Simultaneous determination of heavy metals in drinking waters and wines by methods: Ion pair-reversed phase high performance liquid chromatography and capillary zone electrophoresis

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Abstract: Simultaneous determination of metal ions in drinking waters and wines using ion paired reversed phase high performance liquid chromatography (IP−RPHPLC) and capillary zone electrophoresis (CZE) have been investigated. Co(II), Cu(II), Ni(II), Fe(II) and V(V) are analyzed as their Nitro-PAPS chelates. Reversed flow injection (rFI) is used for in-line complexation prior to their separation by IP-RPHPLC. The optimum mobile phase compositions, obtained from sequential simplex method by carried out on a C18 µBondapak column at a flow rate of 1.0 mL min^{-1} and detection at 570 nm, are 39.7% acetonitrile containing 5.5 mmol L^{-1} acetate buffer 5.0 containing 3.5 mmol L^{-1} tetrabutylammonium bromide (TBABr). Meanwhile, CZE condition is performed on an uncoated fused-silica capillary (30.0 cm effective length) with the electrolyte of 35 mmol L^{-1} borate buffer pH 10.0 in 10% acetonitrile and 0.04 mmol L^{-1} Nitro-PAPS, separation voltage of 15 kV and detection at 250 nm. The analytical performance of both methods are examined and discussed. The analysis time of IP-RPHPLC including in-line complexation by rFI and separation of five metals is 19 min whereas the analysis time of CZE is 7 min. A high risk of iron and copper contamination is found in all drinking water and wine samples studied. The results obtained from both proposed methods are comparable (*t*-test, 95% confidence limit).

Keywords: Reverse-flow injection, ion pair reversed phase high performance liquid chromatography, capillary zone electrophoresis, metal-Nitro-PAPS chelates, simplex optimization, drinking waters, wines

Introduction

Determination of metal ions is still an active topic in analytical science. There are various techniques for the analysis of metal ions. High performance liquid chromatography (HPLC) and capillary electrophoresis (CE) are becoming as the accepted methods used for the analysis of metal ions. Both HPLC and CE have benefit of simultaneous multielement determination. HPLC and CE are known as complementary techniques, they are used in the same manner. Recently, the separation modes of HPLC including ion exchange chromatography (IEC) (Divjak *et al*., 2003; Zhang *et al*., 2003; Kaur *et al*., 2005; Srijaranai *et al*., 2011), reversed phase and ion pair reversed phase high performance liquid chromatography (IP-RPHPLC) (Ma *et al*., 1997; Chen *et al*., 1999; Srijaranai *et al*., 2002, 2006; Matsumiya *et al*., 2004; Butwong *et al*., 2010) whereas capillary zone electrophoresis (CZE) (Chen *et al*., 2002; Wang *et al*., 2004; Santalad *et al*., 2007) mode separation in CE have been widely used for determination of metal ions in various samples. Two main approaches for metals analysis by either HPLC or CE are analysis as their free metal ions and analysis as their suitable chelates. IEC and CZE are commonly used methods for the first approach, where the selectivity based on size to charged ratio of metal ions. In the second approach, the determination of metal ions after the complexation with the appropriate ligands can be performed by either IP-RPHPLC or CZE. Pre-column derivatization can be carried out by either batch (off-line) or on-line complexation in both HPLC and CE. On-line complexation is of interest in HPLC because of ease of operation and high reproducibility. Pre-capillary derivatization is mostly used in CE because the loss of analytes was not observed. The advantages of determination of metal ions as the suitable chelates are increasing selectivity of separation, and increasing sensitivity of detection and ability to do speciation.

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2-(5-nitro-2-pyridylazo)-5-(N-propyl-N-sulphopropyl-amino)phenol or Nitro-PAPS is a pyridylazo compound. Similar to other pyridylazo compound such as 4-(2-pyridylazo)resorcinol (PAR) and 2-(5-bromo-2-pyridylazo)-5-[N-propyl-N-(3-sulfopropyl)amino]phenol (5-Br-PAPS), Nitro-PAPS forms water soluble chelates with many metal ions including Fe(II), Cu(II), Co(ii), Ni(II) and Zn(II) having molar absorptivities of ca. $10^4 – 10^5$ L mol^{-1} cm^{-1} in the visible region (Okutani *et al.*, 1997; Ueno *et al.*; 2000; Burakham *et al.*, 2007). Nitro-PAPS has been used as a highly sensitive chromogenic reagent for spectrometric techniques of several metal ions. Most of the application of Nitro-PAPS were single element analysis with direct colorimetric including iron (Makino *et al.*, 1988; Ringanakos *et al.*, 2003; Internet:GmbH-Germany, 2003) and zinc (Makino, 1991; Internet: Chema diagnostic, 2000). Flow injection couple to spectrophotometry was used for the determination of Fe(II) in salt samples (Yamane *et al.*, 1995). Flow injection analysis of iron and copper at ppb level in water sample were also determined using Nitro-PAPS (Ohno *et al.*, 2002). Although Nitro-PAPS chelates have properties similar to PAR chelates, there were fewer publications on simultaneous analysis of Nitro-PAPS chelates by HPLC and CE. IP-PRHPLC was used for the analysis of Nitro-PAPS chelalates of Cu(II) and Co(II) (Okutani *et al.*, 1997, 2000). The analysis of Pb(II), Cd(II) and Mn(II) was reported using ion chromatography which used Nitro-PAPS as post-column reagent (Yamane *et al.*, 1997). The simultaneous analysis of Cu, Zn and Fe by flow injection was demonstrated using a new design multi-channel micro-cell (Sakai *et al.*, 2000; Teshima *et al.*, 2006).

The proposed of this study was to use Nitro-PAPS as a ligand for simultaneous analysis of Cu(II), Co(II), Ni(II), Fe(II) and V(V) in drinking waters and wines by IP-RPHPLC and CZE. The complexation of metal-Nitro-PAPS chelates was performed on-line by the lab made flow injection system prior to IP-RPHPLC. In CZE, pre-capillary complexation was used. The optimum conditions and analytical characteristics in both IP-RPHPLC and CZE for the analysis of Nitro-PAPS chelates were examined. The results obtained from both proposed method as well as inductive coupled plasma-optical emission spectrometry (ICP-OES) have been compared.

Experimental
Chemical and reagents
All of chemical used were at least analytical reagent grade. 2-(5-bromo-2-pyridylazo)-5-[N-propyl-N-(3-sulfopropyl)amino]phenol (5-Br-

PAPS) or Nitro-PAPS was obtained from Dojindo laboratories (Japan). Tetrabutylammonium bromide (TBABr) and sodium acetate were perchased from Fluka (Switzerland). Methanol and acetonitrile were of HPLC grad and were obtained from Lab-Scan (Ireland). The atomic absorption standard solutions (1000 mg L^{-1}) of metal ions were obtained by Ajax Finechem (Australia). Aqueous solutions were prepared with deionized water from RiO$_s$™ Type I Simplicity 185 (Millipore water, USA). Standard solutions of metal ions were prepared daily by stepwise dilution of 1000 mg L^{-1} stock solution with water. Stock Nitro-PAPS solution (1.0×10-3 mol L^{-1}) was prepared by dissolving an accurately amount of Nitro-PAPS in water and stored in a dark bottle. Working solutions were prepared daily by stepwise dilution.

Instrumentations
The chromatography consisted of a Waters 6000A Dual pump (Waters, USA), a Rheodyne injector with a 20μL sample loop and a Waters 996 photodiode array. The CSW 32 software (Waters) was used for data acquisition. A C18 μBondapak (3.9×300 mm I.D.) column was used. The rFI system used a 505LA peristaltic pump, PFA Teflon tube (1.5 mm I.D.) was employed for the reaction coils (150 cm) and was connected to a six port low-pressure injection valve (Upchurch, USA). A four way switching valve (Upchurch, USA) was used to allow the metal-chelates flow into the HPLC system. A manual operation using a stopwatch (Casio, Japan) was used for time control. The spectra of the metal chelates in batch equipped with a 1 cm quartz cell. The pH measurements were performed on a Model 215 pH meter (Denver Instrument, England). All experimental of CZE were performed on a Beckman P/ACE ™ MDQ Capillary electrophoresis system (Beckman Instrument, Fullerton, USA) equipped with a photodiode array detector. The electropherograms were recorded and integrated by an IBM personal computer with 32 Karat software version 5.0 (Beckman). A fuse silica capillary (Beckman) with a total length of 40.2 cm (30.0 cm effective length)×50 μm I.D. was used. Samples were injected in the hydrodynamic mode at 0.5 psi. All experiments were conducted at 25 °C. An inductively coupled plasma-optical emission spectrometer (Optima 2100 DV, Perkin Elmer, Germany) with Winlab 32™ Instrument control was used for analysis of free metals in the samples.

IP-RPHPLC of metal-Nitro-PAPS chelates
Once the baseline of HPLC was steady, the rFI-HPLC manifold was started. There were four

steps in operating the rFI-HPLC including prefilled, complexation, separation and washing. Operating periods and valve position for rFI-HPLC were slightly adapted from our previous work (Srijaranai *et al*., 2006). There are four operation steps in briefly as follows:

1 (prefilled), 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 the metal solution. During the prefilled step, an aliquot (85 µL) of Nitro-PAPS was filled into the loop.

2 (complexation), the metal ions were merged with Nitro-PAPS to from complexes during their passage through the reaction coil. To avoid dilution zone and to allow only the middle zone of the Nitro-PAPS chelates to pass into HPLC loop, the Nitro-PAPS chelates was passed through the HPLC loop (20 µL) after 15 s of the injection of Nitro-PAPS.

3 (separation), the Nitro-PAPS chelates were introduced into column and then separated in HPLC system.

4 (washing), the step was performed to wash the rFI and HPLC loop before the next analysis.

The optimum mobile phase was studied using the sequential simplex method (Berridge *et al*., 1984; Srijaranai *et al*., 2002). The mobile phases were the mixtures of acetonitrile, acetate buffer and TBABr at the established concentrations from the simplex optimization calculations. A mobile phase flow rate of 1.0 mL min⁻¹ was used throughout. It is worthwhile to ensure that there was neither pairing ion retained nor saturating in the column between run, the column was washed with MeOH:water (50:50) for 15 column volumes and was then equilibrated with the mobile phase being used for 25 column-volumes before injection. The experiments were then performed using the established vertices until no further improvement of the CRF was observed.

CZE of metal-Nitro-PAPS chelates

To obtain the reproducibility, new capillary was initially washed with methanol for 5 min, followed by water for 2 min, 0.1 mol L⁻¹ HCl for 5 min, water for 2 min, 0.1 mol L⁻¹ NaOH for 5 min and water for 2 min. At the beginning for each day, the capillary was rinsed with 0.1 mol L⁻¹ NaOH and water for 5 min, the equilibrated with the being used electrolyte for 20 min. In between all electrophoretic separations, the capillary was automatically rinsed with 0.1 mol L⁻¹ NaOH (3 min), water (3 min) and the electrolyte (5 min). All electrolyte solutions were filtered through a 0.45 µm membrane filter and degassed by sonication

before use.

The metal-Nitro-PAPS chelates were prepared at the metal to ligand ratio (M:L) of 1:2 at pH 5.0. Parameters affected the separation of CZE were studied the composition of electrolytes (pH, type and concentration of buffer and content of organic modifier).

ICP-OES of metal ions

After the complete digestion, sample solutions were analyzed by ICP-OES under the conditions as follows: RF generator power of 1.30 kW, plasma gas flow rate of 15 L min⁻¹, auxiliary gas flow rate of 0.2 L min⁻¹, and pump rate of 1.5 mL min⁻¹. The analytical lines (nm) were 238.8 (Co), 327.3 (Cu), 290.8 (V), 231.6 (Ni) and 238.2 (Fe), respectively.

Analysis of metal ions in samples

The proposed methods were applied to the determination of metal ions in water and wine samples. The studied samples were purchased from the local supermarket. The samples were digested with HNO_3, diluted and complexed with Nitro-PAPS before analyzed by IP-RPHPLC and CZE, whereas another portion of the digested sample solutions can be directly analyzed by ICP-OES.

Wine sample (100.00 mL) was digested with 5.0 mL concentrated HNO_3. After complete digestion (clear solution), the solution was diluted with deionized water and then adjusted to pH 5.0 with NaOH. These solutions were filtered through 0.45 µm membrane filter before analyses.

Water sample was performed in the same manner of wine analysis but use of 500.00 mL representative sample and 2.5 mL concentrated HNO_3 for the digestion.

Results and Discussion

IP-RPHPLC of metal-Nitro-PAPS chelates

The pre-column derivatization by complexation provides the ability and sensitivity of detection of the chelates. The absorption maxima of the studied metal-Nitro-PAPS chelates are overlapped each other in the range of 563 – 593 nm, whereas Nitro-PAPS has the maximum absorption at 454 nm. Therefore, the detection wavelength set at 570 nm was compromised. To successfully achieve the separation by IP–RPHPLC, the metal chelates have o be stable and kinetically inert. For a certain chelating agent (ligand), metal ion to ligand ratio (M:L) and pH are the most important parameters providing the selectivity for each chelate. The conditions for complexation of the studied metal- Nitro-PAPS

chelates were also studied. The mole ratio of metal to Nitro-PAPS of 1:2 and pH 5.0 were obtained. In the present study, the pre-column derivatization of the metals with Nitro-PAPS was performed on-line using a reverse flow injection (rFI) system. The use of the rFI gives the advantages of low background noise baseline and less consumption of Nitro-PAPS. Aliquot of Nitro-PAPS was injected into metal stream and the chelates were formed in a reaction coil before subsequent separation and detection.

Under the proposed IP-RPHPLC, the composition of mobile phase is the crucial parameter to be optimized. There are various factors including pH, buffer concentration, organic modifier and ion pairing agent concentration. The retention behaviors of the metal- Nitro-PAPS are governed by those parameters. In order to obtain the optimum mobile phase within a small number of experiments, the variable size simplex was employed. In this study, three variables (concentration of acetate buffer, concentration of tetrabutyl ammonium bromide (TBABr) and amount of acetonitrile) were investigated at the mobile phase pH of 5.0 throughout. The optimization was initiated by performing the first four experiments using the conditions obtained from the initial vertices. The results were evaluated using chromatography response function (CRF) (Berridge *et al.*. 1984) as follows:

$$CRF = \sum_{i=1}^{L} R_i + L^a - b|T_A - T_L| + c(T_1 - T_0) \quad (1)$$

Where R (the resolution between adjacent peak pairs), L (the number of peaks detected), T_a (a specified analysis time), T_L (the retention time of the last-eluted peak), T_1 (the retention time of the first-eluted peak), T_0 (a specified minimum retention time), while a, b and c are operator-selectable weightings. In the present study the parameters were T_A=20 min, T_L=17 min, while a, b and c are 2, 0.5 and 2, respectively.

The simplex was then moved according to the simplex rule (Srijaranai *et al.*, 2002), in the present study worksheets were applied to calculate for the subsequent vertices. The experiments were then performed using the established vertices until no further improvement of the CRF. The CRF values at the corresponding vertex numbers are shown in Table 1. Although the highest CRF value was obtained at vertex 6 (CRF=108.88), but the analysis time was very long as the same as the conditions of vertex number 9 and 13. The experimental conditions represented by the values of the variables of vertices 14 – 19 were considered more rugged. Therefore,

the optimum conditions of vertex number 14 was acetonitrile-water (39.7:60.3, v/v) containing 5.5 mmol L^{-1} acetate buffer (pH 5.0) and 3.5 mmol L^{-1} TBABr. To improve the separation, TBABr as the ion pairing agent was added to the mobile phase aimed to improve separation of metal-Nitro-PAPS chelates as anionic forms (Okutani *et al.*, 1997). The role of the ion-pairing agent is to provide the exchange sites for the charged species to give some degree of variation in the retention behavior, thus the chromatographic selectivity for separation was expected. In addition, the addition of acetate buffer has been reported that it was able to block the residual silanols and led to the formation of well-defined peak shapes and to enhance peak heights. Using the optimum mobile phase, the separation of five Nitro-PAPS chelates was achieved within 17 min. The elution order was Cu(II),V(V), Co(II), Ni(II) and Fe(II), whereas Nitro-PAPS was eluted at 10.5 min. It showed that resolution for allstudied metal-Nitro-PAPS are higher than 2.0.

Capillary zone electrophoresis of metal-Nitro-PAPS chelates

Although the metal-Nitro-PAPS chelates are colored, the detection in the visible region gave unstable baseline and poor peak shape. Moreover, detection at the visible wavelength provided lover absorption than the detection in UV region. Thus, the detection at 250 nm was selected.

To achieve complete separation of metal-Nitro-PAPS chelates, background electrolyte compositions, buffer pH, concentration of buffer and organic additive were optimized. Preliminary studies with several buffers containing different mixtures of acetate (25 mmol L^{-1}, near pH 5.0), phosphate (25 mmol L^{-1}, near pH 7.0) or borate (25 mmol L^{-1}, near pH 10.0) buffers and 10% acetonitrile in the presence of 4×10 mol L^{-1} Nitro-PAPS were tested by direct hydrodynamic injection (0.5 psi, 5s) of a mixture of the metal-Nitro-PAPS complexes at metal concentration 1.0 mg L^{-1} each. It showed that acetate and phosphate buffers did not lead to complete separation of the chelates, whereas borate buffer provided higher resolution and seems to be as the running buffer for further studies. Then, borate buffer pHs in range of 9.0 − 10.5 were investigated. When pH increased, the long migration time of the chelates was observed. A strongly effect of pH on migration of the chelates was found especially in Ni(II) and Fe(II) complexes (Figure 2). It is clearly seen that at pH>10.0, peaks of Ni(II) and Fe(II) chelates were not resolved as well as at lower pH showed the similar results.

Table 1. Data obtained from simplex optimization of IP-RPHPLC

Vertex No.	Vertex	ACN (%)	concentration of acetate buffer pH 5.0 (mmol L⁻¹)	concentration of TBABr (mmol L⁻¹)	CRF
1	I	40.00	5.00	3.00	63.05
2	I	42.36	5.47	3.23	58.28
3	I	40.59	6.88	3.23	60.62
4	I	40.59	5.47	3.94	63.35
5	R	38.43	6.09	3.55	70.88
6	E	36.46	6.41	3.71	108.88
7	R	38.75	4.16	3.76	71.90
8	C_R	39.21	4.84	3.63	71.96
9	R	37.16	4.59	3.35	90.34
10	R	38.40	3.14	3.66	71.14
11	C_R	38.40	3.88	3.63	71.90
12	R	39.51	5.44	3.68	69.49
13	C_W	38.55	3.71	3.66	88.51
14	R	39.67	5.52	3.49	72.00
15	C_R	39.24	4.80	3.70	69.50
16	R	39.25	5.44	3.69	69.49
17	C_W	39.27	4.12	3.71	67.79
18	R	39.25	5.11	3.70	71.90
19	R	39.63	5.21	3.93	70.88

I = initial vertex; R = reflection vertex; E = expansion vertex; C_R = concentration vertex on the reflection side; C_W = concentration vertex on the worst side

Table 2. The quantitative analysis data for rFI-IPRPHPLC of metal-Nitro-PAPS chelates

Metal	linear equation Y = AX + C	linear range ($\mu g\ mL^{-1}$)	R^2	LOD ($\mu g\ mL^{-1}$)	%RSD peak area	%RSD t_R	% recovery (n=9)
Cu(II)	918.19X+8.80	0.005-1.50	0.9999	0.002	4.8	0.1	102.5±3.2
V(V)	234.11X-21.41	0.100-1.50	0.9949	0.055	3.0	0.1	97.4±4.0
Co(II)	1949.00X-7.59	0.005-1.20	0.9998	0.001	4.1	0.2	106.0±3.4
Ni(II)	414.78X-11.93	0.070-1.00	0.9983	0.006	5.1	0.1	105.7±2.4
Fe(II)	2152.44X-23.72	0.008-1.00	0.9995	0.002	4.9	0.9	99.4±1.3

Table 3. Quantitative data for CZE of metal-Nitro-PAPS chelates

Metal	linear equation Y = AX + C	linear range ($\mu g\ mL^{-1}$)	R^2	LOD ($\mu g\ mL^{-1}$)	%RSD peak area	%RSD t_R	% recovery (n=9)
Cu(II)	$1.201\times10^{-4}X - 0.04211$	0.08-0.70	0.9990	0.06	1.0	0.2	97.9±3.7
V(V)	$0.695\times10^{-4}X + 0.00498$	1.00-10.00	0.9970	1.00	1.4	0.3	98.2±3.2
Co(II)	$1.474\times10^{-4}X + 0.11124$	0.30-1.50	0.9967	0.01	1.5	0.3	98.2±3.3
Ni(II)	$0.946\times10^{-4}X + 0.00753$	0.60-5.00	0.9988	0.60	1.8	0.4	102.1±4.9
Fe(II)	$1.921\times10^{-4}X - 0.02916$	0.10-0.80	0.9964	0.08	1.7	0.2	95.4±2.9

Table 4. The content of Cu(II) and Fe(II) found in water and wine sample using three analytical methods

Sample	Concentration ($\mu g\ mL^{-1}$) IP-RPHPLC Cu(II)	Fe(II)	CE Cu(II)	Fe(II)	ICP-OES Cu(II)	Fe(II)
Water						
MW1	2.60±0.50	11.40±2.28	2.30±1.20	10.13±1.00	< 5.00	9.20±1.20
MW2	3.50±1.45	8.80±1.95	3.10±1.30	10.41±0.90	< 5.00	12.20±2.00
DW1	2.80±0.90	14.20±3.00	2.50±1.00	15.12±1.10	< 5.00	12.80±2.00
DW2	2.20±0.76	6.20±1.55	1.85±0.85	9.22±0.60	< 5.00	8.40±1.60
DW3	3.80±1.02	17.40±3.10	3.55±1.10	19.15±1.30	< 5.00	20.40±2.20
TW1	2.10±1.05	14.80±2.36	1.90±1.25	12.20±1.40	< 5.00	16.40±1.90
Wine						
RW1	12.40±2.30	104.60±3.20	16.50±1.00	103.40±0.90	12.50±2.00	111.00±4.50
RW2	12.30±3.00	55.80±3.00	15.82±1.20	46.43±1.10	18.90±1.80	69.20±2.80
RW3	18.60±3.00	47.40±2.50	14.51±1.50	35.81±0.80	17.53±1.30	40.40±2.30
WW1	14.10±2.80	6.90±1.85	13.12±1.30	8.40±1.00	19.50±1.90	10.90±1.50
WW2	9.50±2.54	8.50±2.00	12.72±1.80	9.23±1.10	15.20±1.70	10.50±1.40

MW: mineral water; DW: drinking water; TW: tap water; RW: red wine; WW: white wine
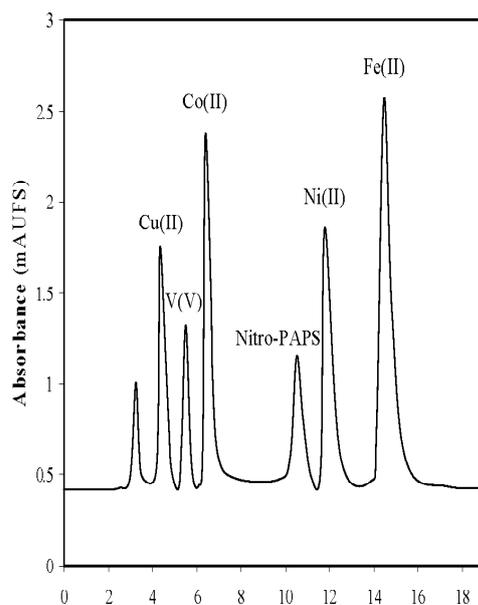
Figure 1. Chromatogram of metal-Nitro-PAPS chelates; condition: C18 column, mobile phase 39.7% acetonitrile, 3.5 mmol L^{-1} TBABr and 5.5 mmol L^{-1} acetate buffer pH 5.0, flow rate of mobile phase 1.0 ml min^{-1}, visible detection at 570 nm; peak: 0.15 µg mL^{-1} Cu(II), 0.50 µg mL^{-1} V(V), 0.08 µg mL^{-1} Co(II), excess nitro-PAPS, 0.30 µg mL^{-1} Ni(II) and 0.20 µg mL^{-1} Fe(II).
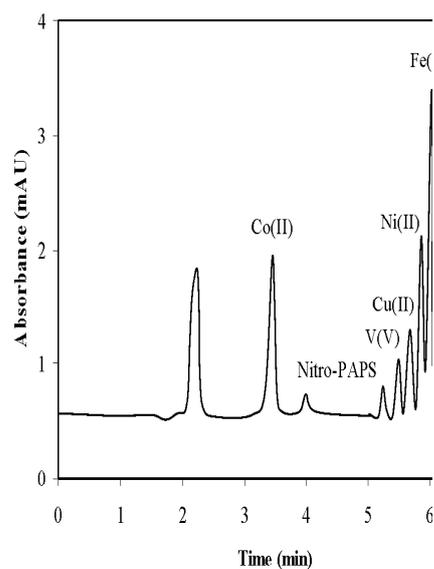
Figure 3. Electropherogram of metal- Nitro-PAPS chelates at the optimum condition: pH 10.0 and the other conditions as the same in Figure 2. Peak: 0.50 µg mL^{-1} Co(II) , excess nitro-PAPS, 2.00 µg mL^{-1} V(V), 0.50 µg mL^{-1} Cu(II), 1.50 µg mL^{-1} Ni(II) and 1.50 µg mL^{-1} Fe(II).
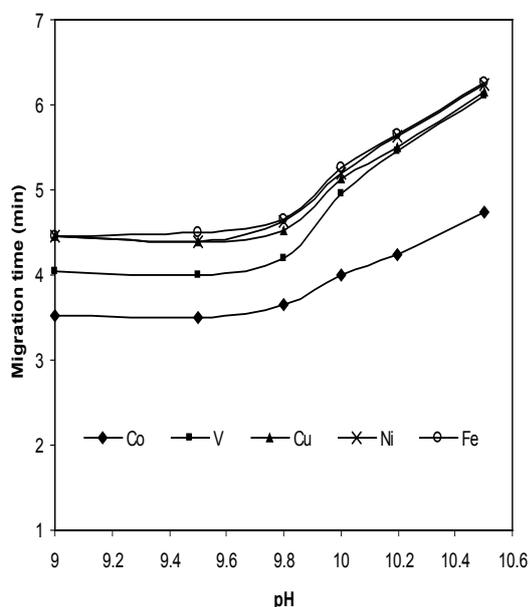
Figure 2. Effect of pH on the migration of the metal- Nitro-PAPS chelates. Electrophoretic condition: 35 mmol L^{-1} borate buffer containing 10% acetonitrile and in the presence of 4×10^{-5} mol L^{-1} Nitro-PAPS, Temperature of 25°C, on-capillary detection at 250 nm.
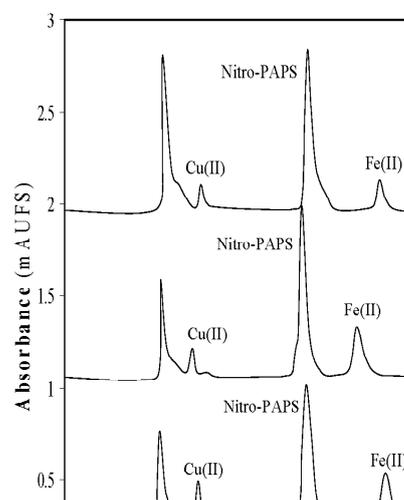
Figure 4. Overlaid typical chromatograms of the representative drinking water and wine sample: (A) DW2, (B) TW1 and (C) RW2. The IP-RPHPLC condition as described in Figure 1.

This behavior may be due to their closed apparent mobilities of the cheates ($\mu_{app,\ Ni(II)}$=2.58×10^{-4} and $\mu_{app,\ Fe(II)}$=2.54×10^{-4} cm^2 V^{-1} s^{-1}, at pH 10.0) under the studied electrophoretic condition ($\mu_{app,\ EOF}$=6.09×10^{-4} cm^2 V^{-1} s^{-1}). To obtain the highest resolution (Rs≈0.7 for Ni(II)&Fe(II)) under the studied condition, pH 10.0 was selected for further studies.

The effect of borate concentrations were then needed to be investigated in range of 15 − 50 mmol L^{-1}. Increasing of borate concentration leads to decrease mobility of the metal chelates that provided better resolution (Rs≈5% increments). However, at concentration of buffer higher than 40 mmol L^{-1} showed no further Rs improvement as well as results in longer analysis time and produces higher current in the electrophoretic system. At concentration of 35 mmol L^{-1} borate was used throughout that provided a proper separation in terms of resolution and analysis time.

In the last studied parameter, organic additive was also investigated to improve the Rs of the chelates over the range of 5 − 15% acetonitrile (ACN). The results showed that no improving resolution of the chelates at the additive content higher than 10% was observed. Morover, it leads to gave long time analysis of the chelates. Thus, the buffer containing 10% ACN was selected as optimum value.

In summary of the electrophoretic condition forseparation of metal-Nitro-PAPS chelates was 35 mmol L^{-1} borate pH 10.0 and 10% ACN containing 4×10^{-5} mol L^{-1} Nitro-PAPS. The separation voltage of 15 kV, temperature of 25°C andon-capillary detection at 150 nm were carried out for evaluation. Under the optimum CZE condition, the metal-Nitro-PAPS chelates separation was achieved within 7 min as shown in Figure 3. It was found that the order of migration for Co(II), V(V) and Cu(II) was observed in different elution from IP-RPHPLC system. The resolutions obtained from the CZE (≤1.0) found to be less than by IP-RPHPLC (≥2.0) especially for Ni(II) and Fe(II) chelates. This behavior may due to no difference charge to mass ratio of their chelates under the electrophoretic conditions.

Analytical performances of IP-RPHPLC and CZE

By using the optimum conditions of rFI-IP-RPHPLC and CZE, the quantitative analysis of the metal ions was evaluated. The analytical validated results obtained from both methods are summarized in Tables 2 and 3, respectively. The proposed method gives linear calibrations for all the studied chelates with the determination coefficient higher than 0.99. The limit of detection (LOD) was deduced as the concentration giving a signal to noise ratio of 3 (S/N=3). As can be seen, LODs obtained from IP-RPHPLC were lower than those obtained from CZE about 18 − 100 times which indicating that the proposed HPLC is superior to CZE and more proper in terms of sensitivity for trace analysis of the metals in the samples.

The mixture solution of Cu(II), V(V), Co(II), Ni(II) and Fe(II) at the concentrations of 0.1, 1.0, 0.05, 0.5 µg mL^{-1}, respectively was injected in nine replicate determinations for IP-RPHPLC. In CZE study, the mixture solution of 0.5 µg mL^{-1} Cu(II) and Co(II), 2.0 µg mL^{-1}, V(V) and 1.5 µg mL^{-1} Ni(II) and Fe(II) was performed in five replicates. The precision (intra-day) in terms of the relative standard deviation (RSD) of peak area and retention time (or migration time) of the five metal chelates studied was listed in Tables 2 and 3. The result showed high precision for both the proposed method with RSD<5.0% and <1.0% for peak area and time, respectively.

In order to test the capability of the proposed method for the real samples, accuracy by means of recovery was examined. In IP-RPHPLC, the test solution was the same concentration as used in the precision study whereas CZE, the mixture solution of 0.30 µg mL^{-1} Co(II), Ni(II) & Fe(II) and 0.70 µg mL^{-1} Cu(II) & V(V) was spiked in to representative samples. High accuracy was observed in both IP-RPHPLC and CZE with recoveries higher than 95% for water and wine samples.
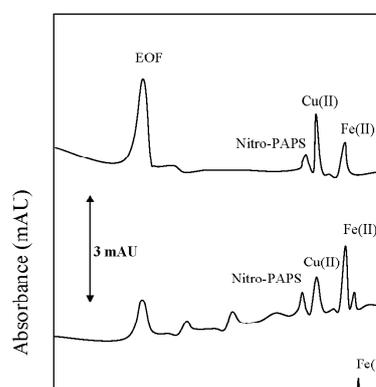
Figure 5. Electropherograms of the studied sample under the optimum condition: (A) DW3, (B) RW1 and (C) WW2. Other condition are the same as in Figure 3.

Analysis of metal ions in water and wine samples

The proposed methods were applied to the determination of metal ions in water and wine samples. Metal ions were identified by comparing either retention times (t_R, from IP-HPLC) or migration time (t_M, from CZE) and their absorption spectra obtained from photodiode array with the standard metal ions and by spiking with known metals and looking for increases in peak height. Co (II), Ni(II), and V(V) were not detected in any sample. A typical chromatogram and electropherogram of the sample studied is illustrate in Figures 4 and 5, respectively. The result of Cu(II) and Fe(II) contamination in the samples are listed in Table 4. As the results from both proposed methods, Cu(II) was found up to 3.8 µg mL^{-1} in drinking waters while it was detected in high risk contamination up to 18.6 µg mL^{-1} in wine samples. Fe(II) was found to be in range 6.2-19.2 µg mL^{-1} for drinking waters, whereas in the studied wine samples were contaminated with high iron in range 6.9-104.6 µg mL^{-1}. Using paired t-test at the confidence level 95%, it was found that the both proposed methods were not significant differences between the used method methods and ICP-OES.

Conclusions

The present study demonstrates the use of Nitro-PAPS for pre-column complexation of metal ions prior to their analysis by IP-RPHPLC and CZE. Simultaneous analysis of Nitro-PAPS chelates of Co (II), Cu(II), Ni(II), Fe(II), and V(V) has been successfully achieved using either rFI-RPHPLC or CZE. The derivatization of the studied metal ions with Nitro-PAPS before the analysis provides both selectivity and sensitivity. The separation mechanism of two methods is based on separation of anionic chelates. Sensitivity obtained from rFI-IP-RPHPLC (20 µL injection) is Ca. 10 times higher than CZE (8.3 nL injection) However, shorter analysis times (ca. 2 times) less waste disposal of CZE are superior over rFI-IP-RPHPLC. The results from the analysis of the samples using both methods are satisfactorily comparable to those obtained by ICP-OES

Acknowledgements

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