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SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF Fe(II) AND Zn(II) IN MULTIVITAMIN SOFT GEL CAPSULE

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ABSTRACT

The present work describes novel spectrophotometric method for the determination of Fe(II) and Zn(II) in multivitamin soft gel formulation. The method is based on formation of coloured complexes of Fe(II) and Zn(II) with 2,2'-Bipyridyl and Dithizone respectively. Fe(II) forms deep red colored chromogen with 2,2'-Bipyridyl having maximum absorbance at 510nm and Zn(II) complexes with dithizone to develop cherry red color having maximum absorbance at 525nm. Beer's law was obeyed in the range of 10 – 35 µg/ml for Fe(II) and 2 – 12 µg/ml for Zn(II). The detection limits were 1.85 µg/ml and 0.11 µg/ml for Fe(II) and Zn(II) respectively. The RSD values were, in all instances less than 2.0%. No interference was observed form common pharmaceutical adjuvants and both Fe(II) and Zn(II) were successfully determined in their dosage form.

Keywords: Fe(II), Zn(II), Soft Gel Capsule, Dithizone, 2,2'-Bipyridyl.

INTRODUCTION

Metals are indispensable constituents of approximately one third of all proteins [1]. As such, metals are involved in virtually all biological processes, including metabolism, energy transduction, gene expression, cell signalling, formation of endo- and exoskeletons, and electron and information transfer [1,2].

Among the techniques suitable for the quantification of metal ions in metalloproteins, inductively coupled plasma mass spectrometry and atomic absorption and emission spectroscopies are likely to be the most widely employed [3]. However, although these techniques are reliable and sensitive, they suffer from the limitation of being rather costly (considering instrument acquisition and maintenance), time-consuming (with respect to sample preparation), and not always readily available [4,5]. Thus, simple spectrophotometric (or spectrofluorometric) techniques, which tend to be less costly and labor-intensive, are viable alternatives to those methods requiring more sophisticated instrumentation. The determination of trace metals by ultraviolet–visible (UV–Vis) 1 spectroscopy typically relies on alterations in the absorption spectrum of a chromophoric chelator on binding the desired metal ion [6].

Iron plays a central role in the biosphere, serving as the active center of proteins responsible for O2 and electron transfer and of metalloenzymes such as oxidases, reductases and dehydrases. Iron deficiency anemia is one of the world's most common nutritional deficiency diseases. Zinc is a representative of the group of microelements, thus in small quantities it is essential for human, animal and plant growth. Zinc influences living processes and displays regulatory functions, as it participates in the content of above 40 enzyme systems. Iron and zinc provided by dietary supplements is often found as Fe(II) fumarate and Zn(II) sulfate is cheaper and is absorbed equally well.

The Pharmacopeias recommends an atomic absorption spectroscopy (AAS) technique or an extractive titrimetric method for determination of Fe(II) and Zn(II) ions. A number of analytical methods for quantitative analysis of iron and zinc have been developed. These methods include spectrophotometry [7, 8] flourimetry [9,

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10], flow-injection analysis [11, 12], voltammetry [13, 14], chemiluminescence [15, 16], capillary electrophoresis [17, 18], atomic emission and atomic absorption spectrometry [19, 20], and chromatography [21, 22]. Although some of these methods are highly sensitive, they have disadvantages such as the necessity for expensive and sophisticated instrumentation. Thus, UV/Visible spectrophotometry has been used because it is relatively cheap, rapid and simple. The UV/Visible spectrophotometry involves the use of ligands which selectively bind to iron (II) and zinc (II) to produce colored complex.

MATERIALS AND METHODS

Apparatus

UV-1800 Shimadzu (Japan), Shimadzu Balance BL-220h. Pure form of Fe(II) and Zn(II) were supplied by GLPL. Marketed formulation of combination was procured from GLPL. All reagents, i.e. Dithizone, 2,2'-Bi-pyridyl, Dibasic Ammonium Citrate of Analytical Grade were used of Loba Chimie. All solvents like Chloroform, Methanol, were Supplied by Merck.

Selection of Chromogen for color development

2, 2' bi-pyridyl was selected as chromogen for Fe(II) and dithizone was selected as chromogen for Zn(II) as both chromogenic reagents did not interfere with estimation of Fe(II) and Zn(II) selectively.

Optimisation of method

For optimsation of 2, 2' bi-pyridyl reagent, 1% solution of reagent was prepared in methanol. The reagent solution was added in increasing volume of 1, 2, 3, 4, & 5 ml to 30 µg/ml of Fe(II) solution in 0.1N HCl. The volume that shows maximum absorbance was choosen as optimsed volume i.e. 2 ml of reagent, as shown in table 30. For optimsation of dithizone 0.01% solution was prepared in chloroform. The reagent was added in increasing volume of 0.5, 1.0, 1.5, 2.0, & 2.5 ml to 6 µg/ml of Zn(II) in 0.1N HCl. But as the volume of dithizone reagent increased the color of complex was quenched by color of dithizone. Hence 1 ml of 0.01% solution of dithizone in chloroform was optimized.

Optimised procedure for colorimetric determination of Fe(II) and Zn(II)

Preparation of stock solution

Stock solution was prepared by dissolving 100 mg of Fe(II) and Zn(II) in 100ml of 0.1N HCl, in respective volumetric flasks. The stock solution was further diluted to get range of 10-35 µg/ml for Fe(II) and 2-12 µg/ml for Zn(II).

Preparation of reagent solutions

a) **Preparation of 2, 2,' bi-pyridyl reagent (1%w/v)**: 1 g of 2, 2' bi-pyridyl was dissolved in 100ml of methanol.

b) **Preparation of Dithizone (0.1% w/v)**: 10 mg of dithizone was dissolved in 100 ml of chloroform.

Preparation of buffer solution (Alkaline ammonium citrate): 5g of dibasic ammonium citrate was dissolved in 100ml of 5% Ammonia solution.

Procedure

For determination of Fe(II) and Zn(II), the mixture containing 15 µg/ml of Fe(II) and 3.3 µg/ml was prepared form the stock solution. To the solution 2 ml of bi-pyridyl reagent was added, followed by 1 ml of buffer solution and 1 ml of dithizone reagent. The solution was shaken vigorously in separating funnel. It was extracted with 15 ml of chloroform 3 times. After separation of two layers, the bottom organic layer was transferred in 50 ml volumetric flask by passing it through anhydrous calcium sulphate. The chloroform layer was diluted upto the mark with chloroform to get desired concentration 3.3 µg/ml for Zn(II). The aqueous layer was diluted with water upto the mark in 10ml volumetric flask to get desired concentration of 15 µg/ml for Fe(II). The absorbances of both layer were taken separately against reagent as the blank, i.e dithizone reagent for chloroform layer and bipridyl reagent for aqueous layer.

Validation of Colorimetric method

a) Linearity and Range

Fe(II) was found to be linear in the range of 10-35 µg/ml. the absorbances of these solutions was measured at 520 nm. Zn(II) was found to be linear in the range of 2-12 µg/ml. The absorbances of these solutions were measured at 525 nm. Calibration curves were plotted using concentration v/s absorbances.

b) LOD and LOQ

Limit of detection can be calculated by using following formula

$$LOD = 3.3\ \sigma / S$$

Limit of quantitation can be calculated based on standard deviation of the response and the slope.

$$LOQ = 10\ \sigma / S$$

Where σ = Standard deviation of the response

S = Slope of the calibration curve.

The concentration found then were injected to detect LOD and LOQ.

c) Precision

Repeatability was done by using 3 replicate readings at 3 concentration levels. For Intra day variability trials are taken in a day and for Inter day variability studies were done on 3 consecutive days. Concentration used for Fe(II) was 10-30 µg/ml and for Zn(II) 2-6 µg/ml.

d) Analysis of formulation (HAEMORICH®)

Twenty Capsules were emptied and oily paste was finely mixed. A quantity of oily paste equivalent to 100 mg

of Fe(II) and 22 mg of Zn(II) was weighed and transferred to 100 ml volumetric flask. 80 ml of 0.1N of HCl was added and sonicated for 22 mins. The volume was made up to 100 ml with 0.1N HCl. The solution was filtered using whatmann filter paper No. 41. From this 1 ml of aliquot was diluted to 10 ml with 0.1N HCl. Form above solution 1.5 ml was pipetted and transferred to 50 ml volumetric flask. To the solution 2 ml of bi-pyridyl reagent was added, followed by 1 ml of buffer solution and 1 ml of dithizone reagent. The solution was shaken vigorously in separating funnel. It was extracted with 15 ml of chloroform 3 times. After separation of two layers, the bottom organic layer was transferred in 50 ml volumetric flask by passing it through anhydrous calcium sulphate. The chloroform layer was diluted upto the mark with chloroform to get desired concentration 3.3 µg/ml for Zn(II). The aqueous layer was diluted with water upto the mark in 10ml volumetric flask to get desired concentration of 15 µg/ml for Fe(II). The absorbances of both layer were taken separately against reagent as the blank, i.e dithizone reagent for chloroform layer and bipridyl reagent for aqueous layer.

e) Recovery study

To study accuracy of the method, recovery studies were carried out by addition of standard drug solution to sample at 3 different levels, 80%, 100% and 120% of the test concentration (test concentration is 15 µg/ml for Fe(II), and 3.3 µg/ml for Zn(II)).

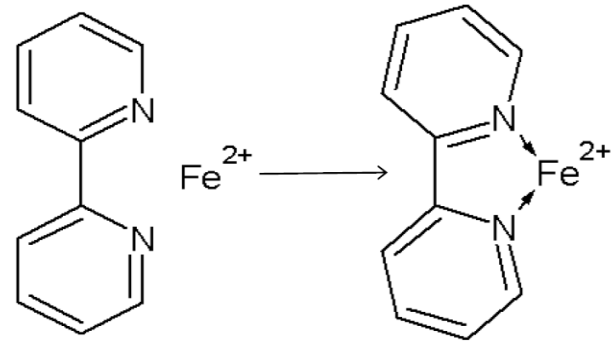
f) Specificity study

Commonly used excipients (Vegetable oil, Soy Lecithin, Hydrogenated soya bean oil, and Yellow wax) were spiked into a pre weighed quantity of drugs. The spectrum was taken by appropriate dilutions and the quantity of each drug was determined.

RESULT AND DISCUSSION

The developed method is based on complexation of Fe(II) with 2,2'- Bipyridyl in acidic condition to develop red colored chromogen having maximum absorbance at 510 nm as shown in figure 1.

Figure 1. Recation of Fe(II) with 2,2'- Bipyridyl



2, 2' bi-pyridyl *Ferrous ion* *Red colored complex*

Firgure 2. Reaction of Dithizone with Zn(II)

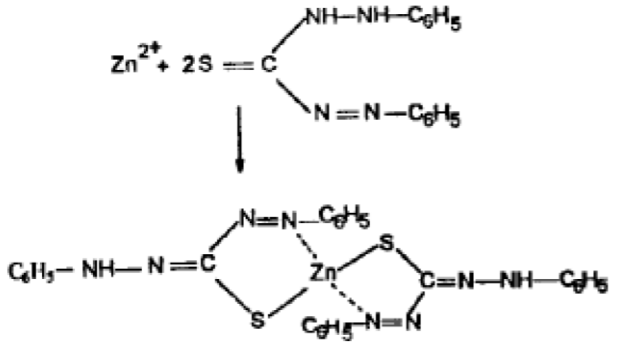


Figure 3. Linearity of Fe(II)

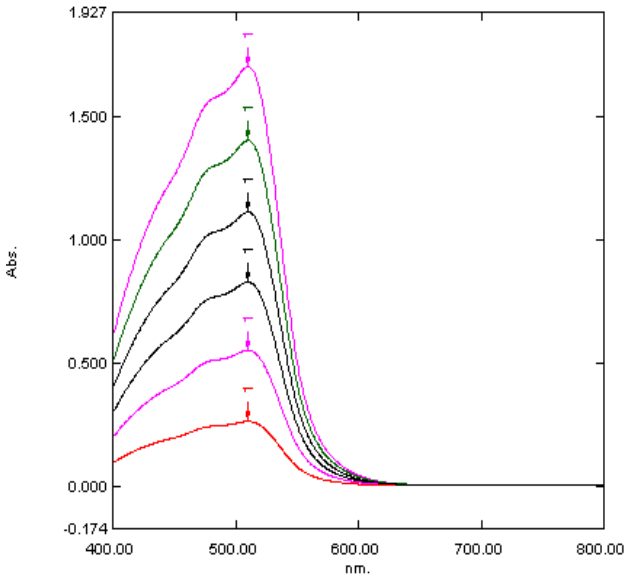


Figure 4. Calibration curve of Fe(II)

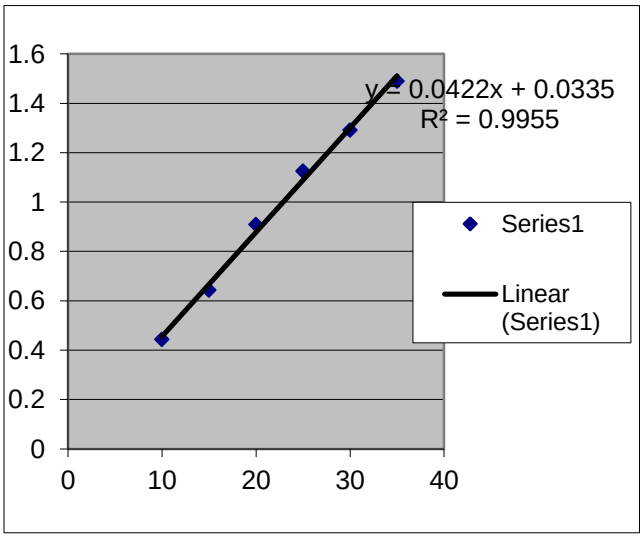


Figure 5. Linearity of Zn(II)

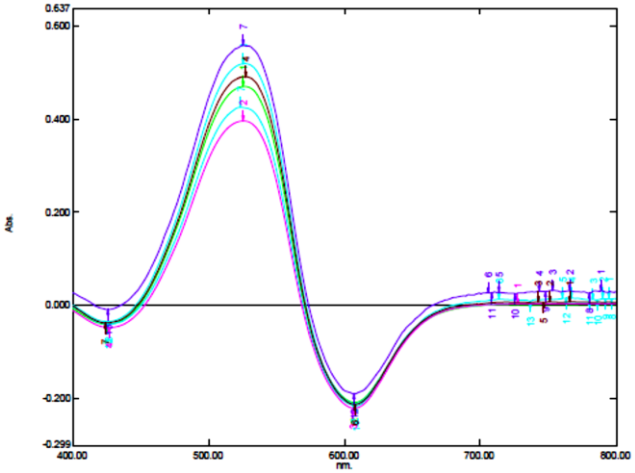


Figure 6. Calibration curve of Zn (II)

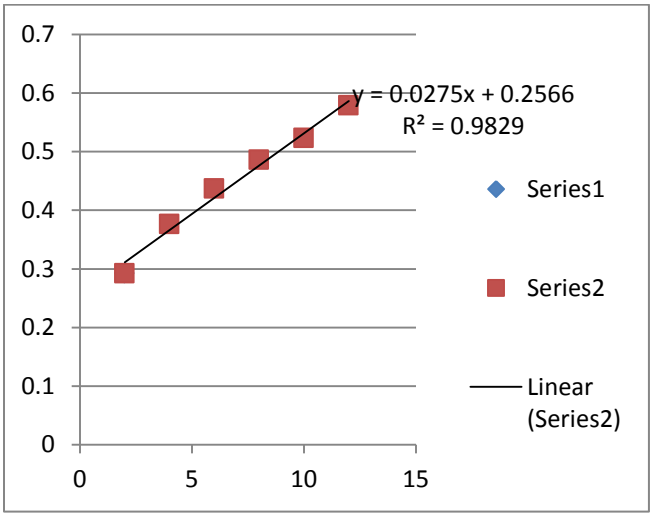


Table 1. Validation summary of Fe(II) and Zn(II)

S.No.	Parameters	Results	
		Fe(II)	Zn(II)
1	λ_{max}	510 nm	525 nm
2	Linearity Range (n=5)	10-35 µg/ml	2-12 µg/ml
3	Co-relation coefficeint	0.9955	0.9829
4	Precision %RSD Intraday (n=3) Interday (n=3)	 0.72 1.29	 1.06 1.07
5	Assay (n=3)	99.23±0.91	99.24±1.84
6	Accuracy (n=3)	98.27±0.54	100.66±0.44
7	LOD	1.85 µg/ml	0.111 µg/ml
8	LOQ	5.62 µg/ml	0.338 µg/ml
9	Specificity	Specific	Specific

Table 2. Analysis of formulation (HAEMORICH® Capsule)

Label Claim (mg)	Concentration estimated (mg/)	% Concentration estimated	% RSD (n=3)
Fe(II) (300)	297.70932	99.23644	0.915121
Zn(II) (66)	65.5030332	99.24702	1.872918

Present work also include estimation of Zn(II) along with Fe(II) in the same formulation. This includes complexation of Zn(II) with Dithizone in presence of alkaline ammonium citrate buffer as shown in figure 2. Zn(II) form red colored complex which is soluble in chloroform and insoluble in water. Hence the method was developed to estimate Fe(II) and Zn(II) from soft gel formulation simultaneously using 2,2'- Bipyridyl and dithizone as chromogen.

Table 3. Accuracy of Fe(II) and Zn(II)

Label claim (mg/capsule)	Amount added (%)	Total Amount (µg/ml)	Amount Found (µg/ml)	% Recovery ± SD
Fe(II) (300)	80	12	27	97.87315±0.394674
	100	15	30	99.03897±0.29082
	120	18	33	97.91038±0.234524
Zn(II) (66)	80	2.64	5.94	100.6428±1.499535
	100	3.30	6.60	100.1286±1.374346
	120	3.96	7.26	101.2105±1.029203

The optical characteristics such as absorption maxima, beer's law limits, and detection limits are presented in table 1. The overlain spectra of Fe(II) and its calibration curve is shown in figure 3 and 4, whereas the overlain spectra of Zn(II) and its calibration curve is shown in figure 5 and 6. The developed method was applied to pharmaceutical formulation Haemorich®, the result obtained are given I table 2. The accuracy of method was ascertained by spiking the formulation with reference standard, the result obtained thereof are mentione d in table 3. The method was found to be specific by carrying specificity in presence of various vitamins and minerals.

CONCLUSION

It could be concluded that the developed method for Fe(II) and Zn(II) is simple, relatively precise, accurate and can be satisfactorily applied to soft gel formulation.

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