Research Article

Method Development and Validation for the Estimation of Anthelmintic Drug (Albendazole) in Tablet Preparations

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ABSTRACT

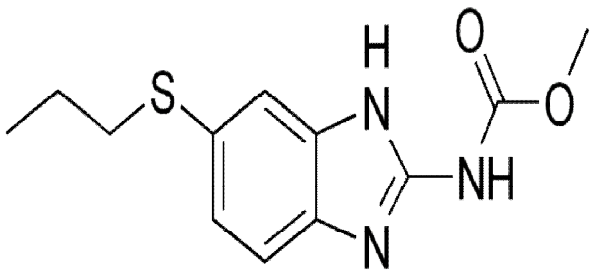
Fixed dose Albendazole is an anthelmintic or anti-worm medication. A simple, precise and specific spectrophotometric method was developed and validated for the estimation of Albendazole in tablet dosage form. Albendazole showed maximum absorbance i.e.; λ_{max} at 308 nm. The drug was derivatized in ethanolic HCl diluents solution. Beer Lambert's law was obeyed at concentration range of 4-10 ppm. A linearity curve was calibrated by concentration versus absorbance. The regression equation of curve was calculated as Y = 0.0813 x + 0.0065, with correlation coefficient r = 0.9993. The accuracy was determined by recovery study and the overall percentage recovery was found to be 99.29% ± 0.943. Precision of the method was found to be 99.53% ± 0.853. SD and % RSD values were less than 1. The LOD and LOQ were calculated as 1.051 ppm, 3.185 ppm respectively. The developed method was validated in terms of linearity, accuracy, precision, limit of detection and quantification, robustness as per ICH guidelines. The method can be successfully applied for quality control analysis of Albendazole in pharmaceutical formulation.

Keywords: Albendazole, Validation, Spectrophotometric Method, Beer's law.

INTRODUCTION

Albendazole is an anthelmintic or anti-worm medication. Chemically it is Methyl 5-propylthio-1H-benzimidazol-2-ylcarbamate. It prevents newly hatched insect larvae from growing or multiplying in your body. It is insoluble in water and soluble in DMF, strong acids, strong bases, methanol, ethanol, ether, chloroform etc.

Albendazole causes degenerative alterations in the tegument and intestinal cells of the worm by binding to the colchicine-sensitive site of tubulin, thus inhibiting its polymerization or assembly into microtubules. The loss of the cytoplasmic microtubules leads to impaired uptake of glucose by the larval and adult stages of the susceptible parasites, and depletes their glycogen stores. Degenerative changes in the endoplasmic reticulum, the mitochondria of the germinal layer, and the subsequent release of lysosomes result in decreased production of adenosine triphosphate (ATP), which is the energy required for the survival of the helminth. Due to diminished energy production, the parasite is immobilized and eventually dies[1].



"Structure of Albendazole"

Literature survey reveals that several uv-spectrophotometric methods have been developed for the estimation of Albendazole in tablet dosage form including uv-spectrophotometer[2,3], RP-HPLC[4], titration[5,6] and flow injection method[7].

Since no analytical method has so far been developed by using ethanolic HCl as diluent solution. Therefore, it was endeavored to develop an accurate, precise and economic spectrophotometric method for the determination of Albendazole from pharmaceutical dosage form.

The aim of the present work is to develop and validate an economical, accurate, precise and reproducible UV-Spectrophotometric method for the determination of Albendazole as in solid dosage form. Drug was found to be freely soluble in ethanolic HCl which was chosen for proceeding study.

MATERIALS AND METHODS

Reagents and Chemicals

The reference standard Albendazole (99.4%) pure was received as a gift sample from Java Pharmaceutical Kot Lakhpat Lahore. Ethanol (AR grade) and other chemicals, HCl, NaOH (AR grade) was procured from Merck Chemical. Sample tablets Zentel (Albendazol label claim 200 mg) were purchased from local market Lahore. Distilled water was used throughout the study.

Apparatus

A single beam UV-spectrophotometer (Cecil CE 2041, 2000 series) with 1 cm thickness of cell was used for the measurement. Analytical balance (JS-110, Japan) was

used to weigh the sample and standard (Albendazole) material.

Method Preparation of Stock and Standard Solution

Accurately weighed 50mg of Albendazole (reference standard) was transferred in a 50 mL volumetric flask and dissolved it in ethanolic HCl. Magnetic stirrer was used for better dissolution.

Make the volume up to the mark to get 1000 ppm stock solution. Further 10 ppm standard dilution was made by taking 1mL of above solution and make up the volume up to 100 mL with 0.1 M NaOH.

Application of the Proposed Procedure for the Determination of Albendazole in Tablets

The proposed method was applied in order to determine the Albendazole in tablets dosage form.

The marketed tablet Albendazole was used for this. Twenty tablets were weighed and average weight was calculated, crushed to fine powder.

The powder equivalent to 50 mg of Albendazole was transferred in a 50 mL volumetric flask and dissolved it in ethanolic HCl diluent solution.

Magnetic stirrer was used for better dissolution. The excipients were separated by filtration, from above filtrate take 1 mL and make the volume up to 100 mL with 0.1 M NaOH to get 10 ppm solution.

Assay Measurement

The mean assay results of three sample tablets were comparable with claimed value. The obtained results are presented in Table-1 and percentage was found to be 99.75%.

Table 1: Assay determination of Albendazole from its tablet

Sample Tablet	λ_{max}	Label Claimed	Amount Found mg/Tab	Mean % Assay
Albendazole	308 nm	200 mg	199.5 mg	99.75%

Method Validation

The proposed method was developed by using specificity, linearity, accuracy, precision, ruggedness and robustness as per ICH guidelines, 1996.

Specificity

The specificity of the method was checked by monitoring the uv spectra of sample tablet and compared it with the standard. The excipients did not show any effect on the estimation of Albendazol. Hence, the determination of Albendazol in the tablet was considered to be free from interference due to these excipients.

Linearity

The linearity of the proposed assay was studied in the concentration range 4-10 ppm at 308 nm. The calibration data showed a linear relationship between concentration and absorbance as shown in Figure 1.

The value of regression equation, regression coefficient and correlation coefficient was found 0.813x + 0.065, 0.9986 and 0.9993 respectively as mention in Table 2.

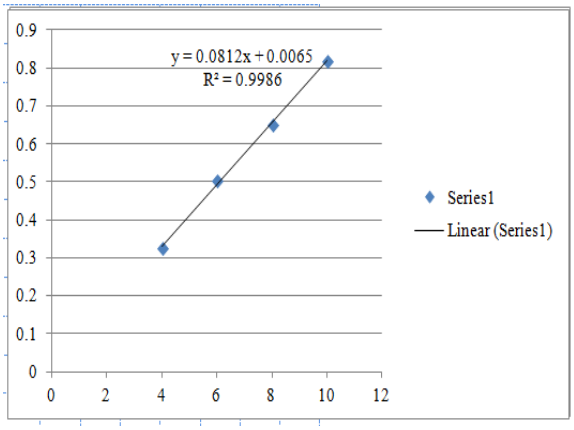


Figure 1: Linearity Study for Albendazol

Table 2: Regression Analysis of linearity curve

Parameters	Results
Regression equation	Y= 0.813x + 0.065
Regression coefficient	R^2 = 0.9986
Correlation coefficient	R = 0.9993

Accuracy

To ensure the accuracy of the method, recovery study was performed by preparing 6 sample solutions and adding a known amount of active drug (10 µg) to each sample solution and measuring the absorbance at 308 nm. The % recovery was calculated along with SD and % RSD as listed on Table 3.

Table 3: % Recovery of Albendazole

Samples after Addition	Absorbance after Addition	% Recovery Assay
Sample 1	0.817	99.88 %
Sample 2	0.808	97.6 %
Sample 3	0.813	99.86 %
Sample 4	0.821	100.2%
Sample 5	0.815	99.23 %
Sample 6	0.820	98.99 %
Mean % Recovery Assay ± SD	**-**	**99.29% ± 0.943**
Mean % Recovery Assay ± %RSD	**-**	**99.29% ± 0.95**

Precision

The intra-day and inter day precision were determined and presented as the % RSD. The mean % assay of tablet - A and tablet- B was found to be **99.57% ± 0.689** and **99.53% ± 0.852** respectively, which was very close to the proposed assay value as shown in Table 4.

Table 4: Assay result of Tablet-A and Tablet-B

Samples	Absorbance of Tablet-A	% Assay of Tablet-A	Absorbance of Tablet-B	% Assay of Tablet-B
Sample tab 1	0.816	99.6 %	0.813	99.26%
Sample tab 2	0.815	99.51 %	0.807	99.53%
Sample tab 3	0.822	100.2 %	0.809	98.77%
Sample tab 4	0.811	99.02%	0.820	100.11%
Sample tab 5	0.823	100.48 %	0.817	99.75%
Sample tab 6	0.808	98.65 %	0.826	100.8%
Mean % Recovery Assay ± SD	-	**99.57% ± 0.689**		**99.53% ± 0.852**
Mean % Recovery Assay ± %RSD	-	**99.57% ± 0.069**		**99.53% ± 0.856**

Table 5: % Assay result of λ_{max} ± 2 Conditions

Samples	Wavelength plus condition λ_{max}: 308nm + 2		Wavelength subtract condition λ_{max}: 308nm - 2	
	Absorbance	% Assay	Absorbance	% Assay
Sample 1	0.815	99.51 %	0.817	99.75%
Sample 2	0.814	99.3 %	0.813	99.26%
Sample 3	0.820	100.12%	0.811	99.02%
Sample 4	0.822	100.36%	0.820	100.98%
Sample 5	0.807	98.53%	0.809	98.77%
Sample 6	0.813	98.26%	0.817	101.3%
Mean % Recovery Assay ± SD	-	**99.34% ± 0.836**		**99.68% ± 0.913**
Mean % Recovery Assay ± %RSD	-	**99.34% ± 0.841**		**99.68% ± 0.915**

Table 6: % Assay result of Day -1 and Day -2

Samples	Day -1		Day -2	
-	Absorbance	% Assay	Absorbance	% Assay
Sample 1	0.818	99.87%	0.809	98.77%
Sample 2	0.814	99.38%	0.830	99.88%
Sample 3	0.823	100.4%	0.826	100.03%
Sample 4	0.815	99.51%	0.817	99.75%
Sample 5	0.811	99.02%	0.814	98.38%
Sample 6	0.804	98.16%	0.816	101%
Mean % Recovery Assay ± SD	-	**99.39% ± 0.763**		**99.68% ± 0.968**
Mean % Recovery Assay ± %RSD	-	**99.39% ± 0.767**		**99.68% ± 0.971**

Robustness

Robustness of analytical method is its capacity to remain unaffected by small but deliberate variations in method parameters. For this purpose sample solution were analyzed at λ_{max} ± 2. With small variation in wavelength % Assay was calculated with in limit 90-110 %.

Results are summarized in Table 5.

Ruggedness

Ruggedness defines the degree of reproducibility of results obtained under a variety of conditions, such as different laboratories, analysts, instruments, environmental conditions, operators and materials.

Ruggedness was determined by analyzing the sample solution at two different days with changed

environmental condition. % Assay was calculated and it was found that the assay was within limit (90-110 %) along with SD and %RSD. Results are summarized in Table-6.

LOD and LOQ

Limit of detection is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Whereas the limit of quantitation refers to the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. Both are expressed in terms of concentration. The LOD and LOQ were calculated by using slope value of linearity curve. The results are enlisted in Table-7.

Table 7: LOD & LOQ Results

Parameters	Results
Slope	0.0813
Standard deviation	0.0259
LOD	1.051 ppm
LOQ	3.185 ppm

Stability

The stability of the developed method was confirmed by assay of the drug formulation at the interval of 1hour and it was determined that there was almost no appreciable change in absorbance up to 8hs at two different temperatures. Percentage purity of the assays was found to be within 99.50 to 101.95%. Thus the proposed method was found to provide high degree of stability.

RESULTS AND DISCUSSION

Analytical method development and validation of Albendazole in tablet formulation was the basic aim of the current research. Fixed dose Albendazole is an anthelmintic or anti-worm medication. A simple, precise and specific spectrophotometric method was developed and validated for the estimation of albendazole in tablet dosage form. Albendazole showed maximum absorbance i.e.; λ_{max} at 308 nm.

The drug was derivatized in ethanolic HCl diluent solution. Beer Lambert's law was obeyed at concentration range of 4-10 ppm. A linearity curve was calibrated by concentration versus absorbance. The regression equation of curve was calculated as Y = 0.0813 x +0.0065, with correlation coefficient r = 0.9993. The accuracy was determined by recovery study and the overall percentage recovery was found to be 99.29% ± 0.943. Precision of the method was found to be 99.53% ± 0.853. SD and % RSD values were less than 1. The LOD and LOQ were calculated as 1.051 ppm, 3.185 ppm respectively. The developed method was validated in terms of linearity, accuracy, precision, limit of detection and quantification, robustness as per ICH guidelines. The method can be successfully applied for quality control analysis of Albendazole in pharmaceutical formulation.

CONCLUSION

Experimental results and discussion show that the developed method is specified and validated in terms of linearity, accuracy, precision, limit of detection and quantification, robustness as per ICH guidelines. The method can be successfully applied in quality control analysis for the estimation of the label claim of Albendazole in Pharmaceutical formulation.

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