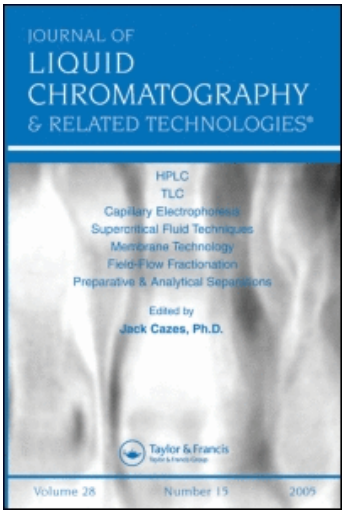

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Online publication date: 28 October 2002

To cite this Article Lian, Hongzhen , Kang, Yufen , Bi, Shuping , Chen, Yijun , Mao, Li , Dai, Lemei , Cao, Mi and Tian, Liching(2002) 'STEPWISE DETERMINATION OF ALUMINUM AND CHROMIUM IN WATER AND BIOMEDICAL SAMPLES BY HPLC-UV, BASED ON THEIR DIFFERENT KINETICS WITH 8-HYDROXYQUINOLINE', Journal of Liquid Chromatography & Related Technologies, 25: 19, 3059 — 3074
To link to this Article: DOI: 10.1081/JLC-120015891
URL: http://dx.doi.org/10.1081/JLC-120015891


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JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES
Vol. 25, No. 19, pp. 3059–3074, 2002

STEPWISE DETERMINATION OF ALUMINUM AND CHROMIUM IN WATER AND BIOMEDICAL SAMPLES BY HPLC-UV, BASED ON THEIR DIFFERENT KINETICS WITH 8-HYDROXYQUINOLINE

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ABSTRACT

A reversed-phase high performance liquid chromatographic (RP-HPLC) method, with spectrophotometric detection for determination of trace amounts of aluminum (Al) and chromium (Cr) in water and biomedical samples, has been developed using 8-hydroxylquinoline (8-HQ) as a precolumn reagent based on the differential chelation kinetics of these two ions. The analysis is achieved with a Spherisorb ODS 2 column, with an eluent consisting of 4.0 mmol L^{-1} acetate buffer (pH 6.0) and

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DOI: 10.1081/JLC-120015891
1082-6076 (Print); 1520-572X (Online)

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3.2 mmol L^{-1} 8-HQ in 57% (v/v) methanol–water, at a flow rate of 1.0 mL min^{-1}. On the basis of the fact that the peaks of Al and Cr chelates appear at the same retention time, the procedure is divided into two stages:

(1) determining Al concentration by peak area after pre-column chelation at ambient temperature;
(2) measuring total peak area of Al and Cr after reaction at 90°C for 30 min, and the concentration of Cr being determined by the difference between Al peak area and the total area.

The response of both Al and Cr is linear from 0.002 to 0.6 μg mL^{-1} with a detection limit of 0.001 μg mL^{-1}. The analysis is free from interference from common ions except 10-fold excess of cobalt. The values obtained by this method are in good agreement with those of ICP-AES or GF-AAS. The proposed method has been successfully applied to the direct determination of Al and indirect determination of Cr in natural water, serum, synthetic renal dialysate, parenteral solution, and pharmaceutical-grade organic acid with the recoveries of over 90%.

INTRODUCTION

Aluminum (Al) is the third most abundant element in the earth's crust. There is a long history of the role of Al in determining toxicity to aquatic and terrestrial biota, and the available evidence points to a strong correlation between Al and toxicity.[1–3] Also, there is considerable concern over the toxicity of Al in living things, especially in man, although its exact biomedical functions are not completely known. In recent years, it is further believed that aluminum is causally implicated in the pathology of Alzheimer's disease, Parkinson's disease, and dialysis encephlopathy, because abnormal amounts of Al have been detected in the brains of dead patients suffering from these diseases.[4–6] Trivalent chromium is considered as an essential trace element, as it is able to coordinate several amino-acid ligands in the human body. The complete structure of the glucose tolerance factor is yet uncertain, as is the exact relationship between chromium (Cr) deficiencies, diabetes, and cardiovascular diseases, such as coronary disease.[7,8] Cr^{3+} is expressed as Cr in later descriptions in this paper. On the other hand, hexavalent chromium is known to be toxic to humans, capable of permeating cell membranes, a powerful mutagen for humans, and a potential carcinogen.[9–11]

The determination of Al and Cr in environmental and biological samples, such as natural and drinking water, renal dialysate, parenteral solution, pharmaceutical ingredients including organic acids, and the like, is very important. Therefore, there is an urgent need for the development of sensitive and reliable techniques to determine them in low concentrations. Many different analytical techniques have been developed to this end. The most commonly used methods are graphite furnace atomic absorption spectrophotometry (GF-AAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES). Airborne dust contamination, matrix interference of biological samples, and insufficient precision in $\mu g\,L^{-1}$ close to detection limit, make determination of Al by GF-AAS rather difficult. Inductively coupled plasma atomic emission spectrometry requires complicated and expensive equipment, of which operation and maintenance are costly. It cannot be used for such samples as serum and the like because of its relative lower sensitivity for Al and Cr.

High performance liquid chromatography of metal chelates has been successfully applied to trace metal analysis using various derivatizing reagents, because the method gives good and wide calibration curves compared to above-mentioned procedures. In addition, sensitivity and reproducibility are also good and the method requires no special technique, except for usual HPLC apparatus. A number of papers were published relative to the determination of Al and/or Cr in form of complexes with ligands by RP-HPLC. However, most authors only made other multi-metals determination, and paid little attention to simultaneous analysis of trace Al and Cr. There have been few reports about the successful examples for the purpose. It is likely because of:

(1) anomalous bandspreading caused by unstable complexes,[12]
(2) more than one peak attributed to different complexing ratio,[13] and
(3) absence of separation owing to the similarity between the two.

8-Hydroxyquinoline has the advantages of high reaction ability, short reaction time, the formation of stable resolvable products with Al and Cr, which are the only two complexes exhibiting good chromatographic behavior.[14] The chelates have high sensitivity for UV-detection. However, in those normal UV analyses of Al and/or Cr after HPLC process using 8-HQ as the chelating reagent, the interference from each other is appreciable. Moreover, no HPLC method giving an acceptable resolution of Al and Cr in mixture and simultaneous determining these two species as 8-HQ chelates, has been reported because of the high degree of overlapping of Al–8-HQ and Cr–8-HQ.[14–28] Kalman filter deconvolution has been used to "calculate" the quantitative results to partial separation of Al and Cr by RP-HPLC on a C_{18} column after precolumn complexation with 8-HQ.[18] The way the baseline is drawn is a critical problem in chromatogram, as it can significantly affect the analytical results. On the other hand, this algorithm is too sophisticated to be applied in practical samples.

Even using expensive acetonitrile and methanol as the mixed organic modifier in mobile phase, Al–8-HQ and Cr–8-HQ were not sufficiently separated by RP-HPLC.[21] Although, the optimized experimental condition allowed reluctant determination at higher concentration, for example, in tannery sludge acid extracts, degree of peak overlap between these two chelates made the trace analysis difficult and unreliable. Therefore, it is not surprising that in most publications about the determination of Al or Cr by HPLC as 8-HQ chelate, Cr or Al is ignored.

It is known that the fast kinetics of Al with 8-HQ allows "on-column" or "on-line" chelation at room temperature. In contract, the complexation of Cr with 8-HQ is rather slow, heating at 90°C for at least 30 min is needed to complete the reaction.[14,18,21] Experimentally making the chromatographic peaks of Al and Cr appear at the same time is an easy thing to do. Thus, it is thought by us, that utilizing this disparity in the reaction rate would provide a good procedure to the problem, which is divided into two stages:

(1) determining Al after precolumn chelation at ambient temperature;
(2) determining the sum of Al and Cr after reaction at 90°C for at least 30 min.

The amount of Cr was calculated by the difference from the total amount. The goal of this paper is to develop a simple, sensitive, and reliable HPLC method for the stepwise determination of Al and Cr, using UV detection with 8-HQ as a precolumn reagent.

EXPERIMENTAL

Reagents and Samples

All reagents used in this study are of analytical-reagent grade unless stated otherwise, and used without further purification, except 8-HQ that is recrystallized twice. High performance liquid chromatography-grade methanol is obtained from Tedia Company, Inc. (Fairfield, OH). Twice distilled water from a quartz device is used for all solutions. Methanol–water (57 + 43, v/v) is used as the mobile phase. It contains 4.0 mmol L^{-1} ammonium acetate–acetic acid buffer (pH 6.0), and 3.2 mmol L^{-1} 8-HQ, unless otherwise indicated. The mobile phase is filtered through a 0.45 μm membrane (Millipore, Bedford, MA) and degassed in an ultrasonic bath prior to use. Aluminum, Cr, and other metal ion standards of a desired concentration, are prepared from ICP-AES grade stock solutions (1000 μg mL^{-1}) provided by ICP-AES laboratory, Center of Materials Analysis, Nanjing University (CMANU). Standard solution of hexavalent chromium is

prepared by appropriate dilution from 1000 μg mL^{-1} stock solution made from potassium dichromate.

Drinking mineral water is purchased from a supermarket in Nanjing. Renal dialysate is prepared in our laboratory. Parenteral solution containing Cr and Rat serum are presented by College of Public Health, Nanjing Medical University. Fumaric acid (*trans*-butenedioic acid) for pharmaceutical purpose is provided by Nanjing Pharmaceutical Plant, of which the purity is analyzed by ion-suppression RP-HPLC method as reported elsewhere.[29]

Apparatus

The chromatographic analysis is performed with a Waters 510 solvent pump, a Waters 486 tunable absorbance detector (Waters, Mildford, MA), and a Rheodyne 7725i injector valve with a 50 μL loop (Rheodyne, Cotati, USA). The analytical column is a Phase Separation Spherisorb ODS 2, 5 μm (15 cm × 4.6 mm I.D.) column from Dalian Johnsson Separation Science & Technology Corporation (Dalian, PRC). A Waters Guard-Pak precolumn module with a Nova-Pak C_{18} insert (Waters) is used to protect the analytical column. The chromatograms are measured with a Model JS-3030 chromatographic working station (Dalian Johnsson), together with a Yokogawa Hokushin Electric Type 3066 pen recorder (Sino-Japanese Sichuan Fourth Meter Factory, Chongqing, PRC).

An inductively coupled plasma quantometer, Model 1100 (Jarrell—Ash Company, Boston, MA), is employed for the determination of the total metals in all samples. An atomic absorption spectrophotometer equipped with a graphite furnace atomizer, Model 3510 (Hewlett-Packard–Shanghai Analytical Products Co., Ltd, Shanghai, PRC), is employed for determining Al concentration below 0.01 μg mL^{-1} and Cr concentration below 0.005 μg mL^{-1} in partial samples.

A Mettler Toledo 320 pH meter equipped with an HA405-K2/120 combination electrode (Mettler-Toledo Instruments Shanghai Co. Ltd, Shanghai, PRC) is used for pH measurement.

All glassware and high-density polyethylene containers are carefully treated with 2 mol L^{-1} nitric acid and rinsed several times with twice distilled water.

General Procedure

An aliquot of metal ion solution is placed in a 10 mL volumetric flask. One milliliter of 1% (m/v) hydroxylamine chloride solution is added. Then, 0.5 mL of 1.0 mol L^{-1} acetate buffer (pH 6.0) and 1.0 mL of 20 mmol L^{-1} 8-HQ solution in methanol are added. The solution is sonicated for 15 min and diluted to the mark with methanol (solution A). 8-Hydroxyquinoline is sparingly soluble in water and

form chelates of metals that are insoluble. The content of methanol is more than 40% (v/v), in order to maintain the solubility of hydrophobic chelates in the resulting aqueous mixture. Meanwhile, the same aliquot of metal ion solution is placed in another 10 mL volumetric flask, and all reagents in solution A are added. Instead of being sonicated, it is heated for more than 30 min at 90°C, carefully, to complete Cr–8-HQ complex formation. After cooling, methanol is added to the mark again (solution B). A part of each solution is filtered through a membrane with 0.45 μm micropore (Milipore). Fifty microliter of the filtered solution A and B are injected onto the column, respectively. The column temperature is 30°C. Elutions are achieved at 1.0 mL min^{-1}. The detection wavelength is 380 nm.

The blank test is necessary to surmount the possible interference from metals, particularly Al, at trace levels in the reaction medium. The concentration of Al is determined from Al–8-HQ peak area of solution A. The peak area in solution B, at the same retention time, represents the total amount of Al and Cr. Consequently, the concentration of Cr is determined by calculating the area deference between solution B and solution A at the corresponding time. Analysis of individual samples is carried out in triplicate. All procedures are carried out in an around 25°C constant room temperature.

The samples considered in this investigation are pretreated as follows: Mineral water—An adequate amount of nitric acid (guaranteed reagent, Shanghai First Chemical Reagent Factory, Shanghai, PRC) is added to 5.0 mL of water sample in 10 mL volumetric flask, the pH of the sample being adjusted to 1.5–2.0. The other procedure for the acidified sample is the same as the preceding description.

Serum, synthetic renal dialysate, and parenteral solution—Sample (1.0 mL) is digested with 5 mL nitric acid and 0.5 mL perchloric acid (guaranteed reagent, Shanghai Taopu Chemical Plant, Shanghai, PRC) for two hours, and the digest is evaporated to near dryness. The cooled residue is diluted with water to 5.0 mL and then transferred to a 10 mL volumetric flask. This solution is treated as indicated above.

Fumaric acid—A 0.15 g powder sample is weighed and digested, and finally dealt with as serum sample.

RESULTS AND DISCUSSION

Chromatographic Separation and Interference Study

Figure 1 shows liquid chromatograms of Al and/or Cr standard solutions generated in this work. The chromatographic patterns are the same for aqueous calibrators and all sample solutions. The peaks at 5.8 min represent the coeluted

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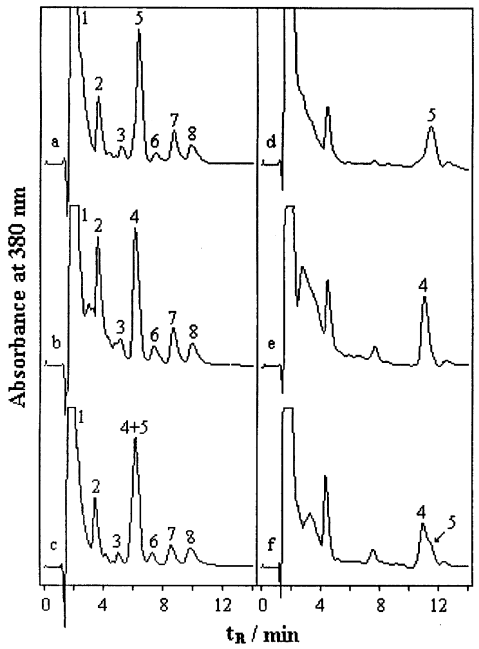

Figure 1. Typical chromatograms for metal-8-HQ chelates. Column: Spherisorb ODS 2, 5 μm, 15 cm × 4.6 mm I.D. Column temperature: 30°C. Mobile phase: methanol–water (v/v) containing 4.0 mmol L^{-1} ammonium acetate–acetic acid (pH 6.0) and 3.2 mmol L^{-1} 8-HQ. The left-hand: 57% methanol–43% water; the right-hand: 50% methanol–50% water. Flow rate: 1.0 mL min^{-1}. Injection volume: 50 μL. Wavelength used for UV detection: 380 nm. a, d. 0.2 μg mL^{-1} Al; b, e. 0.2 μg mL^{-1} Cr; c, f. 0.1 μg mL^{-1} Al + 0.1 μg mL^{-1} Cr. Peaks: 1, 2. 8-HQ; 3. Co–8-HQ + reagent blank; 4. Cr–8-HQ; 5. Al–8-HQ; 6. Reagent blank; 7. Fe–8-HQ; 8. Mn–8-HQ.

Al–8-HQ and Cr–8-HQ, which are distinctly separated and recognized from unreacted ligand, and other metal complexes.

The peaks of Al–8-HQ and Cr–8-HQ emerge at the same time, however, they differ slightly in shape as shown in Fig. 1a and b. The peak of Al–8-HQ is a little wider than that of Cr–8-HQ, presumably because of the degradation of the former on hydrophobic stationary phase during the elution with aqueous mobile phase.[28] Therefore, the quantitation from the peak area should, experimentally, give better accuracy.

Earlier observation[28] has shown that Ca, Mg, Na, K, Si, Sr, Cu, Zn, Fe, and Mn, which are the abundant or sub-abundant elements in drinking and natural waters do not interfere with Al determination in the assay. This is, because no

complexation happens or no coelution with Al–8-HQ occurs. In the present work, standard solutions containing $0.2\,\mu g\,mL^{-1}$ of Al are spiked with Cd, Pb, Co, Ni, Ga, In, La, Y, and Zr at levels of $2.0\,\mu g\,mL^{-1}$ and then analyzed. The only ions that give significant response are Co, Ni, and Ga. Ni–8-HQ covered by excess 8-HQ is far from Al and/or Cr chelates, so Ni does not interfere. The labile Co^{2+}–8-HQ is oxidized to the stable Co^{3+} chelate by air,[14] the peak of which (4.8 min) is close to that of Al and/or Cr chelates, Co displaying interference. This trouble is relieved significantly when the concentration of Co decreases below $2.0\,\mu g\,mL^{-1}$, which is greatly higher than normal levels found in waters and biological samples. Ga–8-HQ (6.1 min) is eluted just after Al and/or Cr chelates and is completely unresolved from the Al and/or Cr chelates once its concentration is over $0.2\,\mu g\,mL^{-1}$, generating a severe interference. Fortunately, it is scarcely present in all samples examined in this work.

Reaction Conditions

It is well known that trivalent Cr forms a more stable chelate with 8-HQ at a pH of around 6.0. $Cr_2O_7^{2-}$ that does not react with 8-HQ, probably owing to extremely high and negative hydration enthalpy ($-1490.3\,kJ\,mol^{-1}$) and free energy ($-1301.1\,kJ\,mol^{-1}$), can be reduced to Cr^{3+} by hydroxylamine chloride and detected with the proposed method. The samples dealt with in this work vary in ionic strength and pH. It is also known Al–8-HQ chelation takes place over wider pH range than Cr–8-HQ. The chromatographic behavior and the UV absorptive spectrum of 8-HQ chelate, as well as unreacted ligand that is an amphoteric substance and produces a group of peaks, are dependent on these parameters. It would be advantageous for the reaction medium to be buffered; therefore, an ammonium acetate concentration of $50\,mmol\,L^{-1}$ at pH 6.0 is selected to accommodate alteration in sample pH and ionic strength during chelation.

In addition, the molar ratio of 8-HQ to Al and/or Cr of 50–500 : 1 is recommended for the chelates formation prior to HPLC analysis, in order to minimize the effect of excess 8-HQ.

Previous study has indicated, that as long as sonication time exceeded 15 min, the concentration of the chelate does not vary, at least not in 30 days, because of the fast kinetics in suitable mediums. This procedure has the advantage of being simple, convenient, and mild, and results in optimum response to Al.[28] However, no Cr–8-HQ chelate is formed under the same condition and even after two hours of sonication at ambient temperature in an ultrasonic bath. The slow kinetics of Cr–8-HQ formation compared to that of Al–8-HQ makes heating necessary to complete complexation of the Cr. Showing the change in detector response with temperature and heating times, Fig. 2 indicates that the

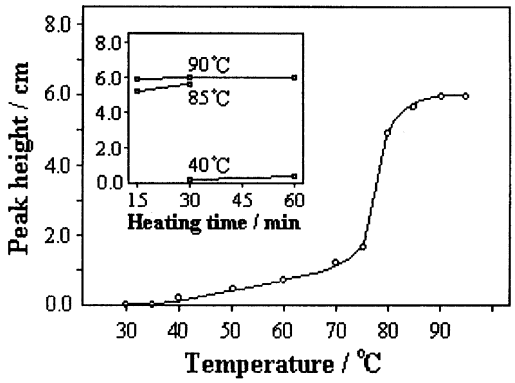

Figure 2. Effects of temperature and time on Cr–8-HQ complexation. Chromatographic conditions as in the left-hand of Fig. 1.

formation of the chelate is mainly dependent on temperature. Although the heating time is one hour, few Cr–8-HQ is produced at 40°C. The main S-shape curve is obtained from the reaction of 30 min at different temperatures. Heating at 90°C for 30 min is chosen for further work, under which conditions the detector response is close to the maximum. In the meantime, we process aqueous solutions containing 0.2 μg mL^{-1} of Al with sonication for 15 min and with heating at 90°C for 30 min, respectively, and analyze them by the recommended method. The Al–8-HQ chelate peaks detected between these two solutions do not differ significantly.

High Performance Liquid Chromatographic Conditions

It is demonstrated that both Al and Cr can form stable chelates with 8-HQ, each of which shows a satisfactorily sharp chromatographic peak when injected individually although Al–8-HQ peak widens slightly. Under all the chromatographic conditions reported previously, however, Al–8-HQ and Cr–8-HQ peaks showed extensive overlapping and gave no acceptable resolution.

The optimization of separation of Al–8-HQ and Cr–8-HQ is attempted employing compositional changes of eluent, i.e., the choice of content of methanol utilized as organic modifier. Usually, the resolution between analysts separated on reversed-phase column should increase when methanol content in mobile phase decreases. However, this effect on the separation of Al–8-HQ and Cr–8-HQ is insignificant. A decrease to 50% (v/v) in methanol concentration

leads to only minor improvement in resolution but serious increase in peak width and unregular deterioration in peak shape, especially for Al–8-HQ as presented in Fig. 1d, e, f. The result further indicates that Al–8-HQ chelate decomposes in a low methanol concentration. The most suitable methanol content in mobile phase is 57% with exact superposition of Al–8-HQ and Cr–8-HQ peaks, which makes simultaneous determination of Al and Cr, by virtue of their different kinetics with 8-HQ, achievable. Also, resolution of Al–8-HQ and Fe–8-HQ is baseline completed (more than 1.5) and that of Al–8-HQ and Co–8-HQ is practically completed (near 1.5). Additionally, this mixture as compared to the reaction medium is enriched in methanol content, in order to minimize the risk of on-column decomposition of the chelates.

The coordinate bonds of Al–8-HQ complexes is prone to hydrolysis in the aqueous elution and easily deteriorates by strong hydrophobic interaction with reversed phase stationary phase, as previously indicated.[28] For this reason, an appropriate amount of the ligand is added in the eluent to eliminate the dissociation. The effect of 8-HQ concentration upon peak height of Al chelate under different methanol content in the mobile phase has been investigated in earlier studies. The finding that the lowest 8-HQ concentration makes the peak height maximum and stability increases with decreasing methanol content implies that these two factors are mutually complementary in maintaining the stability of the chelate. Despite the slow reaction kinetics, Cr–8-HQ exhibits excellent chromatographic behavior for its potent stability, even in the absence of 8-HQ in the mobile phase. However, the presence of $3.2\,\mathrm{mmol\,L^{-1}}$ 8-HQ in the eluent is carried out to have all chelates of various metal ions, including Al, easily chromatographed.

This work is aiming at simultaneous determination of Al and Cr. Chromium forms a more stable chelate with 8-HQ than Al does. On the other hand, changes in pH and ionic strength of the mobile phase can convert existing forms of 8-HQ, so that an erratic baseline and a poor chromatogram take place when an inadequate buffer, e.g., pH < 5 is used. In this case, of course, no variation in the retention times of Al and Cr chelates occurs due to their neutrality and hydrophobicity. Therefore, the final ammonium acetate (pH 6.0) concentration of $4.0\,\mathrm{mmol\,L^{-1}}$ in the mobile phase is chosen for further work. At this concentration, the baseline is stable and flat; the peak height is close to maximum.

Baiocchi[14] supposed that the lability of the Al chelate is attributed to the very small ionic radius of Al and its high and negative enthalpy of hydration. This inference is supported by our experimental results described above. Aluminum has the smallest radius among all the ions mentioned in this paper, except Si. Larger ligands around the "mini" ion tend to repel each other due to space effect. Additionally, enthalpy and free energy of aqueous Al ion formation are almost equal to those of alkali-earth metal ions, which are unable to form chelates with 8-HQ under the present conditions.

Detection Wavelength

The optimum detection wavelength of Al–8-HQ chelates has been reported to be 380 nm. Under the same conditions, the peak height of Cr–8-HQ at the wavelength range of 350–500 nm is shown in Fig. 3. Chromium chelates give an apex at 400 nm that has been employed by all analysts as the detection wavelength. Meanwhile, a high absorption platform appears from 380 to 410 nm, in which region the presence of excess ligand makes for weaker background absorption and insignificantly influences the chelate detection. It has been well known for a long time, that the maximum absorption related to the chelate is around 420 nm by spectrophotometry. In the present mobile phase used, the maximum of Cr–8-HQ is probably shifted towards a shorter wavelength, like Al–8-HQ.[28] As a result of compromise, the wavelength of 380 nm is used in later experiments because the peak height of Al chelate increases by near 40%, but that of Cr chelate decreases only by less than 5% over 400 nm.

Calibration and Detection Limit

Injections covering a wide range of Al or Cr concentrations are made to investigate the linearity of the detector response. The linear relationship of peak area (A_{Al} or A_{Cr}) vs. concentration of standard is maintained by Al or Cr concentration of $0.6\,\mu g\,mL^{-1}$. The equations are $A_{Al} = 301{,}789C_{Al} - 78$ ($r = 0.9998$) and $A_{Cr} = 280{,}694C_{Cr} + 57$ ($r = 0.9996$), respectively. The relative standard deviation (RSD) for five replicate determinations of $0.2\,\mu g\,mL^{-1}$ Cr is 1.6%. At the same time, the linear regression between the difference in peak area (ΔA) vs. concentration of the Cr standard carried out within the range 0.002 to

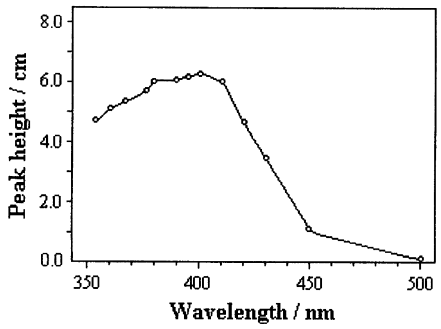

Figure 3. Effect of wavelength on Cr–8-HQ peak height. Chromatographic conditions as in Fig. 2.

Table 1. Determination of Al and Cr in Real Samples ($n = 3$)

	Aluminum Concentration/$\mu g\,mL^{-1}$				Chromium Concentration/$\mu g\,mL^{-1}$			
No.	Detected ± SD	Added	Found ± SD	Other Method	Detected ± SD	Added	Found ± SD	Other Method
1	0.172 ± 0.003	0.150	0.317 ± 0.002	0.203[a]	≤ 0.002	0.020	0.021 ± 0.002	≤ 0.002[b]
2	0.006 ± 0.001	0.005	0.012 ± 0.001	0.006[b]	≤ 0.002	0.015	0.016 ± 0.001	≤ 0.002[b]
3	0.011 ± 0.001	0.020	0.030 ± 0.001	0.010[b]	0.006 ± 0.001	0.020	0.026 ± 0.002	0.005[b]
4	0.158 ± 0.004	0.300	0.451 ± 0.012	0.150[a]	0.100 ± 0.009	0.150	0.269 ± 0.020	0.105[a]
5	0.177 ± 0.002	0.150	0.336 ± 0.004	0.195[a]	0.031 ± 0.002	0.040	0.069 ± 0.005	0.026[a]

Sample solutions: 1. Xindongyang mineral water; 2. Synthetic renal dialysate; 3. Serum; 4. Parenteral solution; 5. Fumaric acid.
[a]Data by ICP-AES.
[b]Data by GF-AAS.

$0.25\ \mu g\ mL^{-1}$ (with the presence of same concentration of Al), is $\Delta A = 285365\ C_{Cr} - 750$ ($r = 0.9996$). In this case, the RSD is 3.9% for $0.2\ \mu g\ mL^{-1}$ Cr ($n = 5$). The slope of curve for ΔA–C_{Cr} is almost the same as that for A_{Cr}–C_{Cr}, indicating that the proposed procedure is feasible. A wider range of Cr concentration is not studied by this differential method, because of the low content in normal samples. The detection limit, calculated as the concentration that gives a signal three times the standard deviations of the blank, is $0.001\ \mu g\ mL^{-1}$ for both Al and Cr (0.05 ng in injection volume) under the optimum chromatographic conditions. The chromatogram of the reagent blank (not shown) exhibits a background of Al or Cr concentration of $0.001\ \mu g\ mL^{-1}$, mainly Al, in 8-HQ and ammonium acetate used. In contrast with the former experiment where the 8-HQ was not pretreated, recrystallization can reduce the blank to at least half.

After reaction with hydroxylamine chloride, the fact that $Cr_2O_7^{2-}$ gives identical peaks both in shape and height as equal molar Cr^{3+} indicates the quantitative conversion towards Cr^{3+}, and makes the indirect determination of hexavalent chromium possible.

Analysis of Real Samples

The analytical results of mineral water and some biomedical samples are listed in Table 1. They are in good agreement with those obtained by ICP-AES or GF-AAS. Typical chromatograms obtained for these samples are not shown. In order to evaluate the validity of analytical data, known amounts of Al and Cr are added to each sample solution and then the analysis is performed in the usual way. The recovery lies between 93.5–116.7% and confirms that there is no problem duo to the matrix effects. The RSDs are 0.65–13.6% for both sample analysis and recovery test. The satisfactory recovery and precision indicated that the proposed method is reliable for simultaneously determining Al and Cr in water and biomedical samples.

ACKNOWLEDGMENTS

This project is supported by the National Natural Science Foundation of China (No. 29777013) and the Analysis and Test Fund of CMANU. The authors thank Waters Collaborating Model Laboratory in CMANU for technical support.

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Received February 16, 2002
Accepted May 23, 2002
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