ISSN 2349-7203

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

HUMAN

Human Journals
Research Article
October 2015 Vol.:4, Issue:3

Spectrophotometric Methods for Determination of Mebendazole in Presence of its Alkaline Induced Degradation Product in Pure Form and Pharmaceutical Preparation

ISSN 2349-7203
IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
HUMAN

Khalid Abdel-Salam M.Attia, Mohammed W.I. Nassar, Mohammed El-Dosoky and Ahmed W. Madkour*

Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Al-Azhar University,11751, Nasr City, Cairo, Egypt.

Submission: 27 September 2015
Accepted: 3 October 2015
Published: 25 October 2015

Keywords: Mebendazole, First derivative, Dual wave length, Area under the curve, Q- Analysis, Absorbance subtraction

ABSTRACT

Five simple, rapid, sensitive, accurate and precise stability-indicating spectrophotometric methods were developed for determination of mebendazole in bulk powder and in pharmaceutical preparation. **Method (A)** First derivative technique (^{1}D); based on measuring peak amplitude of derivatized spectra of mebendazole at 248.2 nm , **Method (B)** Dual wave length method; which depends upon measuring the difference of absorbance of zero order spectra of mebendazole between 241 and 255 nm, **Method (C)** Area under the curve (AUC) method; is used for the determination of mebendazole using the wavelength ranges 230–240 nm and 240–255 nm, **Method (D)** Q-Analysis (QA) method or graphical absorbance ratio (GAR) method; two wavelengths (λ_{iso}) 239.8 nm and (λ_2) 232.2 nm were selected for determination of mebendazole in presence of its degradation product and **Method (E)** Absorbance subtraction (AS) method; two wavelengths (λ_{iso}) 239.8 nm and (λ_2) 330 nm were selected for determination of mebendazole in presence of its degradation product. The calibration graphs were linear in the range of (1−9 µg ml^{-1}) for mebendazole. The obtained results were statistically compared with those of the reported method by applying t-test and F-test at 95% confidence level and no significant difference was observed regarding accuracy and precision.

INTRODUCTION

Mebendazole, chemically Methyl-5-benzoyl-2-benzimidazole carbamate (Fig.1), is used as an anthelmintic agent. The drug is known to act through irreversible inhibition of glucose uptake in the parasite, leading to depletion of glycogen store. This in turn decrease adenosine tri phosphate activity. Only 5–10% of the ingested drug, gets absorbed from the human gastrointestinal tract. The drug is known to be dysmorphonegic in experimental animals [1].

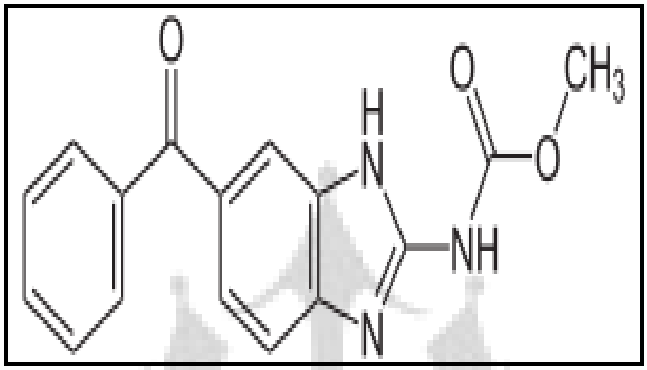

Figure 1: chemical structure of Mebendazole

Different analytical methods reported for the assay of mebendazole in various dosage forms include infrared spectroscopy [2], proton NMR [3], differential pulse polarography [4], fluorescence [5], HPLC methods for determination in plasma[6], HPLC methods for determination in pharmaceutical dosage forms[7],and some colorimetric methods[8,9]. On the other side, few stability-indicating methods have been applied [10].

The aim of current work is to develop and validate a simple, rapid, reliable and precise U.V spectrophotometric stability-indicating methods for analysis of mebendazole in bulk and tablet formulation in presence of its degradation product without previous separation by UV–VIS spectrophotometry.

MATERIALS AND METHODS

Apparatus:

- SHIMADZU dual beam UV-Vis. 1650 Spectrophotometer (Japan). The bundled software, UV-Probe personal spectroscopy software version 2.1 (SHIMADZU) is used.

- Hot plate (Torrey pines Scientific, USA).
- Jenway, 3510 pH meter (Jenway, USA).

Materials and Reagents:

- Mebendazole powder was kindly supplied by MEMPHIS Company for pharmaceutical & chemical industries. Cairo - Egypt. (B. NO.4114937).
- Vermin® 100 mg Tablets. The product of MEMPHIS Company for pharmaceutical &chemical industries. Cairo -Egypt (B.NO.314348).
- Hydrochloric acid, Sodium hydroxide and Methanol (ElNasr Co., Egypt).
- All chemicals are of analytical grade, so no need of prior purification.

Preparation of the degradation product: [10]

10 ml of mebendazole solution (1mg ml^{-1} in 0.1 M methanolic hydrochloric acid) was transferred into 250 ml conical flask. To this 50 ml of 1 M sodium hydroxide solution was added. The flask was heated to boiling under reflux for 30 min, cooled and neutralized with 1M hydrochloric acid. The resulting solution was evaporated to dryness and the degradation product (2-Amino-5-benzoylbenzimidazole) was extracted by methanol, filtered in 100 ml flask and the volume was adjusted with methanol. The obtained solution was claimed to contain (0.1 mg ml^{-1}).

Standard Solutions:

(a) *Stock standard solution of Mebendazole (1 mg/ml)*

0.1 g of mebendazole was accurately weighed in 100 ml volumetric flask, 10 ml of 0.1 M methanolic hydrochloric acid was added, shaken to dissolve and the volume was adjusted to the mark with methanol.

(b) Working standard solution of Mebendazole (100 µg/ml)

10 ml of mebendazole accurately transferred from its stock standard solution into 100 ml volumetric flask, then the volume was completed to the mark with methanol.

(c) Working standard solution of Mebendazole degradation product (100 µg/ml)

The solution obtained from degradation process is used as working standard solution of mebendazole degradation product.

Construction of the Calibration Curves (General Procedures)

Method A (First derivative method):

Individual aliquots mebendazole and mebendazole degradation product each equivalent to (10–90 $\mu g ml^{-1}$), were transferred from their standard working solutions (100 $\mu g\ ml^{-1}$) into two separate series of 10 ml volumetric flasks and volume was made with methanol. The spectra of prepared standard solutions were scanned from 200 - 400 nm against methanol as blank. First derivative (^1D) spectra of mebendazole and its degradation product were recorded. The amplitude at 248.2 nm was measured for each drug concentration. The calibration curve was constructed relating the amplitudes of the first derivative values at 248.2 nm to the corresponding concentrations in $\mu g\ ml^{-1}$ of mebendazole , the regression equation was derived.

Method B (Dual wave length method):

Individually zero order spectra of both mebendazole and mebendazole degradation product in the ranges of (1-9$\mu g ml^{-1}$) were obtained as before, then the difference of absorbance between (241and 255 nm) was found to be zero for degradate. Calibration graph obtained by relating the difference of absorbance between (241and 255nm) of mebendazole spectra to the corresponding concentration of mebendazole, and the corresponding equation was computed.

Method C (Area under curve method):

Individually zero order spectra of mebendazole and mebendazole degradation product in the range of (1-9$\mu g ml^{-1}$) were obtained as before. In this method, the sampling wavelength ranges selected for estimation of mebendazole in presence of degradation product are 230-240 nm (λ_1-λ_2) and 240-255 nm (λ_3-λ_4).The area under the curve (**AUC**) of mebendazole and its degradation product are measured at both wavelength ranges and calibration curves were obtained by plotting the area under curve values against the corresponding concentrations in $\mu g ml^{-1}$. Mixed standards were prepared and their area under the curve values were measured at the selected wavelength ranges. Concentration of mebendazole in mixed standard and the sample solution were calculated using equation (1) and (2).

$A1 = \alpha_1 C_{meb} + \beta_1 C$ …….. (1) at 230-340 nm.

$A2 = \alpha_2 C_{meb} + \beta_2 C$ …….... (2) at 240-255 nm.

Where,α_1And α_2 are absorptivities of mebendazole at (λ_1-λ_2) and (λ_3-λ_4) respectively. β_1 and β_2 are absorptivities of mebendazole degradation product at (λ_1-λ_2) and (λ_3-λ_4) respectively.A_1 and A_2 are area under curve of mixed standard at (λ_1-λ_2) and (λ_3-λ_4) respectively.

Method D (Q-Analysis) or The Graphical Absorption Ratio method:

Individually zero order spectra of mebendazole and mebendazole degradation product in the range of (1-9µgml^{-1}) were obtained as before. The absorbance for both samples were measured at 232.2 nm (λ_{max} of mebendazole) and 239.8nm (λ_{iso} at iso-absorptive point). The absorptivity values for mebendazole and its alkaline degradate at selected wavelengths were calculated. The method employs Q values and the concentrations of the studied drug in the prepared mixed solutions were determined by using the following equations:

$$C_x = [\ (Q_m - Q_y)/(Q_x - Q_y)\ ] \times A_{iso}/a_{iso}\ .$$

Method E (Absorbance Subtraction method):

Individually zero order spectra of mebendazole and mebendazole degradation product in the range of (1-9µgml^{-1}) were obtained as before. In this method the absorbance were measured at 239.8nm (λ_{iso} at isoabsorptive point) and 330 nm (λ_2 at mebendazole degradation product extention area at which no absorbance of mebendazole is observed). The absorptivity values for mebendazole and its alkaline degradate at the selected wavelengths were calculated. The method employs absorbance factor values [A_{iso} / A_2] and the concentration of the studied drug (x) in the prepared mixed solutions was determined by using the absorption value obtained at isoabsorptive point which calculated from the following equations:

$$A_x\ (\text{at } \lambda_{iso}) = [A_{iso}\ (X+Y)\ ] - [(\ A_{iso}/A_2\ )\ \times\ A_2\ (X+Y)]$$

Analysis of pharmaceutical preparation:

Ten **vermin® 100 mg** tablets were accurately weighed and finely powdered. A quantity equivalent to100 mg of mebendazole was shaken three times with 25 ml methanol for 10 minutes and filtered into 100 ml volumetric flask. The volume was adjusted with methanol to obtain a

concentration of (1mg ml^{-1}). The solution was analyzed using procedure described under methods A, B, C, D and E.

RESULTS AND DISCUSSION

Spectral Characteristics:

The zero order (D^0) absorption spectra for both mebendazole and its alkaline degradation product (each 8 µg ml^{-1}) were recorded against methanol as blank over the range of 200 – 400 nm (Fig.2).

A-First derivative method:

The zero order absorption spectrum of mebendazole (8 µg ml^{-1}) and its alkaline degradation product (8 µg ml^{-1}) were recorded against methanol as blank over the range of 200 – 400 nm showing sever overlapping as shown in **(**Fig.2**).** However, this sever overlapping in zero order spectra can be resolved by conversion of zero-order to first derivative spectra of mebendazole and its degradation product and measure amplitude of intact derivatized spectra at specific wavelength at which degradate derivatized spectrum absorbance is equal to zero [11-13].

Mebendazole has a trough at 248.2 nm which shows no interference from the degradation product as shown in (Fig.3). Linear correlation was obtained between the trough at 248.2 nm against the corresponding concentration of mebendazole.

B-Dual wave length method:

The utility of dual wavelength method[14,15] is to calculate the unknown concentration of a component of interest present in a mixture containing unwanted interfering components by measuring the absorbance difference between two points on the mixture spectra. The absorption of interfering component or degradation product between the two points equal zero. For mebendazole calibration curves were prepared by absorbance difference between two wavelengths (241 nm – 255 nm) and the response was found to be linear in the concentration range 1-9 µgml^{-1} (Figure 2).

C-Area under the curve (AUC) method:

Selection of the wavelength region to construct AUC method has a great effect on the analytical parameters such as slope, intercept and correlation coefficient. Different wavelength regions are

tested where the wavelength ranges 230–240nm and 240–255 nm are selected which show good selectivity and percentage recovery, (Fig. 4, 5).

Area under the curve of the absorption spectra in the wavelength ranges 230–240 nm (λ_1-λ_2) and 240–255 nm (λ_3-λ_4) of Mebendazole in the concentration range of 1-9 µg ml^{-1} were calculated. For degradation product area under the curve of the absorption spectra in the wavelength ranges 230–240 nm (λ_1-λ_2) and 240–255 nm (λ_3-λ_4) in the concentration range of 1-9 µg ml^{-1} were also calculated. The absorptivity 'Y' values of Mebendazole and its degradation product were calculated at each wavelength range. The concentrations of mebebdazole can be obtained by applying Cramer's rule and matrices in Eqs. (1) and (2). Concentration of two the drugs in mixed standard and the sample solution are calculated according to the following equations [16,17].

$$A1 = 336.93C_{meb} + 263.93\ C_{deg} \ldots\ldots\ldots\ldots\ldots (1) \text{ at } 230\text{-}340 \text{ nm.}$$

$$A2 = 581.73C_{meb} + 1083.99C_{deg} \ldots\ldots\ldots\ldots\ldots (2) \text{ at } 240\text{-}255 \text{ nm.}$$

Where C_{meb} and C_{deg} are the concentrations of mebendazole and its degradation product in g/100ml respectively. 336.93 and 581.73 are the absorptivity values of mebendazole at (λ_1-λ_2) and (λ_3-λ_4) respectively. 263.93 and 1083.99 are absorptivity values of degradation product at (λ_1-λ_2) and (λ_3-λ_4) respectively. A1 and A2 are the area under the curve of sample solutions at the wave length range (λ_1-λ_2) and (λ_3-λ_4) respectively (Fig.4, 5).

D- Q-Analysis (QA) or The Graphical Absorption Ratio (GAR) method[18,19]:

This method depends on the property that for a substance which obeys Beer's Lambert's law at many wavelengths, the ratio of absorptivity (or absorbance) values at any two wavelengths are constant, independent of the concentration or path length. This ratio is referred as Q-ratio.Two wave lengthes are used to apply this method. One of the two selected wavelengths is an isoabsorptive point (λ_{iso}) 239.8 and the other is wavelength of maximum absorption of the drug (λ_{max}) 232.2.The overlay spectra of mebendazole ,its alkaline degradate and their mixture,(Fig. 6), show isoabsorptive point at 239.8nm. The absorbance values at 239.8nm (λ_{iso}) and 232.2nm (λ_{max}) for mebendazole in the range of 1-9µg ml^{-1}were obtained and similarly for its alkaline induced degradation product absorbance values in the range of 1-9 µg ml^{-1}were measured, absorptivity coefficients were determined for both and the average values were taken. The values and the

absorbance ratio were used to develop the following equation from which the concentration of mebendazole in the sample mixture can be calculated:

$$C_x = [\ (Q_m - Q_y)/(Q_x - Q_y)\ ] \times A_{iso}/a_{iso}$$

$$C_x = [(\ Q_m - 0.8178)/\ (1.1917 - 0.8178)] \times A_{iso}/0.08336$$

where C_x is the concentrations of mebendazole in µg ml^{-1}; Q_m is the absorbance of sample at $\lambda_{232.2}$/absorbance of sample at $\lambda_{239.8}$; Q_x is the mean of absorptivity of mebendazole at $\lambda_{232.2}$/mean of absorptivity of mebendazole at $\lambda_{239.8}$; Q_y is the mean of absorptivity of mebendazole degradate at $\lambda_{232.2}$ /mean of absorptivity of mebendazole degradate at $\lambda_{239.8}$; a_{iso} is the mean of absorptivity of mebendazole at $\lambda_{239.8}$; and A_{iso} is the absorbance of the sample at $\lambda_{239.8}$.

E- Absorbance subtraction (AS) method [20,21] :

This method depends on that if we have amixture of two components (two drugs or drug with its degradation product) X and Y having overlapped spectra , Y has some interference at λ_{iso} and Y has extended absorprion at λ_2 at which X do not show any absorption i.e. abs λ_2 (X+Y) = abs λ_2(Y).

Quantitative estimation of X in the mixture (X+Y) was carried out by subtracting the absorption due to Y at λ_{iso} from absorption due to (X+Y) at λ_{iso} , by using experimentally calculated absorption factor.

$$A_x\ (\text{at } \lambda_{iso}) = [A_{iso}\ (X+Y)\ ] - [(\ A_{iso}\ /A_2\ )\ \times\ A_2\ (X+Y)]$$

Where $A_{iso,}$ A_2 is the absorbance of Y at λ_{iso} and λ_2. (A_{iso} /A_2) is called the absorption factor and it is constant for pure Y and A_{iso} (X+Y) , A_2 (X+Y) are the absorption of the mixture at λ_{iso} and λ_2 . The concentration of X is calculated from the corresponding regression equation (obtained by plotting the absorbance values of zero order curves of X at its λ_{iso} against the corresponding concentrations). For mebendazole and its degradation product mixture λ_{iso} at 239.8 nm, λ_2 at 330 nm (Fig.6) and absorption factor is 1.38232.

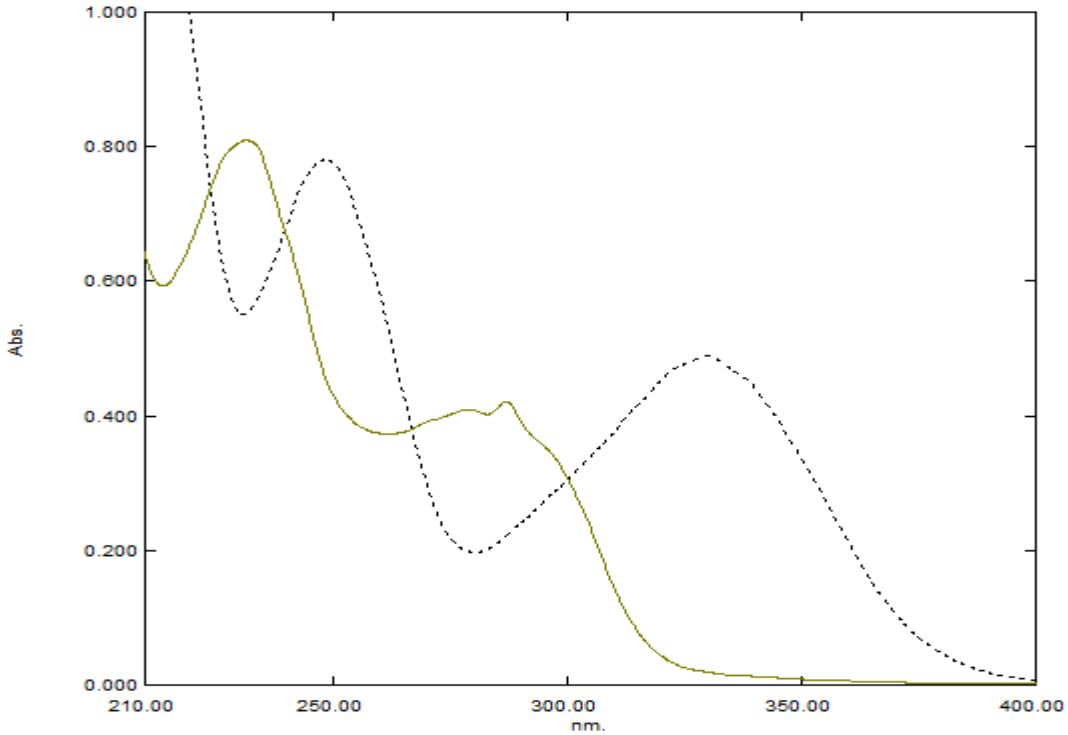

Figure (2): UV- Spectra of intact mebendazole (bold line—) and its degradate (dotted line…..) each at (8µg ml^{-1})

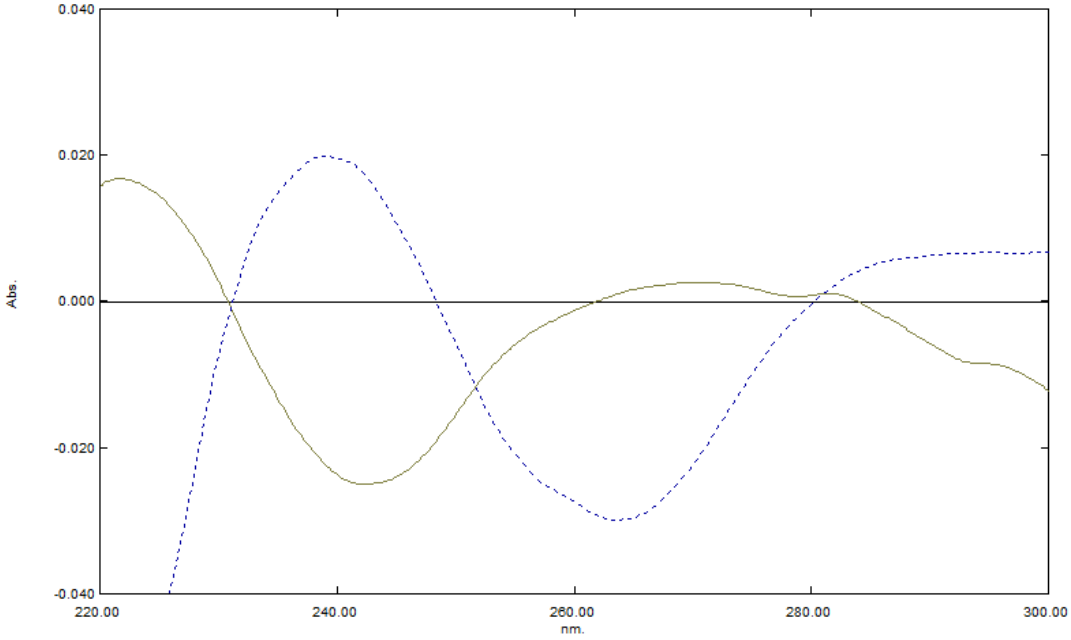

Figure (3): first derivative of intact mebendazole spectrum (8µg ml^{-1})(—) and of its degradate (8µg ml^{-1})(…..)

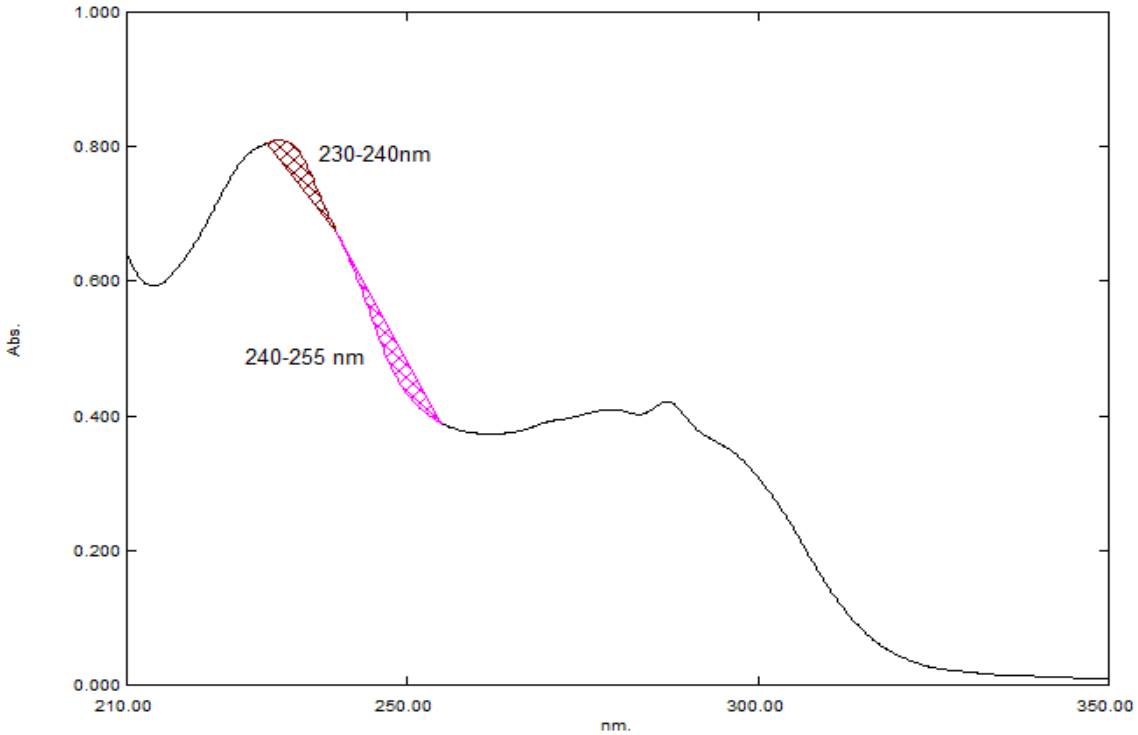

Figure (4): Zero order absorption spectrum of mebendazole (8 µgml^{-1}) showing wavelength ranges for area under curve method using methanol as blank

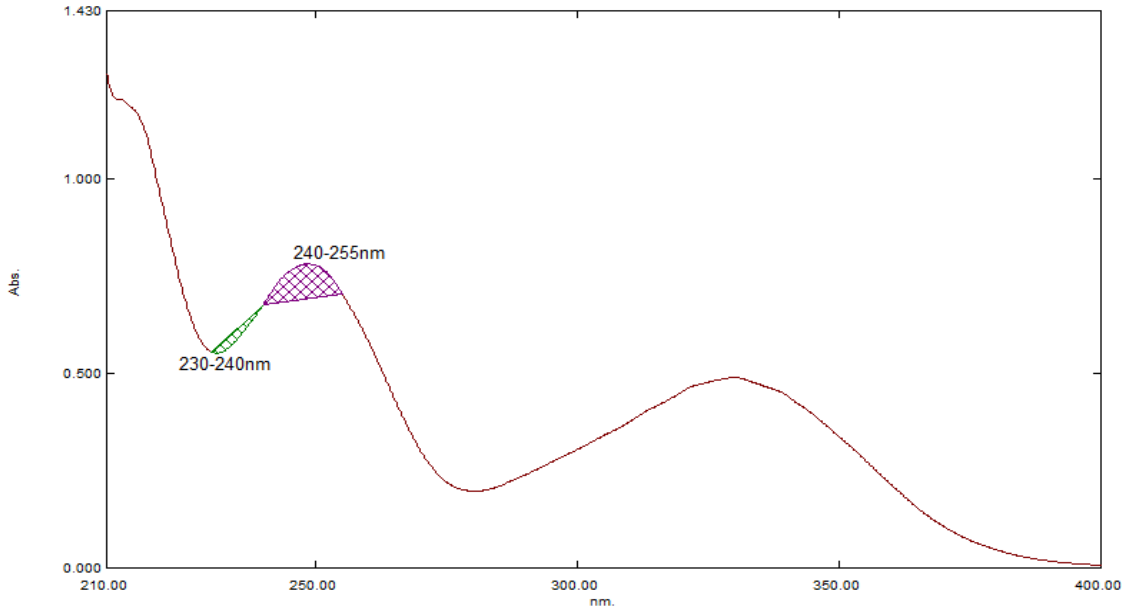

Figure (5): Zero order absorption spectrum of mebendazole degradation product (8 µgml^{-1}) showing wavelength ranges for area under curve method using methanol as blank

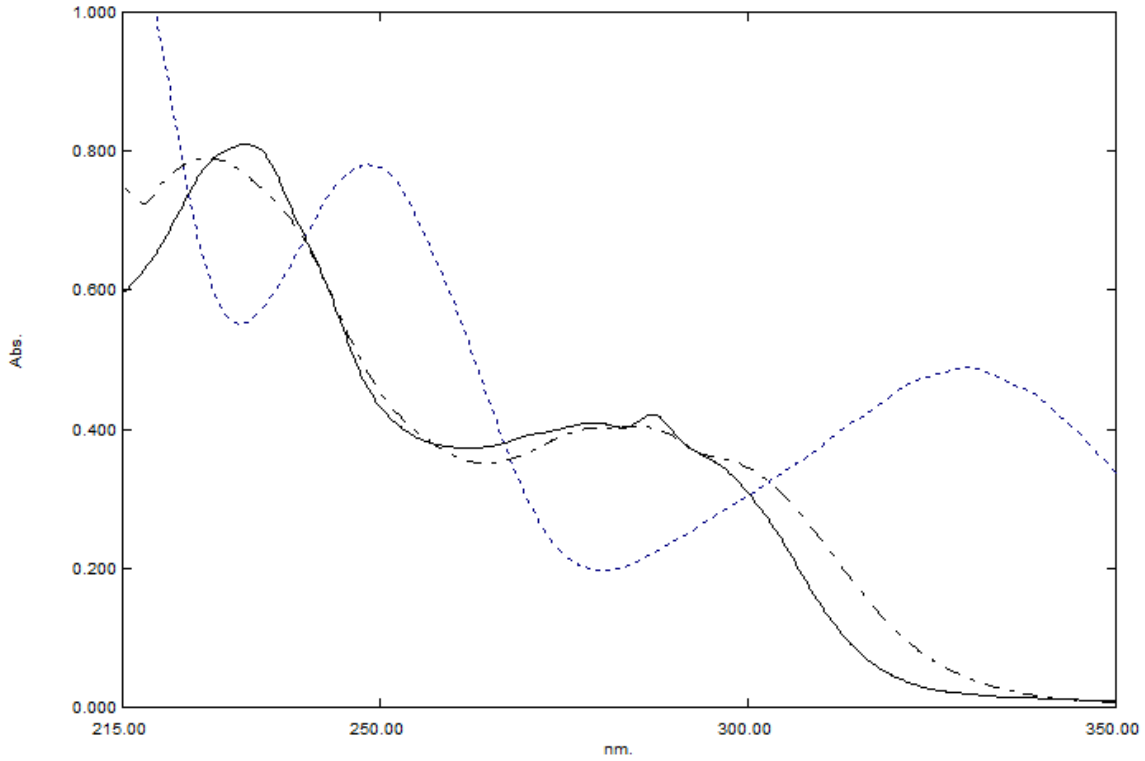

Figure (6): Zero - Order Spectra of Intact mebendazole (8µg ml^{-1})(—), its alkaline degradate (8 µg ml^{-1}) (…..) and their mixture (4 µg ml^{-1} of each) (– . – . –).

Validation of the methods:

Linearity and range:

For first derivative method:

Under the described experimental conditions, the calibration graph for the method was constructed by plotting peak amplitude at 248.2 nm versus concentration in µg ml^{-1}. The regression plot was found to be linear over the range of 1-9 µg ml^{-1}. The linear regression equation for the graph is:

$$P_{248.2\ nm} = 0.0024\ C + 0.0001 \qquad (r^2 = 0.9997)$$

Where C is the concentration of mebendazole in µg ml^{-1}, P is the peak amplitude of the first derivative of zero order spectrum curve at 248.2 nm and r^2 = the correlation coefficient, as shown in table (1).

For Dual wave length method:

Linear correlation was obtained by plotting the differences in absorption at 241 and 255 nm, against the corresponding concentration of mebendazole. Good linearity was obtained in the concentration range of 1-9 µg ml^{-1}.The corresponding regression equation was computed to be:

$$\Delta A_{241-255} = 0.0321C + 0.0006 \qquad (r^2 = 0.9997)$$

Where ΔA is the absorption difference at the selected wavelengths, C is the concentration in µg ml^{-1} and r^2 = the correlation coefficient as shown in table (1).

For Area under the curve method:

The calibration graph for the method was constructed by plotting area under curve of mebendazole versus concentration in µgml^{-1}. The regression plot was found to be linear over the range of 1-9 µgml^{-1}. The linear regression equation for the graph is:

$$P_{AUC} = 0.0336C - 0.0001 \qquad (r^2 = 0.9998)$$

Where P_{AUC} is area under curve at the wavelengths range of 230-240 nm , C is the concentration in µg ml^{-1} and r^2 = the correlation coefficient as shown in table (1).

For Q-Analysis (QA) **or The Graphical Absorption Ratio (GAR) method:**

Linear correlation was obtained between the absorbance value at 232.2 nm versus concentration of mebendazole in µg ml^{-1}.Good linearity is obtained in the concentration range of 1-9 µg ml^{-1}. The linear regression equation for the graph is:

$$A = 0.1022\ C - 0.0099 \qquad (r^2 = 0.9997)$$

Where A is the absorbance at the selected wavelength, C is the concentration in µg ml^{-1} and r^2 = the correlation coefficient as shown in table (1).

For Absorbance subtraction (AS) method:

Linear correlation was obtained between the absorbance value at 239.8 nm versus concentration of mebendazole in µg ml^{-1}.Good linearity is obtained in the concentration range of 1-9 µg ml^{-1}. The linear regression equation for the graph is:

$$A = 0.0860\ C - 0.0077 \qquad (r^2 = 0.9996)$$

Where A is the absorbance at the selected wavelength, C is the concentration in μg ml^{-1} and r^2 = the correlation coefficient as shown in table (1).

Limits of detection and quantitation:

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated according to ICH guidelines [22] from the following equations*:*

$$LOD = 3.3\ S_a / slope$$

$$LOQ = 10\ S_a / slope$$

Where S_a is the standard deviation of y-intercepts of regression lines.

LOD and LOQ values of mebendazole for each method were listed in table (1).

Accuracy and precision:

According to the ICH guidelines [22], three replicate determinations of three different concentrations of the studied drug in pure form within their linearity ranges were performed in the same day (intra-day) and in three successive days (inter-day) for each method. Accuracy as recovery percent (R%) and precision as percentage relative standard deviation (RSD%) were calculated and results are listed in table (2).

Specificity:

The specificity of the proposed methods were assured by applying the laboratory prepared mixtures of the studied drug and its degradate.The results are listed in table (3).

Pharmaceutical Applications:

The proposed methods were applied to the determination of the studied drug in (Vermin ®100mg) tablets. The results were validated by comparison to a previously reported method [9].No significant differences were found by applying t-test and F-test at 95% confidence level[23],indicating good accuracy and precision of the proposed methods for the analysis of the studied drugs in their pharmaceutical dosage form (table 4).

Table (1): Spectral data for determination of mebendazole by the proposed methods:

Parameters	First derivative	Dual wavelength	Area under the curve	Q-analysis	Absorbance subtraction
Wavelength (nm)	248.2	241 & 255	230-240 240-255	232.2 & 239.8	239.8& 232.2
Linearity range (μgml^{-1})	1-9	1-9	1-9	1-9	1-9
LOD (μgml^{-1})	0.275	0.247	0.313	0.151	0.152
LOQ (μgml^{-1})	0.833	0.748	0.951	0.460	0.463
Slope (*a*) **Intercept (*b*)**	0.0024 0.0001	0.0321 0.0006	0.0336 - 0.0001	0.1022 - 0.0099	0.0860 -0.0077
Correlation coefficient (r^2)	0.9997	0.9997	0.9998	0.9997	0.9996

* $y = ax + b$ where y is the response and x is the concentration.

Table (2): Intraday and inter-day accuracy and precision for the determination of the mebendazole by the proposed methods:

Method	Conc. µg.ml^{-1}	Intraday			Inter-day		
		Found Conc. ± SD	Accuracy (R%)	Precision (RSD%)	Found Conc. ± SD	Accuracy (R%)	Precision (RSD%)
First derivative	4	3.92 ± 0.004	98.12	0.106	3.95±0.008	98.92	0.219
	6	6.03±0.014	100.55	0.239	6.01±0.011	100.21	0.183
	8	8.03±0.016	100.39	0.209	8.01±0.019	100.14	0.245
Dual wavelength	4	3.97±0.018	99.48	0.452	4.02±0.047	100.52	1.18
	6	6.06±0.064	101.10	1.06	6.02±0.054	100.41	0.895
	8	8.09±0.065	101.14	0.801	7.99±0.047	99.97	0.595
Area under the curve	4	3.98±0.062	99.52	1.55	3.98±0.017	99.53	0.432
	6	6.00±0.045	100.08	0.757	6.00±0.062	100.07	1.03
	8	8.10±0.06	101.23	0.735	8.01±0.059	100.11	0.743
Q - Analysis	4	3.96±0.019	99.04	0.493	3.97±0.024	99.37	0.619
	6	5.96±0.011	99.40	0.189	5.98±0.020	99.68	0.340
	8	8.03±0.024	100.48	0.306	8.01±0.046	100.11	0.577
Absorption subtraction	4	3.94±0.011	98.72	0.294	3.98±0.023	99.59	0.583
	6	5.91±0.006	98.50	0.113	5.94±0.037	99.02	0.629
	8	8.00±0.029	100.03	0.365	7.99±0.044	99.89	0.551

Table (3): Determination of mebendazole and its degradate in their laboratory mixtures by the proposed methods:

	Intact in (µg ml^{-1})	Degradate in (µg ml^{-1})	Percent of degradate	Intact found in (µgml^{-1})	Recovery % of intact
First derivative	8	1	11.11	7.96	99.58
	7	2	22.22	6.95	99.40
	5	4	44.44	4.93	98.75
	3	6	66.66	2.95	98.33
	Mean ± SD%				99.66±1.105
Dual wavelengt	8	1	11.11	7.95	99.45
	7	2	22.22	7.02	100.31
	5	4	44.44	5.02	100.56
	3	6	66.66	2.94	98.02
	Mean ± SD%				99.75±1.068
Area under the curve	8	1	11.11	7.94	99.31
	7	2	22.22	6.95	99.33
	5	4	44.44	4.93	98.74
	4	5	55.55	3.97	99.33
	Mean ± SD%				99.61±1.011
Q - Analysis	8	1	11.11	8.14	101.79
	7	2	22.22	7.11	101.54
	5	4	44.44	5.00	100.08
	3	6	66.66	2.98	99.46
	Mean ± SD%				100.88±0.824
Absorption subtraction	8	1	11.11	8.07	100.98
	7	2	22.22	6.97	99.57
	5	4	44.44	5.08	101.57
	3	6	66.66	3.02	100.91
	Mean ± SD%				101.03±0.774

Table (4): Determination of mebendazole in Vermin ® 100mg tablets by the proposed and reported method[9]:

	First derivative	Dual wave length	Area under the curve	Q-Analysis	Absorption subtraction	Reported method[9]
N*	5	5	5	5	5	5
$\bar{X}$	99.19	99.82	99.48	99.24	99.35	98.91
SD	0.793	0.848	0.967	0.719	0.978	0.482
RSD%	0.799	0.850	0.972	0.725	0.984	0.488
t****	0.688 (2.306)	2.083 (2.306)	1.178 (2.306)	0.862 (2.306)	0.914 (2.306)	———
F**	2.698 (6.388)	3.090 (6.388)	4.015 (6.388)	2.221 (6.388)	4.105 (6.388)	———

** No. of experimental.*

*** The values in the parenthesis are tabulated values of t and F at (p= 0.05).*

CONCLUSION

The proposed methods are simple, rapid, accurate and precise and can be used for the determination of Mebendazole in pure form and in pharmaceutical dosage form as well as in presence of its degradation product.

ACKNOWLEDGMENT

I am deeply thankful to **ALLAH**, by the grace of whom this work was realized. I wish to express my indebtedness and gratitude to staff members Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy Al-Azhar University, Cairo, Egypt for their valuable supervision, continuous guidance, and encouragement throughout the whole work.

REFERENCES

[1] J.E.F. Reynolds (Ed.), Martindale, The Extra Pharmacopia, 31st edition, The Royal pharmaceutical Society, London, 1996, p. 118.
[2] A.A.Bunaciu, S.Fleschin, H.Y.Aboul-Enein; Analysis of mebendazole polymorphs by Fourier transform IR spectrometry using chemometric methods, Biopolymers 67(2002)56-60.
[3] H.A. Al-Khamees, B.E.D.M. El-Shazly; Determination of mebendazole and its formulations using ^{1}H nuclear magnetic resonance spectrometry, Analyst 113 (1988) 599-602.
[4] A.P.Joshi , C. Jeyaseelan; study of mebendazole by differential pulse polarography, Chem.Tech Research 1(2009)596-601.
[5] W. Baeyens, F. Abdel Fattah, P. DeMoerloose, Fluorimetric analysis of mebendazole and flubendazole with hydrogen peroxide; J. Pharm.Biomed. Anal. 3 (1985) 397-404.
[6] G.Karlaganis,G.J.Munst,J.Bircher;High-pressureliquid-chromatographic determination of the anthelmintic drug mebendazole in plasma, High resolution chromatography 2(2005) 141-144
[7] Z.M.Turabi,O.A.Khatatbeh,D.N.Al-Abed; RP-HPLC Method Development and Validation for the Simultaneous Determination of mebendazole and the two preservatives methylparaben and propylparaben in Pharmaceutical Oral suspension dosage form;pharm.sciences and drug research 6 (2014)70-74.
[8] A.Kar; Spectrophotometric determination of mebendazole in pure and dosage forms by complexation with potassium bismuth(III) iodide, Analst 8(1985) 1031-1033.
[9] N. Swamy , K. Basavaiah ; Selective and sensitive assay of mebendazole in pharma-ceuticals using bromocresol green by spectrophotometry, Thai J. Pharm. Sci. 37 (2013) 171-185.
[10] Z.Al-Kurdi, T.Al-Jallad , A.Badwan, A.M.Y.Jaber; High performance liquid chromatography method for determination of methyl-5-benzoyl-2-benzimidazole carbamate (mebendazole) and its main degradation product in pharmaceutical dosage forms, Talanta 50(1999) 1089-1097.
[11] E. Satana, S. Altmay, N.G. Goger, S.A. Ozkan; Simultaneous determination of valsartan and hydrochlorothiazide in tablets by first derivative ultraviolet spectrophotometry and LC. J. Pharm. Bimed. Anal. 25 (2001) 1009-1013.
[12] H.E. Zaazaa, S.S. Abbas, Z.A. El-Sherif, B. El-Zeany, D.A. El-Haddad; Stability indicating spectrophotometric methods for determination of tiemonium methylsulphate in the presence of its degradation products. J. Appl. Pharm. Sci. 4 (2014) 33-45.
[13] N.A. Elragehy, E.M. Abddel-Moety, N.Y. Hassan, M.R. Rezk; Stability indicating determination of meropenem in presence of its degradation product. Talanta. 77 (2008) 28-36.
[14] N.K. Ramadan, H.M. Mohamed, A.A. Moustafa; Simultaneous determination of rabeprazole sodium and domperidone. J. App. Pharm. Sci. 1 (2011) 73-80.
[15] R.Savithri, N.S. Bindu, P.S. Bhargavi, D.H. Theja, P. Ramalingan; Dual wavelength UV-Spectrophotometric method for simultaneous estimation of atorvastatin and ezetimibe in bulk and their combined tablet dosage form. Der. Pharmacia. Sinica. 2 (2011) 251-258.
[16] S.S. Chitlange, A.L. Mulla , G.R . Pawbake , S.B . wankhede . Simultaneous spectrophotometric estimation of dexrabeprazole and domperidone in capsule dosage form. international journal of pharmaceutical quality assurance. 2(2)(2010) 31-34.
[17] S.L. Prabu ,Shirwaikar , C.D .Kumar , A. Joseph , R . Kumar . Simultaneous Estimation of Esomeprazole and Domperidone by UV Spectrophotometric Method , Indian j pharm sci : 70(1) (2008) 128-131.
[18] N.S.Abdelwahab, Spectrophotometric methods for simultaneous determination of Carvedilol and Hydrochlorothiazide in combined dosage form. Arabian journal of chemistry , 1016 (2011)10-15.
[19] K.P. Bhusari, P.B. Khedkar, S. Dhole, V.S. Banode; Derivative and Q-analysis Spectrophotometric Methods for Estimation of Hydrochlorothiazide and Olmesartan Medoxomil in Tablets India. J. Pharm. Sci., 71(5) (2009) 505–508.

[20] C.V. Patel, A.P. Khandhar, A.D. Captain, K.T. Patel, Validated Absorption Factor Spectorphtometric and Reversed-Phase High-Peroformance Liqid Chromatographic Methods for the Determination of Ramipril and Olmesartan Medoxomil in Pharmaceutical Formulations Eurasian J. Anal. Chem. 2 (3)(2007) 159.
[21] H.M.Lotfy and M.A.Hegazy , Simultaneous determination of some cholesterol-lowering drugs in their binary mixture by novel spectrophotometric methods , Spectrochimica Acta part A , 113(2013) 107-114.
[22] ICH Q2 (R1): Validation of analytical procedure. Text and methodology. Geneva: International conference of Harmonization: 2005.
[23] Armitage P, Berry G. Statistical Methods in Medical Research. 3rd ed. Oxford, UK; Blackwell: 1994.