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Flow-injection in-line complexation for ion-pair reversed phase high performance liquid chromatography of some metal-4-(2-pyridylazo) resorcinol chelates

Supalax Srijaranai [a,*], Saiphon Chanpaka [a], Chutima Kukusamude [a], Kate Grudpan [b]

[a] *Department of Chemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand*
[b] *Department of Chemistry, Faculty of Science, Chaing Mai University, Chaing Mai 50200, Thailand*

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Abstract

Flow injection (FI) was coupled to ion-pair reversed phase high performance liquid chromatography (IP-RPHPLC) for the simultaneous analysis of some metal-4-(2-pyridylazo) resorcinol (PAR) chelates. A simple reverse flow injection (rFI) set-up was used for in-line complexation of metal-PAR chelates prior to their separation by IP-RPHPLC. The rFI conditions were: injection volume of PAR 85 μL, flow rate of metal stream 4.5 mL min^{-1}, concentration of PAR 1.8×10^{-4} mol L^{-1} and the mixing coil length of 150 cm. IP-RPHPLC was carried out using a C_{18} μBondapak column with the mobile phase containing 37% acetonitrile, 3.0 mmol L^{-1} acetate buffer pH 6.0 and 6.2 mmol L^{-1} tetrabutylammonium bromide (TBABr) at a flow rate of 1.0 mL min^{-1} and visible detection at 530 and 440 nm. The analysis cycle including in-line complexation and separation by IP-RPHPLC was 16 min, which able to separate Cr(VI) and the PAR chelates of Co(II), Ni(II) and Cu(II).

Keywords: Flow injection; In-line complexation; Ion pair reversed phase high performance liquid chromatography; Metal-PAR chelates

1. Introduction

Liquid chromatography has been widely recognized as one of the methods for multi-element and sensitive analysis of metal ions. Various modes of liquid chromatography have been used, including normal phase [1–3], reversed phase and ion exchange chromatography (IEC) [4–10]. Since the introduction of ion-pair reversed phase high performance liquid chromatography (IP-RPHPLC) [11,12] for the separation of charged solutes, IP-RPHPLC has gained wide acceptance as an alternative method to IEC for charged analytes, including metal ions. IP-RPHPLC offers multi-element detection capacity, selectivity and sensitivity of analysis. Moreover, the reversed-phase stationary phase has the benefit of lower cost compared to the IEC stationary phase.

Most of the reports on IP-RPHPLC for metal analysis [13–16] are based on the separation as their chelates. Pre-complexation of metal ions with appropriate ligands has many advantages such as increasing selectivity between metal ions, the ability to determine speciation and increasing sensitivity for chelates with high absorptivity. Among the many ligands successfully used for IP-RPHPLC separation of metal ions, 4-(2-pyridylazo) resorcinol (PAR) is one of the most widely used ligands. PAR is an azo dye has been used for the spectrometric determination of over 40 different metals [17]. PAR forms ionic complexes with large absorptivity ($\sim 10^4$ L cm^{-1} mol^{-1}) [18] at about 500 nm. It has been shown to be an effective reagent for the determination of metals using HPLC with either pre-column [19] or post column complexation techniques [20,21].

Typically, complexation of metal ions is performed by batch or external to the chromatographic system before injection. External complexation is time consuming and the large amounts of chemicals used mean more waste to discharge. It is prone to contamination, especially for trace level determinations. Nowadays, the main consideration includes automation of the method, low operating costs, less waste as well as high sample throughput.

Flow injection (FI) has been known with features of a simple operational basis, using inexpensive hardware, straightforward thus leading to convenient operation, high sample throughput, cost effective performance and versatility. FI has been widely

* Corresponding author. Tel.: +66 43 202222/41x2243; fax: +66 43 202373.
E-mail address: supalax@kku.ac.th (S. Srijaranai).

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used as an analytical tool and also combined with the other analytical techniques [22].

Flow injection coupled with HPLC systems is usually intended to improve general features of the analytical process such as sensitivity, precision, rapidity, cost, etc. [23]. FI coupled with HPLC is used in two different modes, i.e., pre- or post-column arrangements. For the pre-column arrangement, as in the present study, the FI port is placed before the HPLC. The specific objectives of pre-column coupling are automation of sample clean-up and/or preconcentration steps, automatic implementation of derivatization reactions and saving reagents. Two methods have been used to couple FI as precolumn of HPLC. The first method, the sample plug is injected through the FI valve and then passed through HPLC loop. In the second method, the sample from the FI system is retained in a precolumn placed in HPLC loop.

Previous work in this laboratory has involved metal analysis by IP-RPHPLC via batch complexation with PAR [24]. In the work described here, a simple FI system was developed as the in-line precolumn for complexation of some metal-PAR chelates before being analysed by IP-RPHPLC. The FI part (rFI) is operated by injecting a PAR reagent solution into a metal ion solution flowing stream. A portion of the PAR-metal mixture zone is then sampled with the HPLC injection valve for subsequent separation and further detection.

2. Experimental

2.1. Chemicals and reagents

All the reagents used were of analytical reagent (AR) grade, 4-(2-pyridylazo) resorcinol and tetrabutylammonium bromide (TBABr) were purchased from Fluka (Switzerland). 2-Diethylaminoethanol was obtained from Merck (Germany). Methanol and acetonitrile were of HPLC grade from Lab-Scan (Thailand). The atomic absorption standard solutions (1000 mg L^{-1}) of Cu(II), Cd(II), Co(II), Hg(II), Zn(II), Fe(III) and Pb(II) were obtained from Ajax Finechem (Australia) whereas Ni(II) was purchased from BDH (England). Cr(VI) oxide was obtained from Merck (Germany). Aqueous solutions were prepared with deionized water obtained from RiOs^{TM} type I simplicity 185 (Millipore Waters, USA) throughout the experiment. Standard solutions of metal ions were prepared daily by stepwise dilution of 1000 mg L^{-1} stock solution with water. Stock PAR solution (0.001 mol L^{-1}) was prepared by dissolving an accurately weighed amount of 4-(2-pyridylazo)resorcinol in water and stored in a dark bottle. Working solution was prepared daily with water and appropriate volume of 2-diethylaminoethanol was added to make the concentration of 2.5×10^{-4} mol L^{-1} in such a PAR solution.

2.2. Instruments

A schematic representation of the rFI coupled with the HPLC system is shown in Fig. 1.

The rFI system used a 505s 505LA peristaltic pump (Watson Marlow, England). PFA Teflon tubes (1.5 mm i.d.) were employed for the reaction coils and were connected to a six-port low-pressure injection valve, four way switching valve (Upchurch, USA) was used to allow the metal-chelates flow to HPLC system. A manual operation using a stopwatch was for time control.

The chromatographic set-up consisted of a Waters 6000A Dual Pump, a Rheodyne injector with 20 μL sample loop and a Waters 484 Tunable Absorbance Detector (Waters, USA) equipped with Waters 740 Data Module Integrator (Waters, USA), and the Millinium 32 Software data acquisition system was used. A 996 photodiode array (Waters, USA) was also used for the study of interferences. A C_{18}-μBondapak (3.9 mm i.d. × 300 mm) coupled to a guard column (Waters, USA) was used as the stationary phase.

The spectra of the metal chelates in batchwise experiments were obtained with a Agilent 8453 (USA) UV–vis spectrophotometer equipped with a 1 cm quartz cell.

2.3. Procedure

Once the baseline of the HPLC was steady, a complete cycle (4 steps) of the rFI-HPLC manifold was started. The 4 steps include prefill, complexation, separation and washing. In the first (prefill) step, the aqueous solution containing metal ions was pumped through the rFI manifold for 30 s, this period was long enough to fill the transmission line with metal solution. The HPLC was in the load mode throughout this step to maintain a steady baseline of mobile phase.

During the prefill step, an aliquot of PAR was filled into the loop connected to V2 (at LOAD position).

Step 2, the complexation step, was started by switching V2 to INJECT position. The metal ions merged with PAR and complexation occurred during their passage through the reaction coil (RC). To avoid a dilution edges of the zone and to allow only the middle zone of the PAR chelates to pass into the HPLC-loop, the valve V3 was switched after 8 s of injection of PAR. The subsequent time period of 2 s, was enough to rinse and fill the HPLC-loop (20 μL).

Then, step 3 (separation step), was initiated via HPLC-valve (V4). The PAR-chelates were introduced and then separated in the HPLC system.

Finally, step 4 (washing step), was to wash the rFI and HPLC-loop for the next analysis, while separation was taking place on the HPLC column.

3. Results and discussion

3.1. Coupling of rFI to IP-RPHPLC

The rFI was chosen instead of normal FI because of its low background noise for HPLC baseline as well as lower PAR consumption. The rFI was coupled to the HPLC by switching valve (V3) shown in the diagram (Fig. 1).

Factorial design was used to investigate the influence of parameters of the rFI system on the peak height (absorbance). The four variables studied were: flow rate of metal ions stream, injection volume of PAR, length of the mixing coils and concen-

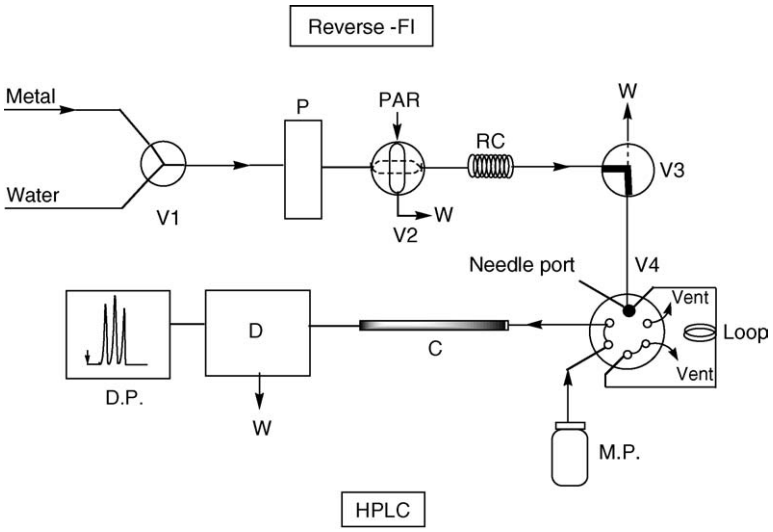

Fig. 1. Diagram of FI-HPLC in-line derivatization system: P, peristaltic pump; RC, reaction coil; V1, three way valve; V2, low pressure injection valve; V3, switching valve; V4, high pressure injection valve; C, analytical column; M.P., mobile phase; D, UV–vis detector/photodiode array detector; D.P., data processor; W, waste.

tration of PAR. A factorial design for four variables at two levels (2^4 resolution, 16 experiments) was performed. According to the results obtained from the factorial design, the chosen parameters to be optimized were the concentration of PAR, injection volume of PAR solution and flow rate of metal stream. The variable size simplex was then employed for optimization. The mixing coil length of 150 cm was used throughout the experiment.

In-line complexation of metal-PAR chelates was performed using the rFI which the optimum conditions were: mixing coil length of 150 cm, injection volume of PAR 85 μL, flow rate of metal stream 4.5 mL min^{-1} and concentration of PAR 1.8×10^{-4} mol L^{-1}. The PAR chelates were then separated via IP-RPHPLC.

Synchronization of the FI manifold and the HPLC is very important to achieve good performance of the coupling system. The time intervals and valve positions of the rFI-HPLC were investigated using the results obtained from the study of the optimization of the rFI. Manual operation of the rFI-HPLC system was found to provide satisfied precision.

The complete cycle of rFI coupling to IP-RPHPLC was 120 s, where as the analysis time of the HPLC was 14 min. Operating periods and valve position for rFI-IPRPHPLC are summarized in Table 1 and Fig. 2.

3.2. IP-RPHPLC of metal-PAR chelates

In IP-RPHPLC, the metal chelates, which are successfully separated, have to be stable and kinetically inert [25]. It is known that the retention behavior of chelates in IP-RPHPLC depends strongly on complex composition (metal:ligand), which is governed by the nature of the central metal ion. The mobile phase composition is also govern the separation. The principal parameters of interest in the mobile phase are pH, buffer (type and concentration), organic modifier and ion pairing agent (long-chain alkyl ions with a charge opposite that of analytes). There are several mechanisms [26–28] explaining the retention behavior of IP-RPHPLC, such as the ion-exchange mechanism, the solvophobic theory and dynamic equilibrium.

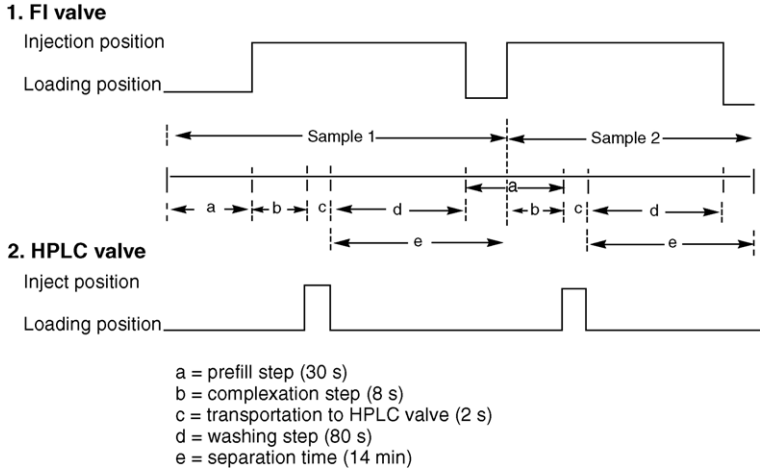

Fig. 2. Diagram of timing control for operation of valves in the FI-HPLC system.

Table 1
Valves positions and operating times for rFI-HPLC

Step	Time (s)	Valve position				In-line complexation operation	
		V1	V2	V3	V4	Medium pump	Stage of operation
Sample 1							
1	30	To V2	Load	To waste	Load	Metals	Prefill
2	38	To V2	Inject	To V4	Load	Metals	Complexation
3	40	To V2	Inject	To V4	Inject	Metals	Separation
4	120	To V2	Load	To V4	Load	Water	Washing
Sample 2							

Typically, PAR forms anionic chelates with metals at the metal to ligand ratio of 1:2 [29]. Thus, cationic ion pairing agent, tetrabutyl ammonium bromide was used. The optimum mobile phase was obtained by slightly adjusting the one obtained in our previous work [24]. The mobile phase composition was 37% acetonitrile, 6.2 mmol L^{-1} TBABr and 3.0 mmol L^{-1} acetate buffer pH 6.0.

Baseline separation of three metal-PAR chelates was achieved within 14 min, with the elution order of Co(II)-PAR, Ni(II)-PAR and Cu(II)-PAR. The excess PAR was detected at the retention time of 9.6 min. The chromatogram is shown in Fig. 3.

Using the optimum mobile phase and the detection at 440 nm, Cr(VI) was retained shortly (4.9 min) after unretained peak (at 3.3 min), as shown in Fig. 4. The spectrochromatogram corresponding to Fig. 4 is shown in Fig. 5. For the condition used, Cr(VI) might present as its oxyanion ($HCrO_4^-$) [30]. Thus, it could interact with ion pairing agent in the same manner to the anionic chelates. The peak at 4.9 min which was identified as Cr(VI) gives the absorption spectrum (as shown in Fig. 6) identical to the spectrum of Cr(VI) detected by UV–vis spectrometer. The resolutions between pairs were as follow: 1.4 for Cr(VI) and Co(II)-PAR, 1.0 for Co(II)-PAR and Ni(II)-PAR, 2.4 for Ni(II)-PAR and excess PAR, and 1.3 for excess PAR and Cu(II)-PAR.

3.3. Performance of rFI-IP-RPHPLC

Quantitative features including linearity and reproducibility for retention time and peak area were studied using the optimum condition. Calibration graphs were prepared by plotting the concentration of each metal ion (μg mL^{-1}) against the peak area. The limit of detection (LOD) was deduced based on three times of baseline signal. The calibration equation, coefficient of correlation (r^2), recovery, reproducibility and LOD are summarized in Table 2.

3.4. Interferences

The effect of interferences on the chromatography of metal-PAR chelates was investigated. The chosen ions are the ions able to form chelate with PAR including Cd(II), Cr(III), Hg(II), Mn(II), Fe(III), Pb(II) and Zn(II). These metal ions were individually injected into the rFI-HPLC. All of the studied ions could not form chelates with PAR under the condition used. Only peak, which was identified as PAR (9.6 min) was observed.

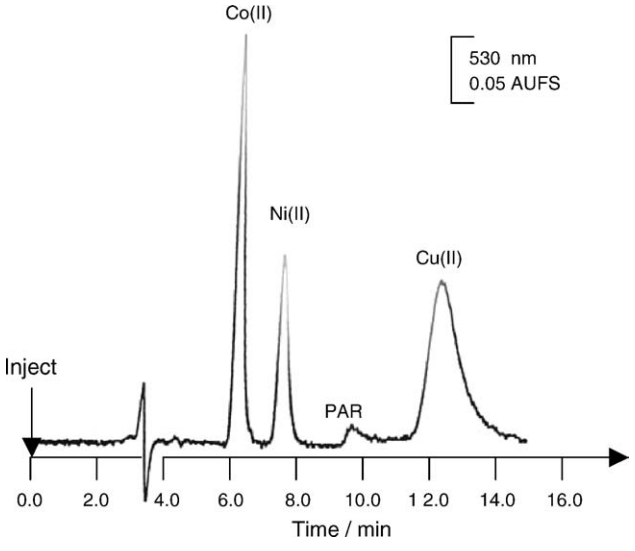

Fig. 3. Chromatogram of metal-PAR chelates; condition: C_{18} column, mobile phase 37% acetonitrile, 6.2 mmol L^{-1} TBABr and 3.0 mmol L^{-1} acetate buffer pH 6.0, flow rate of mobile phase 1.0 mL min^{-1} visible detection at 530 nm; peak: 0.10 μg mL^{-1} Co(II), 0.20 μg mL^{-1} Ni(II), excess PAR and 0.80 μg mL^{-1} Cu(II).

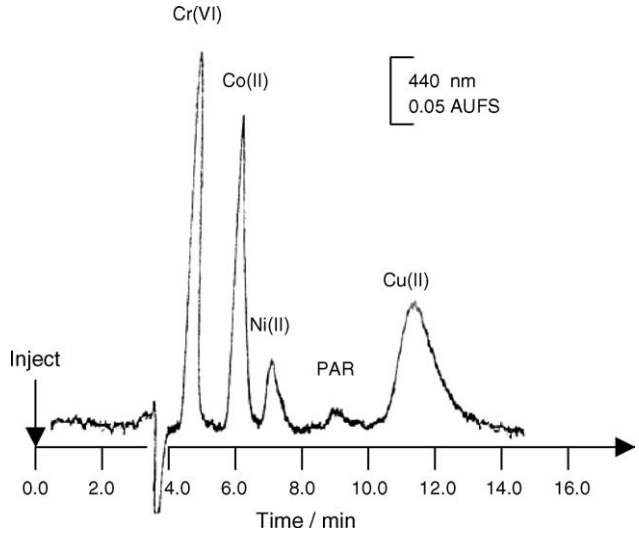

Fig. 4. Chromatogram for Cr(VI) and metal-PAR chelates; peaks: 5.0 μg mL^{-1} Cr(VI), 0.10 μg mL^{-1} Co(II), 0.10 μg mL^{-1} Ni(II), excess PAR and 0.40 μg mL^{-1} Cu(II) (condition as described in Fig. 3, except visible detection at 440 nm).

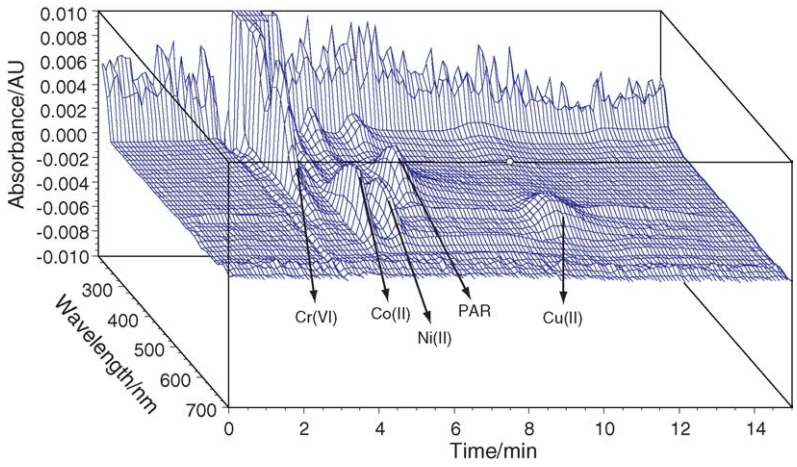

Fig. 5. 3D plot of Cr(VI) and metal-PAR chelates of chromatogram in Fig. 4.

Table 2
The quantitative features of rFIA-HPLC

Chelates	Concentration of metal ion ($\mu g\,mL^{-1}$)	Linear equations $Y = AX + C$	Correlation coefficient (r^2)	R.S.D.[a] (%) ($n = 11$)		LOD (3σ) ($\mu g\,mL^{-1}$)	Percent recovery
				t_R	Area		
Cr(VI)	1.00–6.00	$473.51X - 0.17$	0.9961	2.0	0.94	1.00	113
Ni(II)-PAR	0.01–0.20	$341.66X - 4.73$	0.9983	2.1	0.91	0.02	84
Co(II)-PAR	0.01–0.40	$547.21X - 0.61$	0.9952	0.6	0.97	0.03	96
Cu(II)-PAR	0.05–0.60	$293.80X + 5.49$	0.9986	2.4	0.71	0.15	102

[a] Concentration of each metal was described in Fig. 4.

The study on tolerance level of the metal ions which could not form chelates with PAR was studied by individually spiking the metal ions at difference amounts (ranging from 0.5 to 10.0 $\mu g\,mL^{-1}$) into the mixture of 0.10 $\mu g\,mL^{-1}$ Co(II), 0.20 $\mu g\,mL^{-1}$ Ni(II), 0.40 $\mu g\,mL^{-1}$Cu(II) and 5.0 $\mu g\,mL^{-1}$ Cr(VI). It was found that the presence of the foreign ions did not affect the retention time of the PAR chelates of Co(II), Ni(II) and Cu(II). However, the quantitative signals (both peak height and peak area) were affected by the addition of the foreign ions. Cu(II)-PAR was strongly influenced when the concentrations of the foreign ions increase to 2.5 times resulted in decreased of peak height and peak area. The effect on Ni(II)-PAR was observed when the foreign ions increase to five times greater than Ni(II), resulted in the decreasing of peak height and peak area. This effect was also observed for Co(II) when the concentration of the foreign ion was 10 times to Co(II). The peak area of Cr(VI) was not affected by the addition of the foreign ion. However, the obtained spectra and the 3D plots (results not shown) revealed that neither PAR chelates of the foreign ions nor the ternary complexes were formed. According to the obtained results indicating that in such a condition, quantity of PAR was enough for all of metal ions. Furthermore, to ensure the excess amount of PAR, 10 times higher concentration of PAR, i.e., 1.0×10^{-3} mol L^{-1} was used. Similar results were obtained and large peak of excess PAR overlapped the analyte peaks. The effect of interference on the present method was obviously seen when compared to the previous work [24] on precomplexation of metal-PAR chelates by batch method prior to the analysis by IP-RPHPLC. This may attribute to the nature of the flow system, which a short time that stream of reagents are reacted. Neither physical equilibrium nor chemical equilibrium (i.e., the completeness of reaction) has been attained by the time it was detected.

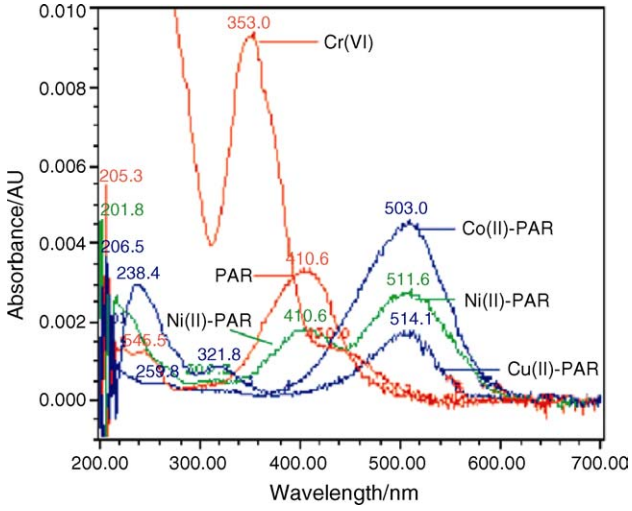

Fig. 6. The absorption spectra of chromatogram in Fig. 4.

3.5. Analysis of real sample

According to the study, it is possible to analyse Cr(VI) simultaneously with Ni(II). The present method was applied to the analysis of chrome plating waste water. The samples were collected from chrome plating plant in Khon Kaen and were

Table 3
Results of analysis of chrome plating waste water

Metal	Concentration ($\mu g\,mL^{-1}$)			
	Sample 1		Sample 2	
	FI-HPLC	AAS	FI-HPLC	AAS
Cr(VI)	984.5 ± 6.4[a]	980.8 ± 7.9[a]	69.3 ± 10.7[a]	72.6 ± 9.3[a]
Co(II)	N.D.[b]	N.D.[b]	N.D.[b]	N.D.[b]
Ni(II)	1574.6 ± 10.2[a]	1580.9 ± 12.0[a]	121.4 ± 7.4[a]	126.8 ± 8.2[a]
Cu(II)	N.D.[b]	N.D.[b]	N.D.[b]	N.D.[b]

[a] S.D. ($n = 3$).
[b] Not detected.

analysed after dilution, pH adjustment and filtration through 0.45 μm membrane. The results obtained are listed in Table 3, which were in good agreement with that of AAS.

4. Conclusion

In the present study, a simple combination of rFI and HPLC resulted in a powerful technique for simultaneous analysis of metal ion as their PAR chelates. The rFI was coupled to HPLC with simple operation. Using the developed rFI for in-line complexation gives benefit of less PAR consumption, less analysis time and less waste disposed comparison to the batch derivatization. The analysis cycle consists of in-line complexation (ca. 2 min) and separation by IP-RPHPLC (14 min). The method was successfully applied for the separation of Co(II), Ni(II) and Cu(II) as their PAR chelates.

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