ANALYTICAL METHOD VALIDATION PROTOCOL FOR ALBENDAZOLE TABLETS USP

ANALYTICAL METHOD VALIDATION PROTOCOL FOR ALBENDAZOLE TABLETS USP 400 MG

Validation Analysis Records for Albendazole Tablets USP

OBJECTIVE: The efficacy & safety of a medicinal product can only be assured by analytical monitoring of its quality.

SCOPE : The scope of analytical validation is to ensure that the procedure under consideration is capable of giving reproducible and reliable results.

Product Name	Albendazole Tablets USP
Ingredient	Albendazole USP
Label Claim	Each uncoated chewable tablet contains Albendazole USP ----------400.0 mg
Test Method	Liquid Chromatography Albendazole

Specificity (**Diluents Interference**)

Placebo Preparation:

A placebo solution was prepared same as the formulation except for the addition of the active ingredients. Here, the product contains no inactive ingredients. So, here the mobile phase is used as the placebo solution. Area at 254 nm, Observation Result: Nil

Conclusion for Specificity:
We observed that at wavelength 254 nm there is no significant area for placebo (Diluents) for Albendazole Tablets USP assay method. Therefore specificity of the method considered acceptable.

System Accuracy:
The system precision of the above method was carried out by taking area for six times of the sample preparation of exact weight.

Text data collection sheet:

Serial No.	Area of Albendazole
1.	
2.	
3.	
4.	
5.	
6.	
Mean	
% RSD	

Acceptance Criteria: RSD is not more than 2.0%.

Linearity/ Accuracy:

Definition:

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The Linearity of an analytical method is its ability to elicit test results that are directly, or by a well defined mathematical transformation, proportional to the concentration of the analyte in samples within a given range. Linearity is usually expressed in terms of the variance around the slope of the regression line calculated according to an established mathematical relationship from test results obtained by the analysis of sample with varying concentration of analyte.

Range:

Definition:

The Range of an analytical method is the interval between the upper & lower level of analyte that have been demonstrated with precision, accuracy & linearity using the method as written. The Range is normally expressed in same units as test results e.g. Percent or Parts per million, obtained by the analytical method.

Assay: Albendazole Tablets USP (Limit: 90.0 % to 110.0 % of the labeled amount)

Chromatographic Condition:

Column: Packing L1, 5µm (25 cm X 4.6 mm)

Column temperature: ambient

Detector : UV 254nm

Flow rate : 2.0ml/min.

Injection volume : 20µl

Mobile phase— Dissolve 0.50 g of monobasic ammonium phosphate in 400 mL of water. Add 600 mL of methanol, mix, and filter, discarding the first 15 mL of the filtrate. Degas the clear filtrate before use. Make adjustments if necessary.

Sulfuric acid in methanol— Prepare a mixture of 1 mL of sulfuric acid and 99 mL of methanol.

Standard preparation—

Transfer----------mg (100 mg) of Albendazole WS, accurately weighed, to a 50-mL volumetric flask. Add 5 mL of Sulfuric acid in methanol and 25 mL of methanol, and shake to dissolve. Dilute with methanol to volume, and mix. Transfer 5.0 mL of this stock solution into 50-mL volumetric flask, dilute with methanol to volume, and mix.

Assay preparation—

Weigh and finely powder not fewer than 20 Tablets. Transfer a required accurately weighed quantity of the powder into a 50-mL volumetric flask. Add 5 mL of Sulfuric acid in methanol and 20 mL of methanol, and shake by mechanical means for about 15 minutes. Dilute with methanol to volume, mix,

and filter, discarding the first 15 mL of the filtrate. Transfer 5.0 mL of the clear filtrate into 50-mL volumetric flask, dilute with methanol to volume, and mix.

Chromatographic system-

Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the tailing factor is not more than 2.0; the column efficiency is not less than 1000 theoretical plates; the resolution between the Albendazole peak and the Parbendazole peak is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—

[Note—Use peak heights where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in %, of $C_{12}H_{15}N_3O_2S$ in the portion of Tablets taken by the formula:

$$\frac{\text{Sample area} \times \text{WS weight} \times \text{Potency of WS} \times \text{Average Weight} \times 100}{\text{Standard area} \times \text{Sample weight} \times 100 \times \text{Claim}} = \quad \%$$

Text data collection sheet:

S.No.	Standards	Area of Albendazole
	Standard-1	
	Standard-2	
	Standard-3	
	Standard-4	
	Standard-5	
	Standard-6	
	Mean	
	% RSD	

Acceptance Criteria: RSD is not more than 2.0%.

Samples	Sample Area of Albendazole	Mean
Sample-A-01 80%		
Sample-A-02 80%		
Sample-A-03 80%		
Sample-B-01 90%		
Sample-B-02 90%		
Sample-B-03 90%		

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Samples	Sample Area of Albendazole	Mean
Sample-C-01 100%		
Sample-C-02 100%		
Sample-C-03 100%		
Sample-D-01 110%		
Sample-D-02 110 %		
Sample-D-03 110 %		
Sample-E-01 120%		
Sample- E-01 120%		
Sample- E-01 120%		

Calculation:

Data Collection:

Concentration (µg/ml)	Concentration in %	Corr. Coefficient	Sample mean area	% Recovery	Corr. Coefficient
	80.0	1.0			
	90.0				
	100.0				
	110.0				
	120.0				

Precision:

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogeneous sample. The precision of the analytical method is usually expressed as Standard deviation or relative standard deviation (coefficient of variation) of a series measurement. The precision may be measured of either the degree of reproducibility or of repeatability of the analytical method on the normal operating condition.

Precision – Method precision:
Albendazole Tablets **(**Limit: 90.0 % to 110.0 % of the labeled amount)

Analyst (I) …….:
Chromatographic Condition:
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Column temperature: ambient

Detector: UV 254 nm

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Injection volume: 20µl

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Sample Dilutions:

(A) Take ----------mg of the sample and proceed as above.

(B) Take ----------mg of the sample and proceed as above.

(C) Take ----------mg of the sample and proceed as above.

(D) Take ----------mg of the sample and proceed as above.

(E) Take ----------mg of the sample and proceed as above.

(F) Take ----------mg of the sample and proceed as above.

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	Standard-1	
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	Standard-3	
	Standard-4	
	Standard-5	
	Standard-6	
	Mean	
	% RSD	

Samples		Sample Area of Albendazole	Mean
Sample A	T1		
	T2		
Sample B	T1		
	T2		
Sample C	T1		
	T2		
Sample D	T1		
	T2		
Sample E	T1		
	T2		
Sample F	T1		
	T2		

Calculation:

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Table for Six Replicate Assays:

Sample Number	Estimated % Amount	Mean	Relative Standard Deviation (% RSD)
Sample A			
Sample B			
Sample C			
Sample D			
Sample E			
Sample F			

Acceptance Criteria: NMT 2% (% of Relative Standard Deviation)

Intermediate Precision: – (Within laboratory variations such as different days, analyst & equipments):

Analyst (II): "……"

Chromatographic Condition:

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(C) Take ----------mg of the sample and proceed as above.

(D) Take ----------mg of the sample and proceed as above.

(E) Take ----------mg of the sample and proceed as above.

(F) Take ----------mg of the sample and proceed as above.

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	Standard-2	
	Standard-3	
	Standard-4	
	Standard-5	
	Standard-6	
	Mean	
	% RSD	

Acceptance Criteria: RSD is not more than 2.0%.

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Samples		Sample Area of Albendazole	Mean
Sample A	T1		
	T2		
Sample B	T1		
	T2		
Sample C	T1		
	T2		
Sample D	T1		
	T2		
Sample E	T1		
	T2		
Sample F	T1		
	T2		

Calculation:

Table for Six Replicate Assays:

Sample Number	Estimated % Amount	Mean	Relative Standard Deviation (% RSD)
Sample A			
Sample B			
Sample C			
Sample D			
Sample E			
Sample F			

Acceptance Criteria: NMT 2% (% of Relative Standard Deviation)

Table for Six Replicate Assays analyst by two different Analysts & days:

Test Data analyst by **…….**-

Table for Six Replicate Assays:

Sample Number	Estimated % Amount	Mean	Relative Standard Deviation (% RSD)
Sample A			
Sample B			
Sample C			
Sample D			
Sample E			
Sample F			

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Test Data analyst by **……:**
Table for Six Replicate Assays:

Sample Number	Estimated % Amount	Mean	Relative Standard Deviation (% RSD)
Sample A			
Sample B			
Sample C			
Sample D			
Sample E			
Sample F			

Acceptance Criteria: NMT 2 % (% of Relative Standard Deviation).

Robustness:

To demonstrate the analytical method is capable to yield reproducibility results under; small but deliberate variations in method parameters during normal usage such as composition & Flow rate of mobile phase.

Procedure:

Perform the robustness study by injecting single of resolution solution & standard solution for six times for the following parameters.

- Change in ratio of the mobile phase. Record the observation in below observation table.
- Change in Flow rate of mobile phase. Record the observation in below observation table.

OBSERVATION TABLE:-

Change ratio in the mobile phase at 254 nm					
Mobile phase		Flow rate ml/min	System suitability		
Buffer	Methanol		Retention time	Theoretical plate	Tailing Factor
390 ml	610 ml	2.0 ml/min.			
400 ml	600 ml	2.0 ml/min.			
410 ml	590 ml	2.0 ml/min.			

Change ratio in the mobile phase at 254 nm					
Mobile phase		Flow rate ml/min	System suitability		
Buffer	Methanol		Retention time	Theoretical plate	Tailing Factor
400 ml	600 ml	1.9 ml/min.			
400 ml	600 ml	2.0 ml/min.			
400 ml	600 ml	2.1 ml/min.			

Acceptance criteria:

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Analytical method validation shall be robust (i.e. Theoretical Plates is not less than 1000 & tailing factor is not more than 2.0).

Analysed By/On: **Checked By/On:**