PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Quality Control	**SOP No.:**
Title: Analytical Method Validation	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1.0 OBJECTIVE:

To lay down a procedure for accurate, reliable and consistent data by the use of validated analytical methods for the assessment of pharmaceutical products.

2.0 SCOPE:

This SOP is applicable for.

(a) Routine Pharmacopoeial analytical methods that are transferred.

(b) Standard chromatographic methods in Quality Control Department.

3.0 **RESPONSIBILITY:**

3.1 Preparation - Executive QC.

3.2 Checking - Assistant Manager QC.

3.3 Approval - Manager QC

4.0 PROCEDURE:

4.1 The analytical parameters for method validation shall be selected as mentioned in the respective tables as follows -

4.1.1 For Methods transferred from PRC - Table - 1

4.1.2 For Pharmacopeial Methods - Table - 2

4.1.3 For In-house developed Methods - Table - 3

Table - 1 (For Transferred Methods)

Category	Parameters to be Performed
	Ruggedness
Assay	√

Table- 2 (For Pharmacopoeial Methods)

Category	Parameters to be Performed			
	Specificity	Accuracy	LOD	LOQ
Assay	√	√	X	X
Chromatographic Purity / RS	√	* √	√	√
Residual Solvent	√	√	√	√
Swab Analysis	√	√	√	√

Note : * only to be carried out when the impurities are known and available.

Table- 3 (For In-house Developed Methods)

Category	Parameters to be Performed							
	Specificity	Precision	Linearity & Range	Accuracy	Ruggedness	Robustness	LOD	LOQ
Assay	√	√	√	√	√	√	X	X
Chromatographic Purity / RS	√	√	√	* √	√	√	√	√
Residual Solvent	√	√	√	√	√	X	√	√
Swab Analysis	√	√	√	√	√	X	√	√

Note : * only to be carried out when the impurities are known and available.

4.2 The Method Validation Protocol shall be prepared as per Annexure -I

4.2.1 Analytical Method Validation Protocol shall be numbered as

AMVP = Analytical Method Validation Protocol

XXX = Serial No. starting from 001.

4.2.2 The records of Analytical Method Validation Protocol Number shall be maintained on Annexure –II

4.3 The experimental design, comprising the tests or methods to be validated, the relevant analytical parameters to be checked, shall be indicated in the protocol on the basis of the category given in the above table.

4.4 Analytical Parameter -

4.4.1 Specificity –

4.4.1.1 Assay

Experimental Design -

- System Suitability determination.
- Diluents / Blank, impurity & placebo interferences.
- Separate injections of individual impurities for identification and purity check by PDA, if required.
- 5 replicates for standard & 6 replicates for test & determination of % RSD of peak area response or absorbance and RT for replicate.

Acceptance Criteria –

- As per respective STP. If not defined/ specified, Theoretical Plates – NLT 2000, Tailing Factor- NMT 2.0, Resolution - NLT 1.0.
- No interference of diluents/ blank & swab at the RT of drug substances for HPLC & for UV- NMT 1.0%. In case of placebo, interference for HPLC- NMT0.1% & for UV- NMT 1%.
- Purity of impurity used should be NLT 90%.
- % RSD: For Area- NMT 2.0%

  For RT- NMT 1.0%

4.4.1.2 Related Impurities/ Chromatographic Purity

Experimental Design -

- 1 to 4 as defined in Assay.
- Calculate RRT of known impurity.
- Determine RRF by slope of Linearity Graph.

Acceptance Criteria -

- 1 to 4 as defined in Assay- except % RSD – For Area- NMT 5%.
- RRT of the individual impurity should be comparable to the corresponding RRT in system precision solution.
- If RRF is between 0.8 to 1.2, calculate the impurities by using the response of the low load of the drug substance.
- If RRF is less than 0.8& more than 1.2, calculate the impurities by using RRF of the respective impurities & by using response of low load of drug substance.
- RRF should be between 0.2 to 5.0.

4.4.1.3 Residual Solvent

Experimental Design -

- 1 to 4 as defined in Assay.

Acceptance Criteria -

- 1 to 4 as defined in Assay - except % RSD - For Area- NMT 15.0%.
- For RT- NMT 2.0% and Interference should be not more than 2.0%.

4.4.1.4 Cleaning Validation (Swab Analysis)

Experimental Details & Acceptance Criteria: Same as defined in Assay.

4.4.2 Linearity and range

4.4.2.1 Assay

Experimental Design -

- Determination of minimum 5 levels of standard in the range of 50 to 150% of the working level concentration including limit level concentration. Carry out linearity for first & last level in 5 replicates and other levels in duplicates & take mean response/ absorbance for calculation
- Determine the precision of lower and highest level in 5 replicates.

Acceptance Criteria –

- Linearity Coefficient of mean response of replicate determination plotted against respective concentration should not be less than 0.99.
- % RSD - For Peak Area lowest & highest level- NMT 2.0%

  For RT- NMT 1.0%

4.4.2.2 Related Impurities/ Chromatographic Purity

Experimental Details & Acceptance Criteria: Same as defined in Assay.

4.4.2.3 Residual Solvent

Experimental Details & Acceptance Criteria: Same as defined in Assay.

4.4.2.4 Cleaning Validation (Swab Analysis)

Experimental Details & Acceptance Criteria: Same as defined in Assay.

4.4.3 Precision

4.4.3.1 Assay

Experimental Design -

- Repeatability of 5 replicates determinations for assay of standard at working level concentration and determination of % RSD of response of standard replicates & retention time.

- Method Precision of at least 6 replicate assay of a sample & determination of % RSD.

Acceptance Criteria –

- System suitability as per specificity.
- % RSD - For Peak Area Response of replicate standards- NMT 2.0%.

  For RT- NMT 1.0%.
- % RSD - For Assay- NMT 2.0%.

4.4.3.2 Related Impurities/ Chromatographic Purity

Experimental Design -

- Repeatability of 6 replicate determinations of Impurity solution for known impurities & drug solutions for unknown impurities at the limit level concentration. Determine % RSD for Peak Area Response & RT.
- Method precision of at least 6 replicate determinations of a sample. Determine % RSD of the content of known impurities, single maximum unknown impurity and total impurities.
- If unknown impurities are not detectable in sample then spike working level concentration of known impurities in sample.

Acceptance Criteria –

- System suitability as per specificity.
- % RSD - For Peak Area Response of replicate standards- NMT 2.0%.

  For RT- NMT 1.0%.
- % RSD - Of known, single maximum unknown impurity & total impurities NMT10.0%.
- % RSD of % Recovery of known impurities- NMT 10%.

4.4.3.3 Residual Solvent

Experimental Design -

- Repeatability of 6 replicate injections of solvent standards at working level Determine % RSD of peak area response & RT of individual solvents.
- Method precision of at least 6 replicate determinations of a sample. Determine % RSD of the content of solvents.
- If unknown impurities are not detectable in sample then spike working level concentration of known impurities in sample.

Acceptance Criteria –

- % RSD - For Peak Area- NMT 15.0%.

  For RT- NMT 2.0%
- % RSD - For Content of Solvent- NMT 15.0%.

- % RSD - Recovery of Solvent- NMT 15.0%.

4.4.3.4 Cleaning Validation (Swab Analysis)

Experimental Design -

- Repeatability of 6 replicate injections of standard at working level. Determine % RSD of peak area response of replicate standards and RT of components.
- Repeatability of the accuracy in the limit level concentration of 6 replicates.

Acceptance Criteria –

- Same as defined in Assay.
- % RSD – Recovery for 6 replicate determinations- NMT 10.0% and individual

  % recovery should be more than 80%.

4.4.4 Accuracy

4.4.4.1 Assay

Experimental Design -

- Inject 5 replicates of standard solution. Determine % RSD of Peak Area Response & retention Time.
- To the placebo, add drug substances corresponding to 50,100 & 150% of the working level concentration & determine the assay. Prepare each level in triplicates & inject once.
- In case of lower dosage form where drug weight equivalent or less than 5.0mg, prepare the standard stock solution & spike this standard in placebo solution.
- In case of higher dosage form where drug weight more than 5.0mg, spike the known amount of working standard in placebo mixture.

Acceptance Criteria –

- % RSD – For Peak Area- NMT 2.0%.

  For RT- NMT 1.0%
- Recovery should be between 98 and 102%.

4.4.4.2 Related Impurity/ Chromatographic Purity

Experimental Design -

- To a sample in which content of impurities is already known, the individual linearity levels of known impurities are added & the recovery is calculated. Carry out this exercise in minimum 3 replicates for at least 3 levels (50% to 150% of limit level).

Acceptance Criteria –

- Recovery should be between 90 & 110%.

4.4.4.3 Residual Organic Solvent Test

Experimental Design -

- Same as defined in Related Impurity/ Chromatographic Purity.

Acceptance Criteria –

- Recovery should be between 85 & 115%.

4.4.4.4 Cleaning Validation (Swab Analysis)

Experimental Design -

- Prepare standard stock solutions of drug substances in presence of placebo. Transfer the known quantity on SS plate, evaporate to dryness on water bath. Swab the surface of the plate with a swab. Transfer the swab in volumetric flask. Add known amount of diluents, swirl the flask & carry out the determination. Prepare the standard solutions of the each level as above without evaporation. Carry out recovery study for at least 3 levels in at least 3 replicates & calculate % recovery.

Acceptance Criteria –

- Recovery should be 80 and 120%.

4.4.5 Reproducibility (Intermediate Precision)

4.4.5.1 Assay

Experimental Design -

- Repeatability of 6 replicate determination of standard at assay level concentration. Determine % RSD of Peak area response & Retention Time.
- Intermediate precision 6 replicates for assay of a sample by a different analyst/ day at normal laboratory condition/ instrument/ different columns of same make. Determine % RSD of assays.

Acceptance Criteria –

- System suitability as per specificity.
- % RSD – For Peak Area of replicate standards – NMT 2.0%

  For RT– NMT 1.0%.
- % RSD – For Assay- NMT 2.0%.
- Cumulative % RSD of Assay- NMT 2.0%.

4.4.5.2 Related Impurity/ Chromatographic Purity

Experimental Design -

- Carry out specificity & system suitability.
- Repeatability of 6 replicate determinations of impurity solution for known impurities & drug solutions for unknown impurities at limit level concentration. Determine % RSD for peak area & retention time.

- Intermediate precision of 6 samples injected once by a different analyst/ day at normal laboratory conditions/ instrument/ different column of same make. Determine % RSD of known, single maximum unknown impurity & total impurities.
- Determine cumulative % RSD of known, single maximum unknown impurity & total impurities.
- If known impurities are not detectable in sample then spike working level concentration of the known impurities in the sample (6 sample preparation and inject once).

Acceptance Criteria –

- System suitability as per specificity.
- % RSD – For peak area response of replicate standards- NMT 5.0%.

  For RT- NMT 1.0%.
- % RSD for known, single maximum unknown impurity & total impurities- NMT 10.0%.
- Cumulative % RSD for known, single maximum unknown impurity & total impurities- NMT 10.0%.
- Cumulative % RSD of % recovery of known impurities – NMT 10.0%. (% Recovery should be more than 90%).

4.4.5.3 Residual Organic Solvent Test

Experimental Design -

- System suitability determination.
- Diluents Interference.
- Repeatability of 6 replicate injections of solvent standard. Determine % RSD for peak area & retention time of individual solvent.
- Intermediate precision of 6 replicate determinations of trace solvents of a sample by different analyst/ day at normal laboratory conditions/ instrument/ different column of same make. Determine % RSD of solvent present in sample & Cumulative % RSD of Content of Solvents I precision study & intermediate precision study together.
- If solvents are not detectable in sample then spike working level concentration the sample. Determine cumulative % RSD of % recovery of solvents I precision study & intermediate precision study

Acceptance Criteria –

- Resolution should be more than 1.0.
- No interference of the diluents. If interference observed, the response of the interference should be NMT 2.0%.
- % RSD – For peak area- NMT 15.0%

  For RT- NMT 2.0%.
- % RSD – For content of solvents- NMT 15.0%.
- Cumulative % RSD for content of solvent- NMT 15.0%

- Cumulative % RSD for % recovery of solvents- NMT 15.0%. (% Recovery should be between 85 to 115%.

4.4.5.4 Cleaning Validation (Swab Analysis)

Experimental Design -

- Repeat the precision study on a different instrument/ different day at normal laboratory temperature/ with different analyst and determine the reproducibility of replicate determinations. Carry out 6 replicate determinations for limit level. Calculate RSD of % recoveries. Also calculate % RSD of % recoveries of precision study & intermediate precision study.

Acceptance Criteria –

- % RSD- For Peak Area - NMT 2.0%.

  For RT- NMT 1.0%.
- % RSD of % recovery of 6 replicate determinations- NMT 10% with individual recovery should be more than 80%.
- Cumulative % RSD – NMT 10.0%.

4.4.6 Robustness

4.4.6.1 Assay

Experimental Design -

- Deliberately alter any 3 critical parameters by minor variation.

  Mobile phase composition (higher concentration by 2% or lower concentration by 5-10%)

  pH of mobile phase / buffer (± 0.2)

  Column oven temperature (± 2.0°C)

  Mobile phase flow rate (± 0.2 ml/min)
- Repeatability of 6 replicate determination of standard at working level concentration. Determine the % RSD of peak area response of replicates and retention time.
- Determine the cumulative % RSD of Assay of precision study and robustness study together.

Acceptance Criteria –

- System Suitability as per specificity.
- % RSD- For Peak Area- NMT 2.0%

  For RT- NMT 1.0%
- % Cumulative RSD- NMT 2.0%

4.4.6.2 Related Impurity/ Chromatographic Purity

Experimental Design -

- Inject 6 replicates of standard & determine the % RSD for peak area response and retention time.
- 2 to 3 same as defined in Assay.

Acceptance Criteria –

- System Suitability as per specificity.
- % RSD- For Peak Area- NMT 5.0%.

  For RT- NMT 1.0%
- % Cumulative RSD- NMT 10.0%

4.4.6.3 Residual Organic Solvent Test

Not Applicable

4.4.6.4 Cleaning Validation (Swab Analysis)

Not Applicable

4.4.7 Limit Of Quantification (LOQ)

4.4.7.1 Assay

Not applicable

4.4.7.2 Related Impurity/ Chromatographic Purity

Experimental Design -

- Determine the signal-to-noise ratio at lower levels (equal to the first level of linearity & higher than LOD). Carry out 6 replicate determinations at this concentration.
- Signal to noise ratio should be calculated by the following formula:

  S / N = 2 H / h

  Where H = height of peak corresponding to the component concerned in the chromatogram obtained with the prescribed reference solution, measured.

  from the maximum of the peak to the extrapolated baseline of the signal observed over a distance equal to 20 times the width at half height.

  h = Range of the background noise in the chromatogram obtained after injection or application of a blank, observed over a distance equal to 20 times

  of the width at half height of the peak in the chromatogram obtained with the prescribed reference solution & if possible, suited equally around the place where peak would be found.
- Determine % RSD for Peak Area response & Retention Time.

Acceptance Criteria –

- The concentration is acceptable as LOQ if the signal-to-noise ratio is equal to or more than 10.

- % RSD- For Peak Area- NMT 5.0%

  For RT- NMT 1.0%

4.4.7.3 Residual Organic Solvent Test

Experimental Design -

- Same as defined in Related Impurity/ Chromatographic Purity.

Acceptance Criteria –

- Same as defined above except % RSD- For Peak Area- NMT 15.0%

  For RT- 2.0%

4.4.7.4 Cleaning Validation (Swab Analysis)

Experimental Design -

- 1 to 3 same as defined in Related Impurity/ Chromatographic Purity.

Acceptance Criteria –

- 1 to 2 same as defined in Related Impurity/ Chromatographic Purity.

4.4.8 Limit Of Detection (LOD)

4.4.8.1 Assay

Not applicable

4.4.8.2 Related Impurity/ Chromatographic Purity

Experimental Design -

- 1 & 2 same as defined in LOQ for Related Impurity/ Chromatographic Purity.

Acceptance Criteria –

- The concentration is acceptable as LOD if the signal-to-noise ratio is equal to or more than 3.

4.4.8.3 Residual Organic Solvent Test

Experimental Design & Acceptance Criteria

- Same as defined in Related Impurity/ Chromatographic Purity.

4.4.8.4 Cleaning Validation (Swab Analysis)

Experimental Design -

- 1 & 2 same as defined in Related Impurity/ Chromatographic Purity.

Acceptance Criteria –

- The concentration is acceptable as LOD if the signal-to-noise ratio is equal to or more than 3.
- The limit should be subsequently validated by the analysis of a suitable number of samples known to be near or prepared at the Quantitation limit.

5.0 SAFETY AND PRECAUTION:

Not Applicable.

6.0 REVISION HISTORY:

Revision No.	Reason for Revision	Superseded from & date
00		

7.0 REFERENCES:

Not Applicable.

8.0 ABBREVIATIONS

SOP : Standard Operating Procedure

% : Quality Control

LOD : Limit of Detection

LOQ : Limit of Quantification

RS : Related Substances

STP : Standard Test Procedure

NMT : Not More Than

NLT : Not Less Than

CU : Content Uniformity

RT : Retention Time

RRT : Relative Retention Time

RRF : Relative Retention Factor

RSD : Relative Standard Deviation

PDA : Photo Diode Array detector

SS : Stainless Steel

9.0 ANNEXURE

Annexure –I : Analytical Method Validation Protocol

Annexure – II : Records of Analytical Method Validation Protocol Number

Annexure- I
ANALYTICAL METHOD VALIDATION PROTOCOL

PROTOCOL FOR ANALYTICAL METHOD VALIDATION

NAME OF THE PRODUCT
METHOD NAME
DOCUMENT NUMBER

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Department: Quality Control	**SOP No.:**
Title: Analytical Method Validation	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

TABLE OF CONTENTS

PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Quality Control	**SOP No.:**
Title: Analytical Method Validation	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1.0 PROTOCOL AUTHOR

Name	Designation	Signature	Date

2.0 PROTOCOL PRE – APPROVAL

Name	Designation	Signature	Date

3.0 VALIDATION TEAM: Validation team shall comprise of representatives from Q.C. and Q.A.

Function	Name	Designation	Signature	Date
Q.C.				
Q.A.				

PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

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Department: Quality Control	**SOP No.:**
Title: Analytical Method Validation	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

4.0 OBJECTIVE & SCOPE:

4.1 OBJECTIVE:

To lay down the procedure for validation of Analytical Methods for Laboratory suitability.

4.2 SCOPE :

This protocol is applicable to Analytical method to be validated when,

- Transferred method. (As technology transfer),
- Pharmacopoeial method,
- In-house Analytical Method (Method developed at PRC).

5.0 RESPONSIBILITIES:

5.1 PROTOCOL DEVELOPMENT:

The Quality Control shall be responsible for protocol development in co-ordination with Quality Assurance department.

5.2 VALIDATION PROGRAMME:

Quality Control Department shall be responsible to carry out successful validation of Analytical method and shall establish the suitability of the method and its subsequent implementation in QC department.

6.0 ACCOUNTABILITY:

6.1 APPROVAL OF PROTOCOL & EVALUATION OF RESULTS FOR FINAL APPROVAL:

The Manager –QC & QA shall be accountable to review all documents related to Analytical method Validation at site and evaluate results and approve its summary report.

7.0 CRITERIA FOR VALIDATION AND REVALIDATION:

Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use.

Method needs to be validated or revalidated.

- Whenever the change in Analytical method

- Change in qualitative composition of ingredients.
- Wherever method is transferred from one site to another site(to be validated for Ruggedness only)

8.0 METHODOLOGY:

Analytical method to be validated when

1 Transferred method either from PRC/LLM to QC.
2 Pharmacopoeial methods.
3 In house developed method

9.0. PRODUCT DETAILS:

Name of the product :

Formulation type :

Label claim :

Batch No. :

Composition of the batch : As per master formula card No.:

S.No.	Name	Unit Quantity (mg)
1.		
2.		
3.		

10.0 INSTRUMENTS, REAGENTS AND WORKING STANDARDS

10.1 INSTRUMENTS :

S.No.	Name of Instrument	Make/Model	Identification No. of Instrument	Status for Calibration and Qualification of instrument.
1				
2				
3				

10.2 REAGENTS:

S.No.	Name of Reagents	Grade
1		
2		
3		

10.3 DETAIL OF WORKING / REFERENCE STD:

Name of working /Ref Std. :

Working / Ref STD .No. :

Potency :

11.0 METHOD (STP'S) AND METHOD PARAMETERS

11.1 INSTRUMENTAL PARAMETER

11.2 PREPARATION OF REAGENTS / BUFFER

11.3 PREPARATION OF STANDARD SOLUTIONS

11.4 PREPARATION OF TEST SOLUTIONS

11.5 PROCEDURE

11.6 CALCULATIONS

11.7 ACCEPTANCE CRITERIA

12 DATA SHEETS

12.1 DATA SHEET FOR SPECIFICITY

12.2 DATA SHEET FOR LINEARITY

12.3 DATA SHEET FOR RANGE

12.4 DATA SHEET FOR PRECISION

12.5 DATA SHEET FOR ACCURACY

12.6 DATA SHEET FOR REPRODUCIBILITY (INTERMEDIATE PRECISION)

12.7 DATA SHEET FOR LOD

12.8 DATA SHEET FOR LOQ:

12.9 DATA SHEET FOR ROBUSTNESS:

Note:-Prepare data sheet for above parameters (as applicable) giving all details with reference to experimental design and acceptance criteria including name of analyst, instrument ID, date of analysis etc. Also include in data sheet observations and calculations as per method.

13.0 SUMMARY REPORT OF METHOD VALIDATION:

S.No.	Method Parameters	Observation	Acceptance Criteria	Remarks

14.0 CONCLUSION:

QC/043/01/00

15.0 APPROVAL:

Approved / Not Approved**:**

Name	Designation	Signature	Date
	AM (Q.C.)		
	MANAGER (Q.C.)		
	MANAGER (Q.A.)		

PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Quality Control	**SOP No.:**
Title: Analytical Method Validation	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

Annexure-II

RECORDS OF ANALYTICAL METHOD VALIDATION PROTOCOL NUMBER

S.No.	Name of Product	Method	Number Assigned	Assigned By