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Spectrophotometric Determination of Zinc in Pharmaceutical Medication Samples Using 8-Hydroxyquinoline Reagent

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Abstract

Simple, rapid, cheap and sensitive spectrophotometric method has been described for the determination of zinc in pharmaceutical samples. The method is based on the formation of zinc- 8-Hydroxy quinoline chelate, the maximum absorption (λ_{max}) at 384 nm. The method obeyed Beer's law in the range 1-5 μg/mL and the corresponding molar absorptivity value is 0.01578×10^3 $L.mol^{-1}.cm^{-1}$. The Sandell sensitivity values of limits of detection (LOD) and quantification (LOQ) was 0.381μg/mL and 1.156 μg/mL respectively. The recovery percentage of zinc was found 98.00 %, 98.96 %,99.91%, 97.50%, 98.5% and 99.30 % for (Capsule-13 mg), (Tablet-20 mg), (Tablet-40 mg),(Capsule-50 mg), (Capsule-50 mg) and (insulin vial-0.025 mg) respectively. All variable parameters has been optimized according to ICH guidelines. The limiting concentrations of some cations for interference by Mn(II), Fe(II), Co(II), Ni(II), Cu(II), Cd(II),Sn(II) ,Pb(II), Mg(II), Ca(II) and Ba(II) are reported. The method accuracy was established by comparison with conventional flame atomic absorption spectrometric method by using t-test, $t_{tab.}$= 2.571, $t_{cal.}$ =0.3231 at 95% confidence level, indicating the absence of systematic errors.

Keywords: spectrophotometric, zinc, pharmaceutical medication, 8-hydroxyquinoline chelate, insulin

1. Introduction

In biology, Zinc is as ordinary as iron (Williams, Phil, & F.R.S., 1984). It is a necessary trace element and plays a vital role in normal growth and development (Favier & Hininger-Favier, 2005), many biological functions such as cellular integrity, protein synthesis and nucleic acid metabolism need Zinc. It plays important role in brain development and also as an antioxidant (Takeda, 2000) and (Tate, Miceli, & Newsome, 1999) Zinc is a fundamental micronutrient (Maret & Sandstead, 2006). It has a connection with diseases, chronic kidney disease (Neto et al., 2016), HIV infection (Siberry, Ruff & Black, 2002), diabetics (Jansen, Karges & Rink, 2009) and cancer risk (Emily, 2004). Zinc is the most familiar element in multimineral and multivitamin preparations with microelements. In biological material, pharmaceutical preparations, and food, marked with the use of, spectrophotometric, (Sabel, Neureuther & Siemann, 2010) and, (Adi et al., 2016), spectrofluorometric (Takahira, Satoshi & Hitoshi, 2003), atomic absorption spectrometry(Sołtyk, et al., 2000), atomic absorption spectrophotometry method for determination of zinc in insulin(Ata et al., 2015), capillary electrophoresis (Wittrisch, et al., 1997), electrochemicals(Kumar, 1997) and thin layer chromatography(Manciri, & Zuanon-Netto,1998) methods.

In pharmaceutical preparations Zn-II has been marked spectrophotometrically, mostly with the employ of azo derivatives (Bhalotra, & Duri, 1999) and (Korn, et al., 1999).

Stable bidentate ligand chelate synthesized with zinc ion, 8-Hydroxyquinoline has atom that replaced by zinc ion and heterocyclic nitrogen atom, which forms with this ion a five membered ring (Kai, et al., 1985) as shown in Fig.1.

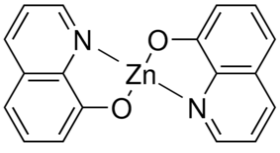

Figure 1. Zn-Bidentate chelate

The target of the study is to find out the validity of (system suitability, accuracy, linearity and precision) of the spectrophotometric method for determination of zinc in pharmaceutical samples by using 8-Hydroxyqinoline reagent.

2. Materials and Methods

Instrumentation

A Shimadzu (UV-1800) double beam UV/VIS spectrophotometer using 10 mm quartz cells and Aurora (Model –AI 1200) flame atomic absorption spectrometer was used, the spectroscopic setting were; band width 0.2 nm; deuterium (D_2) for background correction; integration time was 5 second; the current of the lamp was 5.0 mA lamp and the analyte was detected at 213.9 nm.

Reagent and solutions

All the chemicals used were of analytical reagent grade or highest available purity, deionized water was used. Glassware were cleaned by soaking in acidified solution of potassium permanganate followed by washing with concentrated nitric acid and rinsed with deionized water several times. The chemicals were used without further purification.

8-Hydroxyquinoline solution (C_9H_7NO), (2×10^{-3} M)

Prepared by dissolving 0.0290 g of 8-Hydroxyquinolin (8-HQ) in a volumetric 100 ml filled to the mark with ethanol of 99.2%. More diluted solutions were prepared as needed.

Stock solution of Zinc

Stock solution of Zinc (1000 mg L^{-1}) was purchased from Scharlau (Darmstadt, Germany).Working standard solutions were prepared daily by sequential dilution of the stock solution with deionized water.

Ammonium hydroxide solution

A diluted solution of ammonium was prepared in volumetric flask 100 ml with adding 10 mL of concentrated ammonium solution (28-30% A.C.S grade) to deionized water.

Sulfuric acid solution

A solution 0.0001 M of sulfuric acid was prepared in a volumetric flask 100 mL by serial dilution of concentrated sulfuric acid (98%) with deionized water.

General procedure

A volume of 1- 5 ml of standard solution containing 1-5 μg of zinc in 10 mL volumetric flask was mixed with (preferably) 5.5 mL 8-HQ (1.1×10^{-3} M) followed by addition of 0.1-1 mL (preferably 0.6 ml) of 1×10^{-4} M sulfuric acid. After 1 minute 1 mL of ethanol was added, the mixture was diluted up to the mark with deionized water. The absorbance was measured after waiting for 15 minutes at 384 nm against a corresponding reagent blank. The zinc content in the samples was determined by using the linearity equation of calibration curve.

Procedure for commercial samples

Ten tablets and capsules were weighed accurately and ground into a fine powder. Tablet and capsule powder equivalent to 200 mg was accurately weighted and taken to a beaker and digested according to Ahmed method(Ahmed, et al., 2010).10 mL of concentrated nitric acid was added and to dryness, 10 ml of 20% (v/v) of sulfuric acid solution added. The volume was became 2.5 mL by heating on a hotplate and cooled to room temperature. The solution was neutralized with diluted ammonium solution, the resulting solution filtrated and transferred quantitatively to 50 mL volumetric flask and filled with deionized water to the mark. Humulin N Vial (5 mL, 100 IU/ml) of Lilly Pharmaceuticals, shaken well insulin vial, 5.0 mL taken, digested according to Ahmed method(Ahmed, et al., 2010), the resulting solution transferred quantitatively to 25 mL volumetric flask and filled with deionized water to the mark.

Sample collection

Pharmaceutical samples (tablets and capsules) of different companies were collected and insulin vial from the local pharmacy of Misan province. Samples (tablets and capsules) were homogenized with mortar.

3. Result and Discussion

Optimization of the experimental parameters

Absorption spectra

The UV-VIS absorption spectra of the zinc- 8HQ chelate was shown in Fig.2, acquired from 350-450 nm with a maximum absorbance 384 nm and an average molar absorptivity (0.01578×10^3 L. $mol.^{-1}cm^{-1}$). Free ligand 8-HQ spectra was shown in Fig.3 acquire from 270-450 nm with maximum absorbance 316 nm at room temperature. The

absorption peak of the Zn-8HQ chelate shifted to long wavelength, as a result of a charge transfer from the metal to the ligand (MLCT band) (Zhong, et al., 2008).

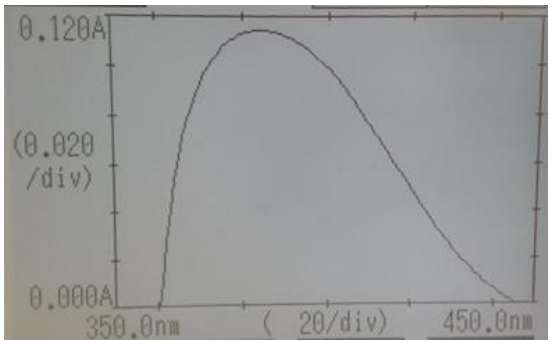

Figure 2. Absorbance spectra of Zn-8HQ chelate (λ_{max} 384 nm)

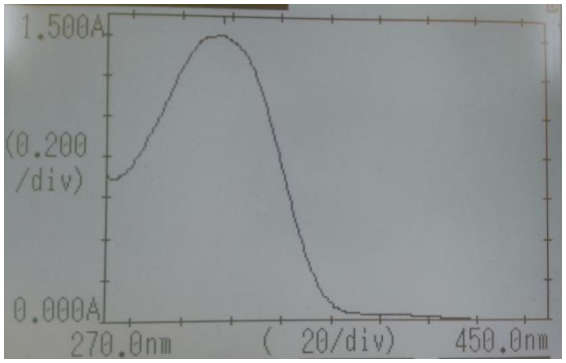

Figure 3. Absorbance spectra of the reagent 8-HQ 2×10^{-3}M (λ_{max} 316 nm)

Effect of solvent

Different volumes (0-8 mL) of ethanol were added to fixed concentration of zinc ions as shown in Fig.4 and the absorbance was measured according to the general procedure. Maximum absorbance was appeared in 10% (v/v) ethanol / water medium, 10% ethnol solution was used in the determination procedure.

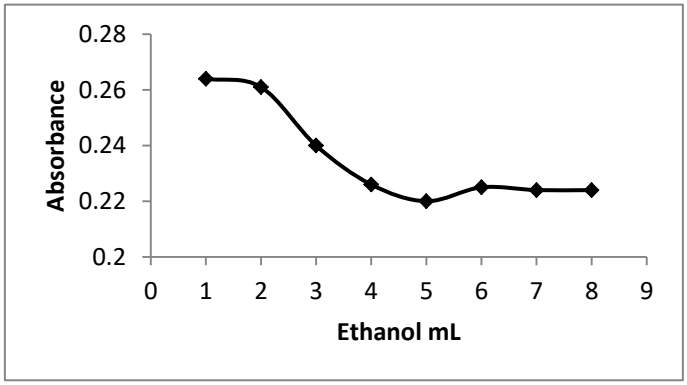

Figure 4. Effect of the ethanol on the absorbance of Zn- 8HQ chelate

Effect of sulfuric acid concentration

The variation of the absorbance was appeared after the addition of (0.1-1.0 mL) of 0.0001 M sulfuric acid at room temperature (25± 5C°) as shown in Fig.5. For all the following measurements 0.6 mL of 0.0001 M diluted sulfuric acid solution was added.

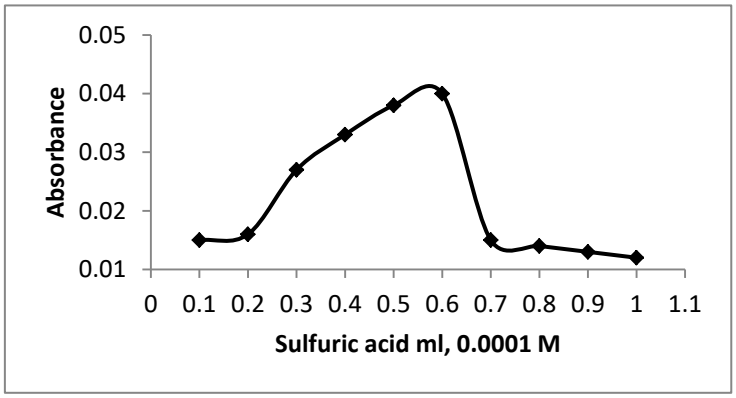

Figure 5. Effect of the sulfuric acid on the absorbance of Zn-8HQ chelate

Effect of the reaction time

The reaction is very fast. A stable maximum absorbance was obtained during interval time (2-16 minutes) as shown in Fig. 6, just after dilution within 15 minutes all subsequent measurements was adjusted.

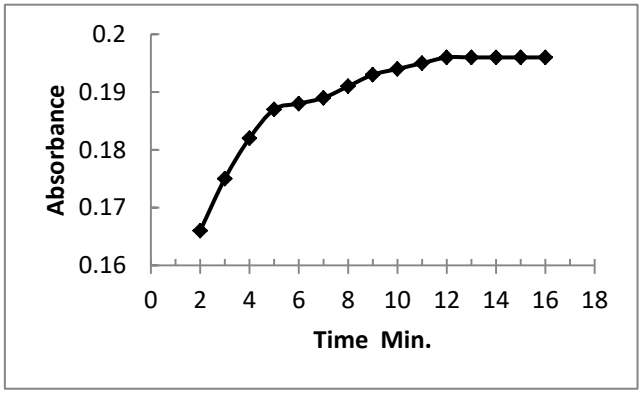

Figure 6. Effect of time on the absorbance of Zn-8HQ chelate

Effect of the 8-HQ reagent concentration

Different molar of 8-HQ were added to a fixed zinc ion (1 μg/mL) and the absorbance was measured according to the general procedure as shown in Fig. 7. The effect of changeable reagent concentration was noticed. For all following measurements, 5.5 mL of 1×10^{-5} M 8HQ reagent was added.

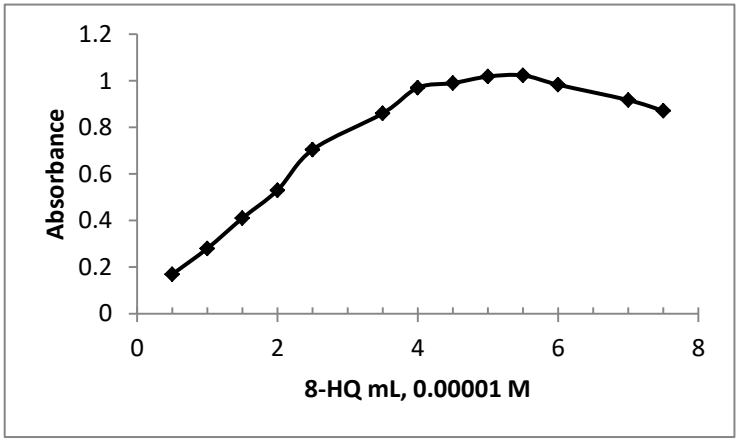

Figure 7. Effect of reagent on the absorbance of Zn-8HQ chelate

Method validation

System suitability

Standard solutions (1.0-5.0 μg/mL) were prepared by using zinc working standard (1.0 μg/mL). System suitability

parameters were evaluated and found to be within the limits. The function of the system suitability test was to make sure that the complete testing system (including instrument, reagent and analyte) is suitable for proposed application. The relative standard deviation RSD% for five absorbance reading of each zinc standard solution is available in Table 1.

Table 1. Evaluation of the spectrophotometric system suitability of zinc analysis

Sr. No	Zinc standard μg / ml	Five absorbance reading					RSD%
1	1.0	0.024	0.025	0.024	0.024	0.025	1.004
2	2.0	0.053	0.052	0.053	0.052	0.051	0.717
3	3.0	0.077	0.077	0.078	0.077	0.078	0.316
4	4.0	0.010	0.090	0.011	0.011	0.010	0.373
5	5.0	0.121	0.122	0.121	0.121	0.122	0.202

Acceptance criteria = The RSD% for zinc absorbance reading should not be further than 2.0%

Linearity

Linearity was evaluated for the spectrophotometric and the flame atomic absorption methods through a graphical representation of concentration versus absorbance. The absorbance was linear and following Beer's law, the concentration of zinc ion was in the range (1-5 μg/mL) at 384 nm, (0.1 – 1.5μg/mL) at 213.9 nm passing through the origin (R^2 = 0.9960), (R^2 = 0.9985) for spectrophotometric and flame atomic absorption methods respectively as shown in Fig.8 and Fig.9.The operating parameters of flame atomic absorption for zinc are summarized in Table 2.

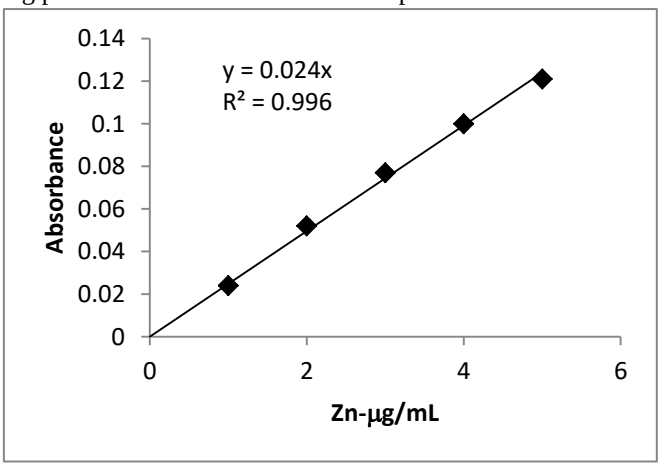

Figure 8. Calibration graph (1-5 μg/mL) of Zinc by spectrophotometric method

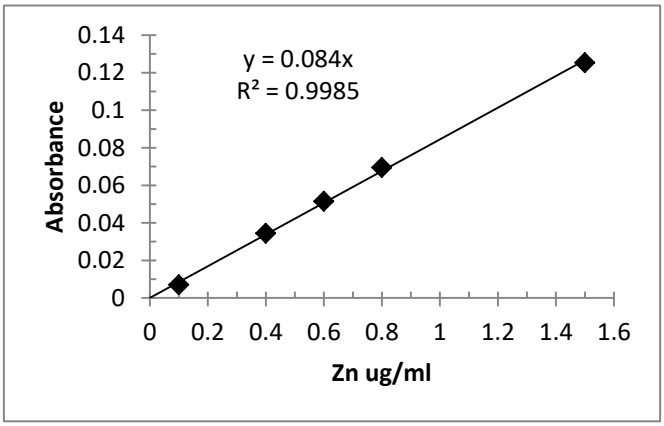

Figure 9. Calibration graph (0.1-1.5 μg/mL) of Zinc by flame atomic absorption method

Table 2. Operating parameters of flame atomic absorption for zinc

Element	Wave length nm	Slit width (nm)	HCL amp current (mA)	Flame
Zinc	213.9	0.2	5.0	Air / acetylene

The optimum analytical parameters are summarized in Table 3 shows the required data for obtaining the calibration curve, slop, linear regression equation and the regression coefficient.

Table 3. Optical characteristic of the spectrophotometric method and the flame atomic absorption method

Parameter	Spectrophtometric method	Flame atomic absorption method
λ_{max}	384 nm	213.9 nm
Beer,s law limits (μg/mL)	1-5 μg/mL	0.1-1.5 μg/mL
Slop	0.024	0.084
Regression equation	y = 0.024x	y = 0.084x
Limit of detection (μg/mL)	0.381	0.216
Limit of quantification (μg/mL)	1.156	0.655
Regression coefficient (R^2)	0.9960	0.9985
ε Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	0.01578×10^3	0.00514×10^3

Accuracy

To provide additional support to the accuracy of the method, different standard concentrations of zinc were added to known concentration of the samples and the total concentration were determined by using the proposed method (n=5).

The recovery % = $[(C_t - C_s) / C_a] \times 100$, where C_t is the total zinc concentration after standard addition, C_s zinc concentration in the samples, C_a zinc concentration added to the samples. Capsule-1, tablet-2, tablet-3, capsule-4, capsule-5 and Insulin vial, containing (13 mg), (20 mg), (40 mg), (50 mg), (50 mg) and (0.025 mg) of labeled zinc respectively as shown in Table 4.

Table 4. Percentage recovery of zinc concentration in samples for accuracy

Sample	Zinc added (mg/mL)	Total zinc (mg/mL)	Total zinc found (mg/mL)	Recovery %
Capsule -1	0.1	2.7	2.666	98.00
Tablet -2	0.1	0.12605	0.12501	98.96
Tablet -3	0.1	2.00476	1.99465	99.91
Capsule -4	0.1	0.2488	0.2463	97.50
Capsule-5	0.1	7.6923	7.6185	98.50
Insulin vial	0.1	1.1	1.0859	99.30

Sensitivity of the method

The LOD and LOQ are the limit of detection and limit of quantification respectively of zinc by the proposed methods were determined using calibration standard. LOD and LOQ were calculated as 3.3 σ /s and 10 σ/s respectively, where s is the slop of the calibration curve and σ the standard deviation of the blank (n=5). LOD and LOQ were found to be (0.381, 0.216 μg /ml) and (1.156, 0.655 μg /ml) for the proposed method and the standard method respectively as shown in Table 3.

Assay of formulation

The mean value of zinc in drug tablet, capsule and insulin vial samples 12.83 mg,19.69 mg,39.79 mg, 49.97 mg,48.88 mg and 0.0245 mg for spectrophotometric method and 12.91 mg, 19.92 mg, 39.62 mg, 49.95 mg, 49.56 mg and 0.024625 mg for standard method respectively. The purity of zinc was calculated (1.73, 3.25, 4.73, 18.60, 18.80 w/w% and 4.98 mg/L) for spectrophotometric method and (1.74, 3.28, 4.71, 18.59, 19.06 w/w %) for standard method respectively as shown in Table 5.

Table 5. Assay of formulation

Sample	Label to content	Direct Method (Found) mg	Purity	Standard Method (Found) mg	Purity w/w%
Capsule -1	13.00 mg	12.83	1.73 w/w%	12.91	1.74%
Tablet -2	20.00 mg	19.69	3.25 w/w%	19.92	3.28%
Tablet -3	40.00 mg	39.79	4.73 w/w%	39.62	4.71%
Capsule -4	50.00 mg	49.97	18.60 w/w%	49.95	18.59%
Capsule -5	50.00 mg	48.88	18.80 w/w%	49.56	19.06%
Insulin vial	0.025 mg	0.0245	4.98 mg/L	0.024625	4.985 mg/L

Effect of foreign cations

A number of divalent cations were studied for their possible interference in the determination of Zn (II) in a pharmaceutical samples under the optimum conditions. The criterion for an interference (Ojeda, et al., 1987) was an absorbance value varying by more than 5% from the estimated value for zinc (II) alone. Interference from theses cations are probably due to the complex formation with 8-hydroxyquinoline, the greater tolerance limits for these cations can be achieved by using several masking agents. Tolerance limits for the determination of 1μg/mL zinc (II) in the concentrations below 16.42 μg/mL Co (II), 82.12 μg/mL Cd(II), 13.14 μg/mL Pb(II), 93.85μg/mL Ni(II), 59.72 μg/mL Mg(II),8.11 μg/mL Ca(II), 109.5 μg/mL Ba(II), both Fe (II) and Cu(II) ,however have 25.27μg/mL and the concentration of Mn(II) and Sn(II) is 50.53 μg/mL.

4. Conclusion

The method using 8-hydroxyquinoline as a spectorophotometric reagent for zinc determination is cheap and simple also can be used in each laboratory. The zinc-8HQ complex formed is stable and shows a good sensitivity. This proposed method was successfully applied for zinc determination in pharmaceutical samples and the results shows a good agreement with certified values and with results obtained by flame atomic absorption method, t-test, $t_{tab.}$= 2.571> $t_{cal.}$ = 0.3231 at 95% confidence level.

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Conflict of interest

All authors have declared that there is no conflict of interests including any financial, personal or other relationships with other people or organizations that can influence their work.

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