ANALYTICAL METHOD VALIDATION

ANALYSIS RECORDS

ALPRAZOLAM TABLETS USP

Quality Control Department

ANALYTICAL METHOD VALIDATION REPORT FOR ALPRAZOLAM TABLETS 0.5 MG

Validation Analysis Records for Alprazolam 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	Alprazolam Tablets USP
Ingredient	Alprazolam USP
Label Claim	Each uncoated tablet contains
	Alprazolam USP----------0.5mg
Test Method	Liquid Chromatography
	Alprazolam USP

Specificity (Diluents Interference)

Placebo Preparation:
A placebo solution was prepared same as the formulation except for the addition of the active ingredients. Here 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 Alprazolam tablets 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.

Test data collection sheet:

Serial No.	Area of Alprazolam
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: **Alprazolam Tablets USP** (Limit: 90.0 % to 110.0 % of the labeled amount).

Condition:-
Column: Packing L3, 5µm (30 cm X 4.6 mm)

Column temperature: ambient

Detector: UV 254nm

Flow rate: 2.0ml/min.

Injection volume: 20µl

Mobile phase:—
Prepare a filtered and degassed mixture of Acetonitrile, chloroform, butyl alcohol, water, and glacial acetic acid (850:80:50:20:0.5). Make adjustments if necessary.

Standard Preparation:—
Dissolve an accurately weighed ----------mg (25.0mg) of Alprazolam WS in 100ml volumetric flask, add 25ml Acetonitrile, shake and dilute with Acetonitrile. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with Acetonitrile to volume, and mix.

Sample Preparation:—
Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed required quantity of the powder to a 200-mL volumetric flask. Transfer 2 mL of water and 20 mL of Acetonitrile, shake vigorously for 10 minutes, dilute with Acetonitrile to volume, and mix.

Chromatographic system: — Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the resolution, R, between the internal standard and alprazolam is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

ANALYTICAL METHOD VALIDATION REPORT FOR ALPRAZOLAM TABLETS 0.5 MG

Procedure— separately injects equal volumes (about 20 µL) of the Standard preparation and the Assay preparation into the chromatograph, records the chromatograms, and measures the area responses for the major peaks. Calculate the quantity, in %, of alprazolam ($C_{17}H_{13}ClN_4$) in the portion of tablets taken by the formula:

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{WS Weight}}{100} \times \frac{5.0}{50} \times \frac{200}{\text{Sample weight}} \times \frac{\text{Potency of WS}}{100} \times \frac{\text{Average Weight}}{\text{Claim}} \times 100 = \%$$

Text data collection sheet:

Sr. No.	Standards	Area of Alprazolam
1	Standard-1	
2	Standard-2	
3	Standard-3	
4	Standard-4	
5	Standard-5	
6	Standard-6	
7	Mean	
8	% RSD	

Samples	Sample Area Alprazolam	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%		
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-02 120 %		
Sample-E-03 120 %		

Calculation:

ANALYTICAL METHOD VALIDATION REPORT FOR ALPRAZOLAM TABLETS 0.5 MG

Data Collection:

Concentration (µg/ml)	Concentration in %	Corr. Coefficient	Sample Mean Area	% Recovery	Corr. Coefficient
	80	1.0			
	90				
	100				
	110				
	120				

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

Alprazolam Tablets USP (Limit: 90.0 % to 110.0 % of the labeled amount).

Analyst (I): ……..

Chromatographic condition:-

Column: Packing L3, 5µm (30 cm X 4.6 mm)

Column temperature: ambient

Detector: UV 254nm

Flow rate: 2.0ml/min.

Injection volume: 20µl

Mobile phase:—

Prepare a filtered and degassed mixture of Acetonitrile, chloroform, butyl alcohol, water, and glacial acetic acid (850:80:50:20:0.5). Make adjustments if necessary.

Standard Preparation:—

Dissolve an accurately weighed ----------mg (25.0mg) of Alprazolam WS in 100ml volumetric flask, add 25ml Acetonitrile, shake and dilute with Acetonitrile. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with Acetonitrile to volume, and mix.

Sample Preparation:—

Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed required quantity of the powder containing 5.0mg of Alprazolam to a 200-mL volumetric flask. Transfer 2 mL of water and 20 mL of Acetonitrile, shake vigorously for 10 minutes, dilute with Acetonitrile to volume, and mix.

Chromatographic system: —

ANALYTICAL METHOD VALIDATION REPORT FOR ALPRAZOLAM TABLETS 0.5 MG

Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the resolution, R, between the internal standard and alprazolam is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure— separately injects equal volumes (about 20 µL) of the Standard preparation and the Assay preparation into the chromatograph, records the chromatograms, and measures the area responses for the major peaks. Calculate the quantity, in %, of alprazolam ($C_{17}H_{13}ClN_4$) in the portion of tablets taken by the formula:

$$\frac{\text{Sample area} \times \text{WS Weight} \times 5.0 \times 200 \times \text{Potency of WS} \times \text{Average Weight} \times 100}{\text{Standard area} \times 100 \times 50 \times \text{Sample weight} \times 100 \times \text{Claim}} = \%$$

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.

Text data collection sheet:

S.No.	Standards	Area of Alprazolam
1	Standard-1	
2	Standard-2	
3	Standard-3	
4	Standard-4	
5	Standard-5	
6	Standard-6	
7	Mean	
8	% RSD	

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

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Samples		Area of Alprazolam	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).

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

Alprazolam Tablets USP (Limit: 90.0 % to 110.0 % of the labeled amount).

Analyst (II): ……..
Chromatographic condition:-
Column: Packing L3, 5µm (30 cm X 4.6 mm)

Column temperature: ambient

Detector: UV 254nm

Flow rate: 2.0ml/min.

Injection volume: 20µl

Mobile phase:—
Prepare a filtered and degassed mixture of Acetonitrile, chloroform, butyl alcohol, water, and glacial acetic acid (850:80:50:20:0.5). Make adjustments if necessary.

Standard Preparation:—

ANALYTICAL METHOD VALIDATION REPORT FOR ALPRAZOLAM TABLETS 0.5 MG

Dissolve an accurately weighed ----------mg (25.0mg) of Alprazolam WS in 100ml volumetric flask, add 25ml Acetonitrile, shake and dilute with Acetonitrile. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with Acetonitrile to volume, and mix.

Sample Preparation:—

Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed required quantity of the powder containing 5.0mg of Alprazolam to a 200-mL volumetric flask. Transfer 2 mL of water and 20 mL of Acetonitrile, shake vigorously for 10 minutes, dilute with Acetonitrile to volume, and mix.

Chromatographic system: —

Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the resolution, R, between the internal standard and alprazolam is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure— separately injects equal volumes (about 20 µL) of the Standard preparation and the Assay preparation into the chromatograph, records the chromatograms, and measures the area responses for the major peaks. Calculate the quantity, in %, of alprazolam ($C_{17}H_{13}ClN_4$) in the portion of tablets taken by the formula:

$$\frac{\text{Sample area} \times \text{WS Weight} \times 5.0 \times 200 \times \text{Potency of WS} \times \text{Average Weight} \times 100}{\text{Standard area} \times 100 \times 50 \times \text{Sample weight} \times 100 \times \text{Claim}} = \%$$

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.

Text data collection sheet:

S.No.	Standards	Area of Alprazolam
1	Standard-1	
2	Standard-2	
3	Standard-3	
4	Standard-4	

5	Standard-5	
6	Standard-6	
7	Mean	
8	% RSD	

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

Samples		Area of Alprazolam	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:

Test Data analyst by ……..-

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

Test Data analyst by "……."

ANALYTICAL METHOD VALIDATION REPORT FOR ALPRAZOLAM TABLETS 0.5 MG

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 in flow rate at 254nm

Mobile phase					Flow rate (ml/min)	System suitability		
Acetonitrile	Butyl alcohol	Chloroform	Water	Glacial acetic acid		Retention Time	Theoretical Plates	Tailing Factor
840ml	53ml	81 ml	21ml	0.5ml	2.0			
850ml	50ml	80 ml	20ml	0.5ml	2.0			
860ml	47ml	79 ml	19ml	0.5ml	2.0			

Change in flow rate at 254nm

Mobile phase					Flow rate (ml/min)	System suitability		
Acetonitrile	Butyl alcohol	Chloroform	Water	Glacial acetic acid		Retention Time	Theoretical Plates	Tailing Factor
850ml	50ml	80 ml	20ml	0.5ml	1.9			
850ml	50ml	80 ml	20ml	0.5ml	2.0			
850ml	50ml	80 ml	20ml	0.5ml	2.1			

Acceptance criteria:

Analytical method validation shall be robust (i.e. Theoretical Plates is not less than 2000 & tailing factor is not more than 2.0).

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