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DEVELOPMENT AND VALIDATION OF NOVEL UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF MEBENDAZOLE IN PHARMACEUTICAL FORMULATION

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Keywords:

Mebendazole, ICH guidelines, Accuracy, Precision, Validation

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ABSTRACT: Mebendazole is a broad-spectrum anthelmintic drug that is widely used in the treatment of helminthic infections. A simple, accurate, and precise UV-visible spectrophotometric method has been developed and validated for the determination of Mebendazole in tablet formulation. The solvent used for the development of the proposed method was Phosphate buffer 6.8 at 278.6 nm. The Beers' law was obeyed in the concentration range 5-30 µg/ml. The developed method was validated as per ICH guidelines such as linearity, accuracy, precision, the limit of detection, the limit of quantification, and robustness. The correlation coefficient was found to be 0.999. The accuracy was found between 99.22 to 99.54%. The % RSD of Mebendazole was found to 0.05 to 0.20 for intraday and 0.05 to 0.15 for inter-day precision. The method was found to be precise as % RSD was found to be less than 2%. The LOD and LOQ were found to be 0.986 µg/ml and 2.988 µg/ml, respectively. The results demonstrated that the excipients in the commercial tablets did not interfere with the method and can be conveniently employed for routine quality control analysis of Mebendazole in pharmaceutical tablets formulations.

INTRODUCTION: Mebendazole (MBZ) is a broad spectrum anthelmintic drug of the benzimidazole class. Chemically, MBZ is methyl-5 - benzoyl benzimidazole - 2 - carbamate. It is available in tablets, syrup, or suspension dosage forms [1-2]. Mebendazole is commonly used to treat pinworm, whipworm, roundworm, and hookworm infections. It has poor aqueous solubility and oral bioavailability [3-4]. It causes many adverse effects such as anemia and liver damage in high doses. It is a well-known anthelmintic drug, but due to its poor aqueous solubility, low bioavailability, and high first-pass metabolism, it has not achieved the therapeutic efficacy of the drug [5-7].

A literature survey had been revealed various analytical methods such as HPLC [7-9], HPTLC [8, 9,] and Spectroscopic [10] methods for the determination of Mebendazole drug in the pharmaceutical formulation alone or combination with other drugs. In this study, efforts were made to develop a simple, easy and economical UV spectrophotometric method using suitable solvent for the determination of Mebendazole in the raw materials as well as in the marketed dosage formulations. The International Conference on Harmonization (ICH) guidelines under section Q2 (R1) was used to validate the developed method [11-13].

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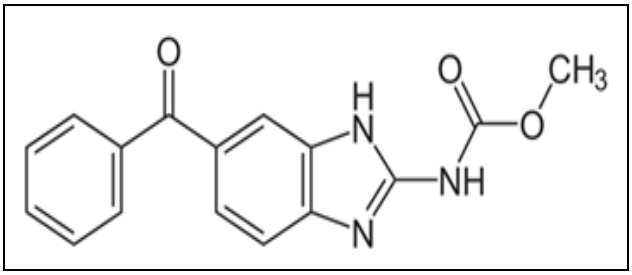

FIG. 1: CHEMICAL STRUCTURE OF MEBENDAZOLE

MATERIALS AND METHODS:

Materials: Mebendazole was obtained from Medopharm, Chennai, Tamil Nadu, India, as a gift sample. All the other chemicals and reagents used were of analytical grade and purchased from Sigma Aldrich. The pharmaceutical tablets of Mebendazole (VERMOX 500) were procured from a local Pharmacy shop.

Instrumentation: Spectroscopic analysis was carried out using Double beam UV-Visible Spectrophotometer-1800, Shimadzu (Kyoto, Japan) with 10 mm path length matched quartz cells.

Selection of Solvent: The criterion for the selection of solvent is based on solubility. The solubility of Mebendazole was determined by using a variety of solvents.

Preparation of Standard Stock Solution and Working Solution: Accurately weighed 100 mg of pure Mebendazole drug and transferred into a 100 ml volumetric flask. A small quantity of Phosphate buffer 6.8 was added to ensure complete solubilization of the drug, and finally, volume was made up to the mark with the same buffer solution. The solution was sonicated for 5 min to dissolve and remove air completely.

The standard stock solution was obtained having a concentration of 1000 µg/ml. From this stock solution, 10 ml was taken into a 100 ml volumetric flask, diluted up to 100 ml with Phosphate buffer 6.8 to get them working solution of 100 µg/ml, and filtered through Whatman filter paper before analyzing.

Preparation of Calibration Curve: From the working solution, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ml solution was transferred into a series of calibrated 10 ml volumetric flasks, and the volume was made up using Phosphate buffer 6.8. The solutions were scanned in the range of 200 to 400 nm against a blank (Phosphate buffer 6.8).

Method Validation: The proposed analytical method has been validated as per the guideline of the International Conference on Harmonization (ICH) under section Q2 (R1) for the following parameters such as linearity, accuracy, precision, the limit of detection (LOD), limit of quantification (LOQ) and robustness [13-16].

Linearity: The linearity of the analytical method is the ability of that method to produce results that are directly proportional to the concentration of the analyte in samples with a given range [17-19]. The serial dilution was prepared in the range of 5-30 µg/ml from the working solution. The samples were analyzed by a UV spectrophotometer using phosphate buffer 6.8 as a blank, and the obtained data were used for the linearity calibration curve.

Accuracy: An accuracy of the proposed method was determined to check the recovery of the test sample by using the standard addition method. The recovery of the method was estimated by spiking the sample solution at three different levels 80, 100, and 120%. The accuracy was reported as % recovery ± (% confidence interval) with % relative error on the base of actual and estimated concentrations [20].

Precision: A precision of the proposed method was performed to check the degree of repeatability of the method. In this method, the sample was measured at least three times on the same day at intervals of an hour for an inter-day study and three different days for inter-day study [21]. The standard deviation (SD) and relative standard deviation (RSD) were calculated.

Limit of Detection (LOD) and Limit of Quantification (LOQ): The LOD and LOQ were performed to check the sensitivity of the method. For this study six replicates of the analyte at the lowest concentration were measured and quantified. LOD measures the limit of the method to detect the minimum concentration of an analyte in the sample; however undoubtedly quantitated as an exact value [20-22]. The LOQ may be defined as the limit of minimum detection capacity of the method to analyze the analyte in a sample which shows the quantitated reliably with the specified level of accuracy and precision [23].

Robustness: The robustness of the proposed method is the ability to remain unaffected by deliberate variations in method parameters. It was determined by altering the λ max of the analysis by ± 2 nm. The % mean recovery (± % confidence interval), as well as % relative error, were determined [23, 24]. The analysis of the standard pure drug of Mebendazole solution in phosphate buffer 6.8 was performed at different wavelengths (± 2 nm).

Ruggedness: The ruggedness of the analytical method is the degree of reproducibility of test results obtained by analysis of the same samples under a variety of normal test conditions on different days and by different analysts [23-25].

Assay of Marketed Formulation: The newly proposed method was applied to analyze the marketed formulation of Mebendazole (VERMOX 500 mg). Ten tablets were weighed and finally powdered in a pestle mortar. The drug powder equivalent to 10 mg was weighed accurately and moved to a 100 ml volumetric flask, dissolved in about 40 ml of Phosphate buffer 6.8, and sonicated for 10 min to get a clear solution of 100 µg/ml. The volume was made up to the mark with the same buffer to obtain a sample solution.

The solution was then filtered through Whatman filter paper #44 [23- 25]. This filtrate was diluted suitably with the same buffer to get the solution concentration of 10 µg/ml. The absorbance of the sample solution was measured at 278.6 nm using Phosphate buffer 6.8 as the blank.

RESULTS AND DISCUSSION: The new analytical method was developed, optimized, validated, and applied for the quantitative analysis of pure drug and marketed tablets formulation.

Method Development: The reported methods or the estimation of Mebendazole are complex, time-consuming, require a large number of solvents, and also require high costly equipment. In the present work, an inexpensive, nontoxic solvent was chosen to get a simple, cost-effective and eco-friendly UV spectrophotometric method for the determination of Mebendazole in a tablet formulation. Based on solubility analysis, Phosphate buffer 6.8 was selected as a solvent for the experiment. The Maximum absorption (λ_{max}) of Mebendazole in Phosphate buffer 6.8 was found to be 278.6 nm.

Method Validation: The proposed method was validated as per ICH guidelines for its linearity, accuracy, precision, robustness, the limit of detection, the limit of quantitation, and robustness. The overall results of the validation parameters were compiled in **Table 1.**

TABLE 1: VALIDATION PARAMETERS FOR DETERMINATION OF MEBENDAZOLE IN PHOSPHATE BUFFER 6.8

S. no.	Parameters	Values
1.	Absorption maxima (nm)	278.6 nm
2.	Linearity range (µg / ml)	5-30
3.	Regression Equation	y = 0.033x + 0.004
4.	Correlation coefficient (r^2)	0.999
5.	Accuracy (% recovery ± SD)	99.22 ± 0.18 to 99.54 ± 0.10
6.	Precision (% RSD)	Intraday assay = 0.05 to 0.20 Interday assay = 0.05 to 0.15
7.	LOD (µg / ml)	0.986
8.	LOQ (µg / ml)	2.988

Linearity: The UV spectrum and the calibration curve of Mebendazole in phosphate buffer 6.8 at 278.6 nm are shown in **Fig 2** and figure 3, respectively. The linearity of Mebendazole was found to be in the range of 5-30 µg/ml with a correlation coefficient of 0.999.

The linearity of Mebendazole was shown in **Table 2**. The significant linear regression is validated by the ANOVA test within the F value. It is observed from the result obtained that the F calculated value is greater than F critical at 278.6 nm.

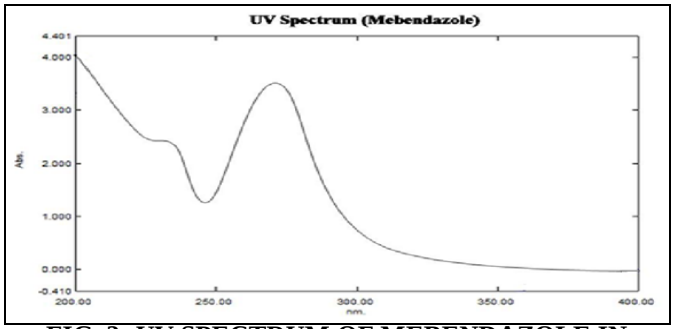

FIG. 2: UV SPECTRUM OF MEBENDAZOLE IN PHOSPHATE BUFFER 6.8 (5µg/ml)

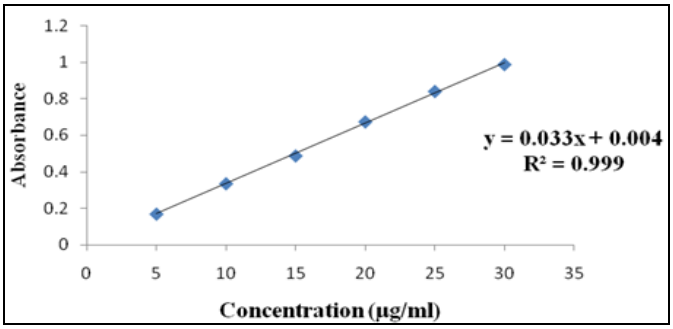

FIG. 3: CALIBRATION CURVE OF MEBENDAZOLE

TABLE 2: LINEARITY OF MEBENDAZOLE

Concentration (µg / ml)	Absorbance Value
5	0.170
10	0.336
15	0.488
20	0.674
25	0.84
30	0.987
Average	0.5825
Regression equation	y = 0.033x + 0.004
r^2	0.999

Accuracy: The accuracy of the proposed method was estimated by a recovery study at the three-level of percentage addition.

As shown in **Table 3**, the percentage recovery of Mebendazole was found to be in the range of 99.22 to 99.54. The results of the recovery studies undoubtedly demonstrate the accuracy of the proposed method.

TABLE 3: ESTIMATION OF ACCURACY BY % RECOVERY METHOD

Concentration (µg/ml)	Level of Addition (%)	Amount of Drug Found (µg) Mean ± SD*	% Mean Recovery ± % RSD
10	80	07.92 ± 0.03	99.22 ± 0.18
	100	09.95 ± 0.01	
	120	11.89 ± 0.01	
15	80	11.86 ± 0.02	99.34 ± 0.08
	100	14.97 ± 0.01	
	120	17.88 ± 0.01	
20	80	15.88 ± 0.04	99.54 ± 0.10
	100	19.95 ± 0.03	
	120	23.91 ± 0.02	

* (n=3) determinations

Precision: The precision of the proposed method was carried out by repeating the complete experiment with different analysts on different days under the same laboratory environment. The % RSD of Mebendazole was found to 0.05 to 0.20 for intraday and 0.05 to 0.15 for inter-day precision. The results of intraday and inter-day precision were shown in **Table 4**. The developed method was found to be precise as the % RSD values of intra-day and inter-day studies were found less than 2%.

TABLE 4: INTRADAY AND INTERDAY PRECISION

Concentration (µg/ml)	Intraday Precision		Inter-day Precision	
	Mean ± SD *	% RSD	Mean ± SD*	% RSD
10	09.96 ± 0.02	0.20	09.92 ± 0.01	0.15
15	14.96 ± 0.01	0.06	14.92 ± 0.01	0.06
20	19.97 ± 0.01	0.05	19.93 ± 0.01	0.05

* (n=3) determinations

Limit of Detection (LOD) and Limit of Quantification (LOQ): The LOD and LOQ study was performed to check the sensitivity of the proposed developed method. The LOD and LOQ were found to be 0.986 µg/ml and 2.988 µg/ml, respectively. From the obtained results, it can be easily interpreted that this UV method is highly sensitive to analyze Mebendazole.

Robustness: The robustness of the developed method shows a non-significant influence of the absorption level through the analysis of the Mebendazole solution in Phosphate buffer 6.8 at different wavelengths (± 2 nm). The result of the robustness study was shown in **Table 5.**

TABLE 5: ROBUSTNESS STUDY

Concentration (µg/ml)	At 276.6 nm			At 280.6 nm		
	Absorbance	Mean ± SD	%RSD	Absorbance	Mean ± SD	%RSD
10	0.327	0.32 ± 0.004	1.423	0.324	0.32 ± 0.003	1.123
	0.321			0.322		
	0.318			0.317		

Ruggedness: As shown in **Table 6**, it was observed that there were no significant changes in the result by changing an analyst, which confirmed that the developed method is rugged.

TABLE 6: RUGGEDNESS STUDY

Conc. (µg/ml)	Analyst 1				Analyst 2			
	Absorbance	Mean ± SD	% RSD	Recovery %	Absorbance	Mean ± SD	% RSD	Recovery %
10	0.336	0.334±	0.96	99.60	0.337	0.335±	0.78	99.70
10	0.331	0.003			0.336	0.002		
10	0.337				0.332			

Assay of Marketed Formulation: The developed and validated method was applied for the estimation of the drug in marketed tablet formulation.

The result of the assay of the marketed formulation was shown in **Table 7**. The % assay was found to be 97.3. The developed method was a good agreement with the label claim.

TABLE 7: RESULTS FOR ASSAY OF MARKETED FORMULATION

Compound Name	Amount Label (mg)	Amount Found (mg)*	%RSD	% Assay
Mebendazole	500	486.54	0.11	97.30%

* (n=3) determinations

CONCLUSION: The UV spectroscopic method for the determination of Mebendazole has been developed and validated as per the guidelines of the International Conference on Harmonization (ICH) under section Q2 (R1). The method found to be simple, reliable, more accurate and precise with lower limits of detection, more specific quantification and sensitivity. It is concluded that from the results obtained, the analysis of validation parameters proved that the method is reproducible and efficient for the determination of Mebendazole pure drug and in tablet formulations without any interferences from excipients. Hence, the method can be used for routine analysis of bulk drug and formulations and can also be used for bioequivalence studies.

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CONFLICTS OF INTEREST: The authors declare no conflicts of interest.

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