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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF MEBENDAZOLE IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

A new simple and sensitive spectrophotometric method in UV region has been developed for the determination of Mebendazole in bulk and in pharmaceutical formulations. Mebendazole exhibits absorption maxima at 234 nm. The method obeys the Beer's law in the concentration range of 1-10 µg/mL. The method is accurate, precise and economical. The percent recovery is near to 100%. This shows that the method was free from the interference of excipients. The results of validation study were analyzed with respect to accuracy, limit of detection, linearity, limit of quantification, precision, ruggedness, robustness and specificity were found to be satisfactory. The proposed method has been applied successfully of drug in bulk and pharmaceutical formulation.

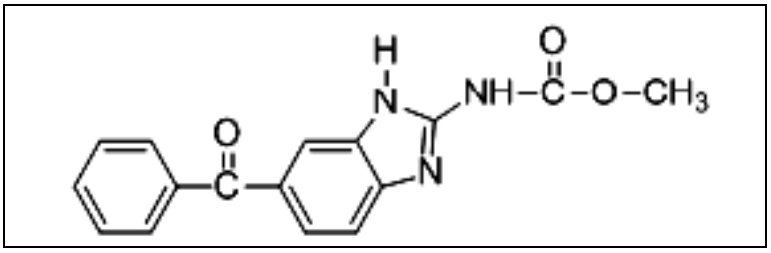
KEYWORDS: Mebendazole, Anthelmintic, UV-Visible spectroscopy, Validation, Recovery.

INTRODUCTION

Mebendazole is broad spectrum anthelmintic agents against nematodes infections including whipworm (*Trichuris trichura*), threadworm (*Enterobius vermicularis*), roundworm (*Ascaris lubricoides*), hookworm (*Ancylostoma duodenale, Necator americanus*),

etc. It is BCS class II drug having low aqueous solubility (71.3 mg/L) and High permeability (Log P = 2.8) ultimately leads to variable absorption of mebendazole. It undergo extensive hepatic first pass metabolism. It has very low bioavailability (5-10%) and maximum amount of drug is protein bound (90-95%).

Analysis of the drug is the most important aspect of formulation development. A suitable method is essential for the estimation of bulk drug, of the drug in formulation, in dissolution studies and in biological samples. Chemically Mebendazole is methyl (5-benzoyl-1H-benzimidazol-2yl) carbamate. It is white to slightly yellow, amorphous powder, almost odourless. It is practically insoluble in water, alcohol, methylene chloride, ether, chloroform and in dilute mineral acids whereas freely soluble in formic acids. It irreversibly block glucose uptake in susceptible helminthes, thereby depleting glycogen stored in parasites. It is official in Chin., Eur., Int., US, British, Indian, and Viet. pharmacopoeia. It is available as chewable tablet, suspension and sachets in market. It is given as 100 mg twice daily for 3 consecutive days. Repeated after 2-3 weeks if needed (to kill ova developed later). There are many reported methods for analysis of Mebendazole either alone or in combination with other drugs in pharmaceutical dosage forms or individually in biological fluids. The quantification of Mebendazole in human plasma by LC-MS has been reported. The estimation of Mebendazole in bulk and in tablet dosage form was done by UV and RP-HPLC method in alone or combination with forced degradation studies. There is a need for a simple, rapid, cost effective and reproducible method for development of Mebendazole. Therefore objective of the study was to develop a simple, accurate, precise, cost effective and reproducible UV-Visible method for estimation of mebendazole as per International Conference on Harmonization (ICH) guidelines Q2(R1).



INSTRUMENT AND MATERIALS

Instrument

A double beam UV-Visible spectrophotometer (Shimadzu UV-1800) and weighing Balance (Shimadzu) was used.

Materials

Mebendazole was gifted from Holden Pharmaceuticals (Sinnar, Nashik). Methanol was purchased from Merck (Mumbai). Aerosil 200 was purchased from Research lab fine chem Industries (Mumbai). Other Solvents were purchased from Fischer scientific co. (India). All the other chemicals and reagents used were of analytical grade.

EXPERIMENTAL

Method Development

Solvent Selection

Various solvents like water, methanol, 0.1 M Hydrochloric acid, chloroform, acetonitrile were selected for the solubility and stability study and it was found that Mebendazole was soluble in the following solvents; formic acid, N, N-dimethyl formamide, 0.5 M Methanolic Hydrochloride, etc. In the present investigation 0.5 M methanolic hydrochloride was selected as a solvent.

Preparation of standard solution

Standard stock solution was prepared by dissolving accurately weighed 10 mg of mebendazole in 50 mL of solvent (0.5 M Methanolic Hydrochloride). Keep it on sonicator bath for 15 min. After 15 minutes, the volume was made up to the mark with 100 mL volumetric flask with same solvent to give a solution of 100 µg/mL.

Selection of analytical wavelength

The standard solution of Mebendazole was scanned in the wavelength range of 200-400 nm. The absorption spectra obtained was showing the wavelength at different value for different solvent.

Selection of analytical concentration range and preparation of calibration curves

From the standard stock solution of mebendazole, respective aliquots was pipette out into 10 mL volumetric flasks and dilutions were made with respective solvent to obtain working standard solution of mebendazole of desired concentrations. The spectrum was measured for three times for each concentration. The Absorbance spectra of each solution of mebendazole were measured at respective wavelength. The stable wavelength of solvent is selected and continues further for validation study.

Analysis of formulation

Weight equivalent to 10 mg was weighed accurately. Transferred it to 100 mL volumetric flask, dissolved in 50 mL of 0.5 M Methanolic Hydrochloride by ultrasonication of the flask for 15 minutes and volume was made up to the mark with respective solvent. The solution was filtered through 0.45 µ filter. An aliquot of sample stock solution was transferred to 10 mL volumetric flask and volume was made up to mark with same solvent.

Validation

Linearity and range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

The linearity of the analytical method was demonstrated over the concentration range of 1-10 µg/mL of the target concentration for both the drugs. Aliquots of 0.1, 0.2, 0.3, 0.4, ……, 1 mL were pipetted out from working standard solution into series of 10 mL volumetric flasks. The volume was made up to the mark with 0.5 M Methanolic Hydrochloride.

Limit of Detection (LOD)

The detection limit is the lowest amount of analyte in a sample which can be detected but not quantitated.

$$LOD = 3.3\ \sigma/S$$

Where,

σ = Standard deviation

S = Slope

Limit of Quantification (LOQ)

The quantitation limit is the lowest amount of analyte in a sample which can be quantitatively determined.

$$LOQ = 10\ \sigma/S$$

Accuracy

The value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. Accuracy of proposed method was ascertained on the basis of recovery study. Recovery studies were carried out by spiking standard working solution to preanalyzed sample solution (formulation) at three different levels 80 %, 100 % and 120%. Final concentrations of mebendazole was determined. At each levels of the amount, three determinations were performed.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. It was ascertained by replicate estimation of samples. It involves intra-day and inter-day precision. For this three replicate of 4.8, 6, 7.2 µg/mL of mebendazole solution were prepared and analysis carried out three times on the same day and different day at each concentration levels. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

Ruggedness

The ruggedness is degree of reproducibility of test results under verify of condition like different analysts, different instruments and different days.

To establish ruggedness of the proposed method the solutions of 6 µg/mL of mebendazole standard solution was prepared and analyzed with the change in the different analyst.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

The robustness of the proposed method the solutions of 6 µg/mL of mebendazole standard solution was prepared and analyzed by changing parameter wavelength. The wavelength was selected $\lambda_{max} \pm 1$ i.e. 233 and 235 nm for mebendazole standard solution.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

Specificity was done by using an excipient, aerosil 200 (adsorbent). The three different concentrations at three levels 80 %, 100 %, 120 % respectively are spiked in standard mebendazole solution (6 µg/mL). At each levels of the amount, three determinations were performed to check effect of Aerosil 200.

RESULTS AND DISCUSSION

Selection of Analytical Wavelength

The standard solution of mebendazole scanned showing the wavelength at different value for 0.5 M Methanolic Hydrochloride as shown below.

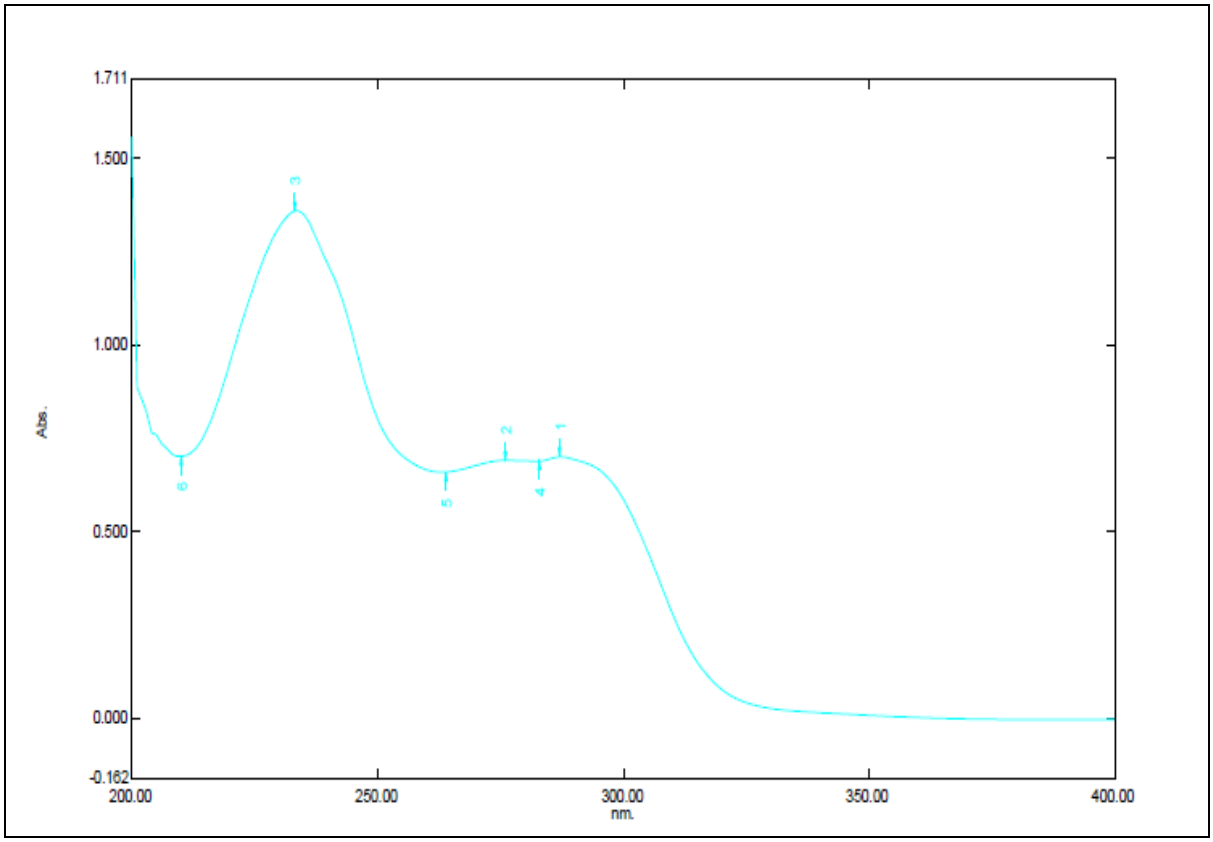


Fig. 1 UV spectrum of drug in 0.5 M methanolic hydrochloride (λ_{max}= 234 nm)

The above spectrum of mebendazole in 0.5 M methanolic hydrochloride showed maximum absorption at 234 nm, which is complying with reported λ_{max}. Hence, it was selected as λ_{max} of mebendazole in 0.5 M methanolic hydrochloride for further use.

Selection of analytical concentration range and preparation of calibration curves

Table 1: Linearity of drug in 0.5 M Methanolic Hydrochloride (λ_{max}= 234 nm)

Sr No.	Conc (µg/mL)	Absorbance
1	1	0.111
2	2	0.173
3	3	0.334
4	4	0.404
5	5	0.511
6	6	0.651
7	7	0.759
8	8	0.909
9	9	1.103
10	10	1.208

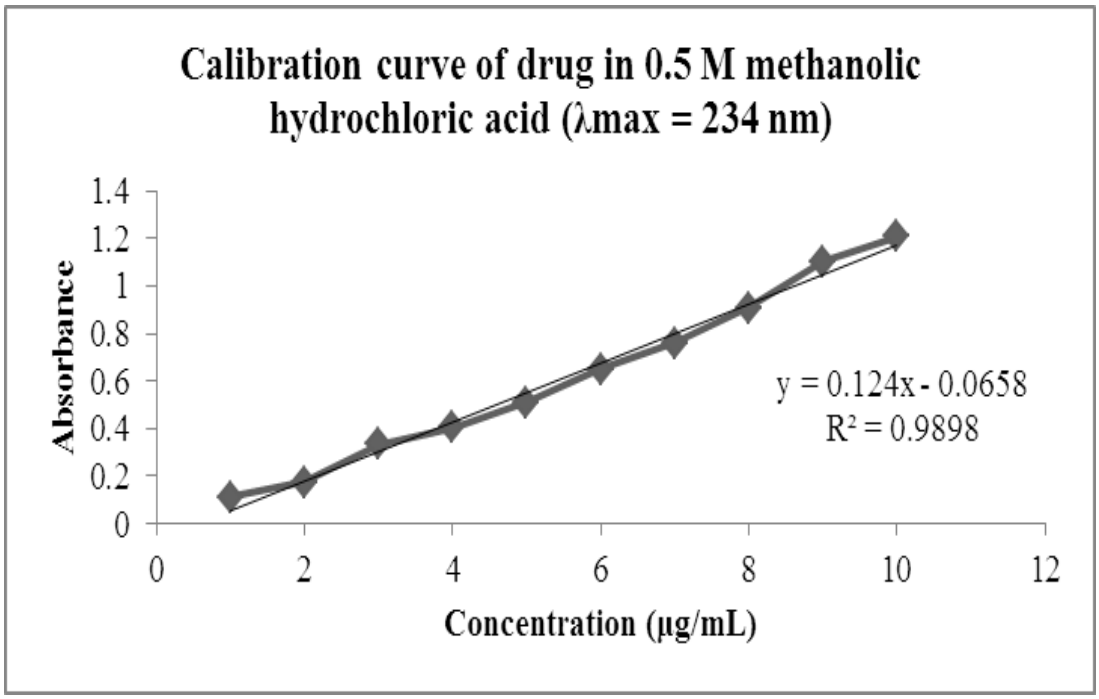


Fig. 2 Calibration curve of drug in 0.5 M Methanolic Hydrochloride (λ_{max} = 234 nm)

Validation

The Validation of drug was performed in 0.5 M Methanolic Hydrochloride depending upon good solubility, stability of wavelength and correlation coefficient.

Linearity and range

For UV-spectrophotometric method was obtained between absorbance and concentration of in the range 1-10 µg/mL with regression coefficient R^2 = 0.9898. The linear regression equation was found to be y = 0.124x-0.0658. Results of linearity data is summarized in **Table 1** and **Fig. 2**.

Limit of Detection (LOD)

The limits of detection (LOD) which represents the sensitivity of the proposed method were determined. The LOD value obtained was 0.1714 µg/mL, which indicates the high sensitivity of the proposed method.

Limit of Quantitation (LOQ)

The limits of quantitation (LOQ) which represents the sensitivity of the proposed method were determined. The LOQ value obtained was 0.5194 µg/mL, which indicates the high sensitivity of the proposed method.

Accuracy

The accuracy of method was ascertained by performing recovery study at three concentration levels i.e. 80%, 100% and 120%. The percent recovery obtained indicates non-interference from the excipients used in the formulation. The results of recovery study are given in **Table 2** and **Table 3**.

Table 2: Recovery study of mebendazole

Level of addition	Standard API (µg/mL)	Formulation stock added (µg/mL)	Total conc.(µg/mL)	Absorbance	Drug recovered (µg/mL)	% Recovery
80%	6	4.8	10.8	1.214	10.321	95.5645
				1.215	10.329	95.6392
				1.216	10.3371	95.7139
100%	6	6	12	1.417	11.9581	99.6505
				1.419	11.9742	99.7849
				1.419	11.9742	99.7849
120%	6	7.2	13.2	1.565	13.1516	99.6334
				1.563	13.1355	99.5112
				1.562	13.1274	99.450147

Table 3: Statistical Validation of Recovery Studies

Level of addition	%Recovery (mean ± SD)*	% RSD	SE
80%	95.6392 ± 0.001	0.0823	0.00058
100%	99.7401 ± 0.00115	0.08141	0.00067
120%	99.5316 ± 0.00153	0.09771	0.00088

*(n=3)

Precision

Precision of proposed method was determined by Intra-day and Inter-day precision and it was expressed in terms of percent relative standard deviation (% RSD). For Intra-day and Inter-day precision % RSD were found in the range of 0.1972-0.3349 and 0.1988-0.3366

respectively. Whereas, standard error were found in the range of 0.00058-0.00153 for both. Results of precision study are summarized in **Table 4** and **Table 5**.

Table 4: Intra-day precision study

Conc. (µg/mL)	Absorbance (mean ± SD)*	SD	%RSD	SE
4.8	0.507	0.001	0.19724	0.00058
6	0.6507	0.00153	0.23475	0.00088
7.2	0.79	0.00265	0.33491	0.00153

*(n=3)

Table 5: Inter-day precision study

Conc. (µg/mL)	Absorbace (mean ± SD)*	SD	%RSD	SE
4.8	0.503	0.001	0.1988	0.00058
6	0.6477	0.0021	0.3214	0.0012
7.2	0.786	0.0026	0.3366	0.00153

*(n=3)

Ruggedness

In the ruggedness study, the influence of small, deliberate variations of the analytical parameters on absorbance of drug was examined. The factor selected was change in analyst. Results of ruggedness study indicate that the selected factor remained unaffected by small variations with % RSD of 0.1943-0.3423, which confirms the ruggedness of method. Results are shown in **Table 6**.

Table 6: Ruggedness data for mebendazole

Sr. No.	Conc. (µg/mL)	Absorbance (mean ± SD)*	%RSD	SE
1	4.8	0.506 ± 0.0017	0.3423	0.001
2	6	0.6527 ± 0.0021	0.3189	0.0012
3	7.2	0.7863 ± 0.0015	0.1943	0.00088

*(n=3)

Robustness

In the robustness study, the influence of small, deliberate variations of the analytical parameters on absorbance of drug was examined. The factor selected was change in wavelength. Results of robustness study indicate that the selected factor remained unaffected by small variations with % RSD of 0.0896-0.2348, which confirms the robustness of method. Results are shown in **Table 7**.

Table 7: Robustness data for mebendazole

Sr. No.	Wavelength (nm)	Absorbance (mean ± SD)*	%RSD	SE
1	233	0.645 ± 0.01	0.155	0.00058
2	234	0.6507 ± 0.0015	0.2348	0.00088
3	235	0.6443 ± 0.0006	0.0896	0.00033

*(n=3)

Specificity

The specificity of proposed method was ascertained by performing study at three concentration levels i.e. 80%, 100% and 120%. The mean recovery of added excipient at each level was found to be 93.9695-95.5376% with standard deviation of 0.00058-0.00153. The % RSD was found to be 0.0912-0.00153. The percent recovery obtained indicates non-interference from the excipients used in the formulation. The results of specificity study are given in **Table 8** and **Table 9.**

Table 8: Specificity study

Level of addition	Standard API (µg/mL)	Aerosil 200 added (µg/mL)	Total conc.(µg/mL)	Absorbance	Drug recovered (µg/mL)	% Recovery
80%	6	4.8	6	0.645	5.7323	95.5376
				0.644	5.7242	95.4032
				0.646	5.7403	95.672
100%	6	6	6	0.641	5.7	95
				0.642	5.7081	95.1344
				0.639	5.6839	94.7312
120%	6	7.2	6	0.634	5.6435	94.0591
				0.633	5.6355	93.9247
				0.633	5.6355	93.9247

Table 9: Statistical validation of specificity studies

Level of addition	%Recovery (Mean ± SD)*	% RSD	SE
80%	95.5376 ± 0.001	0.0155	0.00058
100%	94.9552 ± 0.00153	0.2384	0.00088
120%	93.9695 ± 0.00058	0.0912	0.00033

*(n=3)

The results of validation parameters showed that proposed method was found to be simple, accurate, economic, sensitive, and precise and can be adopted for estimation of mebendazole in bulk and formulation.

CONCLUSION

The developed UV spectrophotometric method was found to be simple, sensitive, accurate, precise, and reproducible. The values of % recovery was close to 100% indicating reproducibility and accuracy of the proposed method shows that the method could find practical application hence, utilized as routine quality control analysis.

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CONFLICT OF INTEREST

Author declares no conflict of interest regarding publication.

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