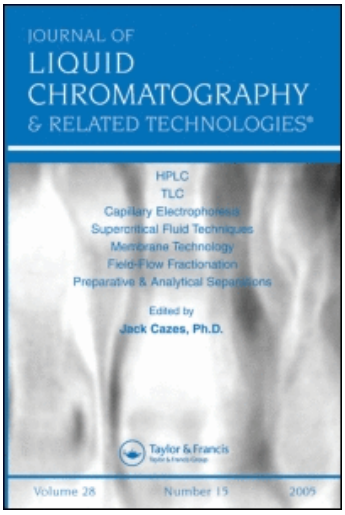

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

SIMULTANEOUS DETERMINATION OF HEAVY AND TRANSITION METALS IN BIOLOGICAL SAMPLES BY CHELATION ION CHROMATOGRAPHY

Haitao Lu[a]; Xizhu Yin[a]; Shifen Mou[b]; J. M. Riviello[c]
[a] Chemical Group, Department of Basic Courses, Laiyang Agricultural College, Shandong Province, P. R. China [b] Research Center for Eco-Environment Sciences, Academia Sinica, Beijing, P. R. China [c] Dionex Corporation, Sunnyvale, CA, U.S.A.

Online publication date: 08 July 2000

J. LIQ. CHROM. & REL. TECHNOL., 23(13), 2033–2045 (2000)

SIMULTANEOUS DETERMINATION OF HEAVY AND TRANSITION METALS IN BIOLOGICAL SAMPLES BY CHELATION ION CHROMATOGRAPHY

Haitao Lu,[1,*] Xizhu Yin,[1] Shifen Mou,[2] J. M. Riviello[3]

[1] Chemical Group, Department of Basic Courses
Laiyang Agricultural College
Shandong Province, 265200, P. R. China

[2] Research Center for Eco-Environment Sciences
Academia Sinica
P.O. Box 2871
Beijing 100871, P. R. China

[3] Dionex Corporation
Sunnyvale, CA 94086, USA

ABSTRACT

A highly sensitive method of simultaneous separation and determination of Pb^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+}, and Ni^{2+} was developed and evaluated by on-line sample pretreatment of chelation ion chromatography. Bulk quantities of anions, alkali metals, alkaline earth metals, tervalence cations, and other interfering components were eliminated by pyrophosphoric acid / ammonium acetate buffer solution (pH 5.5), while retaining heavy and transition metals on iminodiacetate-based resins. Then, they were disabsorbed and transferred to sulfonated cation exchangers.

Finally, the concentrated trace metals were separated on a bifunctional ion exchange column by a concentration gradient of oxalic acid and sodium nitrate eluents, coupled with post-column sepectrophotometric detection with 2-[(5-bromo-2-pyridyl)-azo]-5-diethyl-aminophenol (5-Br-PADAP) at 560 nm. The method

detection limits (signal-to-noise 3:1) were at or below μg L^{-1} levels. This technique was validated by analyzing standard biological references.

INTRODUCTION

Concern over the health hazards caused by heavy and transition metals has received increasing attention in recent years.[1] Some reviews have reported their acute and subacute effects on fish, mechanisms of toxicity, the role of toxicity modifying factions, and various sublethal effects, i.e. hematological and biochemical disorder. Outside of the industrial environment, most of the heavy and transition metals that burdened people were derived from the diet. Information on their contents in animals and plants was of great botanical, zoological, nutritional, and environmental interest.

Ion chromatography (IC) was the common technique that had long been used for the separation of trace metals.[2-10] However, these methods could only be used in simple samples, which had the same magnitude levels of trace metals and interference. The contents of analyte in real samples were usually low and the contents of interference were high. If the ionic strength was too high, salt ions might "swamp" ion-exchange sites. This would affect the separation of heavy and transition metals seriously. Some interference could also develop colors with the derivatizing agent; their peaks might overlap the peaks of analyte. Moreover, the contents of trace metals were often too low to be determined. Thus, a separation (to eliminate alkali metals, alkaline earth metals, and other interfering components presented in complex samples) and preconcentration step prior to ion chromatographic analysis was necessary. In order to deal with these problems, chelation ion chromatography (CIC) was the best choice.

CIC was the developing technique, which combined on-line analyte concentration and matrix elimination.[9,11-16] It could selectively concentrate trace heavy and transition metals first while eliminating high concentrations of anions, alkali metals, alkaline earth metals, and the other interfering components. Then, the quantitatively concentrated metals were separated and determined. In this paper, this technique was applied in biological samples and validated by analyzing standard reference samples.

EXPERIMENTAL

Instrumentation

Chromatographic analyses were performed on a metal-free Dionex DX-300 ion chromatography (Dionex Corp., Sunnyvale, CA, U.S.A) equipped with

two advanced gradient pumps (AGP), a MetPac CC-1 chelation column (50 mm × 4 mm, I.D. packed with styrene-based macroporous 12% cross-linked iminodicacetate-functionalized chelating resin; the particle was 20 μm and the capacity of resin was about 0.9 mequiv.), a TMC-1 concentrator column (25 mm × 3 mm, I.D. containing fully sulfonated PS-DVB cation-exchange resin with high capacity 2.2 mequiv.), an IonPac CG5A Guard column, and an IonPac CS5A analytical column (250 mm × 4.6 mm, I.D., 9 μm bead diameter ethylvinyl benzene functionalized with both quaternary ammonium and sulfonate functional groups), a 3.66 mL injection loop, and a Dionex variable wavelength detector with post-column reactor. MetPac CC-1 and TMC-1 columns were used for sample pretreatment. IonPac CS5A column was used for the separation of heavy and transition metals.

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.

Chemicals and Reagents

Ammonium hydroxide, glacial acetic acid, Triton X-100, sodium hydrogen carbonate, sodium nitrate, ammonium persulfate, silver nitrate, mercury nitrate, ammonium acetate, ethanol, lithium chloride, hydrofluoric acid, perchloric acid, 2-[(5-bromo-2-pyridyl)-azo]-5-diethylaminophenol (5-Br-PADAP), and lithium hydroxide monohydrate were of analytical-reagent grade reagents (Peking Chemical Works, Peking, China). Sodium hydroxide, nitric acid, potassium hydroxide, oxalic acid dehydrate, were of guaranteed-reagent grade reagents (Peking Chemical Works, Peking, China). Pyridine-2,6-dicarboxylic acid (PDCA) was of chromatographic grade reagent (Aldrich, U.S.A). Pyrophosphoric acid was of chemical grade reagent.

Pb^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+}, and Ni^{2+} standard solutions (100 μg mL^{-1}, Research Center for Eco-Environmental Sciences, Academia Sinica, China).

Iminodiacetate-based resins (D401, 50~100 mesh, >0.6 Cu^{2+} meq mL^{-1}) (Chemical Works, Nankai University, China)

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

All solutions were prepared with pretreated water purified with a Milli-Q system (>18MΩ, Millipore, Waters chromatography division, Oslo, Norway).

Eluents and Post-Column Reagent Solution

Eluents: E_1: 21.2 mM $NaNO_3$; E_2: 20 mM $H_4P_2O_7$, 2.0 M NH_4OAc, pH 5.5; E_3: 0.5 M HNO_3; E_4: 106 mM $NaNO_3$; E_5: 168 mM Ox, pH 4.7; E_6: 18MΩ DI water; E_7: 12 mM PDCA , 20 mM LiOH and 100 mM LiCl; "carrier" acid: 0.5 M HNO_3.

E_2: 20 mM pyrophosphoric acid, 2.0 M ammonium acetate, pH 5.5

Dissolve 3.7 g of pyrophosphoric acid in 1 l of 2.0 M ammonium acetate buffer solution, pH 5.5. Since pyrophosphoric acid was not available in ultra-pure grade regent, the trace heavy and transition metals could be removed by using D401 resin. Place 40 g purified D401 resin into the solution. After stirring for 60 min, decant pyrophosphoric acid / ammonium acetate solution into an eluent container.

Post-column reagent: 0.3 mM 5-Br-PADAP, 12.4 mM Triton X-100, 0.5 M ammonium hydroxide and 0.3 M sodium hydrogen carbonate solution.

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.

Samples and Samples Preparation

Samples used in this study were pork liver, tea, mussel, prawn, peach leaves, (Research Center for Eco-Environment Sciences of Academia Sinica). Maojian tea, puer tea, luyinzhen tea (Yunnan Province, China), longjing tea (Zhejiang Province, China), baimudan tea (Fujian Province, China), dried small shrimps, general tobacco (Shandong Province, China). All samples had no water content and was obtained in powered form by a laboratory mill.

0.3 g of powdered sample was weighed. It was added into a closed poly-tetrafluoroethyene (PTFE) beaker and wetted with a small amount of water; 5 mL of concentrated nitric acid was added and heated nearly to dryness. Then 20 mL of concentrated hydrofluoric acid was added and heated to dryness. Furthermore, 5 mL of concentrated perchloric acid was added to the residue and heated until white fumes of perchloric acid appeared. After cooling by standing, 2 mL of concentrated nitric acid was added and evaporated to dryness again. Finally, the residue was dissolved to 10 mL of 0.2 M HNO_3 and 1 mL of 0.1 M $Hg(NO_3)_2$ solution were added and heated to boiling for 5 min. All Cl^- was eliminated. Furthermore, 10 mL of 0.44 M $(NH_4)_2S_2O_8$ solution was added and heated to boiling for 5 min. All Mn^{2+} was oxidized to permanganate (MnO_4^-). Since $(NH_4)_2S_2O_8$ was an unstable compound, it must be newly synthesized.

After cooling by standing, the solution was diluted to 25 mL with 0.1 M HNO_3 solution, then transferred to a closed polytetrafluoroethylene beaker.

$$2Mn^{2+}5S_2O_8^{2-} + 8H_2O \; 2MnO_4^- + 10SO_4^{2-} + 16H^+$$

Before injection, each solution should be further diluted to a proper concentration within its linear range and filtered through a 0.45 μm filter.

RESULTS AND DISCUSSION

Experimental Procedure

The detailed scheme of chelation ion chromatographic system was shown in Figure 1.

AGP1 pretreatment program (shown in Table 1) was entered as a method file in AI-450 operating software and subsequently downloaded onto AGP1. When the operation started, the sample was flushed by acid carrier to a mixing tee, where it was buffered by pyrophosphoric acid / ammonium acetate buffer solution in the pH range of 5 to 6 before entering MetPac CC-1 column. This column contained macroporous iminodiacetate chelating resin, which had high affinity for transition and heavy metals compared to alkali and alkaline earth

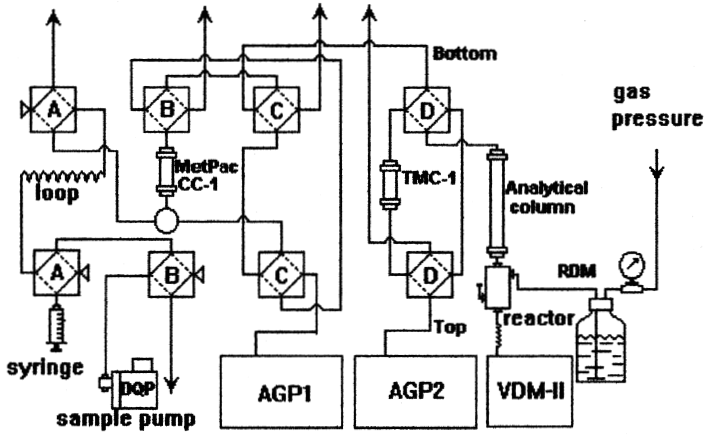

Figure 1. Scheme of chelation ion chromatographic system.

Table 1

Chelation Concentration and Matrix Elimination Operating Conditions for Analysis of Heavy and Transition Metals[a]

Time (Min)	E_1 (%)	E_2 (%)	E_3 (%)	Valve A[b] Valve B[b]	Valve C	Flow Rate (mL min^{-1})	Sample Pump
0.0	0	100	0	off	on	1.0	on
3.0	0	100	0	off	on	1.0	off
3.1	0	100	0	off	on	2.5	off
5.0	0	100	0	off	on	2.5	off
5.1	0	0	100	on	on	2.0	off
8.0	0	0	100	on	on	2.0	off
8.1	100	0	0	off	off	2.5	off
16.0[c]	100	0	0	off	off	2.5	off
16.1	0	100	0	on	off	2.0	off
20.0	0	100	0	on	off	0.0	off
35.0	0	100	0	off	off	2.0	off
40.0	0	100	0	on	off	0.0	off

[a] AGP-1 program. [b] Begin sample analysis. [c] off: real line connected, on: dotted line connected. Sample pump flow rate: 1.5 mL min^{-1}.

metals. All anions and monovalent cations could not be retained. Manganese, which coeluted with cadmium, was oxidized to permanganate before injection and eliminated as the form of anion. A 10 mg amount of Ca^{2+} could be eluted completely with 5 mL of eluent.

The other alkaline earth metals had the same results. Pyrophosphoric acid could selectively bind iron and aluminum, thus preventing uptake by the chelating resin during concentration. This approach not only prevented precipitation of iron and aluminum at high concentrations, but also allowed an effective removal of iron and aluminum from MetPac CC-1 column. The complex formation constants between IDA and Pb^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, were far bigger than those between pyrophosphate and these metals, respectively. But for tervalence cations, the metal-pyrophosphate complexes during the on-line neutralization step were more stable than metal-iminodioacetate complexes. Tervalence cations could not retained. More than 99.8% of a 2 mg amount of iron or aluminum could be eluted with 6 mL of eluent. Thus, alkaline earth metals, iron and aluminum, etc., could be selectively eluted while Pb^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+}, and Ni^{2+} quantitatively remained. Their recoveries were more than 90% at $\mu g\ L^{-1}$ levels. Changes of flow-rate from 1 to 3 mL min^{-1} did not influence the recoveries of retained metals. Before each analysis, the flow paths

should be rinsed by 0.5 M HNO_3, otherwise, the reductive component would reduce permanganate to Mn^{2+}.

All metals retained in a MetPac CC-1 column could be eluted to TMC-1 column by 0.5 M HNO_3 solution. However, before the TMC-1 column was linked with IonPac CS 5A column, it must be converted from the hydrogen form to the sodium form, otherwise, the peaks of Pb^{2+}, Cu^{2+} and Cd^{2+} would be within a big negative peak and could not be determined accurately.

AGP2 concentration gradient program was entered on the AGP2 front panel. Since CS5A column had both anion and cation exchange capacity, metals could be separated by anion and cation exchange mechanisms. Thus, major selective changes could be made by simply changing the eluent. When the metals was separated with Ox eluent, the separation had both anion and cation exchange components. Since Pb^{2+} and Cd^{2+} formed relatively weak complexes with oxalate, they were separated as cations on cation exchange sites. The net charge of Cu^{2+}, Co^{2+}, Zn^{2+}, and Ni^{2+} complexes were negative and separated by anion exchange.[6] The elution order was Pb^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+}, Zn^{2+}, and Ni^{2+}. The use of $NaNO_3$ solution in eluent reduced tailing, improved peak shape, and, obviously, eluted in a shorter time.

From a series of experiments, an optimum condition was chosen (shown in Table 2). A typical chromatogram of synthetic standard solution was illustrated in Figure 2. All individual metal peaks were well separated. Although there were bulk quantities of alkaline earth metals, aluminum, and iron, they could not affect the separation and determination of heavy and transition metals any more.

Table 2

Gradient Separation Program for Analysis of Heavy and Transition Metals[a]

Time (Min)	E_4 (%)	E_5 (%)	E_6 (%)	Valve D[b]
0.2	7	27	66	off
16.1[c]	7	27	66	on
24.0	7	27	66	on
24.1	7	60	33	on
34.0	7	60	33	on
34.1	7	27	66	off

[a] AGP-2 program. [b]Begin sample analysis. [c] off: real line connected, on: dotted line connected.

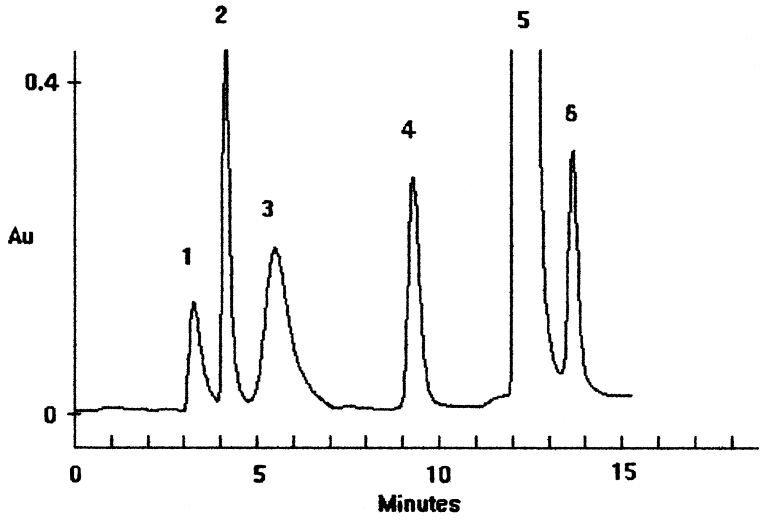

Figure 2. Chromatogram of heavy and transition metals in synthetic standard solution. Peaks: 1) Pb^{2+}, 2) Cu^{2+}, 3) Cd^{2+}, 4) Co^{2+}, 5) Zn^{2+}, 6) Ni^{2+}.

The analytical column had to be eluted frequently using PDCA (E_7) to remove iron and aluminum, which were commonly found in eluents and, if not removed, could lead to column overload.

Accuracy and Detection Limits

Under the optimized concentration gradient programs (shown in Table 1 and 2), it produced sharper peaks with good peak separation and excellent calibration curves. All metals had good linearities whose correlation coefficients were greater than 0.999. The R.S.Ds, based on >10× detection limits concentration, were found to be in the range of 1.8%-5.0%. The detection limits (signal-to-noise ratio 3:1) of this method for Pb^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+}, and Ni^{2+} were 2, 0.2, 0.6, 0.2, and 0.2 μg L^{-1}, respectively.

Analysis of Samples

As a validation of the analytical technique, we analyzed five standard samples, which were well-distributed, stable, and had accurate content. Table 3 showed the comparison of the results between IC and certified values. They were averages of three totally independent analyses involving sample digestion

Table 3

Analytical Results of Standard Animal and Plant Samples

Metal	Peach Leaves		Tea		Mussel		Prawn		Pork Liver	
($\mu g\ L^{-1}$)	IC	Certified	IC	Certified	IC	Certified	IC	Certified	IC	Certified
Pb	1.03	0.99	1.11	1.06	0.24	0.30	0.45	0.54	2.07	1.96
Cu	10.06	10.40	18.10	17.20	4.36	4.66	18.30	17.20	7.11	7.70
Cd	/	/	0.041	0.032	4.87	4.50	0.029	0.023	0.081	0.067
Co	0.22	0.25	3.30	3.20	0.035	0.029	0.12	0.10	0.90	0.94
Ni	/	/	8.15	7.61	/	/	/	/	1.00	1.03

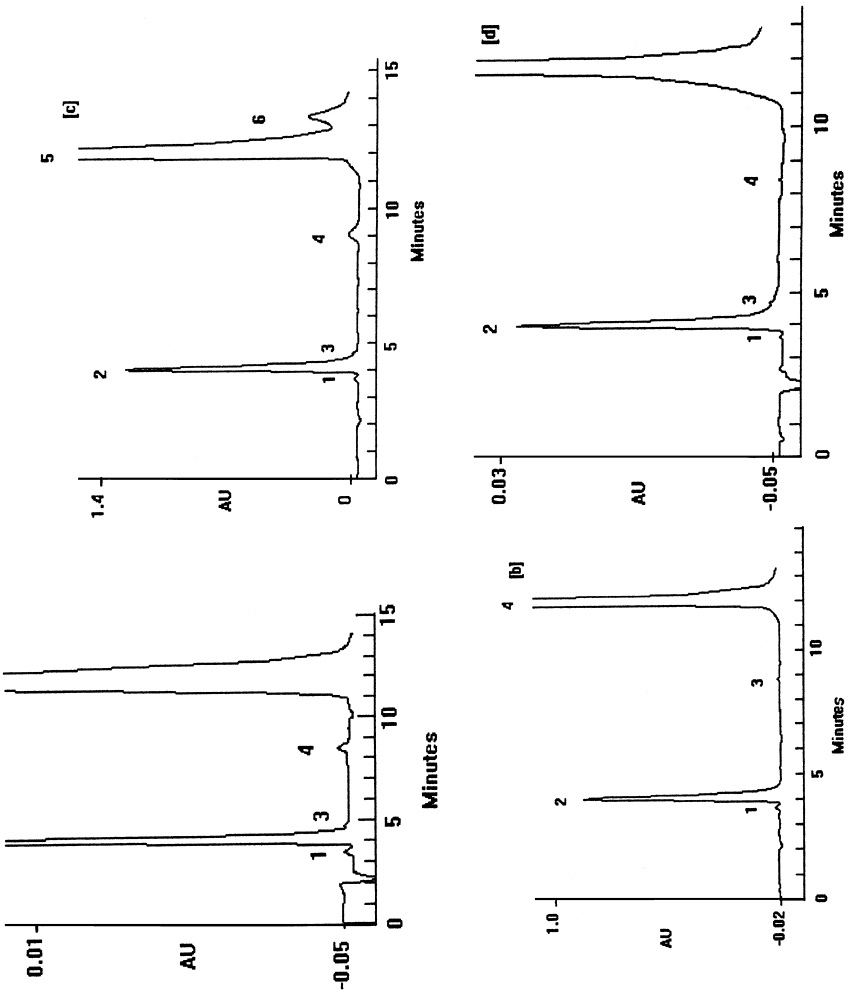

Figure 3. Chromatograms of heavy and transition metals in standard biochemical samples. [a] pork liver [b] peach leave [c] tea [d] shrimp. Peaks: 1) Pb^{2+}, 2) Cu^{2+}, 3) Cd^{2+}, 4) Co^{2+}, 5) Zn^{2+}, 6) Ni^{2+}.

and ion chromatographic procedure. They were obtained based on the system calibration with the standards. It was found that the IC values were in good agreement with certified values. But, some of them had bigger deviations. This was because their concentration in solution was close to their detection limits. Since the concentrations of Ni^{2+} and Cd^{2+} in some samples were lower than their detection limits, they could not be determined. If they had enough concentrations, they would also have good accuracies. Figure 3 were the chromatograms of metals in pork liver, peach leaf, tea, and shrimp samples.

Seven other samples were also analyzed (listed in Table 4).

We also studied the variations in the retention time of these metals and noted very low variations after many analyses were performed. System blanks were well below the detection limits for Pb^{2+}, Cd^{2+}, Co^{2+}, and Ni^{2+}. The major contributors to contamination were Cu^{2+} and Zn^{2+}. These contaminations could be reduced by using ultrapure reagent and water. The ion chromatography 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}). The system was rinsed with 200 mL of deionized water. Then, all 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 of them were dissolved from the system, which could not be eliminated completely. However, their contents were constant, it could not influence their determination. Moreover, there was no indication of any memory effect when a blank was run immediately after a sample with high concentration of heavy and transition metals.

Table 4

Analytical Results of Some Real Samples

Metal ($\mu g\ g^{-1}$)	Maojian	Puer	Luyinzhen	Longjing	Baimudan	Shrimp	Tobacco
Pb	5.82	1.18	3.21	4.49	3.10	0.76	0.58
Cu	15.9	20.3	18.1	16.2	13.7	10.7	15.19
Cd	/	/	/	/	/	/	/
Co	0.461	2.65	3.10	1.70	1.62	0.23	1.01
Ni	3.91	5.45	6.47	5.20	11.6	1.23	3.71

CONCLUSIONS

The CIC method provided accurate and precise analyses of heavy and transition metals in biological samples. It showed high validity. It allowed the analysis of many metals at trace levels and it was possible to eliminate the matrix interference completely. Sample preconcentration and matrix elimination covered a wide sample matrix concentration. One of the most important advantages was that the analytical media could be injected directly for analysis after sample digestion.

REFERENCES

1. J. O.Nriagu, **Changing Metal Cycles and Human Health**, Springer-Verlag Berlin, Heidekery, New York, 1984.

2. P. L. Buldini, S. Cavalli, A. Mevoli, E. Milella, J.Chromatogr.A.,**739**, 131 (1996).

3. P. L.Buldini, S. Cavalli, A. Mevoli , J. Chromatogr. A., **739**, 167 (1996).

4. P. Janvion, S. Motellier, H. Pitsch, J. Chromatogr. A., **715**, 105 (1995).

5. N. Cardellicchio, P. Ragone, S. Cavalli, J. M. Riviello., II CS'96 International Ion Chromatography Symposium. No. 102, The University of Reading, Reading, England, U. K.

6. A. W. Al-Shawi, R. Dahl, II CS'96 International Ion Chromatography Symposium. No. 59, The University of Reading, Reading, England, U. K.

7. A. Rahmalan, M. Z. Abdullah, M. M. Sanagi, M. Rashid, J.Chromatogr. A., **739**, 233 (1996).

8. I. N. Voloschik, M. L. Litvina, B. A. Rudenko, J. Chromatogr. A, **671**, 205 (1994).

9. W. Shotyk, I. Immenhauser-Potthast, J. Chromatogr. A., **706**, 167 (1995).

10. A. R. Timerbaev, G. K. Bonn, J. Chromatogr., **640**, 195 (1993).

11. S. Motellier, H. Pitsch, J. Chromatogr., A, **739**, 119 (1996).

12. R. Caprioli, S. Torcini, J. Chromatogr., **640**, 365 (1993).

13. N. Cardellicchio, S. Cavalli, J. M. Riviello, J. Chromatogr., **640**, 207 (1993).

14. H. T. Lu, S. F. Mou, Y. Yan, S. Y. Tong, J. M. Riviello, J. Chromatogr.A, **800(2)**, 247 (1998).

15. H. T. Lu, S. F. Mou, Y. W. Hou, F. Liu, K. A. Li, S. Y. Tong, Z. L. Li, J. M. Riviello, J. Liq. Chrom. & Rel. Technol., **20(19)**, 3173 (1997).

16. H. T. Lu, S. F. Mou, Y. Yan, F. Liu, K. A. Li, S. Y. Tong, J. M. Riviello, Talanta., **45**, 119 (1997).

Received May 10, 1999 Author's Revisions February 22, 2000
Accepted August 16, 1999 Manuscript 5038