How to Cite:
Abdulhadi, R. Z., & Mohammed, D. H. (2022). New spectrophotometric method for determination of mebendazole by oxidative coupling reaction. *International Journal of Health Sciences*, *6*(S4), 5610–5621. https://doi.org/10.53730/ijhs.v6nS4.9337

New spectrophotometric method for determination of mebendazole by oxidative coupling reaction

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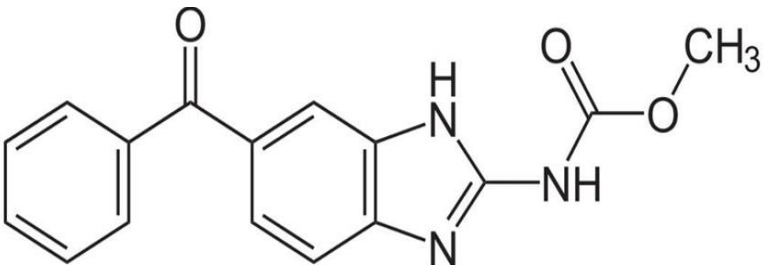
***Abstract*---**A rapid, simple, accurate and sensitive spectrophotometric method was developed for determination of mebendazole (MBL) in pure form and in its pharmaceutical dosages. This method depend on oxidation of MBL with excess amount of N-bromosuccinimide (NBS) in acidic medium with standing for complete the reaction , and the oxidized MBL coupled with Rhodamine B to get red product related with MBL amount and measuring the absorbance of surplus dye at 570 nm. A linear calibration curve was obtained over the concentration range 2.5 – 30 µg.ml-1 with correlation coefficient of 0.9992. The molar absorptivity and sandell's sensitivity index values were determined to be 1.476×10^4 L.mol-1.cm-1 and 0.02 µg.cm-2. The limit of detection (LOD) and quantification (LOQ) were calculated to be 0.469 and 1.982 $\mu g.ml^{-1}$, respectively . The proposed method has been successfully applied to the determination of MBL in available dosage form, the validity proposed method was confirmed by recovery study via standard addition technique.

***Keywords*---**mebendazole, N-bromosuccinimide, determination, spectrophotometry, rhodamine B.

Introduction

Mebendazole (MBL) , methyl [5- benzol- 1H- benzoimidazole- 2- yl] carbamate. Scheme 1. It is a white to slightly yellow powder, Practically water insoluble [1]. Mebendazole is an anthelmintic with an expansive scope of activity that is generally used to treat hookworm, pinworm, roundworm, tapeworm, threadworm,

and blended pervasions. MBL is likewise ordinarily used to treat gastrointestinal in the two people and creatures [2].



Scheme. 1 : chemical structure of mebendazole ($C_{16}H_{13}N_3O_2$)
M.Wt. = 295.293 g /mol

It is mentioned from the so-called (mass dust), as it is a drug with an unfortunate solubility in water at a ratio of (71-3) mg / l and a high permeability (Log p = 2.8), which is for some the degree of solvent in liquor, methyl chloride, diluted Acids , ether are completely soluble in formic acid. [3,4,5]. In view of its enemy of malignant growth impacts, mebendazole has gotten back to the examination spotlight by utilizing it to treat cancer[6].

MBL shows direct cytotoxic action, yet in addition synergizes with radiation and different chemotherapeutic specialists, and animates the counter cancer resistant reaction in the body, Studies have shown that MBL fundamentally makes harm the DNA of a disease cell, hence halting its development and it its spread to decrease. [7,8] .

A survey of the literature showed that several spectrophotometric methods have been reported for the determination of MBL in pharmaceutical doses and biological samples. Most of these methods rely on several interactions between MBL and reagents to create a spectroscopically estimated color product for MBL, of which: (Eosin)[9] , (AgNPs)[10] , (Methanolic Hydrochloride)[11] , (N-Chloro Succinamide)[12] , (DNSA and CA)[13] , (Lanthanum (III))[14] , (H_2SO_4 in methanol)[15] , (1-fluoro 2,4-dinitrobenzene)[16].

The aim of this research is to develop a simple, rapid and sensitive spectrophotometric method for the determination of mebendazole in its pure form and its pharmaceutical preparations based on the oxidation of mebendazole using N-bromosuccinamide and then coupling with the reagent rhodamine in an acidic medium.

Experimental
Apparatus

All absorption spectra and absorbance measurements were done by using a double beam UV-visible spectrophotometer (JASCOV-630) with 1.0-cm quartz cells.

Chemical reagents

All of the chemical compounds utilized in the tests were analytical grade, which meant they didn't need to be purified any further.

MBL stock solution 1000 µg. ml^{-1} : Prepared by dissolving 0.1 g of pure MBL in 10 ml 1 M of NaOH solution and heating for 5 min. , then neutralize the solution by 1M of HCl solution and diluted to 100 ml with distilled water using a volumetric flask. Working standard solution (100 µg. ml^{-1}) (3.386×10^{-4} $mol.L^{-1}$) was produced by diluting the stock solution appropriately. N-bromosuccinamid solution 20 µg. ml^{-1}: It was made by combining 0.002 g of NBS with distilled water and diluting it to 100 ml with distilled water. For at least two days, this solution remained stable.

Rhodamine (RD) stock solution 100 µg. ml^{-1} : It was made by dissolving 0.01 g of dye powder in distilled water and diluting to 100 ml with the same solvent. Working standard solution (10 µg. ml^{-1}) (2.087×10^{-5} $mol.L^{-1}$) was prepared by diluting the stock solution with distilled water to the desired concentration. SDS solution (5×10^{-3} M) : Prepared by dissolving 0.14419 g in 100 ml of distilled water by stirring and heating. hydrochloric acid 1 $mol.L^{-1}$: It was prepared by diluting 8.47 ml of concentrated hydrochloric acid to 100 ml with distilled water.

Initial procedure

In 10 ml volumetric flask we added 1.0 ml of 100 µg. ml^{-1} of MBL, followed by 1.0 ml of 100 g. ml-1 NBS solution and after standing for few minutes we added 1.0 ml of 100 µg. ml^{-1} rhodamine dye and then added 1.0 ml 1 mol.L-1 HCl,. We noticed that a red color gradually appeared over a short period, with the highest absorption peak at 570 nm and stable at room temperature.

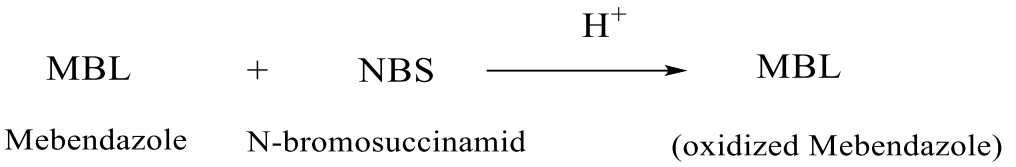
Procedure for dosage forms

MBL tablets (Vermox) solution 100 µg. ml^{-1}: six tablets were weighed, cruched into fine powder. A portion of powder equivalent to 0.025 g was weighed and dissolved in 10 ml 1 M of NaOH solution and heating for 5 min. , then neutralize the solution by 1M of HCl solution and diluted to 100 ml with distilled water using a volumetric flask. Stirring and mixed well with heating , then filtrated using filter paper, the filtrate was transferred into 100 ml volumetric flask and the volume was completed to the mark with distilled water. This solution was treated as in a general procedure.

Results and Discussion

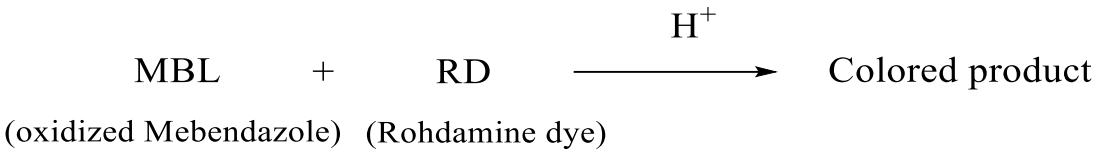
Principle of method and suggested chemical reaction

By following in writing and examining locomotor and systemic responses, we note that NBS is an oxidant and a bromine specialist in the corrosion mechanism of aliphatic and aromatic natural compounds [17]. So make a composite response between MBL and the specific measure of NBS as shown in scheme 1:



Scheme 1

The oxidized MLB then reacts with the RD dye to generate a colored product that was measured at 570 nm and proportional to the MBL concentration, shown in Scheme 2:



Scheme 2

Optimum Reaction Conditions

The following experiments were conducted in 10 ml volumetric flasks with 100 µg of MBL and measuring absorption for colored product at 570 nm .

Choose of oxidant agent :

This study was done by adding 1 ml of (20 µg. ml^{-1}) available oxidizing agents which (N-bromosuccinamid, potassium periodate and Potassium dichromate) into number 10 ml volumetric flasks which contain 1.0 ml of (100 µg. ml^{-1}) MBL and then added 1.0 ml of 1 M HCl. After waiting for a short time added 1 ml of 10 µg. ml^{-1} RD reagent and the volume was completed to the mark with distilled water. The absorbance was measured at 570 nm. (table 1) shows that NBS gives the best results, so it was chosen in the subsequent experiments.

Table 1:choose the type of oxidizing agent

Oxidizing agent 20 µg. ml^{-1}	NBS	KIO_4	$K_2Cr_2O_7$
Absorbance	0.396	0.254	0.178

Amount of oxidant agent

The NBS amount was studied by varying its volume while other factors were held constant. It was found that 1.0 ml of 100 mcg. -1 ml NBS is preferable for subsequent experiments depending on the results in (Fig. 1).

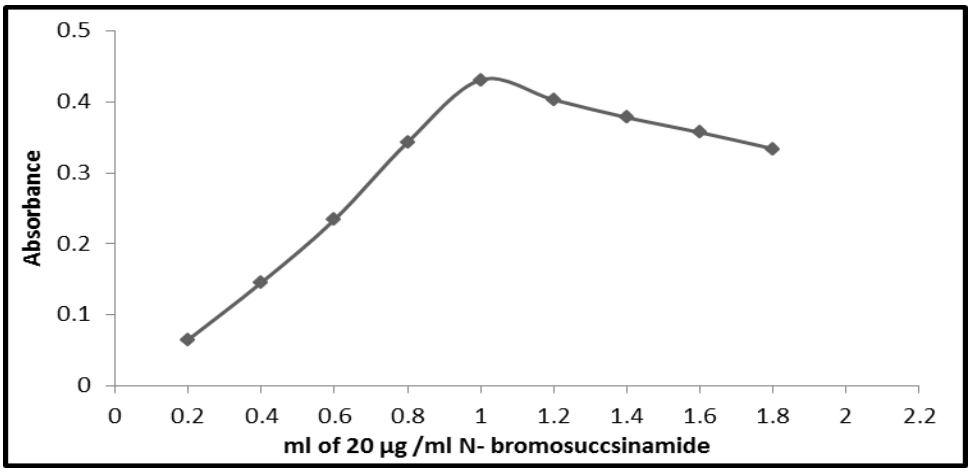


Fig.1: Amount of oxidant agent

Effect of oxidation time and coupling time

Studying the timing of the oxidation reaction is critical as reasonable periods of time were waited for the completion of the oxidation reaction before the addition of the RD reagent, the RD reagent was added and the flasks were shaken and waiting for several minutes to study the conjugation time. (Table 2) shows that 5 min. is the best period to complete the oxidation, and 5 min. is also sufficient to complete the coupling process between the oxidizing MBL and the reagent.

Table 2: Effect of oxidation time and coupling time

Standing time before add RD reagent , min	Absorbance / Standing time after add RD reagent before diluting, min							
	5	10	15	20	30	40	50	60
After addition	0.234	0.246	0.255	0. 269	0.278	0.282	0.280	0.293
5	0.414	0. 411	0. 410	0.411	0.409	0.408	0.409	0.409
10	0.399	0.398	0.397	0.395	0.396	0.399	0.398	0.394
15	0.335	0.356	0.375	0.374	0.373	0.371	0.373	0.375

Effect of acidic medium

In this study, different types of acids were used and H_2SO_4 was found to be an ideal reaction medium (Table 3). In addition, the optimal amount of acid was studied and 0.3 ml of 1 M HCl was chosen as the optimal amount (Fig. 2):

Table 3:choose the type of acid

Acid solution 1.0 ml of (1M)	Absorbance
HCl	0.412
H_2SO_4	0.445
HNO_3	0.298

CH_3COOH	0.147

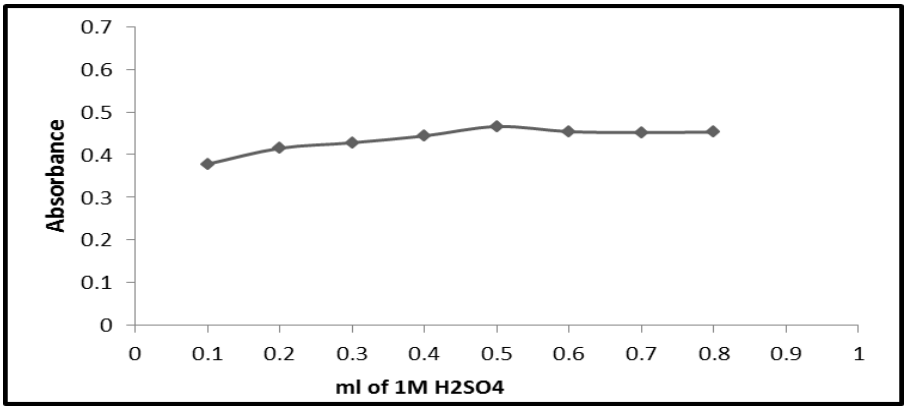


Fig. 2 : amount of acid

Effect of amount of coupling reagent

To get the best amount of coupling reagent, the effect of different amounts of coupling agent RD on the absorption of the colored product was studied using volumes ranging from 0.5 to 2.5 ml of 100 µg. ml^{-1} RD reagent with increasing amounts of MBL and the results are shown in the table 4 :

Table 4 : Effect of amount of condinsation reagent

ml of 100 µg. ml^{-1} reagent	Absorbance / ml of MBL					
	0.5	1	1.5	2.0	2.5	R^2
0.5	0.182	0.389	0.607	0.835	0.972	0.9945
1.0	0.208	0.419	0.626	0.849	1.002	0.9968
1.5	0.235	0.479	0.702	0.939	1.114	0.9971
2.0	0.285	0.517	0.782	1.009	1.218	0.9990
2.5	0.281	0.508	0.778	1.000	1.188	0.9976

From the results in table 1, it is clear that a volume of 2.0 ml of RD reagent at 100 µg. ml^{-1} concentration gives the highest absorption capacity and the best estimate coefficient value (R^2 = 0.9990) , and thus it was selected and accepted in subsequent experiments.

Effect of surfactants

This study was carried out using several different types of surfactants, as well as different amounts of each type and with a concentration of 5×10^{-3} M for each of them, where it was found that the SDS gave an increase in the absorbance of the colored product and that the volume of it was the best amount as shown in (table 5).

Table 5: Effect of surfactants

Surfactant 5×10^{-3} M	Absorbance / ml of Surfactant				
	0.0	0.5	1	2	3
Triton X 100	0.442	0.408	0.435	0.437	0.430
SDS	0.513	0.518	0.539	0.532	0.530
CTAB	0.441	0.401	0.447	0.439	0.436
CPC	0.442	0.440	0.441	0.439	0.430

Effect of temperature and stability

The effect of temperature on the colored product of the reaction was studied, as well as the stability of the colored product at different temperatures, and it was found that the absorbance was stable for at least an hour at room temperature (25Co ± 2), as shown in the fig.5

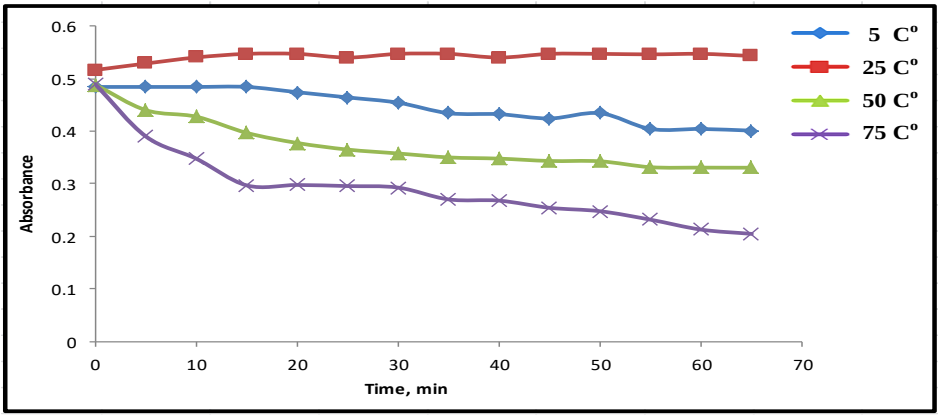


Fig. 5: Effect of heat and time on absorbance of colored product

Sequence of additions

The order of addition of the reaction components was explored to see if the order of addition had an effect on the color intensity of the product and the results are shown in the table 6.

Table 6: Effect of Sequence of additions

N.O	Sequence of additions	Absorbance
1	MBL + H^+ + OX + R + SDS	0.475
2	MBL + OX + H^+ + R + SDS	0.538
3	MBL + OX + SDS + H^+ + R	0.464
4	OX + MBL + H^++ R + SDS	0.421

From the results in table 6 the best sequence of addition was drug + oxidant + Acid + Reagent and the surfactant. Under the same circumstances, other sequences order had lower absorbance.

Final absorption spectrum

Under the optimum reaction conditions 1 ml of 100 µg. ml^{-1} MBL solution oxidized by 1 ml of 20 µg. ml^{-1} NBS in acidic medium and after standing for 5 min 2.0 ml of 100 µg. ml^{-1} RD solution reagent was added, then stand at room temperature for 5 min before complete the volumetric flask to mark with distilled water. The absorbance was measured for this solution against reagent blank similarly prepared without drug at 570 nm. (fig. 6) showing the spectrum of the final product for this procedure .

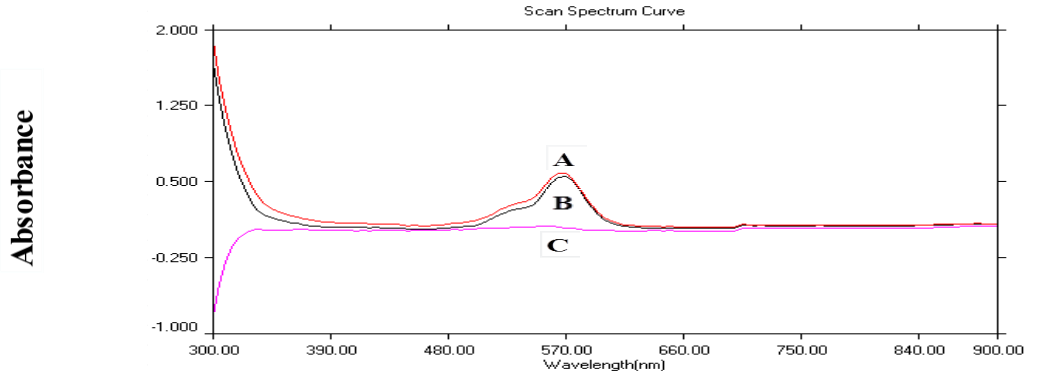


Fig.6: Absorbtion spectrum of 100 µg MBL VS (A) D.W , (B) blank, (C) blanck VS D.W.

Calibration curve

At ideal circumstances a rising volume of 100 µg. ml^{-1} of MBL arrangement was added to a progression of 10 ml volumetric carafes to cover the rang 2.5 - 30 µg.ml^{-1}, , followed by adding 1.0 ml of 100 µg.ml^{-1} NBS arrangement, then 0.5 ml of 1 M H_2SO_4 was added. The arrangements were permitted to remain at room temperature for 5 min, Then 2.0 ml of 100 µg. ml^{-1} RD arrangement was added, then represent 5 min and complete to check with refined water. The absorbance was estimated against reagent clear comparably ready without drug at 570 nm, (fig. 7) is gotten over the reach (2.5 - 30)µg. ml^{-1} with molar absorptivity 1.476 × 10^4 $L.mol^{-1}.cm^{-1}$ and sandell's awareness 0.02 µg.cm^{-2}.

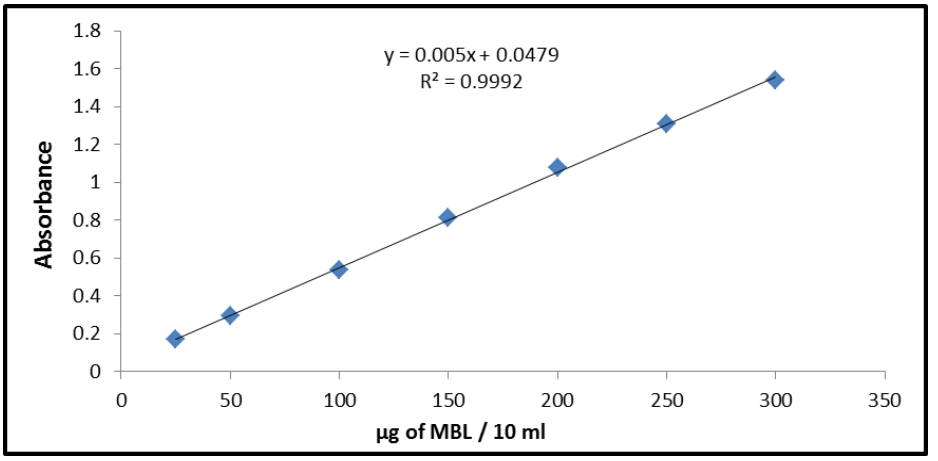


Fig. 7 :Calibration curve of MBL Estimation

Accuracy and precision

To calculate the accuracy and precision of the calibration curve, MBL is determined at two concentrations. The results shown in (Table 5) show that the proposed method is reliable.

Table 5 :accuracy and precision

Amount of MBL µg/10 ml present	Amount of MBL µg/10 ml found	Recovery %	Rrelative error, %*	relative standard deviation, %*
50	49.19	98.38	-1.62	±0.921
150	152.38	101.59	1.59	±1.813

*Average of five determinations

Application of the method

The suggested method was successfully applied to determine the drug in their commercial preparations (tablets). The results in (table 6) indicated that the method is accurate and reproducible.

Table 6: application of the method

Drug	Amount of MBL µg/10 ml	Recovery %	Rrelative error, %*	relative standard deviation, %*
Vermox 100 mg/tablet U.K	100	98.15	1.85	±0.567
	200	100.99	0.99	±1.329
Antiver 100 mg/tablet Egypt	100	102.11	2.11	±0.81
	200	103.14	3.14	±2.302

*Average of three determinations

Evaluation of the suggested method

The standard addition method was applied to confirm the selectivity of this method by adding an appropriate volume of the order containing an appropriate measure of drug readiness to a number of 10 ml volumetric flasks, then adding extended measures of the order of performance and adding the other portions of the methodology. Complete each vial to the imprint with refined water and mix well. After a long period, the absorbance of the arrangements was estimated to be about 570 nm. (Fig. 8) and occurs (Table 7) which shows a good understanding between the standard combination method and the idea strategy.

Table 7 : evaluation of the suggested method

pharmaceutical preparation	Amount of MBL presence µg/10 ml	Amount of MBL measured µg/10 ml	Recovery %
Vermox 100 mg/tablet U.K	50	48.11	99.22
	100	98.80	98.80
Antiver 100 mg/tablet Egypt	50	51.06	102.11
	100	101.18	101.18

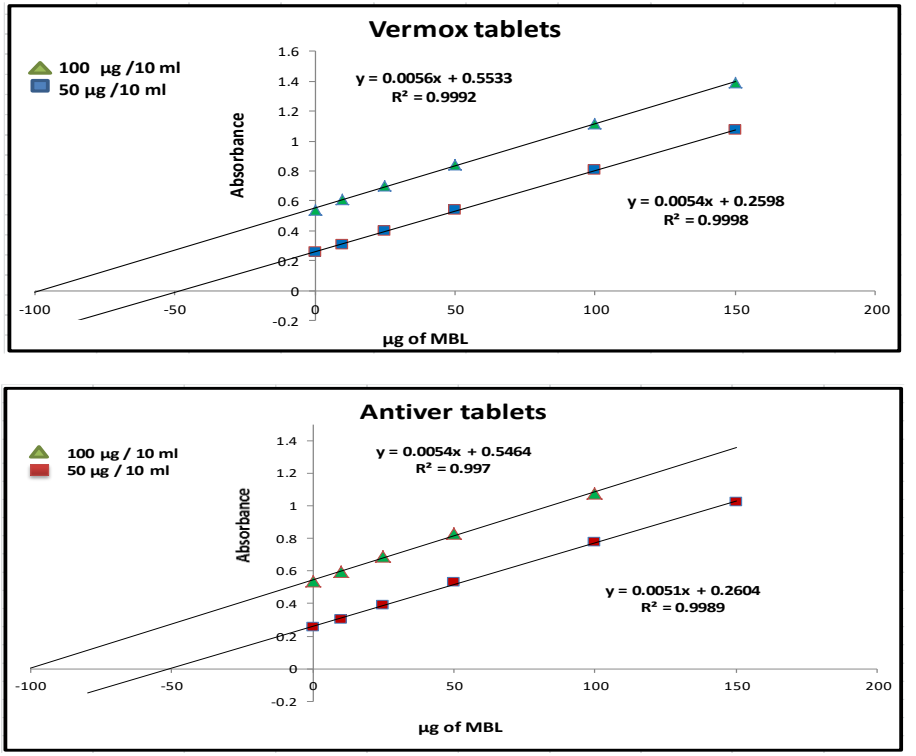


Fig.8: Standared addition curves for estimation of MBL in pharmaceutical preparations

Conclusion

A simple spectrophotometric method was proposed for the determination of mebendazole based on the oxidation of albendazole with N-bromoboxinamide and then conjugation of the oxidized mebendazole with rhodamine reagent, the method was sensitive, with acceptable accuracy and applicable to pharmaceutical preparations.

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