ELSEVIER

Journal of Chromatography A, 788 (1997) 113–119

JOURNAL OF CHROMATOGRAPHY A

Multi-element trace analysis by reversed-phase high-performance liquid chromatography followed by on-line column enrichment as 2-(5-nitro-2-pyridylazo)-5-[N-propyl-N-(3-sulphopropyl)amino] phenol chelates

Tadao Okutani, Yuuichi Tsukada, Akio Sakuragawa*, Takehiro Yamaji, Shiho Morita

Department Industrial Chemistry, College of Science and Technology, Nihon University, 1-8, Kanda-Surugadai, Chiyoda-ku, Tokyo 101, Japan

Received 23 January 1997; received in revised form 26 May 1997; accepted 30 May 1997

Abstract

This paper reports the utilization of 2-(5-nitro-2-pyridylazo)-5-[N-propyl-N-sulphopropyl)amino] phenol (Nitro-PAPS) as a chelating reagent using an ODS column for the preconcentration and the separation of metal ions by reversed-phase high-performance liquid chromatography (RP-HPLC) with ultraviolet-visible detection at 570 nm. Nitro-PAPS chelates were eluted within 12 min with tetrahydrofuran–acetonitrile–water (15:10:75, v/v) containing a 0.01 mol dm^{-3} phosphate buffer solution (pH 6.0) and $2 \cdot 10^{-7}$ mol dm^{-3} Nitro-PAPS as the mobile phase. When the Nitro-PAPS chelates were injected into the RP-HPLC system, they were retained on top of the column and did not travel in the column, with water as the sample solvent. The retained metal chelates were then allowed to travel in the column encountering the stream of the mobile phase. The separation of the peaks on the chromatogram for each component was satisfactory. The determination limits (5σ) were 0.2 ng cm^{-3} for copper and iron, 0.04 ng cm^{-3} for nickel and 0.01 ng cm^{-3} for cobalt. The proposed method was applied to the determination of these metal ions in actual samples, such as rain and river water. © 1997 Elsevier Science B.V.

Keywords: Derivatization, LC; Water analysis; Metal chelates; 2-(5-Nitro-2-pyridylazo)-5-[N-propyl-N-(3-sulfopropyl)amino]phenol; Metal cations

1. Introduction

Recently, some chelating reagents having a sensitivity suitable to atomic absorption spectrometry (AAS) analysis have been synthesized and have been widely used for reversed-phase high-performance liquid chromatography (RP-HPLC) as precolumn chelating reagents for simultaneous determination, spectrophotometric determination and complexometric titration of a trace amount of some metal ions because of their excellent analytical properties. 2-(5-Nitro-2-pyridylazo)-5-[N-propyl - N - (3-sulphopropyl)amino]phenol (Nitro-PAPS) which is a kind of pyridylazo compound, and like 1-(2-pyridylazo)-2-naphthol (PAN) and 4-(2-pyridylazo)resorcinol (PAR), forms water-soluble chelates with many metal ions such as copper(II), cobalt(II), nickel(II) and iron(II), which have high molar absorptivities in the order of 10^4–10^5 dm^3 mol^{-1} cm^{-1} in the visible region [1]. Therefore, the reagent has also been used

*Corresponding author.

0021-9673/97/$17.00
PII S0021-9673(97)00726-7

to determine a trace amount of some metal ions by flow injection analysis (FIA) or HPLC because of its high sensitivity [2,3]. It is, however, difficult to apply the direct determination of trace amounts of metal ions in natural water samples without an effective preconcentration step.

In this instance, the preconcentration methods, including solvent extraction, collection of a membrane filter and adsorption onto silica gel have generally been used for the determination of trace amounts of metal ions in these samples. In the previous paper, it was described that several metal chelates with Nitro-PAPS were extracted into 1,2-dichloroethane to form ion pairs with tetraphenylphosphonium (TPP^+) as a counter cation [4]. The chelates extracted were back-extracted into aqueous phase by adding dodecylbenzensulfonate (DBS^-) as a counter anion for TPP^+. This preconcentration method was applied to the determination of trace metal ions in natural water samples. The method takes some complicated operations and a long period of pretreatment, however, it has the advantages of the high concentration factor and the removal of some interfering ions.

A concentrating method of some metal chelates by RP-HPLC followed by an on-line system using two independent octadecylsilica (ODS) columns as the preconcentration and the separation column was reported [5]. Therefore, the authors examined the utilization of Nitro-PAPS as a chelating reagent using an ODS column for the preconcentration and the separation of metal ions by RP-HPLC. The chromatogram which was obtained by the injection of a large volume (cm^3 level) of a sample solution was almost the same as that obtained with an ordinary volume (mm^3 level) of sample. The proposed method, that is, enables the preconcentration of the metal chelates in the injecting solution, and its concentration factor is about 100 times.

This paper reports an on-line system using one ODS column for the preconcentration and the separation in multi-element analysis with Nitro-PAPS as a chelating reagent. Several metal ions existing at the sub-ng cm^{-3} level in natural water samples can be determined by the proposed method, with good reproducibility.

In this work, the speciation of copper(II), cobalt (II), nickel(II) and iron(II) were reacted with Nitro-PAPS by the precolumn derivatization, and the simultaneous determination of these metal chelates by RP-HPLC with spectrophotometric detection was studied.

2. Experimental

2.1. Reagents

All of the solutions were prepared with ultra-pure water, which was obtained by a Milli-Q SP reagent water system (Millipore). Nitro-PAPS obtained from Dojindo Laboratories (Kumamoto, Japan) was dissolved in ultra-pure water to make a $3.3 \cdot 10^{-5}$ mol dm^{-3} solution. Standard solutions of copper(II), cobalt(II) and nickel(II) were prepared from analytical reagent-grade chemicals as chloride salts, and were kept in hydrochloric acid (pH 1). The concentrations of these solutions were determined by complexometric titration with EDTA. Standard iron(II) solution was prepared from iron(II) ammonium sulfate hexahydrate and standardized by redox titration using a standard potassium permanganate solution. The working solutions of these metal ions were diluted as required. Hydrochloric acid and ammonia water used were of the reagent-grade of poisonous metal analysis (Kanto, Japan). All other reagents used were of the analytical reagent-grade (Wako, Japan). The glass and Teflon wares used were soaked in nitric acid (pH 1) for a long time, and then thoroughly washed with pure water.

For the proposed method, because of its high sensitivity, it is necessary to pay attention in order to avoid contamination with metal.

2.2. Apparatus

The liquid chromatograph consists of a Shimadzu Model LC-6AD computer controlled pump, a Hitachi Model L-4200 UV-visible spectrophotometric detector operated at 570 nm, a Rheodyne Model 7125 syringe-loading injector equipped with a 2.0-cm^3 sample loop. A Capcell PAK C_{18} AG120 (250 mm× 4.6 mm I.D., particle diameter: 5 μm) packed column from Shiseido (Japan) was employed for the preconcentration and the separation of four metal–Nitro-PAPS chelates. For pH measurements, a TOA

Electronics Model HM3OS pH meter was used. The mobile phase was tetrahydrofuran (THF)–acetonitrile (ACN)–water (15:10:75, v/v) containing $2 \cdot 10^{-7}$ mol dm^{-3} Nitro-PAPS, and adjusted to pH:6.0 by the addition of a 0.1 mol dm^{-3} of potassium phosphate buffer solution. The flow-rate of the mobile phase was 1.0 cm^3 min^{-1}.

2.3. Standard procedure

A 20-cm^3 volume of sample solution containing less than 1 μg each of copper(II), cobalt(II), nickel(II) and iron(II) is placed in a 50 cm^3 PTFE beaker, and a 3.0-cm^3 portion of $3.3 \cdot 10^{-5}$ mol dm^{-3} Nitro-PAPS solution is added to the solution. Then, the solution is adjusted to pH 6.0 by the addition of ammonia water solution and diluted to 25 cm^3. A 2.0-cm^3 aliquot of the solution is injected into the column of the RP-HPLC system. The metal–Nitro-PAPS chelates separated are detected at a wavelength of 570 nm. The concentrations of the metal ions are determined by measuring the peak areas and using the peak-area calibration graph.

3. Results and discussion

3.1. Chromatograms of metal–Nitro-PAPS chelates

Absorption maxima of metal–Nitro-PAPS chelates are at 566 nm for copper(II), at 590 nm for cobalt (II), at 568 nm for nickel(II) and at 582 nm for iron(II), and these absorption spectra roughly overlap each other. Zinc(II)–Nitro±PAPS chelate also has an absorption maximum at 566 nm. However, if a solution containing zinc(II)–Nitro-PAPS chelate and an excess of Nitro-PAPS was injected into the column under the same conditions as other metal–Nitro-PAPS chelates, the peak of zinc(II)–Nitro-PAPS chelate was not detected, because of the dissociation of that chelate on the column. On the other hand, the absorption maxima of the metal–Nitro-PAPS chelates were different from that of Nitro-PAPS. Though the mobile phase used contained Nitro-PAPS, some highly sensitive detections of the metal–Nitro-PAPS chelates were expected because of its low base line noise.

Here, a sample containing 4 ng each of copper(II), cobalt(II) and iron(II) and 2 ng of nickel(II) of (a) 20 mm^3 or (b) 2.0 cm^3 was injected into the chromatographic system. The results obtained are shown in Fig. 1.

In spite of the injection of the large volume (2 cm^3) into the column of the chromatographic system, the separation of each component, which showed the peaks on the chromatogram, was satisfactory. The analytical period of time was also short. The peak presented in front of the copper(II)–Nitro-PAPS chelate peak which was shown in Fig. 1(b) is thought to be due to the fact that 2 cm^3 of sample was injected into the column and then it was mixed with the mobile phase, where a mixture of the sample solution and the mobile phase caused a density scattering. However, at present, it is not possible to conclude this.

3.2. Effect of the pH on the formation of the metal–Nitro-PAPS chelates

As shown in Fig. 2, the peak areas of the metal–Nitro-PAPS chelates were of significantly low values under acidic conditions, and they increased along with an increase in the pH; the maximum and the

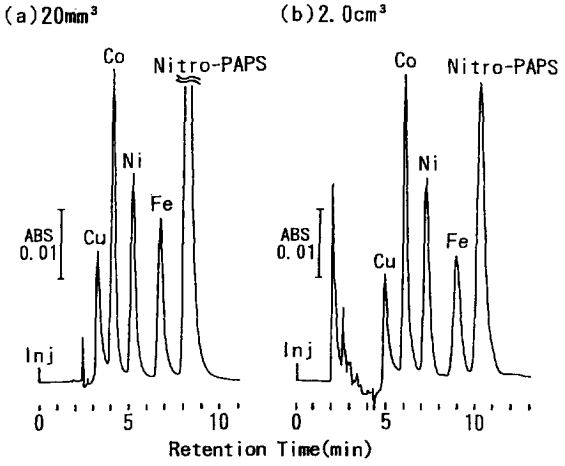

Fig. 1. Effect of the variation in injection volume for the chromatogram using water as injection solvent. Injection volume: (a) 20 mm^3, (b) 2.0 cm^3. Metal, amounts injected (ng): Cu, 4.0; Co, 4.0; Ni, 2.0; Fe, 4.0. Column: Capcell PAK C_{18}. Detection wavelength: 570 nm. Mobile phase: THF–ACN–water (15:10:75, v/v) containing 0.01 mol dm^{-3} KH_2PO_4–K_2HPO_4 and $2 \cdot 10^{-7}$ mol dm^{-3} Nitro-PAPS (pH: 6.0). Flow-rate: 1.0 cm^3 min^{-1}.

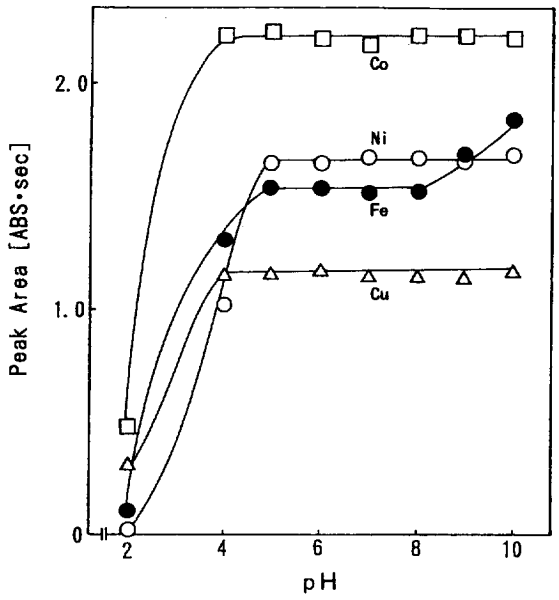

Fig. 2. Effect of the pH on the formation of the metal–Nitro-PAPS chelates. Injection volume: (a) 2.0 cm^3. Metal, amounts injected (ng): (△)=Cu, 20; (□)=Co, 20; (○)=Ni, 10; (●)=Fe, 20. HPLC conditions are the same as those in Fig. 1.

most constant formation of iron(II)–Nitro-PAPS chelate was obtained over a pH range 5–8. For the other chelates they were obtained over a pH range 5–10. The peak area of iron(II)–Nitro-PAPS chelate apparently increased at a pH of 9 or above because the peak of the chelate approached that of Nitro-PAPS and the separation of those peaks was undesirable. Therefore, the optimum working range of pH for the formation of the metal–Nitro-PAPS chelates was 5.5–7.5.

3.3. Injection volume

A large volume of sample (cm^3 level) is not generally injected into the column of a RP-HPLC. That is because it influences the chromatographic separations of several metal chelates and leads to the extension of the band width according to the diffusion of sample solution. The effect of injection volume over a 20 mm^3–3 cm^3 range was investigated by comparing those peak profiles. In all cases, the absolute quantities of metal ions to be injected as the metal–Nitro-PAPS chelates were kept constant. Even though the injection volume was changed over a 20 mm^3–3 cm^3, the peak areas of copper(II)-, cobalt(III)-, nickel(II)- and iron (II)–Nitro-PAPS chelates were almost unchanged. When 2 cm^3 of sample solution is injected, there was a concentration of metal ions about 100 times as compared with the injection of 20 mm^3 of sample solution. However, more than 2 cm^3 of injection volume tended to decrease the peak area of cobalt(III)–Nitro-PAPS chelate. The retention times of the metal–Nitro-PAPS chelates and the reagent were delayed by an increase in injection volume at a rate of 1 min cm^{-3}. In this case, the baseline before the appearance of copper(II)–Nitro-PAPS chelate peak on the chromatogram was unstable because of mixing in the mobile phase and water presented in a sample solution, however, the change of the injection volume did not influence the band width and the separation of each metal–Nitro-PAPS chelate.

3.4. Sample solvent

Recently, the influence of sample solvent on peak profiles was reported [6–8]. In this work, therefore, the effect of sample solvent on peak profiles was examined too.

To study the effect of sample solvent, some experiments were carried out with water or various mixtures of THF, ACN and water as an injection solvent. When the composition of sample solvent was the same as the mobile phase (THF–ACN-water, 15:10:75, v/v), a similar chromatogram to that shown in Fig. 1(a) was obtained by an injection volume of 20 mm^3. However, in the case of a large volume injection such as 2 cm^3, it was difficult to separate the peaks on the chromatogram, and the peak heights according to each metal–Nitro-PAPS chelate decreased. Injection samples prepared using solvents such as methanol–water, THF–water or ACN–water instead of the composition of the mobile phase, also resulted in undesirable peak profiles. Consequently, when a large volume of solution was injected into the chromatographic system, the addition of those solvents to sample solutions did not give a good separation of the metal–Nitro-PAPS chelates. Therefore, a chromatogram which was shown in Fig. 1(b) was obtained by using a com-

position of sample solvent as mentioned in the standard procedure even when an injection volume of 2.0 cm^3 was adopted. The experimental results exhibit that a sharpening of chromatographic peak corresponds to an increase in the polarity of solvents. Thus, it is important for quantitative work to consider the effect of sample solvent on the chromatogram. In RP-HPLC, the sample is prepared as a solution in water or a more highly polar solvent and then it must be injected into the column.

3.5. Effect of counter cations for the adsorption of Nitro-PAPS chelates onto ODS

Copper(II), cobalt(II), nickel(II) or iron(II) form [Cu(Nitro-PAPS)], $[Co(Nitro\text{-}PAPS)_2]^-$, $[Ni(Nitro\text{-}PAPS)_2)^{2-}$ or $(Fe(Nitro\text{-}PAPS)_2]^{2-}$ under the experimental conditions, respectively. And an ion-pair RP-HPLC using a counter cation has usually been employed for the separation and the determination of metal-chelates [3,9,10]. While a good separation for the metal–Nitro-PAPS chelates can be obtained by the proposed method, it is not possible to obtain this when a mobile phase which is free from a potassium phosphate buffer is employed to the proposed RP-HPLC system. The reason for this is considered to be that potassium ions which are contained in the potassium phosphate buffer act as a counter cation. Therefore, the behavior of potassium ion as counter cation on the retention of those metal–Nitro-PAPS chelates was examined. The effect of the concentration of potassium ions in the mobile phase is shown in Fig. 3. An increase in retention time of each metal–Nitro-PAPS chelate was caused by an increase in concentration of that potassium ion. Then the distributions of the metal–Nitro-PAPS chelates between ODS and water were determined by a batch method (Fig. 4). Ten cubic centimeters of aqueous solution containing those metal ions and Nitro-PAPS was taken in a vessel, and an aqueous potassium chloride solution was added to make a certain concentration of potassium cation. Thirty milli-grams of ODS was added to the solution and then the contents were shaken to reach an equilibrium. After the establishment of equilibrium (60 min of shaking), the phases were separated by centrifugation. The concentration of the Nitro-PAPS chelates in the

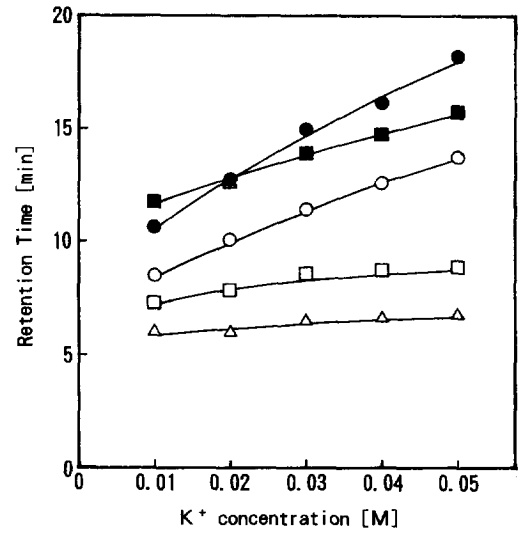

Fig. 3. Effect of the concentraton of K^+ in the mobile phase on the retention time of the metal–Nitro-PAPS chelates. Mobile phase: THF–ACN–water (15:10:75, v/v). Injection volume: 2.0 cm^3. Metal ions: (△)=Cu; (□)=Co; (○)=Ni; (●)=Fe, (■)=Nitro-PAPS.

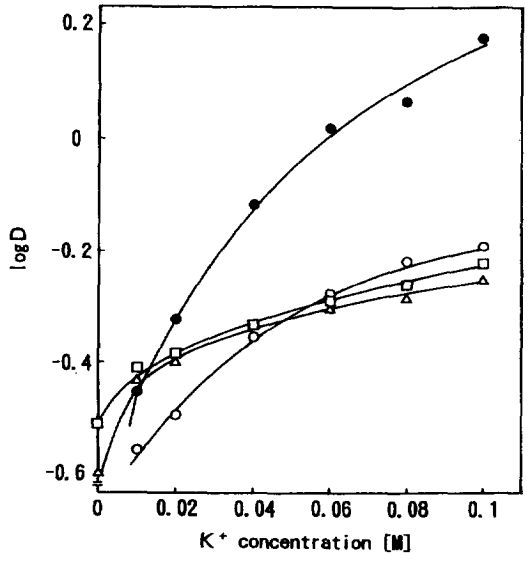

Fig. 4. Relationship between the distribution ratio of the metal–Nitro-PAPS chelates onto ODS and the concentration of potassium cation. D: distribution ratio of metal ions. Nitro-PAPS: $2 \cdot 10^{-5}$ mol dm^{-3}. Metal ions: (□)=Co; (○)=Ni; (●)=Fe, $5 \cdot 10^{-6}$ mol dm^{-3}; and (△)=Cu, $1 \cdot 10^{-5}$ mol dm^{-3}. ODS: 30 mg. Sample solvent: water (pH 6.0).

aqueous phase was determined by a spectrophotometric method. The distributions of the Nitro-PAPS chelates onto ODS also occurred without adding a specific ion pair reagent such as tributylammonium ion (TBA^+). It is thought that the anionic metal–Nitro-PAPS chelates form ion pairs with potassium cations of potassium chloride and then the ion pairs are distributed onto ODS, so that a good separation of those chelates was obtained by the interaction between ODS and the mobile phase.

The phenomena of these results are similar to the chromatographic behaviours of the Nitro-PAPS chelates using a mobile phase which contained potassium ions as counter cation for the chelates. By the proposed method, the polarity of the top of the stationary phase is increased because a large volume of aqueous solution is injected into the column of RP-HPLC. The metal–Nitro-PAPS chelates are unable to travel in the column with water as sample solvent. Thus, since the metal–Nitro-PAPS chelates tend to retain on the stationary phase of RP-HPLC, it is possible to enrich those chelates at the top of the column. Then Nitro-PAPS and the metal–Nitro-PAPS chelates retained are allowed to travel in the column encountering the stream of the mobile phase and they are separated to each component giving a chromatogram, as shown in Fig. 1.

When a mobile phase containing TBA^+ instead of potassium ion was used, a better separation of the Nitro-PAPS chelates than the proposed method was observed. But it had a long retention time, so the pH of the mobile phase, in the proposed method, was adjusted by the use of the potassium phosphate buffer containing potassium ions resulting in the counter cations.

On account of the mechanism of the enrichment and the separation, an increase in retention time of each metal–Nitro-PAPS chelate was caused by an increase in injection volume and also the band widths of peaks might be spread by using a strong organic solvent as a sample solvent.

3.6. Calibration graphs

The calibration graph was linear below 40 ng cm^{-3} for each metal ion. The determination limits (5σ) for the metal ions which were governed by the blank test, were calculated to be 0.01, 0.04, 0.2 and 0.2 ng cm^{-3} for cobalt(II), nickel(II), copper(II) and iron(II), respectively. The reproducibility of the method was also examined for 4 ng cm^{-3} [copper(II), cobalt(II), and iron(II)] and for 2 ng cm^{-3} [nickel (II)]. The relative standard deviations ($n=10$) were 2.5% for copper(II), 2.9% for cobalt(II), 3.2% for nickel(II) and 3.8% for iron(II).

3.7. Effect of foreign ions

The effect of foreign ions usually found in natural water such as rain and river water, was studied by adding each foreign ion, in turn, to the sample. The tolerance limit in 2.0 cm^3 of sample solution was taken as the amount giving an error of ±3% in the recovery. The ions which caused no interference at the 40 μg level were sodium(I) and chloride, those at the 20 μg level were potassium(I), magnesium(II), calcium(II), nitrate, carbonate and sulfate, those at the 2 μg level were manganese(II), fluoride and metasilicate, and those at the 0.2 μg level were aluminum(III) and strontium(II). The tolerance limit of vanadium(V) (added as vanadate) was at the 0.02 μg level. Vanadium(V) was reacted with Nitro-PAPS to form vanadium(V)–Nitro-PAPS chelate and the peak of it appeared between the copper and cobalt peaks on the chromatogram. The peaks were roughly overlapped and a complete separation of these three peaks was not achieved by the proposed chromatographic conditions. The concentration of vanadium in natural waters is several ng cm^{-3}, therefore, the effect of vanadium can be neglected in the analysis of those samples. But more than 0.2 μg level of zinc(II) is apt to decrease the peak area of iron(II)–Nitro-PAPS chelate because Nitro-PAPS is consumed by zinc(II). Therefore, when zinc(II) exists in the sample at more than 0.2 μg, it must be accurately diluted with pure water in advance or an excess Nitro-PAPS added.

The proposed method has the high sensitivity and selectivity of some metal ions, except for a slight interference of zinc(II). Therefore, the method was applied to the determination of copper(II), cobalt(II), nickel(II) and iron(II) in natural water samples.

3.8. Application to water samples

The proposed method was applied to the de-

Table 1
Analytical results of natural water samples

Sample	Ion added (ng cm^{-3})				Ion found (ng cm^{-3})				Recovery (%)				R.S.D. (%, n=6)			
	Cu	Co	Ni	Fe	Cu	Co	Ni	Fe	Cu	Co	Ni	Fe	Cu	Co	Ni	Fe
Rain water[a]					4.0	0.35	2.20	23.5					2.95	3.23	3.61	3.74
	8.0	0.6	4.0	7.2	11.6	1.00	6.18	29.2	97	105	100	95				
River water[b]					1.5	N.D.	0.38	3.5					2.76	–	4.03	4.05
	2.0	0.6	0.5	4.0	3.5	0.62	0.87	7.2	100	103	99	96				

[a] Collected at Kanda-Surugadai, Tokyo (sampled on 11 November 1993).
[b] Collected at Aki river, Tokyo (sampled on 15 November 1993).
N.D., not determined (Co: less than 0.01 ng cm^{-3}). R.S.D.: Relative standard deviation. Injection volume: 2.0 cm^3.

termination of copper(II), cobalt(II), nickel(II) and iron(II) in natural water samples. The pH of the water samples was adjusted to pH 1 by adding hydrochloric acid immediately after the sampling, and suspended particles were removed by using a 0.45-μm membrane filter. The obtained analytical results are listed in Table 1, together with the results of a recovery test. The contents of cobalt(II) in a river water sample was lower than determination limits (0.01 ng cm^{-3}) and the other metal ions were determined at ng cm^{-3} to pg cm^{-3} level. A good recovery for each sample was obtained, as shown in Table 1.

In conclusion, the proposed method was found to be highly useful for multi-element analysis with 2 cm^3 of sample: a larger volume of sample solution can be used. It is also easy to operate this method and not easy to contaminate from the outside because of on-line column enrichment.

The determination of copper(II), cobalt(II), nickel(II) and iron(II) as their Nitro-PAPS chelates using RP-HPLC is highly sensitive. The simultaneous determination of these metal ions at ng cm^{-3} to pg cm^{-3} level can be achieved.

References

[1] K. Kina, M. Kiyonaga, Proceedings of the 35th Annual Meeting of The Japan Society for Analytical Chemistry, Okayama, October 1986, p. 703.
[2] T. Yamane, Y. Yamaguchi, Proceeding of the 53th Symposium on Analytical Chemistry, Akita, May 1992, p. 296.
[3] C. Ohtsuka, K. Matuzawa, H. Wada, G. Nakagawa, Anal. Chim. Acta 256 (1992) 91.
[4] T. Okutani, T. Yamaji, A. Sakuragawa, Anal. Sci. 11 (1995) 765.
[5] S. Ichinoki, M. Yamazaki, J. Chromatogr. Sci. 29 (1991) 184.
[6] K.J. Willians, A.L. Po Wan, W.J. Irwin, J. Chromatogr. 194 (1980) 211.
[7] E. Kaneko, H. Hoshino, T. Yotsuyanagi, Bunseki Kagaku 42 (1993) 753.
[8] E. Kaneko, T. Yotsuyanagi, Proceedings of the 54th Symposium on Analytical Chemistry, Ibaraki, June 1993, p. 441.
[9] Y. Shijo, T. Shimizu, Analyst 1201 (1988) 113.
[10] H. Wang, Y.X. Miao, W.Y. Mou, H.S. Zhang, J.K. Cheng, Mikrochim. Acta 117 (1994) 65.