

逆相 HPLC を用いた環境水中の Cr(VI) の定量

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Determination of hexavalent chromium in environmental water using reverse phase HPLC

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要 旨

Cr⁶⁺を有する重クロム酸イオンを環境水中から逆相HPLCにより定量分析する方法を検討した。重クロム酸イオンは極大波長350nmに強い吸収を有するので、逆相系カラムによる保持時間の相互分離が不十分でも410, 575nmに極大を有するCr³⁺に邪魔されることなく、クロマトグラム上分離が実現できた。検量線は0.01-10mMの範囲で良好な直線性を示し (R²=0.9999, ピーク高さ)、ミネラル水を用いた添加回収試験も回収率90.2-104.7% (ピーク面積) と満足いくものであった。応用例としてCr³⁺を添加した水道水を試料として用いた時、Cr(VI)化合物の生成が時間経過と共にモニター可能であった。

キーワード：重クロム酸, HPLC, 環境水試料

Abstract

We investigated a quantitative method of dichromate ion having hexavalent chromium from environmental water by reverse phase HPLC. Dichromate ion has strong absorption at the maximum wavelength of 350 nm and is different from Cr³⁺ having the maximum wavelength at 410,575 nm. Therefore, even if mutual separation of retention times was insufficient, separation could be realized on the chromatogram without being disturbed by trivalent chromium. The calibration curve shows good linearity in the range of 0.01 to 10 mM (R² = 0.9999, peak height), and the recovery test using mineral water is also satisfactory with a recovery rate of 90.2 to 104.7% (peak area). As an application example, when tap water with Cr³⁺ was used as a sample, generation of hexavalent chromium could be monitored over time.

Key words: dichromate, HPLC, environmental water sample.

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Introduction

Chromium (Cr) has various effects and toxicity¹⁻⁴. For mammals, trivalent chromium (Cr(III)) is associated with glucose metabolism^{1,2}, while hexavalent chromium (Cr(VI)) is known to induce mutations and carcinogenic activity even at low concentrations^{3,4}. Cr exists mainly in these two forms (Cr(III) and Cr(VI)) in environment samples such as river water and biological samples such as blood^{5,6}. Since the difference in these forms greatly affects the action, it is considered that the concentration of Cr is more important by valence number rather than by total amount. Especially Cr(VI) compounds are mainly composed of chromate and dichromate^{3,4}, but due to the seriousness of its toxicity, it is highly necessary to quantify separately from Cr(III)³.

In general, inductively coupled plasma mass spectrometry (ICP-MS) and atomic absorption photometry (AAS) are often used to measure the total amount of Cr. On the other hand, as a quantitative analysis by valence, there is a report applying Cr(III) quantification method⁷. First, Cr(III) is quantitatively determined, then reduction treatment is carried out, and the increase is regarded as Cr(VI). However, this method is complicated, and when the concentrations of both Cr(III) and Cr(VI) are largely different, the measurement error becomes too large and accurate measurement becomes difficult.

Therefore, we examined the method of quantitative analysis of Cr(VI) compound using HPLC in this study. If this method can be used to establish separation conditions properly, Cr(VI) compound should be measured directly and relatively easily. For the HPLC column, we decided to use a highly versatile reverse phase system. Since the main compounds of Cr(VI) are

dichromate under acidic conditions, they were investigated to quantitatively determine them.

Methods

1. Reagents

For potassium chromate and potassium dichromate, special grade reagent manufactured by Wako Pure Chemical Industries (Osaka, Japan) was used. As a sulfuric acid used as an eluent, a special grade reagent manufactured by Wako Pure Chemical Industries was also used. The water used for reagent preparation and HPLC analysis was purified by Advantage (Merck-Millipore, Billerica, MA) to a specific resistance value of 18 MΩ · cm or more.

2. Apparatus

As the HPLC apparatus, a high-performance liquid chromatography Prominence system manufactured by Shimadzu Corporation (Kyoto, Japan) was used. The system was composed of a pump unit LC-20AT (used at 1 mL/min), a low pressure gradient unit LC-20AD/T, a deaerator DGU-20A3, a column oven CTO-20AC (40°C), a photodiode array (PDA) detector SPD-M20A, and an autosampler SIL-10AF, and controlled by the system controller CBM-20A and LC workstation LC Solution Multi-PDA. For the analytical column, Senshu Pak PEGASIL ODS SP 100 AQ (4.6 φ × 250 mm; Senshu, Tokyo, Japan) was used.

A spectrophotometer U-5100 (Hitachi, Ibaraki, Japan) was used for measuring absorption spectrum and absorbance in the preliminary test.

3. HPLC separation of chromium compounds

The compounds to be measured in this study become dichromate ion and chromate ion in water. These are all anionic substances. For analysis by

HPLC, the retention behavior on the analytical column must be varied and time resolvable. In the preliminary test, the mutual separation was confirmed by using an anion exchange column (Senshu Pak SAX-1251-N, 4.6 ϕ \times 250 mm) and a reverse phase column (Senshu Pak PEGASIL ODS SP 100, 4.6 ϕ \times 250 mm) with absorbance detection⁸⁾. The reversed phase type column was more excellent in peak shapes and mutual separations, and it was considered that changing the conditions was also easy. Therefore, we used a reversed phase column in this study, and examined more detailed detection conditions below. We also investigated the quantitiveness of the analytical method determined.

4. Addition of Cr(III) to tap water

As an application example of this analysis method, 0.01 mM Cr³⁺ was added to tap water. The state that it was oxidized by residual chlorine (containing about 0.5 ppm of chlorine) and changed to Cr(VI) was continuously monitored according to the operation and analysis time of HPLC autosampler.

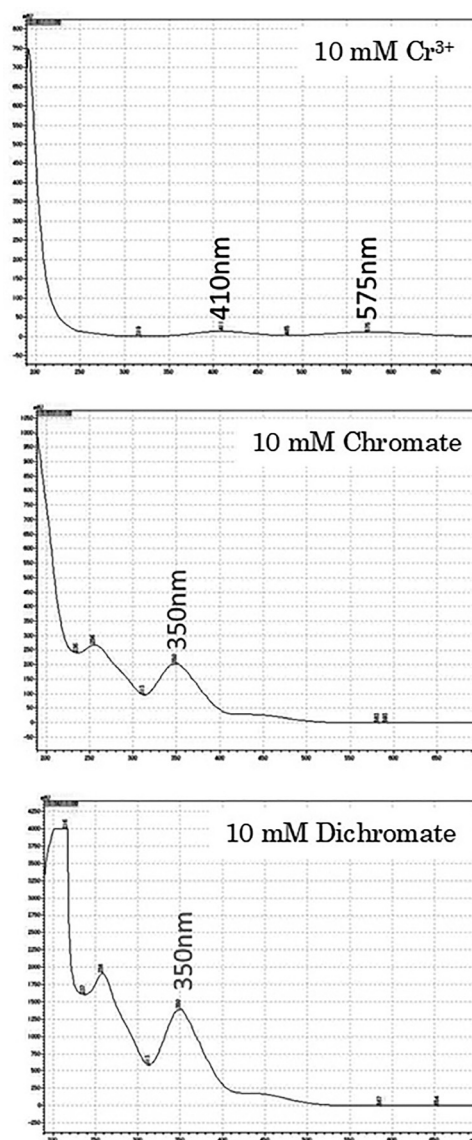
Results and Discussion

1. HPLC conditions

The absorption spectra of Cr³⁺, chromate and dichromate are shown in Fig. 1. Since the absorption maximums of Cr³⁺ and Cr(VI) compounds are different and the absorbance of Cr³⁺ is small, it was possible to selectively detect only two kinds of Cr(VI) compounds by using a detection wavelength of 350 nm. Based on the results of the preliminary tests, the peak shape is good in the reversed phase column, but it is considered that it is not preferable to add the organic solvent into the mobile phase because of its weak retention, so that a 100% water available

column (Senshu Pak PEGASIL ODS SP100 AQ, 4.6 ϕ \times 250 mm) was used in subsequent experiments, and the eluent was further investigated. Although separation of chromate and dichromate could be realized when sulfuric acid was used as eluent (Fig. 2), it changed from chromate to dichromate with pH decrease. In general it is presumed that preservation of actual environmental water samples for the purpose of metal analysis will be carried out under acidic conditions many times, so the subsequent study will be conducted only for dichromate.

Fig. 1 Spectra of Cr³⁺, chromate and dichromate



Measurement was performed by using with the PDA detector of HPLC system.

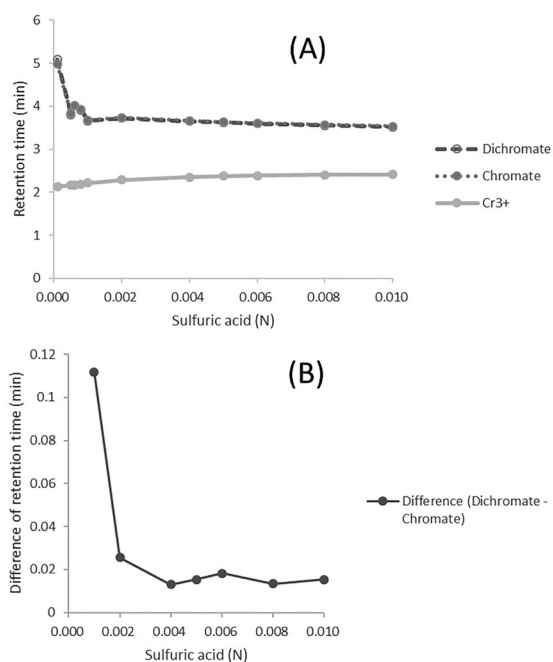


Fig. 2 Retention time (A) and resolution (B) of chromate and dichromate
Injection volume: 10 μ L.

2. Quantitative analysis

A calibration curve of dichromate is shown in Fig.3. At the injection of 10 μ L, the peak height showed good linearity in the range of 0.01–10 mM and the peak area in the range of 0.03–10 mM ($R^2 = 0.9999$ and 0.9998 , respectively). Table 1 shows the results of adding dichromate to mineral water. The recovery rate was good as 90.2–104.7% (depending on the peak area). The lower detection limit was 50 ppb or 5.2 ppb (at sample injection volume of 10 and 100 μ L respectively). Since the environmental standard in the Ministry of the Environment is 50 ppb (concentration in soil eluate) or 500 ppb (wastewater standard), quantification is somewhat difficult but it was found to be detected qualitatively by using with this method without any problems.

3. Application to real samples

A 0.01 mM Cr³⁺ was added to tap water and analyzed with the passage of time using the function of the HPLC autoinjector. Cr(VI)

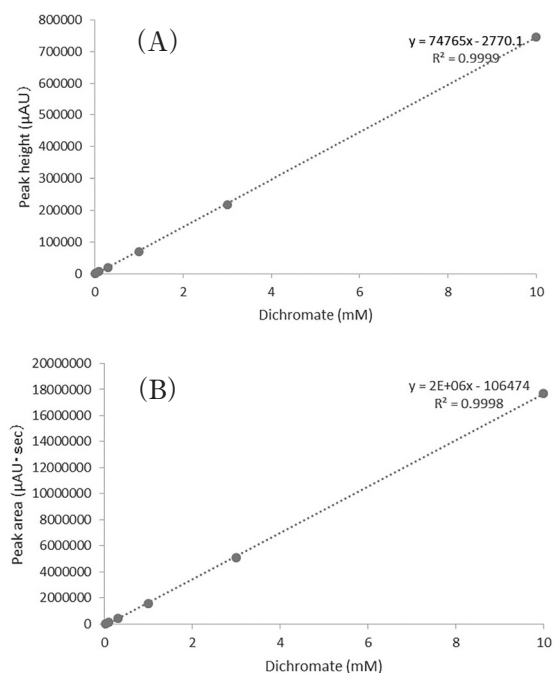


Fig.3 Calibration curve of dichromate ((A) Peak height method, (B) Peak area method)
Eluent: 0.001 M sulfuric acid. Injection volume: 10 μ L.

Table 1 Recovery Test of Dichromate from a Mineral Water

Cr ⁶⁺ (mM)	Peak height		Peak area	
	Recovery (%)	CV (%)	Recovery (%)	CV (%)
0.001	91.8	1.1	90.2	1.5
0.005	98.5	2.2	97.1	1.9
0.009	104.9	2.0	104.7	0.7
0.01	88.6	3.8	90.2	9.7
0.05	100.2	1.0	102.8	1.3
0.09	100.6	0.5	100.4	0.2

Injection volume: 100 μ L. N = 3.

compounds produced by oxidation by residual chlorine contained in tap water were monitored. The results are shown in Fig. 4. Since Cr(VI) compounds were expected to be trace amount, the injection volume to HPLC was 100 μ L. The resulting Cr(VI) is below the range of the calibration curve, so the concentration should be kept informative. The sample used this time is approximately neutral, it is acidified only during

analysis. Therefore, it was considered possible to measure both chromate and dichromate. However, in view of retention time, the compound directly detected was dichromate. From this result it is difficult to declare that only dichromic acid is produced in tap water. It was considered that the change of Cr^{3+} to Cr(VI) compound was easily observed, which is one outcome.

From the above, it is possible to detect Cr(VI) compound in the actual sample although it is not known to details of the form. However, the problem in this study is that the retention in the column is weak. For example, simultaneous analysis with Cr^{3+} and separation from other coexisting substances is incomplete in more complicated matrices. In order to solve this problem, it is considered to connect the columns used this time in series, to extend the retention time by using longer columns, or to connect the columns of the ion exchange and the reversed phase columns in series, are necessary⁹⁾. It will be a future task to further try these to achieve more complete separation and apply it to simultaneous analysis with other components.

Generation of Cr^{6+} was calculated mechanically by peak area method.

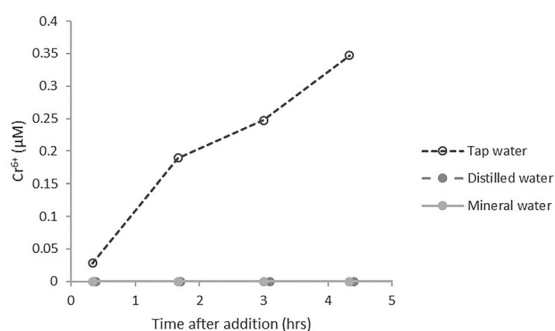


Fig. 4 Generation of Cr^{6+} from Cr^{3+} in a tap water

An original sample: tap water (0.5 ppm residual chlorine included) + 0.01 M Cr^{3+} . Injection volume: 100 μL .

Conclusion

In order to quantify Cr(VI) compound, HPLC method using reversed phase column was studied. Unfortunately, it was considered difficult to quantify chromate and dichromate separately, considering measurement conditions and preservation of samples. This method was comparatively superior in quantitativity and lower detection limit, and was quantifiable even at concentrations below the environmental standard value of wastewater.

References

- 1) Kristin R. Di Bona, Sharifa Love, Nicholas R. Rhodes, DeAna McAdory, Sarmistha Halder Sinha, Naomi Kern, Julia Kent, Jessyln Strickland, Austin Wilson, Janis Beaird, James Ramage, Jane F. Rasco, John B. Vincent (2011) Chromium is not an Essential Trace Element for Mammals: effects of a "low-chromium" diet. *J. Biol. Inorg. Chem.*, 16: 381-390.
- 2) Munehiro Yoshida (2012) Is Chromium an Essential Trace Element in Human Nutrition? *Jpn. J. Hyg.*, 67: 485-491.
- 3) Mehdi Karimi, Alireza Badiei, Ghodsi Mohammadi Ziarani (2016) SBA-15 Functionalized with Naphthalene Derivative for Selective Optical Sensing of $\text{Cr}_2\text{O}_7^{2-}$ in Water. *Anal. Sci.*, 32: 511-516.
- 4) Masato Sasaki, Tokuo Shimizu, Nobuo Uehara (2016) Speciation of Chromium (III) and Chromium (VI) by in situ Extractant Formation Method and Micro Solvent Extraction Method with Hydrophilic Organic Solvent. *Bunseki Kagaku*, 65: 433-438.
- 5) Tetsuo Inui, Kazuhiro Fujita, Masaru Kitano, Toshihiro Nakamura (2010) Determination of Cr(III) and Cr(VI) at sub-ppb levels in water

- with solid-phase extraction/metal furnace atomic absorption spectrometry. *Anal. Sci.*, 26(10): 1093-1098.
- 6) Tetsuo Inui, Wataru Abe, Masaru Kitano, Toshihiro Nakamura (2011) Determination of Cr (III) and Cr (VI) in Water by Wavelength-dispersive X-ray Fluorescence Spectrometry after Preconcentration with an Ion-exchange Resin Disk. *X-Ray Spectrom.*, 40: 301-305.
- 7) Reena Saxena, Niharika Sharma, Shelja Tiwari (2015) Chromium Speciation Using Flow-injection Preconcentration on Xylenol Orange Functionalized Amberlite XAD-16 and Determination in Industrial Water Samples by Flame Atomic Absorption Spectrometry. *Anal. Sci.*, 31: 1303-1308.
- 8) Supachai Maketon, Esther S. Otterson, James G. Tarter (1986) Ion Chromatography with Direct and Indirect Spectrophotometric Detection. *J. Chromatogr.*, 368: 395-400.
- 9) Koji Furukawa, Toshiyuki Kawaguchi, Kiyonobu Kudo, Tomoko Nakazawa, Yuki Yamada, Ryoza Funasaka, Akio Okumura (2016) Analytical Method for Bromic Acid in Drinking Water by LC/MS/MS Using Multimode Column. *Bunseki Kagaku*, 65: 587-592.