QbD Quality by Design	**ANALYTICAL METHOD VALIDATION REPORT 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-Pharmacopoeial Method
Customer Name: Generic Healthcare Pvt. Ltd.	

Prepared By	**Designations**	**Signature**	**Date**
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	For (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
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 Dissolution of Ramipril and Hydrochlorothiazide 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 has been 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 Report.

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

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

4. SAFETY

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

5. STANDARD DETAILS

Standard Name	Batch No./Lot No.
Ramipril Working Standard	RL14203
Hydrochlorothiazide Working Standard	20140403

* Will be mentioned at the time of validation.

6. SAMPLE DETAILS

Sample Name	Batch No./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	Merck
Acetonitrile	HPLC	Finar
Water	MilliQ	Inhouse

8. INSTRUMENT DETAILS

Instrument/ Equipment	Supplier / Manufacturer / Make	Instrument / Equipment In-House No.	Calibration Due Date
HPLC	Thermo	QbD-INST-HPLC-67	24 –Dec-17
HPLC	Agilent	QbD-INST-HPLC-51	05 –Aug-17
Dissolution Apparatus	Lab India	QbD-INST-DISS-09	22-Jul-17
Dissolution Apparatus	Lab India	QbD-INST-DISS-10	22-Jul-17
Analytical Balance	Mettler Toledo	QbD-INST-BAL-12	Daily
Analytical Balance	Mettler Toledo	QbD-INST-BAL-13	Daily
pH Meter	Lab India	QbD-INST-pH-08	Daily

9. METHOD OF ANALYSIS (FOR DISSOLUTION OF RAMIPRIL AND HYDROCHLOROTHIAZIDE)

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 (0.1N 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 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 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 0.45µ 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.

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.

Observations/Results:

Standard Details: (Ramipril 5 mg & Hydrochlorothiazide 12.5 mg & Ramipril 10 mg & Hydrochlorothiazide 25 mg)

Hydrochlorothiazide:

Sr. No.	RT	Area
1	2.153	7760.560
2	2.153	7758.658
3	2.153	7762.480
4	2.153	7763.766
5	2.153	7765.055
Average	2.153	7762.104
SD	0.00	2.54
%RSD	0.00	0.03
System Suitability Results		
% RSD of Area	0.03	NMT 2.0%

Ramipril:

Sr. No.	RT	Area
1	3.937	2089.654
2	3.937	2082.810
3	3.937	2084.558
4	3.937	2085.380
5	3.937	2089.446
Average	3.937	2086.370
SD	0.00	3.05
%RSD	0.00	0.15
System Suitability Results		
% RSD of Area	0.15	NMT 2.0%

Interference Details:

Sample No.	Area of Hydrochlorothiazide		Area of Ramipril	
	RT	Area	RT	Area
Blank Solution	Not Detected	Not Detected	Not Detected	Not Detected
Placebo Solution	Not Detected	Not Detected	Not Detected	Not Detected
Sample Solution	2.157	7546.123	3.940	2212.454

Conclusion:

No interference found due to blank and placebo at the retention time of the Hydrochlorothiazide and Ramipril.

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.

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

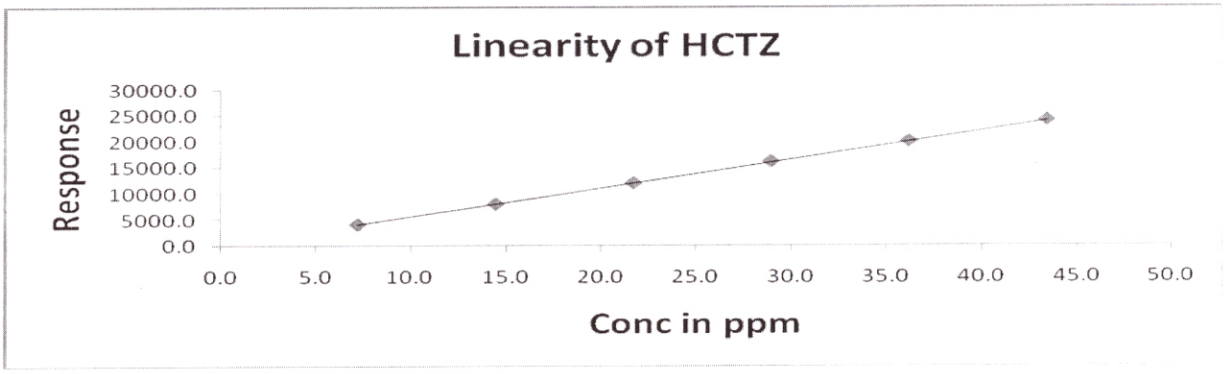
Observations/Results:

Standard details:

Injection No.	Retention Time	Area of Standard
1	2.213	16393.666
2	2.213	16300.718
3	2.213	16265.507
4	2.213	16273.524
5	2.213	16318.812
Average	2.213	16310.446
SD	0.000	51.169
%RSD	0.000	0.314
System Suitability Results		
% RSD of Area	0.31	NMT 1.5%
Tailing Factor	1.19	NMT 2.0
Theoretical Plates	4323	NLT 2000

Linearity for Hydrochlorothiazide:

Level	Linearity level (%)	ml of Standard Stock Solution added	Diluted to volume (mL)	Actual Concentration (ppm)	Area of sample	Average area
1	25	1.50	25	7.2	4108.377 4098.371 4103.076 4102.985 4103.983 4100.051	4102.807
2	50	3.00	25	14.5	8111.711 8058.520 8057.730	8075.987
3	75	4.50	25	21.7	12097.737 12006.775 12006.473	12036.995
4	100	6.00	25	29.0	16233.327 16166.768 16259.276	16219.790
5	125	7.50	25	36.2	20081.864 20099.317 20074.587	20085.256
6	150	9.00	25	43.4	24021.387 40480.045 40458.462 40360.603 40379.248 40288.613	24091.550



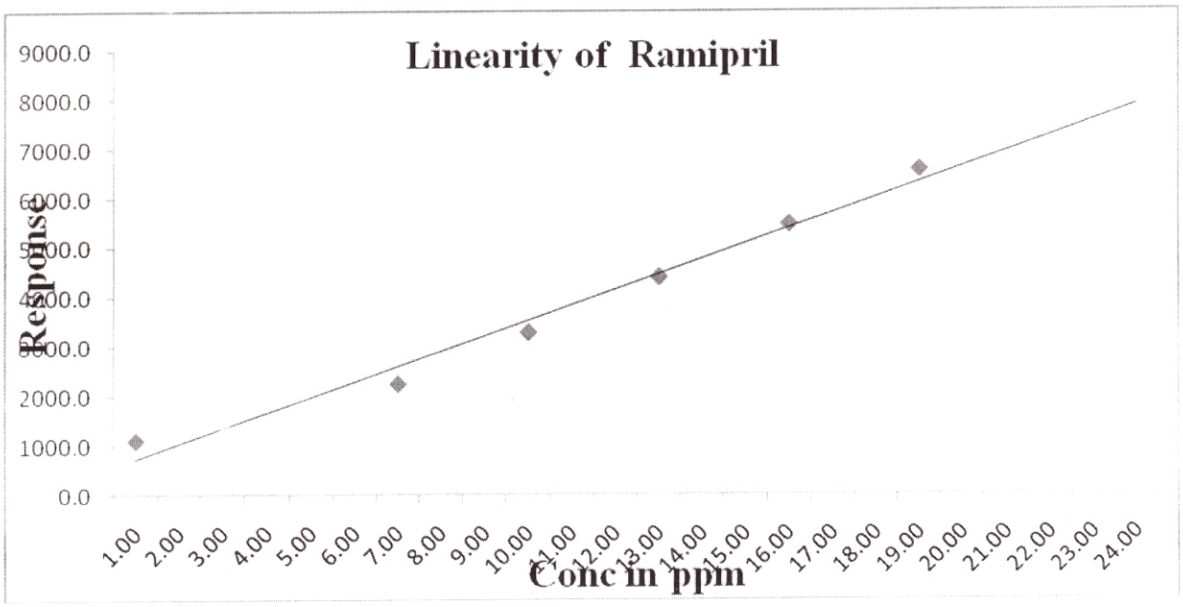
Slope	553.22
y-intercept	86.63
Correleation coefficient	1.0000

Standard details:

Injection No.	Retention Time	Area of Standard
1	4.300	4413.183
2	4.300	4391.896
3	4.300	4387.098
4	4.295	4383.716
5	4.295	4394.023
Average	4.298	4393.983
SD	0.003	11.47
%RSD	0.06	0.26
System suitability results		
% RSD of Area	0.26	NMT 2.0%
Tailing factor	1.02	NMT 2.0
Theoretical plates	5757	NLT 2000

Linearity for Ramipril:

Level	Linearity level (%)	ml of Standard Stock Solution added	Diluted to volume (mL)	Actual Concentration (ppm)	Area of sample	Average area
1	25	0.75	25	3.1	1106.260	1105.106
					1104.019	
					1104.517	
					1106.878	
					1103.886	
					1105.076	
2	50	1.50	25	6.1	2242.621	2238.404
					2235.066	
					2237.525	
3	75	2.25	25	9.2	3288.544	3279.522
					3276.074	
					3273.948	
4	100	3.00	25	12.3	4396.359	4393.220
					4380.366	
					4402.936	
5	125	3.75	25	15.3	5455.809	5458.614
					5461.386	
					5458.646	
6	150	4.50	25	18.4	6544.987	6566.147
					6568.676	
					6568.118	
					6565.379	
					6580.626	
					6569.096	



Slope	355.13
y-intercept	32.22
Correleation coefficient	1.0000

Conclusion:

1. The relative standard deviation for peak area of Ramipril and Hydrochlorothiazide of standard solution is within acceptance criteria.
2. The correlation coefficient obtained from the graph for Hydrochlorothiazide and Ramipril is 1.000.

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.

A) System Precision

Acceptance criteria:

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

Observations/Results:

Standard details (Ramipril 5 mg & Hydrochlorothiazide 12.5 mg & Ramipril 10 mg & Hydrochlorothiazide 25 mg)

Injection No.	Hydrochlorothiazide		Ramipril	
	Retention Time	Area of Standard	Retention Time	Area of Standard
1	2.169	8305.110	4.053	2238.676
2	2.165	8293.775	4.048	2233.925
3	2.165	8308.217	4.053	2235.174
4	2.165	8275.966	4.053	2224.596
5	2.169	8267.204	4.057	2227.548
Average	**2.167**	**8288.405**	**4.054**	**2231.669**
SD	**0.002**	**16.57**	**0.003**	**5.21**
%RSD	**0.10**	**0.20**	**0.08**	**0.23**

System suitability results		
% RSD of Ramipril	0.19	NMT 2.0%
% RSD of HCTZ	0.10	NMT 2.0%

Standard details (Ramipril 10 mg & Hydrochlorothiazide 12.5 mg)
Hydrochlorothiazide:

Injection No.	Hydrochlorothiazide		Ramipril	
	Retention Time	Area of Standard	Retention Time	Area of Standard
1	2.181	3997.841	4.071	5660.518
2	2.183	3996.372	4.072	5660.286
3	2.184	3999.992	4.070	5659.478
4	2.185	3997.232	4.071	5648.703
5	2.181	3994.448	4.071	5649.642
Bkt std	**2.183**	**3997.177**	**4.071**	**5655.725**
Average	**0.002**	**2.03**	**0.001**	**6.00**
SD	**0.08**	**0.05**	**0.02**	**0.11**
%RSD	**2.181**	**3997.841**	**4.071**	**5660.518**

System suitability results		
% RSD of Ramipril	0.11	NMT 2.0%
% RSD of HCTZ	0.05	NMT 2.0%

Conclusion:

Relative standard deviation for five replicate injections of standard solution is within acceptance criteria.

B) Method precision

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%.

Precision sample details (Ramipril 5 mg/Hydrochlorothiazide 12.5 mg):

Sample No.	Sample Weight (mg)	Area of Sample		% Release	
		Hydrochlorothiazide	Ramipril	Hydrochlorothiazide	Ramipril
1	195.75	9447.798	2366.451	99.2	95.4
2	195.21	9695.602	2264.471	101.8	91.3
3	197.28	9840.513	2443.340	103.3	98.5
4	194.84	9638.579	2332.180	101.2	94.0
5	197.65	9607.736	2313.664	100.9	93.3
6	201.33	9660.626	2281.731	101.4	92.0
			Average	101.3	94.1
			SD	1.3	2.6
			%RSD	1.3	2.8

Precision sample details (Ramipril 10 mg/Hydrochlorothiazide 12.5 mg):

Sample No.	Sample Weight (mg)	Area of Sample		% Release	
		Hydrochlorothiazide	Ramipril	Hydrochlorothiazide	Ramipril
1	194.35	4744.433	6247.954	103.9	100.6
2	194.00	4718.604	6213.371	103.3	100.1
3	198.70	4764.531	6356.222	104.3	102.4
4	194.53	4717.303	6301.435	103.3	101.5
5	198.98	4754.034	6300.724	104.1	101.5
6	195.94	4748.065	6274.220	104.0	101.0
			Average	**103.8**	**101.2**
			SD	**0.4**	**0.8**
			%RSD	**0.4**	**0.8**

Precision sample details (Ramipril 10 mg/Hydrochlorothiazide 25 mg):

Sample No.	Sample Weight (mg)	Area of Sample		% Release	
		Hydrochlorothiazide	Ramipril	Hydrochlorothiazide	Ramipril
1	198.94	19198.409	4609.030	101.7	94.1
2	195.86	18912.346	4668.720	100.2	95.3
3	192.33	18907.745	4611.656	100.2	94.2
4	195.52	19545.561	4897.433	103.6	100.0
5	198.97	18810.796	4524.970	99.7	92.4
6	196.19	19192.810	4728.588	101.7	96.6
			Average	**101.2**	**95.4**
			SD	**1.4**	**2.6**
			%RSD	**1.4**	**2.8**

Conclusion:

1. The relative standard deviation for peak area of Ramipril and Hydrochlorothiazide of standard solution is within acceptance criteria.
2. % Release of all six units is within acceptance criteria.
3. The relative standard deviation for % release of six units is within acceptance criteria.

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.

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 5.0%.

Standard Details (Ramipril 5 mg & Hydrochlorothiazide 12.5 mg)

Injection No.	Hydrochlorothiazide		Ramipril	
	Retention Time	Area of Standard	Retention Time	Area of Standard
1	2.181	3975.181	4.071	2865.259
2	2.184	3990.340	4.074	2888.802
3	2.184	4000.298	4.074	2900.784
4	2.184	3994.317	4.074	2896.432
5	2.184	4001.147	4.074	2883.238
Average	**2.183**	**3992.257**	**4.073**	**2886.903**
SD	**0.001**	**10.53**	**0.001**	**13.87**
%RSD	**0.06**	**0.26**	**0.03**	**0.48**

System Suitability Results		
% RSD of Ramipril	0.48	NMT 2.0%
% RSD of HCTZ	0.26	NMT 2.0%

Intermediate Precision Sample Details (Ramipril 5 mg & Hydrochlorothiazide 12.5 mg)

Sample No.	Sample Weight (mg)	Area of Sample		% Release	
		HCTZ	Ramipril	HCTZ	Ramipril
1	198.96	4754.696	3037.586	104.2	95.8
2	198.44	4827.260	3190.934	105.8	100.7
3	199.22	4931.611	3258.585	108.1	102.8
4	198.46	4733.245	3068.377	103.8	96.8
5	198.50	4815.842	3031.977	105.6	95.7
6	196.62	4750.895	3096.239	104.2	97.7
			Average	**105.3**	**98.2**
			SD	**1.6**	**2.9**
			%RSD	**1.5**	**2.9**

Standard Details (Ramipril 10 mg & Hydrochlorothiazide 25 mg)

Injection No.	Hydrochlorothiazide		Ramipril	
	Retention Time	Area of Standard	Retention Time	Area of Standard
1	2.181	8018.445	4.064	5671.704
2	2.182	8039.233	4.069	5666.834
3	2.182	8030.953	4.069	5685.921
4	2.182	8037.891	4.066	5676.504
5	2.181	8032.151	4.068	5670.239
Average	**2.182**	**8031.735**	**4.187**	**5674.240**
SD	**0.001**	**8.24**	**0.002**	**7.40**
%RSD	**0.03**	**0.10**	**0.05**	**0.13**

System Suitability Results		
% RSD of Ramipril	0.13	NMT 2.0%
% RSD of HCTZ	0.10	NMT 2.0%

Intermediate Precision Sample Details (Ramipril 10 mg & Hydrochlorothiazide 25 mg)

Sample No.	Sample Weight (mg)	Area of Sample		% Release	
		HCTZ	Ramipril	HCTZ	Ramipril
1	201.07	9646.399	6057.965	105.1	97.2
2	187.60	9248.398	5925.331	100.8	95.1
3	200.63	9429.118	5943.174	102.7	95.4
4	198.11	9322.973	5928.674	101.6	95.2
5	198.05	9347.578	5931.141	101.9	95.2
6	192..33	9361.114	5934.695	102.0	95.3
			Average	**102.4**	**95.6**
			SD	**1.5**	**0.8**
			%RSD	**1.5**	**0.9**

Standard Details (Ramipril 10 mg & Hydrochlorothiazide 12.5 mg)

Injection No.	Hydrochlorothiazide		Ramipril	
	Retention Time	Area of Standard	Retention Time	Area of Standard
1	3.937	4202.008	2.153	7871.488
2	3.937	4195.907	2.153	7874.765
3	3.933	4200.220	2.153	7888.353
4	3.937	4200.045	2.153	7887.365
5	3.933	4185.474	2.153	7860.188
Average	**3.937**	**4187.947**	**2.153**	**7876.432**
SD	**3.935**	**4193.919**	**2.153**	**7877.421**
%RSD	**0.00**	**6.86**	**0.00**	**11.44**

System Suitability Results		
% RSD of Ramipril	0.11	NMT 2.0%
% RSD of HCTZ	0.05	NMT 2.0%

Intermediate Precision Sample Details (Ramipril 10 mg & Hydrochlorothiazide 12.5 mg)

Sample No.	Sample Weight (mg)	Area of Sample		% Release	
		HCTZ	Ramipril	HCTZ	Ramipril
1	189.4	4514.7847	9278.3255	97.5	101.7
2	200.9	4530.8990	9561.7712	97.8	104.8
3	205.5	4708.3548	9543.7297	101.6	104.6
4	201.1	4621.9735	9390.8676	99.8	102.9
5	200.5	4694.1901	9575.7449	101.3	104.9
6	201.0	4719.9158	9581.1136	101.9	105.0
			Average	**189.4**	**4514.7847**
			SD	**200.9**	**4530.8990**
			%RSD	**205.5**	**4708.3548**

Comparison of Method Precision and Intermediate precision:

i) For Ramipril 5 mg & Hydrochlorothiazide 12.5 mg

Sample No.	Parameter	% Release	
		Hydrochlorothiazide	Ramipril
1	Method precision	99.2	95.4
2		101.8	91.3
3		103.3	98.5
4		101.2	94
5		100.9	93.3
6		101.4	92
1	Intermediate precision	104.2	95.8
2		105.8	100.7
3		108.1	102.8
4		103.8	96.8
5		105.6	95.7
6		104.2	97.7
	Mean	**103.3**	**96.2**
	SD	**2.51**	**3.41**
	%RSD	**2.43**	**3.54**

ii) For Ramipril 10 mg & Hydrochlorothiazide 12.5 mg

Sample No.	Parameter	% Release	
		Hydrochlorothiazide	Ramipril
1	Method precision	103.9	100.6
2		103.3	100.1
3		104.3	102.4
4		103.3	101.5
5		104.1	101.5
6		104	101
1	Intermediate precision	97.5	101.7
2		97.8	104.8
3		101.6	104.6
4		99.8	102.9
5		101.3	104.9
6		101.9	105
	Mean	**101.9**	**102.6**
	SD	**2.41**	**1.81**
	%RSD	**2.37**	**1.76**

iii) For Ramipril 10 mg & Hydrochlorothiazide 25 mg

Sample No.	Parameter	% Release	
		Hydrochlorothiazide	**Ramipril**
1	Method precision	101.7	94.1
2		100.2	95.3
3		100.2	94.2
4		103.6	100
5		99.7	92.4
6		101.7	96.6
1	Intermediate precision	105.1	97.2
2		100.8	95.1
3		102.7	95.4
4		101.6	95.2
5		101.9	95.2
6		102	95.3
	Mean	**101.8**	**95.5**
	SD	**1.52**	**1.86**
	%RSD	**1.50**	**1.95**

Conclusion:

1. The relative standard deviation for peak area of Ramipril and Hydrochlorothiazide of standard solution is less than 2.0%
2. % Release of all six units is more than 75% of labeled amount.
3. The relative standard deviation for % release of six units is less than 5.0%.
4. The difference in the mean value between the % release at method precision and intermediate precision is less than 5.0%.

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.

Acceptance criteria:

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

Calculation:

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

Accuracy for Ramipril 5 mg & Hydrochlorothiazide 12.5 mg/Ramipril 10 mg & Hydrochlorothiazide 25 mg

Standard Details: Hydrochlorothiazide

Sr.No.	RT	Area
1	2.232	17285.375
2	2.228	17282.203
3	2.228	17295.827
4	2.230	17285.744
5	2.232	17282.372
Average	2.230	17286.304
SD	0.00	5.57
%RSD	0.09	0.03
System Suitability Results		
% RSD of Area	0.03	NMT 2.0%
Tailing factor	1.17	NMT 2.0%
Theoretical plates	4075	NLT 2000

Accuracy of Hydrochlorothiazide:

Accuracy Level (%)	Placebo Weight (mg)	mL of Std Added	mL of Volume	Amount Added (ppm)	Area	Amount Recovered (ppm)	% Recovery	Mean % Recovery	% RSD
25	165.07	1.2	750	6.9	4312.542	7.18	103.75	103.7	0.01
	166.34	1.2	750	6.9	4311.647	7.18	103.73		
	166.60	1.2	750	6.9	4311.853	7.18	103.73		
50	168.66	2.5	750	14.4	8853.362	14.75	102.24	102.2	0.03
	167.30	2.5	750	14.4	8854.541	14.75	102.25		
	166.96	2.5	750	14.4	8850.059	14.74	102.20		
100	167.09	5.0	750	28.8	17595.550	29.31	101.60	101.5	0.14
	166.57	5.0	750	28.8	17547.427	29.23	101.32		
	167.06	5.0	750	28.8	17569.828	29.26	101.45		
150	165.74	7.5	750	43.3	25971.796	43.26	99.97	99.9	0.09
	166.21	7.5	750	43.3	25966.558	43.25	99.95		
	165.37	7.5	750	43.3	25930.706	43.19	99.81		
							Mean	101.2	
							SD	1.18	
							% RSD	1.16	

Accuracy of Ramipril:

Accuracy Level (%)	Placebo Weight (mg)	mL of Std Added	mL of Volume	Amount Added (ppm)	Area	Amount Recovered (ppm)	% Recovery	Mean % Recovery	% RSD
25	165.07	1.2	750	3.0	1046.315	2.93	98.5	97.8	0.77
	166.34	1.2	750	3.0	1039.863	2.91	97.9		
	166.60	1.2	750	3.0	1030.405	2.89	97.0		
50	168.66	2.5	750	6.2	2235.645	6.27	101.0	101.0	0.19
	167.30	2.5	750	6.2	2238.134	6.27	101.1		
	166.96	2.5	750	6.2	2229.837	6.25	100.8		
100	167.09	5.0	750	12.4	4477.165	12.55	101.2	100.4	0.96
	166.57	5.0	750	12.4	4456.113	12.49	100.7		
	167.06	5.0	750	12.4	4394.682	12.32	99.3		
150	165.74	7.5	750	18.6	6506.724	18.23	98.0	98.6	0.53
	166.21	7.5	750	18.6	6571.839	18.42	99.0		
	165.37	7.5	750	18.6	6560.354	18.38	98.8		
							Mean	100.0	
							SD	1.23	
							% RSD	1.23	

Accuracy for Ramipril 10 mg & Hydrochlorothiazide 12.5 mg

Standard Details: Hydrochlorothiazide

Sr. No.	RT	Area
1	2.233	8705.757
2	2.233	8705.898
3	2.232	8706.480
4	2.232	8713.060
5	2.230	8708.535
Average	2.232	8707.946
SD	0.001	3.07
%RSD	0.05	0.04
System Suitability Results		
% RSD of Area	0.04	NMT 2.0%
Tailing Factor	1.15	NMT 2.0%
Theoretical Plates	4160	NLT 2000

Accuracy of Hydrochlorothiazide:

Accuracy Level (%)	Placebo Weight (mg)	mL of Std Added	mL of Volume	Amount Added (ppm)	Area	Amount Recovered (ppm)	% Recovery	Mean % Recovery	% RSD
50	165.68	1.2	750	6.92	4212.510	6.96	100.6	100.6	0.04
	166.18	1.2	750	6.92	4212.513	6.96	100.6		
	166.52	1.2	750	6.92	4209.564	6.96	100.5		
100	165.80	2.5	750	14.42	8787.277	14.53	100.7	100.8	0.09
	166.30	2.5	750	14.42	8784.564	14.52	100.7		
	166.78	2.5	750	14.42	8798.959	14.55	100.9		
150	166.99	3.8	750	21.92	13273.398	21.94	100.1	100.0	0.07
	166.59	3.8	750	21.92	13266.470	21.93	100.0		
	166.57	3.8	750	21.92	13253.804	21.91	99.9		
							Mean	100.0	
							SD	1.23	
							% RSD	1.23	

Accuracy of Ramipril:

Accuracy Level (%)	Placebo Weight (mg)	mL of Std Added	mL of Volume	Amount Added (ppm)	Area	Amount Recovered (ppm)	% Recovery	Mean % Recovery	% RSD
50	165.68	2.5	750	6.20	2148.699	6.02	97.1	97.3	0.17
	166.18	2.5	750	6.20	2155.278	6.04	97.4		
	166.52	2.5	750	6.20	2154.722	6.04	97.4		
100	165.80	5.0	750	12.40	4393.667	12.32	99.3	99.0	0.82
	166.30	5.0	750	12.40	4339.654	12.17	98.1		
	166.78	5.0	750	12.40	4407.523	12.36	99.6		
150	166.99	7.5	750	18.61	6466.588	18.13	97.4	98.1	0.60
	166.59	7.5	750	18.61	6538.149	18.33	98.5		
	166.57	7.5	750	18.61	6530.345	18.31	98.4		
							Mean	98.1	
							SD	0.84	
							% RSD	0.86	

Conclusion:

% Recovery at each level is between 95.0 to 105.0%

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.

Acceptance criteria:

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

Hydrochlorothiazide:

Robustness Parameter	% Release	% Difference (NMT 2.0 %)
Precision	100.8	NA
Flow Rate 0.9 mL/min	101.8	1.0
Flow Rate 1.1 mL/min	101.9	1.1
Column temperature 23	102.0	1.2
Column temperature 27	101.8	1.0
Buffer pH 2.3	101.3	0.5
Buffer pH 2.7	100.4	0.3
Low RPM	100.2	0.6
High RPM	100.1	0.7
Low Medium Volume	100.7	0.1
High Medium Volume	98.4	2.4

Ramipril:

Robustness Parameter	% Release	% Difference (NMT 2.0 %)
Precision	99.0	NA
Flow Rate 0.9 mL/min	100.2	1.2
Flow Rate 1.1 mL/min	100.4	1.4
Column temperature 23	99.1	0.1
Column temperature 27	99.4	0.4
Buffer pH 2.3	100.0	1.0
Buffer pH 2.7	98.2	0.6
Low RPM	99.6	0.6
High RPM	100.1	1.1
Low Medium Volume	97.2	1.8
High Medium Volume	96.7	2.3

Conclusion:

The difference in the mean value between the dissolution results at method precision and robustness is less than 5.0%.

10.7. 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.

Acceptance criteria:

Standard Solution Stability:

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

Sample Solution Stability:

1. 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%.

Standard Solution Stability:

Time (hr)	Area of Standard
Initial	4188.6651
5 hrs	4127.1644
6 hrs	4127.2818
12 hrs	4170.3743
15 hrs	4149.0033
18 hrs	4131.2397
21 hrs	4148.5751
25 hrs	4155.0618
Average	4149.6707
SD	21.80
%RSD	0.53

Sample Solution Stability:

Sample No.	Area of Sample	% Release	Difference (NMT 2.0%)
Initial	4399.9892	96.0	**NA**
4 hrs	4389.6772	95.8	0.23
5 hrs	4408.6566	96.2	0.20
11 hrs	4435.2660	96.8	0.80
14 hrs	4423.2373	96.5	0.53
17 hrs	4375.5912	95.5	0.56
20 hrs	4410.6242	96.2	0.24
24 hrs	4377.4795	95.5	0.51

Conclusion:

Standard solution stability:

The relative standard deviation of area of repeatedly determined standard solution over a period of 24 hrs at room temperature is less 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 less than 2.0%.

11. DOCUMENTATION

All validation activities were recorded online in to the laboratory notebook. At the end of the Validation, the report is generated. All results were be discussed, conclusions drawn and no deviations from the protocol observed.

12. REFERENCES

ICH Guidelines

13. REVISION HISTORY

Version	Date	Changes	Reason for change
0		Not Applicable	New