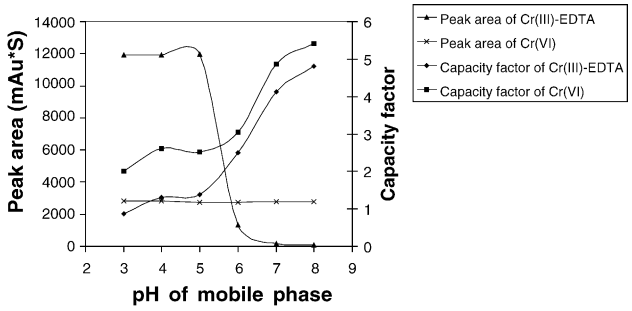

Fig. 5. Effect of pH of mobile phase on capacity factor and peak areas of Cr(III)–EDTA and Cr(VI). The chromatographic conditions were the same as those in Fig. 2.

performance. Therefore, the mobile phase with a pH higher than 5.0 should be avoided for the simultaneous separation of Cr(III) and Cr(VI). Alternatively, Fig. 6 shows an increase of organic modifier concentration resulted in a decreases in retention times of both analytes due to the increase of solvent eluotropic strength [19,25,26].

From the results obtained in this work, the adopted chromatographic condition was expected to constitute an effective alternative for separation and determination of Cr(III) and Cr(VI) in routine analysis. The characteristic of the proposed method (using the optimum chromatographic conditions as described in Fig. 2) is shown in Table 1, revealing the flexible and reliable technique. We have so far applied this method for the simultaneous determination of Cr(III) and Cr(VI) in water sample collected from the developed treatment of chromium removal system using algae *(Spirulina* sp., *Chlorella vulgalis* TISTR 8261 and *Chlorella vulgalis* TISTR 8580) (Table 2). This work was done successfully. Furthermore, this proposed method could also be adapted for the determination of Cr(III) and Cr(VI) in other samples. Fortunately, for sample containing trace level of chromium, the sample preconcentration step must be desired. Many of sample preparation and preconcentration methods have been discussed elsewhere [27–29]. Alternatively, the standard addition method could be carried out for the determination of Cr(III) in samples consisting of the other heavy metals which can also chelate to EDTA.

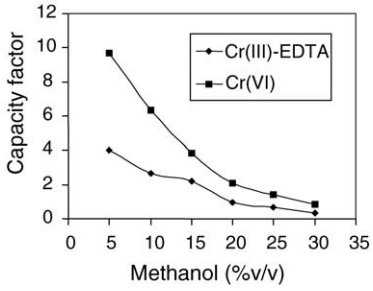

Fig. 6. Effect of % methanol on retention of Cr(III)–EDTA and Cr(VI). The chromatographic conditions were the same as those in Fig. 2.

Table 2
Percentage recoveries and reproducibilities of Cr(III) and Cr(VI) that were spiked to water sample collected from the developed treatment of chromium removal system that were determined using the proposed method

Concentration of Cr spiked (mg l^{-1})		% Recovery ± S.D.[a]	
Cr(III)	Cr(VI)	Cr(III)	Cr(VI)
1.0	1.0	96.10 ± 3.80	98.45 ± 0.60
5.0	5.0	106.32 ± 0.80	98.99 ± 0.60
10.0	10.0	97.30 ± 2.40	97.99 ± 0.90
20.0	20.0	94.30 ± 1.02	102.9 ± 0.11

[a] Standard deviation.

4. Conclusions

The prechelation of Cr(III)–EDTA before analysis by HPLC was carried out at the pH 4.0 and room temperature for 30 min. Under these conditions, Cr(VI) stability did not altered. Ion interaction chromatography has been successfully employed to determine Cr(III) and Cr(VI) simultaneously. This technique was flexible because many of parameters could be readily varied to obtain the good separation result. Importantly, this technique permits the use of the conventional HPLC instrumentation (C_{18} column and UV detector).

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