Spectrophotometric Determination of Mebendazole using Diazotization Reaction and Coupling with m-Aminophenol Reagent

*Rowa F. Mohammed Farha Kh. Omar
Department of Chemistry/ College of Education for Girls/ University of Mosul
*E-mail: rowa.gep15@student.uomosul.edu.iq

ABSTRACT

A simple and sensitive spectrophotometric method has been developed for the determination of mebendazole (MBZ) in its pure and tablet form. The method is based on alkaline hydrolysis of MBZ with sodium hydroxide to give primary amine product which reacts with sodium nitrite in an acidic medium (hydrochloric acid) to yield diazotized mebendazole (D-MBZ) which is coupling with m-aminophenol reagent (mAP) to form an azo dye. The absorbance of the azo dye for this suggested method (yellow dye) has been measured at 360 nm. Beer's law for this proposed method is in the range of (0.5-20 µg.ml⁻¹), the molar absorptivity value is 1.63×10⁴ L.mol⁻¹.cm⁻¹ and the Sandell's value index is calculated and equal to 0.018 µg.cm²⁻¹. Also, the limit of detection and the limit of quantification are calculated and equal to 0.1718 µg.ml⁻¹ and 0.5726 µg.ml⁻¹ respectively. The ratio of the formed azo dye [D-MBZ: mAP] is [2: 1].

Keywords: Spectrophotometric, Diazotization and coupling, Mebendazole, m-Aminophenol reagent.
INTRODUCTION

Mebendazole (MBZ) or methyl N-(6-benzoyl-1H-benzimidazole-2-yl) carbamate Fig. (1) is a synthetic benzimidazole derivate and anthelmintic agent used commonly for roundworm (pinworm and hookworm) infections, trichinosis, capillariasis and toxocariasis and other parasitic worm infections by inhibiting the formation of their cytoplasmic microtubules, thereby selectivity and irreversibly blocking glucose uptake. This eventually causes the helminths death (National Center for Biotechnology Information. PubChem, 2021). In recent times, the drug was shown to display promising antitumor activity, especially in cases of colon cancer, medulloblastoma and glioblastoma (Calvo et al., 2016).

![Chemical structure of mebendazole](image)

**Fig. 1: Chemical structure of mebendazole**

Different analytical methods are reported in literature for the assay of mebendazole as free or in its pharmaceutical formulations such as UV-visible spectrometry (Naguib et al., 2020; Murage et al., 2020; Parakh et al., 2015; Swamy and Basavaiah, 2013; Shah et al., 2015; Attia et al., 2015; Delfino et al., 2016), Hyphenated techniques (De Ruyck et al., 2003), RP-UPLC (Prabhu and Maruthapillai, 2021), high performance liquid chromatography (Naguib et al., 2020; YU et al., 2010) and Electrophoresis Method (Xu et al., 2014).

EXPERIMENTAL

**Apparatus:**
- Shimadzu UV- 1800 pc UV-Visible dual beam spectrophotometry.
- Quartz cells 1-cm (Cuvettes).
- pH meter type inolab pH 7110.
- Electronic balance type ADAM.

**MATERIALS AND SOLUTIONS**

All materials used in this proposed research are of high purity.

1- Stock solution of mebendazole (200 μg .ml⁻¹)

0.05 g of (MBZ) mebendazole was dissolved with 10 ml of NaOH 1M (with heating at boiling point) and the acidity was adjusted to pH=7 with HCl and completed by distilled water in a volumetric flask to 250 ml.

2- Sodium nitrite (3.39×10⁻³ M)

This solution was prepared by dissolving 0.0234 g of sodium nitrite in distilled water then transferred into 100 ml volumetric flasks and completed to the mark with distilled water.

3- Hydrochloric acid (1M)

It was prepared with transfer 8.4ml of concentrated HCl into 100 ml volumetric flask and then completed to 100 ml with distilled water.

4- Diazonium salt reagent (3.39 10⁻⁴ M)

D-MBZ solution was prepared by mixing 50 ml of MBZ (stock solution) with 10 ml of sodium nitrite and 1.5 ml of HCl (1M), after shaking well it has completed to the mark with distilled water.

5- m-Aminophenol reagent (0.1%)

This m-aminophenol solution was prepared by transfer 0.1 g of m-aminophenol (mAP) into 10 ml of ethanol and completed to the mark with ethanol in 100 ml volumetric flask.
6- Potassium hydroxide (1M)
This solution was prepared by dissolving 5.61 g of KOH in distilled water in a volumetric flask and completed to 100 ml with distilled water.

7- Drug solution (Vermox 100 μg MBZ /ml)
Three tablets of MBZ (Vermox 100 mg/tablet) (total weight 1.6992 g) were crushed together and mixed well then weighing 0.0566 g of powder and dissolved with 10 ml of NaOH 1M (with heating at boiling point for 5 minutes) and the acidity was adjusted to pH=7 with HCl then added 10 ml of sodium nitrite (3.39×10^-3 M) and 1.5 ml of HCl (1M) and completed to the mark of the volumetric flask (100 ml) by distilled water.

The Procedure and Calibration Curve:
An aliquot of a sample solution containing (0.05-2.0ml) of 100 µg.ml^-1 diazotized MBZ is transferred into a series of 10 ml volumetric flask then 1.5 ml of (0.1% mAP) followed by 1ml of 1M KOH then diluted to the marks with distilled water and measured at 360 nm against blank solution and the results were as in the Fig. (2).

![Graph showing calibration curve](image)

\[ y = 0.0552x - 0.0045 \]
\[ R^2 = 0.999 \]

molar absorptivity and Sandell's index values are calculated and equal to 1.63×10^4 1 / mol.cm and 0.018 μg/cm^2 respectively.

RESULT AND DISCUSSION

The Optimum Amount of Hydrochloric Acid:
Different amount of 1M HCL solution (0.5-2.0ml) has been studied. 1.5 ml of HCl was enough to obtain a maximum absorbance.

The Effect of Temperature on Diazonium Salt:
The effect of temperature was studied at a various temperature on the diazotization of MBZ (see the results in Table 1).

Table 1: Effect of temperature on diazonium salt.

| Temperature, °C | 0  | 5  | Room temperature (25±2) |
|-----------------|----|----|-------------------------|
| Absorbance      | 0.532 | 0.530 | 0.527  |
From the previous results in (Table 1) the difference in absorbance is small between the different degrees of temperature so and for ease we had chosen room temperature in suffix experiments.

**The Suitable Amount of Reagent (mAP):**
The suitable amount of reagent was checked and gave the following results.

**Table 2: Optimum amount of reagent (mAP)**

| Reagent (0.1%, ml) | Absorbance/ µg.ml⁻¹ of MBZ | \( R^2 \) |
|-------------------|----------------------------|----------|
|                   | 2.5 | 5.0 | 8.0 | 10  | 15  |
| 0.2               | 0.071 | 0.189 | 0.304 | 0.422 | 0.601 | 0.9951 |
| 0.5               | 0.096 | 0.202 | 0.339 | 0.452 | 0.645 | 0.9969 |
| 0.7               | 0.109 | 0.216 | 0.370 | 0.497 | 0.701 | 0.9954 |
| 1.0               | 0.116 | 0.232 | 0.392 | 0.509 | 0.732 | 0.9979 |
| 1.5               | 0.127 | 0.251 | 0.404 | 0.529 | 0.762 | 0.9984 |
| 2.0               | 0.129 | 0.243 | 0.407 | 0.518 | 0.742 | 0.9980 |

According to the value of determination coefficient in (Table 2), 1.5 ml of the reagent was chosen as an optimum amount for the next experiments.

**Selection of the Optimum Type and Amount of Base:**
Many types of base has been checked to make certain of the maximum absorbance (Table 3).

**Table 3: Selection of optimum type of base**

| Base (1M, 2ml) | Absorbance | \( \lambda_{\text{max}} \) |
|----------------|------------|--------------------------|
| NaOH           | 0.517      | 360                      |
| KOH            | 0.530      | 363                      |
| Na₂CO₃         | 0.503      | 359                      |

According to the results in (Table 3) potassium hydroxide was fixed in the next experiments. The optimum amount of KOH was tested also and it showed that 1 ml is a suitable amount according to the highest absorbance of azo dye and it in subsequent experiments (Table 4).

**Table 4: optimum amount of KOH**

| KOH (ml, 1M) | Absorbance |
|--------------|------------|
| 0.2          | 0.527      |
| 0.5          | 0.528      |
| 0.75         | 0.531      |
| 1.0          | 0.533      |
| 1.5          | 0.530      |
| 2.0          | 0.531      |

**Order of Addition:**
The sequence of additives were studied to choose the suitable sequences (Table 5).
Spectrophotometric Determination of

Table 5: Order of additives

| Order of additives | Absorbance |
|--------------------|------------|
| I: MBZ + R + OH^− | 0.534      |
| II: MBZ + OH^− + R| 0.526      |
| III: R + OH^− + MBZ| 0.522      |

From the previous results the order (I) has been chosen for the next experiments because of its high absorbance.

The stability of Azo Dye with Time:

Effect of time on the azo dye has been studied and the result showed that the dye is stable for at least 60 minutes and the result illustrated in (Table 6).

Table 6: The stability of azo dye

| Time    | Absorbance of MBZ (μg/10ml) |
|---------|------------------------------|
|         | 25                           | 50                           |
| Immediately | 0.137                      | 0.279                        |
| 5        | 0.136                        | 0.277                        |
| 10       | 0.136                        | 0.277                        |
| 15       | 0.135                        | 0.276                        |
| 20       | 0.135                        | 0.276                        |
| 25       | 0.135                        | 0.276                        |
| 30       | 0.135                        | 0.275                        |
| 35       | 0.134                        | 0.275                        |
| 40       | 0.134                        | 0.274                        |
| 45       | 0.134                        | 0.275                        |
| 50       | 0.133                        | 0.274                        |
| 55       | 0.133                        | 0.274                        |
| 60       | 0.133                        | 0.274                        |

Absorption Spectra:

Absorption spectra of a yellow-colored solution (Azo dye) was formed by the coupling of diazotized MBZ with mAP reagent in alkaline medium. This Azo dye has given a maximum absorbance at 360nm against blank solution as shown in the Fig. (3).
Fig. 3: Absorbance spectra of (A) Azo dye product from proceeding 100 µg of MBZ measured against blank. (B) Azo dye product against distilled water. (C) Blank against distilled water.

The Nature of the Azo Dye:

The structure of the dye (complex ratio) has been studied using Job's method by preparing a series of volumetric flasks (10ml) contains different amounts (0.25-2.75ml) of D-MBZ (3.39×10^{-4} M) with a complementary amount (2.75-0.25ml) of mAP reagent with the same concentration of D-MBZ and finally added 1 ml of KOH (1M) for each one of flask and completed by distilled water and measured at 360 nm.

Fig. 4: Job's plot for diazotized MBZ coupled with mAP

From the previous figure we conclude that the ratio of azo dye [D-MBZ: mAP] is [2 : 1]. The possible reaction path might be written as a follow (Hussin, 2010).
Accuracy and Precision:

In this study the accuracy and precision has been calculated through the measurements of recovery, percent relative error (RE%) and relative standard deviation (RSD%) values by performing five replicates to three concentrations within the calibration curve. The results listed in (Table 7).

Table 7: Accuracy and precision

| Sample                                      | Amount taken, µg | Found, µg | RE%  | Recovery%* | RSD% |
|---------------------------------------------|------------------|-----------|------|------------|------|
| Standard Mebendazole solution (100µg/ml)    | 30               | 29.68     | -1.07| 98.93      | 2.11 |
|                                             | 60               | 60.20     | +0.33| 100.33     | 1.65 |
|                                             | 100              | 97.13     | -2.87| 97.13      | 1.23 |

Application of the Method:

The application of this suggested method has been performed on tablets of mebendazole pharmaceutical (Vermox 100 mg/tablet) through standard addition method by preparing two series of 10 ml volumetric flasks then added increasing amount (0-1.2ml) of standard D-MBZ (100µg/ml) for each one of these flasks and 0.25ml of drug solution (100µg/ml) for each flask of the first series flasks and 0.5ml of the drug for each of the second series flasks then the flasks proceed as mentioned in the standard and curved working method and measured at 360 nm then it gave the following plot and results.

Fig. 5: Standard addition curve of MBZ
Table 8: Accuracy and precision of mebendazole application

| Drug                      | Amount taken, µg | Found  | RE%   | Recovery* | RSD% |
|---------------------------|------------------|--------|-------|-----------|------|
| Vermox /tablet (JANSSEN)   | 25               | 25.38  | +1.53 | 101.53    | 3.25 |
|                           | 50               | 48.55  | -2.9  | 97.10     | 1.33 |

The previous results are very compatible with manufactured drugs results with accepted analytical error.

Comparison of the Methods
The proposed method was compared to another method from literatures (Table 9).

Table 9: Comparison of mebendazole determination methods

| Analytical parameter                  | Proposed method                  | Literature method (Swamy and Basavaiah, 2013) |
|---------------------------------------|----------------------------------|-----------------------------------------------|
| Type of reaction                      | Diazotization                    | Ion-pair                                      |
| \(\lambda_{\text{max}}\) (nm)         | 360                              | 430                                           |
| Reagent                               | m-aminophenol                    | bromocresole green dye                        |
| Color of the dye                      | Yellow                           | Yellow                                        |
| Molar absorptivity coefficient        | \(1.63 \times 10^4\)            | \(1.55 \times 10^4\)                         |
| Sandell's sensitivity (µg/cm²)        | 0.018                            | 0.019                                         |
| Range of determination (µg. ml⁻¹)     | (0.5-20)                         | (1-20)                                        |
| Application of the method             | Pharmaceutical preparation (tablets) | tablets, suspension and spiked human urine |

Through the results shown in (Table 9) we conclude that the proposed method includes high sensitivity value of MBZ in its drug sample (vermox tablet).

CONCLUSION
After studying the optimal conditions for the proposed method and after applying it to the vermox tablets, we conclude that the proposed method has a high degree of sensitivity in addition to good reproducibility, which confirms its success as a reliable method for the determination of mebendazole in its pure form as well as in the form of a medicinal preparation.

REFERENCES
Attia, K.A.S.M.; Nassar, M.W.I.; El-Dosoky, M.; Madkour, A.W. (2015). Spectrophotometric methods for determination of mebendazole in presence of its alkaline induced degradation products in pure form and pharmaceutical preparation. *Ijppr*, 4(3), 1-19.

Calvo, N.L.; Kaufman, T.S.; Maggio, R.M. (2016). Mebendazole crystal forms in tablet formulations. An ATR-FTIR/chemometrics approach to polymorph assignment. *JPBA*. 122, 157-165.

De Ruyck, H.; Daeseleire, E.; De Ridder, H.; Van Renterghem, R. (2003). Liquid chromatographic electrospray tandem mass spectrometric method for the determination of mebendazol and its hydrolysed and reduced metabolites in sheep muscle. *Analytica Chimica Acta.*, 483 (1-2), 111-123.
Delfino, M.R.; Monzón, C.M.; Jorge, N.L.; Sarno, M.D.C.T. (2016). Mebendazol spectrophotometric determination. Theoretical and experimental study of the interaction with sodium hydroxid. AJST., 7(11), 3948-3953.

Hussin, M.T. (2010). Spectrophotometric determination of nitrazepam by coupling of its diazotized reduced form with m-aminophenol as coupling reagent. RJS., 21(7), 123-140.

Murage, J.K.; Amugune, B.K.; Njogu, P.; Ndwigah, S. (2020). Development and application of a spectrophotometric method in quality evaluation of benzimidazole anthelminthics in Nairobi city county. FJPS., 6(1), 1-7.

Naguib, I.A.; Abdelaleem, E.A.; Hassan, E.S.; Emam, A.A. (2020). Comparative study of eco-friendly spectrophotometric methods for accurate quantification of mebendazole and quinbamid combination; content uniformity evaluation. Spectrochimica. Acta., 235, 118271.

Naguib, I.A.; Hassan, E.S.; Emam, A.A.; Abdelaleem, E.A. (2020). Development and validation of HPTLC and green HPLC methods for determination of a new combination of quinbamid and mebendazole. JCS., 58(1),16-21.

National Center for Biotechnology Information. PubChem Compound Summary for CID 4030, Mebendazole. https://pubchem.ncbi.nlm.nih.gov/compound/Mebendazole. Accessed May 30, 2021.

Parakh, D.R.; Patil, M.P.; Sonawane, S.S.; Jain, C.P. (2015). Developments and validations of Spectrophotometric method for estimation of mebendazol in bulk and pharmaceutical formulation. WJPR., 4(7), 2223-2235.

Prabhu, R.C.; Maruthapillai, A. (2021). New RP-UPLC method development using QbD approach for determination of mebendazole, quinbamid, its impurities and antioxidants in mebendazole and quinbamid fixed dose combinations (FDC). Materials Today: Proceedings., 40, S120-S126.

Shah, U.; Talaviya, T.; Gajjar, A. (2015). Development and validation of derivative spectroscopic Method for the simultaneous estimation of mebendazol and levamisole hydrochloride in pharmaceutical formulations. IJPSR., 2(2), 108-112.

Swamy, N.; Basavaiah, K. (2013). Selective and sensitive assay of mebendazol in pharmaceuticals using bromocresole green by spectrophotometry. Thai. J. Pharm. Sci., 37(4), 171-185.

Xu, L.; Luan, F.; Wang, L.; Liu, H.; Gao, Y. (2014). 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. AOAC International., 97(1), 128-132.

YU, H.J.; FENG, B.; JIANG, C.J.; ZHANG, X. L.; HUI, Y.H.; SHEN, X.S. (2010). Determination of residues of mebendazole and its metabolites in fish/shrimp by solid phase extraction-high performance liquid chromatography. Chinese J. Analysis Laborat., 4, 116023.
التقدير الطيفي للميبيندازول باستخدام تفاعل الأزوتة والاقتران مع الكاشف ميتا-أمينوفينول

الملخص
تم وصف طريقة بسيطة وحساسة لتقدير الميبيندازول في مركب الصيدلاني بيئته النقيبة وببيئة كبسول من خلال تفاعل الأزوتة والاقتران. إذ تم تحليه مائياً في وسط قاعدي ليعطي مجموعة أمين أولي والتي بدورها تتفاعل مع نتريت الصوديوم في وسط حامضي (حامض الهيدروكلوريك) لينتج عن ذلك ملح الديازونيوم والذي ما يليث أن يتفاعل مع الكاشف ميتا-أمينوفينول في وسط قاعدي ليقتنع معه ويئنجه عن صبغة الأزو الصفراء اللون والتي تعطي أعلى امتصاص عند الطول الموجي 360 نانومتر. الاتباع الخطي لقانون بيير كان ضمن المدى الخطي (0.5 - 20 مايكروغرام، ملتر-1) مع عامل تقدير (R² 0.9990) وبمعامل امتصاص مولاري (1.63×10⁻⁴ لتر. مول⁻¹. سم⁻¹) وبدالة ساندل (0.018 ميكروغرام، سم⁻²)، كذلك تم حساب قيم حدود الكشف (0.1718 و 0.5726 مايكروغرام، سم⁻²) وبدالة كامعداد المركب الناتج [D-MBZ : mAP] كانت جزيئتين من الميبيندازول المؤزوت إلى جزيئة واحدة من الكاشف ميتا-أمينوفينول.

الكلمات الدالة: التقدير الطيفي، الأزوتة والاقتران، الميبيندازول، كاشف ميتا-أمينوفينول.