Egypt. J. Chem. Vol. 64, No. 9 pp. 4913 - 4917 (2021)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Indirect Spectrophotometric Determination of Mebendazole Using N-Bromosuccinimide as An Oxidant and Tartarazine Dye as Analytical Reagent

Sumayha M. Abbas, Jasim M. S. Jamur*, Takleef D. Sallal

Department of Chemistry, College of Education for Pure Science (Ibn Al- Haitham), University of Baghdad, Iraq.

Abstract

A simple indirect spectrophotometric method for determination of mebendazol in pure and pharmaceutical formulation was presented in this study. UV-Visible spectrophotometry using the optimal conditions was developed for determination of mebendazole in pure drug and different preparation samples. The method is based on the oxidation of drug by n-bromosuccinimide with hydrochloric acid and the left amount of oxidizing agent was determined by the reaction with tartarazine and the absorbance was measured at 428 nm. Calibration curves were linear in the range of 5 to 30 µg.mL^{-1} with molar absorptivity 8437.2 L.mol^{-1} .cm^{-1}. The limits of detection and quantification were determined and found to be 0.7770 µg.mL^{-1} and 2.3400 µg.mL^{-1} respectively. Accuracy and precision were measured by the relative error and RSD and they were found to be < 5% and < 2 respectively. Indirect analysis of mebendazole in different syrup samples was successfully applied using the proposed method.

Key words: mebendazol; indirect spectrophotometric determination; pharmaceutical preparations

1. Introduction

Spectrophotometric methods can be more useful for determination of environmental samples due to their simple and expensive [1,2]. Anthelmintic drugs can play an important role in addressing the issue of helminth infections due to their containing benzimidazole functional group [3]. Mebendazole (MBZ) is one of the most widely used groups for the treatment of these infections and intestinal infections. MBZ is the anticancer and anti-parasitic agents and has been extensively used as inhibitor for tubulin polymerase [4]. MBZ is also frequently prescribed for anthelmintic and recent work by researchers has shown that it can be used as a therapy of medulloblastoma and glioblastoma [5]. However, the performance of MBZ is limited by bioavailability due to its incomplete absorption by oral administration [6,7]. Chemical structure of MBZ is methyl N-(6-benzoyl-1H-benzimidazol-2yl) carbamate as shown in Figure 1 [8].

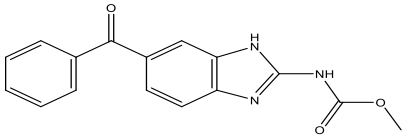

Fig. 1. Chemical Structure of Mebendazol.

A number of analytical methods including voltammetry [9], spectroscopy (FTIR and Raman) [10], spectrophotometry [11,12], chromatography (HPLC) [13], and hyphenated mass spectrometry have been developed for determination of MBZ in different pharmaceutical forms. In a study by Naguib *et al.*, MBZ was determined in pure forms and pharmaceutical formulation using simple and cost-effective UV-VIS spectrophotometer [14]. A method of UV-Visible spectrophotometer has been developed and used to determine MBZ in pure and dosage forms using oxidative coupling reaction with 4-

*Corresponding author e-mail: jasimphduk@gmail.com
Receive Date: 21 March 2021, Revise Date: 15 April 2021, Accept Date: 24 April 2021
DOI: 10.21608/EJCHEM.2021.68614.3509

bromoaniline in the presence of N-bromosuccinimide [15]. MBZ in Vermox Plus tablets was determined by high performance thin layer chromatography (HPTLC) and RP-HPLC using isocratic separation [16]. RP-HPLC method using UV-detection has also been used for determination of MBZ in pure and pharmaceutical tablets [17]. Some physicochemical properties including the behavior of MBZ were described by HPLC method using thermodynamic values [3]. Prabhu and co-worker have developed RP-UPLC method to determine MBZ and its impurities in fixed dose combination [18]. HPLC method with UV detection has also been developed and used to determine MBZ in plasma and muscle samples in Chinese aquaculture [19]. Different forms of MBZ polymorphs were quantified in pharmaceutical raw materials using terahertz-time domain spectroscopy and partial least squares methods [20]. Xu and co-workers have determined MBZ and its metabolites in muscles of bluntnose black bream, eel, turtle and shrimp using liquid chromatography-tandem mass spectrometry (LC-MS/MS) [21]. The objective of this research is to use simple indirect method to determine mebendazol drug in pharmaceutical forms using oxidant reagent.

2. Experimental

2.1. Apparatus

A double beam spectrophotometer UV-Vis instrument was used for all measurements, (Kyoto-Shimadzu Model 1800, Japan). Absorbance was recorded for all samples and blank backgrounds using a 1 mm quartz cell, measuring across 200-800 nm.

2.2. Chemicals and reagents

Pure mebendazol was manufactured by the State for Drugs and Medical Appliances (S.D.I.), Samarra, Iraq. Two mebendazol syrup formulations (20 $\mu g.mL^{-1}$ of mebandazol and Veromx) were supplied by S-awa company, Erbil, Iraq and Belgium respectively.

2.3. Standard and sample preparation

A 0.05 g of MBZ was dissolved in 40 ml ethanol and then diluted to 500 ml distilled water to prepare 100 $\mu g.mL^{-1}$ of stock solution. Tartarazine solution was prepared by dissolving 0.1 g in 100 ml distilled water after it which diluted ten times to be 1.87×10^{-4} M. A solution of 1.12×10^{-2} M of n-bromosuccinimide was prepared by dissolving 0.2 g in 100 ml distilled water. 8.5 ml of hydrochloric acid was diluted to 100 ml distilled water to prepare 1 M. A solution of 100 $\mu g.mL^{-1}$ for both MBZ and veromx syrups was prepared by taking 0.5 ml of each and mixed with 10 ml ethanol and then diluted to 100 ml distilled water.

2.4. General procedure

Different concentrations (5, 10, 15, 20, 25, 30 $\mu g.mL^{-1}$) of MBZ solutions were transferred into six volumetric flasks. 0.1 ml of hydrochloric acid and 0.4 ml N-bromosuccinimide were added to each flask and shaking for 5 minutes, then 1 ml of tartarazine was added to all flasks. All solutions were left for 15 minutes at room temperature after they which diluted with distilled water. The absorbance of left amount of tartarazine was measured at 428 nm.

3. Result and Discussion

Various parameters such as tartarazine volume, n-bromosuccinimide volume, type of acid, concentration of acid and stability of tartarazine were studied to optimise the methodology. The optimal conditions were found to be 1 ml of tartarazine volume and 0.4 ml of NBS that can completely bleach tartarazine dye, Figure 1.

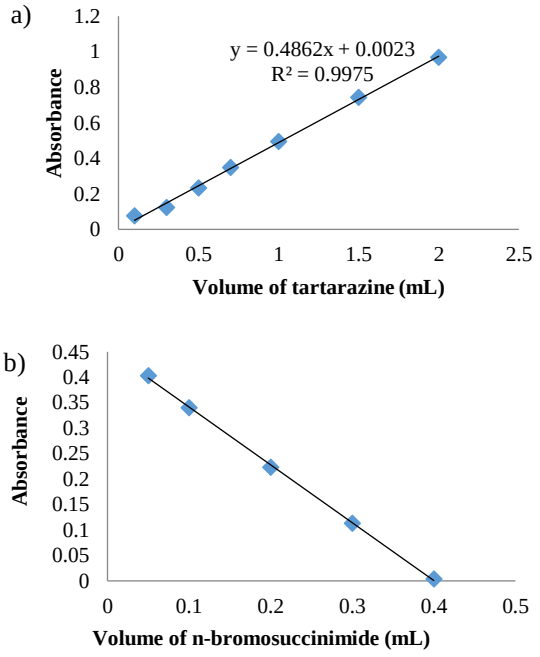

Fig. 1. Optimisation of Reaction Conditions of Volume of Tartarazine (a) and Volume of N-Bromosuccinimide (b)

To identify the optimal acid to analyse the oxidizing processes, different acids were tests: HCL,

H_2SO_4, CH_3COOH and HNO_3. HCL was found to be the suitable for this purpose, Table 1. The stability of tartarazine was studied by measuring the absorbance at different times after oxidizing processes. As shown in Figure 2, the tartarazine was stable with a time of 120 minutes.

Table 1: Effect of Type and Volume of Acid on the Absorbance

Acid type (1 M)	Volume (mL) 0.1	0.2	0.3
	Absorbance		
HCL	0.232	0.227	0.197
H_2SO_4	0.143	0.198	0.226
CH_3COOH	0.206	0.220	0.189
HNO_3	0.214	0.186	0.204

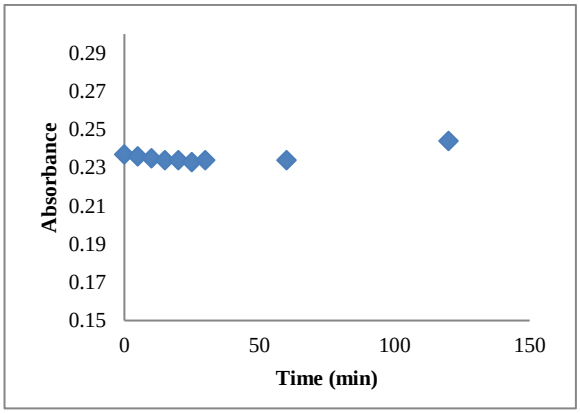

Fig. 2. The Stability of Tartarazine after Oxidizing Processes

MBZ was oxidized by n-bromosuccinimide (NBS) in the presence of HCL and the excess amount of oxidizing agent (NBS) leads to bleaching the tartarazine which was measured at 428 nm using different concentrations of MBZ (5, 10, 15, 20 $\mu g.mL^{-1}$), Figure 3.

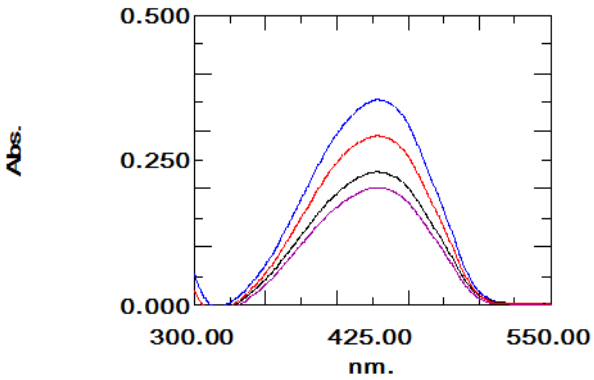

Fig. 3. UV/Vis Spectra of Tartarazin Using Different Concentrations of (20 $\mu g.mL^{-1}$), (15 $\mu g.mL^{-1}$), (10 $\mu g.mL^{-1}$), (5 $\mu g.mL^{-1}$)

3.1. Validation of method

3.1.1. Calibration curve and sensitivity

A positive correlation was found between absorbance and concentration of MBZ with the range 5-30 $\mu g.mL^{-1}$ using the optimal conditions of materials. The residual plot showed random distribution of error and no presence of systematic error, Figure 4. Good analytical characteristics of proposed method were found and were listed in Table 2. The results of this study did not show any significant difference between the present method and the other spectrophotometric methods, Table 3.

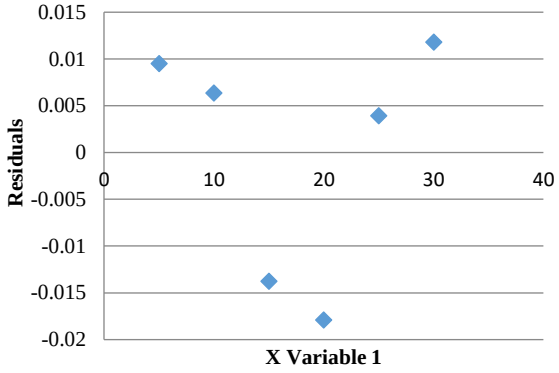

Fig. 4. Residual Plot of Random Distribution of Errors

Table 2: Analytical Characteristics of Proposed Method

Parameter	Proposed method
λmax (nm)	428
Linear range ($\mu g.mL^{-1}$)	5-30
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	8437.2
Regression equation	y = 0.0159x + 0.0754
Sandell's Sensitivity ($\mu g.cm^{-2}$)	0.0633
Correlation coefficient	0.9994
LOD ($\mu g.mL^{-1}$)	0.7770
LOQ ($\mu g.mL^{-1}$)	2.3400

Table 3. Comparison of Analytical Characteristic with Other Spectrophotometric Methods

Reagent	λmax	Beers law	LOD $\mu g.mL^{-1}$	Reference
Bromocresol green	430	1.0-20.0	0.06	11
Bromocresol green in acid	440	0.5-10	0.01	11
Bromocresol green in base	600	0.2-8	0.02	11
Sodium hypochlorite	570	1.25-25	0.11	12
4-bromoanline	434	0.6-2.8	0.04	15

3.1.2. *Selectivity study*

In order to study the selectivity, some of the substances such as vanillin, acacia, cellulose, stearic acid, starch and Na-stearate were added to drug solution (10 µg.mL^{-1}). This is achieved by measure the recovery of tartarazine residual after oxidizing process, Table 4.

Table 4: Effect of Interferences on the Determination of 10 µg.mL^{-1} of MBZ

0.1 ml interfering	MBZ measured (µg.mL^{-1})	% Recovery
Vanillin	9.8	98.0
Acacia	10.9	109
Cellulose	9.9	99.0
Stearic acid	9.4	94.0
Starch	10.1	101
Na-stearate	10.2	102

3.1.3. Accuracy and precision

To investigate the accuracy and precision, different concentrations (5, 10 and 20 µg.mL^{-1}) of pure drug were taken and measured three times using the optimal conditions, Table 5. As indicated in this Table, the proposed method can be used to determine MBZ in pharmaceutical formulations.

Table 5

Statistical Analysis for Determination of MBZ. Where R.E. Is A Relative Error and C.V. Is the Coefficient of Variation

MBZ taken (µg.mL^{-1})	MBZ measured (µg.mL^{-1})	% R.E.	% C.V
5	5.20	4.00	1.99
10	10.3	3.6	1.14
20	24.2	2.6	0.46

3.1.4. Applications of the proposed method in some pharmaceutical formulations

The amount of MBZ drug in different pharmaceutical formulations such as MBZ and veromx syrups was determined using the proposed method. Various concentrations of 5, 10 and 20 µg.mL^{-1} for both dosage formulation solutions (20 mg.ml^{-1}) were spiked to 10 mL volumetric flasks and measured five times at 428 nm. The results as shown in Table 6, indicate that the method can be used to analyse pharmaceutical formulations containing MBZ.

Table 6: UV-Vis Spectrophotometric Determination of MBZ Drug in Pharmaceutical Formulations

Sample (20 mg.mL^{-1})	MBZ taken µg.mL^{-1}	MBZ measured µg.mL^{-1}	% C.V	% Recovery
Mebendazole -S Awa	5 10 20	5.20 10.8 19.8	0.01199 0.01669 0.03568	104 108 99.0
Vermox	5 10 20	5.40 10.6 19.70	0.04762 0.01317 0.05352	108 106 98.5

4. Conclusions

This study has shown that the proposed method is simple, accurate, fast and suitable to determine the mebendazol in pure and pharmaceutical dosage forms. The method using the optimal conditions was applied successfully to quantify mebendazol in different samples such as mebendazole-S Awa and veromx syrups. The method can be applied to other drugs in various formulations such as tablets and ampoules.

5. References

[1] Kandhro, G. A., Soylak, M. & Kazi, T. G. Solid phase extraction of thorium on multiwalled carbon nanotubes prior to UV-Vis spectrophotometric determination in ore samples. *Atomic Spectroscopy* **35**, 270–274 (2014).

[2] Khan, S. *et al.* Cloud point extraction of vanadium in pharmaceutical formulations, dialysate and parenteral solutions using 8-hydroxyquinoline and nonionic surfactant. *Journal of Hazardous Materials* **182**, 371–376 (2010).

[3] Poturcu, K. & Demiralay, E. Ç. Determination of Some Physicochemical Properties of Mebendazole with RPLC Method. *Journal of Chemical and Engineering Data* **64**, 2736–2741 (2019).

[4] Andersson, C. R. *et al.* Mebendazole is unique among tubulin-active drugs in activating the MEK–ERK pathway. *Scientific Reports* **10**, 1–10 (2020).

[5] De Witt, M. *et al.* Repurposing mebendazole as a replacement for vincristine for the treatment of brain tumors. *Molecular Medicine* **23**, 50–56 (2017).

[6] Guerini, A. E. *et al.* Mebendazole as a candidate for drug repurposing in oncology: An extensive

review of current literature. *Cancers* **11**, 1–22 (2019).

[7] Kipper, F. C. *et al.* Vinblastine and antihelmintic mebendazole potentiate temozolomide in resistant gliomas. *Investigational New Drugs* **36**, 323–331 (2018).

[8] Roque-Flores, R. L., de Oliveira, J. F., de Souza Carvalho, F. M. & do Rosário Matos, J. Characterization and Kinetic Studies of the Thermal Degradation of Mebendazole Polymorphs A and C. *Journal of Pharmaceutical Innovation* **15**, 116–124 (2020).

[9] de Macedo, I. Y. L. *et al.* Differential Pulse Voltammetric Determination of Albendazole and Mebendazole in Pharmaceutical Formulations Based on Modified Sonogel Carbon Paste Electrodes with Perovskite-Type LaFeO 3 Nanoparticles . *Journal of The Electrochemical Society* **163**, B428–B434 (2016).

[10] Gunasekaran, S. & Uthra, D. Vibrational spectra and qualitative analysis of albendazole and mebendazole. *Asian Journal of Chemistry* **20**, 6310–6324 (2008).

[11] Swamy, N. & Basavaiah, K. Selective and sensitive assay of mebendazole in pharmaceuticals using bromocresol green by spectrophotometry. *Thai Journal of Pharmaceutical Sciences* **37**, 171–185 (2013).

[12] Swamy, N., Prashanth, K. N. & Basavaiah, K. Spectrophotometric Assay of Mebendazole in Dosage Forms Using Sodium Hypochlorite. *Journal of Applied Spectroscopy* **81**, 525–533 (2014).

[13] Kulik, A., Białecka, W., Podolska, M., Kwiatkowska-Puchniarz, B. & Mazurek, A. HPLC method for identification and quantification of benzimidazole derivatives in antiparasitic drugs. *Acta Poloniae Pharmaceutica - Drug Research* **68**, 823–829 (2011).

[14] Naguib, I. A., Abdelaleem, E. A., Hassan, E. S. & Emam, A. A. Comparative study of eco-friendly spectrophotometric methods for accurate quantification of Mebendazole and Quinfamide combination; Content uniformity evaluation. *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy* **235**, 118271 (2020).

[15] Sallal, T. D. & Abass, S. M. Oxidative coupling reaction for micro trace analysis of mebendazol residual with p-bromoaniline in presence of n-bromosuccinimide. *Baghdad Science Journal* **17**, 780–786 (2020).

[16] Naguib, I. A., Hassan, E. S., Emam, A. A. & Abdelaleem, E. A. Development and Validation of HPTLC and Green HPLC Methods for Determination of a New Combination of Quinfamide and Mebendazole. *Journal of chromatographic science* **58**, 16–21 (2019).

[17] Gopala Krishna, M. & Subba Rao, M. RP-HPLC Technique for the Assurance of Mebendazole In Unadulterated and In Tablet Measurements. *American Journal of PharmTech Research* **10**, 221–230 (2020).

[18] Prabhu, R. C. & Maruthapillai, A. New RP-UPLC method development using QbD approach for determination of mebendazole, quinfamide, its impurities and antioxidants in mebendazole and quinfamide fixed dose combinations (FDC). *Materials Today: Proceedings* 0–6 (2020). doi:10.1016/j.matpr.2020.04.243

[19] Xiao, X., Zhao, Y., Zhou, Y. & Wang, Z. Plasma pharmacokinetics and muscle residue dynamics of mebendazole in Carassius auratus. *Journal of Veterinary Pharmacology and Therapeutics* **40**, 670–674 (2017).

[20] Da Silva, V. H., Vieira, F. S., Rohwedder, J. J. R., Pasquini, C. & Pereira, C. F. Multivariate quantification of mebendazole polymorphs by terahertz time domain spectroscopy (THZ-TDS). *Analyst* **142**, 1519–1524 (2017).

[21] Xu, N. *et al.* Development of a liquid chromatography–tandem mass spectrometry method with modified QuEChERS extraction for the quantification of mebendazole and its metabolites, albendazole and its metabolites, and levamisole in edible tissues of aquatic animals. *Food Chemistry* **269**, 442–449 (2018).