QbD Quality by Design	ANALYTICAL METHOD VALIDATION PROTOCOL FOR DISSOLUTION OF RAMIPRIL AND HYDROCHLOROTHIAZIDE IN RAMITHIAZIDE (RAMIPRIL AND HYDROCHLOROTHIAZIDE TABLETS) BY HPLC METHOD		
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Standard test method code: NA	**Method release date**: NA
Version: 00	**Supersedes Version**: NA
Reference: ICH guideline	**Type of validation**: Validation of Non-Pharmacopoeia method
Customer Name: Generic Healthcare Pvt. Ltd.	

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Abbreviations

Mg	: Milligram
A.R. Grade	: Analytical Reagent Grade
µ	: Micron
ML	: Milliliter
° C	: degree Celsius
µL	: Microliter
g	: Gram
ppm	: Part per million
Std	: Standard
Wt.	: Weight
RSD	: Relative Standard Deviation
Conc.	: Concentration
NMT	: Not more than
NLT	: Not Less Than
QA	: Quality assurance
HPLC	: High Performance Liquid Chromatography

1. OBJECTIVE

The objective of the analytical method validation for Dissolution test for Ramipril and Hydrochlorothiazide content in Ramithiazide Tablets is to demonstrate, that the method will consistently yield results that will accurately reflect the quality characteristics of the product.

2. SCOPE

The validation activity will be carried out on Ramithiazide 5 mg/12.5 mg, Ramithiazide 10 mg/12.5 mg and Ramithiazide 10 mg/25 mg.

3. RESPONSIBILITIES

3.1 It is responsibility of the Manager Analytical, to prepare the Protocol.

3.2 It is the responsibility of the General Manager to review the Protocol.

3.3 It is the responsibility of the Manager QA to approve the Protocol.

4. SAFETY

Laboratory coats, safety gloves and goggles should be worn during this procedure and while handling chemicals. Normal laboratory codes should be followed and appropriate procedures should be adopted for disposal of waste.

5. STANDARD DETAILS

Standard name	Batch no./Lot no.
Ramipril working standard	*
Hydrochlorothiazide working standard	*

Note: Will be mentioned at the time of validation.

6. SAMPLE DETAILS

Sample name	Batch number/Lot no.
Ramithiazide 5 mg/12.5 mg	GS160209
Ramithiazide 10 mg/12.5 mg	GS160210
Ramithiazide 10 mg/25 mg	GS160909
Placebo of Ramithiazide 5 mg/12.5 mg	-

Placebo of Ramithiazide 10 mg/12.5 mg	-
Placebo of Ramithiazide 10 mg/25 mg	-

7. REAGENTS AND CHEMICALS

Reagent	Grade	Manufacturer
Sodium perchlorate	AR	*
Acetonitrile	HPLC	*
Water	MilliQ	Inhouse

*Will be mentioned at the time of validation.

Note: Equivalent reagents, chemicals and solvents may be used provided they meet the specifications.

8. INSTRUMENT DETAILS

Instruments used for the validation will be,

Instrument/ Equipment	Supplier/ Manufacturer / Make	Instrument/ Equipment In-House No.	Calibration due date
HPLC	*	*	*
Analytical Balance	Mettler Toledo	QbD-INST-BAL-12	*
Analytical Balance	Mettler Toledo	QbD-INST-BAL-13	*
pH meter	Lab India	QbD-INST-pH-08	*

* Will be mentioned at the time of validation.

9. METHOD OF ANALYSIS

DISSOLUTION PARAMETERS

Dissolution medium	:	0.1 N Hydrochloric acid
Volume	:	750 mL
Apparatus	:	USP Type 1 (Basket)
Speed	:	100 rpm
Time	:	45 min
Temperature	:	37°C ±0.5°C
Sampling volume	:	10 mL

Preparation of dissolution medium (0.1 N hydrochloric acid):

Dilute 8.5 mL of conc. Hydrochloric acid to 1000 mL with water and mix well.

HPLC Chromatographic Conditions

Column	:	Sunniest C8, 150 mm x 4.6 mm, 5 µm or equivalent
Flow rate	:	1.0 mL/min
Column temperature	:	25°C
LC mode	:	Isocratic
Detection wavelength	:	UV 210 nm
Injection Volume	:	10 µL
Run time	:	10 min

Diluent:

Use 0.1 N hydrochloric acid as blank.

Blank:

Use diluent as blank.

Preparation of buffer:

Dissolve 12.2 g of Sodium perchlorate in 1000 mL water and adjust the pH to 2.5 with diluted orthophosphoric acid. Filter through 0.45 µ membrane filter and degas.

Preparation of mobile phase:

Mix 600 mL of buffer solution and 400 mL of acetonitrile.

Ramipril standard stock solution:

Weigh accurately and transfer 25.0 mg of Ramipril working standard into 250 mL of volumetric flask, add 150 mL of mobile phase, sonicate to dissolve and dilute to 250 mL with mobile phase and mix well.

Hydrochlorothiazide standard stock solution:

Weigh accurately and transfer 30.0 mg of Hydrochlorothiazide working standard into 250 mL of volumetric flask, add 150 mL of mobile phase, sonicate to dissolve and dilute to 250 mL with mobile phase and mix well.

Standard solution: (For Ramithiazide Tablets 5 mg/12.5 mg)

Dilute 1.5 mL of Ramipril standard stock solution and 3 mL of Hydrochlorothiazide standard stock solution to 25 mL with diluent and mix well.

Standard solution: (For Ramithiazide Tablets 10 mg/12.5 mg)

Dilute 3.0 mL each of Ramipril standard stock solution and Hydrochlorothiazide standard stock solution to 25 mL with diluent and mix well.

Standard solution: (For Ramithiazide Tablets 10 mg/25 mg)

Dilute 3.0 mL of Ramipril standard stock solution and 6.0 mL of Hydrochlorothiazide standard stock solution to 25 mL with diluent and mix well.

Sample solution:

Place 750 mL of dissolution medium in the vessel of the apparatus, assemble the apparatus equilibrate the dissolution medium to 37 ± 0.5°C. Place one tablet in each vessel and immediately operate the apparatus at 100 rpm. After 45 minutes withdraw sample from zone midway between the surface of dissolution medium and top of the rotating basket. Filter the sample through 0.45 µ nylon filter.

Procedure:

Separately inject 10 µL of the blank solution, standard solution in five replicate and sample solution into the chromatographic system, record the chromatograph and measure the peak response for the major peaks.

System suitability parameters:

The relative standard deviation for five replicate injections of standard solution should be not more than 2.0%.

Calculations:

Content of Ramipril dissolved

For Ramithiazide Tablets 5 mg/12.5 mg

$$\% \text{ Drug Release} = \frac{AT}{AS} \times \frac{WS}{250} \times \frac{1.5}{25} \times \frac{750}{1} \times \frac{P}{LC}$$

For Ramithiazide Tablets 10 mg/12.5 mg and 10 mg/25 mg

$$\% \text{ Drug Release} = \frac{AT}{AS} \times \frac{WS}{250} \times \frac{3}{25} \times \frac{750}{1} \times \frac{P}{LC}$$

Where,

AT = Area of Ramipril peak obtained from sample preparation.

AS = Average area of Ramipril peak obtained from standard solution.

WS = Weight of Ramipril working standard in mg.

P = %Potency of Ramipril working standard on as is basis.

LC = Label claim of Ramipril per tablet in mg

Content of Hydrochlorothiazide dissolved

For Ramithiazide Tablets 5 mg/12.5 mg and 10 mg/12.5 mg

$$\% \text{ Drug Release} = \frac{AT}{AS} \times \frac{WS}{250} \times \frac{3}{25} \times \frac{750}{1} \times \frac{P}{LC}$$

For Ramithiazide Tablets 10 mg/25 mg

$$\% \text{ Drug Release} = \frac{AT}{AS} \times \frac{WS}{250} \times \frac{6}{25} \times \frac{750}{1} \times \frac{P}{LC}$$

Where,

AT = Area of Hydrochlorothiazide peak obtained from sample preparation.

AS = Average area of Hydrochlorothiazide peak obtained from standard solution.

WS = Weight of Hydrochlorothiazide working standard in mg.

P = %Potency of Hydrochlorothiazide working standard on as is basis.

LC = Label claim of Hydrochlorothiazide per tablet in mg

Limit: Not less than 75% of the labeled amount of Ramipril and Hydrochlorothiazide are dissolved in 45 minutes.

10. VALIDATION PARAMETERS

10.1. Specificity

10.2. Linearity and Range

10.3. Precision

A. System precision

B. Method precision

C. Intermediate precision

10.4. Accuracy

10.5. Robustness

10.6. Solution stability

10.1. SPECIFICITY

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present.

Procedure:

Carry out procedure as per method specified in section Method of analysis.

Placebo preparation:

Place 750 mL of dissolution medium in the vessel of the apparatus, assemble the apparatus equilibrate the dissolution medium to 37 ± 0.5°C. Place one tablet in each vessel and immediately operate the apparatus at 100 rpm. After 45 minutes withdraw sample from zone midway between the surface of dissolution medium and top of the rotating basket. Filter the sample through 0.45 µ nylon filter.

Sequence:

Name	No. of Injection
Blank	1
Standard solution	5
Blank	1
Placebo	1
Sample solution	1
Bracketing standard solution	1

Acceptance criteria:

1. Relative standard deviation for five replicate injections of standard solution should be not more than 2.0%.
2. There should not be any interference of peak due to blank and placebo at the retention time of the Ramipril and Hydrochlorothiazide.

10.2. LINEARITY AND RANGE

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

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Procedure:

Prepare standard solutions as per method specified in section method of analysis.

Preparation of standard stock solution for Linearity:

Ramipril standard stock solution:

Weigh accurately and transfer 25.0 mg of Ramipril working standard into 250 mL of volumetric flask, add 150 mL of mobile phase, sonicate to dissolve and dilute to 250 mL with mobile phase and mix well.

Hydrochlorothiazide standard stock solution:

Weigh accurately and transfer 60.0 mg of Hydrochlorothiazide working standard into 250 mL of volumetric flask, add 150 mL of mobile phase, sonicate to dissolve and dilute to 250 mL with mobile phase and mix well.

Preparation Linearity levels as follows:

Linearity Level	Volume of stock solution (mL)	Diluted to (mL)	Concentration in ppm	
			Ramipril	Hydrochlorothiazide
25%	1.5	50	3.0	7.2
50%	3.0	50	6.0	14.4
80%	4.8	50	9.6	23.0
100%	6.0	50	12.0	28.8
120%	7.2	50	14.4	34.6
150%	9.0	50	18.0	43.2

Sequence:

Name	No. of replicates
Blank	1
Standard Solution	5
Linearity level (25%)	2
Linearity level (50%)	2
Linearity level (80%)	2
Linearity level (100%)	2
Linearity level (120%)	2
Linearity level (150%)	2
Bracketing standard solution	1

Determination:

Plot linearity curve with concentration of the linearity standard solution in ppm on x-axis and corresponding average absorbance response on y-axis. Determine the coefficient of correlation.

Acceptance Criteria:

1. Relative standard deviation for five replicate injections of standard solution should be not more than 2.0%.
2. The value obtained for correlation coefficient (r2) should not be less than 0.98

10.3. PRECISION

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

Precision of the method will be established by carrying out:

A) System Precision

Procedure:

Carry out procedure as per method specified in section method of analysis.

Sequence:

Name	No. of replicates
Blank	1
Standard Solution	6

Acceptance criteria:

Relative standard deviation for five replicate injections of standard solution should be not more than 2.0%.

B) Method precision

Procedure:

Carry out procedure as per method specified in section method of analysis.

Sequence:

Name	No. of replicates
Blank	1
Standard Solution	5
Sample Solution 1	1
Sample Solution 2	1
Sample Solution 3	1

Sample Solution 4	1
Sample Solution 5	1
Sample Solution 6	1
Bracketing standard solution	1

Acceptance criteria:

1. Relative standard deviation for five replicate injections of standard solution should be not more than 2.0%
2. % Release of all six units should not be less than 75% of labeled amount.
3. The relative standard deviation for % release of six units should not be more than 5.0%.

C) Intermediate precision

Intermediate precision of the method will be established by carrying out the analysis on different day, different column with different analyst and different instrument.

Procedure:

Carry out procedure as per method specified in section method of analysis.

Sequence:

Name	No. of replicates
Blank	1
Standard Solution	5
Sample Solution 1	1
Sample Solution 2	1
Sample Solution 3	1
Sample Solution 4	1
Sample Solution 5	1
Sample Solution 6	1
Bracketing standard solution	1

Acceptance criteria:

1. Relative standard deviation for five replicate injections of standard solution should be not more than 2.0%.

2. % Release of all six units should not be less than 75% of labeled amount.
3. The relative standard deviation for % release of six units should not be more than 5.0%.
4. The difference in the mean value between the % release at method precision and intermediate precision should not be more than 5%.

10.4. ACCURACY (RECOVERY)

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 value found.

Procedure:

Carry out procedure as per method specified in section method of analysis.

Preparation of standard stock solution for Linearity:

Ramipril standard stock solution:

Weigh accurately and transfer 90.0 mg of Ramipril working standard into 50 mL of volumetric flask, add 30 mL of mobile phase, sonicate to dissolve and dilute to 50 mL with mobile phase and mix well.

Hydrochlorothiazide standard stock solution:

Weigh accurately and transfer 216.0 mg of Hydrochlorothiazide working standard into 50 mL of volumetric flask, add 30 mL of mobile phase, sonicate to dissolve and dilute to 50 mL with mobile phase and mix well.

For Ramithiazide Tablets 5 mg/12.5 mg and 10 mg/25 mg

Preparation Accuracy levels as follows:

Level	Accuracy Level (%)	Weight of Placebo (mg)	Volume of stock solution (mL)	Diluted to volume (mL)	Conc. (ppm)	
					Ramipril	Hydrochlorothiazide
1	25	Equivalent to 10 mg of Ramipril	1.2	750 mL	2.9	6.9
2	50		2.5		6.0	14.4
3	100		5.0		12.0	28.8
4	150		7.5		18.0	43.2

Each level prepares in triplicate.

For Ramithiazide Tablets 10 mg/12.5 mg

Preparation Accuracy levels as follows:

Level	Accuracy Level (%)	Weight of Placebo (mg)	Volume of stock solution (mL)		Diluted to volume (mL)	Conc. (ppm)	
			Ramipril	Hydrochlor-othiazide		Ramipril	Hydrochlor-othiazide
1	50	Equivalent to 10 mg of Ramipril	2.5	1.2	750 mL	6.0	6.9
2	100		5.0	2.5		12.0	14.4
3	150		7.5	3.8		18.0	21.9

Each level prepares in triplicate.

Sequence:

Name	No. of replicates
Blank	1
Standard Solution	5
Placebo solution	1
Accuracy 25% Level	1
Accuracy 25% Level	1
Accuracy 25% Level	1
Accuracy 50% Level	1
Accuracy 50% Level	1
Accuracy 50% Level	1
Accuracy 100% Level	1

Accuracy 100% Level	1
Accuracy 100% Level	1
Accuracy 150% Level	1
Accuracy 150% Level	1
Accuracy 150% Level	1
Bracketing standard solution	1

Acceptance criteria:

% Recovery for each accuracy level should be between 95.0% to 105.0%.

Calculation:

% Recovery = $\frac{\text{Amount recovered}}{\text{Amount added}} \times 100$

10.5. 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.

Procedure:

Carry out procedure as per method specified in section method of analysis.

The following parameters will be tested for robustness on Dissolution:

Sr. no.	Parameter to be changed	Actual Value	+ (Increase)	- (Decrease)
1	Change in RPM	100 RPM	104 RPM	96 RPM
2	Change in Dissolution medium volume	750 mL	760 mL	740 mL

The following parameters will be tested for robustness on HPLC:

Sr.no.	Parameter to be changed	Actual Value	+ (Increase)	- (Decrease)
1	Change in PH for mobile phase preparation	2.5	2.7	2.3
2	Change in temperature	25°C	27°C	23°C
3	Change in Flow rate	1.0 mL/min	1.1 mL/min	0.9 mL/min

Sequence:

Name	No. of replicates
Blank	1
Standard Solution	5
Sample solution 1	1
Sample solution 2	1
Bracketing standard solution	1

Acceptance criteria:

The difference in the mean value between the dissolution results at method precision and robustness should not more than 5%.

10.6. SOLUTION STABILITY

To evaluate stability of standard solution and sample solution will be prepared as described in the method of analysis and repeatedly will be determined up to 24 hrs from the time of preparation.

Procedure:

Carry out procedure as per method specified in section method of analysis.

Sequence:

Name	No. of replicates
Blank	1
Standard Solution	5
Sample solution	1
Bracketing standard solution	1
Standard solution (Stability interval)	1
Sample solution (Stability interval)	1
Bracketing standard solution	1

Acceptance criteria:

Standard solution stability:

The relative standard deviation of absorbance of repeatedly determined standard solution over a period of 24 hrs at room temperature should not be more than 2.0%.

Sample solution stability:

The absolute difference in the value of % release for sample solution between 0 hr and a specified period of time should be not more than 2.0%.

11. DOCUMENTATION

All validation activities shall be recorded online in to the laboratory notebook. At the end of the Validation, a report shall be generated. All results will be discussed, conclusions drawn and any deviations from the protocol will be documented in the report.

12. REFERENCES

ICH guideline

13. REVISION HISTORY

Version	Date	Changes	Reason for change
0		Not Applicable	New