SPECTROSCOPIC DETERMINATION OF MEBENDAZOLE USING A 1,10 PHENANTHROLINE–IRON COMPLEX**

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Simple, rapid, and sensitive spectroscopic methods have been proposed to determine the antifungal drug mebendazole. These methods adapted the reduction of ferric into ferrous in a 1,10 phenanthroline–iron complex in an acidic medium giving an orange–red ferroin complex. In the first method, the spectrofluorimetric assay was based on mebendazole quenching for the fluorescence of ferric–phenanthroline complex at pH 3.7. The fluorescence difference was quantitated at 409 nm after excitation at 254 nm. The second method involved the spectrophotometric measurement of the formed complex at 510 nm. A linear correlation was found over the concentrations 3.0–17.0 and 5.0–20.0 μg/mL respectively for methods I and II. The correlation coefficient (r) of the two methods is 0.9998. The two methods were successfully utilized for mebendazole determination in tablets. The mechanism of the reaction pathway is represented. Statistical comparison revealed no significant differences between the findings achieved by the proposed and comparison methods.

Keywords: *spectrofluorimetric, spectrophotometric, mebendazole, ferric–phenanthroline complex.*

СПЕКТРОСКОПИЧЕСКИЕ МЕТОДЫ ОПРЕДЕЛЕНИЯ МЕБЕНДАЗОЛА С ПОМОЩЬЮ КОМПЛЕКСА 1,10-ФЕНАНТРОЛИН–ЖЕЛЕЗО

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Предложены простые, быстрые и чувствительные спектроскопические методы определения противогрибкового препарата мебендазола. Методы основаны на восстановлении трехвалентного железа до двухвалентного в комплексе 1,10-фенантролин–железо в кислой среде с образованием оранжево-красного ферроинового комплекса. В методе I спектрофлуориметрический анализ основан на тушении мебендазолом флуоресценции комплекса железа с фенантролином при pH 3.7. Разницу флуоресценции определяли количественно на λ = 409 нм после возбуждения на 254 нм. Метод II базируется на спектрофотометрическом измерении поглощения образовавшегося комплекса при 510 нм. Линейная корреляция обнаружена в диапазонах концентраций 3.0–17.0 и 5.0–20.0 мкг/мл для методов I и II с коэффициентом корреляции (r) 0.9998. Методы успешно применены для определения мебендазола в таблетках. Представлен механизм пути реакции. При сравнении результатов, полученных с помощью предложенных и использованных ранее методов, существенных различий не обнаружено.

Ключевые слова: *спектрофлуориметрический анализ, спектрофотометрическое измерение, мебендазол, комплекс железо–фенантролин.*

Introduction. Mebendazole, named chemically Methyl-5-benzoyl-2-benzimidazole carbamate [1], is a benzimidazole derivative. It interacts with β-tubulin, a eukaryotic cytoskeletal protein, inhibiting its polymerization into microtubules and hampering the cell motility and the intracellular transport of cytoplas-

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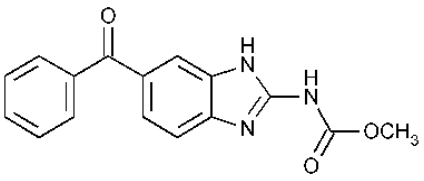
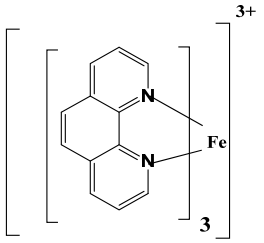
mic particles among other important biochemical functions [2]. Mebendazole was determined by different analytical methods including spectrophotometric [3, 4], spectrofluorimetric [5], and HPLC [6–9]. Five anthelmintic drugs including mebendazole were determined using capillary electrophoresis, with diode array detection [10]:



1,10-Phenanthroline



Ferrin complex

is one of the effective chelating reagents for some metal ions. It has been used for the spectrophotometric determination of ruthenium, sertraline, tocopherols, and captopril [11–14]. A 1,10-phenanthroline-terbium probe was used for the spectrofluorimetric estimation of folic acid in pharmaceutical formulations and biological fluids [15]. 1,10-Phenanthroline was used for the simultaneous determination of tofisopam using spectrophotometric and spectrofluorimetric methods [16]. The reported HPLC method dependent on using tris(1,10-phenanthroline)ruthenium(II) chemiluminescence for the determination of oxalic acid was carried out [17]. We aimed to investigate this reagent for developing simple and selective spectroscopic methods of mebendazole quantitation in its pure form and in different formulations.

Experimental. A Cary Eclipse fluorescence spectrophotometer equipped with a Xenon flash lamp from Agilent Technologies was used. The voltage was 800 V, with a slit width of 5 nm. The wavelengths of excitation and emission were 254 and 409 nm respectively. All figures were obtained with a smoothing factor of 20. A Shimadzu UV-Visible recording spectrophotometer (P/N 206-67001) was used. Recording range was 0–1.0, λ_{max} = 510 nm.

Materials and methods. Distilled water was used throughout the study. Mebendazole was provided from Alexandria Company for Pharmaceuticals and Chemical Industries. Antiver® tablets containing 100 mg of mebendazole/tablet were purchased from a local pharmacy. They are a product of Alexandria Co. for Pharmaceuticals & Chemical Industries. 1,10-phenanthroline-Fe(III) mixture was prepared by dissolving 0.5 g of 1,10-phenanthroline monohydrate and 0.4 g ammonium ferric sulfate in 5 mL of 1 M hydrochloric acid, then diluted to 250 mL with water. The stability of the solution was constant for up to 2 weeks if stored in a refrigerator [16]. The chemicals used for buffer preparation were bought from El-Nasr Pharmaceutical Chemicals Company (ADWIC) (Cairo, Egypt), and the surfactants were bought from ADWIC. (Cairo, Egypt). The organic solvents (spectroscopy grade) were bought from Sigma-Aldrich (Taufkirchen, Germany).

Preparation of standard solutions, buffers, and surfactants. Mebendazole is practically insoluble in water, ethanol, ether, or chloroform but soluble in formic acid [1]. A stock solution containing 100 μg/mL mebendazole was prepared by dissolving 10.0 mg in 5 mL of formic acid and completed to 100 mL with distilled water. 0.4 M acetate buffer solution was prepared using 0.4 M sodium acetate and 0.4 M acetic acid (with the pH ranging from 3.5 to 5.0).

General procedure. In a series of 10-mL volumetric flasks; 1 mL of the ferric–phenanthroline complex was added to aliquots of mebendazole so that it finely matched the concentration range 3–17 μg/mL. The solutions were mixed well, and 1 mL of 0.4 M acetate buffer (pH 3.7) was added. The solutions were diluted with distilled water to the mark, put in a boiling water bath for 30 min, and cooled. The fluorescence intensity was then measured at (λ_{ex} = 254 nm, λ_{em} = 409 nm), and the blank was measured simultaneously. The calibration curve was constructed relating the fluorescence intensity difference (between blank and experiments) at λ_{em} = 409 nm to the corresponding concentrations of mebendazole, and the regression equation was derived. The same procedure was carried out for the spectrophotometric method by adding 1.2 mL of the ferric–phenanthroline complex. The working concentration range was 5–20 μg/mL, and the absorbance was measured at 510 nm. The absorbance was graphed against the final concentration in μg/mL to construct the calibration curve, and the regression equation was derived.

Ten Antiver® tablets were weighed, grinded, and mixed well. A weight of the powder equivalent to 10.0 mg of the drug was taken accurately into a 100-mL volumetric flask, followed by the addition of 5 mL of formic acid to the powder, and mixed well. The solution was then sonicated for 30 min and completed

with distilled water. The working solution containing the required drug concentration was obtained by further dilution of the filtrate. The methods were followed as detailed under "General procedure" considering the calibration range for each method. The tablet content was then calculated either from the calibration curves or from the corresponding regression equations.

Results and discussion. Fluorescence quenching (ΔF) is the decrease in the fluorescence intensity due to different mechanisms such as molecular rearrangements, energy transfer, collision quenching, and electron transfer. Molecular contact between the fluorophore and the quencher should take place [18]. It is found that in proposed method I the fluorescence of the ferric–phenanthroline complex is decreased owing to the formation of a nonfluorescent complex with mebendazole at pH 3.7. The complex formation is due to the reduction of ferric into ferrous in the presence of 1,10-phenanthroline in an acidic medium to give an orange red-colored ferroin complex [16]. Accordingly, the quenching was quantitively measured at an excitation wavelength of 254 nm and an emission wavelength of 406 nm (Fig. 1). In method II, the orange–red complex was measured at 510 nm spectrophotometrically as in Fig. 2. The proposed methods were carried out to match with the green concept as much possible.

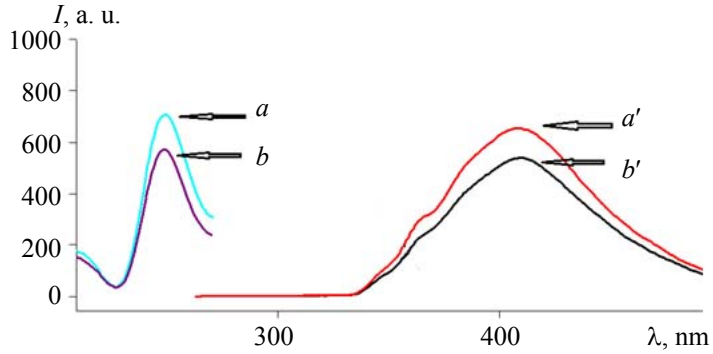


Fig. 1. Excitation and emission spectra induced by the reaction of the studied drugs with the 1,10-phenanthroline–iron complex: (a, a′) blank 1,10-phenanthroline–iron complex in acetate buffer pH 3.7, (b, b′) reaction product of mebendazole with the 1,10-phenanthroline–iron complex.

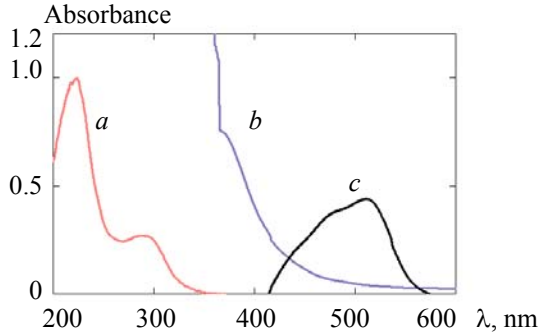


Fig. 2. Absorption spectra of a) mebendazole in an aqueous solution of 5×10^{-3}% formic acid, b) blank solution of an 1,10-phenanthroline–iron complex in acetate buffer at pH 3.7, c) absorption spectrum of the reaction of mebendazole with an 1,10-phenanthroline–iron complex and acetate buffer at pH 3.7.

Optimization of the experimental conditions. The experimental parameters that affect complex formation and stability were carefully investigated and optimized, namely the pH, type and volume of buffer, phenanthroline volume, and heating time. The optimal conditions that resulted in the highest ΔF and A values were 1 or 1.2 mL of ferric–phenanthroline in the spectrofluorimetric and the spectrophotometric methods respectively, 1 mL of 0.4 M acetate buffer (pH 3.7). The solutions were put in a boiling water bath for 30 min. The pH of the acetate buffer was varied and studied using the spectrofluorimetric method over the pH range 3.6–5.5 (Fig. 3a).

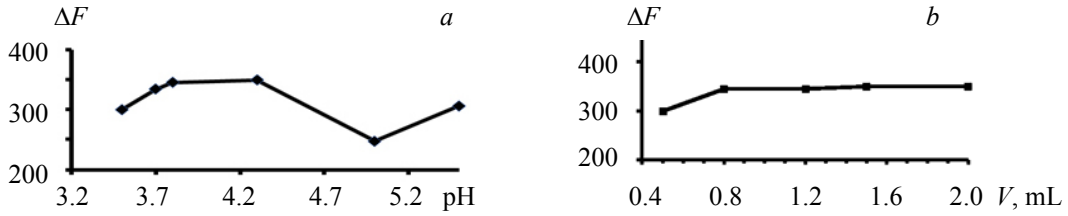


Fig. 3. Effect of (a) pH and (b) volume of 0.4 M acetate buffer on the fluorescence intensity of mebendazole (10 μg/mL) with the 1,10-phenanthroline–iron complex.

The optimal pH that resulted in the highest Δ*F* was 3.7 and increasing the pH above a certain limit caused precipitation of oxides [11]. The optimal Δ*F* was achieved by using 1±0.2 mL of acetate buffer pH 3.7 through this study. Increasing volumes of buffer did not cause any significant increase in Δ*F* (Fig. 3b). By studying different reagent volumes, it was found that 1 and 1.2 mL of 1,10-phenanthroline was enough to produce good stable results for the spectrofluorimetric and spectrophotometric methods respectively (Fig. 4).

It was found that the color reaction is accelerated at elevated temperatures [11]. Different temperatures were tried from 40 to 100ºC using a thermostatic water bath for different time periods where the maximum color intensity was obtained by heating in a boiling water bath for 30–35 min (Fig. 5). Therefore, heating in a boiling water bath for 30 min was optimal.

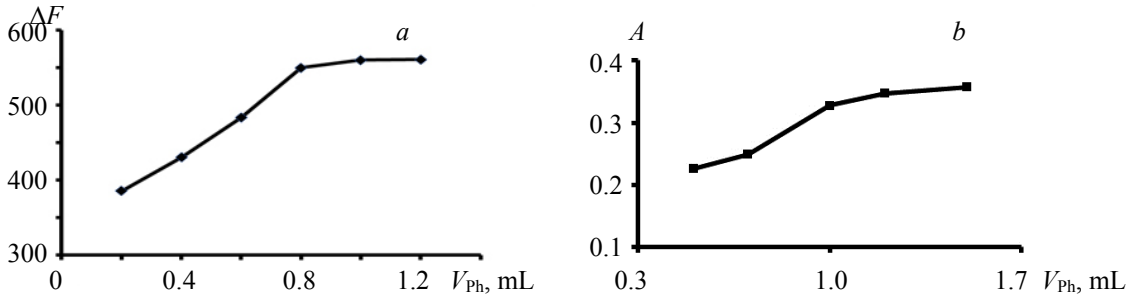


Fig. 4. Effect of volume of 1,10 phenanthroline–iron on the (a) fluorescence and (b) absorbance intensity of mebendazole (10 μg/mL) with the 1,10-phenanthroline–iron complex.

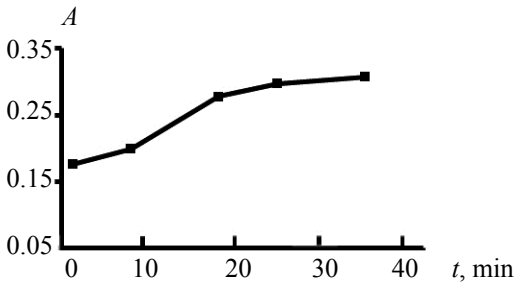


Fig. 5. Effect of heating time on the absorbance intensity of mebendazole (10 μg/mL) with the 1,10-phenanthroline–iron complex.

Analytical performance. A linear response between the quenching of the fluorescence (Δ*F*) or the absorbance and the drug concentration was obtained over the ranges cited in Table 1. Figure 6 illustrates increasing concentrations of mebendazole that react with 1,10-ferric–phenanthroline causing quenching. Linear regression analysis of the data resulted in the following equations: $\Delta F = 38.556C - 19.785$ ($r = 0.9998$) for the spectrofluorometric method; $A = 0.0217C + 0.1122$ ($r = 0.9998$) for the spectrophotometric method, where C is the concentration of the drug (μg/mL) and r is the correlation coefficient.

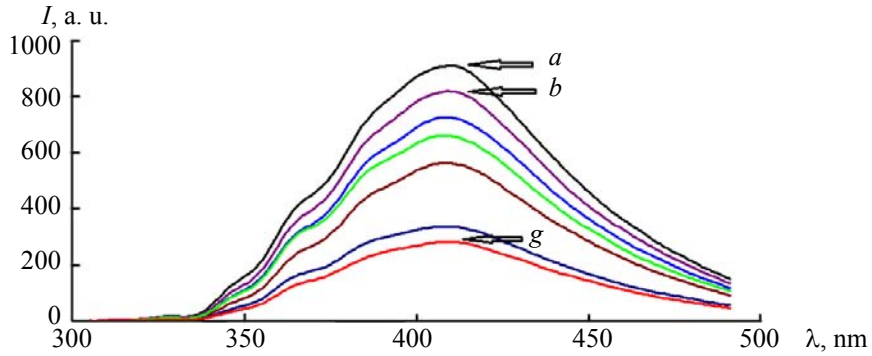


Fig. 6. Emission spectra of the 1,10-phenanthroline–iron complex at 409 nm where (a) is blank of the 1,10-phenanthroline–iron complex, (b–g) reaction products of different concentrations of mebendazole (3–17 μg/ mL) with the 1,10-phenanthroline–iron complex.

TABLE 1. Performance Data of Mebendazole by the Proposed Methods

Parameter	Spectrofluorometric method	Spectrophotometric method
Concentration range, μg/mL	3.0–17.0	5.0–20.0
LOD, μg/mL	0.40	0.025
LOQ, μg/mL	1.19	0.75
Correlation coefficient (r)	0.9998	0.9999
Slope	38.55	0.022
Intercept	–19.78	0.11
$S_{y/x}$	5.32	0.002
S_a	4.60	0.002
S_b	0.43	0.0001
% Error	0.54	0.23
%RSD	1.33	0.56
No. of Experiments	6	6
Mean found, %	99.93	99.85
±SD	1.33	0.56

Linear regression analysis of the data is represented in Table 1. The limits of quantitation (LOQ) and limits of detection (LOD) calculated regarding the ICH Q2 Recommendation [19] were 1.19, 1.37 μg/mL and 0.394, 0.45 μg/mL for methods I and II, respectively.

The proposed methods were evaluated with regard to accuracy and precision using the percentage relative error and percentage standard deviation respectively (Tables 2 and 3). The values of the standard deviation of the residual ($S_{y/x}$), standard deviation of the intercept (S_a), and standard deviation of the slope (S_b) point to the low scattering of the points around the calibration graphs (Table 1).

The repeatability and intermediate precision were evaluated using three concentrations of the drug in pure form at three successive times and on three successive days, as abridged in Table 2.

The results obtained were compared with a reported UV spectrophotometric method for the quantitative determination of mebendazole [4] where the solution of mebendazole in methanol was measured at 246.6 nm. Statistical analysis [20] of the results by a Student's t test and variance ratio F test, showed no significant difference between the performance of the proposed and the comparison methods considering accuracy and precision respectively (Table 3).

The proposed methods offered a wider range, a green solvent as the diluting solvent was distilled water, available reagent, and a higher correlation coefficient in comparison with the reported method. The investigation of the robustness of the method revealed the constancy of ΔF demonstrated with the slight changes in the experimental parameters such as pH 3.8±0.2 and change in the volume of phenanthroline 1.0±0.2 mL.

TABLE 2. Precision Data of the Proposed Methods for the Determination of Mebendazole in Pure Form

Sample concentration	Spectrofluorometric method		Spectrophotometric method	
	%found (repeatability) [a]	%found (intermediate precision) [b]	%found (repeatability)	%found (intermediate precision)
5.0 μg/mL	99.8	100.00	100.50	102.00
	99.9	100.10	102.00	99.60
	100.1	99.90	100.00	100.20
X′	99.93	100.00	100.83	100.60
±SD	0.15	0.10	1.04	1.25
%RSD	0.15	0.10	1.04	1.25
% Error	0.09	0.06	0.60	0.72
10.0 μg/mL	99.90	100.10	100.30	100.30
	99.70	100.30	101.00	99.20
	100.30	99.60	98.20	100.10
X′	99.97	100.00	99.83	99.87
± SD	0.31	0.36	1.46	0.59
%RSD	0.31	0.36	1.46	0.59
% Error	0.18	0.21	0.84	0.34
15.0 μg/mL	100.00	100.3	102.00	98.90
	99.76	100.1	100.50	100.00
	100.20	99.3	100.20	101.00
X′	99.96	99.90	100.90	99.97
± SD	0.27	0.53	0.96	1.05
%RSD	0.27	0.53	0.96	1.05
%Error	0.16	0.31	0.55	0.61

[a] Repeatability refers to the use of the analytical procedure within a laboratory over a short time period using the same analyst with the same equipment.
[b] Intermediate precision (also known as ruggedness) expresses within-laboratory variation, as on different days, or with different analysts or equipment in the same laboratory.

TABLE 3. Application of the Proposed and Comparison Methods for the Determination of Mebendazole in Pure Form and Commercial Tablets

Compound	Spectrofluorometric method		Spectrophotometric method		Comparison method [4]	
	Conc. taken, μg/mL	%found *	Conc. taken, μg/mL	%found	Conc. taken, μg/mL	%found
Mebendazole (pure form)	3.0	98.37	5.0	100.00	3.0	98.73
	5.0	101.56	7.0	98.70	5.0	100
	7.0	100.33	10.0	99.09	7.0	98.93
	10.0	98.78	12.0	98.48		
	15.0	101.29	15.0	99.70		
	17.0	99.29	20.0	98.87		
$X^- \pm$ SD		99.93±1.33		99.85±0.56		99.22±0.68
Student's *t* test		0.87 (2.365)		1.22 (2.365)		
Variance ratio *F* test		1.34 (19.3)		1.47 (5.786)		
Antiver tablets	5.0	100.84	5.0	100	5.0	100.36
	10.0	99.15	10.0	101.43	7.0	98.47
	15.0	100.28	15.0	100	10.0	99.82
$X^- \pm$ SD		100.09±0.86		100.48±0.83		99.55±0.97
Student's *t* test		0.3 (2.776)		1.21 (2.776)		
Variance ratio *F* test		7.22(19.0)		1.37 (19.0)		

N o t e. Values between parentheses are the tabulated *t* and *F* values respectively at $p = 0.05$ [21].
* Each result is the average of three estimations.

Stoichiometry of the reaction. A limiting logarithmic method was adopted to conclude the reaction mechanism [21]. The absorbance of the reaction product was measured using increasing concentrations of either the reagent or mebendazole. Plots of log [drug] *vs* log *A* and log [ferric] *vs* log *A* were constructed; the values of the slopes were 0.63:0.62 for mebendazole:reagent (Fig. 7).

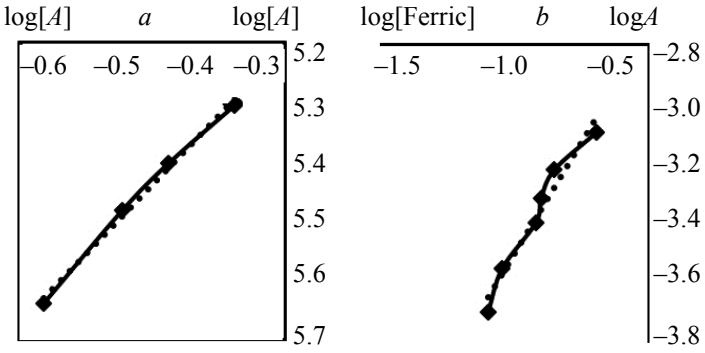
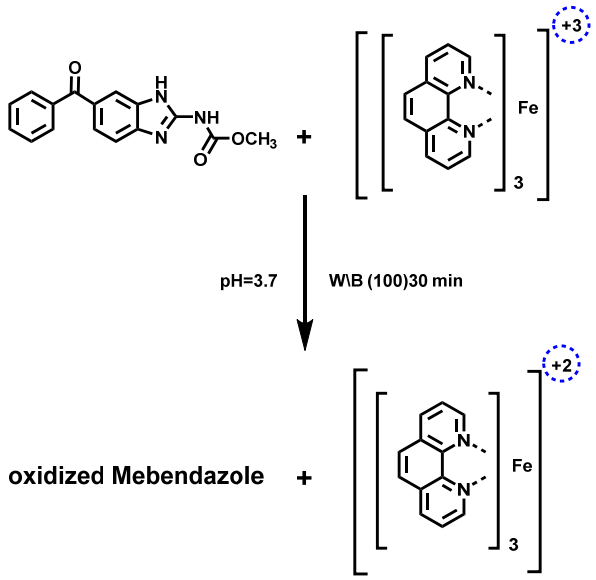


Fig. 7. Stoichiometry of the reaction between mebendazole and the 1,10-phenanthroline–iron complex mixture adopting the limiting logarithmic method, (a) log [*A*] *vs* log *A*, (b) log [ferric] *vs* log *A*, slope (a) 0.62 and (b) 0.63.

The reaction is mainly based on the reduction of Fe(III) to Fe(II) by mebendazole, then complex formation with 1,10-phenanthroline to give the orange–red-colored ferroin complex in acidic pH [16]. Consequently, an equation for the proposed reaction is represented in the following scheme:



Conclusions. This study describes two sensitive effective methods for the estimation of mebendazole. The methods were based on the reduction of ferric into ferrous in a 1,10-phenanthroline–iron complex in an acidic medium giving an orange–red ferroin complex. The proposed methods are simple, rapid, and inexpensive as we used water as the diluting solvent.

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