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SPECTROPHOTOMETRIC DETERMINATION OF Cu(II) USING A SYNTHESIZED AZO QUINOLINE LIGAND AS ANALYTICAL REAGENT

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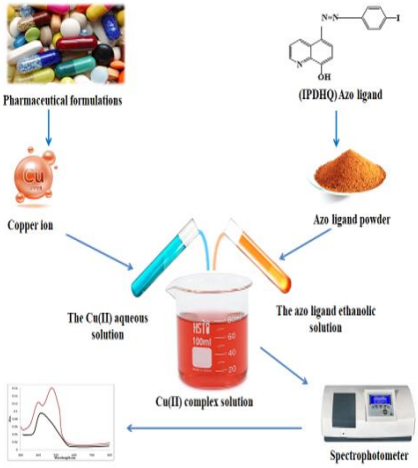
The main purpose of this work is to submit a rapid and simple spectrophotometric determination method for Cu(II) in different pharmaceutical formulations using a synthesized azo reagent, (E)-2-((4-iodophenyl)diazenyl)-8-hydroxyquinoline (IPDHQ), as a bidentate chelation ligand. The submitted method depends on the chelation reaction between Cu(II) and the synthesized ligand to form a orange complex with a maximum absorption at 454 nm. The geometry is octahedral, with a mole ratio of 1:2 (metal:ligand). Under the optimum conditions, the linearity was observed in the concentration range of 0.5–20 μg mL^{-1} and the relative standard deviation for n =10 of 10 μg mL^{-1} of copper was 0.227%. The effect of diverse cations and anions as interferences in the Cu(II) determination was checked. The submitted method was further applied to estimate Cu(II) quantity in different pharmaceutical formulations with satisfactory recovery results.



INTRODUCTION

Azo compounds are an important group of organic compounds due to their containing one or more azo chromophores, giving them different characteristic colors which can be used for various analytical and biological purposes.[1–3] Azo compounds could be used as useful analytical reagents for determination of different elements in micro quantities in multiple specimens.[4,5] This class of chemicals characterized by many advantages, like the ease of preparation, abundant pure product with the desirable stability, in addition to the high selectivity and sensitivity in various speciation analysis. In addition to using them successfully in biological fields as anticancer-treating chemicals,[6–7] antioxidant and antifungal,[8] antibacterial,[9,10] and as a good inhibitor agent of COVID-19.[11]

Copper is one of the trace elements essential for living organisms, the main sources of copper intake being food and drinking water. It plays a significant role in diverse biochemical reactions, such as its role in carbohydrate, lipid, protein, and nucleic acid metabolism.[12,13] The deficiency of copper element

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in the human body causes several diseases, such as biochemical and physiological functional disorders. Nevertheless, the excessive intake of copper can be harmful, causing liver disorder, agitation of the nose and throat, vomiting, diarrhea, and producing harmful free radicals that can damage the DNA molecules.[14,15] Copper ion, like the other heavy metal ions, as a significant environmental contaminant, considered as an increasingly significant environmental issue due to it is non-biodegradable, accumulation in tissues, highly toxic, and hazardous even at low concentrations.[16] It can be spread among the plants and animals then enter the human body through the food chain. In the human body, copper can bind easily to the components of the vital cellular, and lead to serious diseases, organ failures, and disorders such as Alzheimer's disease, cancers, Parkinson's disease, kidney malfunction, and osteoporosis.[17,18] Modern instrumental techniques such as atomic absorption spectrometry (AAS),[19,20] flame atomic absorption spectrometry (FAAS),[21,22] graphite furnace flame atomic absorption spectrometry (GFAAS),[23,24] inductively coupled plasma optical emission spectrometry (ICP-OES),[25,26] inductively coupled plasma mass spectrometry (ICP-MS),[27,28] fluorescence techniques,29,30 electrochemical methods,[31,32] voltammetric methods,[33,34] and potentiometric methods[35,36] have been suggested for the accurate and precise determination of Cu(II) in different samples.

Although the above-mentioned methods are extremely sensitive and selective for the specific ion, they are highly expensive and require qualified laboratory personnel.

In addition, these methods involve tedious processes for sample preparation and multistep procedures for the desirable analyte pre-concentration, and they might not always be available in all analytical laboratories. In contrast, the spectrophotometric methods are economic and low cost, easy in the operation, fast in its analysis ability, simple and robust in the operation. They can easily and sensitively detect the target ions in the visible range by the naked eye; in addition, the sample is not restricted by the aqueous or organic phase during the species determination. Therefore, the spectrophotometric methods have attracted appreciable attention as one of the unremarkably used methods in the determination of Cu(II) in different samples.[37–39] The aim of the presented study is to submit a rapid and sensitive spectrophotometric method for Cu(II) determination using the synthesized quinoline derivative ligand, (E)-2-((4-iodophenyl)diazenyl)-8-hydroxyquinoline (IPDHQ), as an analytical reagent for spectrometric determination at 454 nm. Different analytical conditions were studied to obtain the optimum conditions for the Cu(II) determination. The complex composition was also studied applying the mole ratio method; the interferences effect of diverse cations and anions that may affect the complex stability was checked. The suggested method was employed for the determination of copper ion in different pharmaceutical formulations and the recovery results were satisfactory.

MATERIALS AND METHODS

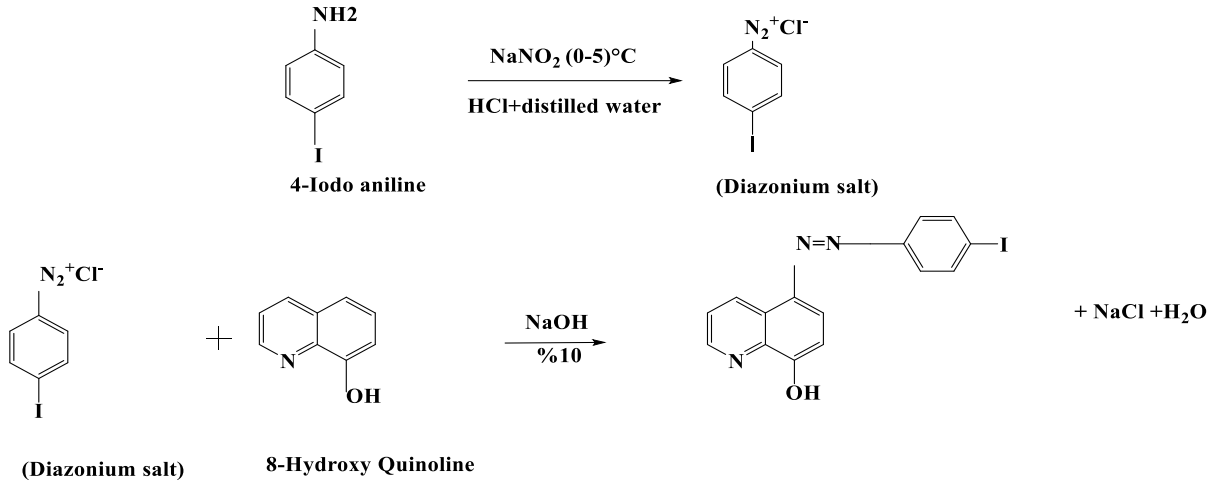
Chemicals and instrumentation

All chemicals were purchased commercially from Merck, Fluka, and Sigma-Aldrich of the highest purity and used without further purification. Shimadzu (UV-1700) spectrophotometer was used to record the UV-Vis spectra. Oakton 2100 Series pH meter was used to carry out the pH measurements; Shimadzu AA-6800 atomic absorption spectrometer equipped with graphite furnace atomizer was used for copper determination in different pharmaceutical formulations.

Preparation of the azo ligand (E)-2-((4-iodophenyl)diazenyl)-8-hydroxyquinoline (IPDHQ)

The iodoaniline diazonium solution was prepared, as mentioned before[40] by dissolving (2.19 g, 0.01 mol) of 4-iodoaniline in a diluted HCl solution (3 mL of concentrated HCl, 20 mL of distilled water), and letting the solution cool to (0–5) °C. The final solution is diazotized at (0–5) °C with aqueous sodium nitrite solution (0.70 g, 0.01 mol). To form the azo ligand, the formed diazonium solution was added with continuous stirring to (1.45 g, 0.01 mol) of 8-hydroxy quinoline

dissolved in a basic alcoholic solution (150 mL of methanol and 5 mL of 10% sodium hydroxide) at (0–5) °C, as shown in Scheme 1. The resulting solution was left for 24 hours, to precipitate the azo crystals; its pH value was adjusted to reach 7 by adding drops of 0.1 N HCl. The formed crystals were then filtered, washed with distilled water, re-crystallized by ethanol, and dried in the air.



Scheme 1 – The IPDHQ ligand preparation steps.

Preparation of the standard and work solutions

The 1000 μg mL^{-1} stock solution of azo ligand IPDHQ was prepared in a 100 mL volumetric flask by dissolving 0.1 g of IPDHQ in 96% ethanol alcohol, then diluting with the same solvent to the mark. To prepare the ligand working solutions, additional dilution procedures were implemented with ethanol. The stock solution of 100 μg mL^{-1} Cu(II) was prepared in a 100 mL volumetric flask by dissolving 0.01 g of $CuCl_2$ in the distilled water and then completing the volume to the mark with the same solvent. To obtain the Cu(II) working solutions, additional dilution procedures were implemented with the same solvent.

The 100 μg mL^{-1} stock solution of different cations and anions was prepared in a 100 mL volumetric flask. The cations stock solutions were prepared by dissolving the appropriate amount of chloride salt of the cations under study with distilled water, then completing the volume to the mark with the same solvent. The anions stock solutions were prepared by dissolving the appropriate amount of the sodium salt of the anions under study with distilled water, then completing the volume to the mark with the same solvent. To obtain the working solutions of different interferences, additional dilution procedures were implemented with the same solvent. To prepare 100 μg mL^{-1} pharmaceuticals stock solution, 10 tablets (caplets, or capsules) of the specified pharmaceuticals were ground and mixed, and an accurate weight from each pharmaceutical containing 0.01 g of Cu(II) was dissolved well in a sufficient amount of distilled water. In a 100 mL volumetric flask, the solution was filtered utilizing Whatman No. 1 filter paper and completed to 100 mL with distilled water.

General determination procedure

Into a series of volumetric flasks (10 mL), different volumes of Cu(II) solution having (5–200 μg) were pipetted, then 0.5 mL of 100 μg mL^{-1} azo solution was added to each one, then completing their volume by distilled water to the mark. The final solution absorbance was measured against the absorbance value of the blank solution at 454 nm. To construct the calibration curve, the measured absorbance values were drawn against the Cu(II) complex concentration values.

RESULTS AND DISCUSSION

UV–Vis spectra of the synthesized azo reagent and the Cu(II) complex

The UV–Vis spectra of the azo ethanolic solution and its Cu(II) complex were recorded at the

wavelength range of 200-700 nm. As shown in Fig. 1, the azo ligand has a maximum absorption peak at 371 nm. Whereas, the Cu(II) complex has a maximum absorption peak at 454 nm. This observed difference in the maximum absorption value indicates a bathochromic shift in the azo ligand absorption spectrum and confirm the formation of a stable Cu(II) complex.

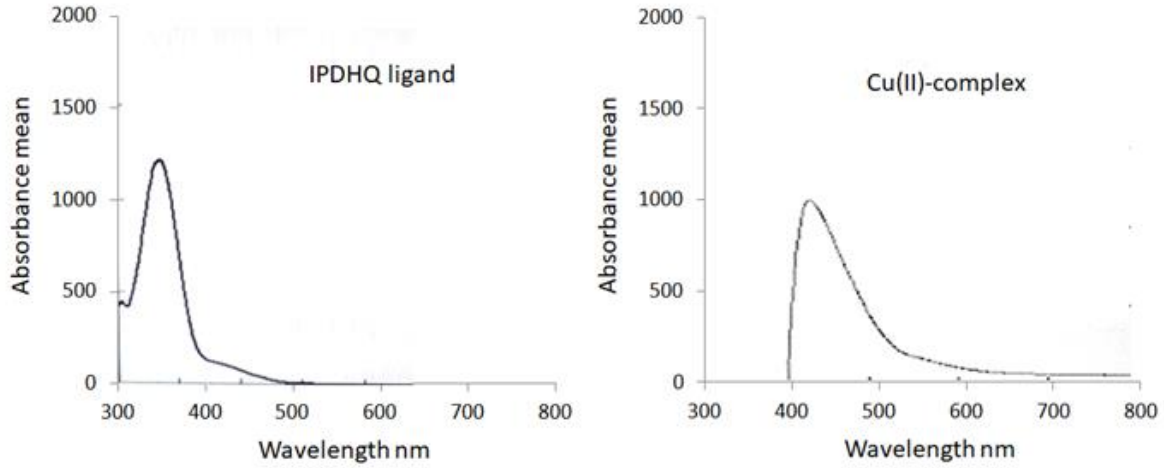


Fig. 1 – UV-Vis spectra for 100 μg mL^{-1} IPDHQ (left), and its complex with 30 μg mL^{-1} Cu(II) (right).

Optimization of analytical variables

Effect of the Cu(II) solution pH value

The effect of the pH value of the Cu(II) aqueous solution on the formation and stability of the Cu(II) complex was tested in the range of 3–9. Using 2 mL of 8 μg mL^{-1} Cu(II) solution and 1 mL of 50 μg mL^{-1} azo ligand solution, the absorbance value of the formed complex was measured at 454 nm at graduated pH values against the blank solution absorbance. The results indicated that the formed complex absorbance increases with the increase of the pH value to 6.7 and then decreases at higher pH values, as shown in Fig. 2a.

Effect of the IPDHQ ligand solution volume

The effect of the azo solution volume on the formation and stability of the Cu(II) complex was tested in the range of 0.2–2.0 mL. Using 2 mL of 8 μg mL^{-1} Cu(II) aqueous solution and different volumes of 50 μg mL^{-1} azo ligand solution, the absorbance value of the formed complex was measured against the blank solution absorbance at 454 nm. The results indicated that the formed complex absorbance reached its maximum value when adding 0.5 mL of the azo solution and then decreased, as shown in Fig. 2b.

Effect of the IPDHQ ligand concentration

The effect of the azo solution concentration on the formation and stability of the Cu(II) complex was tested in the range of 25–200 μg mL^{-1}. Using 2 mL of 8 μg mL^{-1} Cu(II) aqueous solution and 0.5 mL of azo ligand solution with graduated concentrations, the absorbance value of the formed complex was measured against the blank solution absorbance at 454 nm. The results revealed that the formed complex absorbance reached its maximum value when using 100 μg mL^{-1} of the azo solution, as shown in Fig. 2c.

Effect of the Cu(II) solution volume

The effect of the Cu(II) solution volume on the formation and stability of the Cu(II) complex was investigated in the range of 0.5–3.0 mL. Using 0.5 mL of 100 μg mL^{-1} azo ligand solution and 8 μg mL^{-1} Cu(II) solution, the absorbance value was measured against the blank solution at 454 nm. The formed complex absorbance reached its maximum value when the used volume of the Cu(II) solution was 2 mL, as shown in Fig. 2d.

Effect of the time and temperature

Under the optimal conditions obtained in the previous experiments, the effect of the complexation reaction time and temperature on the Cu(II) complex formation and stability was studied in the range of 1–50 min. and 20–60 °C, respectively. The results of the study showed that the formed complex absorbance increased with the formation time reaching to 2 minute to stay at the same value for at least 20 hours, this stability was noticed to be remained almost constant in the temperature range of 20–40 °C.

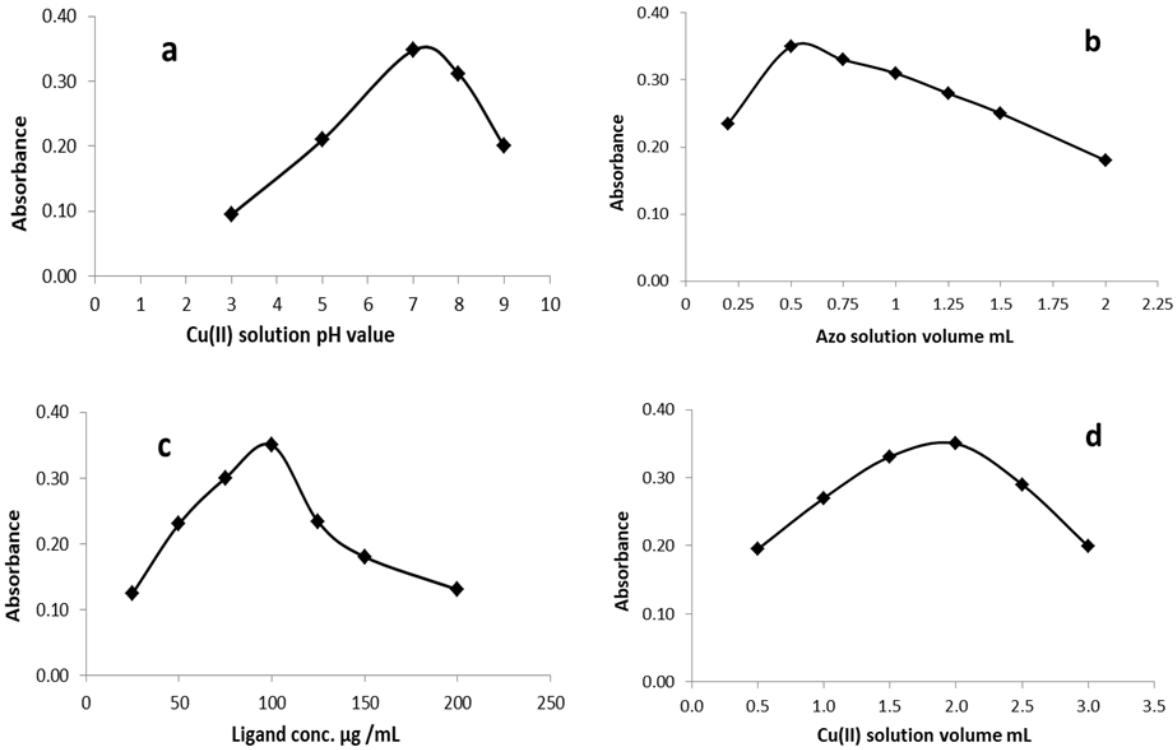


Fig. 2 – Effect of different analytical variables on Cu(II) determination.

Evaluation of the proposed method

As mentioned as optimal conditions obtained in the previous experiments, the calibration curve was constructed by preparing a series of increasing concentrations of the Cu(II) aqueous solution; each has five replicates with a constant concentration of the IPDHQ ligand. The Cu(II) complex absorbance values were measured against the blank absorbance at 560 nm. By constructing the calibration curve between the absorbance and concentration values, the obtained results indicated that the calibration curve obeys Lambert Beer's law in the range of (0.5–20) µg mL^{-1}.

At the optimum conditions, the detection and quantitation limits were calculated to estimate the suggested method's sensitivity. The proposed method's precision was evaluated by the intraday precisions by computing the relative standard deviation value, RSD%, for 10 replicate samples each one containing 10 µg mL^{-1} of Cu(II), the samples were analysed by employing the suggested method in a single day. RSD% value was calculated and found to be equal to 0.227%, indicating that the proposed method is with high precision. The analytical data of the proposed method are listed in Table 1.

Table 1

The analytical data of the suggested method

Parameter	Analytical data
λmax (nm)	454
Regression equation	0.031 x + 0.0229
Specific absorption coefficient (L g^{-1} cm^{-1})	31.0
Molar absorption coefficient (L mol^{-1} cm^{-1})	2.027×10^{3}
Sandell's sensitivity (µg cm^{-2})	3.226×10^{-5}
Correlation coefficient	0.9991
Detection limit (µg mL^{-1})	0.244
Quantitation limit (µg mL^{-1})	0.812
Linear range (µg mL^{-1})	0.5–20
Standard deviation (for n = 10)	0.001
Relative standard deviation% (for n = 10)	0.227
Recovery range %	98.20–102.20

The Cu(II) Complex Composition

The stoichiometric ratio of the prepared Cu(II) complex was found by employing the molar ratio method, which was carried out using a constant volume of the Cu(II) and different volumes of IPDHQ ligand both at the same concentration. As revealed in Fig. 3, The obtained results indicated that the Cu(II) complex has a mole ratio of 1:2 (Metal:Ligand) with the general formula $[Cu(L)_2Cl_2)].H_2O$. Depending on these results and referring to the properties of the imidazole compounds, the complex hybridization is sp^3d^2 with the shape of octahedral, as shown in Scheme 2.

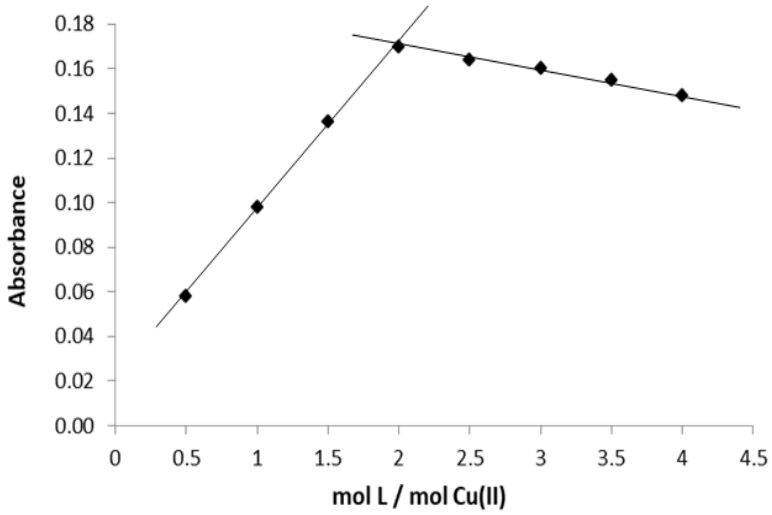
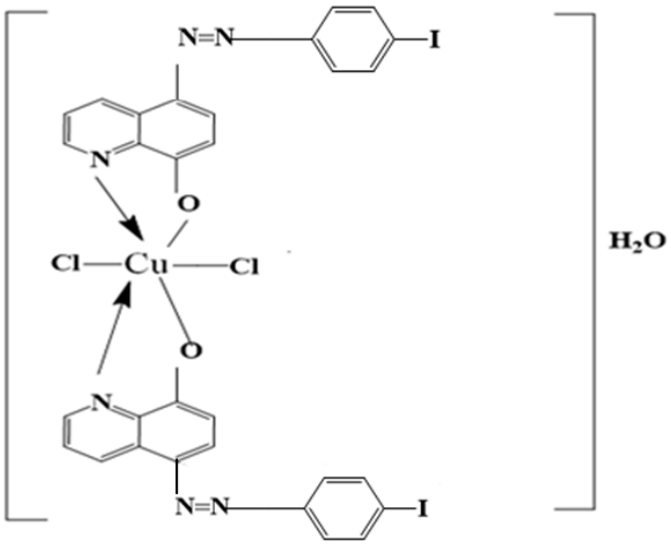


Fig. 3 – Mole ratio method for $[Cu(L)_2Cl_2)].H_2O$ complex.



Scheme 2 – The suggested structure of $[Cu(L)_2Cl_2)].H_2O$ complex.

Effect of the interferences study

The proposed method specificity was evaluated by studying the effect of various ions that combine copper in its pharmaceutical formulations. By following the general procedure for determining 10 µg mL^{-1} Cu(II) in the existence of some metal ions such as Cr(II), Zn(II), Mg(II), Ca(II), and Fe(II) in 1:1 and 1:5 (ion:interference) proportions. The studied cations were found to be affecting the absorbance value due to their competition with the Cu(II) to form a complex with the IPDHQ ligand. To eliminate this effect, masking agents such as NH_3, NaCl, EDTA, $Na_3C_6H_5O_7$, and CH_3COONa in different concentrations were studied. The study results revealed that 100 µg mL^{-1} NaCl is a useful masking agent for Hg(II), while 100 µg mL^{-1}

$Na_3C_6H_5O_7$ was utilized as a masking agent for the other cations, as shown in Table 2. Anions such as, Cl^-, PO_4^{3-}, tartrate ion **(**$C_4H_4O_6^{2-}$), CO_3^{2-}, I^-, gluconate ion ($C_6H_{11}O_7^-$) at the tested concentrations were found to have no interfering effect in the Cu(II) determination.

Table 2

The interference effect of several cations on Cu(II) determination

Interference ion	Interference concentration μg mL^{-1}	[a]Masking reagent volume mL	[b]Rec. %
Cr(III)	10	0.5 $KNaC_4H_4O_6{\cdot}4H_2O$	101.53
	50	1.0 $KNaC_4H_4O_6{\cdot}4H_2O$	99.40
Zn(II)	10	0.2 NaOH	97.70
	50	0.5 NaOH	98.83
Mg(II)	10	1.0 $KNaC_4H_4O_6{\cdot}4H_2O$	97.55
	50	2.0 $KNaC_4H_4O_6{\cdot}4H_2O$	96.57
Ca(II)	10	1.0 $KNaC_4H_4O_6{\cdot}4H_2O$	98.73
	50	2.0 $KNaC_4H_4O_6{\cdot}4H_2O$	102.19
Fe(III)	10	1.0 $KNaC_4H_4O_6{\cdot}4H_2O$	96.97
	50	2.5 $KNaC_4H_4O_6{\cdot}4H_2O$	100.59

[a] the concentration of the masking agent is 100 μg mL^{-1}, [b] Percent recovery for (n = 5)

Applications

The IPDHQ azo ligand was utilized as an analytical reagent for a spectroscopic determination of copper ions in different pharmaceutical formulations. The obtained results were compared with the results of the flame atomic absorption spectrometric method as a reference method to confirm the applicability and effectiveness of the submitted method, and they were in good agreement with them. In addition, the submitted method accuracy was evaluated by calculating the percentage recoveries for five replicates of the tested pharmaceutical samples, and they were found to be in the range of 98.20–102.20%, as shown in Table 3, indicating that the submitted method could be used in copper determination in different pharmaceutical formulations with high precision, accuracy, and sensitivity.

Table 3

Cu(II) determination in various pharmaceutical samples

Sample	Taken value	by the submitted method			by FAAS method		
		found value	[a]E %	Rec. %	found value	[a]E %	Rec. %
Feroglobin B12 (capsules 1 mg / capsule)	10	9.95	–0.50	99.50	9.93	–0.70	99.30
Chlated Copper (vegetarian tablets 2 mg / tablet)	10	9.98	–0.20	99.80	9.88	–1.20	98.80
Skin, Nails & Hair (tablets 2 mg / tablet)	10	9.82	–1.80	98.20	9.7	–3.00	97.00
Ultra vita man (coated caplets / 2 mg / cablet)	10	10.15	1.50	101.50	10.05	0.50	100.50
Centrum (tablets 0.9 mg / tablet)	10	10.22	2.20	102.20	10.08	0.80	100.80
A-Z VITAL (caplets 0.5 mg / caplet)	10	9.85	–1.50	98.50	9.82	–1.80	98.20

[a] Percent error for (n=5)

CONCLUSION

The presented study described the capability to utilize the synthesized azo ligand, (E)-2-((4-iodophenyl)diazenyl)-8-hydroxyquinoline, as an analytical agent for sensitive, inexpensive, and efficient spectrophotometric determination of Cu(II) in pharmaceutical formulations. An orange complex was formed depending on the chelation reaction that occurs between Cu(II) and the

bidentate quinoline azo ligand. The composition of the Cu(II) complex was investigated applying the mole ratio method, the results indicated that the molar ratio of the complex is 1:2 (metal:ligand) and the chemical formula is $[Cu(L)_2Cl_2)].H_2O$ with octahedral geometrical shape. The interference effect of some cations and anions that may be present in the tested samples was checked, to eliminate the interfering effect if it occurred, the appropriate masking agents in the specific concentrations and volumes were used. The suggested method was further applied for the determination of Cu(II) in different pharmaceutical formulations with satisfactory recovery results.

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