INDIRECT SPECTROPHOTOMETRIC DETERMINATION OF MEBENDAZOLE VIA OXIDATION-INDUCED DECOLORIZATION OF NEUTRAL RED DYE: APPLICATION TO PHARMACEUTICAL TABLETS AND HUMAN SERUM

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Received 26.04.2025
Accepted 02.06.2025

Abstract: *The aim of this method is to develop a simple, accurate, sensitive, inexpensive, indirect spectrophotometric assay of mebendazole in pharmaceutical formulation (tablets) and pure form. The suggested technique relies on the oxidation of mebendazole by the addition of a known excess amount of calcium hypochlorite in an acidic medium at room temperature. Then unreacted calcium hypochlorite is reacted with neutral red dye in order to discolor its color. The absorbance was recorded at 525 nm; at concentrations between 2 and 20 µg/ml, a linear association was seen. The calculated molar absorptivity is 12520.42 L/mol.cm, and the Sandell sensitivity value is 0.0236 µg/cm².The limits of detection (LOD) and quantification (LOQ) were determined and found to be 0.089 µg/mL and 0.298 µg/mL, respectively. Absence of additive interference in the dose forms when the proposed method was successfully used to determine MBZ in dose and bulk medication form.*

Keywords: *Mebendazole, Neutral red, Calcium hypochlorite, indirect spectrophotometric determination*
DOI: 10.65382/2221-8688-2026-2-277-287

1. Introduction

The drug mebendazole, identified chemically as methyl-5-benzoyl benzimidazole-2-carbamate (Fig. 1), belongs to the benzimidazole class. It is an amorphous powder that is white to faintly yellow and almost odorless. While it is widely soluble in formic acids, it is nearly insoluble in ethers, chloroform, water, and alcohol [1].

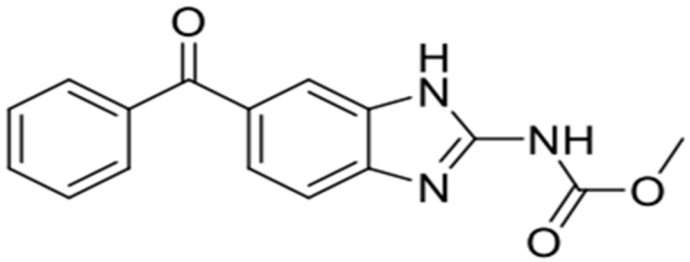

Fig. 1. Chemical composition of mebendazole ($C_{16}H_{13}N_3O_2$). M.wt= 295.293 g/mol

A brief review is given on Mebendazole (MBZ), also known as benzimidazole broad-spectrum anthelmintic, in the treatment of parasitic diseases. These medications are known to limit glucose uptake and transport, which ultimately results in cell death, by blocking mammalian cells' and parasites' microtubule networks [2]. According to the biological drug classification system, this medication is categorized as a second-class medication [3]. Recently, mebendazole has shown increasing interest in research and laboratory studies, as some preliminary studies have shown that it may have an anti-cancer effect [4]. This medication mostly works by interfering with the eukaryotic cytoskeletal protein β-tubulin and preventing it from polymerizing into microtubules. Glycogen storage is depleted as a result of decreased

glucose absorption [5]. Several *in vitro* studies have shown that mebendazole (MBZ) inhibits multiple tumor-promoting processes, including matrix metalloproteinase activity, angiogenesis, prosurvival signaling pathways, tubulin polymerization, and multidrug resistance protein transporters [6]. In addition, ongoing clinical trials and studies have demonstrated its effectiveness in several types of cancer, including acute myeloid leukemia, breast cancer, and gastrointestinal cancer [7]. Some studies suggest that mebendazole may be a promising candidate for monotherapy or for use in combination with ionizing radiation, due to its ability to inhibit tumor angiogenesis [8]. The literature has a variety of analytical techniques for the determination of mebendazole in its free or different formulation dosages, such as UV-visible spectrometry [9-13], high-performance liquid chromatography [14-17], fluorimetry [18, 19], selective sensors [20, 21], cyclic voltammetry [22], and differential pulse polarography [23].

Tissues are stained with neutral red dye. Neutral red dye soluble in water and gave red color solution. It has the ability to shorten its colour when its solution is mixed with oxidizing agents [24]. It has been introduced into this type of reaction in a limited number of studies to estimate pharmaceutical compounds [25-27], so it was used in the current work to estimate mebendazole.

Spectroscopic methods, particularly UV-Vis spectrophotometry, are simple and practical analytical techniques widely used in pharmaceutical quality control. These methods do not require expensive equipment or the use of toxic organic solvents, like chromatography and solvent extraction methods, making them a suitable option for quantitative analysis [28]. The aim of this study is to employ simple indirect spectrophotometric methods for the determination of mebendazole in pure and in pharmaceutical formulation; the process relies on oxidizing MBZ in an acidic media with an excess of the oxidizing agent calcium hypochlorite. and then the unreacted calcium hypochlorite reacts with the neutral red (NR) dye, causing a part of the dye to bleach.

2. Experimental part

Apparatus. SHIMADZU UV-1900i double-beam spectrophotometers UV-Vis was used for all measurements. The pH meter type is a BP3001, and the electronic sensitive balance type is BEL. With two 1 cm plastic cells (cuvettes).

Chemicals and solutions. The chemicals used in this investigation are high-purity reagents. Standard solution of mebendazole (200 µg/mL): this solution was prepared by dissolving 0.0200 g of pure MBZ (manufactured by S.D.I., Samara/Iraq) in 2 mL of (1M) H_2SO_4 and completed to the mark with ethanol in a volumetric flask to 100 mL [12].

Neutral red dye (3.46×10-4 mol/L, 100 µg/mL): This solution was prepared by dissolving the accurately weighed amount of 0.0100 g of neutral red dye in 100 mL of distilled water.

Solution of dilute hydrochloric acid 1 M (approximately): in a volumetric flask, 8.4 mL of concentrated hydrochloric acid was transferred into 91.6 mL of distilled water to create it.

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Solution of calcium hypochlorite (1×10^{-3} mol/L): prepared by dissolving 0.0142 g in 100 mL of distilled water in a volumetric flask.

General procedure. Different concentrations (2, 4, 6, 10, 12, 14, 18, and 20 µg/mL) of MBZ solution were transferred into eight volumetric flasks with a capacity of 10 ml, and 0.5 ml of hydrochloric acid (1M) and 1.4 mL of the oxidizing agent calcium hypochlorite at a concentration of 0.001M were added. The solutions were lifted for 10 min. at room temperature (25±2°C), finally adding 1 mL of NR dye, and then after 10 min. the volumetric flasks were diluted with distilled water to the mark. The wavelength 525 nm was used in measuring the absorbance.

3. Results and discussion

This method is based on the oxidation of MBZ with an excess of $Ca(OCl)_2$, followed by the decolorization of neutral red (NR) dye by the unreacted oxidant. The absorbance of the residual NR dye was measured at 525 nm. The influence of various experimental parameters on the redox reaction was investigated to establish the optimum conditions for the spectrophotometric determination of MBZ in pharmaceutical preparations.

Chosen the optimum concentration of the dye. Different volumes of NR dye ranging from 0.1 to 1.2 mL were taken, and the absorbance was measured at 525 nm against the blank solution, and the results are shown in Fig. 2.

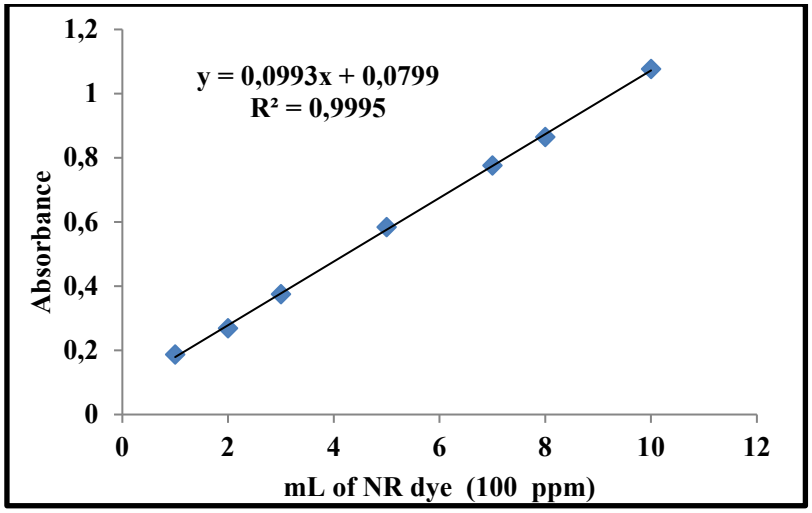

Fig. 2. Calibration curve of neutral red dye

As seen in Fig. 2, 1 ml of NR dye was the best volume; it has good absorbance and an outstanding value of the determination coefficient (R^2).

The effect of oxidizing agents. The effect of various oxidizing agents on bleaching the color of NR dye was studied by preparing a number of oxidizing agents at a concentration of 0.001 M. The oxidizing agents that were used are $K_2Cr_2O_7$, K_2CrO_4, $Ca(OCl)_2$, $NaIO_4$, KIO_4, and NBS by adding 1 mL of the oxidizing agent to a 10 mL volumetric flask containing 1 mL of dye and 0.5 mL of hydrochloric acid. The solutions are left for 5 minutes and then diluted to the mark with distilled water. The absorbance is measured at 525 nm against the blank solution. Calcium hypochlorite proved to be a more effective oxidizing agent than other oxidizing agents and was therefore chosen for subsequent experiments (Fig. 3).

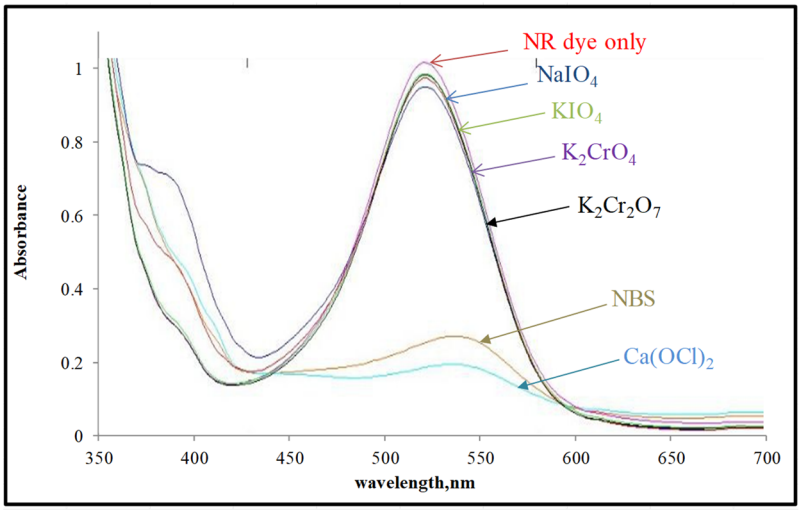

Fig. 3. The impact of different oxidizing agents on dye bleaching

Effect amount of oxidizing agent. After selecting $Ca(OCl)_2$ as the oxidizing agent, its volume was investigated using several amounts ranging from 0.1 to 1.6 mL (0.001 M), and their effect on the absorbance of NR dye was examined. All experiments were conducted in the absence of MBZ. A volume of 1.4 mL of $Ca(OCl)_2$ solution was chosen for subsequent experiments, as Fig. 4 indicates that this amount was sufficient to achieve complete bleaching of the NR dye.

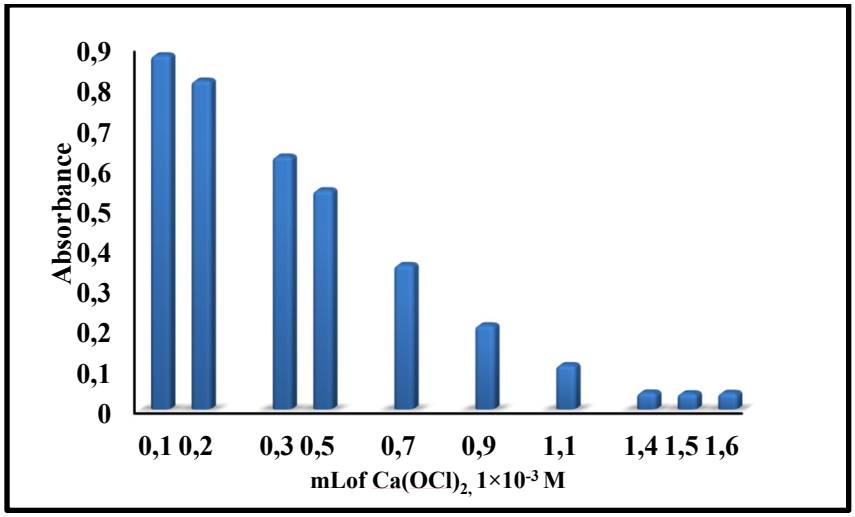

Fig. 4. The optimum amount of calcium hypochlorite

Effect of acidity. The oxidation of MBZ was carried out in acidic medium; therefore, various types of acids were investigated, as shown in Table 1.

Table 1. Choosing of acid type

0.5 mL of 1M acid	Absorbance
HCl	**0.556**
H_2SO_4	0.432
HNO_3	0.458
CH_3COOH	0.202
H_3PO_4	0.347

According to Table 1 data, HCl has the highest absorbance. Additionally, research has been done on the effects of varying amounts of 1M HCl solution. Since 0.5 ml of 1M HCl produced the highest absorbance and highest determination coefficient value (0.9994), it was chosen to be used in the subsequent experiments Table 2.

Table 2. Impact of acid concentration on absorbance

Amount of 1M HCl, ml	Absorbance/µg of MBZ					R^2
	2	**4**	**6**	**10**	15	
0.3	0.182	0.248	0.332	0.467	0.614	0.9967
0.5	**0.211**	**0.292**	**0.374**	**0.563**	**0.786**	**0.9994**
0.7	0.198	0.271	0.366	0.530	0.707	0.9979
0.8	0.209	0.310	0.381	0. 534	0.728	0.9983

Study the sequences of addition. The components of the reaction were added in different sequences in order to determine the optimal procedure for the oxidation of the MBZ compound (Table 3). Various addition orders were tested to achieve the highest absorbance and best analytical performance.

Table 3. The sequence of additions

Reaction component	Order number	Absorbance
MBZ + **H** + **Ox** + NR	**I.**	**0.568**
H+ Ox + MBZ + NR	II.	0.553
MBZ + NR + Ox + H	III.	0.052
NR + Ox + MBZ + H	IV.	0.011

Order I was selected due to give high absorbance.

Study of the oxidation time of MBZ and dye bleaching. The time required for the oxidation of MBZ was studied by adding 0.5 ml of MBZ with 0.5 ml of hydrochloric acid and 1.4 mL of $Ca(OCl)_2$ to volumetric flasks of 10 mL and leaving them for different periods of time. Then 1 mL of NR dye was added before diluting to the mark and also leaving for different periods of time to bleach the dye, and then the absorbance was measured at 525 nm. The result is shown in Table 4.

Table 4. Time's influence on oxidation and bleaching of the dye

Standing time before add NR dye	A/Standing time before dilution, min				
	Immediately	**5**	**10**	**15**	**20**
Immediately	0.204	0.237	0.362	0.487	0.492
5	0.362	0.415	0.547	0.538	0.485
10	0.443	0.557	**0.571**	0.543	0.557
15	0.434	0.498	0.553	0.517	0.466
20	0.419	0.552	0.561	0.549	0.527

According to the results in Table 4, MBZ required a standing time of 10 minutes to completely oxidize, while NR dye needed to bleach for 10 minutes.

Effect stability of remaining dye. The stability of the remaining dye color was studied using two different concentrations of MBZ (10 and 15 μg/mL), and it was observed that the absorbance value of the colored product remained constant for ninety minutes, as shown in Table 5.

Table 5. Effect stability remaining of dye

MBZ μg/mL	Absorbance/time (min.)							
	Immediately	**5**	**10**	**20**	**40**	**60**	**80**	**90**
10	0.568	0.565	0.565	0.561	0.561	0.559	0.557	0.551
15	0.757	0.756	0.756	0.752	0.751	0.753	0.749	0.748

The optimum conditions for the proposed method were collected and listed in Table 6 after being established through previous experiments.

Table 6. Optimum conditions

Variables	Optimal conditions
λ_{max}(nm)	525
Type, amount of acid used (1M)	HCl , 0.5 mL
Type, amount of oxidant (1×10^{-3}M)	$Ca(OCl)_2$,1.4 mL
Amount of (NR) dye (100 ppm)	1 mL
Temperature (ºC)	Room temperature(25 ± 2^0C)
Solvent	Water

Final absorption spectrum. Under optimal conditions (Table 6), 0.5 mL of hydrochloric acid is added to 10 and 16 μg/mL of MBZ (200 μg/mL) in a 10 mL volumetric flask,

followed by the addition of 1.4 mL of $Ca(OCl)_2$ and waiting for 10 minutes at room temperature, after which 1 mL of the NR dye is added and left in the solution for 10 minutes, and the mixture is diluted to the mark with distilled water. The absorption spectrum of the dye remaining against the blank solution NR dye exhibits a maximum absorption at 525 nm, shown in Fig. 5.

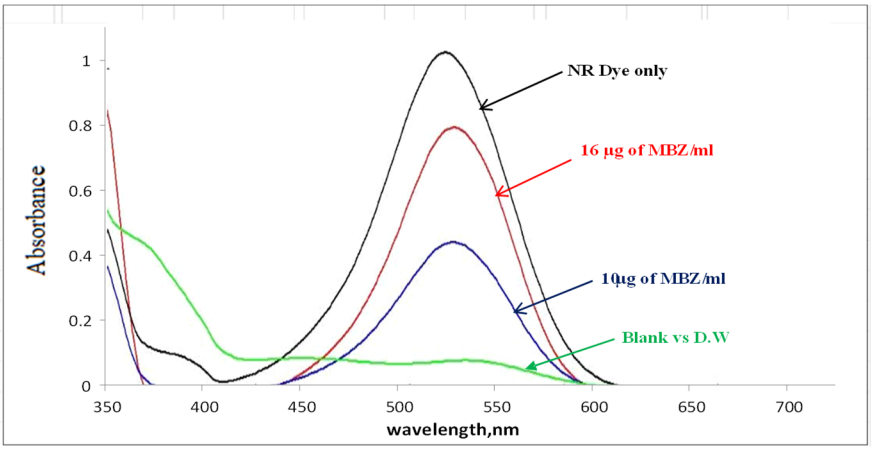

Fig. 5. Final absorption spectrum

Procedure and calibration curve. The calibration curve for MBZ was constructed following the general procedure. As shown in Fig. 6, a linear relationship between absorbance and MBZ concentration was observed in the range of 2–20 µg/mL, while concentrations above 20 µg/mL exhibited a negative deviation from linearity. The calculated molar absorptivity was 12,520.42 $L \cdot mol^{-1} \cdot cm^{-1}$, and the Sandell sensitivity was 0.0236 $\mu g \cdot cm^{-2}$.The limits of detection (LOD) and limits of quantification (LOQ) were determined and found to be 0.089 µg/mL and 0.298 µg/mL respectively.

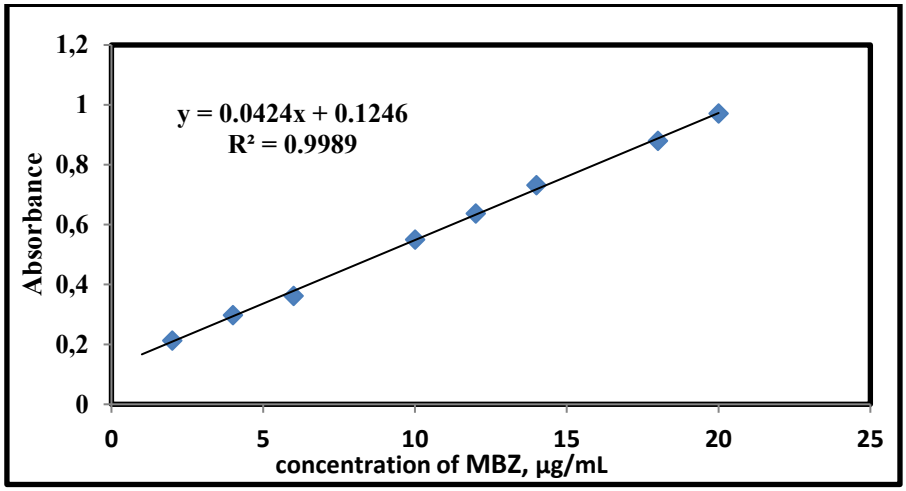

Fig. 6. The calibration curve for MBZ

Accuracy and precision. The suggested method accuracy and precision for MBZ estimation were computed. The recovery percentage, relative error, and relative standard deviation were also calculated. The results obtained in Table 7 indicate that the method has good accuracy and precision.

Table 7. Accuracy and precision

Taken amount of MBZ µg/mL	Found amount of MBZ µg/mL	Recovery %*	Relative error, %*	Relative standard deviation, %*
6	5.99	99.83	- 0.17	1.03

10	9.95	99.50	- 0.50	2.14
15	14.92	99.46	- 0.54	1.89

**Average for five determinations.*

Utilizing the suggested approach. The suggested method was successfully applied for the assay of MBZ in pharmaceutical preparations (each tablet containing 100 mg of the active ingredient). The results shown in Table 8 confirm the applicability and reliability of the method for the determination of MBZ in its pharmaceutical dosage form.

Table 8. Application of the suggested approach

Pharmaceutical preparation	Present amount of MBZ (μg/mL)	Found amount of MBZ(μg/mL)	Recovery %*	Relative error, %*	Relative standard deviation %*
Vermox 100 mg MBZ/tablet Janssen/Belgium	6	5.93	98.83	- 1.17	1.82
	10	9.75	97.50	- 2.50	1.14
Antiver 100 mg MBZ/tablet Alexandria/Egypt	6	5.81	96.83	-3.16	2.24
	10	9.85	98.50	-1.50	1.91

**Average of four determinations*

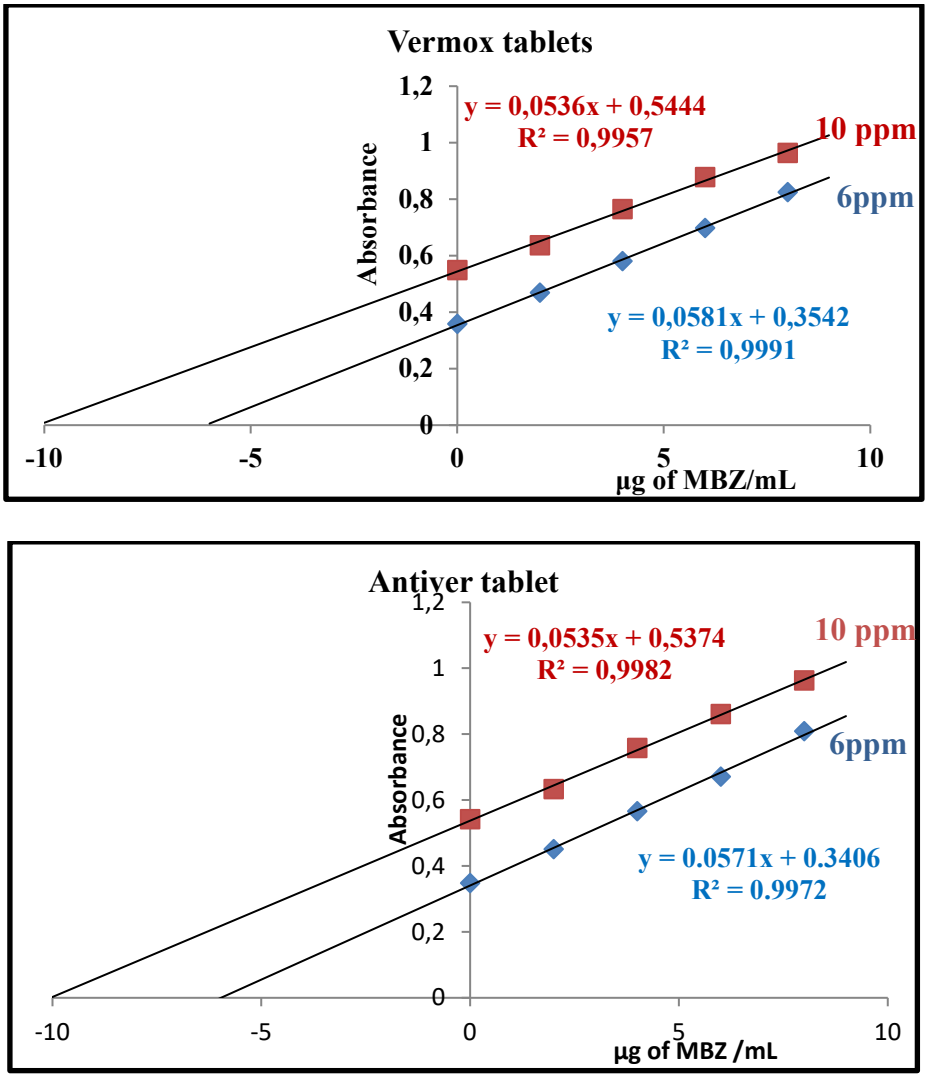

Fig. 7. Standard addition curves of MBZ in pharmaceutical preparations

Standard addition methods. The standard addition method was employed to verify the accuracy of the proposed method and its freedom from interference by the added excipients. Two concentrations (6 and 10 µg/mL) were taken from the pharmaceutical preparations of two different companies. The results, extracted from Fig. 7, are fixed in Table 9.

Table 9. The results of standard addition method

Pharmaceutical preparation	Presence amount of MBZ µg/mL	Measured amount of MBZ µg/mL	Recovery %
Vermox 100 mg MBZ/tablet Janssen/Belgium	6	6.09	101.60
	10	10.15	101.50
Antiver 100 mg MBZ/tablet Alexandria/Egypt	6	5.96	99.33
	10	10.04	100.40

Application the method on human blood serum. Blood samples were collected from healthy individuals who had not previously taken mebendazole. The serum was separated by centrifugation at 4,000 rpm for 15 minutes. MBZ was determined in serum samples by taking 0.2 mL of the serum, followed by the addition of MBZ at concentrations of 6 and 10 µg/mL, 0.5 mL of hydrochloric acid, and 1.4 mL of $Ca(OCl)_2$ (0.001 M). The mixtures were allowed to react for 10 minutes at room temperature (25 ± 2°C), after which 1.0 mL of NR dye was added. After a further 10 minutes, the flasks were diluted to the mark with distilled water. The results presented in Table 10 demonstrate the applicability of the proposed method for the determination of MBZ in human serum samples.

Table 10. Results of application the method on human blood serum

Concentration of MBZ, µg/mL		Recovery %*	RE, %*	RSD%*
Present amount	**Found amount**			
6	5.9	98.62	-1.67	1.82
10	9.76	97.66	- 2.40	1.14

**Average of three determinations*

Table 11. Comparisons of proposed method with other analytical methods

Analytical methods	Suggested method	Literature method [14]	Literature method [11]
λ max (nm)	525	428	570
Type of reaction	Oxidation-reduction	Oxidation-reduction	Formation of complex
Color of dye	Red color	Yellow dye	Blue
Molar absorptivity L/mol.cm	1.25×10^4	8.44×10^4	9.56×10^3
Reagent	Neutral red dye	Tartarazine dye	Sodium hypochlorite
Sandell Sensitivity (µg/cm²)	0.0236	0.0633	0.031
Color stability, min.	90	120	20
LOD, µg/mL	0.089	0.7770	0.11
LOQ, µg/mL	0.298	2.3400	0.33

The findings show that the proposed method is effective and sensitive for identifying mebendazole in pharmaceutical preparations (tablets) (Table 11).

4. Conclusion

An indirect spectrophotometric method was developed, confirming a successful approach for the determination of mebendazole in both pure form and pharmaceutical formulations (tablets). The method is based on the oxidation of MBZ using calcium hypochlorite, with the remaining unreacted $Ca(OCl)_2$ bleaching the color of neutral red (NR) dye.

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