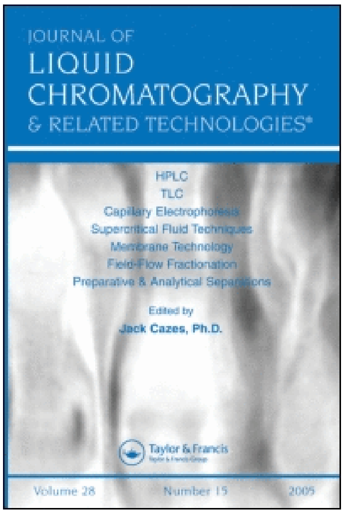

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SEPARATION AND DETERMINATION OF TRACE Cu(II), Zn(II), AND Pd(II) AS THEIR TETRAPHENYLPORPHINE CHELATES BY REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

α,β,γ,δ-Tetraphenylporphine (TPP) was used as a precolumn chelating reagent for reversed-phase high performance liquid chromatographic (HPLC) separation and determination of trace Cu^{2+}, Zn^{2+} and Pd^{2+}. Chelating reactions of TPP with Cu^{2+}, Zn^{2+}, and Pd^{2+} have been optimized in the presence of anionic surfactant sodium dodecyl sulphate (SDS). TPP and its metal chelates were successfully separated on a Nucleosil C_{18} column (150×4.6 mm I.D., 5 μm) with acetone-acetonitrile (37.5: 62.5, v/v) as mobile phase.

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The detection limits (S/N=2) were 3.2×10^{-11} g, 2.2×10^{-11} g and 2.4×10^{-10} g for Cu^{2+}, Zn^{2+}, and Pd^{2+}, respectively. The proposed method was applied to the simultaneous determination of Cu^{2+}, Zn^{2+} in tea water and the results were in agreement with the values obtained by an atomic absorption spectrophotometric method.

INTRODUCTION

In recent years, the application of high performance liquid chromatography (HPLC) in the separation and determination of metal ions has increased rapidly.[1-3] In many papers, trace metals form stable complexes with inorganic or organic reagents. It is well known that porphyrin is a kind of highly sensitive chromogenic reagent. Considering the remarkably high stability of metal porphyrin chelates and the similarity of the chelates absorbance, porphyrin is an ideal chelating reagent for the determination of metal ions by HPLC.[4]

α,β,γ,δ-Tetraphenylporphine (TPP) is a kind of typical porphyrin, which has already been used as a chromogenic reagent in spectrophotometric determination of metals.[5] As TPP and its metal chelates are insoluble in water, chelation can not be directly fulfilled in aqueous solution. This drawback greatly limits the application of TPP from an analytical viewpoint. Up till now, only two papers have been published on quantitative determination of metal ions by HPLC using TPP as a chelating reagent.[6,7] In the mentioned two papers, chelating reactions were accomplished in organic solutions, the chelation and extraction methods were complicated and tedious, errors would be easily brought in.

In this paper, we have successfully developed an HPLC method for the separation and quantitative determination of metal TPP chelates. TPP reacted quantitatively with Cu^{2+}, Zn^{2+}, and Pd^{2+} in aqueous solution in the presence of anionic surfactant sodium dodecyl sulphate (SDS). The sample was directly injected onto the column to be analysed. The proposed method is simple and easy, which can avoid the complicated and tedious extraction step. Such work has not been reported to date.

EXPERIMENTAL

Instrumentation

A Perkin-Elmer Series 3 Liquid chromatograph equipped with an LC-65T UV-VIS detector was used with a 5 μm Nucleosil C_{18} column (150×4.6 mm i.d.), a Perkin-Elmer R100A recorder, a PE-Rheodyne Model 7125 injector, and a Model AA-100-S-2 constant flow pump (Eldex, U.S.A.). A Shimadzu UV-265 spectrophotometer was also used.

Reagents

TPP was synthesized and purified according to the method described by Adler et al.[8] The final product was proved to be TPP of high purity by Elemental analysis, ^{1}HNMR, and HPLC. Its absorption spectrum was the same as reported.

TPP solution (5×10^{-5} mol/L) was prepared by dissolving 7.7 mg of TPP in 25 mL of 1,4–Dioxane and diluting the solution to 250 mL with aqueous 5% SDS solution.

$Cu(Ac)_2 \cdot H_2O$, $ZnSO_4 \cdot 7H_2O$, and $PdCl_2$ were used to prepare the metal ion solutions. All reagents and chemicals were of analytical-reagent grade unless stated otherwise. The water used was distilled three times.

Procedure

A sample solution containing Cu^{2+}, Zn^{2+}, and Pd^{2+} was placed in a 25 mL volumetric flask and 1mL of 0.2% Hydroxylamine hydrochloride, 6 mL of 5×10^{-5} mol/L TPP, 1 mL of HAc-NaAc buffer solution (pH 5.6) were added. The solution was heated in boiling water-bath for 55 min. After cooling to room temperature in running water, the solution was diluted to the mark with water. For HPLC analysis, a 10 µL aliquot of this solution was injected into the LC system. The mobile phase used was a mixture of acetone and acetonitrile at a volume ratio of 37.5 : 62.5. The flow rate was 0.8 mL/min, the detection wavelength was set at 416 nm.

Analysis of Tea-Water Sample

A 1.000 g dry Jasmine tea was put into a plastic flask; 100 mL of boiling water was poured into the flask. After 10 min, the solution was filtered and the obtained tea water was concentrated. Then a mixture of HNO_3 and $HClO_4$ (4+1, v/v) was added. After being digested for 24 hours, the solution was put on a hot plate, gently boiling until the solution was clear. Then the temperature was raised to drive off the white fume of $HClO_4$. The residue was dissolved with water and transferred into a 25 mL volumetric flask, water was added to the mark. The final sample solution was filtered through a 0.45 µm membrane. A 2.0 mL aliquot of the solution was treated by the previously described chelation and HPLC determination procedure.

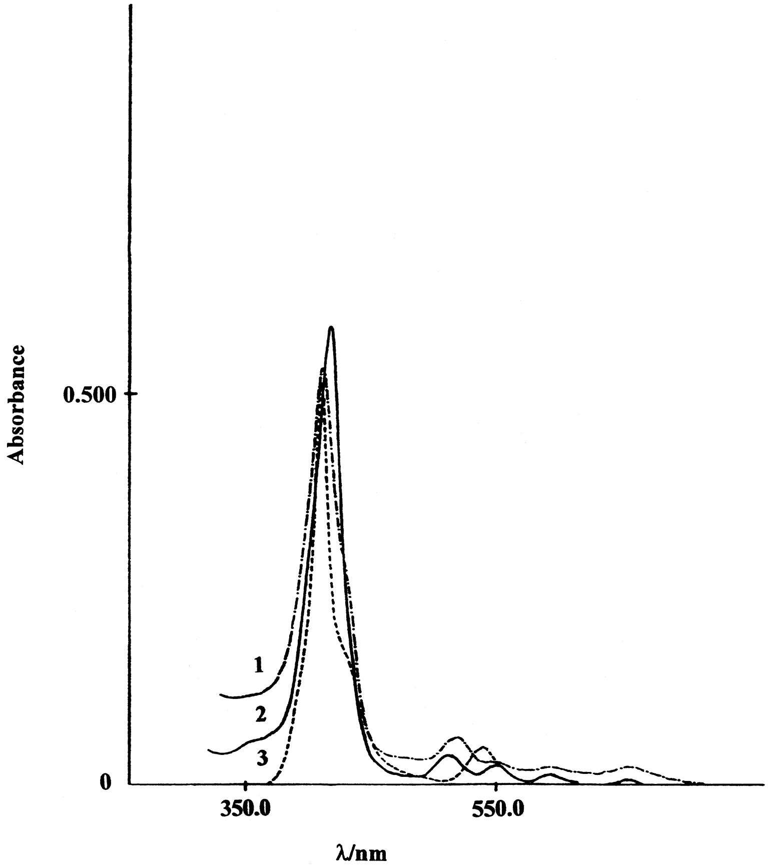

Figure 1. Absorption spectra of metal-TPP chelates, reagent blank as reference. 1. PdTPP; 2. ZnTPP; 3. CuTPP.

RESULTS AND DISCUSSION

Spectral Properties of Metal Chelates

The metal TPP chelates were prepared according to the procedure described in Experimental section. The absorption spectra against reagent blanks are shown in Figure 1. It is evident that the absorption spectra of the chelates overlap closely, which is advantageous for simultaneous separation and determination of metal ions by HPLC.

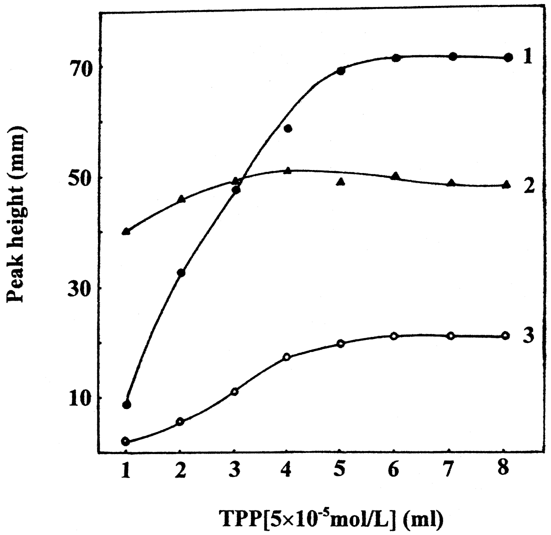

Figure 2. Effect of the quantity of TPP on formation of the chelates. 0.08 µg/mL of Cu^{2+} and Zn^{2+}, respectively; 0.24 µg/ml of Pd^{2+}. 1. ZnTPP; 2. CuTPP; 3. PdTPP.

The maximum absorption for CuTPP, ZnTPP, and PdTPP are 414 nm, 422 nm, and 416 nm, respectively. Molar absorptivities ($L \cdot mol^{-1} \cdot cm^{-1}$) are 4.10×10^5, 4.81×10^5, and 1.13×10^5, respectively. 416 nm was selected for the simultaneous detection of the chelates.

Selection of the Surfactant

As TPP and its metal chelates are insoluble in water, their solubilization with various surfactants was investigated. The result shows that anionic surfactant sodium dodecyl sulphate (SDS) and non -ionic surfactant Triton X-100 are superior to cationic surfactant cetyltrimethylammonium bromide (CTMAB) both in dissolution power for TPP and the chelates, and in the stability of the resulting solution.

SDS is preferable to Triton X-100 because the latter has a cloud point around 70°C, as described below; this creates problems in heating the solution to accelerate the chelation of TPP and metal ions. Accordingly, SDS was employed for further study.

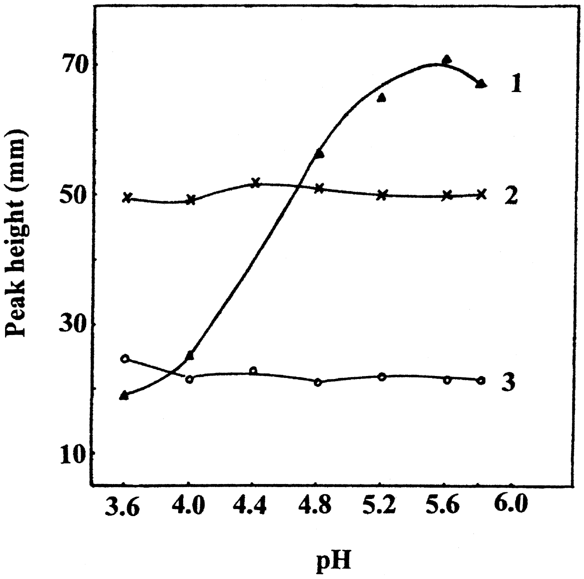

Figure 3. Effect of pH on formation of the chelates. 0.08 μg/mL of Cu^{2+} and Zn^{2+}, respectively; 0.24 μg/mL of Pd^{2+}. 1. ZnTPP; 2. CuTPP; 3. PdTPP.

Accelerative Conditions for Chelate Formation

At room temperature, chelating reaction between metal ion and TPP proceeds slowly. Whereas, it can be accelerated by increasing temperature and pH, the addition of a reducing agent is also effective in accelerating the chelate formation.

In this work, therefore, chelate formation was accelerated by heating the solution at 100°C in the presence of hydroxylammonium chloride.

Effect of the Quantity of TPP on Formation of the Chelates

The effect of quantity of TPP on formation of the chelates was investigated and the results are represented graphically in Figure 2. When the concentration of the metal ions in the final solution are 0.08 μg/mL for Cu^{2+} and Zn^{2+}, 0.24 μg/mL for Pd^{2+}, peak heights of the chelates are fairly constant when the volume of TPP is larger than 5.0 mL. Hence, 6.0 mL of TPP was chosen for further study.

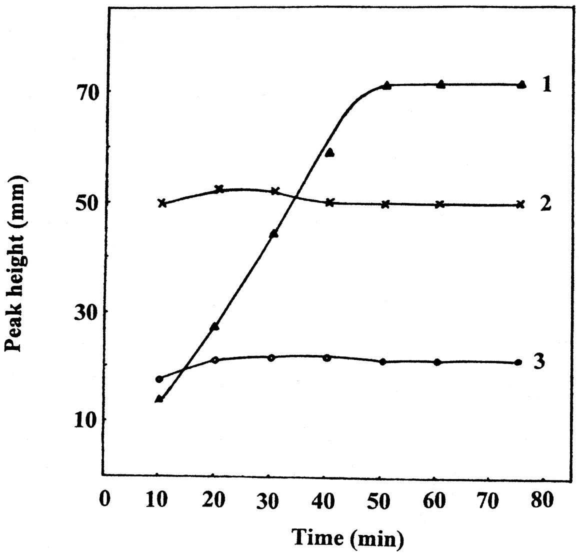

Figure 4. Effect of heating time on formation of the chelates. 1. ZnTPP; 2. CuTPP; 3. PdTPP.

Effect of pH on the Formation of the Chelates

The influence of pH on the formation of the chelates was examined from 3.6 to 6.0. Considering extreme pH such as higher than 7.0 or lower than 2.5 may bring damage to C_{18} column. HAc-NaAc buffer solution was selected for the study. As shown in Figure 3, the influence of pH on peak height of ZnTPP is great compared with those of CuTPP and PdTPP; the peak height of ZnTPP increases with an increase of pH.

Effect of Heating Time on the Formation of the Chelates

Figure 4 shows the effect of heating time on the formation of the chelates. The peak height of CuTPP can reach the maximum value when reaction solution is heated for 3min, while the peak height of ZnTPP increases gradually with the increase of heating time.

When heated for 50 min, constant peak heights of the chelates can be obtained. On the basis of the result, 55 min is selected as the optimum heating time. Once formed, the chelates are stable for at least 24 hours.

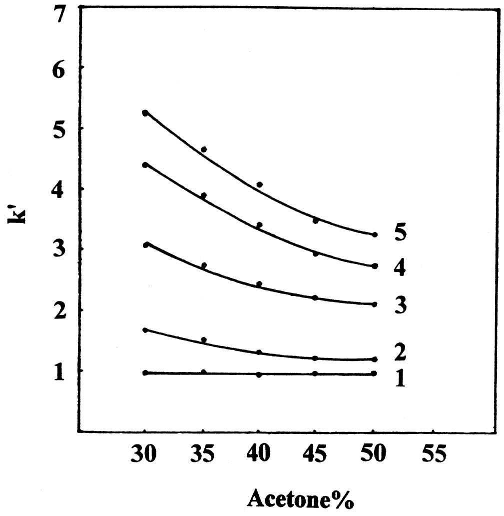

Figure 5. Effect of acetone content in the mobile phase on the retention of chelates. 1. Solvent; 2. ZnTPP; 3.TPP; 4. PdTPP; 5. CuTPP.

Selection of the Mobile Phase and Explanation of the Retention Mechanism of the Chelates

It is known that retention of the metal chelates depends on the stationary phase, mobile phase and the properties of chelating reagent and metal ions. The elution of the chelates was studied on 5 μm Nucleosil C_{18} column using various mobile phases, such as methanol, acetonitrile, methanol-water, methanol-acetone, acetone-acetonitrile. The result shows that TPP and its metal chelates can't be eluted using a mixture of methanol and water as eluent. When using absolute methanol or acetonitrile as mobile phase, long retention time of TPP and its metal chelates will be observed, which is not convenient for rapid separation and determination of the chelates. When employing a mixture of methanol-acetone as mobile phase, there is a negative peak before ZnTPP, which will influence the determination of ZnTPP. Considering all the above factors, a mixture of acetone-acetonitrile was selected as mobile phase.

The effect of volume ratio of acetone to acetonitrile on the retention of TPP and its metal chelates was studied. As shown in Figure 5, the higher the acetone content in the mobile phase, the shorter the retention time of TPP and the chelates is, while the retention of solvent hardly changes. This fact demonstrates that acetone is a strong solvent for TPP and its metal chelates.

Although the relatively higher acetone content in mobile phase is advantageous for shortening the analysis time, when the concentration of acetone is higher than 45%, good separation can not be achieved between solvent and ZnTPP, TPP, and PdTPP, PdTPP, and CuTPP. So the optimum acetone content was selected at 37.5% (v/v) for the advantages of both retention time and resolution.

Although central metal ion is surrounded by macro porphyrin ring, our experiment shows that the retention of the chelates are greatly influenced by central metal ions. When using different kinds of mobile phases, the elution order of TPP and the chelates remains the same: ZnTPP > TPP > PdTPP > CuTPP. This phenomenon can get a good explanation from Horvath's "Solvophobic theory."[9]

According to "Solvophobic theory", there is an equation:

$$\ln k' = \Phi + \frac{1}{RT}\left[\Delta A\left(N\gamma + \alpha\right) + NA_s\gamma\left(X^e - 1\right) + w - \frac{\Delta Z}{\varepsilon}\right] - \ln\frac{RT}{PoV} \qquad (1)$$

Where k' is the capacity factor, $\Phi = \mathrm{In}\varphi$ (φ is the phase ratio), R is the gas constant, N is the Avogadro's number, T is the absolute temperature, γ is the surface tension of the bulk solvent, A_s is the surface area of the solvent molecule, κ^e is a factor for converting the bulk surface to the molecular dimensions, α, w are constants relevant to the solvent, ε denotes the dielectric constant of the solvent, V is the mole volume of the solvent , P_0 denotes the atmosphere pressure.

$\Delta A = A_S + A_L - A_{SL}$, $\Delta Z = Z_{SL} - Z_S - Z_L$, where A_S, A_L , A_{SL} represent the surface areas of solute molecule, nonpolar ligand of the stationary phase and the associated molecule formed between solute and the ligand; Z_S, Z_L, Z_{SL} are the mass electrostatic distributions of solute molecule, nonpolar ligand of the stationary phase, and the associated molecule formed between solute and the ligand to association. In our case, as the relatively small metal ion is surrounded by large porphyrin ring, there is hardly any difference in the surface area of the chelates. So, on a given column, when the solutes are eluted with the same mobile phase, equation (1) can be derived as:

$$\ln k' = \mathrm{constant} + {}^{Z_S}\!/_{RT\varepsilon} \qquad (2)$$

M. Kobayashi et al[10] once mentioned that electrostatic field intensity can be estimated by E_N / r_i , where E_N is Pauling electronegativity of central metal ion, r_i is Pauling radius of metal ion. The attraction between the central metal ion and

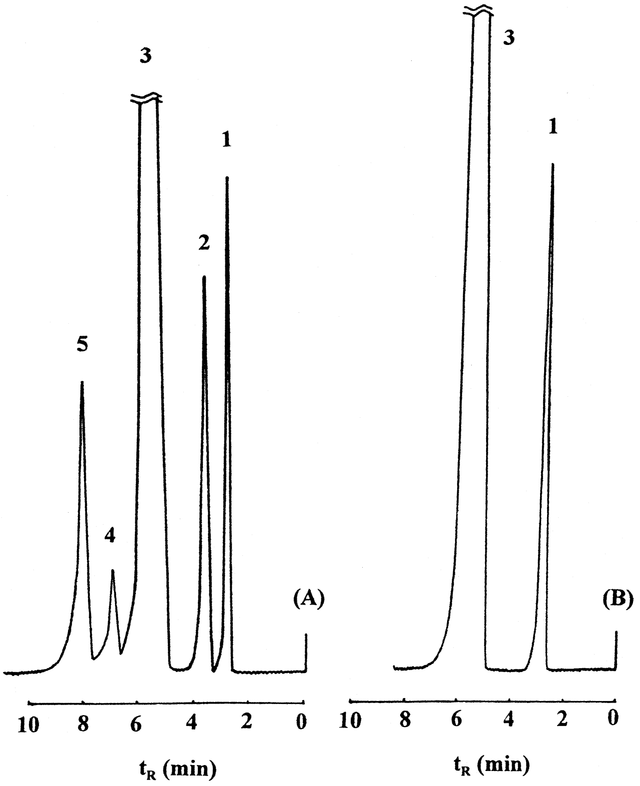

Figure 6. Chromatogram of metal-TPP chelates. (A) TPP and its metal chelates, (B) Reagent blank. 1. Solvent, 2. ZnTPP, 3. TPP, 4. PdTPP, 5. CuTPP.

Table 1

E_N, r_i and E_N/r_i Values of Zn^{2+}, Pd^{2+} and Cu^{2+}

	Zn^{2+}	Pd^{2+}	Cu^{2+}
E_N*	1.65	2.20	2.00
r_i*	74	85	72
E_N/r_i	0.02230	0.02588	0.02778

* E_N, r_i values are referred from reference 11.

Table 2

Reproducibility of Retention Time of TPP and Its Metal Chelates

	Retention Time (min) 1	2	3	4	5	Mean	S.D. (min)	RSD (%)
ZnTPP	3.72	3.70	3.72	3.70	3.72	3.71	0.011	0.30
TPP	5.47	5.44	5.46	5.44	5.47	5.46	0.015	0.28
PdTPP	7.05	7.05	7.04	7.08	7.07	7.06	0.016	0.23
CuTPP	8.13	8.08	8.08	8.13	8.13	8.11	0.027	0.34

S.D. = Standard deviation. RSD = Relative standard deviation.

the electrons in N on porphyrin ring increases with the increase of E_N/r_i value. Higher E_N/r_i value is advantageous for the positive charge in metal ion to distribute onto the whole π electron system of the porphyrin ring, i.e., Z_S value increases with the increase of E_N/r_i value.

The E_N/r_i values of Zn^{2+}, Pd^{2+}, Cu^{2+} are listed in Table 1. From Table 1, it can be seen that E_N/r_i values increase in the order of $Zn^{2+} < Pd^{2+} < Cu^{2+}$.

Based on equation (2), it can be predicted that the retention order of the chelates is CuTPP>PdTPP>ZnTPP, showing good agreement with the experiment result.

Separation of TPP and the Chelates

Figure 6 shows the chromatogram of TPP and its metal chelates under the optimum chromatographic conditions. All the solutes are well resolved, and have relatively good shapes. The retention reproducibility of TPP and its metal chelates are listed in Table 2.

Interferences

Large amounts of Ca^{2+}, Mg^{2+}, Al^{3+}, Pb^{2+}, Cd^{2+}, Co^{2+}, and Mn^{2+} don't interfere with the determination, V^{4+}, V^{5+}, Fe^{2+}, Fe^{3+}, Ni^{2+}, and Cr^{3+} under 100 µg don't interfere with the determination.

The interferences of Ag^+, Hg^{2+} can be eliminated by hydroxylamine hydrochloride. Common anions such as Cl^-, Br^-, I^-, SO_4^{2-}, NO_3^-, ClO_4^- don't interfere with the determination.

Table 3

Regression Analysis of Calibration Graphs and Other Quantitative Data for Metal Ions

	Zn^{2+}	Pd^{2+}	Cu^{2+}
Linear Range (ng)	0.08-1.0	0.6-2.4	0.08-1.0
Calibration Equation[a]	$y=0.5418+8.319x$	$y=-0.3850+1.015x$	$y=-0.1960+6.316x$
Correlation Coefficient	0.9993	0.9975	0.9992
Detection Limits (g)[b]	2.2×10^{-11}	2.4×10^{-10}	3.2×10^{-11}

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[a] $y = A+Bx$, y = peak height (cm), x = amount of metal ion (ng), A = intercept, B = slope. [b] Signal-to-noise ratio = 2.

Table 4

Experimental Accuracy Data

No. Inj.	Peak Height (mm) ZnTPP	 PdTPP	 CuTPP
1	31.2	5.8	17.6
2	32.5	5.4	17.2
3	29.6	5.6	17.5
4	32.5	5.5	16.6
5	32.6	5.8	16.2
6	31.5	5.0	17.0
7	31.4	5.5	17.0
Mean (mm)	31.61	5.51	17.01
S.D. (mm)	1.07	0.27	0.49
RSD (%)	3.37	4.96	2.89

Statistical Analysis

The regression analysis of the calibration graphs and other quantitative data for Cu^{2+}, Zn^{2+} and Pd^{2+} is presented in Table 3. Standard deviations were obtained from seven parallel determinations of 0.3 ng of Zn^{2+}, 0.9 ng of Pd^{2+} and 0.3 ng of Cu^{2+} (Table 4).

Table 5

The Results of the Analysis of Jasmine Tea Water

	AAS Values ($\times 10^{-2}$ μg/mL)	Values Obtained by Proposed Method ($\times 10^{-2}$ μg/mL) 1	2	3	4	Mean	RSD(%)	Concentration ($\times 10^{-2}$ μg/mL) Added	Found[a]	Recovery (%)
Cu^{2+}	4.66	4.91	4.91	4.51	4.91	4.81	4.16	10.00	9.74	97.4
Zn^{2+}	12.66	12.76	13.20	12.46	13.58	13.00	3.78	10.00	10.17	101.7
Pd^{2+}	N.D[b]	N.D	N.D	N.D	N.D	N.D	N.D	25.00	23.50	94.0

[a] Average of three determinations. [b] N.D = not detected.

Application

Jasmine tea water was analysed by the above method and the results are in good agreement with the values obtained by AAS method (Table 5). Recoveries achieved for three additions of the metal ions to tea water samples are shown in Table 5.

CONCLUSION

The established method provides a simple, sensitive and accurate means for the simultaneous determination of Zn^{2+}, Pd^{2+}, and Cu^{2+}. It has been successfully applied to the analysis of the metal ions in tea water. The described reliable HPLC method could be useful for quality control measurements.

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