Original article

Simultaneous spectrophotometric determination of iron (II) and copper (II) in tablets by chemometric methods

Melisew Tadele Alula*, Abdel-Maaboud I. Mohamed and Adnan A. Bekhit

Department of Pharmaceutical Chemistry, School of Pharmacy, Addis Ababa University, Addis Ababa, Ethiopia.

**Corresponding author: P.O.BOX 970, Bahir Dar, Ethiopia*

Tel: +251-918-769973, +251-913-965178; E-mail address: melisewt2004@yahoo.com

Abstract:

The significant spectral overlap $(D_i)^{0.5}$ = 0.5667 which is about 75.3% overlapping of the UV/Visible absorption spectra of iron (II) and copper (II) complexes necessitates chemometric assisted methods for simultaneous analysis of these ions in the pharmaceutical mixture. These metal ions were analyzed simultaneously by UV/Visible spectrophotometric method where 8-hydroxyquinoline was used as a chromogenic reagent. The absorption spectra were recorded in the wavelength range of 350-600 nm digitized at 1 nm. Four chemometrics methods such as first derivative spectrophotometry (^{1}D zero crossing), first derivative ratio (^{1}D ratio), classical least squares (CLS) and principal component regression (PCR) were used to predict the concentrations of the metal ions. For the derivative spectrophotometry, measurements were made at two selected wavelengths. The calibration curves were linear in the range of 2-12 μg/ml for both ions. For classical least squares and principal component regression, the calibration model was obtained and predictions of the unknown concentrations of the ions in the synthetic mixtures and in the dosage form were done. The accuracy and the precision of these methods were determined by calculating the % recovery and the relative standard errors correspondingly. The methods were successfully applied for analyzing synthetic mixtures and commercial pharmaceutical preparation.

Keywords: Classical least squares; Copper; Derivative ratio spectrum; First derivative spectrophotometry; 8-Hydroxyquinoline; Iron; Principal component regression.

Introduction

Trace elements available in human body are necessary for almost every physiological process. They combine with vitamins, form enzymes, interact with deoxyribonucleic acid (DNA) [1] and assist the antioxidative system within the body [2]. A combination of iron and copper is available in different pharmaceutical products. One reason is that, there is a close relationship between the biology of iron and copper; for example, copper deficiency alters body iron metabolism through effects on the ferrioxidase activity of caeruloplasmin, which is essential for iron release from tissues [3]. Iron plays a central role in the biosphere, serving as the active center of proteins responsible for O_2 and electron transfer and of metalloenzymes such as oxidases, reductases and dehydrases [4]. Copper (II) is essential for a variety of biochemical processes in the body, involves in the functioning of the nervous system, maintains the balance of other useful metals in the body and involves in antioxidant functions [5, 6]. The deficiency of both iron and copper results in anemia [7].

The determination of metal ions at trace levels is one component in the field of pharmaceutical analysis. However, their analysis in pharmaceutical preparation is challenging due to complex composition and a wide range of concentrations, which may vary from ppb to percent levels. Although sensitive and selective analytical methods for the determination of metal ions are available, they are too expensive to be used in routine analysis. 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) [8-11] and copper (II) [12-15] to produce colored complex with a higher molar absorptivity. However, the presence of metal ions creates challenges for their quantitative determination, because the absorption spectras overlap and the superimposed curves are not suitable for quantitative evaluation and selectivity is difficult. However, chemometrics assisted UV/Visible spectrophotometric techniques using non-selective or mixed chromogenic reagents can avoid this problem [16,17].

Different chemometric methods have been widely used to determine metal ions in different pharmaceuticals simultaneously without any chemical pre-treatment and during a short period of time [16-20]. Derivative spectrophotometry is an analytical technique used to obtain both qualitative and quantitative information from spectra composed of unresolved bands [21,22]. A number of studies were reported showing the advantage of higher selectivity of derivative spectrophotometry than normal (zero-order) spectrophotometry [23,24]. Derivative spectrophotometry on the basis of zero-crossing measurements involves measurement of the absolute value of the total derivative spectrum at x-axis corresponding to the zero-crossing wavelength of the derivative spectra of individual components which should be only a function of the concentration of other components [21,22]. The derivative ratio technique is based on the use of the first derivative of the ratios of spectra. The absorption spectrum of the mixture is obtained and the amplitudes at appropriate wavelengths are divided by the corresponding amplitudes in the absorption spectrum of a standard solution of one of the components. The first derivative of the ratio spectrum is obtained. The concentration of the other component is then determined from calibration graph [25].

In pharmaceutical application, derivative spectrophotometry has led to significant developments in the analysis of drugs in the presence of their degradation products or in multi-component mixtures [26-31]. Its application to the determination of metal ions in different pharmaceuticals has been reported. For example, first, second and third derivative spectrophotometry methods [32-35] have been used for the determination of two or three metal ions in pharmaceutical formulation using either single or mixed chromogenic reagents.

Multivariate calibration methods have been used for the quantitative analysis of spectral data. They have been shown to improve analysis precision, accuracy, reliability and applicability for spectral analysis relative to the more conventional univariate methods of data analysis [36,37]. The application of quantitative chemometric methods, to multivariate method, needs a calibration step where the relationship between the spectra and the component concentration is deduced

from a set of reference samples, followed by prediction step in which the results of the calibration are used to determine the component concentration from the sample spectrum. These methods have been applied both to zero-order and derivative spectra, depending on the spectral properties of the components to be analyzed and on the type of interferences occurring in the sample [38]. Multivariate calibration methods applied to spectral data are being increasingly used for pharmaceutical analysis [26-29].

Classical least squares (CLS) is the simplest multivariate method that can be performed with easily accessible statistical software such as Excel [26,27] and assumes that responses (absorbance) at each frequency (wavelengths) are proportional to component concentration units. Calibration is realized by recording the spectra at **n** wavelengths of **m** standard mixtures, of known composition of **c** components. The spectra (absorbance) are arranged into the columns of matrix Y (dimensions n × m), with the composition of each mixture forming the columns of concentration matrix X (c × m)

$$Y = K \cdot X \quad (1)$$

With a prior knowledge of X and by recording data for Y, then the matrix of sensitivities, K, can be calculated, but after the rearrangement of equation (1) to the following equation by multiplying the equation components by X^t value as $Y \cdot X^t = K \cdot X \cdot X^t \cdot$ Then;

$$K = (X \cdot X^t)^{-1} \cdot Y \cdot X^t \quad (2)$$

To avoid being under-determined, there must be measurements at more wavelengths than there are components (i.e. n ≥ c). If n > c then the component concentrations in an unknown mixture are obtained from its spectrum by,

$$X_{unknown} = (K^t \cdot K)^{-1} \cdot K^t \cdot Y_{unknown} \quad (3)$$

Principal component regression (PCR) is a factor analysis multivariate statistical tool that involves spectral decomposition. The decomposition is based entirely on spectral variations regardless for the component concentrations and the decomposition is significantly influenced by variations which have no relevance to the analyte concentrations.

The original data obtained in absorbance *A* and concentrations *C* of mixtures would be transformed by mean-centered and scaled (optional) into A_0 and C_0, respectively. This model building procedure has two fundamental concept. Firstly, the computation of eigenvalues and their eigenvectors correspond to the covariance square matrix of the A_0. Secondly, by using cross-validation in the calibration step, the optimal principal components (or the eigenvectors) corresponding to the large eigenvalues are selected.

Materials and Methods

Instruments

Double beam UV/Visible spectrophotometer (Shimadzu 2401 PC) with 1 cm quartz cuvets connected to a computer with UVPC software was used for the spectrophotometric measurements. Data analysis was performed in Pentium IV computer using Excel 2003 (Derivative spectrophotometry and CLS) and VISTA 6 version 6.4.3436-EWU (2001) software (PCR). The pH of the solutions was measured with a model Mettler Toledo 320-S. Glass wares and analytical balance (Mettler Toledo, Tokyo. Japan) were used throughout the laboratory work.

Chemicals

All chemicals used were of analytical grade. Ferrous sulphate (Sigma Aldrich, Germany), cupric sulphates pentahydrate (BDH Laboratory supplied, England) were used as working standards. 8-Hydroxyquinoline (BDH Laboratory supplied, England) was used as a chromogenic reagent. Acetate buffer pH 4.0 was prepared by mixing 15 ml of 0.1 M CH_3COONa solution and 85 ml of 0.1 M CH_3COOH solution. One percent (w/v) ethanolic solution of 8-hydroxyquinoline was prepared by dissolving a gram of it in 100-ml volumetric flask.

Formulation

Maxamin forte® tablets labeled to contain 10 mg

of iron (II) and 1 mg of copper (II) were purchased from the Anglo-French Drugs and Industries, India, and subjected to analysis by the proposed methods.

Solvents

Hydrochloric acid (BDH Laboratory supplied, England), absolute ethanol (Joseph Mills (Denaturants) LTD, Liverpool) and demineralized water were used as solvents through the procedures.

Preparation of stock and working standard solutions

Stock standard solutions of iron (II) (1,000 µg/ml) and copper (II) (1,000 µg/ml) were prepared from their sulphates salts and the salts were dissolved in 0.01 M HCl. Suitable aliquots from the stock solutions were quantitatively used to obtain six serial solutions according to the calibration range.

Sample preparation of the studied drug

Ten tablets were weighed accurately and finely powdered in a mortar. An amount of the powder equivalent to one tablet was transferred quantitatively to 100-ml volumetric flask and then 60 ml of 0.01 M HCl was added. The mixture was shaken well and sonicated for about 15 min. Then the mixture was diluted by 0.01 M HCl solution to the mark and then filtered by Whatman filter paper No. 40. The first portion of filtrate was discarded. The clear solution obtained was used as a stock sample solution and different aliquots of prepared solution were diluted with 0.01 M HCl to produce different concentrations.

General procedures for spectrophotometric determination

Procedure for determination of linearity range of standard solutions

In order to obtain the calibration curve, six solutions of each of the pure components containing appropriate aliquot of studied ions (2-12 µg/ml) were added into 25-ml volumetric flasks and then 1 ml of buffer solution and 1 ml of 8-hydroxyquinoline were added. To these solutions, absolute ethanol was added to the mark giving concentrations range of 2-12 µg/ml. Absorption spectra were recorded between 350-600 nm against a blank solution; the spectra were digitized each 1.0 nm. These ranges were previously verified to obey Beer's law.

Procedure for preparation of laboratory prepared mixture solutions

Laboratory prepared mixtures were prepared by mixing known amounts of working solution of iron (II) with known amount of copper (II) in different proportions in 25-ml volumetric flasks and then 1 ml of buffer solution and 1 ml of 8-hydroxyquinoline were added. Then absolute ethanol was added to the mark giving concentrations of each metal ion in the range of 2-12 µg/ml. Absorption spectra were recorded between 350 and 600 nm against a blank solution.

Procedure for preparation of dosage form solutions

Ten tablets were weighed and powdered finely. An amount of the powder equivalent to one tablet was transferred quantitatively to 100-ml volumetric flask and then 0.01 M HCl was added. The mixture was shaken well and sonicated for about 15 min. Then the mixture was diluted by 0.01 M HCl solution to the mark and then filtered by Whatman filter paper No. 40 and the clear solution obtained was used as a stock sample solution. The stock solution was used for one day. From the stock sample solution, 2.5 ml was taken and added to 25-ml volumetric flask followed by addition of 1 ml of buffer solution and 1 ml of the chromogenic reagent. Then absolute ethanol was added to the mark. Absorption spectra were recorded between 350-600 nm against a blank solution; the spectra were digitized each 1.0 nm.

Results and Discussion

The absorption spectra in ethanolic solution of the individual metal complexes with 8-hydroxyquinoline at pH 4 is shown in Figure 1. It is shown that, the absorption spectra have got a considerable degree of overlapping (wavelength ranges of 350-520 nm) obtained from the $(D_i)^{0.5}$ value [36], 0.5667 that implies there is a 75.3% spectral overlap. Where D_i is the magnitude of the dependency which can be calculated for a two component mixture from the equation:

$$D_i = \frac{\sum (k_1 k_2^t)^2}{\sum k_1 k_1^t \sum k_2 k_2^t} \quad (4)$$

Where k_1 and k_2 are the $l \times n$ matrices of regression coefficients for the studied metal ions complexes and k^t is the transposed k matrix.

The optimum working conditions were studied under the conditions previously established for each metal ion. The effect of 8-hydroxyquinoline concentration was also investigated and acetate buffer solution of pH 4 was selected. The interference of other metal ions such as manganese (II) and zinc (II) were also investigated and it was found that they did not interfere within the specified wavelengths.

Derivative spectrophotometric techniques

Zero-crossing technique

In this work, first derivative spectrophotometry using zero crossing technique has been used for the simultaneous determination of two metal ions, iron (II) and copper (II) in pharmaceutical preparation and the results obtained were accurate and reproducible and no further derivatization (second-derivative) was required.

Derivative spectrophotometry on the basis of zero-crossing measurements involves measurement of the absolute value of the total derivative spectrum at x-axis corresponding to the zero-crossing wavelength of the derivative spectra of individual components which should be only a function of the concentration of other components [22,39].

The derivative spectra of the two metal ions showed good identified zero-crossing points that it can be used for the simultaneous determination of them. The wavelengths of 388 nm and 487 nm were selected for the determination of iron (II) in the presence of copper (II) and wavelengths of 367 nm and 414 nm for the determination of copper (II) in the presence of iron (II) (Figure 2). The spectrophotometric parameters including derivative order, wavelength and $\Delta\lambda$ values should be optimized to obtain maximum resolution, sensitivity and reproducibility [39]. In this study, first-derivative technique, ^{1}D traced with $\Delta\lambda$ = 2 (n=6) was used to resolve the spectral overlapping. The selected wavelengths gave best linear responses and approaching zero intercept values on the coordinate of the calibration graph. The analytical parameters for determination of iron (II) and copper (II) with the proposed derivative spectro-photometric techniques are given in Table 1. The limits of detection (LOD) and quantification (LOQ) were calculated by using the following equations.

$$LOD = 3\sigma/S \quad (5)$$

$$LOQ = 10\sigma/S \quad (6)$$

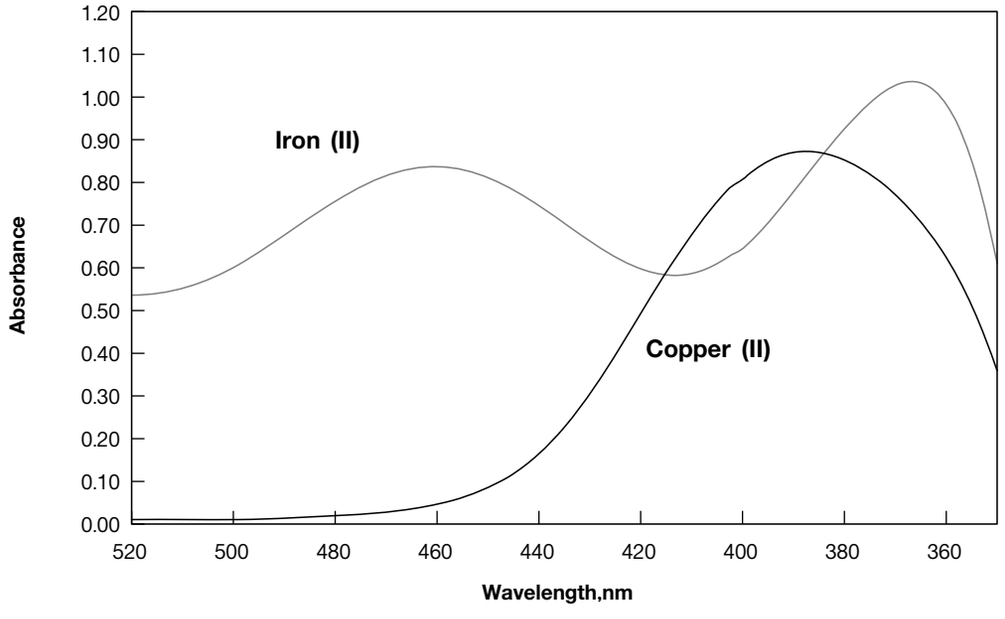

Figure 1 Degree of overlapping for copper (II); 8 μg/ml, and iron (II); 8 μg/ml spectra in 0.01 M HCl solution in the range from 350-520 nm.

Table 1 Analytical parameters for determination of iron (II) and copper (II) with the proposed derivative spectrophotometric techniques

Techniques	Conc. (μg/ml)	Wavelength (nm)	Intercept (a)	Slope (b)	Correlation coefficient (r)	Determination coefficient (r^2)	Limit of detection (LOD, μg/ml)	Limit of quantification (LOQ, μg/ml)
^{1}D zero crossing, Fe (II)	2 - 12	388.0	-0.00047 ± 0.00040	-0.00176 ± 0.00005	0.9983	0.9966	0.684	2.279
	2 - 12	487.0	-0.00051 ± 0.00034	-0.00092 ± 0.00044	0.9955	0.9911	1.113	3.708
^{1}D ratio method, Fe (II)	2 - 12	492.0	0.01195 ± 0.12977	0.42876 ± 0.01666	0.9970	0.9940	0.908	3.027
	2 - 12	503.0	-0.04498 ± 0.10298	-0.37349 ± 0.01322	0.9975	0.9950	0.827	2.757
^{1}D zero crossing, Cu (II)	2 - 12	367.0	0.00032 ± 0.00030	0.00162 ± 0.00004	0.9988	0.9977	0.428	1.427
	2 - 12	414.0	-0.00034 ± 0.00030	-0.00214 ± 0.00004	0.9993	0.9987	0.562	1.874
^{1}D ratio method, Cu (II)	2 - 12	383.0	0.00028 ± 0.00073	0.00344 ± 0.00010	0.9985	0.9971	0.636	2.119
	2 - 12	419.0	-0.00076 ± 0.00179	-0.00645 ± 0.00023	0.9975	0.9949	0.835	2.783

Where σ is the standard deviation of the intercept and S is the sensitivity expressed by the slopes of the calibration curves for each metal ion.

High values of the correlation coefficients of the regression equations confirm the linearity of the calibration graphs and the agreement of the methods to Beer's law. The calibration curves for both metal ions were constructed by plotting ^1D values, at zero crossing values obtained from the first derivatives versus concentration in the range of 2-12 μg/ml.

To check the reproducibility of the method, replicate samples containing iron (II) and copper (II) individually, six laboratory prepared binary mixtures of iron (II) and copper (II) of different ratios were analyzed by the proposed method and the results are given in Table 2. In all cases, recovery values, which actually predicted the accuracy of the proposed method, were in between 98.13 and 102.44% for iron (II) and between 98.23 and 102.08% for copper (II). These satisfactory results demonstrated that the method is effective for the simultaneous determination of them. Plus to this, the good results from the laboratory prepared mixtures confirmed the suitability of the proposed method for the determination of the metal ions mentioned in pharmaceutical preparations and checked the validity of the proposed method.

Derivative ratio technique

The influence of Δλ for obtaining the first derivative of the ratio spectra as well as the effect of divisor concentration on the calibration graphs for the proposed mixture were studied because they affect the quantitative determination of the components [26]. Results indicated that Δλ = 2 nm was considered the most suitable one, while the divisor concentration had no significant effect on the assay results for the studied mixtures and 6 μg/ml was taken in both cases. For the determination of iron (II), the absorption spectra of standard solutions of iron (II) were divided by absorption spectrum of standard solution of 6 μg/ml copper (II) to obtain the corresponding ratio spectra of iron (II).

The first derivative of the obtained ratio spectra for iron (II) was calculated with Δλ = 2 nm (Figure 3). Thus, iron (II) can be determined in the mixture by measuring the amplitude at 492 nm and 503 nm where there is no contribution from copper (II). Similarly, copper (II) was determined; the absorption spectra of copper (II) was divided by that of a standard solution of 6 μg/ml iron (II) resulting ratio spectra and the first derivative of the developed ratio spectra was calculated with Δλ = 2 nm (Figure 4). Thus, determination of copper (II) was made by measuring the amplitude, where iron (II) had no contribution, i.e., 383 nm and 419 nm.

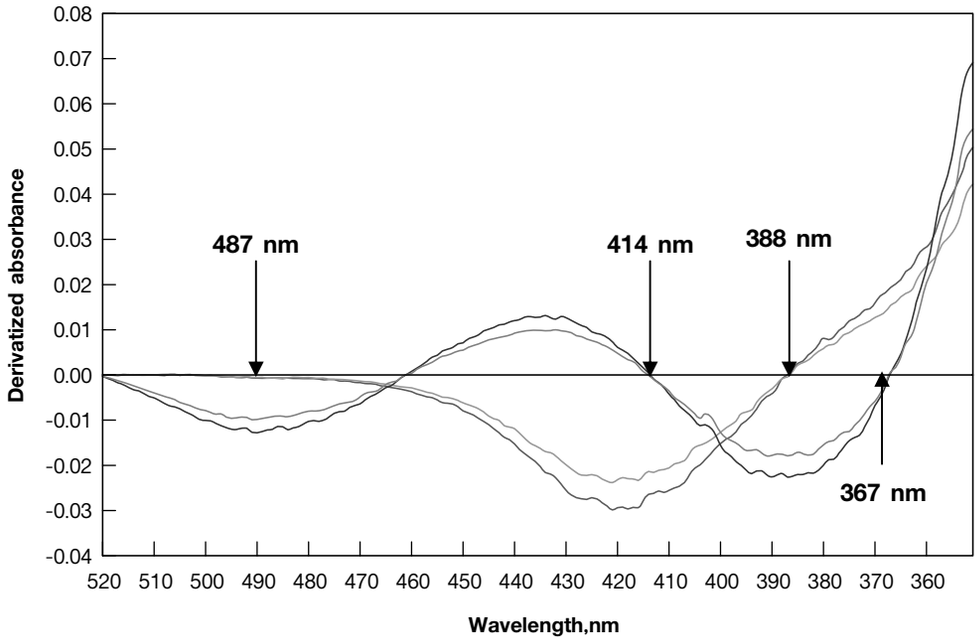

Figure 2 First derivative of copper (II) and iron (II) in 0.01 M HCl solutions; the arrows indicating the measurement wavelengths.

Table 2 Actual and predicted amounts of iron (II) and copper (II) obtained by applying first derivative zero-crossing technique for pure, laboratory-prepared mixtures and commercial dosage form

Real concentrations (μg/ml)		At 388 nm, Found			At 487 nm, Found			Real concentrations (μg/ml)		At 367 nm, Found			At 414 nm, Found		
		μg/ml	Recovery (%)	CV	μg/ml	Recovery (%)	CV			μg/ml	Recovery (%)	CV	μg/ml	Recovery (%)	CV
Pure Fe (II)	2	2.011	100.54	1.67	2.013	100.65	1.32	Pure Cu(II)	2	2.029	101.45	1.54	2.016	100.80	0.87
	4	4.097	102.44	1.22	3.978	99.44	1.76		4	4.048	101.20	2.05	4.016	100.41	0.99
	6	5.969	99.48	1.78	5.899	98.33	1.98		6	5.942	99.03	1.87	5.915	98.58	2.01
	8	8.111	101.39	2.01	7.858	98.23	1.65		8	7.986	99.83	0.98	8.114	101.42	1.65
	10	9.813	98.13	1.55	9.905	99.05	0.97		10	9.945	99.45	1.22	9.921	99.21	1.76
	12	12.141	101.17	2.04	12.037	100.31	1.33		12	12.154	101.28	1.65	12.087	100.72	1.84
Laboratory	2/12	2.008	100.40	1.44	2.019	100.95	1.76	Laboratory	2/12	2.022	101.11	1.85	2.041	102.05	1.55
prepared	4/10	4.059	101.49	1.58	3.977	99.44	1.85	prepared	4/10	4.051	101.28	1.72	3.954	98.86	1.74
mixtures	6/8	6.023	100.38	2.02	6.081	101.35	1.23	mixtures	6/8	5.950	99.19	0.57	6.057	100.96	2.11
Fe(II)/Cu(II)	8/6	8.005	100.07	2.05	7.931	99.14	1.19	Cu(II)/Fe(II)	8/6	8.166	102.08	0.92	8.101	101.26	0.88
	10/4	10.077	100.77	1.77	9.956	99.56	0.96		10/4	10.048	100.48	1.76	9.937	99.37	1.77
	12/2	12.008	100.07	1.86	12.129	101.08	1.43		12/2	11.986	99.89	1.45	11.899	99.16	1.39
Dosage form	11/1	11.062	100.56	1.34	11.188	101.71	1.73	Dosage form	1/11	0.982	98.23	1.88	0.9876	98.76	2.00
Maxamin®								Maxamin®							
Fe(II)/Cu(II)								Cu(II)/Fe(II)							

CV = coefficient of variation

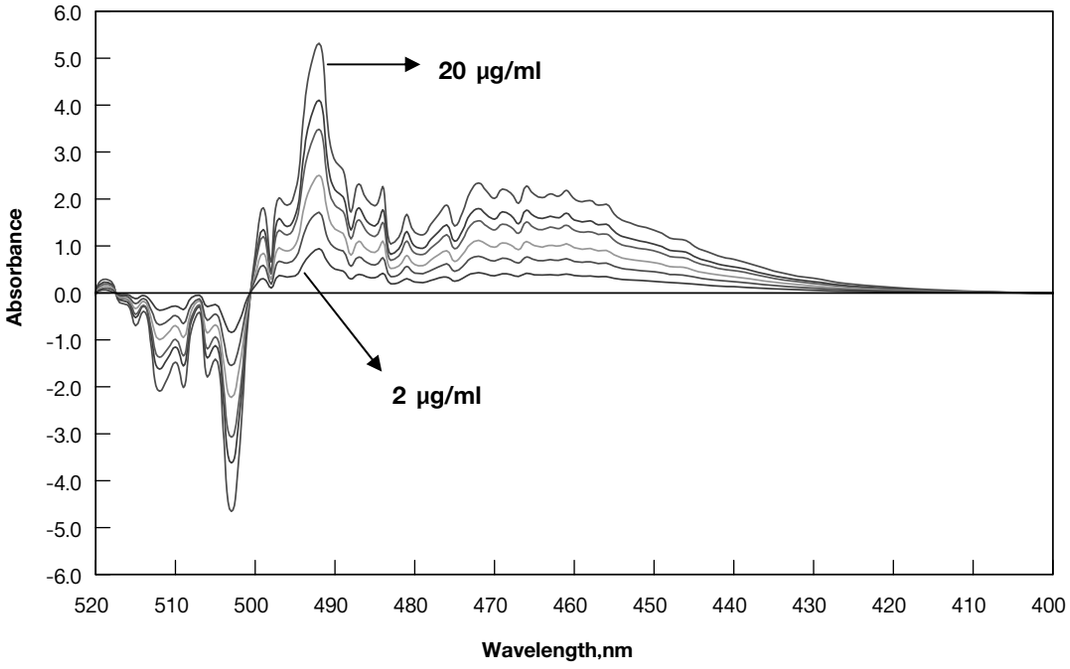

Figure 3 First derivative ratio spectra of iron (II) (2-12 µg/ml); Divisor is 6 µg/ml of copper (II)

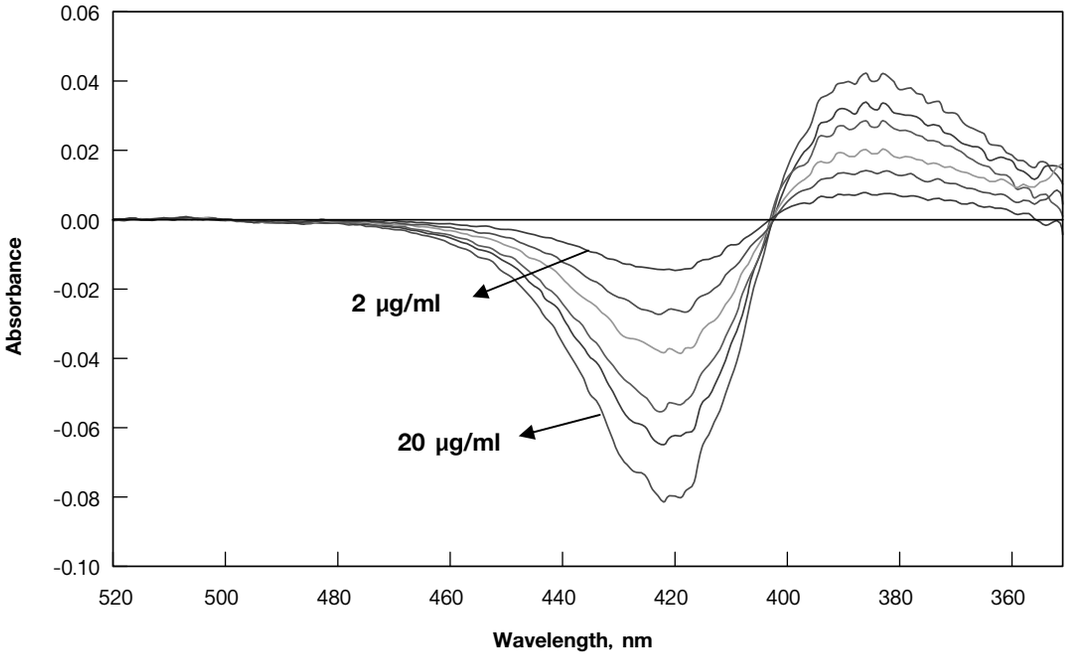

Figure 4 First derivative ratio spectra of copper (II) (2-12 µg/ml); Divisor is 6 µg/ml of iron (II)

Table 3 Actual and predicted amounts of iron (II) and copper (II) obtained by applying first derivative ratio technique for pure, laboratory-prepared mixtures and commercial dosage form

Real concentrations (μg/ml)		At 492 nm, Found			At 503 nm, Found			Real concentrations (μg/ml)		At 383 nm, Found			At 419 nm, Found		
		μg/ml	Recovery (%)	CV	μg/ml	Recovery (%)	CV			μg/ml	Recovery (%)	CV	μg/ml	Recovery (%)	CV
Pure Fe (II)	2	2.034	101.69	2.31	2.030	101.51	1.88	Pure Cu(II)	2	2.047	102.33	1.38	2.031	101.50	1.86
	4	3.982	99.56	1.76	4.014	100.36	1.34		4	3.946	98.65	1.75	3.968	99.19	2.13
	6	5.932	98.86	1.94	5.957	99.28	1.67		6	5.905	98.41	1.12	5.926	98.76	0.95
	8	8.108	101.35	0.84	8.118	101.48	1.52		8	8.142	101.77	1.98	8.161	102.01	0.74
	10	9.947	99.47	0.92	9.875	98.75	0.64		10	9.902	99.02	2.04	9.958	99.58	1.33
	12	12.134	101.12	1.22	12.100	100.83	0.91		12	12.176	101.47	1.45	12.196	101.63	1.56
Laboratory	2/12	2.047	102.34	2.22	2.041	102.03	1.84	Laboratory	2/12	2.039	101.93	1.78	1.975	98.76	1.88
prepared	4/10	4.066	101.66	1.98	4.054	101.35	1.08	prepared	4/10	4.085	102.12	1.11	3.974	99.34	2.06
mixtures	6/8	5.932	98.87	1.87	5.967	99.45	1.55	mixtures	6/8	6.092	101.54	1.78	6.046	100.76	1.45
Fe(II)/Cu(II)	8/6	8.034	100.43	0.93	7.984	99.78	1.87	Cu(II)/Fe(II)	8/6	8.027	100.34	1.54	8.134	101.67	1.22
	10/4	10.089	100.89	0.76	10.047	100.47	2.01		10/4	9.923	99.23	0.95	10.185	101.85	1.77
	12/2	12.185	101.54	1.33	12.146	101.22	0.95		12/2	12.101	100.84	0.88	12.077	100.64	1.53
Dosage form Maxamin Fe(II)/Cu(II)	11/1	11.135	101.23	1.89	11.194	101.76	2.02	Dosage form Maxamin Cu(II)/Fe(II)	1/11	0.988	98.76	1.87	0.987	98.69	2.04

CV = coefficient of variation

The actual and predicted amounts and the relative standard deviation of the concentration of the ions as given by ^{1}D ratio technique for the spectral data are shown in Table 3. The results from the analysis of laboratory prepared synthetic mixtures confirmed the suitability of the proposed method for the simultaneous determination of the studied ions in the pharmaceutical preparations. The concentrations predicted by this method are considerably close to the real ones and recoveries in all the cases were in between 98.75 and 102.34% for iron (II) and between 98.41 and 102.33% for copper (II) which actually showed the accuracy of the proposed method. Therefore, the simultaneous determination of the studied ions in presence of each other and without prior separation of the excipients and additives present in the commercial dosage forms are possible by the proposed method.

Multivariate analysis

In this study two methods namely CLS and PCR were applied. The application of multivariate method, needs a calibration step where the relationship between the spectra and the component concentration is deduced from a set of reference samples, followed by prediction step in which the results of the calibration are used to determine the component concentration from the sample spectrum.

Tables 4 and 5 show the actual and predicted amounts plus errors (%) of the studied metal ions using CLS and PCR methods, respectively. The precision of the proposed methods were evaluated by calculating the relative standard errors which were found to be less than 1.94% and 1.72% for iron (II) and copper (II) respectively. It confirmed a high degree of precision.

The accuracy of the methods were evaluated by taking the recoveries, and the results obtained confirmed a high degree of agreement and indicated the methods are suitable for analysis in the calibration range for each metal ion complex.

Six laboratory prepared binary mixtures were subjected to the CLS and PCR analysis to confirm the suitability of the calibration models in the pharmaceutical samples. The concentrations predicted by the models are very close to the real concentrations taken. The commercial dosage forms also gave very close result to the claimed amount.

Table 4 Actual and predicted amounts of pure, laboratory-prepared mixtures and commercial dosage form of iron (II) and copper (II) by using classical least squares multivariate technique

		Iron (II)					Copper (II)		
Concentrations (μg/ml)		Found (μg/ml)	Recovery (%)	RSE*	Concentrations (μg/ml)		Found (μg/ml)	Recovery (%)	RSE*
Pure	2	2.024	101.22	1.27	Pure	2	2.025	101.23	1.32
	4	4.058	101.45	1.67		4	4.031	100.78	1.45
	6	6.053	100.88	1.33		6	6.050	100.83	1.65
	8	8.046	100.58	1.08		8	8.027	100.34	1.22
	10	9.911	99.11	1.93		10	10.112	101.12	1.56
	12	12.104	100.87	1.64		12	12.199	101.66	1.11
Laboratory	2/12	2.034	101.68	1.56	Laboratory	2/12	2.029	101.45	1.66
prepared	4/10	1.053	101.33	1.94	prepared	4/10	4.030	100.74	1.57
mixtures	6/8	6.044	100.73	1.45	mixtures	6/8	6.047	100.79	1.17
Fe(II)/Cu(II)	8/6	8.091	101.13	1.76	Cu (II)/(Fe(II)	8/6	8.109	101.36	1.72
	10/4	10.072	100.72	1.49		10/4	10.011	100.11	1.34
	12/2	12.113	100.94	1.03		12/2	11.918	99.32	1.48
Dosage form	11/1	11.151	101.37	1.93	Dosage form	1/11	0.991	99.12	1.45
Maxamin®					Maxamin®				
Fe(II)/Cu(II)					Cu (II)/Fe(II)				

*RSE is the relative standard errors calculated from the original matrix data for each concentration.

Table 5 Actual and predicted amounts of pure, laboratory-prepared mixtures and commercial dosage form of iron (II) and copper (II) by using principal component regressions multivariate technique

Iron (II)					Copper (II)				
Concentrations (μg/ml)		Found (μg/ml)	Recovery (%)	RSE*	Concentrations (μg/ml)		Found (μg/ml)	Recovery (%)	RSE*
Pure	2	2.022	101.12	1.65	Pure	2	2.019	100.94	1.67
	4	4.062	101.56	1.88		4	4.047	101.17	1.34
	6	6.019	100.33	1.21		6	6.026	100.43	1.73
	8	8.074	100.92	1.44		8	8.061	100.76	1.98
	10	9.943	99.43	1.07		10	10.123	101.23	1.22
	12	12.080	100.67	1.83		12	12.112	100.93	1.46
Laboratory	2/12	2.023	101.15	1.11	Laboratory	2/12	2.025	101.23	1.22
prepared	4/10	4.070	101.76	1.92	prepared	4/10	4.006	100.16	1.86
mixtures	6/8	6.067	101.11	1.75	mixtures	6/8	6.026	100.44	1.18
Fe(II)/Cu(II)	8/6	8.051	100.64	1.44	Cu (II)/Fe(II)	8/6	8.047	100.59	1.48
	10/4	10.123	101.23	1.67		10/4	10.048	100.48	1.54
	12/2	12.034	100.28	1.88		12/2	11.966	99.72	1.44
Dosage form	11/1	11.188	101.71	1.90	Dosage form	1/11	0.992	99.24	1.78
Maxamin®					Maxamin®				
Fe(II)/Cu(II)					Cu (II)/Fe(II)				

*RSE is the relative standard errors calculated from the original matrix data for each concentration.

Conclusion

The proposed methods can be used for the simultaneous determination of iron (II) and copper (II) found together either in their pure forms or in their pharmaceutical preparation. The methods are precise, accurate and rapid. Moreover, the methods could be suitable for quality control laboratories for analysis of iron (II) and copper (II) in pharmaceutical preparation because the methods do not require separation steps and the instrument used is relatively not expensive and sophisticated.

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