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REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF METAL–BENZYLPROPIONITRILE DITHIOCARBAMATE COMPLEXES

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SUMMARY

A new dithiocarbamate, benzylpropionitrile dithiocarbamate (BPDTC), has been synthesized for use in metal analysis. The high-performance liquid chromatography behavior of metal chelates of BPDTC has been investigated for the simultaneous determination of antimony, cadmium, chromium, copper, mercury, nickel, lead, selenium, thallium, and zinc, all of which are on the U.S. Environmental Protection Agency's list of priority pollutant metals. Metals are extracted into dichloromethane as BPDTC chelates, and then separated on a C_{18} column. Cobalt is added as an internal standard. The effects of pH and of three organic modifiers (methanol, acetonitrile, tetrahydrofuran) of the mobile phase on retention time have been investigated. Addition of dichloromethane to the mobile phase increases solubility and chelate stability, and improves the separation of metal BPDTC complexes. BPDTC is added to the aqueous mobile phase to reduce on-column dissociation of the complexes. Detection limits at 260 nm are in the range of 0.1 to 3 μg/l depending on the metal species.

INTRODUCTION

Dithiocarbamates (DTCs) are useful reagents for the extraction, separation, and spectrophotometric detection of many metals due to their abilities to form stable complexes[1,2]. In recent years, disubstituted DTC chelates have been studied by high-performance liquid chromatography (HPLC), with the resultant separation forming the basis for the simultaneous methods of analysis for the metals[3–13]. A variety of separation conditions, detectors, and a number of different DTCs have been used. Both reversed-phase[3–8] and normal-phase[9,10] chromatographic techniques have been utilized. Electrochemical[11,12], argon-plasma emission spectroscopic[13], and atomic spectrometric detection[14] have been evaluated to some extent, but UV–VIS absorption[3–10] is by far the most used detection method.

Reversed-phase HPLC analysis of metal benzylpropionitrile dithiocarbamate (BPDTC) complexes has been investigated. The metals analyzed in this work are those identified by the U.S. Environmental Protection Agency (EPA) as priority pollutants[15]. Included in the list of pollutant metals are antimony, arsenic, beryllium, cadmium, chromium, copper, mercury, nickel, lead, selenium, silver, thallium and zinc. Beryllium was not evaluated since it has a very limited solubility in water under normal pH conditions due to the formation of insoluble $Be(OH)_2$, and, thus, is associated with the particulate rather than the dissolved components of natural systems[15]. Arsenic and silver were also excluded because of the low complexation ability and high precipitation power with halides, respectively. Since chromium exists in two stable oxidation states in aqueous systems, Cr(VI) and Cr(III) were both evaluated, though Cr(VI) is the most environmentally significant. The most common oxidation states of selenium are +4 and +6, represented respectively by selenite and selenate. However, only selenium(IV) was included in this work as selenium occurs in natural water predominantly in the form of selenite[16]. Similarly, due to low solubility of mercurous salts in water[17], only mercury(II) was included in this work among the two oxidation states of mercury. One ppm of cobalt was added in this work as an internal standard. The very low concentration of cobalt in natural water[18] allows its use as an internal standard.

Organic substituents of DTCs do not influence the metal–sulfur bonding, but change some analytical properties of the complexes. The use of BPDTC chelates offers advantages over the other commonly used DTC derivatives due to the characteristics of the two substituents. First, using a benzyl group as a chromophore in the BPDTC results in higher molar absorptivities. Second, disturbance by an excess of the reagent extracted into the organic phase should not be observed, since a propionitrile group of BPDTC allows the reagent, Na–BPDTC, to be readily soluble in water, but very slightly soluble in the organic solvents such as carbon tetrachloride, chloroform, and dichloromethane.

EXPERIMENTAL

Reagents

All chemicals used were of analytical-grade purity unless otherwise stated. Each stock 1000 mg/l metal ion solution was made by dissolution of the respective metal compound in water or diluted nitric acid. The metal compounds used for the stock solutions consisted of three different chemical forms: oxide forms for chromium(VI), selenium(IV) and zinc(II); chloride forms for antimony(III), cadmium(II) and mercury(II); and nitrate forms for chromium(III), cobalt(II), copper(II), lead(II), nickel(II) and thallium(I). Aqueous solutions were made with distilled water filtered through a Barnstead NANOpure II deionization system (Boston, MA, U.S.A). HPLC-grade solvents (acetonitrile, tetrahydrofuran, methanol, dichloromethane) from Fisher Scientific were employed for the chromatographic work to minimize the background absorbance.

Na–BPDTC was not available commercially but was readily prepared in the laboratory by a modification of the procedure described by Moore[19]. All reagents were chilled below 5°C prior to use. A 31-ml volume of 3-(benzylamino)propionitrile was dissolved in 50 ml of a tretrahydrofuran–acetone (1:1) solution. A solution consisting of 12 ml of carbon disulfide in 13 ml of a tetrahydrofuran–acetone (1:1) solution was slowly added with constant stirring. A 20-ml volume of 5.5 *M* NaOH was added dropwise to the solution, while maintaining the temperature below 5°C, and then the solution was stirred for 30 min. The majority of the solvents (tetrahydrofuran, acetone, water) were removed using a Rotavaporator. A 50-ml aliquot of anhydrous diethyl ether was added and the evaporation is continued. The ether evaporation step was repeated until crystallization occurs. The material was then filtered and washed several times with anhydrous diethyl ether. A 77% yield of pale yellowish white crystals with a melting point of 65–66°C was obtained.

Equipment

Liquid chromatography was carried out using a Hewlett-Packard 1084B liquid chromatograph equipped with an autoinjector and scanning UV–VIS detector. A direct-connect cartridge system (Alltech, Avondale, PA, (U.S.A.) with an Econospere C_{18} reversed-phase cartridge (250 mm × 4.6 mm I.D., 5 μm particle size) was used as the analytical column. The operating conditions were as follows unless otherwise stated: injection volume, 10 μl; detection wavelength, 260 nm; mobile phase temperature, 40°C; column temperature, 35°C. An IBM 9420 spectrophotometer was used to obtain UV–VIS spectra of the complexes and a Perkin-Elmer 4000 Series atomic absorption spectrophotometer was used to evaluate the partition behavior of the metal complexes which were prepared by solvent extraction. A Varian EM-360A NMR spectrometer was used to verify the structure of the BPDTC reagent.

Preparation of metal complexes

Liquid-liquid extraction was used for preconcentration of metals as chelates. Aqueous samples were initially buffered with either 1 *M* phosphate buffer(pH 8) or acetate buffer(pH 5) depending on the metal species. A 1-l aliquot of the sample solution was transferred into a 2-l separatory funnel, to which 1 ml of a 0.2 *M* Na–BPDTC aqueous solution was added. The sample was then agitated for 1 min. For the extraction of Cr(III), the reaction temperature was elevated to 40°C for 15 h. A 1-g amount of sodium nitrate, if needed, was added to the solution to break down colloids which can form in the presence of excess ligand. A 25-ml volume of dichloromethane was then added as the extractant solvent, and the mixture was shaken for 2 min and left to separate for 4–5 min. The organic phase is then recovered and reduced to 1 ml under vacuum. A 10-μl volume of the dichloromethane layer was then injected into the C_{18} column described above. Capacity factors (k') were measured after 1 h of system equilibration. The k' values were calculated assuming that each mobile phase solvent was an unretained substance.

RESULTS AND DISCUSSION

Characterization of BPDTC and metal complexes

The proton NMR spectrum of the free BPDTC has been recorded in deuterated methanol. The resonance signals for phenyl protons in the benzyl group lay in the range δ 7.1–7.3, while the signal for methyl protons in the benzyl group appeared at δ 5.6. The triplet signals for two adjacent CH_2 in the acetonitrile group were in the range δ 2.5–2.9 and δ 3.8–4.2, respectively. The signal appearing at δ 4.67 is due to the trace hydroxyl proton in deuterated methanol or moisture in the sample.

The UV-VIS spectra of free BPDTC solution and metal–BPDTC complexes were obtained in water and dichloromethane, respectively. The molar absorptivities of free BPDTC ligand at 259.0 nm (λ_{max}) was 19,500. The significant differences in wavelength of maximum absorbance of the most intense bands for metal diethyldithiocarbamate (DDTC) complexes have been reported[20,21]. The spectroscopic properties of BPDTC and DDTC complexes were compared in Table I. It is evident that the benzyl group in BPDTC imparts higher absorptivities to the complex compared to DDTC and the absorptivity of each metal BPDTC complex at 260 nm was reasonably consistent from metal to metal. Thus, it was possible to achieve multi-component determinations at one wavelength.

Extraction behavior of BPDTC complexes

Extraction of metal ions by DTCs is known to depend on several factors such as extractant solvent, chemical form of the metal ion, pH of the solution and shaking time[22,23]. Dichloromethane was selected as the extractant solvent because of its high extraction efficiency. The effect of pH on the extraction of metals has been investigated. Copper and cobalt exhibited extraction efficiencies of 100% over the entire pH range. The pH 5 was preferred for the extraction of selenium and chromium(VI), while the optimum pH for quantitative extraction of the remaining metal species investigated was around pH 8. Shaking time for the extraction of metal–DTC complexes into dichloromethane was studied at an adjusted pH as described above. All twelve metal species were extracted sufficiently within 2 min except for chromium(III). The chromium(III)–BPDTC complex was not readily formed under the normal conditions. To produce the Cr(III) complex, the reaction temperature was raised to 40°C at pH 5 and 15 h was allowed for the complex formation reaction to proceed before the addition of the extractant solvent. Under this condition, other group I metal species showed good stabilities. Reaction temperature above 50°C, however, caused decomposition of the chelating agent.

TABLE I

COMPARISON OF UV SPECTROSCOPIC DATA FOR METAL–BPDTC AND -DDTC COMPLEXES

Species	*Molar absorptivity (mol^{-1} cm^{-1} l)*	
	BPDTC at 260 nm	*DDTC at (λ_{max})*
Sb(III)	74 500	25 000 (504 nm)[20]
Cd(II)	128 500	
Cr(VI)	29 590	
Cr(III)	24 600	
Co(II)	38 110	23 300 (323 nm)[21]
Cu(II)	53 800	13 000 (436 nm)[21]
Pb(II)	96 750	
Hg(II)	125 500	
Ni(II)	43 050	34 200 (326 nm)[21]
Se(IV)	137 400	
Tl(I)	90 350	
Zn(II)	58 650	

Eluent behavior of BPDTC complexes

An initial chromatogram of BPDTC complexes of twelve metal species was obtained using the binary system methanol–water (75:25), which did not sufficiently resolve the first five metal peaks (antimony, cadmium, lead, thallium, zinc). The solvent strength of the mobile phase was increased in order to improve the separation of the five metals which overlapped at the beginning of the chromatogram. However, the late eluting metals such as chromium(III), mercury and copper exhibited poor peak shape. To adequately separate all twelve species, it was necessary to divide them in two groups as follows: group I [chromium(III), chromium(VI), cobalt, copper, mercury, nickel, selenium], group II (antimony, cadmium, lead, thallium, zinc).

Chromium(VI), which reacted quickly with BPDTC, produced two peaks. However, using the conditions described earlier under which chromium(III) formed a complex, the chromium(III) produced only one peak. The appearance of the second peak of chromium(VI) is presumably because a fraction of the dichromate ion is reduced by the chelating agent to chromium(III) ion which forms a BPDTC complex during the extraction. According to Hope *et al.*[24] the first peak is expected as $Cr(S_2CNRR')_2(OS_2CNRR')$ and the second peak is $Cr(S_2CNRR')_3$, where R is the acetonitrile group (CH_2CH_2CN) and R′ is the benzyl group ($CH_2C_6H_5$). Experimentally, the second peak was identified as Cr(III)–BPDTC complex directly made from Cr(III). The standard curve for two peaks from Cr(VI) and one peak from Cr(III) showed good linearity. Since the ratios between the areas of two peaks from Cr(VI) were constant at different concentrations, the area of the first peak from Cr(VI) can be used to extrapolate the contribution to the second peak area and subsequently determine the real amount of Cr(III) in the mixture sample of Cr(VI) and Cr(III).

The separation of the metal complexes was assessed using a variety of methods. The effect of the pH of mobile phase was examined. Even though the retention time of group I was independent of the pH of the mobile phase, the change in pH produced a great effect on the group II metal complexes. Decreasing the pH of the mobile phase resulted in increasing retention time of group II and so exhibited more desirable results. Although lower pH was preferred for the separation of group II, a mobile phase pH less than 4.5 resulted in reagent dissociation[25]. A pH of 4.9 exhibited the best separation for the group II metal species.

The effect of organic solvent as a mobile phase was studied to determine the best chromatographic selectivities according to Snyder[26,27]. A single-solvent system consisting of methanol, acetronitrile or tetrahydrofuran, a binary solvent system (methanol–acetonitrile, acetonitrile–tetrahydrofuran, tetrahydrofuran–methanol) and a ternary solvent system (methanol–acetonitrile–tetrahydrofuran) were investigated, while the volume fraction of organic solvents and water was set to keep the solvent strength[28] of the mobile phase constant. An 0.05 *M* acetate buffer, pH 4.9, was used as an aqueous mobile phase. Using a different solvent system as a mobile phase was effective for the separation of group I metals as shown in Fig. 1. Although large changes in selectivity values among the various mobile phase were exhibited, either single or binary organic solvent systems did not provide the solvent power to separate all of the components in the mixture. Increasing acetonitrile content in the mobile phase improved the separation of mercury–chromium(III), mercury–selenium, and copper–selenium, while increasing tetrahydrofuran content contributed to

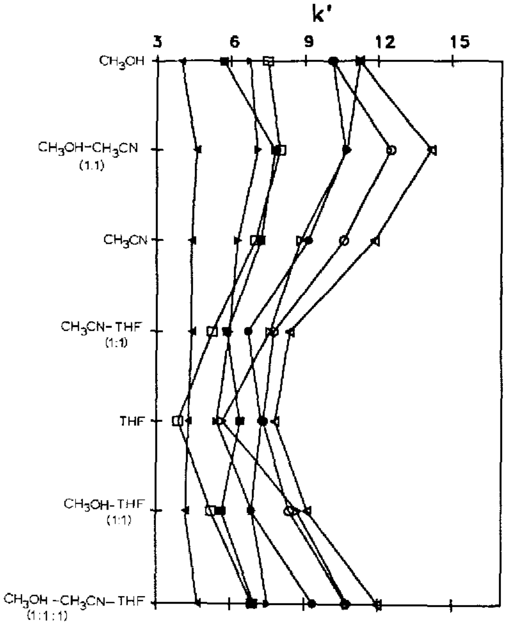

Fig. 1. Effect of organic solvent as a mobile phase on capacity factor for BPDTC complexes of group I metals. THF = Tetrahydrofuran. Metals: ○ = Co; ● = Cu; △ = Hg; ▲ = Se; □ = Ni; ■ = Cr(VI); ▽ = Cr(III); ▼ = decomposition product.

reducing capacity factors of nickel and mercury. Separation of the first three metal species [chromium(VI), selenium, nickel] was improved by increasing methanol content in the mobile phase. A ternary organic solvent system was therefore desirable for the separation of group I metal species. Although using a different solvent system as a mobile phase was effective for the separation of group I metals, it yielded poor results for the separation of group II. However, the peak shapes of group II metal complexes, which were broad and tailed with methanol–water as mobile phase system, were improved with the addition of small volume of acetonitrile and tetrahydrofuran in the mobile phase.

Ichinoki and Yamazaki[8] used chloroform as a stabilizer of some hexamethyleneammonium hexamethylenedithiocarbamate (HMAHMDTC) chelates for the separation of HMAHMDTC chelates of Cd, Ni, Pb, Zn, Cu, Hg, Co and Bi. Dichloromethane was used in this work because of its lower absorptivity at 260 nm. The effect of dichloromethane content in the mobile phase on elution behavior for BPDTC complexes of group I and group II metals is shown in Figs. 2 and 3, respectively. It is apparent that the addition of DCM in the mobile phase is very effective for the separation of BPDTC complexes of both group I and II. For the best separation, the preferred composition of the mobile phase includes 12% DCM in the organic mobile phase for group I and 6% for group II.

The effect of BPDTC in the mobile phase was also examined. The chromatograms with and without BPDTC under the same conditions were compared. The addition of BPDTC to the mobile phase in the concentration range of 0.004 M–0.006

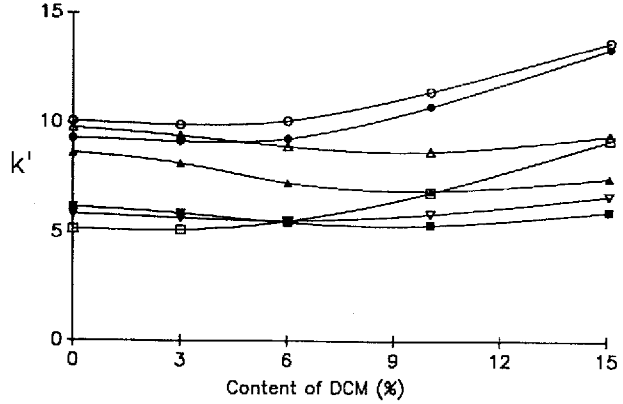

Fig. 2. Effect of dichloromethane (DCM) content in the mobile phase on the capacity factor for BPDTC complexes of group I metals. Mobile phase: methanol–water–DCM ($75-0.75x{:}25{:}0.75x$) (x = percent of dichloromethane in methanol). Other conditions are given in the text. Metals: ○ = Cr(III); ● = Co(II); △ = Hg(II); ▲ = Cu(II); □ = Cr(VI); ■ = Ni(II); ▽ = Se(IV).

M enhanced the peak reproducibilities and peak shapes of the group II metal complexes which was relatively poor initally with the methanol–water mobile phase system. It is concluded that addition of BPDTC to the mobile phase prevents on-column decomposition of the complexes. However, a high concentration of BPDTC (more than 0.01 *M*) made chelate detection difficult at 260 nm.

The final separation involved an aqueous mobile phase containing 0.005 *M* BPDTC and 0.01 *M* acetate buffer (pH 4.9), and an organic mobile phase of ternary solvents (methanol, acetonitrile, tetrahydrofuran) with additional 12% (v/v) dichloromethane for group I and 6% for group II. Column temperature was kept at 35°C for both group metal species. When a mobile phase of methanol–acetonitrile–tetrahydrofuran–dichloromethane-water (47.6:10.2:2.04:8.16:32.0) was used, group I

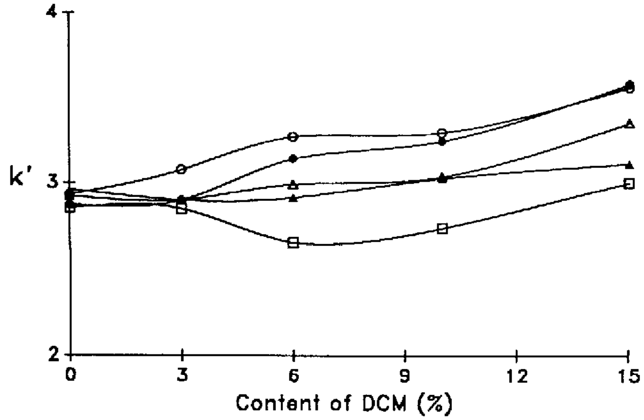

Fig. 3. Effect of dichloromethane content in the mobile phase on capacity factor for BPDTC complexes of group II metals. Conditions as in Fig. 2. Metals: ○ = Tl(I); ● = Sb(III); △ = Zn(II); ▲ = Pb(II); □ = Cd(II).

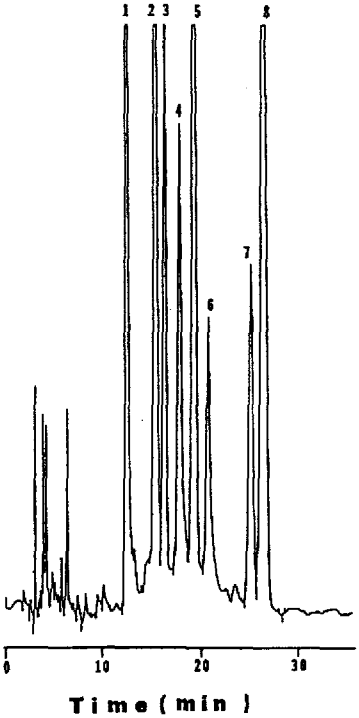

Fig. 4. Separation of a mixture of group I metals. Mobile phase: methanol–acetonitrile–tetrahydrofuran–dichloromethane–water (47.6:10.2:2.04:8.16:32.0) Peaks: 1 = Se; 2 = Ni; 3 = decomposition product; 4 = Cu; 5 = Cr(VI); 6 = Hg; 7 = Co; 8 = Cr(III).

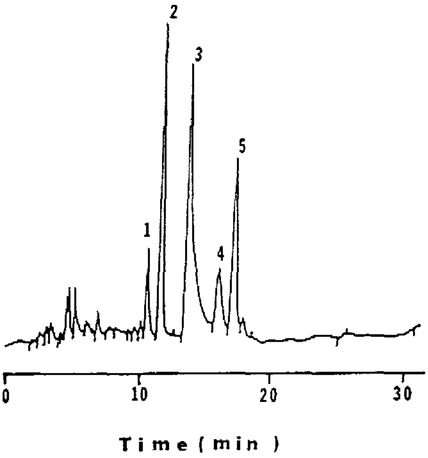

Fig. 5. Separation of a mixture of group II metals. Mobile phase: methanol–acetonitrile–tetrahydrofuran–dichloromethane–water (39.68:12.4:6.2:3.72:38.0). Peaks: 1 = Cd; 2 = Pb; 3 = Zn; 4 = Sb; 5 = Tl.

TABLE II

THE NUMBER OF THEORETICAL PLATES (N) AND THE CAPACITY FACTORS (k') FOR GROUP I AND GROUP II METAL SPECIES

Group	*Species*	*N*	*k'*
I	Se(IV)	3338	3.09
	Ni(II)	4356	4.05
	Cu(II)	4773	4.87
	Cr(VI)	5720	5.38
	Hg(II)	7744	5.86
	Cr(III)	8710	7.75
II	Cd(II)	5518	2.49
	Pb(II)	4910	3.07
	Zn(II)	2566	3.39
	Sb(III)	6084	3.97
	Tl(I)	6720	4.32

chelates were well resolved (Fig. 4). Group II metals were separated using the mobile phase composed of methanol–acetonitrile–tetrahydrofuran–dichloromethane-water (39.68:12.4:6.2:3.72:38.0) (Fig. 5). The number of theoretical plates and the capacity factors of eleven metal species are listed in Table II.

It is clearly evident that simultaneous determination is possible for each of the two groups (six metal species in one group and five in the other group) of BPDTC complexes. Although two injections of the sample mixture under two different mobile phase conditions were required for the determination of eleven pollutant metal species, the separation of each group of metal species were achieved within 30 min without having any interference of other group metal presence. The linearity for each metal species was determined over the range of its detection limit to 5 ppm, which is the concentration range of interest in many environmental samples. The detection

TABLE III

DETECTION LIMITS AND AMBIENT WATER CRITERIA FOR THE METAL SPECIES INVESTIGATED

Species	*Detection limit (μl/g)*	*Ambient water criteria (μl/g)*
Sb(III)	0.2	146
Cd(II)	0.1	10
Cr(III)	3.0	170 000
Cr(VI)	0.7	50
Cu(II)	0.1	1 000
Pb(II)	0.2	50
Hg(II)	0.7	0.14
Ni(II)	0.2	13.4
Se(IV)	0.4	10
Tl(I)	0.2	13
Zn(II)	0.3	5 000

TABLE IV

ANALYTICAL RESULTS OF STANDARD REFERENCE MATERIAL WATER SAMPLE FROM NATIONAL BUREAU OF STANDARDS

ND = Not detected.

Metal	*Concentration reported (μl/g)*	*Concentration found[a] (μl/g)*	*Relative error (%)*
Sb	0	ND	–
Cd	94.0	93.6 ± 20.8	−0.43
Cr	18.6	17.8 ± 5.9	−4.30
Cu	21.9	20.9 ± 5.0	−4.50
Pb	23.7	24.4 ± 7.5	+2.95
Hg	0	ND	–
Ni	49.0	50.6 ± 13.4	+3.27
Se	9.7	10.2 ± 1.7	+5.15
Tl	8.0	8.3 ± 2.0	+3.75
Zn	66.0	62.4 ± 15.6	+2.95

[a] 95% Confidence limits, $n = 3$.

limit of the method was expressed as the amount of metal required to give a peak area of 2000 units which could provide a detectable signal-to-noise ratio of 2 for the last eluting compound [chromium(III)], using a 10-μl injection. The EPA's ambient water criteria[29] for the protection of human health from the toxic properties of pollutant metals ingested through water and contaminated aquatic organisms are compared with the detection limits of our methods in Table III. The detection limits of our method are lower than the current ambient water criteria in all cases except mercury(II). Analytical results of the Standard Reference Material (1643b) issued by the National Bureau of Standards (Washington, DC, U.S.A.) are summarized in Table IV. Results were in good agreement with the standard concentrations, with the average relative error of ± 2.95%.

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