

QbD RESEARCH AND DEVELOPMENT LAB PVT. LTD.

	ANALYTICAL METHOD VALIDATION REPORT FOR ASSAY AND UNIFORMITY OF CONTENT OF RAMITHIAZIDE (RAMIPRIL AND HYDROCHLOROTHIAZIDE TABLETS) BY HPLC METHOD		
	Report Number	QBD-REP-332	Page No.

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
Sachin Pujari	Manager Analytical (QbD research & Development Lab)		10-Aug-17
Reviewed By	Designation	Signature	Date
Satish Sawant	GM Analytical (QbD research & Development Lab)		10-Aug-17
Approved By	Designation	Signature	Date
Nargis penkar	QA Manager (QbD research & Development Lab)		10-Aug-17
Approved By	Designation	Signature	Date
prabhakar Raut	For (Generic Healthcare Pvt. Ltd.)		10.08.2017

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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

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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 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	RL-14203
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	-

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7. REAGENTS AND CHEMICALS

Reagent	Grade	Manufacturer
Sodium Perchlorate	AR	Finar
Acetonitrile	HPLC	Finar
Water	MilliQ	Inhouse

8. INSTRUMENT DETAILS

Instrument/ Equipment	Supplier/ Manufacturer / Make	Instrument/ Equipment In- House No.	Calibration Due Date
HPLC	Agilent	QbD-INST-HPLC-51	AUG-17
HPLC	Thermo	QbD-INST-HPLC-67	DEC-17
HPLC	Shimadzu	QbD-INST-HPLC-42	SEP-17
HPLC	Agilent	QbD-INST-HPLC-04	JAN-18
HPLC	Agilent	QbD-INST-HPLC-05	JAN-18
Dissolution Tester	Labindia	QbD-INST-DISS-09	OCT-17
Dissolution Tester	Labindia	QbD-INST-DISS-10	OCT-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 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 volumes of water, 45 volumes of Acetonitrile and 0.1 volume of triethylamine. Adjust pH 3.0 with dilute phosphoric acid.

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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).


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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 μ L 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.

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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.

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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

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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

$$\begin{aligned} \text{\% Content of Ramipril} &= \frac{\text{AT}}{\text{AS}} \times \frac{\text{WS}}{25} \times \frac{2}{100} \times \frac{250}{1} \times \frac{\text{P}}{\text{LC}} \\ \text{\% Content of HCTZ} &= \frac{\text{AT1}}{\text{AS1}} \times \frac{\text{WS1}}{25} \times \frac{5}{100} \times \frac{250}{1} \times \frac{\text{P1}}{\text{LC1}} \end{aligned}$$

For Ramithiazide Tablets 10 mg/12.5 mg

$$\begin{aligned} \text{\% Content of Ramipril} &= \frac{\text{AT}}{\text{AS}} \times \frac{\text{WS}}{25} \times \frac{2}{100} \times \frac{250}{1} \times \frac{10}{5} \times \frac{\text{P}}{\text{LC}} \\ \text{\% Content of HCTZ} &= \frac{\text{AT1}}{\text{AS1}} \times \frac{\text{WS1}}{25} \times \frac{2.5}{100} \times \frac{250}{1} \times \frac{10}{5} \times \frac{\text{P1}}{\text{LC1}} \end{aligned}$$

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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

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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

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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. 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.

Observations/Results:

Standard Details:

Injection No.	Retention Time	Area of Hydrochlorothiazide Standard	Retention Time	Area of Ramipril Standard
1	2.10	27140.044	3.65	6928.843
2	2.10	27182.012	3.65	6943.844
3	2.10	27163.638	3.65	6935.208
4	2.10	27160.989	3.65	6940.622
5	2.10	27129.962	3.65	6923.226
6	2.10	27176.337	3.65	6945.313
Average	2.10	27158.830	3.65	6936.176
SD	0.000	20.29	0.000	8.77
%RSD	0.00	0.07	0.00	0.13
System Suitability Results				
% RSD of Area of Hydrochlorothiazide			0.07	NMT 2.0%

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% RSD of Area of Ramipril	0.13	NMT 2.0%
Theoretical plates of Ramipril	10410	NMT 2.0
Theoretical plates of Hydrochlorothiazide	10705	NMT 2.0
Tailing factor of Ramipril	1.00	NLT 2000
Tailing factor of Hydrochlorothiazide	1.04	NLT 2000

Interference Details:

Sample	Sample Weight (mg)	Retention Time (min)	Area of Hydrochlorothiazide	Retention Time (min)	Area of Ramipril
Blank Solution	NA	Not detected	Not detected	Not detected	Not detected
Standard Solution	Ramipril 25.9mg and 25.0mg of Hydrochlorothiazide	2.10	27140.044	3.65	6928.843
Ramipril Identification Solution	25.9mg	3.65	6928.843	3.65	6910.967
Hydrochlorothiazide Identification Solution	25.0mg	2.10	26857.995	Not detected	Not detected
Placebo Solution	Not detected	Not detected	Not detected	Not detected	Not detected
Sample Solution	486.6	2.10	26811.141	3.65	6690.018

Conclusion:

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

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10.2. SPECIFICITY BY FORCED DEGRADATION:**Observations/Results:****Standard Details:**

Injection No.	Retention Time	Area of Hydrochlorothiazide Standard	Retention Time	Area of Ramipril Standard
1	2.15	26533.941	4.15	7131.027
2	2.14	26555.379	4.15	7078.064
3	2.14	26620.305	4.15	7124.869
4	2.14	26611.268	4.15	7131.529
5	3.31	26610.794	5.32	7109.973
6	2.14	26650.629	4.15	7131.090
Average	2.34	26597.053	4.34	7117.759
SD	0.48	43.64	0.48	21.12
%RSD	20.42	0.16	10.99	0.30
System Suitability Results				
% RSD of Area of Hydrochlorothiazide			0.16	NMT 2.0%
% RSD of Area of Ramipril			0.30	NMT 2.0%
Tailing factor of Hydrochlorothiazide			1.06	NMT 2.0
Tailing factor of Ramipril			1.09	NMT 2.0
Theoretical plates of Hydrochlorothiazide			5235	NLT 2000
Theoretical plates of Ramipril			6328	NLT 2000

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Sample Degradation Details (For Hydrochlorothiazide):**Observations/Results:**

Sample Type	Sample	Area of	% Assay	%	Peak
Untreated sample	494.71	26437.902	100.6	NA	999
Acid treated sample (5 mL of 1N HCl, 20 min at 70°C)	493.79	26539.404	101.2	-0.6	999
Base treated sample (5 mL of 1N NaOH, 20 min at 70°C)	493.79	26555.589	101.2	-0.6	999
Hydrogen peroxide treated sample (5 mL of 30% H ₂ O ₂ ,	494.31	26541.634	101.1	-0.5	999
Acid treated sample (2 mL of 1N HCl, 20 min at 70°C)	493.96	26502.271	101.0	-0.4	999
Base treated sample (2 mL of 1N NaOH, 20 min at 70°C)	494.26	26475.967	100.8	-0.2	999
Sample solution exposed to Heat	493.70	26918.472	102.6	-2.0	999

Sample Degradation Details (For Ramipril):

Sample Type	Sample Weight (mg)	Area of Sample	% Assay	% Degradation	Peak purity Match
Untreated sample	494.71	6990.951	99.3	NA	999
Acid treated sample (5 mL of 1N HCl, 20 min at 70°C)	493.79	6975.559	99.3	0.0	999
Base treated sample (5 mL of 1N NaOH, 20 min at	493.79	6896.102	98.2	1.1	999
Hydrogen peroxide treated sample (5 mL of	494.31	6921.174	98.4	0.9	999
Acid treated sample (2 mL of 1N HCl, 20 min at 70°C)	493.96	6963.870	99.1	0.2	999
Base treated sample (2 mL of 1N NaOH, 20 min at	494.26	6918.869	98.4	0.9	999
Sample solution exposed to Heat	493.70	7082.866	100.8	-1.5	999

Conclusion:

From the above data it is observed that product is not degraded in above conditions.

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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.

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

Observations/Results:

Standard Details:

Injection No.	Retention Time	Area of Hydrochlorothiazide Standard	Retention Time	Area of Ramipril Standard
1	2.113	27192.801	3.753	7007.386
2	2.113	27239.583	3.753	7018.219
3	2.117	27322.059	3.753	7031.934
4	2.117	27238.009	3.757	7008.798
5	2.117	27175.641	3.757	6989.347
6	2.113	27184.405	3.753	6992.041
Average	2.115	27225.416	3.754	7007.954
SD	0.00	54.63	0.00	16.01
%RSD	0.10	0.20	0.06	0.23
System Suitability Results				
% RSD of Area of Hydrochlorothiazide			0.20	NMT 2.0%
% RSD of Area of Ramipril			0.23	NMT 2.0%

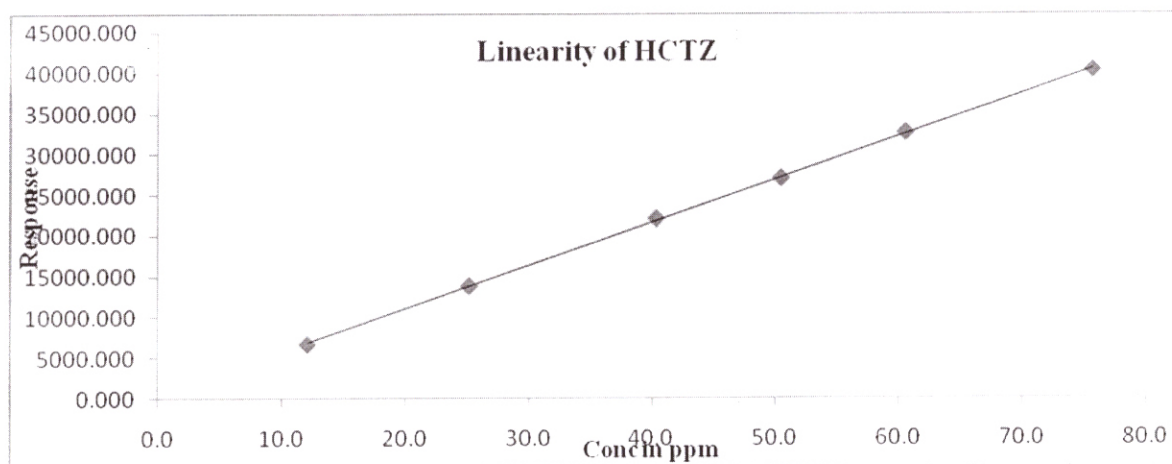
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Tailing factor of Hydrochlorothiazide	1.09	NMT 2.0
Tailing factor of Ramipril	1.00	NMT 2.0
Theoretical plates of Hydrochlorothiazide	10362	NLT 2000
Theoretical plates of Ramipril	9677	NLT 2000

Linearity Determination 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	1.2	100	12.1	6759.543	6761.625
					6742.148	
					6761.846	
					6740.715	
					6798.745	
2	50	2.5	100	25.3	6766.757	13905.135
					13907.523	
					13902.865	
3	75	4.0	100	40.4	13905.017	22179.585
					22197.151	
					22158.106	
4	100	5.0	100	50.5	22183.498	27122.050
					27155.943	
					27084.756	
5	125	6.0	100	60.6	27125.449	32723.721
					32725.509	
					32723.357	
6	150	7.5	100	75.8	32722.296	40390.549
					40376.322	
					40480.045	
					40458.462	
					40360.603	
					40379.248	
					40288.613	

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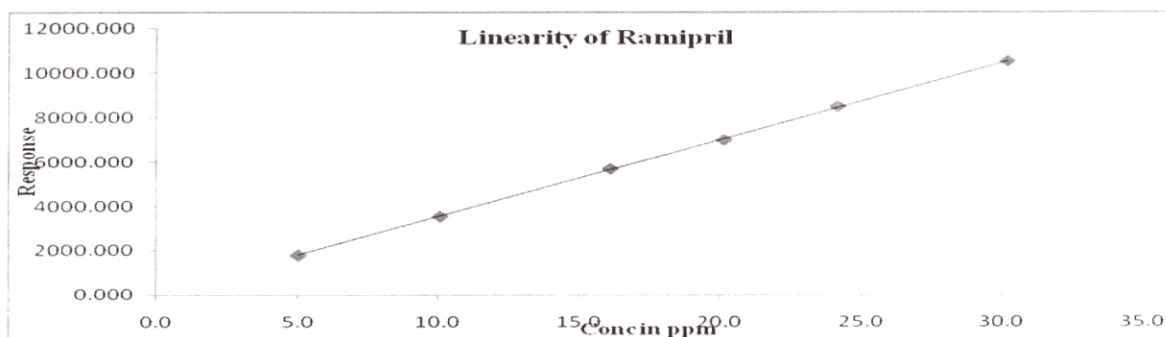
Slope	528.69
y-Intercept	530.01
Correlation Coefficient	0.9999

Linearity Determination 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	0.5	100	5.0	1793.527	1789.150
					1792.789	
					1785.713	
					1780.852	
					1795.589	
					1786.431	
2	50	1.0	100	10.1	3531.387	3531.410
					3535.378	
					3527.465	
3	75	1.6	100	16.1	5672.921	5674.802
					5674.689	
					5676.795	
4	100	2.0	100	20.1	6998.638	6984.227
					6976.426	
					6977.616	

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5	125	2.4	100	24.2	8484.320	8483.543
					8476.854	
					8489.456	
6	150	3.0	100	30.2	10516.722	10517.807
					10531.706	
					10542.009	
					10499.757	
					10505.742	
					10510.904	



Slope	347.37
y-Intercept	43.12
Correlation Coefficient	0.9999

Conclusion:

1. The relative standard deviation for peak area of Ramipril and Hydrochlorothiazide of standard solution is within acceptance criteria.
2. The tailing factor for Ramipril and Hydrochlorothiazide peak in standard solution is within acceptance criteria.
3. The theoretical plates for Ramipril and Hydrochlorothiazide peak in standard solution is within acceptance criteria.
4. The correlation coefficient obtained from the graph for Hydrochlorothiazide is 0.9999 and for Ramipril is 0.9999.

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10.4. PRECISION

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

A) System Precision

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

Observations/Results:

Standard Details (Ramipril 5 mg & Hydrochlorothiazide 12.5 mg & Ramipril 5 mg & Hydrochlorothiazide 12.5 mg)

Injection No.	Retention Time	Area of Hydrochlorothiazide Standard	Retention Time	Area of Ramipril Standard
1	2.10	27140.044	3.65	6928.843
2	2.10	27182.012	3.65	6943.844
3	2.10	27163.638	3.65	6935.208
4	2.10	27160.989	3.65	6940.622
5	2.10	27129.962	3.65	6923.226
6	2.10	27176.338	3.65	6945.313
Average	2.10	27158.830	3.65	6936.176
SD	0.000	20.29	0.000	8.77
%RSD	0.00	0.07	0.00	0.13
System Suitability Results				
% RSD of Ramipril			0.13	NMT 2.0%
% RSD of Hydrochlorothiazide			0.07	NMT 2.0%
Theoretical plates of Ramipril			10410	NMT 2.0
Theoretical plates of Hydrochlorothiazide			10705	NMT 2.0
Tailing factor of Ramipril			1.00	NLT 2000
Tailing factor of Hydrochlorothiazide			1.04	NLT 2000

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Standard Details (Ramipril 10 mg & Hydrochlorothiazide 12.5 mg)

Injection No.	Retention Time	Area of Hydrochlorothiazide Standard	Retention Time	Area of Ramipril Standard
1	2.10	14123.816	3.65	7005.022
2	2.10	14086.982	3.65	6985.895
3	2.10	14137.983	3.65	7006.536
4	2.10	14224.821	3.65	7039.733
5	2.10	14154.966	3.65	7024.769
6	2.10	14132.359	3.65	6996.007
Average	2.10	14143.488	3.65	7009.660
SD	0.000	45.78	0.000	19.57
%RSD	0.00	0.32	0.00	0.28
System Suitability Results				
% RSD of Area of Hydrochlorothiazide			0.32	NMT 2.0%
% RSD of Area of Ramipril			0.28	NMT 2.0%
Tailing factor of Hydrochlorothiazide			10768	NMT 2.0
Tailing factor of Ramipril			10338	NMT 2.0
Theoretical plates of Hydrochlorothiazide			1.06	NLT 2000
Theoretical plates of Ramipril			0.98	NLT 2000

Conclusion:

1. The relative standard deviation for peak area of Ramipril and Hydrochlorothiazide of standard solution is within acceptance criteria.
2. The tailing factor for Ramipril and Hydrochlorothiazide peak in standard solution is within acceptance criteria.
3. The theoretical plates for Ramipril and Hydrochlorothiazide peak in standard solution is within acceptance criteria.

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.

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I) Method Precision For Assay:
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%

i) Precision Sample Details (Ramipril 5 mg & Hydrochlorothiazide 12.5 mg):

Sample No.	Sample Weight (mg)	Area of Sample		% Assay	
		Hydrochlorothiazide	Ramipril	Hydrochlorothiazide	Ramipril
1	975.1	26742.050	6512.104	100.8	99.5
2	975.0	26540.983	6430.609	100.0	98.2
3	974.5	26760.319	6552.363	100.9	100.1
4	974.2	26787.668	6502.765	101.1	99.4
5	975.1	26969.459	6559.458	101.7	100.2
6	974.3	26818.478	6507.422	101.2	99.5
		Mean		100.9	99.5
		SD		0.53	0.71
		%RSD		0.52	0.71

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ii) Precision Sample Details (Ramipril 10 mg & Hydrochlorothiazide 12.5 mg):

Sample No.	Sample Weight (mg)	Area of Sample		% Assay	
		Hydrochlorothiazide	Ramipril	Hydrochlorothiazide	Ramipril
1	498.5	13979.161	6902.898	98.2	101.3
2	525.1	14777.095	7296.229	98.6	101.6
3	508.5	14296.125	7057.916	98.5	101.5
4	502.1	14110.117	6959.222	98.4	101.4
5	501.3	14161.119	6957.830	98.9	101.5
6	496.7	13971.434	6884.103	98.5	101.4
		Average		98.5	101.5
		SD		0.24	0.13
		%RSD		0.24	0.13

iii) Precision Sample Details (Ramipril 10 mg & Hydrochlorothiazide 25 mg):

Sample No.	Sample Weight (mg)	Area of Sample		% Assay	
		Hydrochlorothiazide	Ramipril	Hydrochlorothiazide	Ramipril
1	486.6	26811.141	6690.018	99.4	100.5
2	483.8	26836.422	6700.093	100.1	101.2
3	494.5	27483.766	6868.453	100.3	101.5
4	491.9	26804.727	6712.113	98.3	99.7
5	497.8	27713.304	6920.976	100.4	101.6
6	489.2	26963.565	6753.191	99.4	100.9
		Average		99.6	100.9
		SD		0.79	0.71
		%RSD		0.79	0.71

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Conclusion:

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 is within acceptance criteria.
3. The theoretical plates for Ramipril and Hydrochlorothiazide peak in standard solution is within acceptance criteria.
4. % Assay value of Ramipril and Hydrochlorothiazide for sample solution is within acceptance criteria.
5. % RSD of Assay value of Ramipril and Hydrochlorothiazide for six sample solution is within acceptance criteria.

II) Method Precision for Content Uniformity:**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%


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

Injection No.	Retention Time	Area of Hydrochlorothiazide Standard	Retention Time	Area of Ramipril Standard
1	2.11	26693.654	3.75	6998.101
2	2.11	26623.399	3.75	6978.537
3	2.11	26584.575	3.75	6964.311
4	2.11	26571.035	3.74	6962.451
5	2.11	26622.841	3.74	6983.342
6	2.11	26563.407	3.74	6955.304
Average	2.11	26609.819	3.74	6973.674
SD	0.00	48.33	0.01	15.91
%RSD	0.00	0.18	0.14	0.23
System Suitability Results				
% RSD of Area of Hydrochlorothiazide			0.18	NMT 2.0%
% RSD of Area of Ramipril			0.23	NMT 2.0%
Tailing factor of Hydrochlorothiazide			1.08	NMT 2.0
Tailing factor of Ramipril			1.03	NMT 2.0
Theoretical plates of Hydrochlorothiazide			10583	NLT 2000
Theoretical plates of Ramipril			11081	NLT 2000

Standard Details (Ramipril 10 mg & Hydrochlorothiazide 12.5 mg)

Injection No.	Retention Time	Area of Hydrochlorothiazide Standard	Retention Time	Area of Ramipril Standard
1	2.11	13589.204	3.73	7021.068
2	2.11	13576.131	3.73	7015.366
3	2.11	13614.588	3.73	7032.524
4	2.11	13634.116	3.73	7052.893
5	2.11	13630.656	3.73	7047.321
6	2.11	13610.292	3.73	7014.270
Average	2.11	13609.164	3.73	7030.573
SD	0.00	22.82	0.00	16.55
%RSD	0.00	0.17	0.00	0.24
System Suitability Results				
% RSD of Area of Hydrochlorothiazide			0.24	NMT 2.0%
% RSD of Area of Ramipril			0.17	NMT 2.0%
Tailing factor of Hydrochlorothiazide			1.14	NMT 2.0
Tailing factor of Ramipril			1.04	NMT 2.0
Theoretical plates of Hydrochlorothiazide			10841	NLT 2000
Theoretical plates of Ramipril			11040	NLT 2000


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**i) Precision sample details for Hydrochlorothiazide(Ramipril 5mg & Hydrochlorothiazide
12.5mg):**

Sample	Tablets Weight (mg)	Peak Area of Sample Hydrochlorothiazide	% Content
1	920.47	27629.543	103.9
2	926.28	27305.449	102.7
3	905.59	27466.866	103.3
4	920.14	27673.941	104.1
5	930.56	27967.369	105.2
6	904.67	27599.831	103.8
7	912.88	27678.173	104.1
8	927.36	27388.930	103.0
9	892.39	27595.452	103.8
10	895.88	27537.136	103.6
		Min	102.7
		Max	105.2
		Mean	103.8
		SD	0.68
		% RSD	0.66
		AV Value	3.9

ii) Precision sample details for Ramipril (Ramipril 5 mg & Hydrochlorothiazide 12.5 mg):

Sample	Tablets Weight (mg)	Peak Area of Sample Ramipril	% Content
1	920.47	6757.220	100.0
2	926.28	6609.463	97.8
3	905.59	6679.661	98.8
4	920.14	6508.468	96.3
5	930.56	6675.710	98.8
6	904.67	6809.909	100.8
7	912.88	6762.906	100.1
8	927.36	6639.258	98.2
9	892.39	6503.552	96.2
10	895.88	6475.370	95.8
		Min	95.8
		Max	100.8
		Mean	98.3
		SD	1.74
		% RSD	1.77
		AV Value	1.8


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iii) Precision Sample Details for Hydrochlorothiazide (Ramipril 10 mg & Hydrochlorothiazide 12.5 mg):

Sample	Tablets Weight (mg)	Peak Area of Sample	% Content
1	920.47	13862.943	102.0
2	926.28	14268.605	105.0
3	905.59	14036.682	103.2
4	920.14	14088.776	103.6
5	930.56	14075.274	103.5
6	904.67	14061.901	103.4
7	912.88	14062.203	103.4
8	927.36	14006.217	103.0
9	892.39	14186.247	104.3
10	895.88	13900.783	102.2
		Min	102.0
		Max	105.0
		Mean	103.4
		SD	0.88
		% RSD	0.85
		AV Value	3.7

iv) Precision Sample Details for Ramipril (Ramipril 10 mg & Hydrochlorothiazide 12.5 mg):

Sample	Tablets Weight (mg)	Peak Area of Sample	% Content
1	920.47	6934.208	101.8
2	926.28	6830.801	100.3
3	905.59	6903.521	101.3
4	920.14	6837.118	100.4
5	930.56	6885.831	101.1
6	904.67	6841.661	100.4
7	912.88	6953.359	102.1
8	927.36	6896.770	101.2
9	892.39	6814.032	100.0
10	895.88	6888.926	101.1
		Min	100.0
		Max	102.1
		Mean	101.0
		SD	0.68
		% RSD	0.67
		AV Value	1.6


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v) Precision sample details for Hydrochlorothiazide (Ramipril 10 mg & Hydrochlorothiazide 25 mg):

Sample	Tablets Weight (mg)	Peak Area of Sample	% Content
1	920.47	26198.918	98.6
2	926.28	27036.530	101.7
3	905.59	27303.232	102.7
4	920.14	27055.505	101.8
5	930.56	27397.083	103.1
6	904.67	27495.587	103.4
7	912.88	27296.913	102.7
8	927.36	27487.205	103.4
9	892.39	27138.350	102.1
10	895.88	27202.516	102.3
		Min	98.6
		Max	103.4
		Mean	102.2
		SD	1.41
		% RSD	1.38
		AV Value	1.6

vi) Precision sample details for Ramipril (Ramipril 10 mg & Hydrochlorothiazide 25.5 mg):

Sample	Tablets Weight (mg)	Peak Area of Sample	% Content
1	920.47	6248.850	92.5
2	926.28	6572.144	97.3
3	905.59	6775.708	100.3
4	920.14	6445.797	95.4
5	930.56	6596.430	97.6
6	904.67	6675.208	98.8
7	912.88	6676.491	98.8
8	927.36	6699.905	99.1
9	892.39	6531.341	96.6
10	895.88	6570.784	97.2
		Min	92.47
		Max	100.26
		Mean	97.4
		SD	2.21
		% RSD	2.27
		AV Value	7.5

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Conclusion:

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 is within acceptance criteria.
3. The theoretical plates for Ramipril and Hydrochlorothiazide peak in standard solution is within acceptance criteria.
4. The acceptance value of Ramipril and Hydrochlorothiazide for ten sample solution is within acceptance criteria.

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:**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%


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Observations/Results:

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

Injection No.	Retention Time	Area of Hydrochlorothiazide	Retention Time	Area of Ramipril Standard
1	2.225	29779.744	3.882	6982.083
2	2.225	29910.378	3.883	6990.418
3	2.227	29895.007	3.885	6982.654
4	2.227	29870.134	3.885	6978.374
5	2.225	29937.184	3.883	6982.466
6	2.225	29970.544	3.883	6990.327
Average	2.226	29893.832	3.884	6984.387
SD	0.001	65.75	0.001	4.89
%RSD	0.05	0.22	0.03	0.07
System Suitability Results				
% RSD of Area of Hydrochlorothiazide			0.22	NMT 2.0%
% RSD of Area of Ramipril			0.07	NMT 2.0%
Tailing factor of Hydrochlorothiazide			6040	NMT 2.0
Tailing factor of Ramipril			8584	NMT 2.0
Theoretical plates of Hydrochlorothiazide			1.15	NLT 2000
Theoretical plates of Ramipril			1.06	NLT 2000

Standard details (Ramipril 10 mg & Hydrochlorothiazide 12.5 mg)

Injection	Retention	Area of	Retention	Area of Ramipril
1	2.223	15522.964	3.882	7108.906
2	2.225	15529.755	3.882	7106.701
3	2.225	15525.629	3.883	7101.858
4	2.223	15531.716	3.882	7102.031
5	2.223	15526.224	3.882	7098.623
6	2.223	15534.615	3.882	7101.488
Average	2.224	15528.484	3.882	7103.268
SD	0.001	4.32	0.000	3.79
%RSD	0.05	0.03	0.01	0.05
System Suitability Results				
% RSD of Area of Hydrochlorothiazide			0.03	NMT 2.0%
% RSD of Area of Ramipril			0.05	NMT 2.0%
Tailing factor of Hydrochlorothiazide			5865	NMT 2.0
Tailing factor of Ramipril			8371	NMT 2.0
Theoretical plates of Hydrochlorothiazide			1.13	NLT 2000
Theoretical plates of Ramipril			1.06	NLT 2000

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i) Intermediate precision sample details (Ramipril 5 mg & Hydrochlorothiazide 12.5 mg):

Sample No.	Sample Weight (mg)	Area of Sample		% Assay	
		Hydrochlorothiazide	Ramipril	Hydrochlorothiazide	Ramipril
1	995.72	29565.134	7130.849	99.8	100.4
2	994.71	29626.454	7135.219	100.1	100.5
3	995.09	29548.641	7138.142	99.8	100.5
4	995.33	29467.116	7116.769	99.5	100.2
5	995.73	29706.000	7160.037	100.3	100.8
6	995.17	29503.873	7124.497	99.6	100.3
		Mean		99.8	100.5
		SD		0.29	0.20
		%RSD		0.29	0.20

ii) Intermediate precision sample details (Ramipril 10 mg & Hydrochlorothiazide 12.5 mg):

Sample No.	Sample Weight (mg)	Area of Sample		% Assay	
		Hydrochlorothiazide	Ramipril	Hydrochlorothiazide	Ramipril
1	494.49	15452.158	7121.486	100.3	98.5
2	494.59	15470.739	7129.698	100.4	98.6
3	494.95	15490.150	7139.473	100.5	98.7
4	495.81	15432.785	7115.849	99.9	98.2
5	495.66	15588.560	7154.534	101.0	98.7
6	494.98	15574.824	7135.676	101.0	98.6
		Average		100.5	98.5
		SD		0.41	0.20
		%RSD		0.41	0.20

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iii) Intermediate precision sample details (Ramipril 10 mg & Hydrochlorothiazide 25 mg):

Sample No.	Sample Weight (mg)	Area of Sample		% Assay	
		Hydrochlorothiazide	Ramipril	Hydrochlorothiazide	Ramipril
1	493.22	29503.345	7122.542	98.7	99.3
2	493.35	29483.484	7119.263	98.6	99.3
3	493.63	29462.967	7116.801	98.4	99.2
4	493.27	29499.358	7130.428	98.6	99.4
5	493.44	29512.245	7118.175	98.6	99.2
6	493.83	29534.488	7120.850	98.6	99.2
		Average		98.6	99.3
		SD		0.08	0.10
		%RSD		0.08	0.10

Comparison of Method Precision and Intermediate precision:

i) For Ramipril 5 mg & Hydrochlorothiazide 12.5 mg

Sample No.	Parameter	% Assay	
		Hydrochlorothiazide	Ramipril
1	Method precision	100.8	99.5
2		100.0	98.2
3		100.9	100.1
4		101.1	99.4
5		101.7	100.2
6		101.2	99.5
1	Intermediate precision	99.8	100.4
2		100.1	100.5
3		99.8	100.5
4		99.5	100.2
5		100.3	100.8
6		99.6	100.3
	Mean	100.4	100.0
	SD	0.72	0.71
	%RSD	0.71	0.71


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ii) For Ramipril 10 mg & Hydrochlorothiazide 12.5 mg

Sample No.	Parameter	% Assay	
		Hydrochlorothiazide	Ramipril
98.2	Method precision	98.2	101.3
98.6		98.6	101.6
98.5		98.5	101.5
98.4		98.4	101.4
98.9		98.9	101.5
98.5		98.5	101.4
100.3	98.5	100.3	98.5
100.4		100.4	98.6
100.5		100.5	98.7
99.9		99.9	98.2
101		101	98.7
101		101	98.6
99.5	100.0	99.5	100.0
1.09	1.52	1.09	1.52
1.10	1.52	1.10	1.52

iii) For Ramipril 10 mg & Hydrochlorothiazide 25 mg

Sample No.	Parameter	% Assay	
		Hydrochlorothiazide	Ramipril
1	Method precision	99.4	100.5
2		100.1	101.2
3		100.3	101.5
4		98.3	99.7
5		100.4	101.6
6		99.4	100.9
1	Intermediate precision	98.7	99.3
2		98.6	99.3
3		98.4	99.2
4		98.6	99.4
5		98.6	99.2
6		98.6	99.2
	Mean	99.1	100.1
	SD	0.76	0.98
	%RSD	0.77	0.98

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Conclusion:

1. The relative standard deviation for peak area of Ramipril and Hydrochlorothiazide of standard solution is within acceptance criteria.
2. The tailing factor for Ramipril and Hydrochlorothiazide peak in standard solution is within acceptance criteria.
3. The theoretical plates for Ramipril and Hydrochlorothiazide peak in standard solution is within acceptance criteria.
4. % Assay value of Ramipril and Hydrochlorothiazide for sample solution is within specified limit.
5. % RSD of Assay value of Ramipril and Hydrochlorothiazide for six sample solution is within acceptance criteria.
6. The cumulative RSD for % Assay of Ramipril and Hydrochlorothiazide for twelve samples in method precision and intermediate precision is within acceptance criteria.

II) Intermediate precision for Content Uniformity:**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%

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

Injection No.	Retention Time	Area of Hydrochlorothiazide Standard	Retention Time	Area of Ramipril Standard
1	2.107	26757.552	3.973	7000.661
2	2.110	26742.164	3.977	7017.555
3	2.107	26729.583	3.974	7006.084
4	2.112	26720.862	3.972	7009.540
5	2.107	26715.221	3.973	7010.196
6	2.112	26708.079	3.972	6990.409
Average	2.109	26728.910	3.974	7005.741
SD	0.00	18.34	0.00	9.33
%RSD	0.12	0.07	0.05	0.13
System Suitability Results				
% RSD of Area of Hydrochlorothiazide			0.07	NMT 2.0%
% RSD of Area of Ramipril			0.13	NMT 2.0%
Tailing factor of Hydrochlorothiazide			1.08	NMT 2.0
Tailing factor of Ramipril			1.03	NMT 2.0
Theoretical plates of Hydrochlorothiazide			10583	NLT 2000
Theoretical plates of Ramipril			11081	NLT 2000

Standard details (Ramipril 10 mg & Hydrochlorothiazide 12.5 mg)

Injection No.	Retention Time	Area of Hydrochlorothiazide Standard	Retention Time	Area of Ramipril Standard
1	2.118	13481.003	3.944	7266.084
2	2.115	13300.734	3.942	7175.544
3	2.113	13314.339	3.946	7203.315
4	2.118	13327.831	3.944	7248.287
5	2.117	13325.621	3.944	7168.018
6	2.118	13327.657	3.945	7219.029
Average	2.117	13346.198	3.944	7213.380
SD	0.00	66.87	0.00	39.04
%RSD	0.10	0.50	0.03	0.54
System Suitability Results				
% RSD of Area of Hydrochlorothiazide			0.50	NMT 2.0%
% RSD of Area of Ramipril			0.54	NMT 2.0%
Tailing factor of Hydrochlorothiazide			1.13	NMT 2.0
Tailing factor of Ramipril			1.04	NMT 2.0
Theoretical plates of Hydrochlorothiazide			5865	NLT 2000
Theoretical plates of Ramipril			8371	NLT 2000

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i) Intermediate precision sample details for Hydrochlorothiazide (Ramipril 5 mg & Hydrochlorothiazide 12.5 mg):

Sample	Tablets Weight (mg)	Peak Area of Sample	% Content
1	199.98	26738.909	101.7
2	195.70	26777.155	101.8
3	199.17	26455.656	100.6
4	194.89	26811.343	101.9
5	196.42	26641.010	101.3
6	197.84	26754.995	101.7
7	195.91	26962.827	102.5
8	197.34	26777.232	101.8
9	197.05	26783.011	101.8
10	197.94	26719.267	101.6
Min			100.6
Max			102.5
Mean			101.7
SD			0.49
% RSD			0.48
AV Value			1.4

ii) Intermediate precision sample details for Ramipril (Ramipril 5 mg & Hydrochlorothiazide 12.5 mg):

Sample	Tablets Weight (mg)	Peak Area of Sample	% Content
1	199.98	6811.163	100.7
2	195.70	6536.646	96.7
3	199.17	6440.453	95.2
4	194.89	6638.071	98.2
5	196.42	6489.863	96.0
6	197.84	6595.169	97.5
7	195.91	6659.570	98.5
8	197.34	6602.860	97.6
9	197.05	6587.300	97.4
10	197.94	6578.018	97.3
Min			95.2
Max			100.7
Mean			97.5
SD			1.49
% RSD			1.53
AV Value			4.6

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iii) Intermediate precision sample details for Hydrochlorothiazide (Ramipril 10 mg & Hydrochlorothiazide 12.5 mg):

Sample	Tablets Weight (mg)	Peak Area of Sample	% Content
1	195.98	13502.588	99.0
2	201.22	14463.008	106.0
3	195.81	13649.699	100.0
4	200.20	13756.050	100.8
5	195.30	13677.799	100.2
6	199.39	13619.010	99.8
7	197.59	13654.006	100.1
8	199.74	13587.821	99.6
9	198.10	13745.397	100.7
10	200.49	13510.451	99.0
Min			99.0
Max			106.0
Mean			100.5
SD			2.02
% RSD			2.01
AV Value			6.0

iv) Intermediate precision sample details for Ramipril (Ramipril 10 mg & Hydrochlorothiazide 12.5 mg):

Sample	Tablets Weight (mg)	Peak Area of Sample	% Content
1	195.98	7096.170	101.7
2	201.22	7127.199	102.2
3	195.81	7127.021	102.2
4	200.20	7085.981	101.6
5	195.30	7018.480	100.6
6	199.39	6980.559	100.1
7	197.59	7081.134	101.5
8	199.74	7101.660	101.8
9	198.10	6986.968	100.1
10	200.49	7089.246	101.6
Min			100.1
Max			102.2
Mean			101.3
SD			0.78
% RSD			0.77
AV Value			1.9

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v) Intermediate precision sample details for Hydrochlorothiazide (Ramipril 10 mg & Hydrochlorothiazide 25 mg):

Sample	Tablets Weight (mg)	Peak Area of Sample	% Content
1	199.97	25578.397	95.5
2	201.56	26562.750	99.2
3	197.78	26813.157	100.1
4	195.75	27476.889	102.6
5	200.69	26955.541	100.7
6	199.87	28934.875	108.1
7	199.91	26756.172	99.9
8	198.52	26881.100	100.4
9	196.46	27172.322	101.5
10	202.30	26817.056	100.1
		Min	95.5
		Max	108.1
		Mean	100.8
		SD	3.14
		% RSD	3.11
		AV Value	7.5

vi) Intermediate precision sample details for Ramipril (Ramipril 10 mg & Hydrochlorothiazide 25.5 mg):

Sample	Tablets Weight (mg)	Peak Area of Sample	% Content
1	199.97	6379.967	91.9
2	201.56	6778.099	97.6
3	197.78	6965.385	100.3
4	195.75	6849.165	98.6
5	200.69	6779.973	97.6
6	199.87	7308.197	105.2
7	199.91	6841.221	98.5
8	198.52	6855.122	98.7
9	196.46	6839.633	98.5
10	202.30	6756.875	97.3
		Min	91.88
		Max	105.24
		Mean	98.4
		SD	3.26
		% RSD	3.31
		AV Value	7.9

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Comparison of content uniformity:**i) For Hydrochlorothiazide:**

Sr.No.	Lable claim (mg)	Test	% Mean Content	% mean Difference of content
1	Ramithiazide tablets 5mg/12.5mg	Precision	103.8	2.1
		Intermediate precision	101.7	
2	Ramithiazide tablets 10mg/12.5mg	Precision	103.4	2.9
		Intermediate precision	100.5	
3	Ramithiazide tablets 10mg/25mg	Precision	102.2	1.4
		Intermediate precision	100.8	

ii) For Ramipril:

Sr. No.	Lable claim (mg)	Test	% Mean Content	% mean Difference of content
1	Ramithiazide tablets 5mg/12.5mg	Precision	98.3	0.8
		Intermediate precision	97.5	
2	Ramithiazide tablets 10mg/12.5mg	Precision	101.0	0.4
		Intermediate precision	101.3	
3	Ramithiazide tablets 10mg/25mg	Precision	97.4	1.1
		Intermediate precision	98.4	

Conclusion:

1. The relative standard deviation for peak area of Ramipril and Hydrochlorothiazide of standard solution is within acceptance criteria.
2. The tailing factor for Ramipril and Hydrochlorothiazide peak in standard solution is within acceptance criteria.
3. The theoretical plates for Ramipril and Hydrochlorothiazide peak in standard solution is within acceptance criteria.
4. The acceptance value of Ramipril and Hydrochlorothiazide for ten sample solution is within acceptance criteria.
5. % Difference of mean of content uniformity of Ramipril and Hydrochlorothiazide for Method precision and Intermediate precision is within acceptance criteria.

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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.

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%.

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

Accuracy of Hydrochlorothiazide:

Accuracy level (%)	Sample	Placebo weight (mg)	Amount added(ppm)	Area	Amount recovered (ppm)	% Recovery	Mean % Recovery	% RSD
50	Sample 1	468.98	26.29	7354.546	26.65	101.4	101.2	0.41
	Sample 2	464.81	25.36	7050.771	25.55	100.8		
	Sample 3	464.26	26.64	7464.556	27.05	101.5		
100	Sample 1	465.19	50.07	14024.138	50.82	101.5	101.6	0.17
	Sample 2	465.46	50.56	14206.420	51.48	101.8		
	Sample 3	463.59	50.12	14046.120	50.90	101.6		
150	Sample 1	463.84	75.06	20957.790	75.95	101.2	101.3	0.18
	Sample 2	465.65	74.95	20998.029	76.10	101.5		
	Sample 3	463.80	75.06	20970.367	76.00	101.3		

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Accuracy of Ramipril:

Accuracy level (%)	Sample	Placebo weight (mg)	Amount added(ppm)	Area	Amount recovered (ppm)	% Recovery	Mean % Recovery	% RSD
50	Sample 1	468.98	10.67	4897.906	10.79	101.1	101.1	0.51
	Sample 2	464.81	10.50	4799.096	10.57	100.6		
	Sample 3	464.26	10.71	4944.512	10.89	101.7		
100	Sample 1	465.19	19.82	8820.949	19.43	98.1	98.5	0.50
	Sample 2	465.46	19.86	8925.371	19.66	99.0		
	Sample 3	463.59	19.83	8855.191	19.51	98.4		
150	Sample 1	463.84	30.22	13783.487	30.36	100.5	100.7	0.22
	Sample 2	465.65	30.08	13782.042	30.36	100.9		
	Sample 3	463.80	30.14	13787.558	30.37	100.7		

Accuracy for Ramipril 10 mg & Hydrochlorothiazide 12.5 mg:**Accuracy of Hydrochlorothiazide:**

Accuracy level (%)	Sample	Placebo weight (mg)	Amount added(ppm)	Area	Amount recovered (ppm)	% Recovery	Mean % Recovery	% RSD
50	Sample 1	467.98	12.69	3507.590	12.57	99.0	99.4	0.31
	Sample 2	465.83	12.70	3523.651	12.62	99.4		
	Sample 3	466.32	12.66	3522.441	12.62	99.7		
100	Sample 1	464.60	25.29	7032.703	25.20	99.6	99.7	0.07
	Sample 2	465.01	25.19	7014.152	25.13	99.8		
	Sample 3	465.13	25.24	7029.037	25.18	99.8		
150	Sample 1	464.49	37.56	10364.95	37.13	98.9	99.3	0.36
	Sample 2	466.10	37.50	10418.45	37.33	99.5		
	Sample 3	464.27	37.46	10393.90	37.24	99.4		

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Accuracy of Ramipril:

Accuracy level (%)	Sample	Placebo weight (mg)	Amount added(ppm)	Area	Amount recovered (ppm)	% Recovery	Mean % Recovery	% RSD
50	Sample 1	467.98	10.30	4774.975	10.47	101.6	101.5	0.31
	Sample 2	465.83	10.19	4701.765	10.31	101.1		
	Sample 3	466.32	10.36	4807.576	10.54	101.7		
100	Sample 1	464.60	20.06	9199.755	20.17	100.6	100.4	0.18
	Sample 2	465.01	20.11	9192.101	20.15	100.2		
	Sample 3	465.13	20.08	9191.341	20.15	100.3		
150	Sample 1	464.49	30.06	13497.348	29.59	98.4	98.5	0.36
	Sample 2	466.10	30.05	13552.427	29.71	98.9		
	Sample 3	464.27	30.10	13483.137	29.56	98.2		

Conclusion:

1. The relative standard deviation for peak area of Ramipril and Hydrochlorothiazide of standard solution is within acceptance criteria.
2. The tailing factor for Ramipril and Hydrochlorothiazide peak in standard solution is within acceptance criteria.
3. The theoretical plates for Ramipril and Hydrochlorothiazide peak in standard solution is within acceptance criteria.
4. % Recovery at each accuracy level is within acceptance criteria.
5. Mean % Recovery of all level is within acceptance criteria.

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:

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

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- The tailing factor for Ramipril and Hydrochlorothiazide peak in standard solution should be not more than 2.0
- The theoretical plates for Ramipril and Hydrochlorothiazide peak in standard solution should be not less than 2000
- % Difference for % Assay of sample solution in robustness and % Assay of sample solution in method precision should be not more than 2.0%

System suitability details:

Robustness parameter	% RSD of Ramipril	% RSD of HCTZ	Theroretical plates of Ramipril	Theroretical plates of HCTZ	Tailing factor of Ramipril	Tailing factor of HCTZ
	Limit					
	NMT 2.0%	NMT 2.0%	NLT 2000	NLT 2000	NMT 2.0	NMT 2.0
Precision	0.13	0.07	10410	10705	1.00	1.04
Flow Rate 0.9 mL/min	0.16	0.11	11245	11488	0.96	1.02
Flow Rate 1.1 mL/min	0.22	0.15	10098	10341	0.95	1.03
Organic content decrease	0.40	0.11	5847	3881	1.13	1.30
Organic content increase	0.66	0.18	5015	3757	1.16	1.26
Buffer pH 2.3	0.21	0.22	4532	3390	1.07	1.19
Buffer pH 2.7	0.24	0.11	5019	3475	1.10	1.19

Robustness study details:

For Hydrochlorothiazide:

Robustness parameter	% Assay	% Difference (NMT 2.0 %)
Precision	99.6	NA
Flow Rate 0.9 mL/min	100.1	0.5
Flow Rate 1.1 mL/min	99.9	0.3
Organic content decrease	98.9	0.7
Organic content increase	98.4	1.2
Buffer pH 2.3	98.4	1.2
Buffer pH 2.7	98.3	1.3

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Robustness study details:**For Ramipril:**

Robustness parameter	% Assay	% Difference (NMT 2.0 %)
Precision	100.9	NA
Flow Rate 0.9 mL/min	100.9	0.0
Flow Rate 1.1 mL/min	100.8	0.1
Organic content decrease	100.1	0.8
Organic content increase	100.7	0.2
Buffer pH 2.3	100.0	0.9
Buffer pH 2.7	101.7	0.8

Conclusion:

1. The relative standard deviation for peak area of Ramipril and Hydrochlorothiazide of standard solution is within acceptance criteria.
2. The tailing factor for Ramipril and Hydrochlorothiazide peak in standard solution is within acceptance criteria.
3. The theoretical plates for Ramipril and Hydrochlorothiazide peak in standard solution is within acceptance criteria.
4. % Difference for % Assay of sample solution in robustness and % Assay of sample solution in method precision is within acceptance criteria.

10.7. FILTER STUDY**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%


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

Injection No.	Retention Time	Area of Hydrochlorothiazide Standard	Retention Time	Area of Ramipril Standard
1	2.225	29779.744	3.882	6982.083
2	2.225	29910.378	3.883	6990.418
3	2.227	29895.007	3.885	6982.654
4	2.227	29870.134	3.885	6978.374
5	2.225	29937.184	3.883	6982.466
6	2.225	29970.544	3.883	6990.327
Average	2.226	29893.832	3.884	6984.387
SD	0.001	65.75	0.001	4.89
%RSD	0.05	0.22	0.03	0.07
System Suitability Results				
% RSD of Area of Hydrochlorothiazide			0.22	NMT 2.0%
% RSD of Area of Ramipril			0.07	NMT 2.0%
Tailing factor of Hydrochlorothiazide			1.15	NMT 2.0
Tailing factor of Ramipril			1.06	NMT 2.0
Theoretical plates of Hydrochlorothiazide			6040	NLT 2000
Theoretical plates of Ramipril			8584	NLT 2000

Filter study details:

For Hydrochlorothiazide:

Robustness parameter	% Assay	% Difference (NMT 2.0 %)
Centrifuged	100.8	-
Filtered through 0.22 u filter	100.8	0.02
Filtered through 0.45 u filter	100.9	0.15

Filter study details:

For Ramipril:

Robustness parameter	% Assay	% Difference (NMT 2.0 %)
Centrifuged	101.5	-
Filtered through 0.22 u filter	101.5	0.08
Filtered through 0.22 u filter	101.5	0.03

 Quality by Design	ANALYTICAL METHOD VALIDATION REPORT FOR ASSAY AND UNIFORMITY OF CONTENT OF RAMITHIAZIDE (RAMIPRIL AND HYDROCHLOROTHIAZIDE TABLETS) BY HPLC METHOD		
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Conclusion:

1. The relative standard deviation for peak area of Ramipril and Hydrochlorothiazide of standard solution is within acceptance criteria.
2. The tailing factor for Ramipril and Hydrochlorothiazide peak in standard solution is within acceptance criteria.
3. The theoretical plates for Ramipril and Hydrochlorothiazide peak in standard solution is within acceptance criteria.
4. %Difference between filtered solution through different filters and centrifuged sample solution is within acceptance criteria.

10.8. SOLUTION STABILITY**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%

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

Injection No.	Retention Time	Area of Hydrochlorothiazide Standard	Retention Time	Area of Ramipril Standard
1	2.097	27140.043	3.650	6928.843
2	2.097	27182.012	3.650	6943.845
3	2.097	27163.638	3.650	6935.208
4	2.097	27160.989	3.650	6940.622
5	2.097	27129.962	3.650	6923.226
6	2.097	27176.337	3.650	6945.313
Average	2.097	27158.830	3.650	6936.176
SD	0.000	20.29	0.000	8.77
%RSD	0.00	0.07	0.00	0.13


**ANALYTICAL METHOD VALIDATION REPORT FOR ASSAY AND
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System Suitability Results		
% RSD of Area of Hydrochlorothiazide	0.07	NMT 2.0%
% RSD of Area of Ramipril	0.00	NMT 2.0%
Tailing factor of Hydrochlorothiazide	1.04	NMT 2.0
Tailing factor of Ramipril	1.00	NMT 2.0
Theoretical plates of Hydrochlorothiazide	10705	NLT 2000
Theoretical plates of Ramipril	10410	NLT 2000

Solution stability standard:

Time (hr)	Area of Standard for HCTZ	Area of Standard for Ramipril	%RSD (NMT 2.0%) HCTZ	%RSD (NMT 2.0%) Ramipril
Initial	27140.044	6928.843	NA	NA
3 hrs	27272.086	6969.910	0.34	0.42
5 hrs	27477.861	7028.509	0.62	0.72
13 hrs	27578.480	7048.360	0.72	0.78
19 hrs	27900.150	7159.078	1.07	1.25
25 hrs	28227.280	7213.154	1.47	1.55
28 hrs	28438.752	7265.373	1.76	1.79

Sample solution stability details:

Sample details	Area of Sample		% Assay		%RSD (NMT 2.0%)	%RSD (NMT 2.0%)
	HCTZ	Ramipril	HCTZ	Ramipril	HCTZ	Ramipril
Initial	26811.141	6690.018	98.8	98.6	NA	NA
4 hrs	26774.333	6684.338	98.7	98.5	0.14	0.08
7hrs	26925.700	6714.042	99.2	99.0	0.42	0.35
10 hrs	26949.482	6741.319	99.3	99.4	0.51	0.76
16 hrs	27177.318	6797.521	100.2	100.2	1.35	1.58
22 hrs	27285.001	6819.047	100.6	100.5	1.75	1.90
25 hrs	27424.241	6854.231	101.1	101.0	2.26	2.42

Conclusion:**Standard solution stability:**

The standard solution is stable upto 28hrs.

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Sample solution stability:

Sample solution is stable upto 22 hrs.

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

INVOICE

(ORIGINAL FOR RECIPIENT)

QbD Research And Development Lab Pvt Ltd

2nd Floor, TSSIA House,
Plot No. P-26, Road No.16 / T
Wagle Ind Estate, Thane 400604
CIN No.U73100MH2012PTC232153
GSTIN/UIN: 27AAACQ2815J1ZG
E-Mail : lab@qbdresearch.com

Invoice No.
QbD/117/2017-18
Supplier's Ref.

Dated
27-Jul-2017
Other Reference(s)

Buyer's Order No.

Dated

Buyer

Generic Healthcare Pvt Ltd

City Square, 29/A, Office No- 205, 2nd Floor, Behind
Hotel Pride, Shivaji Nagar, Pune-, 411005, K/A Mr Palve Sir,
State Name : Maharashtra, Code : 27
GSTIN/UIN : 27AADCG9221D1ZU

SI No.	Description of Goods	Quantity	Rate	per	Disc. %	Amount
1	Ramithiazide Tablet <i>Analytical Method Validation For Assay and Uniformity of Content Of Ramithiazide by HPLC Method</i>	1 No.	4,00,000.00	No.		4,00,000.00
	SGST Output			9 %		36,000.00
	CGST Output			9 %		36,000.00

Total **1 No.** **₹ 4,72,000.00**
E. & O.E

Amount Chargeable (in words)

Indian Rupees Four Lakh Seventy Two Thousand Only

HSN/SAC	Taxable Value	Central Tax Rate	Central Tax Amount	State Tax Rate	State Tax Amount	Total Tax Amount
998346	4,00,000.00	9%	36,000.00	9%	36,000.00	72,000.00
Total	4,00,000.00		36,000.00		36,000.00	72,000.00

Tax Amount (in words) : **Indian Rupees Seventy Two Thousand Only**Company's PAN : **AAACQ2815J**

Declaration

1. Payment Terms : Payment should be made within 10 days on receipt of invoice copy.

2. Interest at 21% will be charged on this bill if not paid within one month.

Company's Bank Details

Bank Name : **TJSB Sahakari Bank Ltd-CC-47**A/c No. : **CC A/c No.013130100000047**Branch & IFS Code : **Mulund (W) & TJSB0000013**

for QbD Research And Development Lab Pvt Ltd



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