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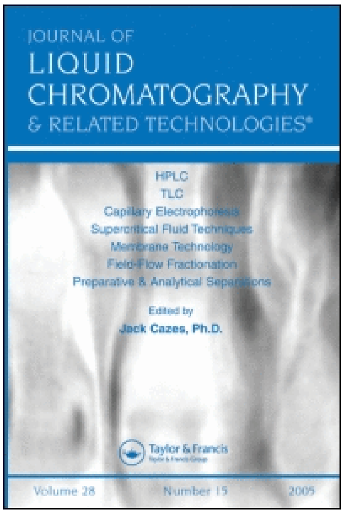

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Giovannetti, Rita and Bartocci, Vito(1998) 'High-Performance Liquid Chromatographic Determination of Mn(II), Co(II), Zn(II), Ni(II), Cu(II) As Coproporphyrin-I Complexes', Journal of Liquid Chromatography & Related Technologies, 21: 17, 2607 — 2617

To link to this Article: DOI: 10.1080/10826079808003410

URL: http://dx.doi.org/10.1080/10826079808003410

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J. LIQ. CHROM. & REL. TECHNOL., 21(17), 2607–2617 (1998)

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF Mn(II), Co(II), Zn(II), Ni(II), Cu(II) AS COPROPORPHYRIN-I COMPLEXES

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ABSTRACT

The mobilities of 3,8,13,18-tetrametyl-21H,23H-porphine-2,7,12,17-tetrapropionic acid or coproporphyrin-I (CPI) and five metal-CPI complexes were measured by reversed-phase high performance liquid chromatography (RP-HPLC) on a octadecyl-bonded silica gel (C_{18} silica) with developing solvent (69:31 v/v) methanol-buffer at pH 7 (buffer ionic strength 0.270 mol L^{-1}: 0.018 mol L^{-1} boric, phosphoric and acetic acid, and 0.036 mol L^{-1} sodium hydroxide), and 0.050 mol L^{-1} of NaCl, with UV detection at 374 and 458 nm.

The mobilities of the metals decrease in the following order of complexed metal ions: Co(II) ≈ Mn(II) > Zn(II) > CPI > Ni(II) > Cu(II). The complexes were completely separated, with exception of Co(II) and Mn(II), and the linear calibration range and detection limits have been obtained. The method has been applied with success to the determination of the mentioned metal ions in natural water samples.

INTRODUCTION

A wide number of papers have been published regarding the analytical methods for the determination of metal ions by ion-chromatography (IC) using different separation mechanisms. These methods are becoming increasingly popular for the separation of ionic species, reversed-phase liquid chromatography (RP-HPLC) with different complexing agents to optimize several separations.[1,2]

Among the numerous organic complexing agents, porphyrins are interesting compounds, from an analytical point of view, for their very high molar absorbivity, that is on the order of several hundred thousands dm^3 mol^{-1} cm^{-1} in a 400-500 nm field (the so-called Soret band).[1] These, as complexing reagents, have been extensively studied for spectrophotometric and kinetic determinations of several metal ions, because they form very stable complexes.[4-6]

Several authors have observed that porphyrin chelates of some metal ions can be suitably separated by HPLC using both normal and reversed phase separation modes; also thin-layer chromatography (TLC) has been applied to preliminary identification of porphyrins and high-performance TLC (HPTLC) has been used with good results for the separation of metalloporphyrins.[7-12] Considering the remarkably high stability of these metal-chelates, the porphyrins are suitable chelating reagents for the determination of metal ions by HPLC. Several reviews have been published on the determination of metal ions with organic chelating reagents by HPLC, but only a few papers regarded the application of porphyrins to the separation and determination of metal ions by HPLC.[13]

In the present paper, the RP-HPLC separation of metal complexes of Mn(II), Co(II), Zn(II), Ni(II), Cu(II) with 3,8,13,18 - tetramethyl - 21H,23H - porphine-2,7,12,17-tetrapropionic acid or coproporphyrin-I (CPI) (Figure 1), by octadecylsilica gel (C_{18}) column, and the determination at ppb levels of these metals as CPI chelates, has been studied. This technique has been successfully used for the determination of these metal ions in natural water samples.

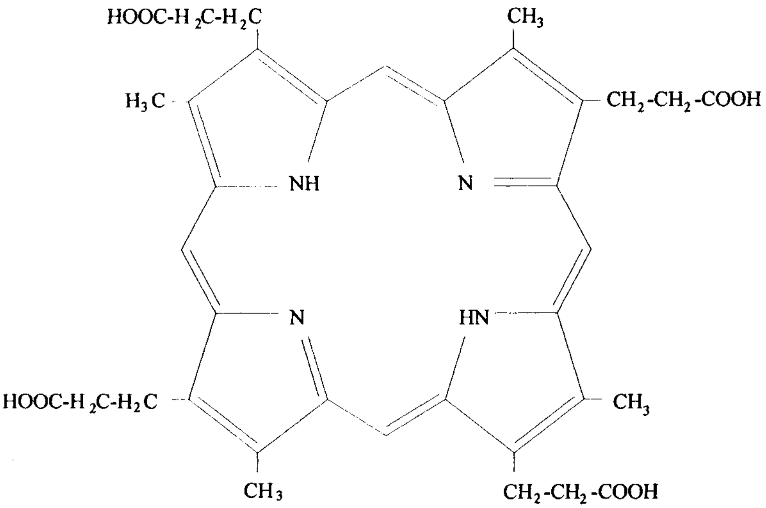

Figure 1. Structural formula of CPI.

EXPERIMENTAL

Reagents

Coproporphyrin-I (CPI) solution $4.08\text{x}10^{-4}$ mol L^{-1}: dissolved in 74.0 mg of CPI-dihydrochloride in 500 mL of $4.81\text{x}10^{-3}$ mol L^{-1} sodium hydroxide solution.

Mn(II), Co(II), Zn(II), Ni(II), Cu(II) standard solutions: dissolved in 364 mg of $MnCl_2$ or 225 mg of $CoCl_2$ or 339 mg of $Zn(CH_3COO)_2$, or 626 mg of $Ni(ClO_4)_2$ or 395 mg of $CuSO_4$, in 100 mL of water and standardized by Inductively Coupled Plasma.

Buffer solution: mix 300 mL of H_3PO_4, CH_3COOH and H_3BO_3 0.67 M, 80.8 mL of NaOH 5 M and dilute to 1000 mL with water (total ionic strength of the buffer 3.030 mol L^{-1}).

The standard solutions of Mn(CPI), Co(CPI), Zn(CPI), Ni(CPI), Cu(CPI) chelates were prepared by adding 10 mL of CPI solution to the appropriate volume of each metal standard solution; boil the mixture for 20 min, allow to cool, and dilute to 25 mL with water for finishing of the reactions.

Table 1

Visible Absorption Spectral Characteristics for CPI and its Metal Complexes

Component	λ (nm)	$\varepsilon \times 10^{-5}$ (L mol^{-1}cm^{-1})
CPI	392	1.072 ± 0.001
MnCPI	366	0.782 ± 0.001
	458	0.381 ± 0.004
ZnCPI	404	1.963 ± 0.045
NiCPI	386	1.001 ± 0.011
CoCPI	412	1.010 ± 0.050
CuCPI	394	2.030 ± 0.020

The obtained solutions contained 9.62 μg/mL of Co(II), 8.97 μg/mL of Mn(II), 10.67 μg/mL of Zn(II), 9.58 μg/mL of Ni(II), and 10.37 μg/mL of Cu(II). All reagents (Merck, Aldrich), were of analytical-reagent grade and used without further purification, de-ionized and redistilled water was used throughout.

Apparatus

Absorption spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer with a 1cm quartz cell connected to a Lauda K2R thermostat. The pH values were determined with a Metrohm 655 pH meter with a combined electrode (Inlab 413). Metal solutions were standardized by an Inductively Coupled Plasma Jobin Yvon JY 24R.

The RP-HPLC separation was obtained on a Dionex 4500i (loop 40 μL), with a variable spectrophotometric detector Dionex AD20 fitted with a 7.5 μL (6 mm) quartz flow cell. A C_{18} column 250 x 4.6 mm, 5 μm particle size (Varian) was used for the separations.

Recommended Procedure for Determination of Metal-Ions as CPI Chelates by RP-HPLC.

To perform a calibration graph place up to 1 mL of each metal-CPI standard solution in a 10 mL volumetric flask and dilute with water; place 7 mL

Table 2

Retention Times for CPI and its Metal-Complexes for Different Conditions*

	Retention Time (min)				
	MnCPI CoCPI	**ZnCPI**	**CPI**	**NiCPI**	**CuCPI**
I = 0.270 (mol L^{-1}) **CH$_3$OH (%, v/v)**					
60	4.45	8.96	13.65	20.59	37.00
68	4.45	7.04	8.19	10.75	16.64
69	4.43	5.92	7.55	8.64	12.13
70	4.40	4.96	6.21	7.68	9.07
CH$_3$OH 69% **I (mol L^{-1})**					
0.192	4.25	5.00	6.43	8.37	10.11
0.244	4.43	5.92	7.55	8.64	12.13
0.270	4.43	5.84	7.31	8.37	11.65
0.296	4.50	7.01	9.95	14.40	28.50
0.348	4.59	9.28	15.39	26.50	42.21
I = 0.270 (mol L^{-1}) CH$_3$OH 69% **NaCl (mol L^{-1})**					
0	4.43	6.24	7.63	9.12	13.28
0.0126	4.40	7.09	8.51	11.23	17.57
0.0252	4.43	6.00	8.64	11.39	17.73
0.0504	4.51	7.95	11.79	16.61	28.27

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* RP-HPLC (Dionex 4500I); (250 x 4.6 mm) Varian C$_{18}$ Column (5 μm). Buffer strength (I).

of sample solution in a 25 mL beaker, add 3 mL of CPI solution, boil the mixture for 20 min, allow to cool, then dilute to 10 mL with water and inject all the solutions for the RP-HPLC determination.

RESULTS AND DISCUSSION

CPI forms, at pH 7, stable 1:1 inner complexes with Mn(II), Co(II), Zn(II), Ni(II), Cu(II) that present absorption maxima with very high molar absorptivities (Soret band) as shown in Table 1. Beer's law was obeyed up to a final concentration of 8.796×10^{-6} mol L^{-1}.

Mn(CPI), Co(CPI), Zn(CPI), Ni(CPI), Cu(CPI), and CPI are easily eluted from the C_{18} column and different measurements have been carried out changing the composition of the mixture, or the ionic strength (NaCl or buffer concentrations) at pH 7, in order to find the best conditions for the elution. In fact, at pH's too low, demetallation of the complexes occurred; that is an undesirable phenomenon for a successful chromatographic process. The results observed are: (a) the decrease of the retention times against the addition of methanol in the eluent mixture, (*b*) the change of the retention times with the buffer concentration, and (c) the increase of the retention times with NaCl concentration. Shown in Table 2 are the summarized, tested conditions.

The best chromatogram has been obtained when the elution was effected with a binary mixture of methanol-buffer (69:31 v/v) and 0.050 mol L^{-1} of NaCl and at buffer ionic strength of 0.270 mol L^{-1} (0.018 mol L^{-1} boric, phosphoric, and acetic acid, and 0.036 mol L^{-1} sodium hydroxide). In Figure 1 is shown the corresponding chromatogram in which may be observed the complete separation of the components of the mixture, with the exclusion of Mn(CPI) and Co(CPI).

The overlap of the peaks of Mn(II) and Co(II) chelates with tetra (*p*-sulfophenyl) porphine has been also observed by Lei et al. and has been solved by addition of pyridine to the mobile phase.[14] The elution order depended on the polarity of the components the mixture and did not change with the eluent composition. The different polarities were in relation with the acidity of the metal-ions; in fact more acid is metal, more polar is the complex. The mobilities of the metal complexes decreased in the following order: Mn(CPI) $\approx$ Co(CPI) > Zn(CPI) > CPI > Ni(CPI) > Cu(CPI); the same order in the separation has been observed by Adachi et al.[15] with Pheophorbide, although in different conditions of the elution.

When an electrolyte such as sodium chloride was added to the developing solvent at concentrations greater than 0.050 mol L^{-1} the signal of Zn(CPI) split in two peaks (see Figure 2); this result implies the formation of two complexes

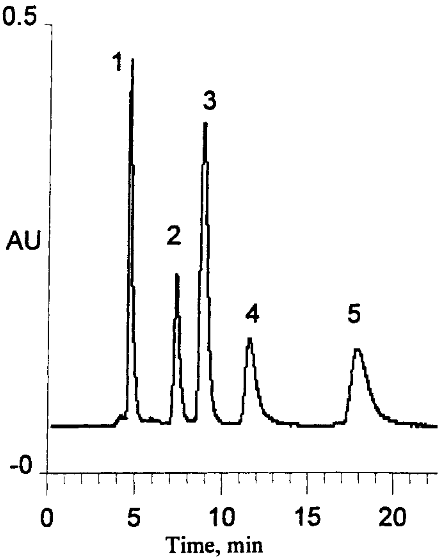

Figure 2. HPLC separation of (peak 1) Mn(CPI)+Co(CPI), (peak 2) Zn(CPI), (peak 3) CPI, (peak 4) Ni(CPI) and (peak 5) Cu(CPI), on a column (250 x 4.6 mm) Varian C_{18} (5 μm). Mobile phase: methanol-buffer pH 7 (0.018 M H_3PO_4, CH_3COOH and H_3BO_3, 0.036 M NaOH) (69:31, v/v) in 0.025 M NaCl; flow-rate 0.5 mL min^{-1}. Detection at 374 nm.

Table 3

Detection Limits (D.L.) for the Metals at Different Wavelengths

Metal Ions	λ (nm)	r	D.L. (ng)	$λ^a$ (nm)	r	D.L. (ng)
Co + Mn	374	0.999	----	412^b	0.999	0.06
Mn	458	0.999	0.14	366	0.999	0.08
Zn	374	0.994	0.09	404	0.994	0.03
Ni	374	0.999	0.15	386	0.999	0.11
Cu	374	0.997	0.26	394	0.997	0.13

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[a] Values obtained from injection of single component.

[b] Only Co(II).

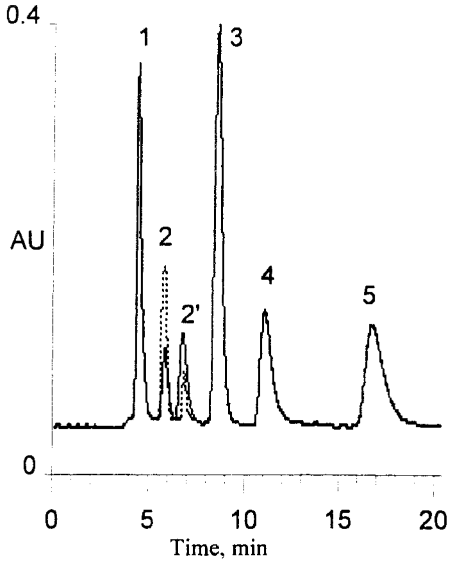

Figure 3. HPLC separation of (peak 1) Mn(CPI)+Co(CPI), (peak 2) Zn(CPI), (peak 3) CPI, (peak 4) Ni(CPI) and (peak 5) Cu(CPI), on a column (250 x 4.6 mm) Varian C_{18} (5 μm). Mobile phase: methanol-buffer pH 7 (0.018 M H_3PO_4, CH_3COOH and H_3BO_3, 0.036 M NaOH) (69:31, v/v) in 0.050 M NaCl; flow-rate 0.5 mL min^{-1}. Detection at 374 nm.

during the migration process in which the dissociation or exchange of Cl^- with the central metal ion of the Zn(CPI) occurs; consequently the metal complex is destabilized when sodium chloride is added to the developing solvent. The splitting of the peak of Zn(II) complexes with other porphyrins has been previously observed by other authors and assigned to the isomer or allomer of the substance.[15] We have attributed the new peak to the complex [Zn(CPI)Cl] in which the Cl^- is bonded with the central metal ion; in fact, as it may be observed in Figure 3, the peak 2 increases while peak 2' decreases with NaCl concentration.

In order to verify the possibility of employing this separation for the determination of the metal ions studied, calibration graphs of the mixture of metal-complexes are performed by plotting the dependence of the peak area on the quantities of each metal injected; this dependence is linear in the range 1 - 56 ng for all metals. The correlation coefficients were always greater than 0.994, while the relative standard deviations were within 1% (5 replicates). The detection limit measuring at least three times the background noise was included between 0.03 and 0.26 ng per injection. Shown in Table 3 are the correlation

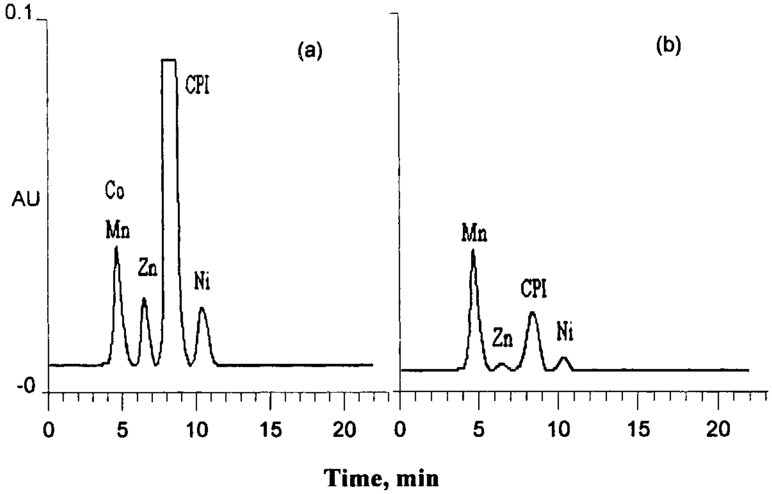

Figure 4. HPLC separation of CPI and metal complexes of a natural water sample on a column (250 x 4.6 mm) Varian C_{18} (5 μm). Mobile phase: methanol-buffer pH 7 (0.018 M H_3PO_4, CH_3COOH and H_3BO_3, 0.036 M NaOH) (69:31, v/v) in 0.025 M NaCl; flow-rate 0.5 mL min^{-1}. Detection at (a) 374 nm and (b) 458 nm.

Table 4

Determination of Metal Ions in Natural Water Sample

	Co(II) (ng/mL)	**Mn(II) (ng/mL)**	**Zn(II) (ng/mL)**	**Ni(II) (ng/mL)**
RP-HPLC	328.11	39.81	320.12	43.11
ICP	321.23	40.72	323.45	44.00

coefficients (r), and the detection limits (D.L.) either at 374 nm, or at the wavelengths corresponding to the absorbance maxima of each metal complex. As it is to be expected, at these values, lower detection limits have been obtained.

For visualizing the Mn(CPI) peak without the interference of Co(CPI) we have operated with detection at 454 nm. At this wavelength the absorption of Co(CPI) is almost negligible; so as to obtain the calibration curve for Mn(II) alone and to determine Co(II) by difference. (See, also, Figure 4).

To evaluate the analytical potentiality of this method in the simultaneous determination of several metals, the recommended procedure has been applied for the analysis of the metal composition of a natural water sample. The sample has been injected and the resulting chromatograms at two detector wavelengths (374 and 458 nm) for the same sample are shown in Figure 4; Mn(II) was determined from the calibration graph at 458 nm and Co(II) for the difference. The result of the analysis was in accordance within 2.5% with that obtained by ICP-AES, as may be observed in Table 4. The detection limits are much lower than those obtained with tetrakis (4-methoxyl-3-sulfophenyl) porphine[16] and comparable with those obtained with tetrakis (3-bromo-4-sulfophenyl) porphine.[17] Other metal ions usually present in natural water, such Ca(II), Mg(II), Sr(II), Ba(II), Si(II) do not interfere; Cl- at concentration greater of 0.05 mol L^{-1} provocates the split of Zn(CPI) peak.

These results indicate that the proposed method is simple and rapid for the determination of Co(II), Mn(II), Zn(II), Ni(II), and Cu(II) within 20 min and can be easily used for the simultaneous determination of the tested metals in a natural water sample.

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Received November 20, 1997
Accepted January 12, 1997
Manuscript 4669