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

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ABSTRACT

A simple and sensitive spectrophotometric method has been developed for the determination of mebendazole (MBZ) in it's 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 $(0.5\text{-}20 \ \mu g.ml^{-1})$, the molar absorptivity value is 1.63×10^4 $L.mol^{-1}.cm^{-1}$ and the Sandell's value index is calculated and equal to $0.018 \ \mu g.cm^{-2}$. Also, the limit of detection and the limit of quantification are calculated and equal to 0.1718 $\mu g.ml^{-1}$ and $0.5726 \ \mu g.ml^{-1}$ 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).
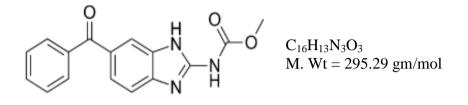

$C_{16}H_{13}N_3O_3$
M. Wt = 295.29 gm/mol

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).
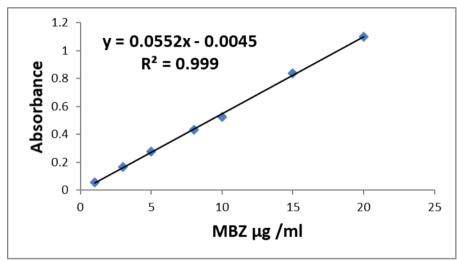

$$y = 0.0552x - 0.0045$$
$$R^2 = 0.999$$

Fig. 2: Calibration curve of MBZ proceed according to suggested method

molar absorptivity and Sandell's index values are calculated and equal to 1.63×10^4 l / 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	λ_{max}
NaOH	0.517	360
KOH	0.530	363
Na$_2$CO$_3$	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).

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).
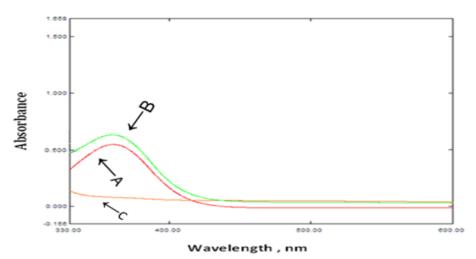
Fig. 3: Absorbance spectra of (A) Azo dye product from proceeding 100 µg of MBZ measured against blank. (B) Azo dye product against distilled water. (C) Blank against distilled water.

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.
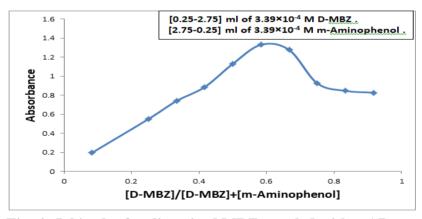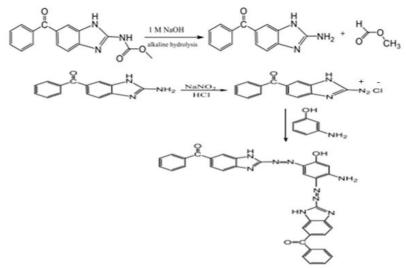

Fig. 4: Job's plot for diazotized MBZ coupled with mAP

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.
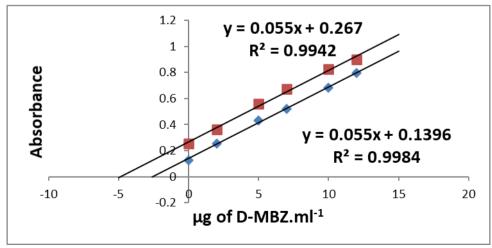

Fig. 5: Standard addition curve of MBZ

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
λmax (nm)	360	430
Reagent	m-aminophenol	bromocresole green dye
Color of the dye	Yellow	Yellow
Molar absorptivity coefficient (L.mole^{-1}.cm^{-1})	1.63×10^4	1.55×10^4
Sandell's sensitivity (µg/cm^2)	0.018	0.019
Range of determination (µg. ml^{-1})	(0.5-20)	(1-20)
Application of the method	Pharmaceutial 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.

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التقدير الطيفي للميبيندازول باستخدام تفاعل الأزوتة والاقتران مع الكاشف ميتا-أمينوفينول

الملخص

تم وصف طريقة بسيطة وحساسة لتقدير الميبيندازول في مركبه الصيدلاني بهيئته النقية وبهيئة كبسول من خلال تفاعل الازوتة والاقتران. إذ تم تحلله مائياً في وسط قاعدي ليعطي مجموعة أمين أولي والتي تتفاعل بدورها مع نتريت الصوديوم في وسط حامضي (حامض الهيدروكلوريك) لينتج عن ذلك ملح الديازونيوم والذي ما يلبث أن يتفاعل مع الكاشف ميتا- امينوفينول في وسط قاعدي ليقترن معه وينتج عنه صبغة الأزو الصفراء اللون والتي تعطي أعلى امتصاص عند الطول الموجي 360 نانوميتر. الاتباع الخطي لقانون بيير كان ضمن المدى الخطي (0.5 – 20 مايكروغرام. مللتر$^{-1}$) بمعامل تقدير (R^2) 0.9990 وبمعامل امتصاص مولاري ($63\times10^4$1. لتر. مول$^{-1}$. سم$^{-1}$) وبدلالة ساندل (0.018 ميكروغرام. سم$^{-2}$). كذلك تم حساب قيم حدود الكشف والتقدير فكانت 0.1718 مايكرو غرام. مللتر$^{-1}$ و 0.5726 مايكروغرام. مللتر$^{-1}$ على التوالي. نسبة التعقيد للمركب الناتج [D- mAP : MBZ] كانت جزيئتين من الميبيندازول المؤزوت إلى جزيئة واحدة من الكاشف ميتا- امينوفينول.

الكلمات الدالة: التقدير الطيفي، الازوتة والاقتران، الميبيندازول، كاشف ميتا- امينوفينول.