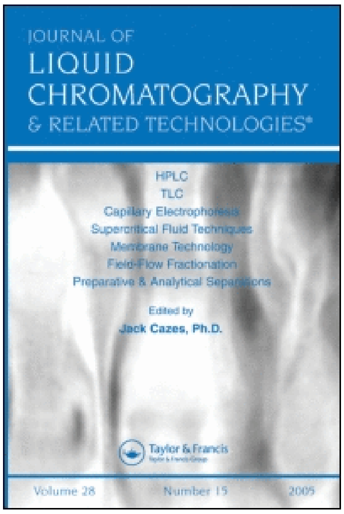

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DETERMINATION OF TRACE METAL IONS USING PORPHYRINS AS CHELATING AGENTS BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY-DENSITOMETRY

Serap Seyhan Bozkurt[a]; Ipek Kaynak Çavdar[a]; H. Mine Kurtbay[a]; Melek Merdivan[a]
[a] Dokuz Eylul University, Faculty of Arts and Sciences, Chemistry Department, Buca İzmir, Turkey

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Serap Seyhan Bozkurt, Ipek Kaynak Çavdar,
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Dokuz Eylul University, Faculty of Arts and Sciences, Chemistry Department,
Buca İzmir, Turkey

☐ *A method was developed for separation and quantitative determination of mercury, copper, cobalt, and zinc as porphyrin complexes using newly synthesized tetra-(3-bromo-4-hydroxyphenyl) porphyrin (TBHPP), tetra-(4-phenoxyphenyl) porphyrin (TPPP) and tetra-p-chloromethylphenyl porphyrin (CMPP) in aqueous medium by HPTLC densitometry. The method involves enrichment of metal ions as metal complexes with octadecyl cartridges and elution using tetrahydrofuran. The analysis was performed on HPTLC silica gel plates with acetone-chloroform (2:8, v/v) as the mobile phase for TBHPP and TPPP quantification and dichloromethane-chloroform-hexane (1:1:3, v/v) for CMPP quantification. Quality parameters were confirmed and detection limits were ranged from 0.16 to 0.92 ngμL^{-1}. Detection was performed densitometrically in absorption-reflection mode around 420 nm. The method was found to be simple, precise and accurate for the determination of these metal ions in water and food sample.*

Keywords high performance thin layer chromatography, metal, porphyrin, solid phase extraction

INTRODUCTION

Thin layer chromatography has found wide spread use in clinical laboratories, in the drug industry and many biochemical and biological studies. Nowadays, it also finds widespread use in the industrial laboratories and in the analytical chemistry for quantitative trace metal analyses by densitometry, despite the ability of a great number of analytical methods such as atomic absorption spectrometry, plasma emission spectrometry, x-ray fluorescence spectrometry, and voltammetry. Many complexing agents which form chelates that can be separated chromatographically and

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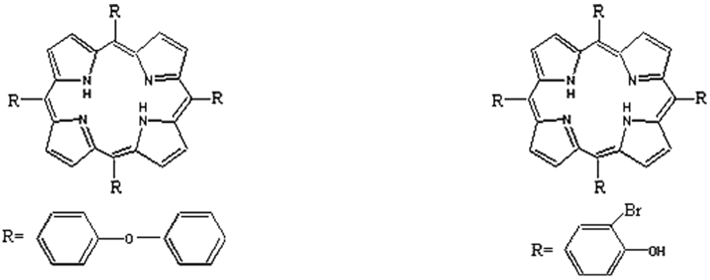
Address correspondence to Melek Merdivan, Dokuz Eylul University, Faculty of Arts and Sciences, Chemistry Department, 35160, Buca İzmir, Turkey. E-mail: melek.merdivan@deu.edu.tr

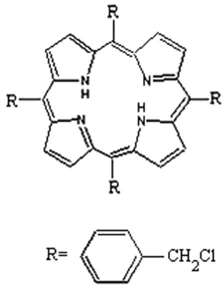
determined analytically, can be used successfully such as dithiocarbamates, dithiozonates, dioxime, oxines, thiooxinates, β-diketones, benzoylthioureas, pyridylazo-resorcinol.[1–7]

Porphyrins are a class of naturally occurring macrocyclic compounds, which contain four pyrrole rings linked via sp–hybrid carbons and p-electrons are delocalized over the molecule by conjugation. The porphyrin ring system is very stable and exhibits aromatic character.[8] In general, porphyrins possess a strong absorption band around 400 nm (Soret band) with molar absorptivities of the order of 10^5 as well as several less intense bands between 450 and 700 nm. If a porphyrin reacts easily and quantitatively with a metal ion, the porphyrin must be useful as a sensitive spectrophotometric reagent for the determination of metal ions.[9–12] However, a problem occurs due to overlapping of Soret bands of porphyrins and metalloporphyrins. To solve this problem, chromatographic techniques are the best choice. Most separations have so far focused on the complexes of porphyrins with metal ions using high performance liquid chromatography and high performance thin layer chromatography;[13–17] only a few papers



5,10,15,20-tetra-(4-phenoxyphenyl)porphyrin (TPPP)
compound 1
(a)

5,10,15,20-tetra-(3-bromo-4-hydroxy henyl)porphyrin (TBHPP)
compound 2
(b)



5,10,15,20-tetra-p-chloromethylphenyl porphyrin (CMPP)
compound 3
(c)

FIGURE 1 Structures of the synthesized porphyrins reagents.

have been presented involving the quantitative analysis of metals using chelating agents by HPTLC.[18–21]

The purpose of this study was to develop a fast, selective, cheap, and simple possible method of separation and simultaneous determination of mercury, zinc, copper, and cobalt in aqueous solutions by HPTLC with the basis of solid phase extraction using TBHPP, TPPP or CMPP (Figure 1) as complexing agents. By this proposed method, the analysis of metal ions in water and food samples has also been successful.

EXPERIMENTAL

Chemicals and Apparatus

All solutions were prepared with ultra-pure water (Milli-Q Millipore $18.2\,\mathrm{M\Omega\,cm^{-1}}$ resistivity). All chemicals used were of analytical grade. For each element, a stock solution of $1000\,\mathrm{ng\,\mu L^{-1}}$ and a working solution of $1\,\mathrm{ng\,\mu L^{-1}}$ were prepared. A buffer solution of pH 10 was prepared from $0.5\,\mathrm{mol\,L^{-1}}$ pyrrolidine-acetic acid. A 2.0% (v/v) TritonX-100 alcoholic solution as a surfactant was used for TPPP and CPMM. The glass and teflon wares were soaked in $4\,\mathrm{mol\,L^{-1}}$ HNO_3 overnight and then thoroughly washed with distilled water.

TLC was performed on 10 × 10 cm and 10 × 20 cm glass packed silica gel 60 F_{254} HPTLC plates, all purchased from Merck. Before use, silica gel plates were activated at 110°C for 30 min and then cooled in a desiccator.

Solutions ($1\,\mathrm{ng\,\mu L^{-1}}$) of all test substances and samples were applied to chromatographic plates as bands by Linomat V semi automatic sample applicator (Camag, Switzerland) and developed in twin through or horizontal chambers from Camag. The bands on the HPTLC plates were quantitated with a densitometer Camag TLC-Scanner 3 in the absorbance-reflection mode, using a tungsten source. The automatic scanning was controlled by the winCATS 1.4.2 software. The slit dimensions were set at 4.0 mm in length and 0.3 mm in width, with a scanning rate of $20\,\mathrm{mm\,s^{-1}}$. All the plates were scanned and measured within 15 min following detection.

Synthesis of Porphyrin Reagents

TBHPP

A 0.170 g of tetrahydroxyphenylporphyrin was mixed with 1.2 mL concentrated H_2SO_4 and 150 mL of tetrahydrofuran (THF) in a round-bottomed flask and refluxed for 3 h at 70°C. The reaction mixture was left to cool, and 5 mL of 0.6 M NaOH solution and 250 µL of bromine were added drop by drop. The mixture was refluxed for 1 h at 70°C and left to cool, then 5 mL of 1.0 M H_2SO_4 was also added and mixed for 30 min. The reaction mixture was extracted with diethyl ether and the THF in the upper phase was evaporated by a rotary evaporator. After 10 mL of distilled water was added, the formed residue was filtered off and finally dried under vacuum. The yield of TBHPP was 75%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 9.88 ppm (s, 4H), 7.55 ppm (s, 2H), 7.05–6.70 ppm (m, 20H). FTIR (KBr): 3125 cm^{-1} (C-OH), 897 cm^{-1}(C-Br). Elemental analysis, expected (calculated): C-49.55 (49.67), H-2.76 (2.82), N-5.01 (5.26).

TPPP

A 0.2 g of tetrahydroxyphenylporphyrin was stirred in 15 mL dimethylformamide with 0.120 g of crushed sodium hydroxide. Chlorobenzene, 0.4 g, was added over 1 h using a dropping funnel. After 30 h, 10 mL of ethanol was added to the solution, followed by 80 mL of distilled water. The product was filtered off and washed with absolute ethanol and then dried. It was chromatographed on alumina with chloroform. TPPP was eluted with the solvent front and separated easily from any unreacted starting material. The yield of TPPP was 82%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.30 ppm (s, 2H), 8,28–7,77 ppm (m, 44H). FTIR (KBr): 2100 cm^{-1} (Ar-O-Ar). Elemental analysis, expected (calculated): C-77.49 (77.86), H-4.65 (4.28), N-5.16 (5.82).

CMPP

A 0.123 g of tetraphenylporphyrin was mixed with 0.002 g of *p*-formaldehyde, 0.027 g of anhydrous $ZnCl_2$ powder, and 150 mL of THF in a round-bottomed flask and refluxed for 30 min at 75°C. Ten mL of 1.0 M HCl was added to the reaction mixture drop by drop and refluxed for more than 15 min. Then the reaction mixture was transferred to a separatory funnel and washed with 2.0–3.0 mL of cold distilled water, 2.0–3.0 mL of saturated Na_2CO_3 solution, and 2.0–3.0 mL of distilled water. After filtration of the mixture, the product was dried under vacuum. The yield of CMPP was 71%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.28 ppm (s, 2H), 8,05–7,54 ppm (m, 34H). FTIR (KBr): 2920 cm^{-1} (Ar-CH_2-Cl), 895 cm^{-1}(C-Cl). Elemental analysis, expected (calculated): C-65.52 (65.97), H-4.20 (4.54), N-5.66 (5.26).

TLC Analysis

Sample and standard material zones were applied as bands (1/0.8 μL) with the following settings: band length, 6 mm; band width, 0.3 mm;

distance between bands, 9.2 mm; distance from the plate side edge, 10 mm; and distance from the bottom of the plate, 15 mm.

The plates were developed to a distance of 50 mm in a horizontal or twin through chamber with (2:8, v/v) acetone-chloroform for TBHPP and TPPP and (1:1:3, v/v) dichloromethane-chloroform-hexane for CMPP and their metal chelates. The chamber was equilibrated with 10 mL of mobile phase for 20 min prior to inserting the plate. After each analysis, the mobile phase was removed and substituted by a fresh one.

The plates were scanned at 420 nm with slit dimension of 4.0 mm × 0.3 mm, a scanning rate of 20 mm s^{-1}; the HPTLC plates absorption images were documented. All the plates were scanned and measured within 15 min.

After the development, bands were identified by matching their retention factor (hR_F, $100 \times R_F$) values and absorption spectra with those obtained for standards.

Standard Procedure

An appropriate volume (not more than 7.5 mL) of 1 ng μL^{-1} standard or sample solution was transferred into a 25 mL of volumetric flask. For the blank test, no standard or sample solution was added. Three mL of 1×10^{-3} mol L^{-1} porphyrin solution, 5 mL of buffer solution (pH 10 for TPPP and CMPP, and pH 7 for TPPP), and 3 mL of 2% Triton X-100 solution (for TBHPP and CMPP) were added. The solution was diluted to volume with water and heated in a boiling water bath for 20 min. The prepared metal chelate solutions were passed through the C_{18} cartridges (AccuBond ODS-C18, 200/500 mg/3 mL) using an Alltech model vacuum manifold. After enrichment, the retained metal chelate was eluted with 2.5 mL of THF. Each sample and standard solution were added as 1 or 0.8 μL volumes in triplicate on the plates and were dried, respectively. The chromatograms were developed with the proper mobile phase for each porphyrin and evaluated as given above.

Method Validation

The analytical methods were validated according to the guidelines of IUPAC.[22] Linearity, precision, limit of detection (LOD), and quantitation (LOQ) were determined using solutions of each metal chelate in ethanol for TBHPP and CMPP and in THF for TPPP. Concentration ranges for calibration solutions of metal of each studied porphyrin at 420 nm were selected with respect to linear range of signal versus concentration of each metal. For calibration, standard solutions containing 6 ng metal μL^{-1} for each porphyrin were prepared by the procedure given above and an increasing volume of standard metal complex solution from 0.1 to 10 µL was applied on the plate to get 10 calibration points.

Precision of analysis was performed in various ways. For repeatability of the method, the standard solutions containing various amounts of metal (6 and 12 ng μL^{-1}) for all studied ligands were analyzed on two different plates on the same day to calculate the relative standard deviation of the mean values from each plate. For intermediate precision, the same standard solutions on three different plates were developed at three different days and the results were compared.

The LODs were estimated based on three times of the ratio of the standard deviation of the response (SD) to the slope of the calibration curve at low calibration points (0.1–0.3 ng μL^{-1}). The standard deviation of the response was determined using the standard deviation of the y-intercepts of regression lines.[23] Similarly, the LOQs were calculated using the calibration lines with the formula: 10(SD/S).

RESULTS AND DISCUSSION

Porphyrins and Their Metal Chelates Formation

The optimal condition for the reaction of Zn(II), Ni(II), Cu(II), Co(II), Cd(II), Hg(II), Pb(II), Mn(II), Pt(IV), and Pd(II) with TBHPP, TPPP, and CMPP was in a neutral or basic medium. The experiments show that the chelation pH was 10 (0.5 mol L^{-1} pyrrolidine-acetic acid) for TBHPP and CMPP and 7 (phosphate buffer) for TPPP. By the experiments, it was recommended that 3 mL of 1×10^{-3} mol L^{-1} porphyrin solution was sufficient, even to overcome foreing ions in real samples. It was found that the reaction was completed for 20 min at 40°C. The solubilities of metal -TPPP and -CMPP were increased using Triton X-100 as surfactant.

Solid Phase Extraction

The retention of metal chelates using 50–500 mg of C_{18} solid phase extraction columns were tested. The optimized capacity of enrichment cartridge for transport of the metal chelates was determined as 200 mg of C_{18} for TBHPP and TPPP 500 mg of C_{18} for CMPP. In enrichment and elution steps, the flow rates were optimized as 2.5 mL min^{-1} and 1 mL min^{-1}, respectively. Organic solvents such as THF, chloroform, acetone, and ethanol were tested to get a proper elution solvent for the retained metal complexes. Within these solvents, 2.5 mL of THF was quantitatively eluted the sorbed metal chelates.

Chromatographic Separation

The metal chelates of Zn(II), Ni(II), Cu(II), Co(II), Cd(II), Hg(II), Pb(II), Mn(II), Pt(II), and Pd(II)–TBHPP and –TPPP with acetone-chloroform (2:8, v/v) and –CMPP with dichloromethane-chloroform-hexane (1:1:3, v/v) showed good chromatographic beheviour with reproducible hR_F values as seen in Table 1. Within these results, the best separation for Cu-, Co-, Zn-, Hg-TBHPP, and Hg-TPPP and for Cu-CMPP succeeded relative to their chelating agents and their other metal chelates (Figure 2). The hR_F values of metal chelates were found higher than their chelating agents because of the bonding between the metal cation and the four nitrogen atoms in the chelating agent.

Spectrophotometric Properties

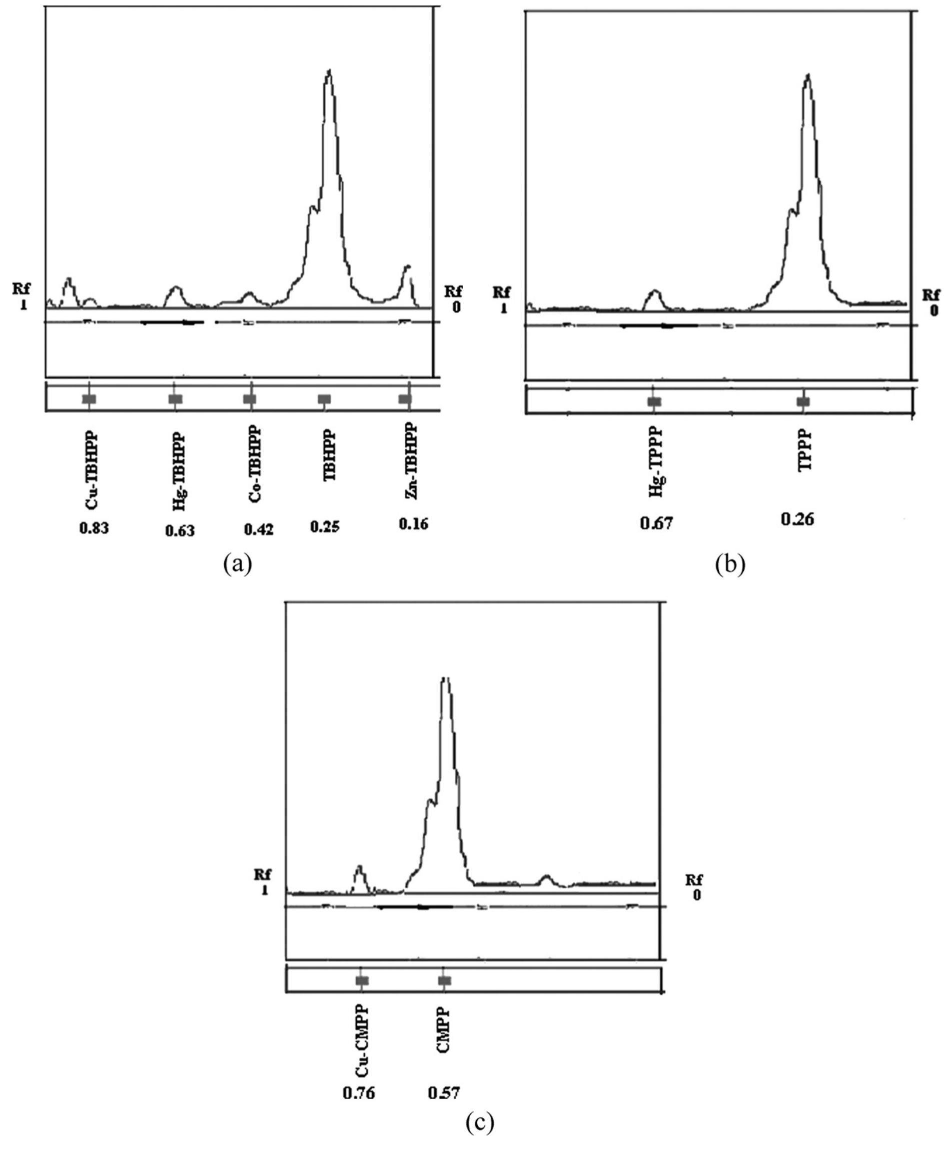
The absorption spectra of metal-TBHPP, -TPPP, and -CMPP chelates were obtained from the three-dimensional chromatograms as illustrated in Figure 3. The results show that the maximum absorption is 427 nm for Zn-TBHPP chelate, 421 nm for Cu-TBHPP chelate, 418 nm for Hg-TBHPP chelate, 419 nm for Co-TBHPP chelate, 423 nm for Hg-TPPP chelate, and 414 nm for Cu-CMPP chelate. During analysis, each metal-TBHPP, -TPPP, and -CMPP chelate was detected at its maximum absorption wavelength.

Quality of Parameters

Under the optimum conditions, the linear ranges were established for each metal chelate from the curves constructed using a linear least squares

TABLE 1 The hR_F Values of Zn(II), Ni(II), Cu(II), Co(II), Cd(II), Hg(II), Pb(II), Mn(II), Pt(IV), Pd(II)-TBHPP, TPPP and CMPP on Silica Gel

Developer	Zn(II)	Ni(II)	Cu(II)	Co(II)	Cd(II)	Hg(II)	Pb(II)	Pd(II)	Mn(II)	Pt(IV)
Acetone-chloroform (2:8)										
TBHPP (25 ± 3)	16 ± 3	25 ± 3	83 ± 3	45 ± 2	25 ± 3	68 ± 4	65 ± 4	28 ± 2	58 ± 1	25 ± 4
TPPP (26 ± 4)	25 ± 1	20 ± 0	20 ± 0	23 ± 3	21 ± 3	68 ± 1		25 ± 2	76 ± 2	
Dichloromethane-chloroform-hexane (1:1:3)										
CMPP (57 ± 3)	53 ± 1	54 ± 3	76 ± 1	51 ± 1	57 ± 2	55 ± 3	55 ± 3	54 ± 2	57 ± 2	57 ± 1

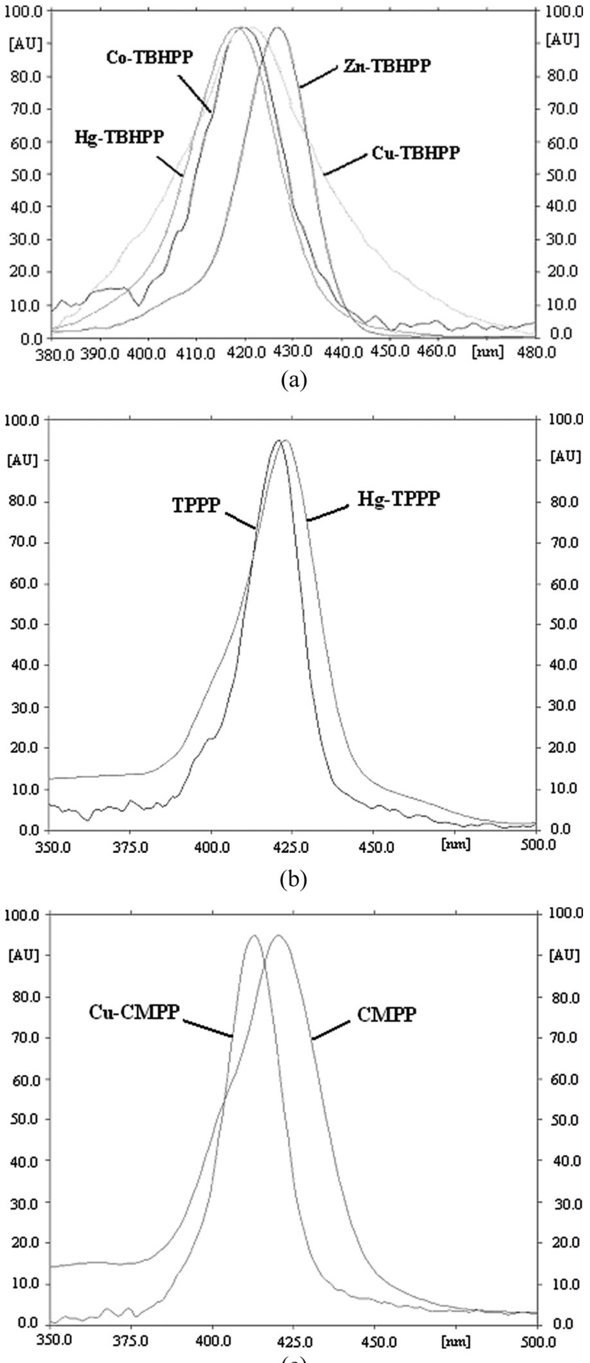


(a) (b)

(c)

FIGURE 2 Chromatograms of metal-TBHPP, -TPPP and –CMPP. Hg(II), Zn(II), Cu(II), and Co(II)-TBHPP complexes in acetone-chloroform (2:8) on HPTLC-silica gel 60 F_{254} plate. Hg(II)-TPPP complexes in acetone-chloroform (2:8) on HPTLC-silica gel 60 F_{254} plate. Cu(II)-CMPP complexes in dichloromethane-chloroform-hexane (1:1:3) on HPTLC-silica gel 60 F_{254} plate.

of regression of the peak areas as a function of metal ion concentration with a correlation coefficients (R^2) better than 0.99. The within day and between day precision were evaluated for 6 and 12 ng μL^{-1} by performing 5 replicate determinations. The LODs and LOQs were estimated from their regression lines. The results were summarized in Table 2.



(a)

(b)

(c)

FIGURE 3 Absorption spectra of a) TBHPP, b) TPPP and c) CMPP on HPTLC-silica gel 60 F_{254} plate.

TABLE 2 Summary of Analytical Statistical Data

	Hg-TBHPP	Zn-TBHPP	Cu-TBHPP	Co-TBHPP	Hg-TPPP	Cu-CMPP
Linear range (ng μL^{-1})	3.6–60	3.6–30	1.2–30	0.6–30	0.6–30	2.4–60
LOD (ng μL^{-1})	0.90	0.92	0.36	0.19	0.16	0.41
LOQ (ng μL^{-1})	3.01	3.06	1.11	0.54	0.54	1.36
Repeatibility (RSD%, n = 5) (6 ng μL^{-1})	3.09	3.67	2.66	1.87	1.69	0.16
Intermediate precision (RSD%, n = 5) (6 ng μL^{-1})	3.55	4.25	3.20	3.85	2.25	0.45
Linear regression equation	$y = 16.11 \times + 43.40$	$y = 20.41 \times - 25.70$	$y = 42.24 \times + 7.39$	$y = 50.93 \times + 16.52$	$y = 28.76 \times + 41.24$	$y = 37.22 \times + 36.18$
Regression coefficient (R^2)	0.9908	0.9904	0.9944	0.9961	0.9972	0.9942
Retention factor (hR_F)	68	16	83	42	67	76

Interferences

Under the complexation conditions, the foreign ions of Mn(II), Pt(IV), Pd(II), Sn(IV), Cd(II), Cr(III), Pb(II), Ni(II), Mg(II), Fe(III), U(VI), Th(IV) can react with porphyrins to form color chelates. The U(IV), Ag(I), Mg(II), Fe(III), and Sn(II) did not interfere due to uncomplexation of related reagents with these ions. Ni(II), Cd(II), Mn(II), Pb(II), Pd(II), and Pt(IV) for TBHPP; Zn(II), Co(II), and Hg(II) as extra metal ions for CMPP and Zn(II), Cu(II), and Co(II) as extra metal ions for TPPP, form chelates but their retention factors were different than the studied ones as seen in Table 1. However, in the complextion step the reagents were added in excess, due to the possibility of presence of some chelating ions in analytical matrices.

Application to Water Samples and Dried Tomato Powder

A 0.25 g of dried and homogenized tomato powder sample, which was purchased from the local market, was treated with 2 mL of concentrated nitric acid in a PTFE beaker and the mixture was heated until dryness. This procedure is repeated until using 10 mL of concentrated nitric acid to achieve complete solubility.[24] Then, the volume was made up to 50 mL with water.

All water samples (tap water, geothermal waters, river waters) were filtered through 0.45 μm filter paper (Millipore Millex-HV, Hydrophilic PVDF). An appropriate volume of sample solutions were used for Hg(II), Zn(II), Cu(II), and Co(II) determination using reagents of TBHPP, TPPP, or CMPP by the given procedure before. Amount of metals was calculated from the regression equations obtained from calibration curves. The results were given in Table 3, together with the results of recoveries.

TABLE 3 Determination Results of Zn(II) and Co(II) in the Samples with this Method

	Sample (ng μL^{-1})					
Metals	Water Tap	Geoethermal 1	Geoethermal 2	River 1	River 2	Tomato Dried, Powder
With TBHPP						
Zn(II)						
Present	–	0.26 ± 0.03	–	–	–	–
Added	4.80	4.80	4.80	4.80	4.80	4.80
Found	4.88 ± 0.14	5.10 ± 0.12	4.60 ± 0.09	4.99 ± 0.18	4.70 ± 0.22	4.99 ± 0.18
Avg. recovery (%)	102	101	96	104	98	104
Cu(II)						
Present	0.24 ± 0.02	–	–	0.30 ± 0.05	–	0.13 ± 0.02
Added	4.80	4.80	4.80	4.80	4.80	4.80
Found	5.04 ± 0.10	4.92 ± 0.20	4.80 ± 0.16	5.10 ± 0.10	4.99 ± 0.25	5.14 ± 0.17
Avg. recovery (%)	100	102	100	100	104	104
Co(II)						
Present	–	–	–	0.06 ± 0.01	–	0.05 ± 0.03
Added	4.80	4.80	4.80	4.80	4.80	4.80
Found	4.91 ± 0.14	4.88 ± 0.24	4.82 ± 0.22	5.23 ± 0.24	4.89 ± 0.27	5.27 ± 0.25
Avg. recovery (%)	102	102	100	108	102	109
Hg(II)						
Present	–	–	–	–	–	–
Added	4.80	4.80	4.80	4.80	4.80	4.80
Found	4.89 ± 0.09	4.86 ± 0.15	4.86 ± 0.20	5.03 ± 0.19	4.85 ± 0.24	4.94 ± 0.16
Avg. recovery (%)	100	102	100	100	104	102
With TPPP						
Hg(II)						
Present	–	–	–	–	–	–
Added	4.80	4.80	4.80	4.80	4.80	4.80
Found	4.85 ± 0.25	4.88 ± 0.18	4.86 ± 0.32	4.99 ± 0.15	4.82 ± 0.28	4.97 ± 0.33
Avg. recovery (%)	101	102	101	100	100	103
With CMPP						
Cu(II)						
Present	0.21 ± 0.03	–	–		–	
Added	6.00	6.00	6.00		6.00	
Found	6.42 ± 0.38	6.14 ± 0.30	6.04 ± 0.28		6.18 ± 0.14	
Avg. recovery (%)	103	102	100		103	

CONCLUSION

The proposed method revealed that thin layer chromatography with silica plates and densitometry is ideally suited for the analysis of metal ions. The advantages of thin layer chromatography over the other methods are due to simultaneous determination of metals and being free via the selective complexation of metals. TBHPP, TPPP. and CMPP were synthesized and used as chelating agents. It can be applied in water and food samples to separate and determine metal ions in one chromatographic run. Quality parameters have shown that a good precision and low detection limits can be obtained, providing an easy and rapid procedure for the determination of metal ions using solid phase extraction involved porphyrin reagents.

Due to its simplicity, rapidity, and reliability, it can be a good alternative way to save money and time with respect to atomic spectrometric and liquid chromatographic techniques for the analysis of metal ions in aqueous medium.

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