STANDARD OPERATING PROCEDURE	
Department: Quality Assurance	**SOP No.:**
Title: Analytical Method Validation	**Effective Date:**
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1.0 PURPOSE

To define the characteristics for consideration during the validation of an analytical method of drug product (Pharmacopoeial or in-house).

2.0 SCOPE

2.1 This procedure describes the typical characteristics to be selected for validation of an analytical method, the process for their determination and the acceptance criteria applicable.

3.0 REFERENCE(S) & ATTACHMENTS

3.1 References

3.1.1 Validation of Analytical Procedures: Text and Methodology – Q2 (R1)

3.1.2 Chapter <1225> Validation of Compendial Procedures; USP.

3.1.3 Chapter <1092> The Dissolution procedure: Development and Validation.

3.2 Attachments

3.2.1 Nil

4.0 DEFINITION & ABBREVIATION(S)

4.1 Definitions

4.1.1 **Specificity**:

Specificity is the ability of the analytical method to assess unequivocally the analyte in the presence of components that may be expected to be present in the sample.

4.1.2 **Limit of detection:**

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value (s).

4.1.3 **Limit of quantitation:**

The Quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy.

4.1.4 **Linearity**:

The linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration of analyte in the sample.

4.1.5 **Precision**:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple samplings of the same homogeneous sample

under the prescribed conditions.

4.1.6 **Repeatability**:

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

4.1.7 **Intermediate Precision:**

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

4.1.8 **Accuracy:**

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the found value.

4.1.9 **Robustness:**

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

4.2 Abbreviations

4.2.1 GC: Gas Chromatography

4.2.2 HPLC: High Performance Liquid Chromatography

4.2.3 LOQ: Limit of Quantification

4.2.4 LOD: Limit of Detection

4.2.5 QA: Quality Assurance

4.2.6 QC: Quality Control

4.2.7 R&D: Research and Development

4.2.8 RSD: Relative Standard Deviation

4.2.9 Spec.: Specifications

4.2.10 STP: Standard Testing Procedure

4.2.11 UV: Ultra Violet.

5.0 RESPONSIBILITY

5.1 Corporate Quality Assurance

5.1.1 To ensure implementation of the procedure

5.2 Quality Control Analyst:

5.2.1 To ensure availability of required reagents, working standard/ reference standard/ column/ instrument.

5.2.2 To perform and record the analytical method validation.

5.3 Head QC or designee

5.3.1 To review and ensure correct analytical method validation.

5.3.2 To provide the required reagents/material/column/instruments.

5.3.3 To approve the analytical method validation report.

5.3.4 To ensure implementation of system as per guideline.

5.4 Quality Assurance Head:

5.4.1 To ensure implementation of procedure.

6.0 Distribution:

I. Quality Assurance

II. Quality Control

7.0 PROCEDURE:

7.1 ANALYTICAL METHOD VALIDATION FOR ASSAY/PRESERVATIVE TEST:

7.1.1 **Parameters to be considered for an In-house method**

7.1.1.1 Specificity

7.1.1.2 Solution stability

7.1.1.3 Filter compatibility

7.1.1.4 Filter saturation

7.1.1.5 Linearity and range

7.1.1.6 Precision (System precision, Repeatability, Intermediate precision)

7.1.1.7 Accuracy

7.1.1.8 Robustness

7.1.1.9 System suitability

7.1.2 **Parameters to be considered for a Pharmacopoeial method**

7.1.2.1 Specificity

7.1.2.2 Solution stability

7.1.2.3 Filter compatibility

7.1.2.4 Filter saturation

7.1.2.5 Precision (System precision, Repeatability, Intermediate precision)

7.1.2.6 Linearity and range

7.1.3 **SPECIFICITY:**

7.1.3.1 **Interference study:**

Determination:

Prepare and analyse blank solution (diluent), placebo solution, known impurities solutions (as included in specifications) at specification limit, process impurities according to the peak response, sample solution and standard solution to check the interference. For preservative test perform only placebo interference.

For multiple strength, select

- Higher strength if dosage formula is linear
- If in case, different colours of material used for coating and/or formulation, inject each placebo or placebo mixed with all colours and measure the absorbance /peak responses.

Acceptance criteria:

For HPLC method

No peak shall be observed due to blank solution, placebo solution and known impurity solution at the retention time of principle peak as observed in the standard solution and sample solution.

For UV method

Interference due to placebo solution at the wavelength of determination shall not be more than 2.0%.

7.1.3.2 **Forced degradation study (For HPLC methods):**

Perform forced degradation studies to demonstrate that the analytical method is stability indicating.

Follow the respective guideline for forced degradation study for further details.

For multiple strength, select

- Higher strength sample shall be use for degradation if it is linear dosage form
- Lower Drug placebo ratio of dosage form if average weight is same for all strengths.
- If different colours of material used for coating and/or formulation, subject to degradation study of individual placebo and sample shall be of higher strength.

Acceptance criteria:

The peak of interest shall be spectrally pure and no co-elution shall be observed with blank peak(s) and /or impurity peak(s).

7.1.4 **SOLUTION STABILITY:**

Determination:

Prepare standard and sample solutions as described in the method to be validated.

If multiple strength, select

- Higher strength if dosage formula is linear
- Lower Drug placebo ratio of dosage form if average weight is same for all strength.

Keep the prepared solutions tightly closed and store at room temperature and at 10°C. Analyze the standard and sample solution up to 24 h of both the condition. (If possible 48 h), if the drug product tends to be stable in solution (Based on the information obtained from development report).

Analysis may also be done at intermediate time intervals. However, report the results of initial and actual time point.

Determine the absolute percentage difference in assay/response at a particular time point with respect to the initial assay/response forstandard solution and test solution.

Acceptance criteria (For HPLC & UV methods):

The absolute % difference in assay/ response with respect to initial at each time point shall not be more than 2.0.

If it is outside the set criteria, make appropriate recommendations.

7.1.5 **FILTER COMPATIBILITY:**

Determination:

Prepare the sample solution as described in the method to be validated.

If multiple strength, select higher strength if dosage form is linear.

At the filtration stage, filter the sample solution through Whatman GF/C filter, Whatman No.41, Whatman No.42; discard about 10 mL sample solution from each filter.

For 0.45µm membrane filter and 0.45µm PVDF discard about 5 mL sample solution, collect the sample solutions for further analysis.

Centrifuge the same (unfiltered) sample solution. Analyse all the solutions as described in the test procedure and calculate the assay results.

Determine the absolute difference in the results obtained for the filtered solutions and the centrifuged solution.

Acceptance criteria (For HPLC & UV method):

The absolute difference in assay results obtained for the filtered solutions and the centrifuged solution shall not be more than 2.0. If it is out of the set criteria, make appropriate recommendations.

7.1.6 **FILTER SATURATION:**

Determination:

Prepare the sample solution as described in the method to be validated.

If multiple strength, select higher strength if dosage form is linear.

At filtration stage, when Whatman filter paper no.41, Whatman filter paper no.42 and GF/C filter is recommended for use in Filter compatibility study filter 10.0 mL and 20.0 mL sample solution and discard using three separate filters, followed by filtration of further 10 mL aliquots. Collect the filtrates each in separate test tubes. When 0.45µm PVDF filter and 0.45µm nylon membrane filter is recommended for use in Filter compatibility study filter suitable volume (1mL, 3mL and 5mL) of sample solution and discard using separate filters, followed by filtration of further 10 mL aliquots. Collect the filtrates each in separate test tubes.

Analyse the thus obtained solutions as described in the method to be validated and calculate the assay results. Determine the absolute difference in the assay result obtained in two consecutive aliquots.

Acceptance criteria (For HPLC & UV methods):

The absolute difference in the assay result obtained for two consecutive aliquots shall be not more than 2.0. If it is out of the set criteria, make appropriate recommendations.

7.1.7 **LINEARITY AND RANGE:**

Determination:

Prepare linearity solutions from the stock solution of standard, to obtain the solutions at 50 % to 150 % of the working concentration by preparing minimum five concentrations. Inject all the prepared solutions in duplicate.

Plot a graph of corrected peak areas vs. concentration (ppm). Determine and report the slope, intercept, and correlation coefficient of the regression line and residual sum of squares.

For range, record the concentration levels over which the results are linear.

Acceptance criteria (For HPLC & UV methods):

Correlation Coefficient shall be not less than 0.999

7.1.8 **PRECISION:**

7.1.8.1 **System precision:**

Determination:

Prepare the standard solution as described in the method to be validated and inject the obtained solution in six replicates. Calculate the relative standard deviation of the responses.

Acceptance criteria (For HPLC & UV methods):

Relative standard deviation shall not be more than 2.0 %

7.1.8.2 **Repeatability:**

Determination:

Repeatability shall be performed in the following way, for multiple strengths.

- If Sample used for crushed powder for sample preparation, select only higher strength.
- If sample used for intact tablets for sample preparation, all strength to be performed.
- If dilution of different strength varies, all the strength to be performed.

Prepare six different sample solutions, as directed in the method to be validated, from the same homogeneous sample and analyse over a short period of time by the same analyst, on the same equipment and on the same day.

Calculate the assay results. Determine the relative standard deviation and 95 % Confidence interval of the assay results obtained from the six preparations.

Acceptance criteria (For HPLC & UV methods):

Relative standard deviation of the assay results obtained from six preparations shall not be more than 2.0 %.

7.1.8.3 **Intermediate precision:**

Determination:

Prepare six different sample solutions, as directed in the method to be validated, from the same homogeneous sample and analyzed by a different analyst, on different equipment, on different day.

Calculate the assay results. If multiple strength, select only higher strength. Determine the relative standard deviation and 95 % Confidence interval of the assay results obtained from the six preparations.

Calculate the absolute difference in the assay results obtained in Repeatability (mean value of six results) and Intermediate precision (mean value of six results).

Acceptance criteria (For HPLC & UV methods):

- Relative standard deviation of the assay results obtained from six preparations shall be not more than 2.0 %
- The absolute difference in the assay results obtained in the Repeatability study (mean value of six results) and Intermediate precision study (mean value of six results) shall be not more than 2.0.

7.1.9 **ACCURACY (RECOVERY):**

Determination:

Prepare recovery solutions by spiking the drug substance in to the volumetric flask containing placebo powder, to obtain the solutions at 50 %, 100 % and 150 % of target concentration of drug substance as in sample solution described in the method to be validated.

If multiple strength with different colour, select worst condition of placebo as below and perform the accuracy.

- Lower Drug: placebo ratio if average weight of the dosage basis is same.
- If different colour of material is used for formulation and /or coating material's mixed with excipient proportionally in the individual dosage form.

The quantity of placebo in recovery solutions at 50 %, 100 % and 150 % shall remain constant as in sample solution described in the method to be validated. If the amount of drug substance to be spiked is less than 10 mg, then prepare a stock solution of the drug substance and use this for spiking to prepare the recovery solutions. Prepare the recovery solutions at all the three concentration levels in triplicate with duplicate injection and analyse as per the method to be validated.

Calculate the quantity recovered in mg or µg and the % recovery, for each level and the mean recovery for all nine solutions.

Acceptance criteria (For HPLC & UV methods):

All the individual recoveries shall be within 97.0 % to 103.0 % and the mean recovery shall be within 98.0 % to 102.0 %.

7.1.10 **ROBUSTNESS:**

Determination:

Carry out one set of analysis, using the same homogeneous sample. If multiple strength, select only higher dosage form. By making individual small deliberate changes in the analytical procedure. Select the changes to be made in the analytical procedure from the below list, as applicable.

- Change in pH of buffer (pH specified in method ± 0.2).
- Change in pH of mobile phase (pH specified in method ± 0.2).
- Change in mobile phase composition of each component (Absolute 2 % or 30% relative whichever is larger).
- Change in column oven temperature (Temperature specified in method ± 5°C).

Calculate the result of assay for each set of analysis. Determine the absolute difference in the results obtained in Robustness study and Repeatability study (sample 1).

Acceptance criteria (For HPLC & UV methods):

- The absolute difference in the results obtained in robustness study and repeatability study (sample 1) shall not be more than 2.0.
- Shall meet all the requirements of system suitability.

7.1.11 **SYSTEM SUITABILITY**

Determination:

Perform System suitability before performing any parameter.

Acceptance criteria (For HPLC & UV methods):

The system suitability shall comply as per methodology.

7.2 ANALYTICAL METHOD VALIDATION FOR RELATED SUBSTANCES TEST OF DRUG PRODUCT:

7.2.1 **Parameters to be considered for In-house method**

7.2.1.1 Specificity

7.2.1.2 Limit of detection and Limit of Quantitation

7.2.1.3 Solution stability

7.2.1.4 Filter compatibility

7.2.1.5 Filter saturation

7.2.1.6 Linearity and range

7.2.1.7 Precision (System precision, Repeatability, Intermediate precision)

7.2.1.8 Accuracy

7.2.1.9 Robustness

7.2.1.10 Relative response factor

7.2.1.11 System suitability

7.2.2 **Parameters to be considered for Pharmacopoeial method**

7.2.2.1 Specificity

7.2.2.2 Solution stability

7.2.2.3 Filter compatibility

7.2.2.4 Filter saturation

7.2.2.5 Precision (System precision, Repeatability, Intermediate precision)

7.2.2.6 Limit of Quantitation

7.2.3 **SPECIFICITY:**

7.2.3.1 **Interference Study:**

Determination:

Prepare blank solution (diluent), placebo solution, known impurities solutions at specification limit, process impurities according to the peak response standard solution and sample solution to check for interference.

In case of multiple strength dosage form inject all placebo and sample preparation individually and check the interference study.

Acceptance criteria:

The peaks due to blank solution, placebo solution, principal peak and process impurities shall not interfere at the retention time of any of the known impurities (those included in the specification), unknown impurities and main peak and all the known impurity peaks (those included in the specification) shall be well resolved from each other.

7.2.3.2 **Forced degradation study (Applicable to HPLC method):**

Perform forced degradation studies to demonstrate that the analytical method is stability indicating. Follow the respective guideline for the forced degradation study for further details.

For multiple strength, select

- Higher strength sample shall be used for degradation if linear dosage form
- Lower Drug placebo ratio of dosage form if average weight is same for all strength.
- If different colours are used in dosage form, all individual and/or formulation, subject to degradation study of individual placebo and sample shall be of higher strength.

Acceptance criteria:

The peaks of interest shall be spectrally pure and no co-elution shall be observed with blank peak(s) and/or impurity peak(s).

7.2.4 **Limit of Detection and Limit of Quantitation Determination:**

Either of the following shall determine LOD & LOQ values.

7.2.4.1 **Signal to noise ratio Method:**

Signal to noise is performed by comparing measured signals from sample with known low concentration of analyte with that blank sample and establishing minimum concentration at which the analyte can be reliably detected or quantified.

Acceptance criteria:

Signal to noise ratio is 10:1 for LOQ and 3:1 for LOD.

7.2.4.2 **Standard deviation of response and slope method:**

Prepare a series of solutions by quantitative dilutions of the stock solution of standards to obtain solutions of suitable concentrations (at least six different concentrations) from 5 % to 30 % of specification limit. However, when poor peak response is observed, solutions may be prepared up to 50 % of specification limit. Inject each solution into the chromatograph in duplicate (inject only once if impurity solutions are not stable) and calculate the mean peak areas and corrected peak areas.

Determine the slope and residual standard deviation for each standard using the corrected peak areas and concentration (ppm). Calculate the value of limit of detection and limit of quantitation for each peak using the following formula:

Calculation:

$$LOD = \frac{3.3 \times \sigma}{S} \qquad LOQ = \frac{10 \times \sigma}{S}$$

Where,

σ = Residual Standard Deviation

S = Slope

LOD = Limit of detection

LOQ = Limit of quantitation

Prepare a solution at LOQ level for each standard and inject in six replicates. Calculate the relative standard deviation of the peak areas for each peak.

Acceptance criteria:

Relative standard deviation of the peak areas due to each LOQ level standard injected in six replicates shall not be more than 10.0 %.

7.2.5 **SOLUTION STABILITY:**

Determination:

- If no known impurity is given in the specification, prepare sample solution as it is.
- If known impurity is given in the specification and is above LOQ level, prepare sample solution as it is.

- If known impurity is given in specification and is below LOQ level, spike the impurity at the specification limit in the sample solution.

Prepare the standard solutions and sample solution as described in the method to be validated. If multiple strength, select

- Higher strength sample shall be used if linear dosage form
- Lower Drug placebo ratio of dosage form if average weight is same for all strength.

Keep the prepared solutions tightly closed and store at room temperature and at 10°C. Analyse the standard and sample solution up to 24 hours of both the condition (if possible 48 h).

If the drug product tend to be stable in solution (based on the information obtained from development report / data), analysis may also be done at intermediate time intervals. However, report the results of initial and actual time point.

Determine the absolute % difference in assay /response of standard peak and % impurity for sample solution at a particular time point with respect to the initial % assay/response of standard peak and % impurity for sample solution.

Acceptance criteria:

- The absolute difference in assay / response of standard peaks with respect to initial at each time point shall not be more than 5.0. If it is outside the set criteria, make appropriate recommendations.
- The absolute difference of impurity results in the sample solution at each interval with respect to initial shall not be more than 0.02.

7.2.6 **FILTER COMPATIBILITY:**

Determination

- If no known impurity is given in the specification, prepare sample solution as it is.
- If known impurity is given in the specification and is above LOQ level, prepare sample solution as it is.
- If known impurity is given in specification and is below LOQ level, spike the impurity at the specification limit in the sample solution.

Prepare the sample solution and placebo as described in the methodology. If multiple strength, select

- Higher strength, if linear dosages form.
- Lower Drug placebo ratio of dosage form, if average weight is same for all strength.

At filtration stage, filter solution through Whatman GF/C filter, Whatman No.41, Whatman No.42; discard about 10 mL sample solution from each filter. For 0.45µm membrane filter and 0.45µm PVDF discard about 5 mL sample solution, collect the sample solutions for further analysis. Centrifuge the same (unfiltered) sample solution. Analyse all the solution thus obtained and calculate the % impurity results. Determine the absolute difference in the % impurity result obtained from filtered solution and centrifuged solution.

Each of the sample solutions thus obtained shall be analyzed as described in the methodology. The % impurities shall be calculated. The absolute difference in the results obtained from filtered solutions and centrifuged solution shall be calculated.

Acceptance criteria:

The absolute difference in % impurity result obtained from filter solution and centrifuge solution shall not be more than 10 % of specification limit. If it is out of the set criteria, appropriate recommendation shall be made.

7.2.7 **FILTER SATURATION:**

Determination:

- If no known impurity is given in the specification, prepare sample solution as it is.
- If known impurity is given in the specification and is above LOQ level, prepare sample solution as it is.
- If known impurity is given in specification and is below LOQ level, spike the impurity at the specification limit in the sample solution.

Prepare the sample solution and placebo as described in the methodology. If multiple strength, select

- Higher strength sample, if linear dosage forms.
- Lower Drug placebo ratio of dosage form, if average weight is same for all strengths.

At filtration stage, when Whatman filter paper no.41, Whatman filter paper no.42 and GF/C filter is recommended for use in Filter compatibility study, filter 10.0 mL and 20.0 mL sample solution and discard using three separate filters, followed by filtration of further 10 mL aliquots. Collect the filtrates each in separate test tubes. When 0.45µm nylon membrane filter and 0.45µm PVDF filter is recommended for use in Filter compatibility study filter suitable volume of sample solution (1 ml,3 ml, & 5 ml) and discard using separate filters, followed by filtration of further 10 mL aliquots. Collect the filtrates each in separate test tubes.

Analyse these solutions as described in the method to be validated and calculate the % impurity results. Determine the absolute difference in the results obtained from two consecutive aliquots.

Acceptance criteria:

The absolute difference in % impurity result obtained in two consecutive filtrations shall not be more than 10 % of specification limit. If it is out of the set criteria, make appropriate recommendations.

7.2.8 LINEARITY AND RANGE:

Determination:

Prepare linearity solutions from the stock solution of standards to obtain solutions at LOQ to 250% of the specification limit by preparing minimum five concentration level. Inject each solution into the chromatograph in single injection; calculate the peak area and corrected area.

Plot a graph of corrected areas vs. concentration (ppm) for each solution. Determine and report the slope, intercept, correlation coefficient of the regression lines and residual sum of squares. For range, record the concentration levels over which the results are linear.

Acceptance criteria:

Correlation Coefficient for each impurity shall be not less than 0.995.

7.2.9 PRECISION:

7.2.9.1 System precision:

Determination:

Prepare the standard solutions as described in the method to be validated and inject the obtained solutions in six replicates. Calculate the relative standard deviation of the responses.

Acceptance criteria (For HPLC)

Relative standard deviation shall not be more than 5.0 % or as specified in the method.

7.2.9.2 Repeatability:

Determination:

- If no known impurity is given in the specification, prepare sample solution as it is.
- If known impurity is given in the specification and is above LOQ level, prepare sample solution as it is.
- If known impurity is given in specification and is below LOQ level, spike the impurity at the specification limit in the sample solution.

Repeatability shall be performed in the following way, for multiple strength.

- If Sample is used for crushed powder for sample preparation, select only higher strength.
- If sample is used for intact tablets for sample preparation, all strengths to be performed.
- If sample dilution varies, repeatability shall be performed on all strengths.

Prepare six different sample solutions and placebo as described in the methodology and analyse using the method to be validated, over a short period of time by same analyst, on same equipment, on same day. Calculate the % impurity results. Determine the relative standard deviation and 95% confidence interval of the results obtained from the six preparations.

Acceptance criteria:

Relative standard deviation of individual and total impurities results obtained in six preparations shall not be more than 15.0 %.

7.2.9.3 **Intermediate precision:**

Determination:

- If no known impurity is given in the specification, prepare sample solution as it is.
- If known impurity is given in the specification and is above LOQ level, prepare sample solution as it is.
- If known impurity is given in specification and is below LOQ level, spike the impurity at the specification limit in the sample solution.

Prepare six different sample solutions and placebo as described in the Repeatability study and analyse using the method to be validated, by different analyst, on different equipment, on different day. Calculate the % impurity results. Determine the relative standard deviation and 95% confidence interval of the results obtained from the six preparations. Only higher strength to be selected for intermediate precision.

Determine the relative standard deviation of the individual and total impurity results obtained from twelve preparations of Repeatability and Intermediate precision.

Acceptance criteria:

- Relative standard deviation of individual and total impurities results obtained in six preparations shall not be more than 15.0 %.
- Relative standard deviation of individual and total impurities results obtained in twelve preparations (Repeatability and Intermediate precision) shall not be more than 15.0 %.

Note: The % Impurities below 0.05%; acceptance criteria shall not be applicable for it.

7.2.10 **ACCURACY (RECOVERY):**

Determination:

- If no known impurity is present, spike diluted standard at specification limit in placebo.
- For, known impurity spike the impurity in the sample solution at the specification limit.
- For, known impurities and unknown impurities, spike the known impurities and diluted standard in placebo at the specification limit.

Prepare the test solution as described in methodology in triplicate and analyse. If multiple strength of different colour, select worst condition of placebo which are,

- Lower Drug: placebo ratio if all strengths are linear.
- If different colour of material used for formulation and/ or coating, placebo shall be mixed with all colours at proportionally.

Prepare the recovery solutions to obtain the solutions at LOQ to 250 % of specification limit by preparing minimum three concentration level. Prepare the recovery solution at concentration levels in triplicate with single injection and analyse the same. Calculate the mean areas of the peaks and the % results.

Acceptance criteria:

Recovery at LOQ to 250% shall be within 80.0 % to 120.0 %.

7.2.11 **ROBUSTNESS:**

Determination:

Prepare one sample solution and placebo as described in the Repeatability study and carry out analysis by making individual, small, deliberate changes in the analytical procedure. Select the changes to be made in the analytical procedure from the below list, as applicable.

- Change in pH of buffer (pH specified in method ± 0.2)
- Change in pH of mobile phase (pH specified in method ± 0.2)
- Change in mobile phase composition (Absolute 2 % or 30% of relative, whichever is larger).
- Change in flow rate (Flow rate specified in method ± 0.2)
- Change in column oven temperature (Temperature specified in method ± 5°C)

The result of % impurity shall be calculated for each set of analysis. The absolute difference in % impurity result obtained in robustness study and method precision (sample-1) shall be calculated.

Acceptance criteria:

The absolute difference in the results obtained in robustness study and Repeatability study (Sample-1) shall not be more than 15 % of specification limit.

7.2.12 **RELATIVE RESPONSE FACTOR:**

Note: The relative response factor study may be carried out in the validation of an in-house related substances method. The relative response factor allows for the quantitation of a known impurity against the diluted standard of the drug substance, eliminates the need of using an impurity standard for every analysis. In the impurity calculation formula, use the inverse of the relative response factor i.e. the correction factor.

Determination:

Calculate the relative response factor from the value of slope obtained in the linearity study using the following formula:

$$\text{Relative Response Factor} = \frac{\text{Slope obtained for impurity}}{\text{Slope obtained for drug substance}}$$

Calculate the Correction Factor as follows:

$$\text{Correction Factor} = \frac{1}{\text{Relative Response Factor}}$$

Acceptance criteria:

Record the value of correction factor and use in the impurity calculation.

7.2.13 **SYSTEM SUITABILITY:**

Determination:

Perform System suitability before performing any parameter.

Acceptance criteria (For HPLC methods):

The system suitability shall comply as per methodology

7.3 ANALYTICAL METHOD VALIDATION FOR DISSOLUTION TEST OF DRUG PRODUCT (IMMEDIATE RELEASE):

7.3.1 **Parameters to be considered for an In-house method:**

7.3.1.1 Specificity

7.3.1.2 Solution stability

7.3.1.3 Filter compatibility

7.3.1.4 Filter saturation

7.3.1.5 Linearity and range

7.3.1.6 Precision (System precision, Repeatability, Intermediate precision)

7.3.1.7 Accuracy

7.3.1.8 Robustness

7.3.1.9 System suitability

7.3.2 **Parameters to be considered for a Pharmacopoeial method:**

7.3.2.1 Specificity

7.3.2.2 Solution stability

7.3.2.3 Filter compatibility

7.3.2.4 Filter saturation

7.3.2.5 Precision (System precision, Repeatability, Intermediate precision)

7.3.3 **SPECIFICITY:**

Determination:

Prepare a blank solution (diluent), placebo solution, standard solution and sample solution and analyse to check for interference.

Acceptance criteria:

For HPLC method:

No peak shall be observed due to blank solution and placebo solution at the retention time of principle peak as observed in the standard solution and sample solution.

For UV method:

Interference due to placebo solution shall not be more than 2.0 %.

7.3.4 **SOLUTION STABILITY:**

Determination:

Prepare standard solution and sample solution (equivalent to target concentration) as described in the method to be validated. Keep the prepared solutions tightly closed and store at room temperature and at 10°C. Analyse the standard and sample solution up to 8 hours (if estimation method is by UV spectrophotometry) or analyse the standard and sample solution up to 24 hours of both the condition (if possible 48 h) (if estimation methods is

HPLC) if the drug product tends to be stable in solution (based on the information obtained from development report / data), analysis may also be done at intermediate time intervals. However, report the results of the initial and actual time point. Determine the absolute % difference in assay at the particular time point the initial assay for standard and % Dissolution release in test solution.

Acceptance criteria (For HPLC & UV methods):

The absolute % difference in assay/ % release with respect to initial at each time point shall not be more than 2.0. If it is out of the set criteria, make appropriate recommendations.

7.3.5 **FILTER COMPATIBILITY:**

Determination:

Prepare the sample solution (equivalent to target concentration) as described in the method to be validated. If multiple strength, select higher strength if dosage formula is linear.

At the filtration stage, filter the sample solution through Whatman GF/C filter, Whatman No.41, Whatman No.42; discard about 10 mL sample solution from each filter. For 0.45µm membrane filter and 0.45µm PVDF discard about 5 mL sample solution, collect the sample solutions for further analysis. Centrifuge the same (unfiltered) sample solution. Analyse all the solutions and calculate the results. Determine the absolute difference in the results obtained for the filtered solution and centrifuged solution.

Acceptance criteria (For HPLC & UV methods):

The absolute difference in results obtained for the filtered solutions and the centrifuged solution shall not be more than 2.0. If it is out of the set criteria, make appropriate recommendations.

7.3.6 **FILTER SATURATION:**

Determination:

Prepare the sample solution (equivalent to target concentration) as described in the method to be validated. If multiple strength, select higher strength if dosage formula is linear. At filtration stage, when Whatman filter paper no.41, Whatman filter paper no.42 and GF/C filter is recommended for use in Filter compatibility study, filter 10.0 mL and 20.0 mL sample solution and discard using three separate filters, followed by filtration of further 10 mL aliquots. Collect the filtrates each in separate test tubes. When 0.45µm nylon membrane filter and 0.45µm PVDF filter is recommended for use in Filter compatibility study filter suitable volume (1mL, 3mL and 5mL) of sample solution and discard using separate filters, followed by filtration of further 10 mL aliquots. Collect the filtrates each in separate test tubes.

Analyse the thus obtained solutions as described in the method to be validated and calculate the results. Determine the absolute difference in the results obtained for two consecutive aliquots.

Acceptance criteria (For HPLC & UV):

The absolute difference in the result obtained for two consecutive filtered aliquots shall not be more than 2.0. If it is out of the set criteria, make appropriate recommendation.

7.3.7 **LINEARITY AND RANGE:**

Determination:

Prepare linearity solutions from the stock solution of standard to obtain the solutions at 30 % to 150 % of the working concentration by preparing minimum 5 concentration level. In case of multiple strength of dosage form, shall cover from 30 % of lower strength and up to 150 % of higher strength by preparing minimum 5 concentration level. Inject all the prepared solutions in single injection.

Plot a graph of corrected area vs. concentration (ppm). Determine and report the slope, intercept, and correlation coefficient of the regression line and residual sum of squares. For range, record the concentration levels over which the results are linear.

Acceptance criteria (For HPLC & UV methods):

Correlation Coefficient shall be not less than 0.995

7.3.8 **PRECISION:**

7.3.8.1 **System precision:**

Prepare the standard solution as described in the method to be validated and inject the obtained solution in six replicates. Calculate the relative standard deviation of the responses.

Acceptance criteria (For HPLC & UV methods):

Relative standard deviation shall not be more than 2.0 %.

7.3.8.2 **Repeatability:**

Determination:

Perform the dissolution test for six dosage units using the method under validation and analyzed by same analyst, on the same equipment, on same day. For multiple strength, repeatability, perform all the strengths and calculate the dissolution results. Determine the relative standard deviation and 95 % Confidence interval of the dissolution results (six dosage units).

Acceptance criteria (For HPLC & UV methods):

Relative standard deviation of dissolution results (six dosage units) shall not be more than 5.0 %

7.3.8.3 **Intermediate precision:**

Determination:

Perform the dissolution test on six dosage units using the method under validation and analyzed by a different analyst, on different equipment, on different day. Calculate the dissolution results. Determine the relative standard deviation and 95 % Confidence interval of the dissolution results (six dosage units). Only higher strength to be selected for intermediate precision.

Calculate the absolute difference in the results obtained in Repeatability (mean value of six dosage units) and Intermediate precision (mean value of six dosage units).

Acceptance criteria (For HPLC & UV methods):

Relative standard deviation of dissolution results (six dosage unit) shall not be more than 5.0 %. The absolute difference in the dissolution results obtained in Repeatability (Mean result of six dosage units) and Intermediate precision (Mean result of six dosage units) shall not be more than 5.0 %.

7.3.9 ACCURACY (RECOVERY):

Determination:

Prepare recovery solutions by spiking the drug substance in to the dissolution vessel containing placebo powder equivalent to one dosage unit to cover from 50 % to 120 % of target concentration by preparing minimum 3 concentration level.

If multiple strength select

- Higher strength if dosage formula is linear. If the formula is linear and coating material are of different colors use placebo mixed with all the color proportionally.
- Lower drug placebo ratio of dosage form if average weight is same for all strengths.

Analyse the solutions as described in the method under validation.

If the amount of drug substance to be spiked to each dissolution vessel is less than 10 mg or if the drug substance floats in the dissolution medium or if the drug substance is poorly soluble, then prepare a stock solution of drug substance for spiking into the dissolution vessels.

Calculate the recovery in mg and as % recovery for each level and mean recovery of all six solutions.

Acceptance criteria (For HPLC & UV methods):

All the individual recoveries shall be within 95.0 % to 105.0 %.

The relative standard deviation for % recovery shall not be more than 5.0 %.

7.3.10 ROBUSTNESS:

Determination:

Carry out one set (dissolution test of six dosage units) of analysis using the same batch of the product, by making individual, small, deliberate changes in the analytical procedure. Select the changes to be made in the analytical procedure from the below list, as applicable.

- Change in medium volume (± 1%).
- Change in pH of dissolution medium (pH specified in dissolution medium ± 0.2).
- Change in strength of the dissolution medium (specified morality ± 0.02 M).
- Change in pH of mobile phase (pH specified in method ± 0. 2).
- Change in mobile phase composition (Absolute 2% or 30% of relative, whichever is larger).

Calculate the dissolution results for each set of analysis. Determine the absolute difference between the results obtained in Robustness study (Mean dissolution) and Repeatability study (Mean dissolution).

Acceptance criteria (For HPLC & UV methods):

The absolute difference in the results obtained in Robustness study (Mean dissolution) and Repeatability study (Mean dissolution) shall not be more than 5.0%.

7.3.11 **SYSTEM SUITABILITY**

Determination:

Perform System suitability before performing any parameter.

Acceptance criteria (For HPLC & UV methods):

The system suitability shall comply as per methodology.

7.4 ANALYTICAL METHOD VALIDATION FOR DISSOLUTION TEST OF DRUG PRODUCT (MODIFIED RELEASE).

7.4.1 **Parameters to be considered for an In-house method:**

7.4.1.1 Specificity

7.4.1.2 Solution stability

7.4.1.3 Filter compatibility

7.4.1.4 Filter saturation

7.4.1.5 Linearity and range

7.4.1.6 Precision (System precision, Repeatability, Intermediate precision)

7.4.1.7 Accuracy

7.4.1.8 Robustness

7.4.1.9 System suitability

7.4.2 **Parameters to be considered for a Pharmacopoeial method:**

7.4.2.1 Specificity

7.4.2.2 Solution stability

7.4.2.3 Filter compatibility

7.4.2.4 Filter saturation

7.4.2.5 Precision (System precision, Repeatability, Intermediate precision)

7.4.3 **SPECIFICITY:**

Determination:

Prepare a blank solution (diluent), placebo solution, standard solution and sample solution and analyse to check for interference.

Use placebo in the finished dosage form and inject at each interval

Acceptance criteria:

For HPLC method:

No peak shall be observed due to blank solution and placebo solution at the retention time of principle peak as observed in the standard solution and sample solution.

For UV method:

Interference due to placebo solution shall not be more than 2.0 %.

7.4.4 **SOLUTION STABILITY:**

Determination:

Prepare Standard solution and sample solution (at 100 % level) as described in method under validation. Keep the prepared solutions tightly closed and store at room temperature, 10°C and at 37°C and analyse at the intervals specified in methodology and at 6 hours beyond the last time point of all condition. Determine the absolute % difference in assay/% dissolution release at each time point with respect to the initial assay/% dissolution release for standard and test solutions

Acceptance criteria (for HPLC & UV methods):

The absolute difference in assay, with respect to initial, at each time point shall not be more than 2.0. If it is out of the set criteria, make appropriate recommendations

7.4.5 **FILTER COMPATIBILITY:**

Determination:

Prepare the sample solution at concentration equivalent to the limit specified in methodology at the first time point and last time point. At the filtration stage, filter the solution through Whatman GF/C filter, Whatman No.41, Whatman No.42; discard about 10 mL sample solution from each filter. For 0.45µm membrane filter and 0.45µm PVDF discard about 5 mL sample solution, collect the sample solutions for further analysis. Centrifuge the same (unfiltered) sample solution. Analyse all the solutions and calculate the results. Determine the absolute difference in the results obtained for the filtered solutions and the centrifuged solutions.

Acceptance criteria (For HPLC & UV methods):

The absolute difference in result obtained from filtered solution and centrifuged solution shall not be more than 2.0. If it is out of the set criteria, make appropriate recommendations.

7.4.6 **FILTER SATURATION:**

Determination:

Prepare the sample solution at concentration equivalent to limit specified in methodology for the first time point and last time point. At filtration stage, when Whatman filter paper no. 41, Whatman filter paper no.42 and GF/C filter is recommended for use in Filter compatibility study filter 10.0 mL and 20.0 mL sample solution and discard using three separate filters, followed by filtration of further 10 mL aliquots. Collect the filtrates each in separate test tubes. When 0.45µm nylon membrane filter and 0.45µm PVDF filter is recommended for use in Filter compatibility study filter suitable volume of sample solution (1 ml, 3 ml & 5ml) and discard using separate filters, followed by filtration of further 10 mL aliquots. Collect the filtrates each in separate test tubes. Analyse the thus obtained solutions as described in the method under validation and calculate the results. Determine the absolute difference in the results obtained for two consecutive aliquots.

Acceptance criteria (For HPLC & UV methods):

The absolute difference in result obtained in two consecutive filtrations shall not be more than 2.0. If it is out of the set criteria, make appropriate recommendations.

7.4.7 **LINEARITY AND RANGE:**

Determination:

Prepare linearity solutions from the stock solution of standard to obtain the solutions at 30% of limit specified at first time point to 150% of the limit specified at last time point. This range must contain minimum 9 linearity points. Inject all the prepared solutions in single injection.

Plot a graph of corrected area vs. concentration (ppm). Determine and report the slope, intercept, and correlation coefficient of the regression line and residual sum of squares. For range, record the concentration levels over which the results are linear.

Acceptance criteria (For HPLC & UV methods):

Correlation Coefficient shall be not less than 0.995

7.4.8 **PRECISION:**

7.4.8.1 **System precision:**

Prepare the standard solution as described in the method to be validated and inject the obtained solution in six replicates. Calculate the relative standard deviation of the responses.

Acceptance criteria (For HPLC & UV methods):

Relative standard deviation shall not be more than 2.0 %.

7.4.8.2 **Repeatability:**

Determination:

Perform the dissolution test for six dosage units using the method under validation and analyzed by same analyst, on the same equipment, on the same day. For multiple strength, precision shall carried out at all the strengths and Calculate the dissolution results at each time point. Determine the relative standard deviation and 95% Confidence interval of the dissolution results (six dosage units) for each time point.

Acceptance criteria (For HPLC & UV methods):

Relative standard deviation of dissolution results (six dosage unit) at each time point shall not be more than 10.0 %.

7.4.8.3 **Intermediate precision:**

Determination:

Perform the dissolution test on six dosage units using the method under validation and analyzed by a different analyst, on different equipment, on different day. For multiple strength only higher strength shall be selected and Calculate the dissolution results for each time point. Determine the relative standard deviation of the dissolution results (six dosage units) for each time point.

Calculate the absolute difference in the results obtained in Repeatability (mean value of six dosage units) and Intermediate precision (mean value of six dosage units) for each time point.

Acceptance criteria (For HPLC & UV methods):

Relative standard deviation of dissolution results (six dosage unit) at each time point shall not be more than 10.0 %.

The absolute difference in the dissolution results at each time point obtained in Method precision (Mean results of six dosage units) and Intermediate precision (Mean results of six dosage units) shall not be more than 10.0 %.

7.4.9 **ACCURACY (Recovery):**

Determination:

Prepare recovery solutions by spiking the drug substance in to the dissolution vessel containing placebo powder equivalent to one dosage unit to obtain the solutions at 50% of limit specified at first time point, 100% of limit specified at last time point and 120% of limit specified at last time point, in triplicate.

Analyse the solutions as described in the method under validation.

If the amount of drug substance to be spiked to each dissolution vessel is less than 10 mg or if the drug substance floats in the dissolution medium or if the drug substance is poorly soluble, then prepare a stock solution of drug substance for spiking into the dissolution vessels.

Calculate the recovery in mg and as % recovery for each level and mean recovery of all solutions.

Acceptance criteria (For HPLC & UV methods):

All the individual recoveries shall be within 95.0 % to 105.0 %.

The relative standard deviation for % recovery shall not be more than 5.0 %.

7.4.10 **ROBUSTNESS:**

Determination:

Carry out one set (dissolution test of six dosage units) of analysis using the same batch of the product, by making individual, small, deliberate changes in the analytical procedure. Select the changes to be made in the analytical procedure from the below list, as applicable.

- Change in medium volume (±1 %).
- Change in pH of mobile phase (pH specified in mobile phase ± 0.2)
- Change in strength of the dissolution medium (specified molarity ± 0.02 M)
- Change in mobile phase composition (Absolute 2% or 30% relative, whichever is larger.)

Calculate the dissolution results for each set of analysis. Determine the absolute difference between the results obtained at each time point in Robustness study (mean dissolution) and Repeatability study (mean dissolution).

Acceptance criteria (For HPLC & UV methods):

The absolute difference in the results obtained in Robustness study (mean dissolution) and Repeatability study (Mean dissolution) at each time point shall not be more than 10.0.

7.5 ANALYTICAL METHOD VALIDATION FOR RESIDUAL SOLVENTS TEST OF DRUG PRODUCT.

Parameters to be considered for an in House method.

- Specificity
- Linearity and range
- Precision (System precision, Method precision, Ruggedness)
- Accuracy
- Robustness
- System suitability

7.5.1 **SPECIFICITY**

7.5.1.1 **Interference study**

Determination:

Drug products: Prepare the blank (diluent), individual known residual solvents, sample solution and six injections of standard as described in the methodology and inject into the GC system. Record the retention time (RT) of All peaks observed in the resulting chromatograms. Inject placebo to check the interference.

Acceptance criteria:

No interference of solvents from each other at the retention time of analyte.

7.5.2 **LIMIT OF DETECTION & LIMIT OF QUANTITATION:**

Prepare a series of equivalent lowest concentration solutions by quantitative dilutions of the standard stock solution of residual solvents. Inject each solution and record the peak areas.

Determine the slope and standard deviation of the response by plotting a graph of peak area vs. concentration (ppm).

Determine the value of LOD and LOQ using the following formula:

Calculation:

$$LOD = \frac{3.3 \times STEYX}{Slope} \qquad LOQ = \frac{10 \times STEYX}{Slope}$$

Where,

STEYX = Residual sum of squares

LOD = Limit of detection

LOQ = Limit of quantitation

Prepare the solution at LOQ level and inject six injections and calculate the relative standard deviation of the peak

areas.

Acceptance criteria:

% RSD of the peak area of residual solvents in six injections of LOQ and LOD is not more than 15.0 and 33.0 respectively.

7.5.3 **LINEARITY AND RANGE:**

Determination:

Prepare linearity solutions by quantitative dilutions of the stock solution of standards to obtain solutions at LOQ and minimum five levels between 50 % and 150 % level of the specification limit and inject each solution into the gas chromatogram in triplicate and calculate the mean peak area of each solvents.

Plot a graph of mean peak areas vs. concentration (ppm) and determine the equation of regression line. Report the slope, intercept and correlation coefficient of the regression lines and residual sum of squares. For range, record the concentration levels over which the results are linear.

Acceptance criteria:

Correlation Coefficient shall not be less than 0.995.

7.5.4 **PRECISION**

7.5.4.1 **System precision:**

To check the system precision, prepare the standard solution as per the methodology and record the peak areas of six injections of standard solution. Calculate the mean, standard deviation and % RSD of each residual solvent.

Acceptance criteria:

System suitability criteria shall meet

7.5.4.2 **Repeatability:**

Determination:

If any analyte is present in the sample prepare six-test solution.

if any analyte is not present in the sample, prepare six test solutions followed by six test solutions by spiking with standard solution at 100 % level of the specification limit.

Calculate the mean, standard deviation and % RSD of residual solvent results.

Acceptance criteria :

The % RSD for each residual solvent results in method precision is not more than 15.0.

7.5.4.3 **Ruggedness:**

Determination:

If any analyte is present in the sample prepare six-test solution.

If any analyte is not present in the sample, prepare six test solutions followed by that six test solutions by spiking with standard solution at 100 % level of the specification limit.

Calculate the mean, standard deviation and % RSD of residual solvent results.

Acceptance criteria:

The overall % RSD of the residual solvent results obtained from method precision and ruggedness is not more than 15.0.

7.5.5 ACCURACY (Recovery)

Determination:

Prepare three test solution as per the methodology and record the areas.

Spike the test sample with standard solution, at three levels in the range of 50%, 100% and 150% of specification limit.

Calculate mean recovery, standard deviation and the % RSD of recovery results.

Acceptance criteria:

The % Recovery of each residual solvent at 50%, 100% and 150% levels of the specification limit shall be within the range 80 % to 120%.

7.5.6 ROBUSTNESS:

Determination:

Carry out GC analysis as per method precision by making the following changes in the chromatographic conditions (±10%).

If any analyte is present in the sample prepare two test solutions.

If any analyte is not present in the sample, prepare two test solutions by spiking with standard solution at 100% level of the specification limit.

- Change the flow rate of carrier gas (Flow rate specified in method ± 10% variation).
- Change the temperature of the column oven (initial column oven temperature specified in method ± 10% variation).
- Change the Headspace Equilibration temperature. (Equilibration temperature specified in the method ± 10% variation).

Acceptance criteria:

Shall meet all the requirement of system suitability

7.6 Documents and reporting of results of analytical method validation

7.6.1 All method validation activities shall be recorded on the raw data sheet.

7.6.2 After completion of analysis, the analyst shall sign in the Record of analysis and submit the same to the Head Q.C or designee.

7.6.3 Head Q.C his designee shall check all documentation, calculations and sign in the Record of analysis in the checked by column.

7.7 Method validation report.

7.7.1 Method Validation protocol shall be prepared for each individual test /product before the method validation study to be started.

7.7.2 Method Validation report shall be prepared once the analytical activity of method validation study is completed, all the relevant raw data are verified, and all the calculations are checked.

7.7.3 A summary shall be mentioned of method validation report followed by recommendation if any.

7.7.4 Method Validation report shall clearly identify the critical parameter, shall suggest the precautions to be taken care while actual use of methodology and shall suggest any modifications to be done in methodology.

7.7.5 Method Validation report shall be checked, reviewed and approved by the respective authorities.

7.7.6 Original copy of method validation protocol and validation report shall be filed together with proper labeling along with all the relevant raw data.

8.0 REVISION HISTORY

Version No.	00	Effective Date	
Details of revision: New SOP Prepared			