Journal of Chromatography, 640 (1993) 127–134
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2751

Ion-pair reversed-phase high-performance liquid chromatography for trace metal preconcentration followed by ion-interaction chromatography

Corrado Sarzanini*, Giovanni Sacchero, Maurizio Aceto, Ornella Abollino and Edoardo Mentasti

Department of Analytical Chemistry, Via P. Giuria 5, 10125 Turin (Italy)

ABSTRACT

Ion-interaction chromatography of Plasmocorinth B (a disulphonated azo dye) complexes of Co(III), Cu(II), Fe(III), Ga(III), In(III), Ni(II), V(V) and Zr(IV) was studied. The behaviour of two different reversed-phase C_{18} columns (5 and 10 μm) was compared and an on-line enrichment procedure was developed following the optimization of eluent (pH, ligand concentration, ionic strength and organic modifier). The described technique, applied to the analysis of metal ions at μg/l levels in natural waters, gave satisfactory precision and accuracy in comparison with inductively coupled plasma atomic emission spectroscopic results.

INTRODUCTION

Direct and reversed-phase high-performance liquid chromatography (HPLC) procedures have been developed for the determination of metal ions [1–3]. Complex formation followed by extraction [4,5] or by sorption on coated or uncoated columns and pre-column or on-column on-line formation of chelates [6–10] are the commonest methods of achieving preconcentration and separation of metals.

The objective of this study was to investigate the feasibility of using metal complexes, obtained by reaction with a sulphonato azo dye (Plasmocorinth B) in ion-pairing reactions for metal preconcentration and separation by HPLC. The effect of different parameters, namely pH, ligand and ion-pairing concentration, ionic strength and stationary phase size, was assessed.

The optimization of the method enabled analyte metals to be separated from alkaline and alkaline earth elements and the interfering matrix ions that are not complexed and which pass through the column unretained. The preconcentration procedure furnished enrichment factors of up to 900, and the technique has been applied successfully to samples of river water. Detection limits are in the range 15–90 ng/l.

EXPERIMENTAL

Apparatus and materials

The liquid chromatograph used for this work was a Varian Model LC 5000 equipped with a Rheodyne injection valve (100-μl sample loop inserted), a Vista 401 Data Station, gradient programmer and a Varian UV-100 detector (Varian, Walnut Creek, CA, USA). The analytical columns were reversed-phase LiChrospher 100 RP-18 5 μm and 10 μm (250 × 4 mm I.D.) columns. The operating conditions (at 25°C) were: mobile phase flow-rate 1.0 ml/min, complex detection at $\lambda = 270$ nm. For trace enrichment, a chemically inert Model DQP-1 pump (Dionex, Sunnyvale, CA, USA) was used.

High-purity acids were obtained with a sub-boil-

* Corresponding author.

0021-9673/93/$06.00

ing quartz still (K. Kurner, Rosenheim, Germany).

An Orion EA 920 pH-meter equipped with a glass-calomel electrode was used for pH measurements.

Plasmocorinth B was obtained from Aldrich, and tetrabutylammonium hydroxide was a Fluka product (Buchs, Switzerland); all other chemicals were of analytical–reagent grade (Merck, Darmstadt, Germany).

Standard metal solutions, 1000 mg/l (Merck), were diluted daily to obtain reference and working solutions. The chelates were obtained by mixing the aqueous analyte solution with the reagent at the appropriate concentrations (see below). All solutions were prepared with high-purity water (HPW; Milli-Q water system, Millipore). Analytical reagent-grade methanol and HPW were filtered through a 0.45-μm filter and used as mobile phase solvents.

All glass and polyethylene or polypropylene labware were cleaned with nitric acid in a microwave oven (2450 MHz) for 4 min, power 540 W, and then repeatly rinsed with HPW.

Chelates preparation

The method is based on the formation of metal complexes by reaction with the ligand Plasmocorinth B. The chelating agent 3-(5-chloro-2-hydroxyphenylazo)-4,5-dihydroxynaphthalene-2,7-disulphonic acid, acts like tridentate planar ligand [11] and is characterized by the presence of two hydroxy substituents in the *ortho* position relative to the azo group and two sulphonato groups not involved in the complexation. Plasmocorinth B was selected because of its ability to form thermodynamically stable metal ion complexes [12] and to form ion pair with proper ion-interaction reagents, *i.e.*, the tetrabutylammonium (TBA) cation. Fig. 1 shows schematically the rections between Plasmocorinth B and metal ions and ion-pairing reactions involved in the considered system. In order to achieve metal complex formation, samples containing 1–10 mg/l of metals or mixtures of metals containing 0.80 m*M* Plasmocorinth B were prepared, buffering (acetic acid, sodium hydroxide) the solution to eluent pH. In preliminary experiments the samples (100 μl) were introduced into the chromatographic system as such.

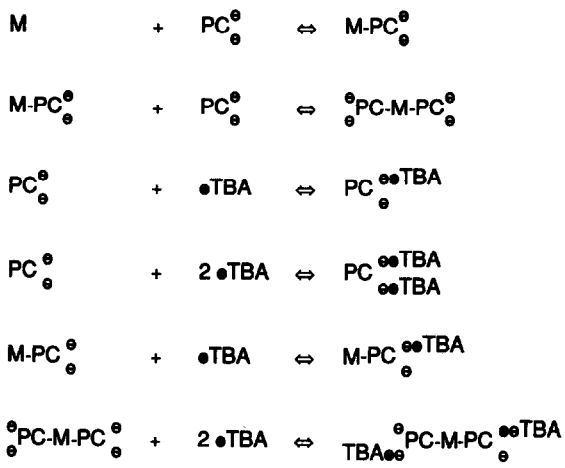

Fig. 1. Complexation and ion-pairing reactions involved in the system (M = metal ion, PC = Plasmocorinth B, TBA = tetrabutylammonium ion; metal charges and functional groups omitted).

Chromatographic study

A chromatographic study was developed to optimize the solvent system, enabling the separation of all metal–Plasmocorinth B complexes. For these studies analyte samples were prepared as mentioned above. Metal mixtures, Al(III), Co(III), Cu(II), Fe(III), Ga(III), In(III), Ni(II), V(V) and Zr(IV), and individual metal samples were prepared to verify specific peaks in each chromatogram.

The eluents investigated were methanol–water solutions. Methanol–water was used as the ionic strength (sodium nitrate), ion-pairing (TBA hydroxide) and pH (acetic acid, sodium hydroxide) adjusting solvent. Different concentrations of Plasmocorinth B in the eluent were also investigated.

The behaviour of two kinds of column, 5-μm and 10-μm LiChrospher 100 RP-18 (250 × 4 mm I.D.) columns, was evaluated.

RESULTS AND DISCUSSION

The reactions involved in the proposed method, shown schematically in Fig. 1, involve bi- and trivalent metal ions, their complexes with Plasmocorinth B and related ion pairs with TBA. Considering the nature of these compounds, the chromatographic mechanisms operating could be modified by varying the methanol (organic modifier) and TBA concentration as well as the ionic strength and pH.

The first experiments evaluated the effect of the methanol concentration in the eluent. Dramatic fluctuations in the capacity factor for metal–Plasmocorinth B complexes were found when the methanol concentration was reduced below 48% (v/v). In addition, methanol concentrations greater than 50% decreased the retention times of trivalent metal ions too much. A concentration of 50% (v/v) resulted the optimum compromise between good resolution and capacity factors.

The effect of Plasmocorinth B concentration in the eluent was investigated. Concentrations of 0–40 μM did not affect the capacity factors of metal chelates (see Fig. 2), but the presence of Plasmocorinth B, even at low concentrations, optimized the system performance. In fact, the baseline was also stabilized for injection of samples at high Plasmocorinth B concentrations and peak reproducibility improved as a result. Taking into account these considerations and to avoid an unfavourable increase in eluent absorbance owing to high Plasmocorinth B concentration, 2.0 μM PC was selected for eluent composition.

In ion-interaction chromatography an important role is usually played by the ion-pairing agent. Fig. 3 shows the effect of different concentrations of TBA on the capacity factor of considered complexes. Concentrations below 1.6 mM result in peak overlapping, while higher concentrations give too large capacity factors. Thus 1.6 mM was selected as the working concentration. In the eluent optimization, to evaluate the ionic strength effectiveness, 0–250 mM sodium nitrate was investigated. The results in Fig. 4 show that low concentrations gave too high retention times, but poor resolution was obtained at concentrations higher than 25 mM. It seems remarkable that the complexes most affected by ionic strength modification were complexes of trivalent metal ions. This may be explained by considering that Plasmocorinth B complexes of these metals exhibit a 2:1 (ligand to metal) molar ratio, which could result in ion pairs containing one (metal-Plasmocorinth B–TBA) or more, usually two (metal-Plasmocorinth B–TBA_2), molecules of TBA. The ionic strength enhancement improves the competition of NO_3^- for TBA, and probably only metal-Plasmocorinth B–TBA is formed, which shows reduced retention times. In the subsequent experiments 10.0 mM sodium nitrate, which gave good separations, was used unless stated otherwise.

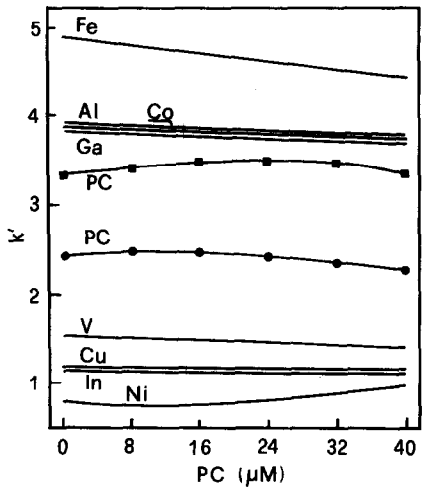

Fig. 2. Effect of Plasmocorinth B (PC) concentration on capacity factor (k') of metal chelates. Chromatographic conditions: mobile phase, methanol–water (50:50, v/v) containing 10 mM acetic acid, 15 mM sodium nitrate, 1.6 mM TBA hydroxide, PC as shown and sodium hydroxide up to pH 6.3; flow-rate, 1.0 ml/min. Sample volume, 100 μl; 0.80 mM Plasmocorinth B, 2–6 mg/l metals.

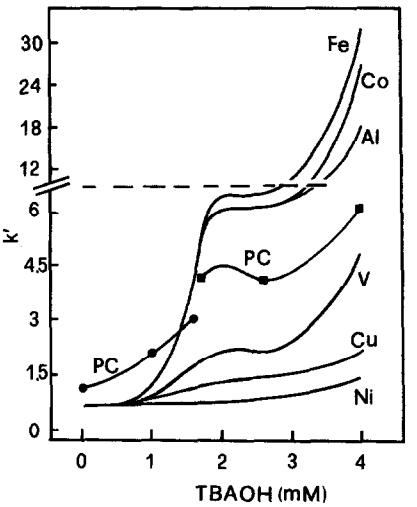

Fig. 3. Effect of TBA concentration on capacity factor (k') of metal chelates. Chromatographic conditions: mobile phase, methanol–water (50:50, v/v) containing 10 mM acetic acid, 15 mM sodium nitrate, 2.0 μM Plasmocorinth B (PC), TBA hydroxide (TBAOH) as shown and sodium hydroxide up to pH 6.3; flow-rate, 1.0 ml/min. Sample volume, 100 μl; 0.80 mM Plasmocorinth B, 2–6 mg/l metals.

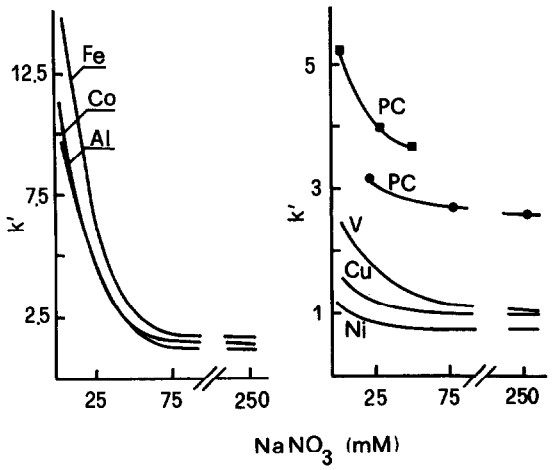

Fig. 4. Dependence of the capacity factor (k') of metal chelates on ionic strength (sodium nitrate). Chromatographic conditions: mobile phase, methanol–water (50:50, v/v) containing 10 mM acetic acid, 2.0 μM Plasmocorinth B (PC), 1.6 mM TBA hydroxide, sodium nitrate as shown and sodium hydroxide up to pH 6.3; flow-rate, 1.0 ml/min. Sample volume, 100 μl; 0.80 mM Plasmocorinth B, 2–6 mg/l metals.

It should be pointed out that Figs. 2, 3 and 4 show two different lines for Plasmocorinth B ion pairs. This is because, as a function of eluent composition, two peaks can be ascribed to the ligand. The structure of Plasmocorinth B suggests that the two sulphonato groups present on the ligand can form 1:1 or 1:2 (ligand to ion-pairing ratio) ion pairs. In particular (Fig. 4), when high concentrations of Na^+ and NO_3^- are present the competition for SO_3^- and TBA^+ ions reduces the capacity of ion-pairing available to form Plasmocorinth B–TBA_2 (1:2 stoichiometry) ion pairs and only the peak of Plasmocorinth B–TBA (1:1 stoichiometry) appears at lower retention time.

The behaviour of capacity factors for some of the considered metal–Plasmocorinth B complexes (Fig. 5) showed that low pH values affect the separation performance, with great fluctuations in retention times resulting from small variations in pH. In the next experiments a pH value of 6.3 was adopted to ensure good separation of peaks, a reduced chromatographic time and better reproducibility of data.

The concentration of ligand in the sample solutions was also investigated. Fig. 6 shows the behaviour of peak area as a function of Plasmocorinth B concentration, from which a good reproducibility could be expected for Plasmocorinth B concentrations of 0.4 mM or more. Also of interest (Fig. 7) is the behaviour of the asymmetry factor (AF10) as a function of the ratio of ligand to stoichiometric ligand concentration (L/L_s), where the stoichiometric term is related to metal concentration.

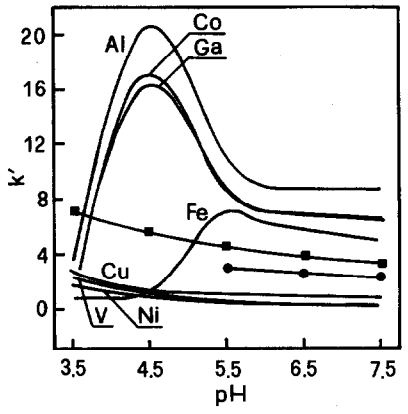

Fig. 5. Effect of pH on capacity factor (k') of metal chelates. Chromatographic conditions: mobile phase, methanol–water (50:50, v/v) containing 2.0 μM Plasmocorinth B (PC), 1.6 mM TBA hydroxide, pH as shown; flow-rate, 1.0 ml/min. Sample volume, 100 μl; 0.80 mM Plasmocorinth B, 2–6 mg/l metals.

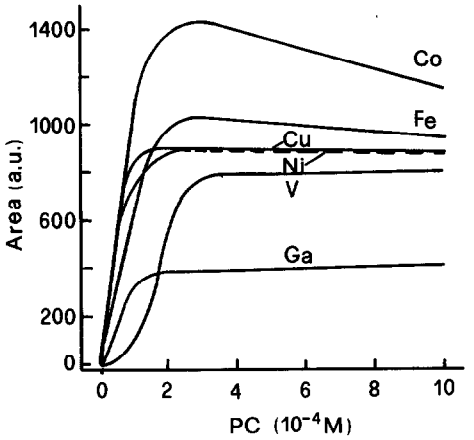

Fig. 6. Dependence of peaks area on Plasmocorinth B (PC) concentration of the sample. Chromatographic conditions: mobile phase, methanol–water (50:50, v/v) containing 10 mM acetic acid, 2.0 μM Plasmocorinth B, 1.6 mM TBA hydroxide, 10 mM sodium nitrate and sodium hydroxide up to pH 6.3; flow-rate, 1.0 ml/min. Sample volume, 100 μl; 2–6 mg/l metals, Plasmocorinth B as shown.

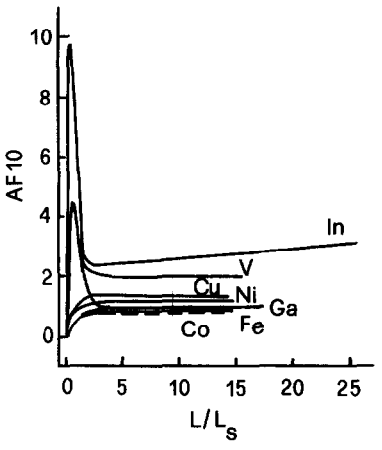

Fig. 7. Effect of the ratio of the ligand to stoichiometric ligand concentration (L/L_s) on asymmetry factor (AF10). Chromatographic conditions as in Fig. 6.

Taking into account these data and the fact that the method will used exclusively for the preconcentration of metal ions at trace levels, a 0.8 m*M* Plasmocorinth B concentration was chosen, which corresponds to values of the L/L_s ratio of 10–22.5, enabling the determination of total metal ions from ng/l up to mg/l concentration levels.

Standard metal ion solutions containing Ca^{2+}, Mg^{2+}, Na^+ and K^+ (30, 2, 2 and 2 mg/l, respectively), usually present in mineral waters, or 0.5 *M* sodium chloride (which simulates seawater), were analysed with the developed method. The agreement between chromatograms, and the results (peak area) confirm that the method is reproducible in the same standard deviation range (see below).

Columns and gradient elutions

The isocratic procedure optimized on a 10-μm LiChrospher RP-18 column was also tested on a 5-μm column of the same type. The chromatograms of a mixture of metals, *i.e.*, Ni(II), Cu(II), V(V), Co(III) and Fe(III) at 0.40, 0.50, 0.80, 0.70 and 0.80 mg/l, respectively, were compared (see Fig. 8). The 10-μm column shows a better performance in terms of resolution of peaks at shorter retention times; however, separation with the 5-μm column is

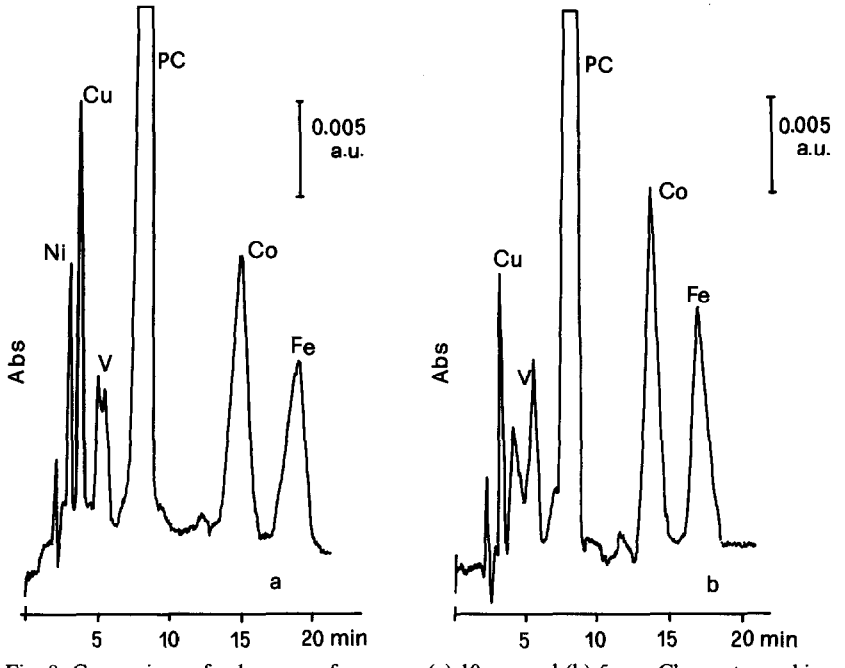

Fig. 8. Comparison of columns performance: (a) 10 μm and (b) 5 μm. Chromatographic conditions: mobile phase, methanol–water (50:50, v/v) containing 10 m*M* acetic acid, 2.0 μ*M* Plasmocorinth B (PC), 1.6 m*M* TBA hydroxide, 12.5 m*M* sodium nitrate and sodium hydroxide up to pH 6.3; flow-rate, 1.0 ml/min. Sample volume, 100 μl; 0.80 m*M* Plasmocorinth B, metals 0.40 mg/l Ni, 0.50 mg/l Cu, 0.80 mg/l V and Fe, 0.70 mg/l Co. Detection 270 nm.

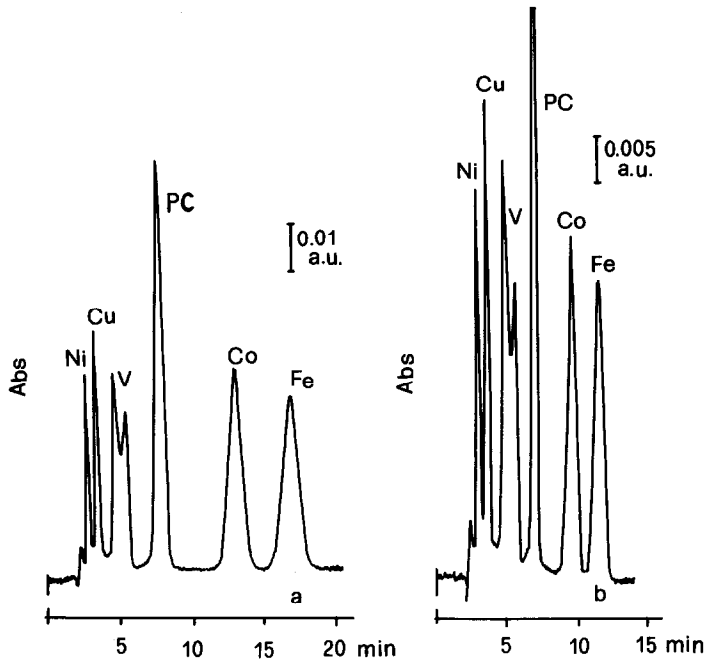

Fig. 9. Comparison of isocratic and flow-rate gradient elution. Chromatographic conditions: mobile phase, methanol–water (50:50, v/v) containing 10 m*M* acetic acid, 2.0 μM Plasmocorinth B (PC), 1.6 m*M* TBA hydroxide, 8.75 m*M* sodium nitrate and sodium hydroxide up to pH 6.3. (a) Isocratic flow-rate, 1.0 ml/min. (b) Gradient flow-rate (time: 0–5, 5–6, 6–12, 12–13 min; flow: 1, 1–2 linear; 2, 2–1 linear ml/min). Sample volume, 100 μl; 0.80 m*M* Plasmocorinth B, metals 1.0 mg/l Ni, 1.5 mg/l Cu, 3.5 mg/l V, 2.0 mg/l Co, 3.0 mg/l Fe. Detection 270 nm.

more sensitive for trivalent metals with longer retention times.

Taking into account the mechanism involved in the separation, the effectiveness of gradient elutions, namely flow-rate, ionic strength and organic modifier, were also evaluated. Flow-rate gradient elutions resulted in unchanging baseline, good resolution and shorter retention time. Chromatograms for the same sample obtained by isocratic and flow-rate gradient elution are shown in Fig. 9.

An equilibration period is not required to restore the starting conditions between consecutive injections. Similar results, good separation and reduced retention times were obtained with organic modifier and ionic strength gradient elutions. But, in these cases, 30 min are required to restore the starting conditions and the whole procedure was more time-consuming than isocratic elution.

Preconcentration procedure

The microcolumns used for preconcentration based on the developed precomplexation and ion-pairing method were evaluated. The 100-μl loop on the Rheodyne valve was replaced by a 5-μm or 10-μm LiChrospher 100 RP-18 (4 × 4 mm I.D.) microcolumn. Samples of 100 ml were fluxed through the microcolumn with the aid of a Dionex pump (Model DQP-1). A flow-rate of 4.0 ml/min was selected for the loading procedure because recovery yields were unchanged up to this value and higher rates resulted in counterpressure phenomena.

Preconcentration recoveries were evaluated by comparing the peak areas obtained by direct injection (100-μl loop) of samples (Co, Cu, Fe, Ga, Ni concentration: 3 mg/l each) with those obtained by loading the cartridge with 100.0-ml aliquots of mixtures (3 μg/l of each metal ion).

Because preliminary investigations showed that metal–Plasmocorinth B complexes are not retained by the microcolumn, an alternative procedure was adopted. Taking into account the hydrophobic nature of cartridges and the lower charge of ion pairs

compared with metal chelates, the ion-pairing reaction was carried out directly on the samples. An investigation was also made on the efficiency of Plasmocorinth B and TBA sample concentrations. L/L_s values (see above) of 10 or more and 0.32 m*M* TBA produced the best concentrations. This TBA concentration, which seems very high in comparison with eluent composition taking into account the preconcentration step, is required to enable the formation of ion pairs of all metal complexes in the microcolumn. The preconcentration procedure involved a washing step after the sample loading. A 10.0-ml volume of water removed the matrix and excess TBA. The washing volume must be well defined and taken into account when evaluating blanks.

Table I compares the recovery yields obtained for 5-μm and 10-μm microcolumns, before the optimization of Plasmocorinth B concentration, with the enrichment on a 10-μm column after optimization at lower metal ion concentration. Fig. 10 shows a representative chromatogram obtained using preconcentration and flow-rate gradient elution procedures.

Linear range and detection limits

The optimized procedure was used to determine the linear range: samples of increasing concentration were prepared until the chromatographic peak areas exhibited non-linearity. Calibration ranges within

TABLE I

PRECONCENTRATION RECOVERY YIELD (%) WITH DIFFERENT COLUMNS AND AFTER OPTIMIZATION

Chromatographic conditions: mobile phase, methanol–water (50:50, v/v) containing 10.0 m*M* acetic acid, 2.0 μ*M* Plasmocorinth B, 1.6 m*M* TBA hydroxide, 12.5 m*M* sodium nitrate and sodium hydroxide up to pH 6.3; flow-rate, 1.0 ml/min; sample, 100 ml; pH 6.3; 0.8 μ*M* Plasmocorinth B; 0.32 m*M* TBA; loading flow-rate 4.0 ml/min; detection 270 nm. Preconcentrators: LiChroCART 100 RP-18 (4 × 4 mm I.D.).

Element	Recovery yield (%)		
	5 μm[a]	10 μm[a]	10 μm[b]
Co	26.2	47.9	95.7
Cu	28.5	83.0	95.8
Fe	26.8	40.7	94.9
Ga	10.0	15.0	94.8
Ni	–	38.2	72.0

[a] $L/L_s = 2.5$; metals 3.0 μg/l.
[b] $L/L_s = 10.0$; metals 0.8 μg/l Cu, Ga and Ni, 0.5 μg/l Co and Fe.

TABLE II

CALIBRATION RANGE AND DETECTION LIMITS FOR METAL DETERMINATION

Element	Linearity range (mg/l)	R^a	Detection limit (μg/l)[b]
Al	0.1 –2.0	0.991	50
Co	0.1 –10	0.999	30
Cu	0.2 –10	0.990	60
Fe	0.05–10	0.999	15
Ga	0.2 –10	0.998	60
Ni	0.2 –10	0.999	65
V	0.5 –10	0.994	30
Zr	0.2 –4	0.998	150

[a] R = Linear regression coefficient.
[b] Detection limits of procedure defined as three times absolute blank.

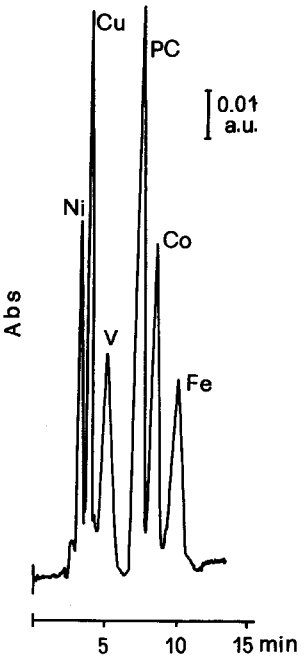

Fig. 10. Preconcentration followed by gradient (flow-rate) elution. Chromatographic conditions as in Fig. 9. Sample volume 100 ml; pH 6.3; 0.32 m*M* TBA; 0.80 μ*M* Plasmocorinth B (PC); Ni, Cu, V, Co and Fe 3 μg/l; preconcentration flow-rate, 4.0 ml/min; 10-μm column.

TABLE III

RETENTION TIME AND PEAK AREA REPRODUCIBILITY FOR METAL IONS BY OPTIMIZED PROCEDURE

Metals 3 μg/l.

Element	Retention time (min)[a]	Area (μV s)[a]
Co	8.5 ± 1.7	1252 ± 1
Cu	3.8 ± 0.7	939 ± 7
Fe	10.2 ± 2.1	868 ± 6
Ni	3.2 ± 1.1	363 ± 11
V	5.2 ± 3.1	784 ± 9

[a] Mean ± standard deviation (%).

the coefficient of linear regression and detection limits (DL) are listed in Table II. Obviously, the developed preconcentration procedure resulted in a greater reduction of DL, and the resulting values ranged between 15 and 90 ng/l. Reproducibilities achieved for retention times and peak areas are summarized in Table III.

Real sample

The results of the analysis of Po river water samples are given in Table IV. The river water data can be compared with the results of direct analysis by graphite furnace atomic absorption spectrometry with Zeeman-effect background correction, and with those obtained with a different on-line preconcentration method using an XAD-2 microcolumn functionalized with 1-(2-thiazolylazo)-2-naphthol and inductively coupled plasma [13]. The good agreement between the results confirms that the method is suitable for the analysis of natural samples.

ACKNOWLEDGEMENTS

Financial support from Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST, Rome) and from the Italian National Research Council (CNR, Rome) is gratefully acknowledged.

TABLE IV

ANALYTICAL DATA FOR THE ANALYSIS OF A RIVER WATER SAMPLE

HPLC–ion-interaction chromatography: developed method; ion-exchange chromatography–inductively coupled plasma atomic emission spectroscopy; see ref. 13; GF Zeeman AAS: graphite furnace atomic absorption spectrometer Zeeman-effect background correction. Standard deviations within 10%.

Element	Concentration (μg/l)		
	HPLC–ion-interaction chromatography	Ion-exchange chromatography–inductively coupled plasma atomic emission spectroscopy	GF Zeeman AAS
Co	<DL	–	<DL
Cu	1.5	1.3	1.5
Fe	5.7	5.9	5.4
Ni	5.6	5.5	5.4

REFERENCES

1 S. Dilli, P. R. Haddad and A. K. Htoon, *J. Chromatogr.*, 500 (1990) 313.
2 A. R. Timerbaev, O. M. Petrukhin, I. P. Alimarin and T. A. Bol'shova, *Talanta*, 38 (1991) 467.
3 K. Robards, P. Starr and E. Patsalides, *Analyst*, 116 (1991) 1247.
4 H. Osashi, N. Vehara and Y. Shijo, *J. Chromatogr.*, 539 (1991) 225.
5 H. Shofstahl and J. K. Hardy, *J. Chromatogr. Sci.*, 28 (1990) 225.
6 Y. Shito, K. Tanaka and N. Nehara, *Anal. Sci.*, 7 (1991) 507.
7 C. Lin, X. Zhang and X. Liu, *Analyst*, 116 (1991) 277.
8 E. A. Jones, H. S. Bezuidenhout and J. F. Van Staden, *J. Chromatogr.*, 537 (1991) 277.
9 G. Sacchero, O. Abollino, V. Porta, C. Sarzanini and E. Mentasti, *Chromatographia*, 31 (1991) 539.
10 M. V. Main and J. S. Fritz, *Talanta*, 38 (1991) 253.
11 H. Hoshino, K. Nakano and T. Yotsuyanagi, *Analyst*, 115 (1990) 33.
12 C. Sarzanini, O. Abollino, M. De Luca and E. Mentasti, *Anal. Sci.*, 8 (1992) 201.
13 V. Porta, C. Sarzanini, O. Abollino, E. Mentasti and E. Carlini, *J. Anal. Atom. Spectrom.*, 7 (1992) 19.