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RESEARCH ARTICLE

Direct and Derivative Spectrophotometric Determination of Zn(II) in Pharmaceuticals

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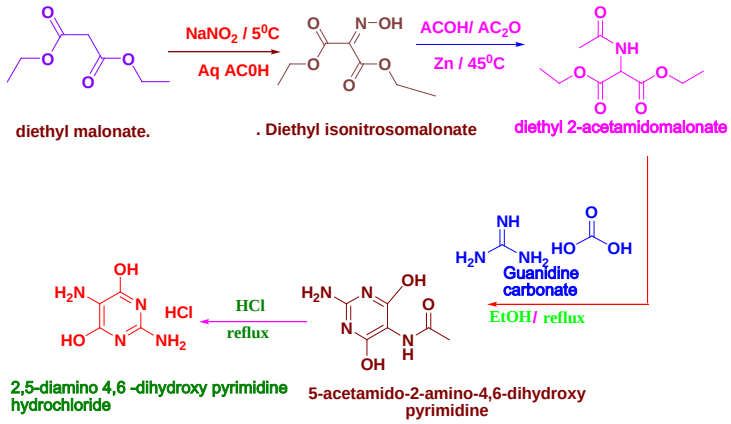
Abstract: Heterocyclic compound diamino and dihydroxy- pyramidine was synthesized and proposed as chelating agent for the determination of trace amounts of zinc. Zinc(II) forms the ripen-mango-colour complex with DADHP (Diamino dihydroxypyrimidine) in 1:3 stoichiometric ratio at pH-6 in acetic acid and sodium acetate buffer in the presence of pyrideniumchloride as salting out agent. The maximum absorbance is observed at 480 nm. The Beer's law is obeyed in the range of 1-6 µg. The molar absorptivity **(ε)** and Sandell's sensitivity of the complex is 0.1484×10^4 L mol^{-1} cm^{-1} and 0.04545 µg cm^{-1} respectively. The method was successfully applied for the quantification of Zn(II) in pharmaceutical multi vitamin preparations in the presence of other trace elements. The statistical data evaluated reveals the sensitivity and accuracy of the method than the other methods reported.

Keywords: Zinc determination, Direct and derivative spectrophotometry, DADHP

Introduction

Zinc is an essential trace element to flora and fauna[1,2], that can cause symptoms of deficiency[3,4] and can be toxic when exposures exceed physiological needs[5,6]. It is an indispensable constituent of approximately one third of all proteins[7]. Zinc will play fundamental roles in all replications, Gene expression, energy transduction, cell signaling, formation of endo and exo skeletons and information transfer[8,9]. Monitoring of zinc in pharmaceuticals and other real samples is very important, commonly the analytical methods for the determination of zinc are neutron activation analysis (NAA), Atomic absorption and emission spectrophotometry, Inductively coupled plasma spectrophotometry (ICPMS) are the most widely employed[10]**.** However, although these techniques are reliable and sensitive they suffer from the limitation of being rather expensive, time consuming and not always readily available[11-12]. In order to achieve accurate, reliable and sensitive results, spectrophotometric techniques which tend to be less expensive and labor intensive are available alternatives, to those methods requiring more sophisticated instruments. Further, the development of derivative spectrophotometry is very useful approach for determining the concentration of components in the mixtures with the overlapping spectra as it eliminates much of the interference[13-16]. Further, it was widely used in the analysis of pharmaceutical samples protein analysis, environmental sample analysis[17].

The determination of trace amounts of Zinc(II) by ultra violet and visible spectrophotometry typically relies on four distinct characteristics[9] apart from the divalent cations. First when coordinated by ligand in any Geometry it has stereo chemical flexibility, second in terms of the hard-soft acid-base theory it has amphoteric property, third divalent zinc(II) has no redox activity[18]. Finally Zn^{2+} has chemical stability and it undergoes the complexation with selected sensitive chromophoric chelator[19]. Among the many reagents reported so far with heterocyclic rings[20-24] the present heterocyclic pyramidine (Scheme 1) has been shown to serve as an excellent chromophor for the quantification of zinc ions in aqueous solution. The complexation is found to be quantitative in sodium acetate and acetic acid buffer in the presence of pyridinum chloride [pyridine+2 M HCl] as salting out agent.



Scheme1. Formation of 2,5-diamino-4,6-dihydroxypyrimidine

Experimental

2,5-Diamino-4,6- dihydroxypyrimidine was prepared in three steps by the methods reported in the literature[25-26]. Diethylmalonote (47.4 mL) was mixed with the mixture of glacial acetic acid and water (1:1.5) and transferred in to 500 mL round bottom flask fitted with mechanical stirrer and thermometer. The flask was cooled in an ice bath 0.65 g of $NaNO_2$ was added in portions by maintaining the temperature about 5 ^{0}C with continuous stirring. After the addition of total $NaNO_2$ the ice bath was removed stirring was continued for four hours. The reaction mixture was extracted with ether and evaporated to procure diethyl-isonitroso malonote.

Diethyl isonitroso malonote mixed with1:3 mixture of acetic anhydride and glacial acetic acid in a three necked round bottom flask fitted with mechanical stirrer, thermometer and dropping funnel. 78.5 g of Zn dust was added in small portions by maintaining the temperature at 40-50 ^{0}C followed by intermittent cooling. The reaction mixture was filtered with suction, the filtrate and washings were evaporated on the steam bath and then cooled in an ice bath by rapid stirring, diethyl acetamido malonote separates out as a white crystalline solid. The yield was 35- 40 g, M.P was (95-97 ^{0}C).

Diethyl acetamido malonote was mixed with guanidinium carbonate and EtOH refluxed for 36 h, then the reaction mixture was filtered by suction. The filtrate and the washings were evaporated and cooled in an ice bath to separate out the 5- acetamido-2-amino-4,6-hydroxypyrimidine. This was refluxed with 100 mL of 1 M HCl for 1 h then cooled in an ice bath filtered followed by washings with HCl and acetone then air dried at 40 ^{0}C to procure the

2,5-diamino-4,6-dihydroxypyrimidine (25 g). The structure was confirmed from Mass, IR, H^1 NMR Spectral studies.

Preparation of solutions

All the chemicals were of AnalaR grades from Fisher Scientific Qualigens India.

Zn(II)-solution

Stock standard Zn(II) solution was prepared by dissolving 0.4397 g of Zn(II) sulphate hepta hydrate in double distilled water containing 1000 µg/mL. The solution was standardized by complexometric titration using EDTA[27]. The working standard solutions were prepared by suitable dilution of the stock solution.

Buffer solutions

Buffer solutions were prepared[28] by employing 0.1 M Acetic acid, 0.1 M sodium acetate in the pH range 2-8.

Solutions of diverse ions

Solutions of diverse ions containing 1000 µg/mL were prepared by dissolving required amounts of salts of the corresponding ions in double distilled water.

Ligand solution

The reagent stock solution (0.1 M) was prepared by dissolving 1.421 g of DADHP in ethylene glycol. This was diluted to the required concentration using ethylene glycol.

Instruments

Elico micro processor based double beam UV- visible spectrophotometer SL 210 equipped with 1 cm quartz cells were used for spectrophotometric measurements. The pH measurements are made with Elico digital pH meter L.1 127 model.

General procedure to evaluate different parameters

Direct spectrophotometry

To evaluate the optimum conditions for the determination of Zn(II) an aliquots of solution containing microgram quantities of Zinc(II) were taken in a series of comparison tubes followed by the addition of 2 mL of pyridine and 1 mL of 2 M HCl, then the pH was adjusted to 6 using acetic acid and sodium acetate buffer. The solutions are equilibrated with 5 mL of DADHP (3×10^{-3} M) reagent solution and made up to 20 mL with double distilled water. The absorption of the ripen-mango-colour complex was measured at 480 nm against a similarly prepared reagent blank.

The composition of the complex was ensured by mole ratio, slope ratio and Job's continuous variation methods. The calibration plot was obtained in the range 1-6 µg. obeying the Beer's law.

Derivative spectrophotometry

For the above solutions the derivative spectras (1^{st}, 2^{nd} & 3^{rd} order) were recorded with group size 9 and degree of freedom 5 in the wavelength range 400-600 nm. The derivative peaks were measured by the peak zero method at respective wave length. The peak heights were plotted against the amount of zinc to obtain the calibration. The amount of zinc present in the pharmaceutical sample solutions were computed from the calibration plots, both in direct and derivative spectrophotometry. The calibration plots follow the straight line equation

Y=A+Bx where x is the concentration of solution, Y is the measured absorbance or peak height, A is the intercept and B is the slope. By substituting the experimental data in least square analysis, the equations were calculated as Y=-0.01143+0.0309x for zero order spectrophotometry $\partial A/\partial\lambda$=0.00396+0.00469x for first order derivative, $\partial^2 A/\partial\lambda^2$ = 0.00329+ 0.00247x for second order derivative and $\partial^3 A/\partial\lambda^3$ = 0.00362+0.00200x for the third order derivative spectrophotometry respctively.

Results and Discussion

The ripen-mango-colour complex of [Zn(II) -DADHP] absorption spectra was examined in the wave length range 400-600 nm against the reagent blank. The complex shows the maximum absorption at 480 nm in acetic acid and sodium acetate buffer (Figure 1).

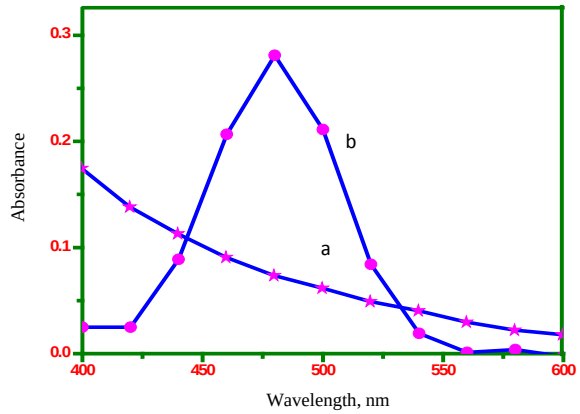


Figure 1. Absorption spectra of (a) DADHP *vs*. buffer blank (b) Zn(II)-DADHA complex *vs*. regent blank Zn(II) = 7.645x10^{-5} M (100 µg) DADHP = 7.62x10^{-5} M

Selection of pH

The colour and the absorption of the Zn(II) -DADHP complex depends on the pH of the solution. The maximal and reproducible absorbance was obtained at pH-6 in acetic acid and sodium acetate buffers, both in direct and derivative spectrophotometry (Figure 2).

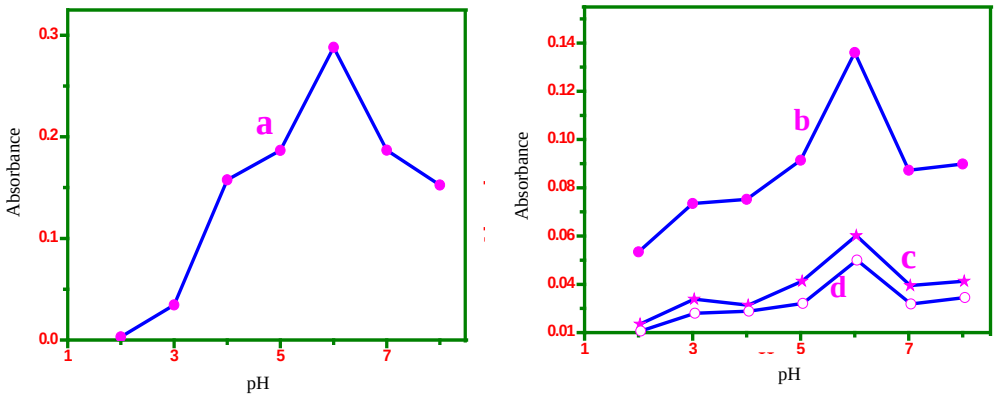


Figure 2. Influence of pH on the absorbance of Zn(II)-DADHP complex (a) Direct spectrophotometry (b) First derivative (c) Second derivative and (d) Third derivative spectrophometry Zn(II) =7.645x10^{-5} (M (100 µg); DADHP =7.645x10^{-4} M

Effect of salting out agent

The complexation selectivity was investigated in different volumes of pyridine in the presence of HCl solution. It was observed that the high intensive colour and maximum absorbance with 2 mL of pyridine in the presence of 1 mL of 2 M HCl (Figure 3).

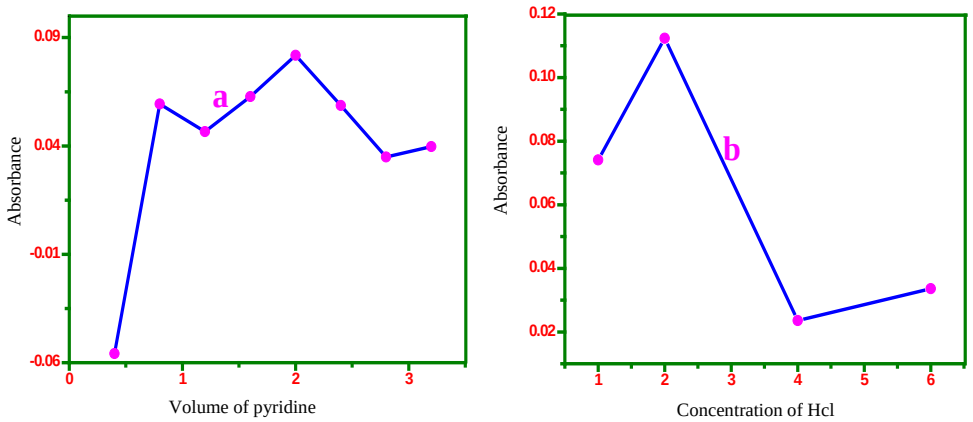


Figure 3. Effect of salting out agent on the absorbance of Zn(II)-DADHP complex (a) Effect of pyridine; (b) Effect of HCl concentrartion Zn(II) =7.645x10^{-6} M (10 µg); DADHP = 7.645x10^{-4} M

Effect of reagent concentration

To achieve the complete complexation of Zn(II) and for the maximum colouration hundred folds excess of the reagent was necessary. The ripen-mango- colour formation between the Zn(II) and the reagent was instantaneous and the colour was stable for more than 48 h, (Figure 4).

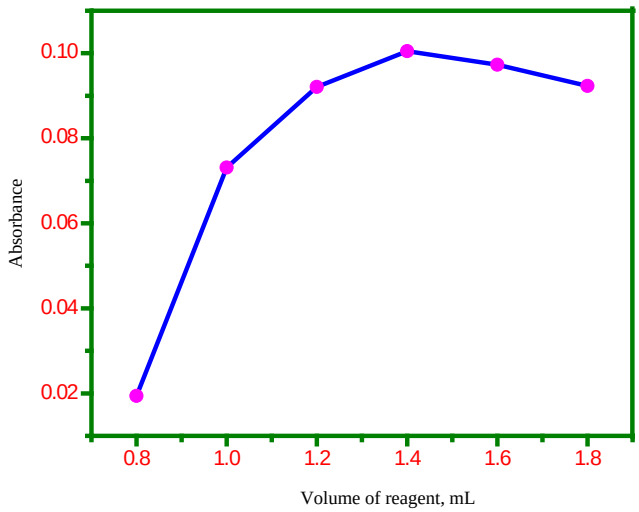


Figure 4. Effect of reagent on the absorbance of Zn(II)-DADHP complex Zn(II) =7.645x10^{-6} M (10 µg); DADHP = 7.645x 10^{-4} M

Composition of the complex

Elating of experimental observations in mole ratio, Job's continuous variation method and slope ratio method confirms Zn(II) forms the 1:6 complex with the reagent and the stoichiometric ratio is 1:3. So, it was confirmed that the reagent is a bidentate ligand, Figure 5.

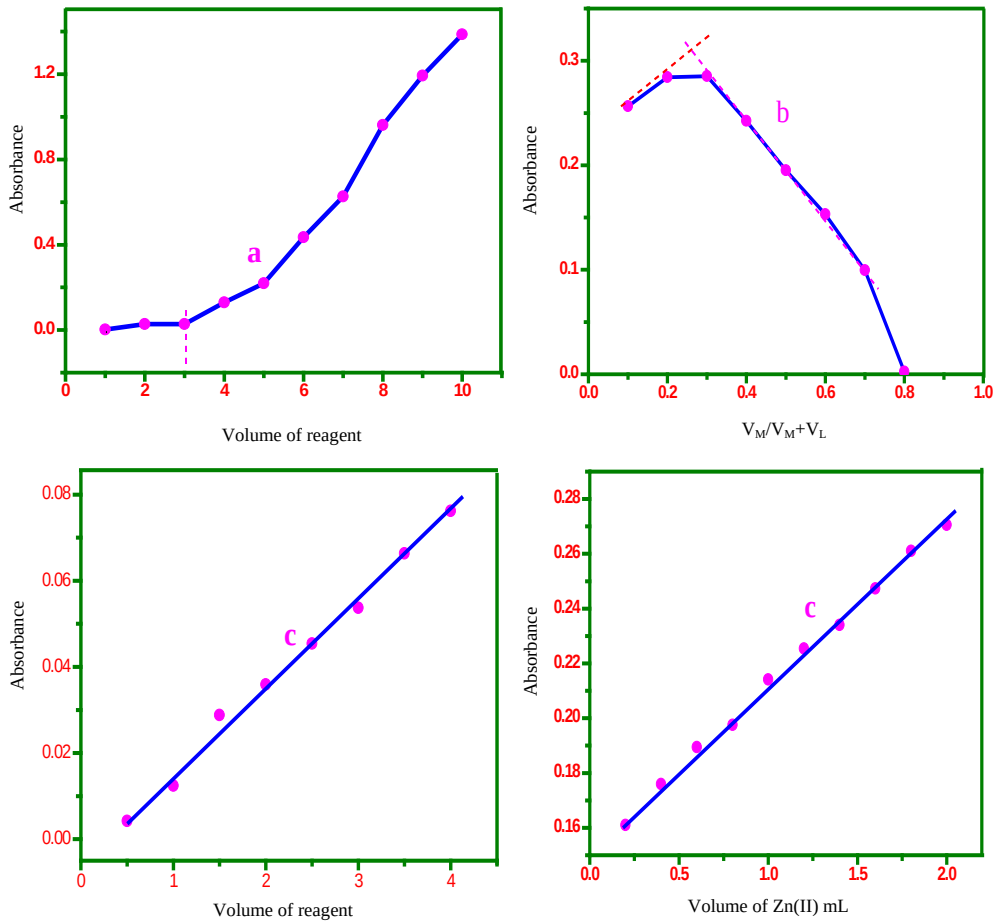


Figure 5. Evaluation of the composition of Zn(II)-DADHP complex; (a) Mole ratio method; (b) Job's continuous variation method; (c) Slope ratio method; Zn(II) =3x10^{-3} M = DADHP

Performance for the calibration of proposed method

Calibration plots constructed in the range 1-6 µg obeying the Beer's law for both direct and derivative spectrophotometry, the molar absorptivity of the complex is 0.1484x10^4 L.mole^{-1}.cm^{-1} and the Sandell's sensitivity of the method was 0.04545 µg cm^{-2}. In the derivative spectrophotometry it was clearly reflects the peak height is proportional to the amount of Zn(II) present in the solution (Figure 6). The standard deviation correlation co-efficient and other statistical parameters of the method were evaluated to ten replicate determinations (Table 1).

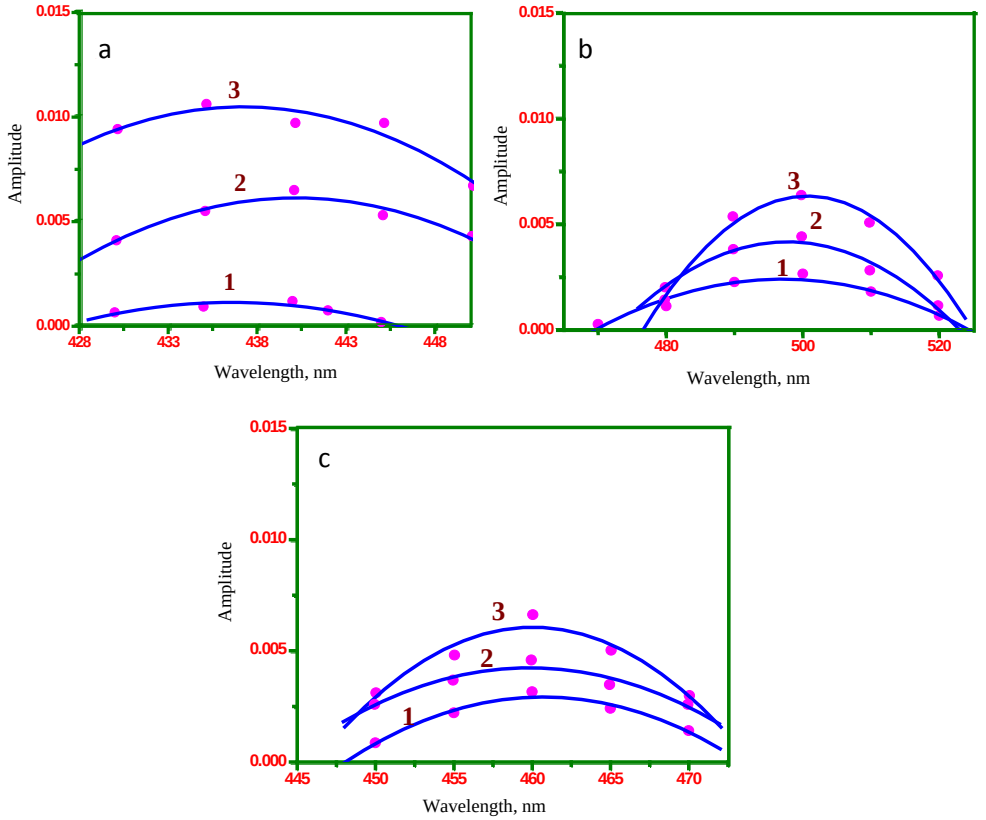


Figure 6. Derivative spectra of [Zn (II)-DADHP] system (a) First order; (b) Second order; (c) Third order Zn(II) µg/mL (1) 0.2; (2) 0.4; (3) 0.6

Table 1. Performance data for the calibration proposed method

Concentration Range, µg/mL	Least square equation Y = A + Bx A = Intercept B = Slope	Correlation Coefficient (r)	Standard Deviation	RSD %	REP %	Amount Determined in Ten replicate measurements, µg / mL
0.5 – 6.0	Y = -0.0114 + 0.0309x	1.1281	0.1400	4.4217	0.6378	3.3404,3.1262,3.0254,2.9630 3.2412,3.1502,3.2502,3.3215 2.9764,3.2676.

Pharmaceutical samples

The pharmaceutical samples are prepared by an established procedure to destroy the organic matters present[29-31] based on the use of 0.01 M hydrochloric acid for repeated evaporation. Finally, the residue left over was shaken well with double distilled water, sonicated and filtered. The filtrate was diluted to 100 mL, 2 mL of aliquots of the solution was used for the quantification of zinc. The measured absorbance and amplitude values are compiled with calibration plots (Figure 7) and the results are summarized in Table 2.

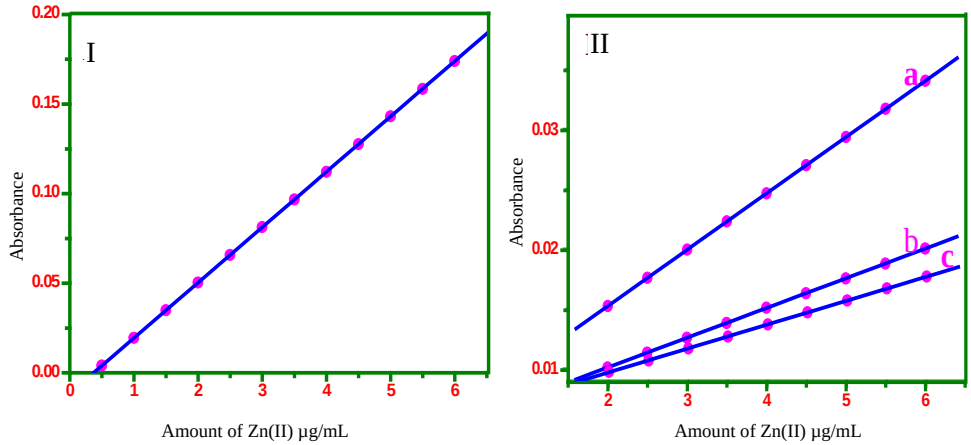


Figure 7. Calibration curve of Zn(II) obeying the Beer's law (0.5-6.0 µg/mL) (I) Direct spectrophotometry; (II) a- First; b- Second; c-Third derivative spectrophotometry

Table 2. Determination of zinc in pharmaceutical preparations

Pharmaceuticals	Form	Certified value mg / tablet	Found mg /tablet	Recovery %	RMSEP	REP %	RSD	*t* – test
ZINCOVIT			23.10	105.001	0.1632	3.6546	0.5487	0.1123
1st derivative	$ZnSO_4.H_2O$	22	18.467	83.940	0.1313	3.9901	0.7108	1.9595
2nd derivative			17.34	78.818	0.3656	3.7235	2.107	0.1902
3rd derivative			22.104	100.473	0.7066	3.6106	0.4777	0.2448
ZEVIT			43.161	104.25	0.0574	1.3769	0.1330	0.8718
1st derivative	$ZnSO_4.H_2O$	41.4	41.931	101.28	0.1580	1.3321	0.3768	0.5324
2nd derivative			43.199	104.34	0.3379	0.2578	0.7823	0.5000
3rd derivative			41.401	100.003	0.6230	0.0734	1.3213	0.2228
ANOFER			27.028	108.111	0.0116	3.9035	0.0312	1.1709
1st derivative	ZnO	25	24.363	97.456	0.2269	0.3121	0.9316	0.0195
2nd derivative			26.132	104.528	0.2276	0.0805	0.8709	1.5728
3rd derivative			19.854	79.460	0.1414	1.2811	7.1199	2.5591
ZINKĈ VIT			22.632	102.87	0.11907	0.7312	0.5260	0.1779
1st derivative	$ZnSO_4.H_2O$	22	17.9146	81.43	0.2488	0.7365	1.3888	0.2466
2nd derivative			14.6972	66.80	0.2593	0.6491	3.3204	0.2329
3rd derivative			13.6539	62.06	0.1359	0.0002	0.9955	0.2164
BECOZINC			54.73	99.63	0.1426	0.6889	0.2605	0.6652
1st derivative	$ZnSO_4.H_2O$	54.93	42.619	77.58	0.5704	5.9147	1.3465	0.0078
2nd derivative			52.076	94.80	0.3376	4.7426	0.6483	1.5960
3rd derivative			54.657	99.50	0.4944	1.1406	0.9045	2.6869
MULTIRICH			30.37	97.97	0.2662	2.4333	0.8770	0.3825
1st derivative	$ZnSO_4.H_2O$	31	32.55	105.00	0.5935	6.4662	1.2731	0.1097
2nd derivative			31.22	100.7	0.5092	7.7580	1.6359	0.0776
3rd derivative			26.43	85.25	0.7151	1.0382	2.7050	0.5081

Contd…

ZINCOFER			54.386	98.87	0.3316	5.642	0.6096	1.1500
1st derivative	$ZnSO_4.H_2O$	55	47.19	85.80	0.2306	0.1493	0.4883	0.4705
2nd derivative			28.43	51.59	0.3590	1.7599	1.2627	0.02113
3rd derivative			30.74	55.89	0.7148	1.2634	2.3248	0.2432
BECOSULES			41.61	100.52	0.0581	0.9657	0.1360	1.4526
1st derivative	$ZnSO_4.H_2O$	41.4	41.38	99.95	0.4788	1.0049	1.1570	0.0575
2nd derivative			38.84	93.81	0.4982	1.6505	1.2860	0.3586
3rd derivative			21.69	52.39	1.0096	0.5968	2.6301	0.2156

Effect of diverse ions

The effect of various anions and cations on the determinations of Zn(II) (4 µg/mL) in the developed optimal conditions was examined and the tolerance limits were defined as the concentration of added solution causing less than ±2% relative error on the absorbance. The results are presented in the Table 3. The interference due to Cu^{2+}, Ni^{2+}, Cd^{2+}, Mn^{2+}, Fe^{2+} and Ag^{+} was eliminated using thiourea, citrate, ascorbic acid, fluoride as appropriate masking agent. But it was noticed that there was strong interference of EDTA in the determination of Zn(II).

Table 3. Tolerance limits of diverse ions in the determination of Zn(II) (4 µg/mL)

Ion	Tolerance limit, µg/mL	Ion	Tolerance limit, µg/mL
Na^{+}	2000	Sulphate	5000
NH_4^{+}	2000	Thiourea	3000
K^{+}	1500	Thiosulphate	3000
Mg^{2+}	1000	Nitrate	2000
Ca^{2+}	1000	Fluoride	2000
Ba^{2+}	1000**	Chloride	2000
Al^{3+}	1000	Ascorbic acid	1000
Cr^{2+}	1000	Iodide	1000
Co^{3+}	1000	Citrate	1000
Pb^{2+}	600	Meta phosphate	1000
Hg^{2+}	500	Phosphate	200
Bi^{2+}	500		
Ni^{2+}	100**		
Fe^{2+}	100**		
Mn^{2+}	100*		
Pd^{2+}	100***		
Ag^{+}	100**		
Cu^{2+}	100**		
Cd^{2+}	100***		

**In the presence of thiourea 500µg, **In the presence of fluoride, ***In the presence of 500 µg citrate.*

Conclusion

The results shown in the Table (4) indicates the recovery of Zn(II) from the pharmaceutical samples.

In most of the cases the recoveries are less than 100%, in 1st, 2nd and 3rd derivative spectrophotometric measurements. Only in few it is greater than 100%. Further, the 3rd derivative spectrophotometric method was found to be more sensitive and accurate than direct, 1st and 2nd derivative methods, in which the recoveries are equal to 100% but greater than 50%. The lost of the target compounds could be caused by several factors. The most important one is, the samples were measured after digestion process with acids, meanwhile the measurements of standard solutions of Zn(II) for preparing the calibration curves were carried out without any digestion process. The main advantage of the proposed method is flexibility of the system, fully automated and very simple to assemble and operate. Satisfactory recoveries were observed in the analysis of pharmaceutical preparations, exclusively in 3rd derivative method the results are good agreement with the certified values

Table.4. Comparison of direct and derivative spectrophotometry

Pharmaceuticals	Direct	First derivative	Second derivative	Third derivative
ZINCOVIT	105.001	83.940	78.818	100.473
ZEVIT	104.25	101.28	104.34	100.003
ANOFER	108.111	97.456	104.528	79.460
ZINKĈ VIT	102.87	81.43	66.80	62.06
BECOZINC	99.63	77.58	94.80	99.50
MULTIRICH	97.97	105.00	100.7	85.25
ZINCOFER	98.87	85.80	51.59	55.89
BECOSULES	100.52	99.95	93.81	52.39

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