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ORIGINAL ARTICLE

Indirect Spectrophotometric Determination of Mebendazole Using Methyl Orange Dyein the Presence of the Oxidizing Agent NBS

RASHA Z. ABDULHADI[1], DAWOOD HABBOO MOHAMMED[2]
[1-2]Department of Chemistry/ College of Education for Girls / University of Mosul
Correspondence to: Rasha Z. Abdulhadi, Email: rashazbar2016@gmail.com

ABSTRACT

Aim: To develop a swift , simple, accurate and sensitive spectrophotometric method for determination of mebendazole (MBZL) in pure and pharmaceutical formulations.

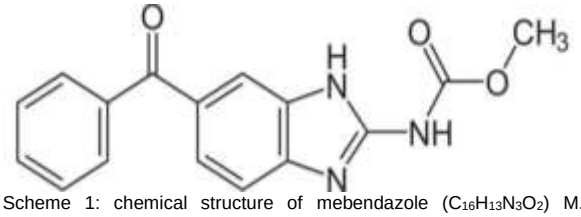
Methods: The suggested method depend on oxidation of MBZL with known excess amount of N-bromosuccinimide (NBS) in acidic medium and after reaction is insured to be complete , the surplus NBS is estimated by de colorization of methyl orange (MO) dye and measuring the absorbance of surplus dye at 508 nm. A linear calibration curve was obtained over the concentration range 2.5– 25 µg.ml^{-1} with correlation coefficient of 0.9997. The molar absorptivity and sandell's sensitivity index values were determined to be 1.505 × 10^{4} L.mol^{-1}.cm^{-1} and 0.019607 µg.cm^{-2}. The limit of detection (LOD) and quantification (LOQ) were calculated to be 0.3736 and 2.553 µg.ml^{-1}, respectively. The proposed method has been successfully applied to the determination of MBZL in available dosage form, the validity proposed method was confirmed by recovery study via standard addition technique .

Keywords: mebendazole ; N-bromosuccin**i**mide ; Determination **;**Spectrophotometry ; methyl orange

INTRODUCTION

Mebendazole (MBZL) , methyl [5- benzol- 1H- benzoimidazole- 2-yl] carbamate. Scheme 1 It is a white to slightly yellow powder. Pleasant taste. Practically water insoluble[(1)].

Mebendazole is an anthelmintic with a broad range of action that is commonly used to treat hookworm, pinworm, roundworm, tapeworm, threadworm, and mixed infestations. MBZL is also commonly used to treat gastrointestinal helminths in both humans and animals [(2)].



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

This drug is classified in the biological drug classification system, as a second-class drug. It is included in the medicines called (brick dust), as it is a medicine with poor solubility in water with a ratio of (71-3) mg / liter and a high permeability (Log p = 2.8), and it is partially soluble in alcohol, methyl chloride, dilute acids, The ether is completely soluble in formic acid [(3,4,5)].

Because of its anti-cancer effects, mebendazole has returned to the research spotlight by using it to treat cancer[(6)]. MBZL not only shows direct cytotoxic activity, but also synergizes with radiation and various chemotherapeutic agents, and stimulates the anti-tumor immune response in the body, Studies have shown that MBZ significantly causes damage to the DNA of a cancer cell, thus stopping its growth and reducing its spread. [(7,8)] .

After searching the literature survey revealed that various spectrophotometric procedures have been reported for the determination of MBZL in pharmaceutical formulations and biological samples. Most of these methods based on reaction between MBZL and several reagents to create a colored product of which (1-fluoro 2,4-dinitrobenzene)[(9)] , (AgNPs)[(10)] , (Methanolic Hydrochloride)[(11)] , (N-Chloro Succinamide)[(12)] , (DNSA and CA)[(13)] , (Lanthanum (III))[(14)] , (H_2SO_4 in methnol)[(15)] , (Eosin)[(16)].

However, several of these procedures requires expensive equipment and skilled operation. The goal of this research is to develop a swift, simple, accurate and sensitive spectrophotometric method for the estimation of mebendazole in pure form and it's pharmaceuticals based on the oxidation of mebendazole by using N-bromosuccinamid the color palace of methyl orange dye by the unreacted N-bromosuccinamid.

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.

MBZL stock solution 500 µg. ml^{-1} : Prepared by dissolving 0.125 g of pure MBZL 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 100 µg. ml^{-1}: It was made by combining 0.01 g of NBS with distilled water and diluting it to 100 ml with distilled water. For at least two days, this solution remained stable.

Methyl orange(MO) stock solution 1000 µg. ml^{-1} : It was made by dissolving 0.1 g of dye powder in distilled water and diluting to 100 ml with the same solvent**.** Working standard solution **(100 µg. ml^{-1}) (3.055×10^{-4} mol.L^{-1})** was prepared by diluting the stock solution with distilled water to the desired concentration.

hydrochloric acid 1 mol.L^{-1}: It was was prepared by diluting 8.47 ml of concentrated hydrochloric acid to 100 ml with distilled water.

Initial procedure: A series of 10 ml volumetric flasks were filled with 0.5 ml of 1 mol.L-1 HCl, followed by 1.0 ml of 100 g. ml-1 NBS solution. An increasing volume of 10 µg. ml^{-1} of MBZL solution was added to cover the rang 2.5 - 25 µg.ml^{-1}. The solutions were allowed to stand at room temperature for 15 min, then 1.0 ml of 3.055×10^{-4} mol.L^{-1} MO solution was added, and stand for 5 min and complete to mark with distilled water. The absorbance was measured against reagent blank similarly prepared without drug at 508nm.

Procedure for dosage forms

MBZL 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: Frome follow – up to the literature and research of kinetic and mechanism reactions, we note that NBS is an oxidizing agent and a

bromination agent in the acid medium of aliphatic and aromatic organic compounds[17]. So soppose a chemical reaction between MBZL and calculated amount of NBS:

$$\underset{\text{(excess)}}{\text{MEZL} + \text{NBS}} \xrightarrow{H^+} \underset{\text{(oxidized)}}{\text{MEZL}} + \underset{\text{(unreacted)}}{\text{NBS}}$$

Scheme 1:

Then the unreacted NBS is react with MO dye and oxidized a fixed amount of MO dye to colorless result . Finally measured the residual amount of dye at 508 nm which is proportional to concentration of MBZL :

$$\underset{\text{(unreacted)}}{\text{NBS}} + \underset{\text{(excess)}}{\text{MO}} \xrightarrow{H^+} \underset{\text{(oxidized)}}{\text{MO}} + \underset{\text{(unreacted)}}{\text{MO}}$$

Scheme 2:

Optimum Reaction Conditions: The following experiments were conducted in 10 ml volumetric flasks with 100 µg of MBZL and measuring MO dye absorption at 508 nm .

Amount of MO dye: To find the largest amount of MO dye that can be used in the determination of MBZL which that followed the Bear law, increasing volumes of 3.055×10^{-4} mol.L^{-1} MO dye solution were added to 10 ml volumetric flask contained 0.5 ml of (1M) hydrochloric acid. The volume was completed to the mark with distilled water and the absorbance was measured at 508 nm. The standard curve as in (fig. 1)shows that 1.0 ml of dye is the best volume that gives highs absorbance within the linear relationship.

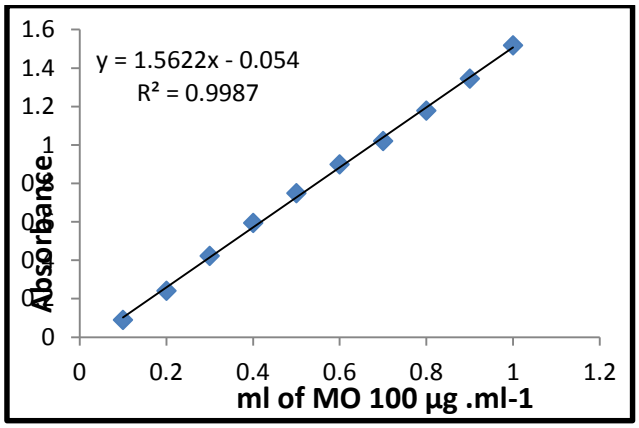


Fig.1: Standard curve for MO dye

Choose of oxidant agent: This study was done by adding 1 ml of available oxidizing agents that decolorized MO dye (N-bromosuccinamid, Potassium dichromate and Potassium iodate) wit conc.(100 µg. ml^{-1}) of each one into 10 ml volumetric flask which contain 1.0 ml of 3.055×10^{-4}mol.L^{-1} MO dye and 0.5 ml of 1 M HCl, then volume was completed to the mark with distilled water. The solution was stand for 15 min. and the absorbance was measured at 508 nm. (fig. 2) shows that NBS gives the best results, so it was chosen in the subsequent experiments.

Amount of oxidant agent: NBS volume has been studied by changing the reagent volume while the other factors were constant. It found that 1.0 ml of 100 µg. ml^{-1} NBS is sufficient for decolorization of MB dye (fig. 3).

Effect of acidity: Various types of acids were studied and HCl was found to be an ideal reaction mediator (Table 1). In addition, the optimal amount of acid was studied and 0.3 ml of 1 M HCl was chosen as the optimal amount (Fig. 4)**:**

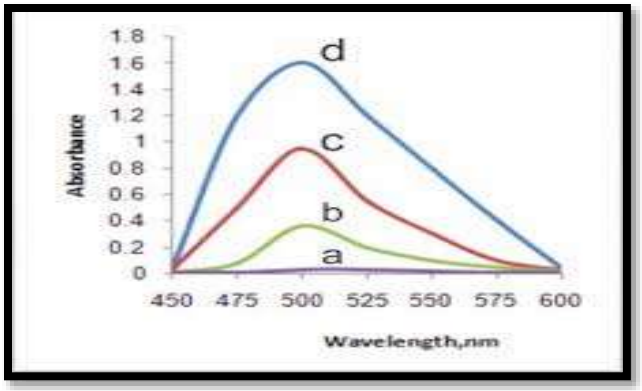


Fig 2: d MO dye without oxidant (c) MO dye with Potassium dichromate, (b) MO dye with Potassium naitrat (a) MO dye with N-bromosuccinamid

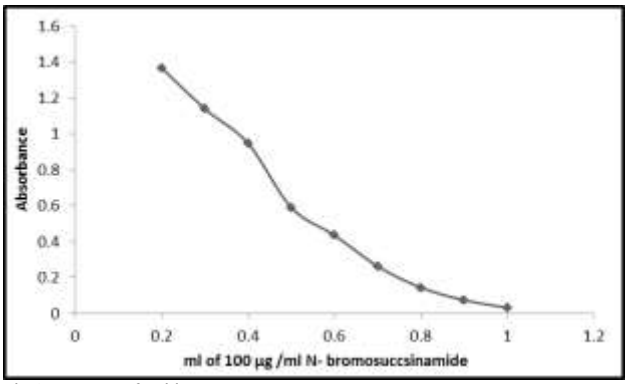


Fig.3: Amount of oxidant agent

Table 1: choose the type of acid

Acid solution 0.5 ml of (1M)	Absorbance
HCl	0.543
H_2SO_4	0.485
HNO_3	N.R
CH_3COOH	0.247

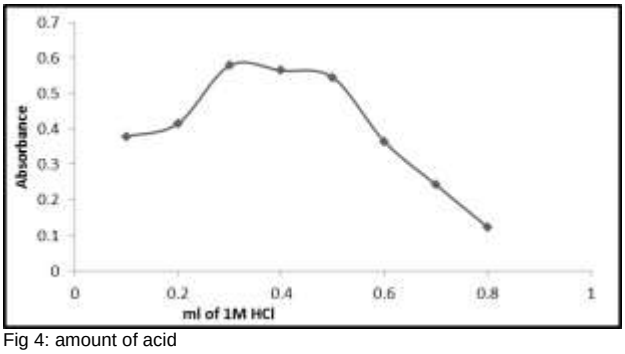


Fig 4: amount of acid

Effect of oxidation time: The study of the oxidation reaction's timing is crucial. Before adding MO dye, it was determined by adding reaction components. MO dye was added after shaking the flasks and after a reasonable amount of time. To complete the oxidation reaction, shake the flasks again for several minutes. (table 2) reveals that 15 minutes is the best period for MO dye oxidation.

Effect of temperature and stability: The stability of reaction was studied in different temperatures and the shows that the absorbance is stable for one hour at room temp.(25C^o ± 2).

Table 2: Effect of oxidation time

Standing time before add M.O , min	Absorbance / Standing time after add M.O and 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	0.493	0. 494	0. 495	0.501	0.499	0.504	0.501	0.506
10	0.519	0.538	0.577	0.575	0.576	0.579	0.578	0.577
15	0.535	0.556	0.575	0.574	0.573	0.571	0.573	0.575
20	0.499	0.494	0.493	0.494	0.483	0.481	0.473	0.469

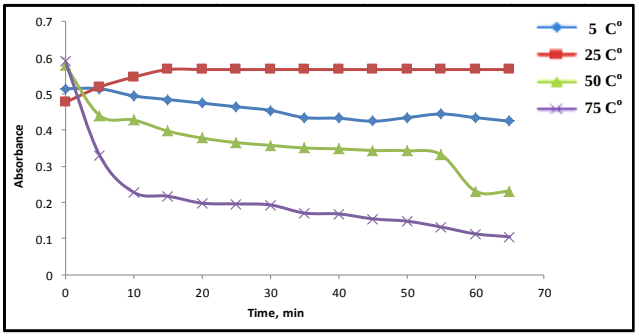


Fig 5: Effect of heat and time on absorbance of condensation dye

Sequence of additions: Different orders of addition of the reaction components were explored to see if the order of addition had an effect on the color intensity of the result

Table 3: Effect of Sequence of additions

N.O	Sequence of additions	Absorbance
1	MEZL + H^+ + OX + MO	0.575
2	MEZL + OX + H^+ + MO	0.558
3	MEZL + OX + MO + H^+	0.564
4	OX + MO + MEZL + H^+	0.561

From the results in table 3 the best sequence of addition was drug +Acid + oxidant and the methyl orange dye. Under the same circumstances, other sequences had lower absorbance.

Effect of surfactants: Several types of surfactants have been studied, as it was found that these substances reduce absorption as in (Table 4), so this study was excluded.

Table 4: Effect of surfactants

Surfactant	Absorbance / ml of Surfactant				
1×10^{-3} M	0.0	0.5	1	2	3
Triton X100	0.577	0.198	0.200	0.297	0.298
SDS	0.575	0.488	0.489	0.492	0.488
CTAB	0.574	0.201	0.200	0.199	0.196
CPC	0.578	0.190	0.191	0.186	0.180

Final absorption spectrum: Under the optimum reaction conditions 1 ml of 100 µg. ml^{-1} MBZL solution oxidized by excess of 100 µg. ml^{-1} NBS in acidic medium and after standing for 10 min 1.0 ml of 3.055×10^{-4}mol.L^{-1} MO solution was added, then stand at room temperature for 15 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 508 nm. A (fig. 6) showing the spectrum of the final product for this procedure .

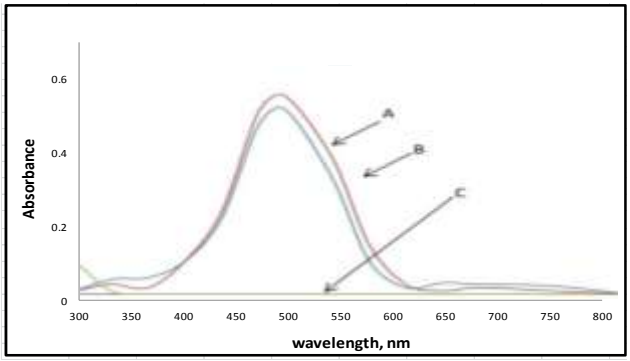


Fig 6: Absorbtion spectra of(A) MO dye only ,(B) MO dye with MBZL VS blank, (C) blanck VS D.W.

Calibration curve: At optimum conditions an increasing volume of 100 µg. ml^{-1} of MEZL solution was added to a series of 10 ml volumetric flasks to cover the rang 2.5 - 25 µg.ml^{-1}, then 0.3 ml of 1 M HCl was added, followed by adding 1.0 ml of 100 µg. ml^{-1} NBS solution.. The solutions were allowed to stand at room temperature for 10 min, Then 1.0 ml of 3.055×10^{-4}mol.L^{-1} MO solution was added, then stand for 15 min and complete to mark with distilled water. The absorbance was measured against reagent blank similarly prepared without drug at 508 nm, (fig. 7) is obtained over the range (2.5 – 25)µg. ml^{-1} with molar absorptivity 1.505×10^4 L.mol^{-1}.cm^{-1} and sandell's sensitivity 0.019607 µg.cm^{-2} .

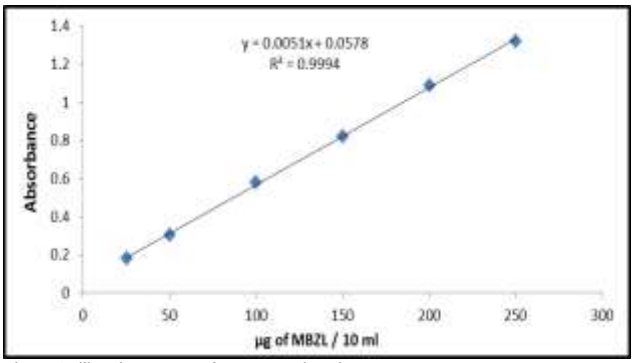


Fig 7: Calibration curve of MBZL Estimation

Accuracy and precision: To calculate the accuracy and precision of the calibration curve, MBZL 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 MBZL µg/10ml present	Amount of MBZL µg/10ml found	Recovery %	Rrelative error, %*	relative standard deviation %*
100	98.44	98.44	-1.56	±0.745
150	151.45	100.97	0.97	±0.608

Average of five determinations

Table 6: effect of interferences

Interference	Recovery (%) of 100 µg MBZL / 10ml of Interference			
	20	40	100	200
Starch	99.39	99.22	99.02	98.44
Glucose	99.61	99.41	97.27	97.46
Arabic gum	99.41	100.19	100.03	100.01
Succrose	99.62	99.39	99.22	99.31
Lactose	99.37	100.07	100.11	99.62

Effect of interferences: The effect of the presence of some drug additives usually found in pharmaceutical preparations under optimal conditions was studied by adding different amounts of these additives to 100 µg MBZL/10 ml. The results obtained in (Table 6) demonstrate the absence of a significant overlap of these substances, which indicates the efficiency and selectivity of the method for its pharmaceutical applications.

Application of the method: The suggested method was successfully applied to determine the drug in their commercial preparations (tablets). The results in (table 7) indicated that the method is accurate and reproducible.

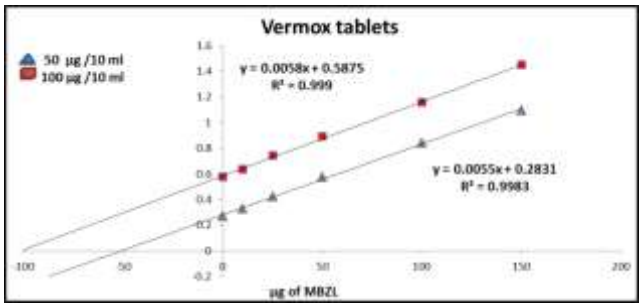
Table 7: application of the method

Drug	Amount of MBZLµg/10 ml	Recovery %	Rrelative error, %*	relative standard deviation, %*
Vermox 100 mg/tablet U.K	100	97.34	2.66	±0.48
	200	101.45	1.45	±0.192
Antiver 100 mg/tablet Egypt	100	102.27	2.27	±1.34
	200	104.24	4.24	±1.171

Average of three determinations

Table 8: evaluation of the suggested method

pharmaceutical preparation	Amount of MBZL presence µg/10 ml	Amount of MBZL measured µg/10 ml	Recovery %
Vermox 100 mg/tablet U.K	50	51.47	102.94
	100	101.29	101.29
Antiver 100 mg/tablet Egypt	50	48.42	96.84
	100	103.22	103.22



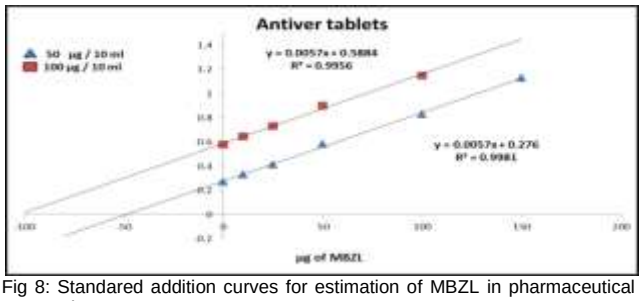


Fig 8: Standared addition curves for estimation of MBZL in pharmaceutical preparations

Evaluation of the suggested method: The standard addition procedure was applied to confirm the validity of the method by adding a fixed volume of the solution containing a fixed amount of the pharmaceutical preparation to a series of 10 ml volumetric vials, then adding increasing amounts of the working solution and adding the rest of the components of the procedure. Complete each vial to the mark with distilled water and mix well. Finally, the absorbance of the solutions was measured at 508 nm. (fig. 8) and results in (Table 8) which show a good agreement between the standard summation method and the suggestion method.

CONCLUSION

An indirect spectrophotometric technique using N-bromosuccinamid and Methyl orange dye for the dedication of mebendazol has been proposed. The method exceedingly selective and touchy and it characterised by simplicity as it did not need to be organized the temperature and extraction steps and the method has been successfully carried out to pharmaceutical preparations with desirable accuracy and precision.

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