GUIDELINE FOR ANALYTICAL METHOD VALIDATION

1.0 OBJECTIVE:

This guideline defines the characteristics for consideration during the validation of an analytical procedure. It describes the typical characteristics to be selected for validation of an analytical procedure, the process for their determination and the acceptance criteria applicable.

2.0 SCOPE:

This procedure is applicable to all the analytical method validations carried out in Quality control Department.

3.0 RESPONSIBILITY:

All the analysts of the validation group

Head – Quality Control

4.0 PROCEDURE:

4.1. DEFINITIONS:

Certain terms used in this guideline are defined as follows:

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.

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.

4.1.5.1 Repeatability:

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

4.1.5.2 **Intermediate Precision:**

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

4.1.6 **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.

4.1.7 **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 ANALYTICAL METHOD VALIDATION OF ASSAY / PRESERVATIVE CONTENT / CONTENT UNIFORMITY / BLEND UNIFORMITY OF DOSAGE FORM FOR DRUG PRODUCT:

Parameters to be considered for an In-house method

- Specificity
- Solution stability
- Filter compatibility
- Filter saturation
- Linearity and range
- Precision (System precision, Repeatability, Intermediate precision)
- Accuracy
- Robustness
- System suitability

Parameters to be considered for a Pharmacopoeial method

- Specificity
- Solution stability
- Filter compatibility
- Filter saturation
- Precision (System precision, Repeatability, Intermediate precision)

- Accuracy

4.2.1 Specificity:

4.2.1.1 **Interference study:**

Determination:

Prepare and analyse blank solution (diluent), placebo solution, known impurities solutions (as per Drug product and Drug Substance specifications) at specification limit, all impurities according to the peak response, sample solution and standard solution to check the interference.

If method is isocratic, to show the specificity studies by extending run time of method.

For multiple strength, select

- Higher strength of dosage form if linear formula of multiple strength dosage forms.
- If in case, different colours are used in Drug product, inject each placebo and measure the absorbance / peak response.

Acceptance criteria:

- **For HPLC method**

  No peak should 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.

- **For UV method**

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

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

Note: For preservative / Content Uniformity test it is not applicable, only Interference study will be performed.

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

For multiple strength, select

- Higher strength of dosage form if linear formula of multiple strength dosage forms.

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

Acceptance criteria:

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

4.2.2 **Solution stability:**

Determination:

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

If multiple strength, select

1) Higher strength if dosage formula is linear.

2) Lower Drug placebo ratio of dosage form if average weight is same for all strength.

Keep the prepared solutions tightly closed and store at controlled room temperature (RT) (20 to 25°C) and at temperature below RT (as per STP recommendation like 2-8°C, 10°C or 15°C). Analyse the standard and sample solution up to 48 hrs 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 interval condition. Determine the absolute difference in assay / % difference in response at a particular time point with respect to the initial for standard solution and test solution.

Acceptance criteria (For HPLC & UV methods):

The absolute difference in assay / preservative content / % difference in response with respect to initial at each interval time point should not be more than 2.0. If it is outside the set criteria, make appropriate recommendations.

4.2.3 **Filter Compatibility:**

Determination:

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

If multiple strength select higher strength of dosage form if formula is linear or Lower Drug placebo ratio of dosage form if average weight is same for all strength.

At the filtration stage, filter the sample solution through Whatman No.41 and Whatman No.42; discard about 10 mL sample solution from each filter if used. For Whatman GF/C filter, 0.45µm nylon filter and 0.45µm PVDF filter 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 with the centrifuged solution.

If methodology itself contains sample to be centrifuged and then filtered, then consider centrifuge sample as 'as is sample' and same sample solution to be filtered through different filter.

Acceptance criteria (For HPLC & UV method):

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

4.2.4 **Filter Saturation:**

Determination:

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

If multiple strength select higher strength of dosage form if formula is linear or Lower Drug placebo ratio of dosage form if average weight is same for all strength.

At filtration stage, when Whatman filter paper no.41 and Whatman filter paper no.42 is recommended for use in filter of choice filter 10.0 mL and 20.0 mL sample solution and discard using each separate filters, followed by filtration of further 10 mL aliquots. Collect the filtrates each in separate test tubes. Whatman GF/C filter, 0.45µm PVDF filter and 0.45µm Nylon filter is recommended for use in Filter compatibility study filter suitable volume (1mL, 3mL and 5mL) of sample solution and discard using separate filters, 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 / preservative content result obtained for two consecutive aliquots should be not more than 2.0. If it is out of the set criteria, make appropriate recommendations.

4.2.5 **Linearity and range:**

Determination:

Analyse linearity solutions from the stock solution of standard, to cover at 50 % to

150% of the working concentration by preparing minimum 5 Concentrations. Inject all the prepared solutions in duplicate.

Plot a graph of peak areas vs. corrected 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 should be not less than 0.999.

4.2.6 **Precision:**

4.2.6.1 **System precision:**

Determination:

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

Acceptance criteria (For HPLC & UV methods):

Relative standard deviation should not be more than 2.0 % or as specified in the method.

4.2.6.2 **Repeatability:**

Determination:

Repeatability shall perform on all the strength if multiple strength dosage forms.

Prepare six different sample solutions for Assay and ten determination for Content Uniformity test, 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 / preservative content results obtained from the six preparations.

Acceptance criteria (For HPLC & UV methods):

- Relative standard deviation of the assay / preservative content results obtained from six preparations should not be more than 2.0 %.
- The Requirement for Dosage Uniformity are met if the acceptance value of the first 10 dosage unit is less than or equal to 15.0.If the acceptance value is greater than 15.0 test the next 20units, and calculate the acceptance value. The requirement are met if the final acceptance value of 30dosage unit is less than or equal to 15.0 and no individual content of any dosage units is less than

0.75M nor more than 1.25M as specified in calculation of Acceptance value.

4.2.6.3 **Intermediate precision:**

Determination:

Intermediate precision shall be performed only on higher strength if multiple strength dosage forms.

Prepare six different sample solutions for Assay / preservative test and ten determinations for Content Uniformity test, as directed in the method to be validated from the same homogeneous sample and analyse by a different analyst, on different equipment, on different day. Calculate the assay results. 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 / preservative content results obtained from six preparations should be not more than 2.0 %.
- The Absolute difference in the assay / preservative content results obtained in the Repeatability study (mean value of six results) and Intermediate precision study (mean value of six results) should be not more than 2.0.
- The Requirement for Dosage Uniformity are met if the acceptance value of the first 10 dosage unit is less than or equal to 15.0.If the acceptance value is greater than 15.0test the next 20units and calculate the acceptance value. The requirement are met if the final acceptance value of 30dosage unit is less than or equal to 15.0 and no individual content of any dosage units is less than 0.75M nor more than 1.25M as specified in calculation of Acceptance value.
- The Absolute difference in the content uniformity results obtained in the Repeatability study (mean value of ten results) and Intermediate precision study (mean value of ten results) should be not more than 3.0.

4.2.7 **Accuracy (Recovery):**

Determination:

Prepare recovery solutions by spiking the drug substance into 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.

In case of multiple strengths, Accuracy shall be performed by.

1) Higher strength dosage form, if formula is linear with same placebo material.
2) If different colour of multiple strength, Accuracy shall be performed individually.

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 and the % recovery, for each level and the mean recovery for all nine solutions.

Acceptance criteria (For HPLC & UV methods):

- For assay all the individual recoveries should be within 97.0 % to 103.0% and the mean recovery should be within 98.0 % to 102.0 %.
- For Preservative content all individual recovery is should be with 85.0% to 115.0%.

4.2.8 **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 mobile phase / buffer (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).
- Change in flow rate (Flow rate specified in method ± 0.2. In case, flow rate specified ≥1.0mL, change flow rate ± 0.2 and for <1.0mL, change flow rate ± 0.1.)

Calculate the result of assay for each set of analysis. Determine the absolute difference in the results obtained in Robustness study and Mean value of Repeatability study.

Acceptance criteria (For HPLC & UV methods):

- The Absolute difference in the results obtained in robustness study and Mean value of Repeatability study should not be more than 2.0.
- Should meet all the requirements of system suitability.

4.2.9 **System Suitability**

Determination:

Perform System suitability before performing any parameter.

Acceptance criteria (For HPLC & UV methods):

- The system suitability should comply as per methodology.

Note 1: In Assay / preservative method validation, sample solutions are injected in duplicate for all validation parameter, except specificity studies in which single injection of sample solution. Single injection shall be performed for Content Uniformity / blend Uniformity test validation.

Note 2: Generally, analytical methodology (Chromatographic condition / UV) and sample concentration of content uniformity / Blend Uniformity test remaining same as per assay method and to conduct validation study only minimum parameter like precision and accuracy. If chromatographic condition and sample concentration are different from assay determination, to conduct all validation parameter as described. If intact tablets are used for assay sample preparation, Content Uniformity validation need not be performed.

4.3 ANALYTICAL METHOD VALIDATION FOR RELATED SUBSTANCES TEST OF DRUG PRODUCT

Parameters to be considered for In-house method

- Specificity
- Limit of detection and Limit of Quantitation
- Solution stability
- Filter compatibility
- Filter saturation
- Linearity and range
- Precision (System precision, Repeatability, Intermediate precision)
- Accuracy
- Robustness

- Relative response factor
- System suitability

Parameters to be considered for Pharmacopeial method

- Specificity
- Solution stability
- Filter compatibility
- Filter saturation
- Precision (System precision, Repeatability, Intermediate precision)
- Limit of detection and Limit of Quantitation
- Accuracy
- Linearity and range

4.3.1 **Specificity:**

4.3.1.1 **Interference Study:**

Determination:

Prepare blank solution (diluent), placebo solution, known impurities solutions at specification limit, process impurities according to the peak response, standard solution to check for interference. In case of multiple strength dosage form, inject all individually, and check the interference.

Acceptance criteria:

- The peaks due to blank solution, placebo solution, and process impurities should 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.

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

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

For multiple strength, select

1) Higher strength sample will be used for degradation if formula is linear.
2) Lower Drug placebo ratio of dosage form if average weight is same for all strength.

3) If different colors are used in dosage form, all individual colored placebos are subjected to degradation study and degradation will be performed only on higher strength.

Acceptance criteria:

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

4.3.2 **Limit of Detection and Limit of Quantitation:**

Determination: Either of the following will determine LOD & LOQ values:

Signal to Noise Ratio Method:

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

Acceptance criteria:

- A signal to noise ratio 10:1 for LOQ and 3:1 for LOD.

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. (Solutions may be prepared up to 50 % of specification limit if peak response is less). 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 should be not more than 10.0 %.

4.3.3 **Solution stability:**

Determination:

a) If no known impurity is given in the specification, prepare sample solution as it is.

b) If known impurity is given in the specification and is above LOQ level, prepare sample solution as it is.

c) If known impurity is given in specification and is below or equal to 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 strengths, select

1) Higher strength sample will be used if formula is linear.
2) Lower Drug placebo ratio of dosage form if average weight is same for all strength.

Keep the prepared solutions tightly closed and store at controlled room temperature and at temperature below RT (as per STP recommend like 2-8°C, 10°C or 15°C). Analyse the standard and sample solution up to 24 hrs 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 interval condition. Determine the % response / Assay of standard peak and % impurity for sample solution at a particular time point with respect to the initial % response / Assay of standard peak and % impurity for sample solution.

Acceptance criteria:

- The difference in % response of standard peaks with respect to initial at each time point should 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 should not be more than 10 % of specification limit.

4.3.4 **Filter Compatibility:**

Determination

a) If no known impurity is given in the specification, prepare sample solution as it is.

b) If known impurity is given in the specification and is above LOQ level, prepare sample solution as it is.

c) If known impurity is given in specification and is below or equal 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 strengths exist, select higher strength dosage form.

At filtration stage, filter solution through, Whatman No.41, Whatman No.42; discard about 10 mL sample solution from each filter. For Whatman GF/C filter, 0.45µm Nylon 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.

Acceptance criteria:

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

4.3.5 **Filter Saturation:**

Determination:

a) If no known impurity is given in the specification, prepare sample solution as it is.

b) If known impurity is given in the specification and is above LOQ level, prepare sample solution as it is.

c) If known impurity is given in specification and is below or equal 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 dosage form.

At filtration stage, when Whatman filter paper no.41, and Whatman filter paper no.42 is recommended for use in Filter compatibility study, discard 10.0 mL and 20.0 mL sample solution and discard using each separate filters, followed by filtration of further 10 mL aliquots. Collect the filtrates each in separate test tubes. Whatman GF/C filter,

0.45µm nylon filter and 0.45µm PVDF filter is recommended for use in Filter compatibility study filter suitable (1 mL, 3mL and 5 mL) volume of sample solution and discard using separate filters, Collect the filtrates each in separate test tube.

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 should not be more than 10 % of specification limit. If it is out of the set criteria, make appropriate recommendations.

4.3.6 **Linearity and range:**

Determination:

Prepare set of linearity solutions of impurity standard and main drug standard from the stock solution to obtain solutions at LOQ to 250% of the specification limit by preparing minimum 5 concentrations level. Inject each solution into the chromatograph in single injection.

Plot a graph of areas vs. corrected 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.and calculate Relative Response Factor.

Acceptance criteria:

- Correlation Coefficient for each impurity and main drug standard should be not less than 0.99.

4.3.7 **Precision:**

4.3.7.1 **System precision:**

Determination:

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

Acceptance criteria (For HPLC):

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

4.3.7.2 **Repeatability:**

Determination:

a) If no known impurity is given in the specification, prepare sample solution as it is.

b) If known impurity is given in the specification and is above LOQ level, prepare sample solution as it is.

c) If known impurity is given in specification and is below LOQ level or equal, spike the impurity at the specification limit in the sample solution.

Repeatability shall perform on all the strengths, if multiple strength dosage forms.

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 should be not more than 15.0 %.

Note: Record the impurities below 0.05 %,acceptance criteria will not be applicable for it

4.3.7.3 **Intermediate precision:**

Determination:

a) If no known impurity is given in the specification, prepare sample solution as it is.

b) If known impurity is given in the specification and is above LOQ level, prepare sample solution as it is.

c) If known impurity is given in specification and is below LOQ level or equal 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 select 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 should be not more than 15.0 %.

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

4.3.8 **Accuracy (Recovery):**

Determination:

a) If no known impurity is present, spike standard at specification limit in placebo.

b) For, known impurities and unknown impurities spike the known impurities and diluted standard in placebo at the specification limit.

c) For known impurity, spike impurity standard in Sample solution at the specification limit.

In case of multiple strengths with different color, select worst condition of placebo as below and perform the accuracy.

1. Lower Drug: placebo ratio if average weight of all strength is same.
2. If different colours used in drug product, all colouring material mixed with excipients at proportionally and perform accuracy.

Prepare the recovery solutions and cover the range from LOQ to 250 of specification limit by preparing minimum 4-concentration level. Prepare the recovery solution at different concentration levels in triplicate with single injection and analyse the same. Calculate the areas of the peaks and the % results.

Acceptance criteria:

- Recovery should be between 80% and 120%. If it is out of the set criteria, appropriate recommendation shall be made.

4.3.9 **Robustness:**

Determination:

Prepare Standard, sample solution and placebo as described in the methodology and carry out 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 mobile phase / buffer (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. In case, flow rate

specified ≥1.0mL, change flow rate ± 0.2 and for <1.0mL, change flow rate ± 0.1.)

- 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 shall be calculated.

Acceptance criteria:

- The absolute difference in the results obtained in robustness study and Repeatability study should be not more than 15 % of specification limit.

4.3.10 **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.*

Determination:

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

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

Average value of Relative Response Factor will be reported.

Acceptance criteria:

- Record the value of Relative Response factor and use in the impurity calculation.

4.3.11 **System Suitability**

Determination:

Perform System suitability before performing any parameter.

Acceptance criteria (For HPLC).

- The system suitability should comply as per methodology.

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

Parameters to be considered for an In-house method:

- Specificity
- Solution stability
- Filter compatibility
- Filter saturation
- Linearity and range
- Precision (System precision, Repeatability, Intermediate precision)
- Accuracy
- Robustness
- System suitability

Parameters to be considered for a Pharmacopoeial method:

- Specificity
- Solution stability
- Filter compatibility
- Filter saturation
- Precision (System precision, Repeatability, Intermediate precision)
- Accuracy

4.4.1 **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 should 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 should not be more than 2.0 %.

4.4.2 **Solution stability:**

Determination:

Prepare standard solution and sample solution as equivalent to target concentration as mentioned in the method to be validated and keep the prepared solutions tightly closed and store at controlled room temperature (20°C to 25°C). Analyse the

standard and sample solution up to 24 hours (if estimation method by UV spectrophotometry) or store at controlled room temperature (20°C to 25°C) and at temperature below RT (as per STP recommend like 2-8°C, 10°C or 15°C). Analyse the standard and sample solution up to 48 hrs (Estimation methods by HPLC) 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 intervals conditions. Determine the absolute difference in % assay at the particular time point with respect to the initial assay for standard solution 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 interval point should not be more than 2.0. If it is out of the set criteria, make appropriate recommendations.

4.4.3 **Filter Compatibility:**

Determination:

Prepare the sample solution as equivalent to target concentration in the method to be validated. If multiple strength, select higher strength dosage form. At the filtration stage, filter the sample solution through Whatman No.41, Whatman No.42; discard about 10 mL sample solution from each filter. For Whatman GF/C filter, 0.45µm Nylon filter and 0.45µm PVDF filter 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 should not be more than 2.0. If it is out of the set criteria, make appropriate recommendations.

4.4.4 **Filter Saturation:**

Determination:

Prepare the sample solution as equivalent to target concentration in the method to be validated.

If multiple strength, select higher strength dosage form.

At filtration stage, when Whatman filter paper no.41 and Whatman filter paper no.42

is recommended for use in Filter compatibility study filter 10.0 mL and 20.0 mL sample solution and discard using each separate filters, followed by filtration of further 10 mL aliquots. Collect the filtrates in each separate test tube. When 0.45µm nylon filter, GF/C 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):

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

4.4.5 **Linearity and range:**

Determination:

Analyse linearity solutions from the stock solution of standard covering from 30 % to 150 % of the working concentration by preparing minimum 5-concentration level. In case of multiple strength of dosage form, will 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 area vs. corrected 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 should be not less than 0.995.

4.4.6 **Precision:**

4.4.6.1 **System precision:**

Prepare the standard solution as described in the method to be validated and inject / measure the obtained solution as mentioned in the method. Calculate the relative standard deviation of the responses / absorbance.

Acceptance criteria (For HPLC)

- Relative standard deviation should not be more than 2.0 %.

4.4.6.2 **Repeatability:**

Determination:

Perform the dissolution test for six dosage units using the method under validation and analyse by same analyst, on same equipment, on same day. For multiple strength, repeatability to be perform all the strength 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) should not be more than 5.0 % or should meet as per Specifications.

4.4.6.3 **Intermediate precision:**

Determination:

Perform the dissolution test on six dosage units using the method under validation and analyse 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 select 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) should 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) should not be more than 5.0.

4.4.7 **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 materials are of different colors, recovery shall perform individually.

Operate the dissolution tester, prepare further solutions and analyse the same, as described in 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 should be within 95.0 % to 105.0 %.
- The relative standard deviation for % recovery should not be more than 5.0 %.

4.4.8 **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 dissolution parameter

- Change in dissolution medium volume (± 1%).
- Change in RPM ((± 4%).
- 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 chromatographic parameter, If chromatographic condition is different from assay

- Change in pH of mobile phase / buffer (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. In case, flow rate specified ≥1.0mL, change flow rate ± 0.2 and for <1.0mL, change flow rate ± 0.1.)

- Change in column oven temperature (Temperature specified in method ± 5°C).
- Change in Wave length (± 2) only for UV- Visible spectrophotometry method.

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) should not be more than 5.0.

4.4.9 **System Suitability**

Determination:

Perform System suitability before performing any parameter.

Acceptance criteria (For HPLC & UV methods):

- The system suitability should comply as per methodology.

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

Parameters to be considered for an In-house method:

- Specificity
- Solution stability
- Filter compatibility
- Filter saturation
- Linearity and range
- Precision (System precision, Repeatability, Intermediate precision)
- Accuracy
- Robustness
- System suitability

Parameters to be considered for a Pharmacopoeial method:

- Specificity
- Solution stability
- Filter compatibility

- Filter saturation
- Precision (System precision, Repeatability, Intermediate precision)
- Accuracy

4.5.1 **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 injected at each interval.

Acceptance criteria:

- **For HPLC method:**

  No peak should 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 should not be more than 2.0 %.

4.5.2 **Solution stability:**

Determination:

Prepare Standard solution and sample solution (at 100 % level) in the method to be validated and Keep the prepared solutions tightly closed and store at controlled room temperature (20°C to 25°C) and analyse at each intervals specified in the methodology and at 24Hrs beyond the last time point with intermediate time points (if estimation method by UV spectrophotometry) or store at controlled room temperature (20°C to 25°C) and at temperature below RT (as per STP recommend like 2-8°C, 10°C or 15°C) (If estimation methods by HPLC). Analyse at each intervals specified in methodology and at 48 hours beyond the last time point with intermediate time points. Determine the absolute difference in assay for standard solution and % dissolution release for test solution at the particular time point with respect to initial.

Acceptance criteria (for HPLC & UV methods):

- The absolute difference in assay for standard solution and % dissolution release for test solution at each time point should not be more than 2.0 %. If it is out of the set criteria, make appropriate recommendations.

4.5.3 **Filter Compatibility:**

Determination:

Prepare the sample solution at concentration equivalent to the limit specified in methodology at the last time point. At the filtration stage, filter the solution through Whatman No.41 and Whatman No.42; discard about 10 mL sample solution from each filter. For Whatman GF/C filter, 0.45µm Nylon 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 filter solution and centrifuge solution should not be more than 2.0. If it is out of the set criteria, make appropriate recommendations.

4.5.4 **Filter Saturation:**

Determination:

Prepare the sample solution at concentration equivalent to limit specified in methodology for the last time point. At filtration stage, when Whatman filter paper no.41and Whatman filter paper no.42 and is recommended for use in Filter compatibility study filter10.0 mL and 20.0 mL sample solution and discard using separate filters, followed by filtration of further 10 mL aliquots. Collect the filtrates in each separate test tube. When GF/C filter, 0.45µm nylon filter and 0.45µm PVDF filter is recommended for use in Filter compatibility study filter (1 mL, 3mL and 5mL) volume of sample solution and discard using separate filters, followed by filtration of further 10 mL aliquots. Collect the filtrates in test tubes. Analyse the 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 should not be more than 2.0. If it is out of the set criteria, make appropriate recommendations.

4.5.5 **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 6 linearity points. Inject all the prepared solutions in single.

Plot a graph of area vs. corrected 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 should be not less than 0.995.

4.5.6 **Precision:**

4.5.6.1 **System precision:**

Prepare the standard solution as described in the method to be validated and inject / measure the obtained solution as mentioned in the method. Calculate the relative standard deviation of the responses / absorbance.

Acceptance criteria (For HPLC & UV methods):

- Relative standard deviation should not be more than 2.0 %.

4.5.6.2 **Repeatability:**

Determination:

Perform the dissolution test for six dosage units using the method under validation and analyse by same analyst, on same equipment, on same day. For multiple strength, precision will carried out all the strength and Calculate the dissolution results for each time point. Determine the relative standard deviation of 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 should not be more than 10.0 % or meet as per specification.

4.5.6.3 **Intermediate precision:**

Determination:

Perform the dissolution test on six dosage units using the method under validation and analyse by a different analyst, on different equipment, on different day. For multiple strength only higher strength will be select 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 should 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) should not be more than 10.0.

4.5.7 **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 should be within 95.0 % to 105.0 %.
- The relative standard deviation for % recovery should not be more than 5.0 %.

4.5.8 **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 dissolution parameter

- Change in dissolution medium volume (± 1%).
- Change in RPM ((± 4%).
- 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 chromatographic parameter, If chromatographic condition is different from assay

- Change in pH of mobile phase / buffer (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. In case, flow rate specified ≥1.0mL, change flow rate ± 0.2 and for <1.0mL, change flow rate ± 0.1.)
- Change in column oven temperature (Temperature specified in method ± 5°C).
- Change in Wave length (± 2) only for UV- Visible spectrophotometry method.

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).

4.5.9 **System Suitability**

Determination:

Perform System suitability before performing any parameter.

Acceptance criteria (For HPLC & UV methods):

- The system suitability should comply as per methodology.

4.6 ANALYTICAL METHOD VALIDATION FOR RESIDUAL SOLVENTS TEST OF DRUG PRODUCT

Parameters to be considered for an In-house method

- Specificity
- Limit of Detection And Limit of Quantification
- Linearity and range
- Precision (System precision, Method precision, Ruggedness)

- Accuracy
- Robustness
- System suitability

4.6.1 **Specificity:**

Interference study:

Determination:

Drug products: Prepare the blank (Diluent), individual known residual solvents, sample solution and six injections of standard solution 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 at the retention time of analyte.

4.6.2 **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 % RSD of the peak areas.

Similarly prepare the solution at LOD level and inject six injections and calculate the % RSD 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.

4.6.3 **Linearity & Range:**

Determination:

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

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

Acceptance criteria:

- Correlation coefficient should not be less than 0.99.

4.6.4 **Precision:**

4.6.4.1 **System precision:**

Determination:

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 should meet.

4.6.4.2 **Repeatability:**

Determination:

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

1. If any analyte is not present in the sample, prepare six-test solutions followed by that prepare 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 result in method precision is not more than 15.0.

4.6.4.3 **Ruggedness:**

Determination:

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

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

Acceptance criteria:

- The Overall % RSD of the residual solvents results obtained from method precision and ruggedness is not more than 15.0.

4.6.4.4 **Accuracy (Recovery):**

Determination:

1. Spike the Test sample with standard solution, at three levels in the range of 50%, 100% and 150% of specification limit.
2. Calculate mean recovery, standard deviation and the % RSD of recovery results.

Acceptance criteria:

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

4.6.5 **Robustness:**

Determination:

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

1. If any analyte is present in the sample prepare two test solutions.
2. 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 temp. (Equilibration temp specified in the method + 10% variation).

4.6.6 **Acceptance criteria:**

- Should meet all the requirements of system suitability.

- The overall %RSD of the residual solvents result obtained from method Precision and Robustness is not more than 15.0.

NOTE :

Execution of Analytical method validation of drug substance and drug product has to be carried out according to methodology mentioned in respective method validation protocol.

4.7 REVALIDATION / PARTIAL VALIDATION OF ANALYTICAL METHODOLOGY FOR DRUG PRODUCT:

Revalidation of analytical methodology will perform in the following circumstances:

- Method parameters have been changed.
- Scope of the method has been changed.
- Changes in the synthesis of drug substances.
- Changes in the composition of the drug products.
- Impurity profile has been changed.
- Dissolution parameter / medium changes**.**

5.0 ANNEXURE (S):

Nil

6.0 REFERENCE (S):

6.1 **SOP:** Preparation, approval, distribution, control, revision and destruction of Standard Operating Procedure (SOP).

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

6.3 **Guidance for Industry:** Analytical Procedures and Methods Validation Chemistry, Manufacturing and Controls Documentation (Draft, August 2000) – CDER.

6.4 Reviewer Guidance: Validation of Chromatographic Methods (November 1994) – CDER.

6.5 Chapter <1225> Validation of Compendial Procedures; USP 32.

6.6 **The Dissolution Procedure:** Development and Validation – Proposed USP General Chapter (Pharmacopeial Forum, Vol. No. 30(1) [Jan-Feb 2004] Page 351.

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

6.8 Compliance handbook of pharmaceuticals, medical devices and biologics by Carmen

Medina.

6.9 Chapter <905> Uniformity of Dosage form; USP 32.

6.10 Agencia Nacional de Vigilancia Sanitaria (ANVISA) Guideline, Resolution –RE n.899, of May 29 2003.

6.11 Uniformity of Content of single dosage preparation. European Pharmacopeia chapter<2.9.6>.

7.0 ABBREVIATION (S)/DEFINITION (S):

ARD : Analytical Research And Development.

HPLC: High Performance Liquid Chromatography.

UV : Ultra Violet.

RPM : Rotations Per Minute.

GC : Gas Chromatography.

RSD : Relative Standard Deviation.

LOQ : Limit of Quantification.

LOD : Limit of Detection.

TLC : Thin layer Liquid Chromatography

REVISION CARD

S.No.	REVISION No.	REVISION DATE	DETAILS OF REVISION	REASON (S) FOR REVISION	REFERENCE CHANGE CONTROL No.
01	00	---	---	New SOP	---