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Short communication

Use of ion chromatography for the determination of heavy and transition metals in biochemical samples

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Abstract

A novel, highly sensitive method for simultaneous separation and determination of Cu^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, Mn^{2+} and Pb^{2+} in biochemical samples was developed and evaluated by ion chromatography. All of these metals were well separated on a bifunctional ion-exchange column by a concentration gradient of oxalic acid and sodium chloride eluents, coupled with spectrophotometric detection after post-column derivatization with 2-[(5-bromo-2-pyridyl)azo]-5-diethylaminophenol at 560 nm. The method detection limits (signal-to-noise 3:1) were at $\mu g\ l^{-1}$ levels. The calibration graphs were linear ($r^2 > 0.999$) over two-orders of magnitude. This technique was optimized and validated by analyzing five standard biochemical references.

Keywords: Derivatization, LC; Food analysis; Heavy metals; Transition metals; Metal cations; Bromopyridyl-azodiethylaminophenol

1. Introduction

Heavy and transition metals are of special importance from an ecotoxicological point of view, both because of the high toxicity of compounds containing these metals and because of their accumulation in various organisms [1–3]. Information on their content in plants and animals is of great botanical, nutritional and environmental interest. Some of the heavy and transition metals are toxic when their concentrations exceed certain values.

Ion chromatography (IC) is a useful tool for the separation and determination of trace metals. A bifunctional ion-exchange column (CS 5 or CS 5A column) was found to be the most effective analytical column to separate heavy and transition metals. These columns have both anion- and cation-exchange capacity. Metals can be separated by both cation- and anion-exchange mechanisms. The common eluent is pyridine-2,6-dicarboxylic acid (PDCA) [4–9] or oxalic acid (Ox) [4,10]. When Ox is used, cadmium and manganese co-elute. Iron and aluminum cannot be eluted from the analytical column. When PDCA is used, heavy and transition metals can be well separated, but sensitivities are reduced, because the metal–PDCA complexes are more stable than the corresponding metal–Ox complexes and more difficult to derive with color-developing reagents.

In this paper, a novel color-developing and eluent system is described. All the metals can be separated

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well by a concentration gradient of oxalic acid and sodium chloride eluent. The metals reacted with 2-[(5-bromo-2-pyridyl)azo]-5-diethylaminophenol (5-Br-PADAP) as post-column reagent and are detected at 560 nm. The detection limits are greatly improved. This method is successfully applied to determine heavy and transition metals in biochemical samples.

2. Experimental

2.1. Instrumentation

Chromatographic analyses were performed on a metal-free Dionex DX-300 ion chromatograph (Dionex, Sunnyvale, CA, USA) equipped with an advanced gradient pump, an IonPac CG 5A guard column, an IonPac CS 5A analytical column (250× 4.6 mm I.D., 9 μm bead diameter ethylvinylbenzene functionalized with both quaternary ammonium and sulfonate functional groups), a 500-μl injection loop and a Dionex variable-wavelength detector with post-column reactor.

All samples were injected at least in triplicate and filtered through a 0.45-μm filter prior to injection. All measurements were made at room temperature.

Data collection and operation of all components in the system were controlled by Dionex AI-450 chromatographic software interfaced via an ACI-2 advanced computer interface to an AST Power Premium 3/33 computer.

The eluent flow-rate was 1.0 ml min^{-1}; the flow-rate of post-column reagent was 0.6 ml min^{-1}; the total flow-rate (1.6 ml min^{-1}) was checked at the exit of the waste line.

2.2. Reagents and standards

Ammonium hydroxide, nitric acid, Triton X-100, hydrofluoric acid, sodium hydrogencarbonate, ethanol, perchloric acid, sodium chloride and 5-Br-PADAP were of analytical-reagent grade (Peking Chemical Works, Peking, China), oxalic acid dehydrate (Ox) was of guaranteed-reagent grade (Peking Chemical Works).

Working standard solutions were prepared daily by standards (100 mg l^{-1}) which were obtained from the Research Center for Eco-Environment Sciences of Academia Sinica (China).

All standards, samples and reagents were stored in polyethylene bottles cleaned and conditioned following suggested procedures for trace metals determination [11,12].

The post-column reagent solution consisted of 0.3 m*M* 5-Br-PADAP, 0.8% Triton X-100, 0.5 *M* ammonium hydroxide and 0.3 *M* sodium hydrogencarbonate solution.

2.3. Samples and samples preparation

The pork liver, prawn, tea, peach leave and mussel used in this study were from the Research Center for Eco-Environment Sciences of Academia Sinica.

A 0.3-g amount of powdered sample was placed in a closed polytetrafluoroethyene (PTFE) beaker and wetted with a small amount of water. Then, 5 ml of concentrated nitric acid was added and the beaker was heated nearly to dryness. Then, 20 ml of 50% (w/w) hydrofluoric acid was added and the beaker was heated to dryness. Furthermore, 5 ml of 60% (w/w) perchloric acid was added to the residue and the beaker was heated until a white fume of perchloric acid appeared. After standing to cool, 2 ml of concentrated nitric acid was added and evaporated to dryness again. Finally, the residue was dissolved to 10 ml with 0.01 *M* HNO_3.

3. Results and discussion

3.1. Chromatographic separation

Since the IonPac CS 5A column had an anion- and cation-exchange capacity, metals could be separated by cation- and anion-exchange mechanisms. Hydrated and weakly complexed metals could be separated as cations on cation-exchange sites. By adding chelating carboxylic acid to the eluent, the net charge on the metal was reduced. Also, if the chelating agent concentration was high and the β constant was $>10^3$, then the net charge of the metal complex was negative and the metal complex could be separated by anion-exchange. When the metals were separated by oxalic acid and sodium chloride eluents, Pb^{2+} and Cd^{2+} formed relatively weak

Table 1
Optimum concentration gradient program for the separation of metals

Time (min)	E_1[a] (%)	E_2[a] (%)	E_3[a] (%)
0.0	15	15	70
15.9	15	15	70
16.0	40	20	40
29.9	40	20	40
30	90	10	0
44.9	90	10	0
45	15	15	70

[a] E_1: 0.5 *M* NaCl; E_2: 100 m*M* Ox; E_3: deionized water.

complexes with oxalate and were separated by cation-exchange, Cu^{2+}, Co^{2+}, Zn^{2+} and Ni^{2+} formed relatively stable complexes with oxalate and were separated by anion-exchange. Furthermore, Cl^- could also form complexes with some metals. Since all these metals had different conditional formation constants and distribution of species, they could be separated well by cation- and anion-exchange components. The elution order was Cu^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, Mn^{2+} and Pb^{2+}. A series of separation conditions was tested. From these, an optimum concentration gradient program was chosen (shown in Table 1).

When the concentrations of Mg^{2+} and Ca^{2+} were lower than 0.1 g l^{-1} and 0.2 g l^{-1}, respectively, they could not affect the separation and determination of heavy and transition metals. But if their concentrations were relatively high, they would precipitate on the column as insoluble oxalate complexes. Furthermore, Fe^{3+} and Al^{3+} often existed in the eluent and could not be eluted from the column. The column would be overloaded soon and worse separation would result. Thus, we used 450 m*M* NaCl and 10 m*M* Ox to elute them out at the end of each analysis.

From the above experiment, we could not determine Fe^{2+} and Fe^{3+} simultaneously. The previous papers had reported that two kinds of elution system were needed to separate all these metals. So, we studied the elution system further and found a new elution system that could separate well all these metals (shown in Fig. 1). Table 2 shows the optimum concentration gradient program.

Fig. 1 shows that all nine metals were well separated simultaneously. If this method could be used in real samples, it would improve the development of determining heavy and transition metals. But, there was one problem that we had no way to resolve. When the concentration of Mg^{2+} or Ca^{2+} was more than 10 mg l^{-1} or 50 mg l^{-1} in the samples, respectively, Cu^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+} and

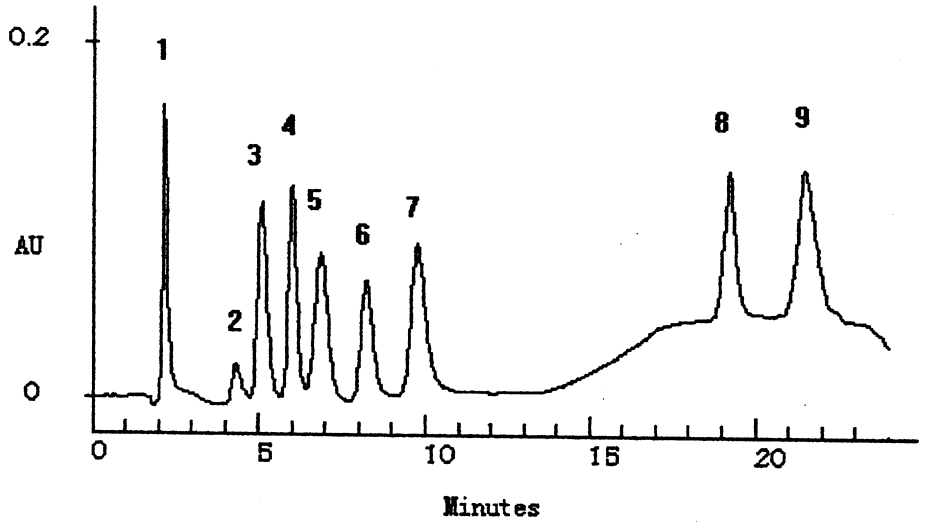

Fig. 1. Chromatogram showing the separation of nine metals on the CS 5A column, using a concentration gradient program as in Table 2. Peaks: 1=Cu^{2+} (100 μg l^{-1}); 2=Cd^{2+} (40 μg l^{-1}); 3=Ni^{2+} (300 μg l^{-1}); 4=Zn^{2+} (120 μg l^{-1}); 5=Co^{2+} (200 μg l^{-1}); 6=Fe^{2+} (200 μg l^{-1}); 7=Mn^{2+} (70 μg l^{-1}); 8=Fe^{3+} (300 μg l^{-1}); 9=Pb^{2+} (1200 μg l^{-1}).

Table 2
Optimum concentration gradient program for the separation of nine metals

Time (min)	E_1[a] (%)	E_2[a] (%)	E_3[a] (%)
0	70	0	30
10	70	0	30
20	0	100	0
30	0	100	0
30.1	70	0	30

[a] E_1: 28 m*M* Ox, 45 m*M* NaCl, 115 m*M* $NaNO_3$, 40 m*M* HCl; E_2: 28 m*M* Ox, 45 m*M* NaCl, 265 m*M* $NaNO_3$, 40 m*M* HCl; E_3: deionized water.

Co^{2+} would elute together. They could not be separated and determined. Maybe this problem could be resolved by other methods, such as on-line sample pretreatment, etc. This will be studied in the next work. In this paper, we used the elution program shown in Table 1 to separate the metals in biochemical samples.

3.2. Optimization of spectrophotometric detection

A number of post-column derivatizing agents were often used for the determination of heavy and transition metals with high sensitivity, e.g., dithizone [13], 4-(2-pyridylazo)resorcinol (PAR) [4–7,9] and 8-hydroxyquinoline-5-sulfonate (HQS) [14]. However, dithizone and HQS could not be used under the same conditions for different metals. Therefore, they were not suitable for the simultaneous detection of metals in chromatographic analysis. PAR was a commonly used derivatizing agent and could develop color with most metals under the same conditions. However, its molar absorptivities were not very high ($>2\cdot10^4$). 5-Br-PADAP was a novel derivatizing agent whose metal complexes showed relatively high molar absorptivities ($>10^5$) in the range 550 to 570 nm and had a relatively low background absorbance [15,16]. Its concentration had a great influence on the absorbance of the complexes. Since Triton X-100 has the functions of solubilization, sensibilization, dispersion and stabilization to the color-development system, it could be used to enhance the sensitivity and solubility of spectrophotometric detection for the metals in aqueous process. The pH could influence the 5-Br-PADAP complexation process. A high pH increased the ionization of 5-Br-PADAP, thus increasing its complexing capabilities. However, the background absorbance would also be high. Moreover, hydrolysis of metals could be observed along with the simultaneous disappearance of their signals, whereas very low pH values could decrease its complexing capabilities. Thus, we chose 0.3 m*M* 5-Br-PADAP, 0.8% Triton X-100, 0.5 *M* ammonium hydroxide and 0.3 *M* sodium hydrogencarbonate solution as the optimum post-column derivatizing reagent. For the best signal-to-noise ratio, the optimum wavelength was 560 nm.

3.3. Accuracy and detection limits

In this work, an optimized concentration gradient program was selected (shown in Table 1). It produced sharper peaks with good peak separation and excellent calibration curves. The use of a large loop did not have any detrimental effect on peak efficiency or asymmetry. The linear ranges, correlation coefficients, relative standard deviations (RSDs) and detection limits are listed in Table 3. It can be seen that all metals had good linearities with correlation coefficients greater than 0.999. The RSDs based on $>10\times$ detection limits concentration were found to be in the range 2.1 to 3.4% and the detection limits (signal-to-noise ratio 3:1) of this method were at the $\mu g\ l^{-1}$ level. The data confirmed that the precision of this method was good.

3.4. Analysis of samples

As a validation of the analytical technique, we analyzed five standard biochemical samples, which were well distributed, stable and had accurate content. Table 4 shows the comparison of the results between IC and certified values. They were averages of three totally independent analyses involving sample digestion and IC. They were obtained based on the system calibration with our standards. It was found that the IC values were in good agreement with standard values. The typical recovery ranges were 95–107%. The concentrations of Pb^{2+}, Co^{2+}, Ni^{2+} and Cd^{2+} were below their detection limits and, therefore, they could not be determined in any sample (Pb^{2+}) or only in part of the samples (Co^{2+}, Ni^{2+}, Cd^{2+}). If their concentrations would be high

Table 3
Linear ranges, correlation coefficients, relative standard deviations (RSDs) and detection limits

Metal	Linear range ($\mu g\ l^{-1}$)	Correlation coefficients (r)[a]	RSD (%)[b]	Detection limit ($\mu g\ l^{-1}$)[c]
Cu	0.8–200	0.9999	2.3	0.8
Ni	20–1200	0.9990	3.4	9
Zn	1–300	0.9998	3.2	1.1
Cd	4–1000	0.9999	2.1	2.5
Co	20–600	0.9999	3.1	2.0
Mn	4–250	0.9998	2.3	1.2
Pb	40–1000	0.9999	2.9	40

[a] Diluted 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256.
[b] Concentration>10× detection limit (n=7).
[c] Signal-to-noise ratio: 3:1.

enough, they would also show good agreement. In this paper, a novel, highly sensitive method of simultaneous separation and determination of Cu^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, Mn^{2+} and Pb^{2+} in biochemical samples was developed. Fig. 2 shows the chromatograms of metals in peach leave and mussel samples.

We also studied the variation in the retention time of these metals and noted a very low variation after many analyses were performed. System blanks were well below the detection limits for Pb^{2+}, Cd^{2+}, Co^{2+} and Ni^{2+}. A major contribution to contamination arose from Cu^{2+} and Zn^{2+}. Using ultrapure reagent and water could reduce these contamination. Furthermore, the ion chromatograph used was a polymeric metal-free system for the entire flow path. Trace metal impurities were removed from the chromatographic system by flushing the flow path, pumps and columns with 0.2 *M* oxalic acid for 3 h (1.0 ml min^{-1}). Then, the system was rinsed with 200 ml of deionized water. Furthermore, the columns were removed and the flow path was washed again with 6.0 *M* HNO_3 for 3 h before the final rinse with deionized water. But some Cu^{2+} and Zn^{2+} were dissolved from the system, which could not be eliminated completely. However, their contents were

Table 4
Analytical results of five standard samples

Sample		Cu	Ni	Zn	Cd	Co	Mn	Pb
Mussel	IC value[a]	7.31	1.00	131.4	4.37	0.91	9.80	–[b]
	Certified[a]	7.70	1.03	138.0	4.50	0.94	10.2	1.96
Prawn	IC value[a]	4.63	–	60.4	–	–	2.09	–
	Certified[a]	4.66	0	60.8	0.023	0.029	1.96	0.30
Pork liver	IC value[a]	17.1	–	16.80	–	–	8.50	–
	Certified[a]	17.2	0	17.2	0.067	0.10	8.32	0.54
Tea	IC value[a]	15.9	7.2	37.7	–	0.22	794.4	–
	Certified[a]	16.2	7.61	38.7	0.032	0.20	766	0.28
Peach leave	IC value[a]	9.97	–	21.6	–	–	79.5	–
	Certified[a]	10.4	0	22.8	0.018	0.25	75.4	0.99

[a] mg l^{-1}.
[b] –: Not detected (n=3).

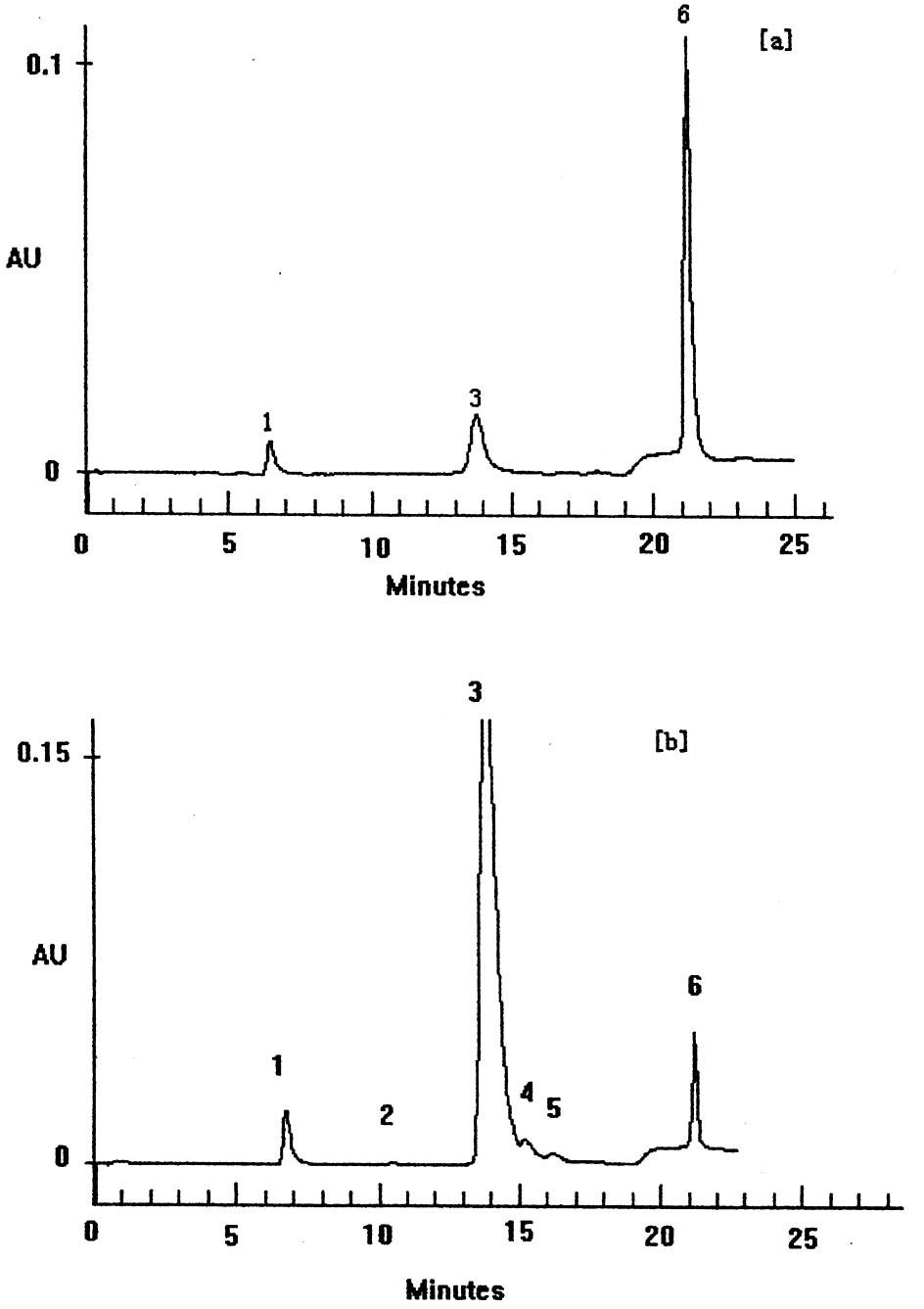

Fig. 2. Chromatograms of metals determined in peach leave and mussel prepared as described in Experimental. Chromatographic conditions as in Table 1. (a) Peach leave: peaks: 1=Cu^{2+} (9.97 mg l^{-1}); 3=Zn^{2+} (21.6 mg l^{-1}); 6=Mn^{2+} (79.5 mg l^{-1}). (b) Mussel: peaks: 1=Cu^{2+} (7.31 mg l^{-1}); 2=Ni^{2+} (1.00 mg l^{-1}); 3=Zn^{2+} (131.4 mg l^{-1}); 4=Cd^{2+} (4.37 mg l^{-1}); 5=Co^{2+} (0.91 mg l^{-1}); 6=Mn^{2+} (9.80 mg l^{-1}).

constant, they could not influence their determination. Moreover, there was no indication of any memory effect when a blank was run immediately after a sample with a high concentration of metals.

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