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ORIGINAL ARTICLE

Spectrophotometric Method for the Determination of Mebendazol in Different Formulation

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ABSTRACT
This research presents a new spectrophotometric method used to determine mebendazole using oxidation and coupling reaction. This method is based on using potassium chromate as an oxidizing agent for catechol, which is then combined with imibendazole to form a stable product with brown color. The highest absorption is achieved at 402 nm. Moreover, absorbance coefficient is 1556.0794 l.mol-1.cm-1. Beer's law limits were (4-140) µg/ml.
Keywords: Spectrophotometry, mebendazol , Oxidative coupling

INTRODUCTION
Mebendazole or methyl (6-benzoyl-1-hydrobenzimidazole-2-yl) carbamate or methyl 5-)-benzoyl-1-hydrobenzimidazole-2-yl) carbamate has a molecular formula $C_{16}H_{13}N_3O_3$) [1] with a molecular weight of 295.30 g/mol [2]. It is worth noting that it can be in the form of an almost amorphous powder, odorless and with a slightly yellowish-white color [3], and has the following structural formula as shown in Fig. (1) [4]. Mebendazole is one of the most important (broad-spectrum) drugs that can be used for the treatment of parasitic worms [5]. The unintentional ingestion of the eggs of Ascariasis (roundworm) or Trichuris Trichora (the whipworm) and the eggs and larvae of Ancylostoma duoctenale (which may infect the duodenum), The main cause of infection with this disease [6]. Mebendazol has been determined by different kinds of analytical techniques in various formulations and some biological liquids. This can include spectrophotometric methods [7]–[11], Chromatography methods [12]–[15] and electric ways [16]–[20].

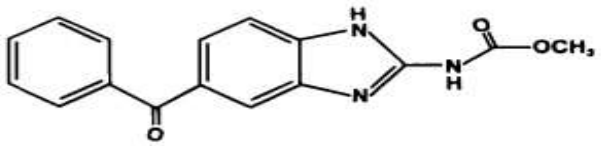

Fig 1: Mebendazol

Experiments
Apparatus: A Shimadzu UV-VIS 1800 digital double-beam recording spectrophotometer (Kyoto, Japan) is used to measure all spectral and absorbance levels with matched 1-cm quartz cells
Reagents: All Chemicals included in the experiments are of the highest purity available.

Mebendazole (MBZ) solution: (100) micro g/ml: This solution is prepared by dissolving 0.01 g of mebendazole in 10 ml of (1M) sodium hydroxide with heating at boiling point for five minutes, then the pH value is adjusted at PH=7 with hydrochloric acid (1M) in addition to fill the volume with distilled water to the mark in a 100ml volumetric vial.
Potassium dichromate solution (5 x 10^{-3} M): This solution is prepared by dissolving 0.07 g of dichromate in 2.5 ml of sulfuric acid at a concentration of 1 M, the volume is then filled with distilled water in a volumetric bottle of 50 ml.
Hydrochloric acid solution: A diluted (0.05M) was used.
Catechol (100 µg/ml): Catechol is prepared by dissolving 0.01 g of catechol in 100 ml distilled water.
Pharmaceutical solution: The contents of ten tablets of the medicinal preparation Albendazole (albena) is weighed, crushed and mixed well, then weighed the equivalent of one tablet (200 mg of albendazole). This is hydrolyzed and then processed in the same manner as described in the tablet analysis.

RESULTS AND DISCUSSION
2 ml of catechol concentration (100 µg/ml) is added to a series of 25 ml titrated flask and 1.5 ml of potassium chromate (5 x 10^{-3} M), this is then followed by the addition of increasing volumes of mebendazole solution (100 µg/ml). This is completed to the mark with distilled water and the reaction mixture was left for 5 min. The absorbance of each solution was measured at 402 nm against the vacuum prepared in the same way but without mebendazole.
Study of the Optimum Reaction Conditions: The effects of different parameters in addition to its relation with the mentioned product is studied and presented in this section. Moreover, the optimum conditions are provided.
Effect of the potassium chromate: The effect of potassium chromate was studied and the absorbance was measured at 402nm versus blank

Table 1: Effect of the potassium chromate

ml of K_2CrO_4 (5×10^{-3} M)	Absorbance/min.						Blank
	0	5	10	15	20	30	
1	0.322	0.321	0.320	0.322	0.321	0.321	0.153
1.5	0.432	0.433	0.432	0.433	0432	0.431	0.123
2	0.402	0.401	0.402	0.403	0.401	0.402	0.251
2.5	0.354	0.354	0.353	0.355	0.351	0.351	0.145
3	0.212	0.213	0.211	0.210	0.211	0.212	0.095

The result shows that the dye formation reached the maximum with 1.5 ml of potassium chromate.
Effect of reagent concentration: Different volumes of catechol (1000 ppm) are added into a series of 25ml calibrated flask to present the effect of reagent concentration. The absorbance was measured at 402 nm versus blank. The results included in Table (2) indicate that the use of 2 ml of (1000 ppm) catechol reagent gives the maximum color intensity.
The result shows that the dye formation reached the maximum with 2ml of catechol.

Table 2: Effect of the concentration of reagent on absorbance.

Reagent conc.(ml)	Absorbance
0.5	0.123
1	0.234
1.5	0.342
2	0.432
2.5	0.375

Effect of temperature: This study provides the effect of temperature on the absorbance of the coloured product. This was

implemented by placing three 25ml calibrated flasks, 2ml of (100ppm) catechol, 1.5 ml of (5×10^{-3}M) potassium chromate, followed by 2ml of (100μgml^{-1}) Mebendazol solution. The solution was diluted to the mark with distilled water and the first flask was allowed to be put up with increasing time at room temperature, the second was at 5°C and the third in water bath at 50°C. The absorbance was measured at 402nm at different periods versus blank prepared in the same way but containing no Mebendazol. The results included in Table (3) indicate that the absorbance of the coloured product was decreased when the reaction was carried out at 0°C or 50°C. Therefore, it is recommended that the reaction mixture should be carried out at room temperature.

Table 3: Effect of temperature on absorbance of colored product.

Temp. ºC	Absorbance/minutes							
	0	5	10	15	20	25	30	40
5	0.021	0.027	0.032	0.034	0.038	0.041	0.048	0.059
R.T.	0.432	0.431	0.433	0.432	0.431	0.433	0.432	0.433
50	0.502	0.572	0.552	0.542	0.537	0.514	0.501	0.488

R.T.=Room temperature=25 C

Stability of the product: The stability of the product was studied by placing 2ml of (100ppm) catechol, into a series of 25ml graded flasks, followed by 2 ml of (5×10^{-3}M) potassium chromate and 2ml of (100μgml^{-1}) Mebendazol. The solution was diluted to the mark with distilled water and the absorbance was measured at 402nm at different periods versus reagent blank. The results included in Table (4) show that the product needs 5 minutes to attain maximum absorbance and it remains stable for about 50 minutes.

Table 4: Rate of reaction and stability of product.

Time (min)	0	5	15	20	25	30	35	40	45	50	55	65
Absorbance	0.432	0.433	0.433	0.432	0.433	0.432	0.431	0.433	0.434	0.433	0.430	0.427

Order of addition of reagents: The reagent 2ml of (100ppm) catechol (R), the oxidant 1.5ml of (5×10^{-3}M) (ox) and the sample 2ml of (100μgml^{-1}) solution Mebendazol, were mixed in various orders as shown in Table (4).

Table 4: Effect of order of addition on the absorbance of the coloured product.

Reaction components	Order number	Absorbance at 402nm
R+OX+S	I	0.432
R+S+OX	П	0.031
S+R+OX	Ш	0.152
OX+S+R	IV	0. 093

The results indicate that order (I) gives the highest absorbance of the product. Therefore, this is considered in all subsequent experiments.

Final absorption spectra: Mebendazol complex is formed based on the optimum conditions described above. An absorption spectrum is ranging between 200 and 650 nm with a maximum absorption at 402nm. This is different in comparison with the reagent blank which shows small absorption at λ max. Therefore, the maximum absorption at 402nm is considered in the subsequent experiments.

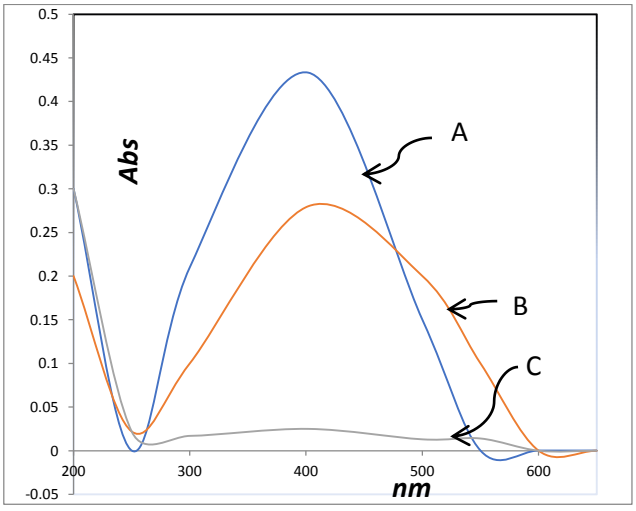

Fig 2: Absorption spectra of 8μgml-1 Albendazol measured, (A) sample against blank, (B) sample against distilled water, (C) Blank against distilled water

Quantification: A calibration graph is presented based on two main parameters including: absorbance versus concentration. Moreover, Beer's law is provided in the range of (4-140) μg /ml of the solution as shown in Fig (3).

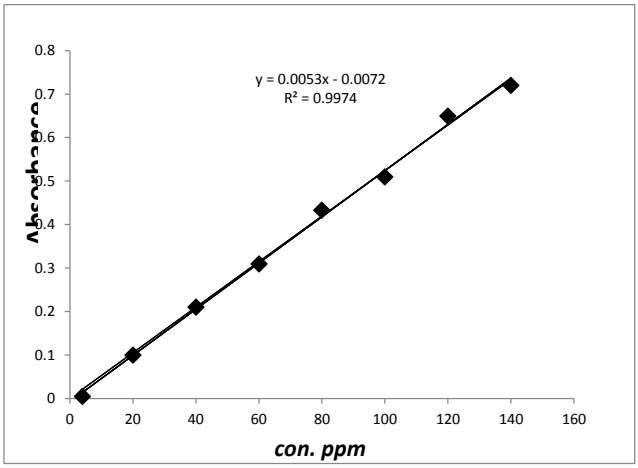

Fig 3: Calibration graph for the determination of Mebendazole

Accuracy and precision of the method :Mebendazol is determined at three concentrations in order to present the accuracy and precision of the method. The results included in Table (8) indicate that the method is performing well.

Table 8: Accuracy and precision of the method

Amount of taken, μg/ Mebendazol ml	Amount of Mebendazol faund, μg/ml	Relative error, % *	Relative standard deviation, % *
20	19.8	+1.0	±10.94
80	80.12	-0.15	±0.316
120	120.9	-0.75	±0.193

* Rate 6 replicates

Nature of the product: The stoichiometry of the reaction between Mebendazol and catechol in the presence of potassium chromate is investigated based on the mole–ratio method. In this experiment, 1.5 ml of potassium chromate (5×10^{-3}M) was added into a series of 25ml calibrated flask followed by increasing volumes of catechol (100ppm) and 2 ml of Mebendazol. The solutions are diluted to the mark with distilled water. Then after, the

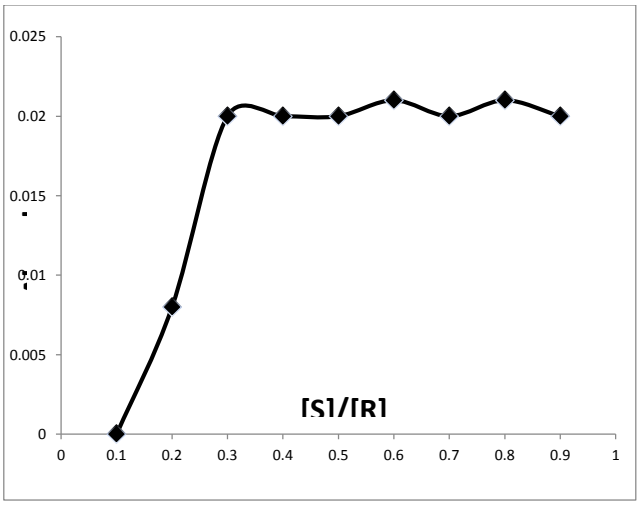

Fig 4: Mole–ratio plot for Mebendazol: catechol reagent in the presence of potassium chromate

reaction mixture was put up for 5 minutes. The absorbance of each solution was measured at 402nm versus blank. The results shown in Fig. (4) exhibit the existence of a 1:1 Mebendazol.: catechol.

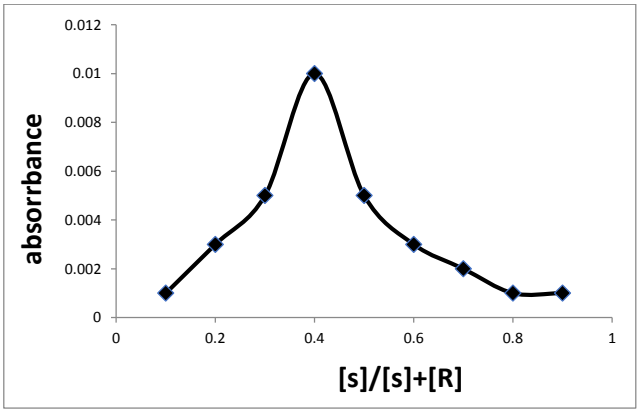

Fig 5: Jops method for Mebendazol: catechol reagent in the presence of potassium chromate.

Application of the method:

Table 9: Accuracy and precision of application of the method on the pharmaceutical drug

Pharmaceutical formulation	Amount of Mebendazol taken, µg	Amount of Mebendazol faund, µg/ml	Relative error, % *	Relative standard deviation, % *
tablets Mebendazol (Albena)	20	19.7	+1.5	±0.325
	80	79.2.	+1.0	±0.491
	100	99.5	+0.5	±0.412

* Rate 6 replicates

CONCLUSION

A new spectrophotometric method was proposed to determine aqueous Mebendazol solution. The method was based on the coupling of Mebendazol with catechol reagent in the presence of potassium chromate to present a colored dye which provided maximum absorption at 402 nm. The molar absorptivity is 1556.0794 $l.mol^{-1}cm^{-1}$. The proposed method was applied for the determination of benzocaine in two synthetic pharmaceuticals.

REFERENCES

1 N. C. for B. Information, "PubChem Compound Summary for CID 4030, Mebendazole," National Center for Biotechnology Information. https://pubchem.ncbi.nlm.nih.gov/compound/Mebendazole.

2 A. A. Al-Badr and M. Tariq, "Mebendazole," in Analytical Profiles of Drug Substances, vol. 16, Elsevier, 1987, pp. 291–326.

3 D. R. Parakh, M. P. Patil, S. S. Sonawane, C. P. Jain, and others, "Development and validation of spectrophotometric method for estimation of mebendazole in bulk and pharmaceutical formulation," World J. Pharm. Res., vol. 4, no. 7, pp. 2223–2235, 2015.

4 A. E. Guerini et al., "Mebendazole as a candidate for drug repurposing in oncology: An extensive review of current literature," Cancers (Basel)., vol. 11, no. 9, p. 1284, 2019.

5 S. Xu et al., "In vitro efficacies of solubility-improved mebendazole derivatives against Echinococcus multilocularis," Parasitology, vol. 146, no. 10, pp. 1256–1262, 2019.

6 S. A. Silber et al., "Efficacy and safety of a single-dose mebendazole 500 mg chewable, rapidly-disintegrating tablet for Ascaris lumbricoides and Trichuris trichiura infection treatment in pediatric patients: a double-blind, randomized, placebo-controlled, phase 3 study," Am. J. Trop. Med. Hyg., vol. 97, no. 6, p. 1851, 2017.

7 N. R. Ahmad, F. K. Omar, and others, "A New Method For Estimation Mebendazole In Its Pharmaceutical Preparations And In Camel Urine," Basrah J. Vet. Res., vol. 17, no. 2, 2018.

8 S. M. Derayea, H. R. H. Ali, A. A. Hamad, R. Ali, and others, "Application of silver nanoparticles for the spectrophotometric determination of three benzimidazole anthelmintic drugs in their pharmaceutical preparations," J. Appl. Pharm. Sci., vol. 7, no. 02, pp. 76–82, 2017.

9 A. G. Dhandar, S. B. Ganorkar, A. S. Patil, and A. A. Shirkhedkar, "Development and validation of UV spectrophotometric method for simultaneous estimation of Quinfamide and Mebendazole in in-house pharmaceutical formulation," 2018.

10 S. M. Abbas, J. Jamur, and T. D. Sallal, "Indirect spectrophotometric determination of Mebendazole using n-bromosuccinimide as an oxidant and tartarazine dye as analytical reagent," Egypt. J. Chem., vol. 64, no. 9, pp. 1–2, 2021.

11 R. M. Alghanmi and L. Y. Alhazmi, "Spectrophotometric determination of mebendazole through charge transfer interaction," J. pharm. sci. res, vol. 10, no. 5, pp. 2504–2515, 2019.

12 R. Prasad Pawar et al., "Use of Micellar Liquid Chromatography to Determine Mebendazole in Dairy Products and Breeding Waste from Bovine Animals," Antibiotics, vol. 9, no. 2, p. 86, 2020.

13 I. A. Naguib, E. A. Abdelaleem, A. A. Emam, N. W. Ali, and F. F. Abdallah, "Development and validation of HPTLC and green HPLC methods for determination of furosemide, spironolactone and canrenone, in pure forms, tablets and spiked human plasma," Biomed. Chromatogr., vol. 32, no. 10, p. e4304, 2018.

14 S. A. Dar et al., "Determination of benzimidazoles in fish plasma by chromatographic method and their effects on metabolic and antioxidative enzymes activity," Aquaculture, vol. 486, pp. 57–63, 2018.

15 M. R. Delfino, C. M. Monzón, N. L. Jorge, and M. del C. T. Sarno, "Mebendazole spectrophotometric determination: theorical and experimental study of the interaction with sodium hydroxide," 2016.

16 L. Xu, F. Luan, L. Wang, H. Liu, and Y. Gao, "Development of a capillary zone electrophoresis method for determination of mebendazole and levamisole hydrochloride in a combined tablet and a comparison with a LC method," J. AOAC Int., vol. 97, no. 1, pp. 128–132, 2014.

17 D. V. Thomaz et al., "Electrochemical Study o f Albendazole and Mebendazole Adsorbates on Activate d Charcoal Modified Electrode," RJLBPCS, vol. 4, pp. 156–163, 2018.

18 M. Ghalkhani and S. Maghsoudi, "Development of an electrochemical differential pH sensing system based on mebendazole and potassium ferrocyanide," J. Mater. Sci. Mater. Electron., vol. 28, no. 18, pp. 13665–13672, 2017.

19 M. Ghalkhani, J. Beheshtian, and M. Salehi, "Electrochemical and DFT study of an anticancer and active anthelmintic drug at carbon nanostructured modified electrode," Mater. Sci. Eng. C, vol. 69, pp. 1345–1353, 2016.

20 J. S. Lee et al., "A Liquid Chromatography--Tandem Mass Spectrometry Approach for the Identification of Mebendazole Residue in Pork, Chicken, and Horse," PLoS One, vol. 12, no. 1, p. e0169597, 2017.