QbD Quality by Design	**ANALYTICAL METHOD VALIDATION PROTOCOL FOR ASSAY AND UNIFORMITY OF CONTENT OF RAMITHIAZIDE (RAMIPRIL AND HYDROCHLOROTHIAZIDE TABLETS) BY HPLC METHOD**		
	Protocol number	QbD-PROTO-322	**Page no.** 1 of 25

Standard test method code: NA	**Method release date**: NA
Version: 00	**Supersedes Version**: NA
Reference: ICH guideline	**Type of validation**: Validation of Non-Pharmacopoeial method
Customer Name: Generic Healthcare Pvt. Ltd.	

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	For (Generic Healthcare Pvt. Ltd.)		

INDEX

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
HPLC	: High Performance Liquid Chromatography
Conc.	: Concentration
NMT	: Not more than
NLT	: Not Less Than
QA	: Quality assurance

1. OBJECTIVE

The objective of the analytical method validation for Assay and Uniformity of content of 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	*

* Will be mentioned at the time of validation.

6. SAMPLE DETAILS

Sample name	Batch number/Lot no.
Ramithiazide 5 mg/12.5 mg	GP170401
Ramithiazide 10 mg/12.5 mg	GP170402
Ramithiazide 10 mg/25 mg	GP170403
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 (FOR ASSAY AND CONTENT UNIFORMITY)

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:

A mixture of 55 volume of water, 45 volume of acetonitrile and 0.1 volume of triethylamine. Adjust pH 3.0 with dilute phosphoric acid.

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 0.45µ membrane filter and degas.

Preparation of mobile phase:

Mix 500 mL of buffer solution and 500 mL of acetonitrile.

Ramipril standard stock solution:

Weigh accurately and transfer 25.0 mg of Ramipril working standard into 25 mL of volumetric flask, add 10 mL of diluent, sonicate to dissolve and dilute to 25 mL with diluent and mix well. (Conc.: 1 mg/mL).

Hydrochlorothiazide standard stock solution:

Weigh accurately and transfer 25.0 mg of Hydrochlorothiazide working standard into 25 mL of volumetric flask, add 10 mL of diluent, sonicate to dissolve and dilute to 25 mL with diluent and mix well.(Conc.: 1 mg/mL).

Standard solution (For Ramithiazide Tablets 5 mg/12.5 mg and 10 mg/25 mg):

Dilute 2 mL of Ramipril standard stock solution and 5 mL of Hydrochlorothiazide standard stock solution to 100 mL with diluent and mix well. (Conc. 0.02 mg/mL of Ramipril and 0.05 mg/mL of Hydrochlorothiazide).

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

Dilute 2 mL of Ramipril standard stock solution and 2.5 mL of Hydrochlorothiazide standard stock solution to 100 mL with diluent and mix well. (Conc. 0.02 mg/mL of Ramipril and 0.025 mg/mL of Hydrochlorothiazide).

Sample solution (For Ramithiazide Tablets 5 mg/12.5 mg and 10 mg/25 mg):

Weight and powder 20 tablets. Weight accurately a quantity of powder containing about 25 mg of Ramipril, disperse in 5 mL of water, add 150 mL of diluent and sonicate for 15 min with intermittent shaking. Cool, dilute to 250 mL with diluent and mix well. Filter the solution through 0.45 µ nylon syringe filter. Dilute 5 mL of this solution to 25 mL with diluent and mix well. (Conc. 0.02 mg/mL of Ramipril and 0.05 mg/mL of Hydrochlorothiazide).

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

Weight and powder 20 tablets. Weight accurately a quantity of powder containing about 25 mg of Ramipril, disperse in 5 mL of water, add 150 mL of diluent and sonicate for 15 min with intermittent shaking. Cool, dilute to 250 mL with diluent and mix well. Filter the solution through 0.45 µ nylon syringe filter. Dilute 5 mL of this solution to 25 mL with diluent and mix well. (Conc. 0.2 mg/mL of Ramipril and 0.025 mg/mL of Hydrochlorothiazide).

Procedure:

Separately inject 10 µL of the blank solution, standard solution in five replicate and sample solution in duplicate 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%. The theoretical plates of the principal peaks should be not less than 2000 and the tailing factor should be not more than 2.0.

Calculations:

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

% Content of Ramipril

$$\% \text{ Content} = \frac{AT}{AS} \times \frac{WS}{25} \times \frac{2}{100} \times \frac{250}{WT} \times \frac{25}{5} \times \frac{P}{LC} \times \text{Average weight (mg)}$$

%Content of Hydrochlorothiazide

$$\% \text{ Content} = \frac{AT1}{AS1} \times \frac{WS1}{25} \times \frac{5}{100} \times \frac{250}{WT1} \times \frac{25}{5} \times \frac{P1}{LC1} \times \text{Average weight (mg)}$$

For Ramithiazide Tablets 10 mg/12.5 mg

% Content of Ramipril

$$\% \text{ Content} = \frac{AT}{AS} \times \frac{WS}{25} \times \frac{2}{100} \times \frac{250}{WT} \times \frac{25}{5} \times \frac{P}{LC} \times \text{Average weight (mg)}$$

%Content of Hydrochlorothiazide

$$\% \text{ Content} = \frac{AT1}{AS1} \times \frac{WS1}{25} \times \frac{2.5}{100} \times \frac{250}{WT1} \times \frac{25}{5} \times \frac{P1}{LC1} \times \text{Average weight (mg)}$$

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.

WT = Weight of sample in mg.

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

LC = Label claim of Ramipril per tablet in mg

AT1 = Area of Hydrochlorothiazide peak obtained from sample preparation.

AS1 = Average area of Hydrochlorothiazide peak obtained from standard solution.

WS1 = Weight of Hydrochlorothiazide working standard in mg.

WT1 = Weight of sample in mg.

P1 = % Potency of Hydrochlorothiazide working standard on as is basis.

LC1 = Label claim of Hydrochlorothiazide per tablet in mg

UNIFORMITY OF CONTENT:

Note: HPLC Chromatographic conditions, Diluent, mobile phase, preparation of standard solutions are same as mention in assay method.

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

Add intact tablet into 250 mL volumetric flask. Disperse in 5 mL of water, add 150 mL of diluent and sonicate for 20 min with intermittent shaking. Cool and dilute to 250 mL with diluent and mix well. Filter the solution through 0.45 µ nylon syringe filter. (Conc. 0.2 mg/mL of Ramipril and 0.05 mg/mL of Hydrochlorothiazide).

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

Add intact tablet into 250 mL volumetric flask. Disperse in 5 mL of water, add 150 mL of diluent and sonicate for 20 min with intermittent shaking. Cool and dilute to 250 mL with diluent and mix well. Filter the solution through 0.45 µ nylon syringe filter. Dilute 5 mL of this solution to 10 mL with diluent and mix well. (Conc. 0.2 mg/mL of Ramipril and 0.025 mg/mL of Hydrochlorothiazide).

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

Add intact tablet into 250 mL volumetric flask. Disperse in 5 mL of water, add 150 mL of diluent and sonicate for 20 min with intermittent shaking. Cool and dilute to 250 mL with diluent and mix well. Filter the solution through 0.45 µ nylon syringe filter. Dilute 10.0 mL of this solution to 20 mL with diluent and mix well. (Conc. 0.2 mg/mL of Ramipril and 0.05 mg/mL of Hydrochlorothiazide).

Procedure:

Separately inject 10 µL of the blank solution, standard solution in six replicate and ten sample solutions of uniformity of content 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%. The theoretical plates of the principal peaks should be not less than 2000 and the tailing factor should be not more than 2.0.

Calculations:

For Ramithiazide Tablets 5 mg/12.5 mg

$$\% \text{ Content of Ramipril} = \frac{AT}{AS} \times \frac{WS}{25} \times \frac{2}{100} \times \frac{250}{1} \times \frac{P}{LC}$$

$$\% \text{ Content of HCTZ} = \frac{AT1}{AS1} \times \frac{WS1}{25} \times \frac{5}{100} \times \frac{250}{1} \times \frac{P1}{LC1}$$

For Ramithiazide Tablets 10 mg/12.5 mg

$$\% \text{ Content of Ramipril} = \frac{AT}{AS} \times \frac{WS}{25} \times \frac{2}{100} \times \frac{250}{1} \times \frac{10}{5} \times \frac{P}{LC}$$

$$\% \text{ Content of HCTZ} = \frac{AT1}{AS1} \times \frac{WS1}{25} \times \frac{2.5}{100} \times \frac{250}{1} \times \frac{10}{5} \times \frac{P1}{LC1}$$

For Ramithiazide Tablets 10 mg/25 mg

$$\% \text{ Content of Ramipril} = \frac{AT}{AS} \times \frac{WS}{25} \times \frac{2}{100} \times \frac{250}{1} \times \frac{10}{5} \times \frac{P}{LC}$$

$$\% \text{ Content of HCTZ} = \frac{AT1}{AS1} \times \frac{WS1}{25} \times \frac{5}{100} \times \frac{250}{1} \times \frac{10}{5} \times \frac{P1}{LC1}$$

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.

WT = Weight of sample in mg.

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

LC = Label claim of Ramipril per tablet in mg

AT1 = Area of Hydrochlorothiazide peak obtained from sample preparation.

AS1 = Average area of Hydrochlorothiazide peak obtained from standard solution.

WS1 = Weight of Hydrochlorothiazide working standard in mg.

WT1 = Weight of sample in mg.

P1 = % Potency of Hydrochlorothiazide working standard on as is basis.

LC1 = Label claim of Hydrochlorothiazide per tablet in mg

10. VALIDATION PARAMETERS

10.1. Specificity

10.2. Specificity by forced degradation

10.3. Linearity and Range

10.4. Precision

A. System precision

B. Method precision

C. Intermediate precision (Ruggedness)

10.5. Accuracy

10.6. Robustness

10.7. Filter study

10.8. Solution stability

10.1. SPECIFICITY

Specificity is the ability to asses 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.

Ramipril Identification solution:

Weigh accurately and transfer 25.0 mg of Ramipril working standard into 25 mL of volumetric flask, add 10 mL of diluent, sonicate to dissolve and dilute to 25 mL with diluent and mix well. Dilute 2 mL of this solution to 100 mL with diluent and mix well.

Hydrochlorothiazide Identification solution:

Weigh accurately and transfer 25.0 mg of Hydrochlorothiazide working standard into 25 mL of volumetric flask, add 10 mL of diluent, sonicate to dissolve and dilute with diluent and mix well. Dilute 5 mL of this solution to 100 mL with diluent and mix well.

Placebo solution (For Ramithiazide Tablets 5 mg/12.5 mg and 10 mg/25 mg):

Weight accurately a quantity of placebo powder containing about 25 mg of Ramipril, add 150 mL of diluent and sonicate for 15 min with intermittent shaking. Cool and mix well, dilute to 250 mL with the diluent and filter the solution through 0.45 µ nylon syringe filter. Dilute 5 mL of this solution to 25 mL with diluent and mix well.

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

Weight accurately a quantity of powder containing about 25 mg of Ramipril, add 150 mL of diluent and sonicate for 15 min with intermittent shaking. Cool and mix well, dilute to 250 mL with the diluent and filter the solution through 0.45 µ nylon syringe filter. Dilute 5 mL of this solution to 25 mL with diluent and mix well.

Determination:

1. Inject blank solution.
2. Inject standard solution in six replicate.
3. Inject placebo solution.
4. Inject individual Identification solution.
5. Inject sample solution.
6. Inject bracketing standard.

Acceptance criteria:

1. The relative standard deviation for peak area of Ramipril and Hydrochlorothiazide of standard solution should be not more than 2.0%
2. The tailing factor for Ramipril and Hydrochlorothiazide peak in standard solution should be not more than 2.0
3. The theoretical plates for Ramipril and Hydrochlorothiazide peak in standard solution should be not less than 2000

Acceptance criteria for specificity:

There should not be any interference of peak due to blank and placebo at the retention time of the Ramipril and Hydrochlorothiazide peak.

10.2. SPECIFICITY BY FORCED DEGRADATION

Sample will be stressed by acid, base, oxidation and thermal to assess stability indicating aspect of the method.

The degraded samples will be analyzed using a photodiode-array detector.

Procedure:

Carry out determination as per method specified in section Method of Analysis.

10.3. 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:

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

Ramipril standard stock solution:

Weigh accurately and transfer 25.0 mg of Ramipril working standard into 25 mL of volumetric flask, add 10 mL of diluent, sonicate to dissolve and dilute to 25 mL with diluent and mix well. (Conc.: 1 mg/mL).

Hydrochlorothiazide standard stock solution:

Weigh accurately and transfer 25.0 mg of Ramipril working standard into 25 mL of volumetric flask, add 10 mL of diluent, sonicate to dissolve and dilute to 25 mL with diluent and mix well. (Conc.: 1 mg/mL).

Preparation Linearity levels as follows:

Sr No	Linearity level (%)	mL of stock solution of Ramipril diluted to	mL of stock solution of HCTZ diluted to	Diluted to volume (mL)	Concentration of Ramipril (ppm)	Concentration of HCTZ (ppm)
1	25	0.5	1.2	100	5.0	12.0
2	50	1.0	2.5	100	10.0	25.0
3	80	1.6	4.0	100	16.0	40.0
4	100	2.0	5.0	100	20.0	50.0
5	120	2.4	6.0	100	24.0	60.0
6	150	3.0	7.5	100	30.0	75.0

Plot linearity curve with concentration of the linearity levels in ppm on x-axis and corresponding average peak area response on y-axis. Determine the correlation coefficient.

Determination:

1. Inject blank solution.
2. Inject standard solution in six replicate.
3. Inject 25% and 150% linearity level in six replicate.
4. Inject other linearity level in three replicate.
5. Inject bracketing standard.

Acceptance criteria:

1. The relative standard deviation for peak area of Ramipril and Hydrochlorothiazide of standard solution should be not more than 2.0%
2. The tailing factor for Ramipril and Hydrochlorothiazide peak in standard solution should be not more than 2.0
3. The theoretical plates for Ramipril and Hydrochlorothiazide peak in standard solution should be not less than 2000
4. The correlation coefficient obtained from the graph should be NLT 0.99

10.4. PRECISION

Precision of the method shall be established by carrying out as System precision, Method precision and Intermediate precision.

A) System Precision

Procedure:

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

Determination:

1. Inject blank solution.
2. Inject standard solution in six replicate.

Acceptance criteria:

1. The relative standard deviation for peak area of Ramipril and Hydrochlorothiazide of standard solution should be not more than 2.0%
2. The tailing factor for Ramipril and Hydrochlorothiazide peak in standard solution should be not more than 2.0
3. The theoretical plates for Ramipril and Hydrochlorothiazide peak in standard solution should be not less than 2000

B) Method 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.

I) Method precision For Assay:

Procedure:

Carry out procedure as per method specified in section method of analysis. Prepare 6 sample solutions for Assay.

Determination:

1. Inject blank solution.
2. Inject standard solution in six replicate.
3. Inject sample solution (Six sample solutions).
4. Inject bracketing standard.

Acceptance criteria:

1. The relative standard deviation for peak area of Ramipril and Hydrochlorothiazide of standard solution should be not more than 2.0%
2. The tailing factor for Ramipril and Hydrochlorothiazide peak in standard solution should be

not more than 2.0

3. The theoretical plates for Ramipril and Hydrochlorothiazide peak in standard solution should be not less than 2000
4. % Assay value of Ramipril and Hydrochlorothiazide for sample solution should be within specified limit.
5. % RSD of Assay value of Ramipril and Hydrochlorothiazide for six sample solution should be not more than 2.0%

II) Method precision for Content Uniformity:

Procedure:

Carry out procedure as per method specified in section method of analysis. Prepare 10 sample solutions for content uniformity.

Determination:

1.Inject blank solution.

2. Inject standard solution in six replicate.
3. Inject sample solution (ten sample solutions).
4. Inject bracketing standard.

Acceptance criteria:

1. The relative standard deviation for peak area of Ramipril and Hydrochlorothiazide of standard solution should be not more than 2.0%
2. The tailing factor for Ramipril and Hydrochlorothiazide peak in standard solution should be not more than 2.0
3. The theoretical plates for Ramipril and Hydrochlorothiazide peak in standard solution should be not less than 2000
4. The acceptance value of Ramipril and Hydrochlorothiazide for ten sample solution should be not more than 15.0

C) Intermediate precision

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

I) Method precision For Assay:

Procedure:

Carry out procedure as per method specified in section method of analysis. Prepare 6 sample solutions for Assay.

Determination:

1. Inject blank solution.
2. Inject standard solution in six replicate.
3. Inject sample solution (Six sample solutions).
4. Inject bracketing standard.

Acceptance criteria:

1. The relative standard deviation for peak area of Ramipril and Hydrochlorothiazide of standard solution should be not more than 2.0%
2. The tailing factor for Ramipril and Hydrochlorothiazide peak in standard solution should be not more than 2.0
3. The theoretical plates for Ramipril and Hydrochlorothiazide peak in standard solution should be not less than 2000
4. % Assay value of Ramipril and Hydrochlorothiazide for sample solution should be within specified limit.
5. % RSD of Assay value of Ramipril and Hydrochlorothiazide for six sample solution should be not more than 2.0%
6. The cumulative RSD for % Assay of Ramipril and Hydrochlorothiazide for twelve samples in method precision and intermediate precision should be not more than 2.0%

II) Method precision for Content Uniformity:

Procedure:

Carry out procedure as per method specified in section method of analysis. Prepare 10 sample solutions for content uniformity.

Determination:

1.Inject blank solution.

2. Inject standard solution in six replicate.
3. Inject sample solution (ten sample solutions).
4. Inject bracketing standard.

Acceptance criteria:

1. The relative standard deviation for peak area of Ramipril and Hydrochlorothiazide of standard solution should be not more than 2.0%
2. The tailing factor for Ramipril and Hydrochlorothiazide peak in standard solution should be not more than 2.0
3. The theoretical plates for Ramipril and Hydrochlorothiazide peak in standard solution should be not less than 2000
4. The acceptance value of Ramipril and Hydrochlorothiazide for ten sample solution should be not more than 15.0
5. % Difference of mean of content uniformity of Ramipril and Hydrochlorothiazide for Method precision and Intermediate precision should be not more than 3.0%

10.5. ACCURACY (RECOVERY)

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

Procedure:

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

Preparation of Accuracy levels as follows:

Accuracy for 10/25 mg,

Accuracy level (%)	Placebo (mg)	Ramipril std. (mg)	HCTZ std. (mg)	Diluted to volume (mL)	Further Diluted	Diluted to volume (mL)	Conc. of Ramipril (ppm)	Conc. of HCTZ (ppm)
50	Eq to 25 mg of Ramipril	12.5	31.25	250	5	25	10	25.0
100		25.0	62.5	250	5	25	20	50.0
150		37.5	93.75	250	5	25	30	75.0

Accuracy for 10/12.5 mg,

Accuracy level (%)	Placebo (mg)	Ramipril std. (mg)	HCTZ std. (mg)	Diluted to volume (mL)	Further Diluted	Diluted to volume (mL)	Conc. of Ramipril (ppm)	Conc. of HCTZ (ppm)
50	Eq to 25 mg of Ramipril	12.5	15.62	250	5	25	10	12.5
100		25.0	31.25	250	5	25	20	25.0
150		37.5	46.87	250	5	25	30	37.5

Each level prepare in triplicate.

Determination:

1. Inject blank solution.
2. Inject standard solution in six replicate.
3. Inject all accuracy levels.
4. Inject bracketing standard.

Acceptance Criteria:

1. The relative standard deviation for peak area of Ramipril and Hydrochlorothiazide of standard solution should be not more than 2.0%
2. The tailing factor for Ramipril and Hydrochlorothiazide peak in standard solution should be not more than 2.0
3. The theoretical plates for Ramipril and Hydrochlorothiazide peak in standard solution should be not less than 2000
4. % Recovery at each accuracy level should be between 98.0% and 102.0%.
5. Mean % Recovery of all level should be between 98.0% and 102.0%.

10.6. 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 as:

Sr. no.	Parameter to be changed	Actual Value	+ (Increase)	- (Decrease)
1	pH	2.5	2.3	2.7
2	Flow Rate	1.0 mL\minute	1.1 mL\minute	0.9 mL\minute
3	Mobile phase composition	50:50	52:48	48:52

Determination:

1. Inject blank solution.
2. Inject standard solution in six replicate.
3. Inject sample solution (Duplicate).
4. Inject bracketing standard.

Acceptance criteria:

1. The relative standard deviation for peak area of Ramipril and Hydrochlorothiazide of standard solution should be not more than 2.0%
2. The tailing factor for Ramipril and Hydrochlorothiazide peak in standard solution should be not more than 2.0
3. The theoretical plates for Ramipril and Hydrochlorothiazide peak in standard solution should be not less than 2000
4. % Difference for % Assay of sample solution in robustness and % Assay of sample solution in method precision should be not more than 2.0%

10.7. FILTER STUDY

Procedure:

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

Determination:

1. Inject blank solution.
2. Inject standard solution in six replicate.
3. Inject Centrifuged sample (Centrifuged at 2500 rpm for 5 mins).
4. Inject Sample filtered through two different filter papers.
5. Inject bracketing standard.

Acceptance criteria:

1. The relative standard deviation for peak area of Ramipril and Hydrochlorothiazide of standard solution should be not more than 2.0%
2. The tailing factor for Ramipril and Hydrochlorothiazide peak in standard solution should be not more than 2.0
3. The theoretical plates for Ramipril and Hydrochlorothiazide peak in standard solution should be not less than 2000
4. %Difference between filtered solution through different filters and centrifuged sample solution should be NMT 2.0%

10.8. SOLUTION STABILITY

Procedure:

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

Determination:

1. Inject blank solution.
2. Inject standard solution in six replicate.
3. Inject sample solution.
4. Inject bracketing standard.
5. Inject standard solution (Stability interval)
6. Inject sample solution (Stability interval)
7. Inject bracketing standard.

Acceptance criteria:

Standard solution stability:

The RSD of area response of repeatedly injected standard solution over a period of 24 hrs should be not more than 2.0%

Sample solution stability:

The RSD of area response of repeatedly injected sample solution over a period of 24 hrs should be not more than 2.0%

10 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.

11 REFERENCES

ICH guidelines

12 REVISION HISTORY

Version	Date	Changes	Reason for change
0		Not Applicable	New