ANALYTICAL METHOD VALIDATION

FOR

GLIMEPIRIDE (CLEANING VALIDATION)

PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

ANALYTICAL METHOD VALIDATION FOR CLEANING VALIDATION

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ANALYTICAL METHOD VALIDATION FOR CLEANING VALIDATION

1.0 Protocol Approval Sheet:

Prepared By (Name & Designation)	Signature	Date
Quality Control		

Checked By (Name & Designation)	Signature	Date
Quality Control		
Quality Assurance		

Approved By (Name & Designation)	Signature	Date
Quality Assurance		

ANALYTICAL METHOD VALIDATION FOR CLEANING VALIDATION

2.0 Objective: To perform the validation of analytical method for determination of traces of API content of glimepiride in swab by HPLC, And documented evidence and provide the procedure for the same.

3.0 Scope: The scope should evaluate the acceptability of an analytical method for determination of trace of API content in swab by HPLC. This protocol should define the procedure, documentation, reference acceptance criteria and result evaluated for determination of trace of API content in Swab by HPLC.

4.0 Introduction and Overview:

4.1 This protocol provides detailed information on all the aspects of validation of the method of analysis for trace of API Content.

4.2 All instrument/equipment shall be qualified and validated.

4.3 Acceptance criteria and rationale for assessing validation are as per ICH guideline Q2(R1)

5.0 Validation Team:

S.No.	Responsibility	Department
1.	Development of Validation Protocol	QC
2.	Execution of Validation Protocol	QC
3.	Review and assembling of data into final Protocol	QC
4.	Approval Protocol	QA

6.0 Methodology:

Chromatographic Conditions:

Column Stainless steel column 125 x 4.6 mm, packed with octadecylsilane bonded to porous silica 5 µm or equivalent

Flow Rate : 1.0 ml/min

Detector : 228 nm

Temperature : 25ºC

Injection Volume : 20 µL

Run Time : 12 min

Diluent: Prepaid a mixture of 80 volume acetonitrile and 20 volume of water.

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Preparation of Mobile Phase:

Prepare a mixture of 50 volume of a solution containing 0.5 g of monobasic sodium phosphate in 500 ml water, adjusted to Ph 2.1 with orthophophoric acid and 50 volume of acetonitrile.

Preparation of Standard Solution:

Weigh accurately about 20 mg of Glimepiride working standard into a 100 mL volumetric flask add 10 ml of diluent and sonicate to dissolve. Dilute to volume with same solvent. Mix well Filter the solution through 0.45 µ nylon membrane filter. Take 1 ml of solution into 100 ml volumetric flask and dilute to volume with Diluent and mix well. Diluted 1.0 ml of this solution to 10 ml with diluent.

Procedure:

Inject 20 µl of blank, standard solution (five replicate injections), sample solution (in duplicate). Record the chromatograms and measure the peak areas.

Evaluation of System Suitability:

1. % RSD of Glimepiride and peak for 5 replicate injection of standard solution is not more than 2.0.
2. The column efficiency as determined from the Glimepiride peak is not less than 1500.
3. The tailing factor for Glimepiride peak is not more than 2.0.

Calculations:

Glimepiride (%) labeled amount

$$\frac{A_T}{A_S} \times \frac{W_S}{100} \times \frac{1}{100} \times \frac{1}{10} \times \frac{P}{100} \times 1000 = \text{----------ppm}$$

A_T = Area count of Glimepiride peak in sample solution

A_S = Average area count of Glimepiride peak in standard solution

W_S = Weight of Glimepiride working standard taken in mg

P = Purity of Glimepiride working standard used (On as is basis)

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Equipment's and Materials:
Equipment's

Instrument Name	Instrument ID	Make
HPLC	---	Shimadzu
Analytical Balance	---	Sartorius
Analytical Balance	---	Sartorius
pH Meter	---	V-Tech

Table-1

Standards, Chemicals, Column and Samples

1.	Glimepiride working standard No.	---
2.	Potency of Glimepiride Working Standard	98.96%
3.	Acetonitrile (HPLC Grade)	---
4.	Orthophophoric acid	---
5.	Sodium Dihydrogen orthophosphate	---

7.0 Method Validation parameters:

Analytical method has to be validated as per protocol on validation of API Swab method of Glimepiride in Glimepiride Swab for the analytical parameters of System Suitability, Specificity, System Precision, Precision, Linearity and Range, Accuracy.

8.0 System Suitability:

8.1 Objective: To demonstrate and verify that the system suitability parameters of the chromatographic system are adequate for the subjected analysis.

8.2 Procedure: Inject standard solution (Six replicates) as described under Standard Test Procedure

The results shall be tabulated in table-2

Table-2

System Suitability

Replicates	Glimepiride
1	`
2	54496
3	54687
4	54883
5	54959
6	55402
Avg.	54837
Std. Dev.	326.273
% RSD	0.59
Theoretical Plates	4083
Tailing Factor	0.93

Acceptance Criteria: Relative standard deviation for peak areas of Glimepiride should not be more than 2.0%, tailing factor should be more than 2.0 and theoretical plates should not be less than 1500.

9.0 Specificity:

9.1 Objective: To demonstrate the ability of the analytical method to separate the analyze and there is no interference in the peaks of analyze due to other component that may be present in the sample matrix.

9.2 Procedure: Peak purity analysis shall be done by injecting one injection of blank, one injection of placebo preparation (blank with swab stick), and one injection of standard preparation in to the HPLC using chromatographic condition as per methodology.

Table-3

S.No.	RT	Peak Purity	Purity Threshold
Blank	NA	NA	NA
Placebo	NA	NA	NA
Standard of Glimepiride	6.41	NA	NA

Acceptance Criteria: No any peak inference shall be observed at retention time Glimepiride and peak purity analysis for sample shall pass.

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10.0 Accuracy:

10.1 Objective: To study of the reliability, suability, and Accuracy of the method recovery experiment were carried out for cleaning method validation for residual determination of glimepiride. Accuracy is the analytical procedure is the closeness of the test result obtained by that procedure to the true value.

Stock Solution: Weigh accurately about 20 mg of Glimepiride working standard into a 100 mL volumetric flask add 10 ml of diluent and sonicate to dissolve. Dilute to volume with same solvent. Mix well Filter the solution through 0.45 µ nylon membrane filter.

Standard solution: Take 1.5 ml of stock solution into 100 ml volumetric flask and dilute to volume with Diluent and mix well.

- **Accuracy Solution for 50% I**: Select clean and dried 10 cm x 10 cm surface area stain less steel plates spread 0.75 ml spiking solution on dried 10 cm x10 cm surface stain less steel plate, taking utmost care to avoid any spillage. Dry the plate at room temperature using 10 ml of accurately measured diluent recovered the test sample from 10 cm x10 cm surface area stain less steel plate, by gently swirling filter and inject into HPLC. Finally recorded the area of test sample in swab recovery on stainless steel plate calculate the % swab recovery.

  Accuracy Solution for 50% II: Select clean and dried 10 cm x 10 cm surface area stain less steel plates spread 0.75 ml spiking solution on dried 10 cm x10 cm surface stain less steel plate, taking utmost care to avoid any spillage. Dry the plate at room temperature using 10 ml of accurately measured diluent recovered the test sample from 10 cm x10 cm surface area stain less steel plate, by gently swirling filter and inject into HPLC. Finally recorded the area of test sample in swab recovery on stainless steel plate calculate the % swab recovery.

- **Accuracy Solution for 50% III**: Select clean and dried 10 cm x 10 cm surface area stain less steel plates spread 0.75 ml spiking solution on dried 10 cm x10 cm surface stain less steel plate, taking utmost care to avoid any spillage. Dry the plate at room temperature using 10 ml of accurately measured diluent recovered the test sample from 10 cm x10 cm surface area stain less steel plate, by gently swirling filter and inject into HPLC. Finally recorded the area of test sample in swab recovery on stainless steel plate calculate the % swab recovery.

- **Accuracy Solution for 100% I**: Select clean and dried 10 cm x 10 cm surface area stain less steel plates spread 0.75 ml spiking solution on dried 10 cm x10 cm surface stain less steel plate, taking utmost care to avoid any spillage. Dry the plate at room temperature using 10 ml of accurately measured diluent recovered the test sample from 10 cm x10 cm surface area stain less

steel plate, by gently swirling filter and inject into HPLC. Finally recorded the area of test sample in swab recovery on stainless steel plate calculate the % swab recovery.

- **Accuracy Solution for 100% II**: Select clean and dried 10 cm x 10 cm surface area stain less steel plates spread 1.5 ml spiking solution on dried 10 cm x10 cm surface stain less steel plate, taking utmost care to avoid any spillage. Dry the plate at room temperature using 10 ml of accurately measured diluent recovered the test sample from 10 cm x10 cm surface area stain less steel plate, by gently swirling filter and inject into HPLC. Finally recorded the area of test sample in swab recovery on stainless steel plate calculate the % swab recovery.
- **Accuracy Solution for 100% III**: Select clean and dried 10 cm x 10 cm surface area stain less steel plates spread 1.5 ml spiking solution on dried 10 cm x10 cm surface stain less steel plate, taking utmost care to avoid any spillage. Dry the plate at room temperature using 10 ml of accurately measured diluent recovered the test sample from 10 cm x10 cm surface area stain less steel plate, by gently swirling filter and inject into HPLC. Finally recorded the area of test sample in swab recovery on stainless steel plate calculate the % swab recovery.
- **Accuracy Solution for 150% I**: Select clean and dried 10 cm x 10 cm surface area stain less steel plates spread 2.25 ml spiking solution on dried 10 cm x10 cm surface stain less steel plate, taking utmost care to avoid any spillage. Dry the plate at room temperature using 10 ml of accurately measured diluent recovered the test sample from 10 cm x10 cm surface area stain less steel plate, by gently swirling filter and inject into HPLC. Finally recorded the area of test sample in swab recovery on stainless steel plate calculate the % swab recovery.
- **Accuracy Solution for 150% II**: Select clean and dried 10 cm x 10 cm surface area stain less steel plates spread 2.25 ml spiking solution on dried 10 cm x10 cm surface stain less steel plate, taking utmost care to avoid any spillage. Dry the plate at room temperature using 10 ml of accurately measured diluent recovered the test sample from 10 cm x10 cm surface area stain less steel plate, by gently swirling filter and inject into HPLC. Finally recorded the area of test sample in swab recovery on stainless steel plate calculate the % swab recovery.

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- **Accuracy Solution for 150% III**: Select clean and dried 10 cm x 10 cm surface area stain less steel plates spread 2.25 ml spiking solution on dried 10 cm x10 cm surface stain less steel plate, taking utmost care to avoid any spillage. Dry the plate at room temperature using 10 ml of accurately measured diluent recovered the test sample from 10 cm x10 cm surface area stain less

steel plate, by gently swirling filter and inject into HPLC. Finally recorded the area of test sample in swab recovery on stainless steel plate calculate the % swab recovery.

Table-8a

Recovery

Level No./Level in %	Actual Amount of Glimepiride added in mg	Amount of Glimepiride found in mg	% Recovery
Level-1 (50%)	37.76	36.86	98.30
	37.77	37.25	98.63
	37.68	37.66	99.94
Level-2 (100%)	50.38	50.36	99.67
	50.85	50.54	99.70
	50.32	50.05	99.46
Level-3 (150%)	75.64	74.47	98.45
	75.8	74.98	98.92
	75.88	74.84	98.63

Acceptance Criteria: The recovery obtained should be within range 80-100%.

11.0 Linearity and Range:

11.1Linearity: To demonstrate that the analytical method is capable to obtain test results, which are directly proportional to the concentration of analyze.

11.2 Range: Provide acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of analyze within or at the extreme of the specified range of the analytical procedure.

11.3 Procedure:

Stock Solution: Weigh accurately about 25 mg of Glimepiride working standard into a 100 mL volumetric flask add 10 ml of diluent and sonicate to dissolve. Dilute to volume with same solvent. Mix well Filter the solution through 0.45 µ nylon membrane filter.

A. **25 %:** Dilute 1 ml of stock solution in 100 ml volumetric flask and makeup with mobile phase.
B. **50 %:** Dilute 2 ml of stock solution in 100 ml volumetric flask and makeup with mobile phase.
C. **75 %:** Linearity Solution: Dilute 3.0 ml stock solution with 100 ml mobile phase

D. **100%:** Dilute 4 ml of stock solution in 100 ml volumetric flask and makeup with mobile phase

E. **125%:** Dilute 5 ml of stock solution in 100 ml volumetric flask and makeup with mobile phase.

F. The results of linearity shall be tabulated in table-9

Table-9
Linearity

S.No.	Target Concentration in %	Area of Glimepiride
1.	25	159814
2.	50	327259
3.	75	477501
4.	100	645380
5.	125	803938
Correlation Coefficient		0.9998

Acceptance Criteria: Correlation Coefficient should not be less than 0.99.

12.0 LOD and LOQ

LOD is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions.

Limit of detection (LOD) =3.3 x (residual standard deviation) /slope

LOQ is the lowest amount of analyte in a sample that can be quantitated with acceptable precision, under the stated experimental conditions.

Limit of quantification (LOQ) = (10 x residual standard deviation)/slope

Preparation of LOD solution = about 0.2 ml of standard stock solution in 100 ml volumetric flask and diluted up to mark with diluent. Injected in triplicate. The LOD experimental result are recorded.

Table-10

LOD STD

Trial	Area of Glimepiride
1	15959
2	16072
3	15824

Preparation of LOQ solution = about 0.2 ml of standard stock solution in 100 ml volumetric flask and diluted up to mark with diluent. Injected in triplicate. The LOD experimental result are recorded.

Table-11

LOQ STD

Replicates	Glimepiride
1	54596
2	54496
3	54687
4	54883
5	54959
6	55402
Avg.	54837
Std. Dev.	326.273
% RSD	0.59
Theoretical Plates	4083
Tailing Factor	0.93

12.0 Overall Conclusion:

Cleaning method has been validated for clean for Glimepiride. To validate the method used, System suitability, Accuracy, and Linearity and range has been performed and results obtained found within the specified limits of each parameters.

Hence based on above it is concluded that the cleaning method has been validated successfully and used for clean for Glimepiride.

13.0 Abbreviation:

SD	Standard Deviation
RSD	Relative Standard Deviation
S. No	Serial Number
QC	Quality Control
QA	Quality Assurance
Mg	Milligram
ICH	International conference on harmonization
HPLC	High Performance Liquid Chromatography
µl	Microliter
ml/min	Millimeter per minute
%	Percentage
AR	Analytical Reagent

14.0 Post Approval Sheet:

Executed By (Name & Designation)	Signature	Date
Quality Control		

Reviewed By (Name & Designation)	Signature	Date
Quality Assurance		

Approved By (Name & Designation)	Signature	Date
Quality Assurance		