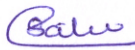
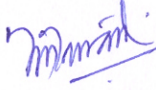
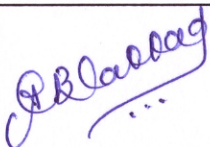


QbD RESEARCH AND DEVELOPMENT LAB PVT. LTD.

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	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	Designation	Signature	Date
Satish Doodale	Manager Analytical (QbD Research & Development Lab)		25-Apr-19
Reviewed By	Designation	Signature	Date
Satish Sawant	GM Analytical (QbD Research & Development Lab)		25-Apr-19
Approved By	Designation	Signature	Date
Detecor Kathirige	QA Manager (QbD Research & Development Lab)		27-Apr-19
Approved By	Designation	Signature	Date
prabhakar Raut	For (Generic Healthcare Pvt. Ltd.)		27.04.2019

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
Report Number	QbD-REP-592	Page No	Page 2 of 52

INDEX

1. OBJECTIVE	4
2. SCOPE	4
3. RESPONSIBILITIES	4
4. SAFETY	4
5. STANDARD AND IMPURITY DETAILS	4
6. SAMPLE AND PLACEBO DETAILS	4
7. DETAILS OF SPECIFICATIONS	5
8. REAGENTS AND CHEMICALS	5
9. INSTRUMENT DETAILS	5
10. METHOD OF ANALYSIS	5
HPLC CHROMATOGRAPHIC CONDITIONS	5
11. VALIDATION PARAMETERS	9
11.1. SPECIFICITY	10
11.2. LIMIT OF DETECTION AND LIMIT OF QUANTITATION	12
11.3. LINEARITY AND RANGE	23
11.4. PRECISION	31
11.5. ACCURACY	38
11.6. ROBUSTNESS	42
11.7. SOLUTION STABILITY	44
11.8. FILTER STUDY	47
12. BATCH ANALYSIS RESULTS	50
13. REFERENCES	51
14. REVISION HISTORY	52
15. INCIDENCES	52
16. OVERALL CONCLUSION	52


**ANALYTICAL METHOD VALIDATION REPORT FOR RELATED
SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD**
Report Number

QbD-REP-592

Page No

Page 3 of 52

Abbreviations

Mg	: Milligram
A.R. Grade	: Analytical Reagent Grade
μ	: Micron
mL	: Milliliter
° C	: degree Celsius
nm	: Nanometer
Min	: Minute
μL	: Microliter
G	: Gram
ppm	: Part per million
LC	: Label claim
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
ND	: Not Detected

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 4 of 52

1. OBJECTIVE

The report summarizes the results of the analytical method validation for Related Substances in Ramithiazide 10/25 mg is to demonstrate, that the method is consistently yield the results which reflects the quality characteristics of the product.

2. SCOPE

The validation activity carried out on Ramithiazide Tablet 10/25 mg and Batch analysis activity carried out on Ramithiazide Tablet 5/12.5 mg and Ramithiazide Tablet 10/12.5 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 QA Manager to approve the Report.

4. SAFETY

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

5. STANDARD AND IMPURITY DETAILS

Standard/Impurity name	Batch number no.	Potency (% as is basis)
Ramipril working standard	WS005	99.64
Hydrochlorothiazide working standard	FF-6043CHR11	99.24
	CL-HCTZ-WS-01	99.86

6. SAMPLE AND PLACEBO DETAILS

Sample name	Batch no./Lot no.
Ramithiazide 10/25 mg	GP180301
Ramithiazide 5/12.5 mg	GP181203
Ramithiazide 10/12.5 mg	GP181206
Placebo of Ramithiazide 10/25 mg	NM

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 5 of 52

7. DETAILS OF SPECIFICATIONS

Name of Components	Limit
Impurity A	NMT 2.0%
Impurity D	NMT 6.0%
Impurity E	NMT 2.0%
Single maximum unknown impurity	NMT 0.5%
Total impurities	NMT 6.0%

8. REAGENTS AND CHEMICALS

Reagent	Grade	Manufacturer	Batch no./ Lot no.
Triethylamine	AR	Finar	547671208KQ
Phosphoric Acid	AR	SDFCL	A18A/1017/2108/31
Acetonitrile	HPLC	Finar	249451101CR
Water	Milli-Q	---	---

9. INSTRUMENT DETAILS

Instrument/ Equipment	Supplier/ Manufacturer / Make	Instrument/ Equipment In-House No.	Calibration Due Date
HPLC	Agilent	QbD-INST-HPLC-67	24-Jun-19
HPLC	Shimadzu	QbD-INST-HPLC-42	20-Jul-19
HPLC	Agilent	QbD-INST-HPLC-04	20-Jun-19
pH meter	Lab India	QbD-INST-pH-08	Daily
pH meter	Elmetron	QbD-INST-pH-86	Daily
Analytical Balance	Mettler Toledo	QbD-INST-BAL-12	Daily
Analytical Balance	Mettler Toledo	QbD-INST-BAL-13	Daily

10. METHOD OF ANALYSIS

HPLC Chromatographic conditions

Column	:	Inertsil ODS 3V (250 X 4.6 mm, 5 µm), C18 or equivalent
Flow rate	:	1.0mL/min
Column oven temperature	:	25°C
LC mode	:	Gradient
Detection wavelength	:	210 nm
Injection volume	:	30 µL
Run time	:	30 minutes

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 6 of 52

Preparation of buffer solution:

Dissolve 12.2987 g of sodium perchlorate in water to make 1000 mL, adjust pH 2.5 with diluted phosphoric acid and mix well. Filter it through 0.45µ membrane filter and degas.

Mobile phase A:

Use buffer solution as Mobile phase A

Mobile phase B:

Acetonitrile

Gradient program:

Time (min)	Mobile phase A (%v/v)	Mobile phase B (%v/v)
0.0	70	30
7.0	70	30
9.0	50	50
25.0	50	50
30.0	70	30

Diluent:

Water : Acetonitrile : Triethylamine (550:450:1 V/V), Mix well and adjust the pH to 3.0 with diluted phosphoric acid.

Preparation of Ramipril standard stock solution:

Weigh accurately and transfer about 25 mg of Ramipril working standard in 25mL of volumetric flask and add 10mL of diluent and sonicate to dissolve and dilute to volume with diluent.

Preparation of Hydrochlorothiazide standard stock solution:

Weigh accurately and transfer about 25 mg of Hydrochlorothiazide working standard in 25 mL of volumetric flask and add 10 mL of diluent and sonicate to dissolve and dilute to volume with diluent.

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 7 of 52

Preparation of Standard solution:

Pipette out 2.0 mL of Ramipril standard stock solution and 2.5 mL of Hydrochlorothiazide standard stock solution and transfer in to 100 mL volumetric flask and dilute to volume with diluent.

Further dilute 2.0 mL of above solution to 20 mL and dilute to volume with diluent.

Placebo solution:

Transfer an accurately weighed portion of the placebo powder equivalent to 20 mg of Ramipril to a 20mL volumetric flask. Add 12mL of diluent, and 1 mL water sonicate for 15 minutes and dilute with diluent to make volume, mix well. Filter through 0.45µm nylon filter.

Sample solution:

Take 20 tablets, determine the average weight of tablet and finely powder. Transfer an accurately weighed portion of the powder equivalent 20 mg of Ramipril to a 20 mL volumetric flask. Add 12mL of diluent, and 1 mL water sonicate for 15 minutes and dilute with diluent to make volume, mix well. Filter through 0.45µm nylon filter.

Procedure:

Separately inject 30 µL of the blank solution, standard solution in six replicate, placebo solution and sample solution into the chromatography system, record the chromatograph and measure the peak response for the major peaks.

System suitability parameters:

The relative standard deviation for six replicate injections of standard solution should be not more than 5.0%.

Note: Disregard any peak due to blank and placebo.

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 8 of 52

Calculations:

Calculate the results by using the following formula

%Known impurities:

$$= \frac{AT1 \times WS \times 2 \times 2 \times 20 \times P \times \text{Average weight (mg)}}{AS \times 25 \times 100 \times 20 \times WT \times 100 \times LC} \times 100 \times RRF$$

Where,

AT1 = Area of known impurity 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.

RRF = Relative response factor.

LC = Label claim in mg.

%Unknown impurities:

$$= \frac{AT2 \times WS \times 2 \times 2 \times 20 \times P \times \text{Average weight (mg)}}{AS \times 25 \times 100 \times 20 \times WT \times 100 \times LC} \times 100$$

Where,

AT2 = Area of unknown impurity 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 in mg.

Total impurities: Sum of % of all known Impurities + Sum of % of all unknown Impurities.

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 9 of 52

11. VALIDATION PARAMETERS

11.1. Specificity

A. System suitability

B. Specificity

11.2. Precision

A. System precision

B. Method precision

C. Intermediate precision

11.3. LOD and LOQ

11.4. Linearity and Range

11.5. Accuracy

11.6. Robustness

11.7. Solution stability

11.8. Filter study

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 10 of 52

11.1. SPECIFICITY

LNB/Pg. No: (QbD-LNB-697, Pg. No-106)

Acceptance criteria and System Suitability:

1. The relative standard deviation for six replicate injections of standard solution should be not more than 5.0%.
2. There should be no peaks in the blank and placebo chromatograms at the retention time of Ramipril, Ramipril impurity A, Ramipril impurity D and Ramipril impurity E.

Standard details:

Ramipril		
Sr. No.	Retention Time	Peak Area
1	16.123	2191.679
2	16.113	2225.651
3	16.110	2208.984
4	16.113	2185.436
5	16.117	2181.993
6	16.117	2165.894
Average	16.116	2193.273
SD	0.0045	21.1470
%RSD	0.03	0.96

System suitability results		
% RSD of area	0.96	NMT 5.0%
% RSD of Area with Bkt Standard	0.97	NMT 5.0%

Hydrochlorothiazide		
Injection No.	Retention Time	Peak Area
1	5.140	4377.810
2	5.147	4276.994
3	5.147	4333.180
4	5.143	4271.156
5	5.147	4370.513
6	5.147	4271.229
Average	5.145	4316.814
SD	0.0030	50.2399
%RSD	0.06	1.16

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
Report Number	QbD-REP-592	Page No	Page 11 of 52

System suitability results		
% RSD of area	1.16	NMT 5.0%
% RSD of Area with Bkt Standard	1.13	NMT 5.0%

Interference details:

Sample	RT (Individual impurity)	RT (As such Sample)	RT (Spiked Sample)
Blank Solution	ND	ND	ND
Hydrochlorothiazide	5.140	5.250	5.133
Ramipril Impurity E	14.063	14.067	14.057
Ramipril Impurity A	14.640	ND	14.647
Ramipril	16.123	15.977	15.980
Ramipril Impurity D	23.887	23.870	23.847

Conclusion:

1. The relative standard deviation for six replicate injections for Ramipril were 0.96% & Hydrochlorothiazide were 1.16%.
2. No peaks in the blank and placebo chromatograms at the retention time of Ramipril, Ramipril impurity A, Ramipril impurity D and Ramipril impurity E.

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 12 of 52

11.2. LIMIT OF DETECTION AND LIMIT OF QUANTITATION

11.2.1 LOD/LOQ establishment:

LNB/Pg. No: (QbD-LNB-697, Pg. No-115)

Acceptance criteria:

1. The relative standard deviation for six replicate injections is not more than 5.0%.
2. LOD/LOQ should be established.

Standard details:

Ramipril		
Injection No.	Retention Time	Peak Area
1	16.130	2072.058
2	16.130	2066.687
3	16.123	2045.282
4	16.120	2068.645
5	16.120	2068.921
6	16.107	2048.324
Average	16.122	2061.653
SD	0.0085	11.6704
%RSD	0.05	0.57

System suitability results for Ramipril		
% RSD of area	0.57	NMT 5.0%
% RSD of Area with Bkt Standard	1.23	NMT 5.0%

Hydrochlorothiazide		
Injection No.	Retention Time	Peak Area
1	5.163	4391.925
2	5.160	4424.471
3	5.153	4407.649
4	5.153	4401.394
5	5.153	4391.609
6	5.157	4392.136
Average	5.157	4401.531
SD	0.0043	12.9821
%RSD	0.08	0.29

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 13 of 52

System suitability results for Hydrochlorothiazide		
% RSD of area	0.29	NMT 5.0%
% RSD of Area with Bkt Standard	0.29	NMT 5.0%

LOD and LOQ Estimation details for Ramipril:

Weight of standard (mg)	Diluted (mL)	Concentration (ppm)
24.99	25.0	10.00
5.0	100.0	
10.0	50.0	

mL of standard solution for LOD/ LOQ	Diluted to mL	Concentration (ppm)	Area
0.5	25.0	0.20	199.723
1.0	25.0	0.40	424.234
1.5	25.0	0.60	708.012
2.0	25.0	0.80	952.111
2.5	25.0	1.00	1180.251
SLOPE		1244.96	
INTERCEPT		-53.81	
CORRELATION COEFFICIENT		0.999	
LOD Concentration (ppm)		0.04	
LOQ Concentration (ppm)		0.14	

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 14 of 52

LOD and LOQ Estimation details for Ramipril Impurity A:

Weight of standard (mg)	Diluted (mL)	Concentration (ppm)
2.76	10.0	276.0

mL of Standard Solution for LOD/ LOQ	Diluted to mL	Concentration (ppm)	Area
0.2	25.0	2.21	2119.833
0.4	25.0	4.42	4424.131
0.6	25.0	6.62	7068.418
0.8	25.0	8.83	9241.745
1.0	25.0	11.04	11490.971
SLOPE		1067.02	
INTERCEPT		-198.95	
CORRELATION COEFFICIENT		0.999	
LOD Concentration (ppm)		0.43	
LOQ Concentration (ppm)		1.30	

LOD and LOQ Estimation details for Ramipril Impurity E:

Weight of standard (mg)	Diluted (mL)	Concentration (ppm)
2.41	10.0	241.0

mL of Standard Solution for LOD/ LOQ	Diluted to mL	Concentration (ppm)	Area
0.2	25.0	1.93	84.998
0.4	25.0	3.86	190.999
0.6	25.0	5.78	296.087
0.8	25.0	7.71	384.274
1.0	25.0	9.64	469.278
SLOPE		49.89	
INTERCEPT		-3.42	
CORRELATION COEFFICIENT		0.999	
LOD Concentration (ppm)		0.62	
LOQ Concentration (ppm)		1.87	

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 15 of 52

LOD and LOQ Estimation details for Ramipril Impurity D:

Weight of standard (mg)	Diluted (mL)	Concentration (ppm)
2.95	50.0	59.0

mL of Standard Solution for LOD/ LOQ	Diluted to mL	Concentration (ppm)	Area
2.5	25.0	5.90	5908.573
5.0	25.0	11.80	11701.418
7.5	25.0	17.70	17427.816
10.0	25.0	23.60	23357.930
12.5	25.0	29.50	29194.393
SLOPE		986.92	
INTERCEPT		49.58	
CORRELATION COEFFICIENT		1.000	
LOD Concentration (ppm)		0.20	
LOQ Concentration (ppm)		0.61	

Conclusion:

1. The relative standard deviation for six replicate injections of Ramipril were 0.57% & Hydrochlorothiazide were 0.29%.
2. From the slope LOD and LOQ values for Ramipril were 0.04 ppm and 0.14 ppm respectively, LOD and LOQ values for Ramipril Impurity A were 0.43 ppm and 1.30 ppm respectively, LOD and LOQ values for Ramipril Impurity E were 0.62 ppm and 1.87 ppm respectively and LOD and LOQ values for Ramipril Impurity D were 0.20 ppm and 0.61 ppm respectively.

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 16 of 52

11.2.2 LOD/LOQ verification:

LNB/Pg. No: (QbD-LNB-697, Pg. No-120)

Acceptance criteria:

1. The relative standard deviation for six replicate injections is not more than 5.0%.

Limit of detection:

The peak at LOD concentration should be consistently detected.

Limit of quantitation:

The relative standard deviation for peak area at LOQ concentration should be not more than 15.0%.

Standard details:

Ramipril		
Injection No.	Retention Time	Peak Area
1	16.090	2074.376
2	16.113	2074.863
3	16.113	2070.281
4	16.123	2061.625
5	16.133	2060.275
6	16.143	2041.123
Average	16.119	2063.757
SD	0.0184	12.7093
%RSD	0.11	0.62

System suitability results for Ramipril		
% RSD of area	0.62	NMT 5.0%
% RSD of Area with Bkt Standard	0.64	NMT 5.0%

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 17 of 52

Hydrochlorothiazide		
Injection No.	Retention Time	Peak Area
1	5.137	4364.101
2	5.140	4355.765
3	5.140	4367.057
4	5.140	4353.821
5	5.143	4376.926
6	5.150	4379.743
Average	5.142	4366.236
SD	0.0045	10.6369
%RSD	0.09	0.24

System suitability results for Hydrochlorothiazide		
% RSD of area	0.24	NMT 5.0%
% RSD of Area with Bkt Standard	0.25	NMT 5.0%

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 18 of 52

LOD Verification details for Ramipril:

Weight of standard (mg)	Diluted (mL)	Concentration (ppm)
25.08	25.0	10.03
5.0	100.0	
10.0	50.0	

mL of standard solution for LOD	Diluted to mL	Concentration (ppm)	Area
0.5	25.0	0.20	189.107
			183.371
			193.123

LOQ Verification details for Ramipril:

mL of standard solution for LOQ	Diluted to mL	Concentration (ppm)	Area
1.5	25.0	0.60	710.818
			682.600
			706.233
			698.371
			714.039
			712.611
		Average	704.110
		SD	11.97
		% RSD	1.70

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 19 of 52

LOD Verification details for Ramipril Impurity A:

Weight of standard (mg)	Diluted (mL)	Concentration (ppm)
2.76	10.0	276.0

mL of standard solution for LOD	Diluted to mL	Concentration (ppm)	Area
0.2	25.0	2.21	2161.573
			2130.477
			2152.114

LOQ Verification details for Ramipril Impurity A:

mL of standard solution for LOQ	Diluted to mL	Concentration (ppm)	Area
0.6	25.0	6.62	7095.934
			7070.364
			7057.275
			7055.150
			7080.709
			7067.198
		Average	7071.110
		SD	15.30
		% RSD	0.22

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
Report Number	QbD-REP-592	Page No	Page 20 of 52

LOD Verification details for Ramipril Impurity E:

Weight of standard (mg)	Diluted (mL)	Concentration (ppm)
2.41	10.0	241.0

mL of standard solution for LOD	Diluted to mL	Concentration (ppm)	Area
0.2	25.0	1.93	107.247
			100.797
			98.731

LOQ Verification details for Ramipril Impurity E:

mL of standard solution for LOQ	Diluted to mL	Concentration (ppm)	Area
0.6	25.0	5.78	305.458
			303.043
			307.965
			301.744
			309.413
			315.821
		Average	307.24
		SD	5.10
		% RSD	1.66

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 21 of 52

LOD Verification details for Ramipril Impurity D:

Weight of standard (mg)	Diluted (mL)	Concentration (ppm)
2.95	50.0	59.0

mL of standard solution for LOD	Diluted to mL	Concentration (ppm)	Area
2.5	25.0	5.90	5782.628
			5799.560
			5841.659

LOQ Verification details for Ramipril Impurity D:

mL of standard solution for LOQ	Diluted to mL	Concentration (ppm)	Area
7.5	25.0	17.70	17415.295
			17398.399
			17417.627
			17356.165
			17367.636
			17401.610
		Average	17392.790
		SD	25.32
		% RSD	0.15

Conclusion:

1. The relative standard deviation for six replicate injections of Ramipril were 0.62% & Hydrochlorothiazide were 0.24%.

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 22 of 52

Limit of detection:

Peak of Ramipril, Ramipril Impurity A, Ramipril Impurity E and Ramipril Impurity D at LOD concentration were consistently detected.

Limit of quantitation:

The relative standard deviation for peak area at LOQ concentration for Ramipril, Ramipril Impurity A, Ramipril Impurity E and Ramipril Impurity D were 1.70%, 0.22%, 1.66% and 0.15% respectively.

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
Report Number	QbD-REP-592	Page No	Page 23 of 52

11.3. LINEARITY AND RANGE

LNB/Pg. No: (QbD-LNB-697, Pg. No-123)

Acceptance criteria:

1. The relative standard deviation for six replicate injections is not more than 5.0%.
2. The correlation coefficient obtained from the graph should not be less than 0.99.

Standard details:

Ramipril		
Injection No.	Retention Time	Peak Area
1	16.133	2091.691
2	16.150	2087.062
3	16.147	2059.705
4	16.147	2077.548
5	16.140	2089.534
6	16.130	2076.330
Average	16.141	2080.312
SD	0.0082	11.9015
%RSD	0.05	0.57

System suitability results for Ramipril		
% RSD of area	0.57	NMT 5.0%
% RSD of Area with Bkt Standard	0.53	NMT 5.0%

Hydrochlorothiazide		
Injection No.	Retention Time	Peak Area
1	5.163	4302.226
2	5.173	4297.689
3	5.177	4308.221
4	5.180	4292.629
5	5.183	4306.128
6	5.180	4289.416
Average	5.176	4299.385
SD	0.0072	7.4768
%RSD	0.14	0.17

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 24 of 52

System suitability results for Hydrochlorothiazide		
% RSD of area	0.17	NMT 5.0%
% RSD of Area with Bkt Standard	0.39	NMT 5.0%

Linearity details for Ramipril:

Weight of standard (mg)	Diluted (mL)	Concentration (ppm)
25.49	25.0	20.39
5.0	100.0	
10.0	25.0	

Linearity data for Ramipril:

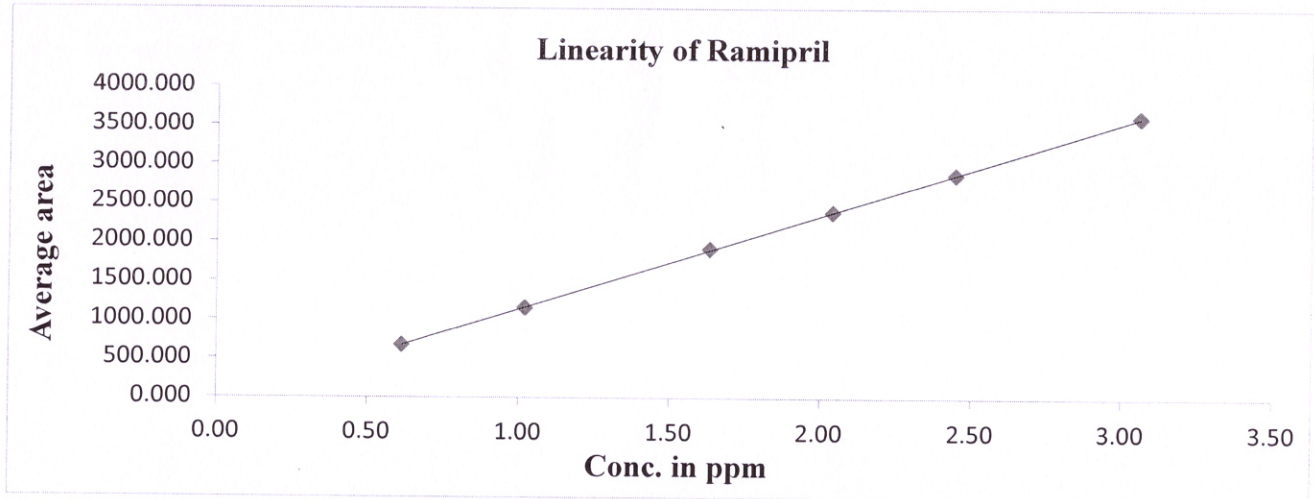
Level	Linearity level (%)	Volume (mL) of Standard stock solution	Diluted to volume (mL)	Concentration (ppm)	Area	Mean area
1	LOQ Level	0.75	25.0	0.61	684.597	671.024
					673.977	
					654.499	
2	50	1.25	25.0	1.02	1181.781	1151.984
					1134.645	
					1139.525	
3	80	2.00	25.0	1.63	1918.400	1907.225
					1890.667	
					1912.607	
4	100	2.50	25.0	2.04	2407.134	2383.395
					2366.662	
					2376.389	
5	120	3.00	25.0	2.45	2883.472	2865.842
					2871.566	
					2842.489	
6	150	3.75	25.0	3.06	3632.981	3607.607
					3593.575	
					3596.265	


**ANALYTICAL METHOD VALIDATION REPORT FOR RELATED
SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD**
Report Number

QbD-REP-592

Page No

Page 25 of 52

Linearity Graph:

Slope	1199.91
y-intercept	-63.54
Correlation coefficient	1.0000
RRF	1.00

Linearity details for Ramipril Impurity A:

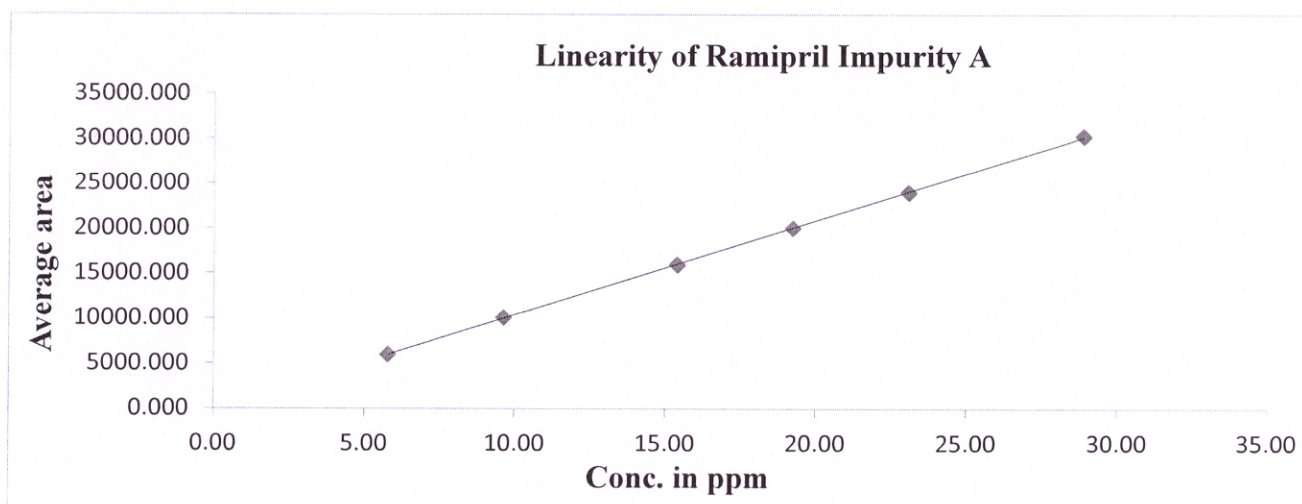
Weight of standard (mg)	Diluted (mL)	Concentration (ppm)
4.81	20.0	240.5
1.0	1.0	

Linearity data for Ramithiazide Impurity A:

Level	Linearity level (%)	Volume (mL) of Standard stock solution	Diluted to volume (mL)	Concentration (ppm)	Area	Mean area
1	LOQ Level	0.6	25.0	5.77	5993.015	5985.763
					5971.960	
					5992.314	
2	50	1.0	25.0	9.62	10103.214	10107.600
					10114.870	
					10104.716	

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 26 of 52

3	80	1.6	25.0	15.39	15989.488	15988.854
					15988.949	
					15988.124	
4	100	2.0	25.0	19.24	20077.899	20052.914
					20061.336	
					20019.508	
5	120	2.4	25.0	23.09	23979.960	23995.800
					23997.252	
					24010.189	
6	150	3.0	25.0	28.86	30264.148	30284.844
					30290.129	
					30300.255	

Linearity Graph:

Slope	1047.310
y-intercept	-63.410
Correlation coefficient	0.9999
RRF	1.15

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 27 of 52

Linearity details for Ramipril Impurity D:

Weight of standard (mg)	Diluted (mL)	Concentration (ppm)
11.99	20.0	599.5
1.0	1.0	

Linearity data for Ramithiazide Impurity D:

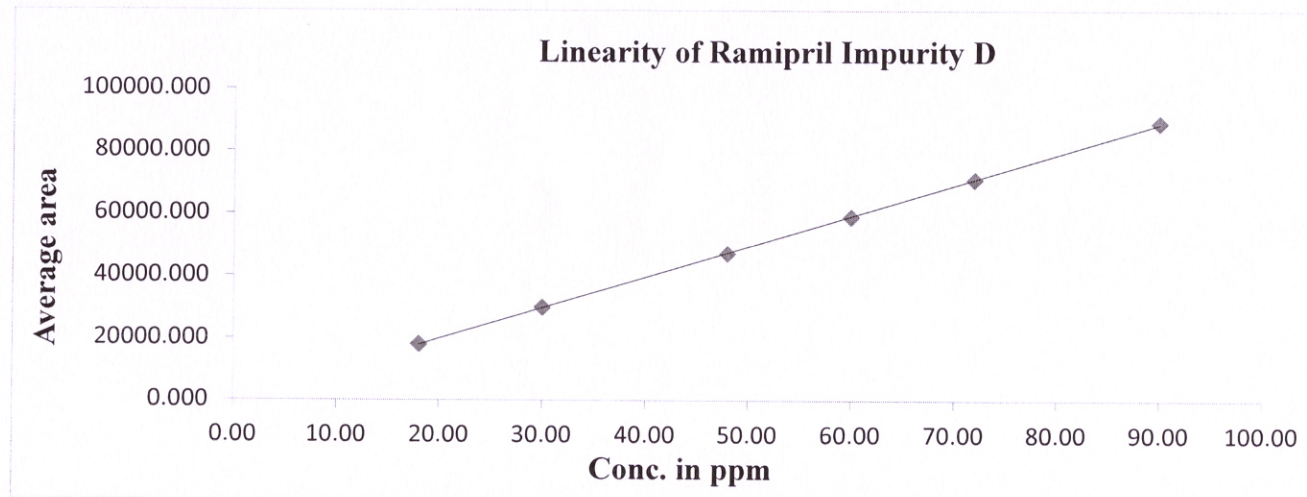
Level	Linearity level (%)	Volume (mL) of Standard stock solution	Diluted to volume (mL)	Concentration (ppm)	Area	Mean area
1	LOQ Level	0.75	25.0	17.99	18113.100	18069.201
					18029.972	
					18064.532	
2	50	1.25	25.0	29.98	29918.477	29916.060
					29912.041	
					29917.663	
3	80	2.00	25.0	47.96	47276.501	47216.881
					47211.271	
					47162.870	
4	100	2.50	25.0	59.95	58928.184	58947.361
					58983.775	
					58930.123	
5	120	3.00	25.0	71.94	70886.768	70918.114
					70900.499	
					70967.075	
6	150	3.75	25.0	89.93	88810.638	88923.318
					89011.311	
					88948.005	


**ANALYTICAL METHOD VALIDATION REPORT FOR RELATED
SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD**
Report Number

QbD-REP-592

Page No

Page 28 of 52

Linearity Graph:

Slope	982.74
y-intercept	290.19
Correlation coefficient	1.0000
RRF	1.22

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 29 of 52

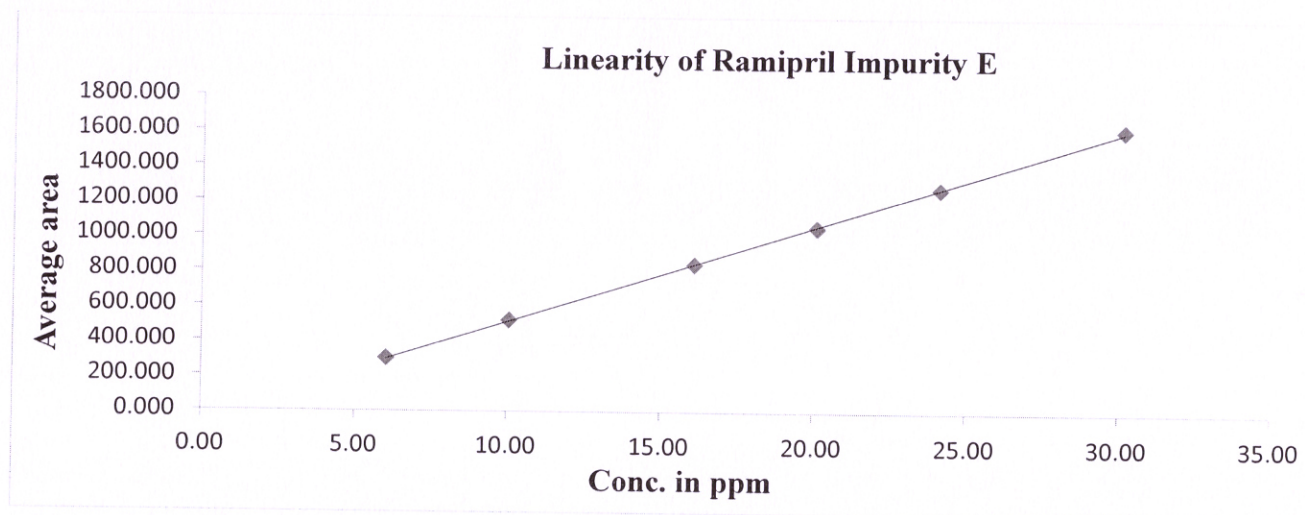
Linearity details for Ramipril Impurity E:

Weight of standard (mg)	Diluted (mL)	Concentration (ppm)
5.03	20.0	251.5
1.0	1.0	

Linearity data for Ramithiazide Impurity E:

Level	Linearity level (%)	Volume (mL) of Standard stock solution	Diluted to volume (mL)	Concentration (ppm)	Area	Mean area
1	LOQ Level	0.6	25.0	6.04	294.601	298.975
					300.922	
					301.402	
2	50	1.0	25.0	10.06	520.246	518.168
					514.168	
					520.090	
3	80	1.6	25.0	16.10	836.407	838.060
					839.639	
					838.133	
4	100	2.0	25.0	20.12	1057.842	1050.973
					1049.836	
					1045.242	
5	120	2.4	25.0	24.14	1268.187	1274.700
					1273.170	
					1282.744	
6	150	3.0	25.0	30.18	1608.814	1615.688
					1620.526	
					1617.725	

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 30 of 52

Linearity Graph:

Slope	54.30
y-intercept	-32.27
Correlation coefficient	0.9999
RRF	22.09

Conclusion:

1. The relative standard deviation for six replicate injections of Ramipril were 0.57% & Hydrochlorothiazide were 0.17%.
2. The correlation coefficient obtained for Ramipril, Ramipril Impurity A, Ramipril Impurity D and Ramipril Impurity E obtained from the graph were 1.0000, 0.9999, 1.0000 and 0.9999 respectively.

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 31 of 52

11.4. PRECISION

A) System Precision

LNB/Pg. No: (QbD-LNB-718, Pg. No-008)

Acceptance criteria:

- The relative standard deviation for six replicate injections is not more than 5.0%.

Standard details:

Ramipril		
Sr. No.	Retention Time	Peak Area
1	16.355	2334.829
2	16.365	2488.392
3	16.373	2334.761
4	16.373	2378.015
5	16.377	2342.541
6	16.375	2371.960
Average	16.370	2375.083
SD	0.0083	58.5876
%RSD	0.05	2.47

System suitability results		
% RSD of area	2.47	NMT 5.0%
% RSD of Area with Bkt Standard	2.18	NMT 5.0%

Hydrochlorothiazide		
Injection No.	Retention Time	Peak Area
1	5.178	3920.732
2	5.180	3922.535
3	5.187	3926.765
4	5.188	3920.454
5	5.188	3918.578
6	5.188	3928.551
Average	5.185	3922.936
SD	0.0046	3.9080
%RSD	0.09	0.10

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 32 of 52

System suitability results		
% RSD of area	0.10	NMT 5.0%
% RSD of Area with Bkt Standard	0.38	NMT 5.0%

Conclusion:

1. The relative standard deviation for six replicate injections for Ramipril were 2.47% & Hydrochlorothiazide were 0.10%.

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 33 of 52

B) Method precision

LNB/Pg. No: (QbD-LNB-718, Pg. No-008)

Acceptance Criteria:

1. The relative standard deviation for six replicate injections is not more than 5.0%.
2. The relative standard deviation for single maximum unspecified impurity and known impurities from six sample preparations should be NMT 15.0% and for total impurities should be NMT 10.0%

Standard details:

Ramipril		
Sr. No.	Retention Time	Peak Area
1	16.355	2334.829
2	16.365	2488.392
3	16.373	2334.761
4	16.373	2378.015
5	16.377	2342.541
6	16.375	2371.960
Average	16.370	2375.083
SD	0.0083	58.5876
%RSD	0.05	2.47

System suitability results		
% RSD of area	2.47	NMT 5.0%
% RSD of Area with Bkt Standard	2.18	NMT 5.0%

Hydrochlorothiazide		
Injection No.	Retention Time	Peak Area
1	5.178	3920.732
2	5.180	3922.535
3	5.187	3926.765
4	5.188	3920.454
5	5.188	3918.578
6	5.188	3928.551
Average	5.185	3922.936
SD	0.0046	3.9080
%RSD	0.09	0.10

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
Report Number	QbD-REP-592	Page No	Page 34 of 52

System suitability results		
% RSD of area	0.10	NMT 5.0%
% RSD of Area with Bkt Standard	0.38	NMT 5.0%

Sample details:

Sample	Ramipril Impurity E	Ramipril Impurity D	% Total Impurities
1	0.85	4.45	5.30
2	0.85	4.45	5.30
3	0.84	4.49	5.33
4	0.83	4.46	5.29
5	0.85	4.50	5.35
6	0.85	4.49	5.34
Average	0.85	4.47	5.32
SD	0.0100	0.0200	0.0200
%RSD	1.18	0.45	0.38
Limit	NMT 15 %		NMT 10 %

Conclusion:

1. The relative standard deviation for six replicate injections for Ramipril were 2.47% & Hydrochlorothiazide were 0.10%.
2. The relative standard deviation for Ramipril Impurity E, Ramipril Impurity D and Total impurities were 1.18%, 0.45% and 0.38% respectively for six sample solutions.

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 35 of 52

C) Intermediate precision

LNB/Pg. No: (QbD-LNB-703, Pg. No-102)

Acceptance Criteria:

1. The relative standard deviation for six replicate injections of standard solution should be not more than 5.0%.
2. Known impurity, single maximum unknown impurity and total impurities should meet the specification limits.
3. The relative standard deviation for single maximum unspecified impurity and known impurities from six sample preparations should be NMT 15.0% and for total impurities should be NMT 10.0%
4. Difference between average impurities results of two analysts,

Level of impurities, degradants (% Individually specified and total sum) (%, relative to active)	Acceptance criteria
<0.1	≤ 0.05% absolute
0.10 to 0.15	≤ 0.05% absolute
>0.15 to 0.30	≤ 0.10% absolute
>0.30 to 0.50	≤ 0.15% absolute
>0.50 to 0.80	≤ 0.20% absolute
>0.80 to 1.0	≤ 0.25% absolute
>1.0 to 5.0	≤ 20% relative
>5.0	≤ 10% relative

Standard details:

Ramipril		
Sr. No.	Retention Time	Peak Area
1	16.153	2465.087
2	16.191	2227.525
3	16.222	2303.521
4	16.156	2248.426
5	16.164	2276.692
6	16.115	2310.580
Average	16.167	2305.305
SD	0.0364	84.4570
%RSD	0.23	3.66


**ANALYTICAL METHOD VALIDATION REPORT FOR RELATED
SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD**
Report Number

QbD-REP-592

Page No

Page 36 of 52

System suitability results

% RSD of area	3.66	NMT 5.0%
% RSD of Area with Bkt Standard	3.41	NMT 5.0%

Hydrochlorothiazide

Injection No.	Retention Time	Peak Area
1	5.166	4480.832
2	5.177	4451.909
3	5.202	4419.675
4	5.176	4535.536
5	5.164	4382.866
6	5.135	4409.777
Average	5.170	4446.766
SD	0.0218	55.2311
%RSD	0.42	1.24

System suitability results

% RSD of area	1.24	NMT 5.0%
% RSD of Area with Bkt Standard	1.18	NMT 5.0%

Sample details:

Sample	Ramipril Impurity E	Ramipril Impurity D	% Total Impurities
1	0.95	4.13	5.08
2	0.99	4.12	5.11
3	0.97	4.15	5.12
4	0.95	4.12	5.07
5	0.98	4.11	5.09
6	0.96	4.14	5.10
Mean	0.97	4.13	5.10
SD	0.0200	0.0100	0.0200
%RSD	2.06	0.24	0.39
Limit	NMT 15 %		NMT 10 %

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 37 of 52

Difference between average impurities results of two analysts,

Name	Ramipril Impurity E	Ramipril Impurity D	% Total Impurities
Difference between method precision and intermediate precision	0.12	0.34	0.22

Conclusion:

1. The relative standard deviation for six replicate injections for Ramipril were 3.66% & Hydrochlorothiazide were 1.24%.
2. The relative standard deviation for Ramipril Impurity E, Ramipril Impurity D and Total impurities were 2.06%, 0.24% and 0.39% respectively for six sample solutions.
3. Difference between method precision and intermediate precision for Ramipril Impurity E, Ramipril Impurity D and Total impurities were 0.12%, 0.34% and 0.22% respectively.

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
Report Number	QbD-REP-592	Page No	Page 38 of 52

11.5. ACCURACY

LNB/Pg. No: (QbD-LNB-718, Pg. No-001)

Acceptance criteria:

1. The relative standard deviation for six replicate injections is not more than 5.0%.
2. % Recovery for LOQ level should be between 70.0% to 130% and other accuracy level should be between 80.0% to 120.0%.

Standard details:

Ramipril		
Sr. No.	Retention Time	Peak Area
1	16.127	2099.722
2	16.107	2079.038
3	16.100	2083.587
4	16.127	2104.412
5	16.133	2086.427
6	16.127	2090.498
Average	16.120	2090.614
SD	0.0133	9.7393
%RSD	0.08	0.47

System suitability results		
% RSD of area	0.47	NMT 5.0%
% RSD of Area with Bkt Standard	0.87	NMT 5.0%

Hydrochlorothiazide		
Injection No.	Retention Time	Peak Area
1	5.147	4385.000
2	5.150	4397.046
3	5.150	4397.991
4	5.150	4388.904
5	5.153	4387.979
6	5.153	4367.186
Average	5.151	4387.351
SD	0.0023	11.1549
%RSD	0.04	0.25

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 39 of 52

System suitability results		
% RSD of area	0.25	NMT 5.0%
% RSD of Area with Bkt Standard	0.28	NMT 5.0%

As such sample area details for Ramithiazide Impurity A:

Sample	Area
Sample 1	ND
Sample 2	ND
Sample 3	ND
Average	ND

Recovery details for Ramithiazide Impurity A:

Accuracy level (%)	Sample	Amount added (ppm)	Area	Corrected area	Amount recovered (ppm)	% Recovery	Mean % Recovery
LOQ	Sample 1	7.11	6182.408	ND	6.80	95.6	95.6
	Sample 2		6172.445	ND	6.80	95.6	
	Sample 3		6178.683	ND	6.80	95.6	
50	Sample 1	11.85	10248.555	ND	11.30	95.3	95.3
	Sample 2		10218.073	ND	11.30	95.3	
	Sample 3		10210.747	ND	11.30	95.3	
100	Sample 1	23.71	20487.535	ND	22.70	95.8	95.6
	Sample 2		20471.264	ND	22.60	95.3	
	Sample 3		20504.030	ND	22.70	95.8	
150	Sample 1	35.56	30939.252	ND	34.20	96.2	96.3
	Sample 2		30962.018	ND	34.30	96.5	
	Sample 3		30950.782	ND	34.20	96.2	


**ANALYTICAL METHOD VALIDATION REPORT FOR RELATED
SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD**
Report Number

QbD-REP-592

Page No

Page 40 of 52

As such sample area details for Ramithiazide Impurity E:

Sample	Area
Sample 1	418.297
Sample 2	414.109
Sample 3	425.468
Average	419.291

Recovery details for Ramithiazide Impurity E:

Accuracy level (%)	Sample	Amount added (ppm)	Area	Corrected area	Amount recovered (ppm)	% Recovery	Mean % Recovery
LOQ	Sample 1	5.92	687.928	268.637	5.70	96.3	99.1
	Sample 2		704.799	285.508	6.10	103.1	
	Sample 3		692.966	273.675	5.80	98.0	
50	Sample 1	9.86	903.913	484.622	10.30	104.4	105.4
	Sample 2		914.204	494.913	10.50	106.5	
	Sample 3		908.864	489.573	10.40	105.4	
100	Sample 1	19.73	1447.351	1028.060	21.80	110.5	110.7
	Sample 2		1440.957	1021.666	21.70	110.0	
	Sample 3		1452.617	1033.326	22.00	111.5	
150	Sample 1	29.59	1993.535	1574.244	33.50	113.2	113.9
	Sample 2		2015.388	1596.097	33.90	114.6	
	Sample 3		2005.915	1586.624	33.70	113.9	

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 41 of 52

As such sample area details for Ramithiazide Impurity D:

Sample	Area
Sample 1	38262.601
Sample 2	38302.940
Sample 3	38341.274
Average	38302.272

Recovery details for Ramithiazide Impurity D:

Accuracy level (%)	Sample	Amount added (ppm)	Area	Corrected area	Amount recovered (ppm)	% Recovery	Mean % Recovery
LOQ	Sample 1	17.55	56269.142	17966.870	21.10	120.2	120.0
	Sample 2		56204.114	17901.842	21.00	119.7	
	Sample 3		56268.997	17966.725	21.10	120.2	
50	Sample 1	29.25	67111.806	28809.534	33.80	115.6	115.8
	Sample 2		67149.189	28846.917	33.90	115.9	
	Sample 3		67186.512	28884.240	33.90	115.9	
100	Sample 1	58.49	94831.406	56529.134	66.30	113.4	113.6
	Sample 2		94865.375	56563.103	66.40	113.5	
	Sample 3		95030.461	56728.189	66.60	113.9	
150	Sample 1	87.74	122365.078	84062.806	98.70	112.5	112.6
	Sample 2		122440.906	84138.634	98.70	112.5	
	Sample 3		122567.945	84265.673	98.90	112.7	

Conclusion:

1. The relative standard deviation for six replicate injections for Ramipril were 0.47% & Hydrochlorothiazide were 0.25%.
2. % Recovery for Ramipril Impurity A, Ramipril Impurity E and Ramipril Impurity D for LOQ level to 150% level was within acceptance criteria.
3. From the above data, it can be concluded that the method is accurate over the range of LOQ to 150% for the determination of organic impurities test.

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 42 of 52

11.6. ROBUSTNESS

LNB/ Pg. No: (QbD-LNB-718, Pg. No-021)

Acceptance criteria:

1. The relative standard deviation for six replicate injections is not more than 5.0%.
2. Difference between average impurities results of Robustness data and Precision data,

Level of impurities, degradants (% Individually specified and total sum) (%, relative to active)	Acceptance criteria
<0.1	≤ 0.05% absolute
0.10 to 0.15	≤ 0.05% absolute
>0.15 to 0.30	≤ 0.10% absolute
>0.30 to 0.50	≤ 0.15% absolute
>0.50 to 0.80	≤ 0.20% absolute
>0.80 to 1.0	≤ 0.25% absolute
>1.0 to 5.0	≤ 20% relative
>5.0	≤ 10% relative

Standard details for Ramipril:

Robustness parameter	% RSD	% RSD with Bkt Standard
Flow rate (0.9 mL/min)	0.80	0.78
Flow rate (1.1 mL/min)	1.85	1.73
Buffer pH (2.4)	0.30	0.34
Buffer pH (2.6)	0.36	0.33

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 43 of 52

Standard details for Hydrochlorothiazide:

Robustness parameter	% RSD	% RSD with Bkt Standard
Flow rate (0.8 mL/min)	0.15	0.20
Flow rate (1.2 mL/min)	0.17	0.15
Buffer pH (2.4)	0.12	0.13
Buffer pH (2.6)	0.07	0.07

%Impurities details:

Robustness parameter	Ramipril Impurity E	Ramipril Impurity D	Total % Impurities
Precision	0.85	4.47	5.32
Flow rate (0.8 mL/min)	0.81	4.13	4.94
Flow rate (1.2 mL/min)	0.84	4.17	5.01
Buffer pH (2.4)	0.79	4.20	4.99
Buffer pH (2.6)	0.79	4.21	5.00

% Difference:

Robustness parameter	Ramipril Impurity E	Ramipril Impurity D	Total % Impurities
Precision	NA	NA	NA
Flow rate (0.8 mL/min)	0.04	0.34	0.38
Flow rate (1.2 mL/min)	0.01	0.30	0.31
Buffer pH (2.4)	0.06	0.27	0.33
Buffer pH (2.6)	0.06	0.26	0.32

Conclusion:

1. The relative standard deviation for six replicate injections was not more than 5.0%.
2. The difference between average results of method precision and robustness data was within acceptance criteria.
3. The above results indicate that the analytical method is robust for all variable conditions outlined in the above table.

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 44 of 52

11.7. SOLUTION STABILITY**LNB/Pg. No: (QbD-LNB-718, Pg. No-008)****Acceptance criteria:**

1. The relative standard deviation for six replicate injections is not more than 5.0%.
2. RSD of area response of repeatedly injected standard solution over a period of 24 hrs should be not more than 5.0%.
3. Difference between impurities results of Initial sample and repeatedly injected sample solution over a period of 24 hrs,

Standard details:

Ramipril		
Injection No.	Retention Time	Peak Area
1	16.355	2334.829
2	16.365	2488.392
3	16.373	2334.761
4	16.373	2378.015
5	16.377	2342.541
6	16.375	2371.960
Average	16.370	2375.083
SD	0.0083	58.5876
%RSD	0.05	2.47

System suitability results for Ramipril		
% RSD of area	2.47	NMT 5.0%
% RSD of Area with Bkt Standard	1.82	NMT 5.0%

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 45 of 52

Hydrochlorothiazide		
Injection No.	Retention Time	Peak Area
1	5.178	3920.732
2	5.180	3922.535
3	5.187	3926.765
4	5.188	3920.454
5	5.188	3918.578
6	5.188	3928.551
Average	5.185	3922.936
SD	0.0046	3.9080
%RSD	0.09	0.10

System suitability results for Hydrochlorothiazide		
% RSD of area	0.10	NMT 5.0%
% RSD of Area with Bkt Standard	0.52	NMT 5.0%

Standard solution stability:

Time intervals	Standard Area
Standard (Initial)	2353.728
Standard (10 Hrs)	2396.874
Standard (16 Hrs)	2355.192
Standard (21 Hrs)	2348.193
Standard (27 Hrs)	2338.447
Average	2358.487
SD	22.4423
%RSD	0.95

Sample solution stability:

Time intervals	Ramipril Impurity E	Ramipril Impurity A	Ramipril Impurity D
Initial	0.88	1.12	4.50
10 Hrs	0.89	1.12	4.51
16 Hrs	0.89	1.12	4.54
21 Hrs	0.90	1.12	4.52
27 Hrs	0.88	1.12	4.54

 Quality by Design	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No

% Difference:

Time intervals	Ramipril Impurity E	Ramipril Impurity A	Ramipril Impurity D
Initial	NA	NA	NA
10 Hrs	0.01	0.00	0.01
16 Hrs	0.01	0.00	0.04
21 Hrs	0.02	0.00	0.02
27 Hrs	0.00	0.00	0.04

Conclusion:

1. The relative standard deviation for six replicate injections for Ramipril were 2.47% & Hydrochlorothiazide were 0.10%.
2. The related standard deviation of area response of repeatedly injected standard solution over a period of 27 hrs were 0.95%.
3. Difference between impurity results of initial samples and repeatedly injected sample solution over a period of 27 hrs. for % Ramipril Impurity E, Ramipril Impurity A and Ramipril Impurity D were 0.00%, 0.00% and 0.03% respectively.
4. The above results indicate that the Standard and sample solution is stable for 27 hrs at room temperature.

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 47 of 52

11.8. FILTER STUDY

LNB/Pg. No: (QbD-LNB-718, Pg. No-008)

Acceptance criteria:

1. The relative standard deviation for six replicate injections is not more than 5.0%.
2. Difference for impurities between filtered solution through different filters and centrifuged sample,

Level of impurities, degradants (% Individually specified and total sum) (%, relative to active)	Acceptance criteria
<0.1	< 0.05% absolute
0.10 to 0.15	< 0.05% absolute
>0.15 to 0.30	< 0.10% absolute
>0.30 to 0.50	< 0.15% absolute
>0.50 to 0.80	< 0.20% absolute
>0.80 to 1.0	< 0.25% absolute
>1.0 to 5.0	< 20% relative
>5.0	< 10% relative

Standard details:

Ramipril		
Injection No.	Retention Time	Peak Area
1	16.355	2334.829
2	16.365	2488.392
3	16.373	2334.761
4	16.373	2378.015
5	16.377	2342.541
6	16.375	2371.960
Average	16.370	2375.083
SD	0.0083	58.5876
%RSD	0.05	2.47

System suitability results for Ramipril		
% RSD of area	2.47	NMT 5.0%
% RSD of Area with Bkt Standard	2.06	NMT 5.0%

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 48 of 52

Hydrochlorothiazide		
Injection No.	Retention Time	Peak Area
1	5.178	3920.732
2	5.180	3922.535
3	5.187	3926.765
4	5.188	3920.454
5	5.188	3918.578
6	5.188	3928.551
Average	5.185	3922.936
SD	0.0046	3.9080
%RSD	0.09	0.10

System suitability results for Hydrochlorothiazide		
% RSD of area	0.10	NMT 5.0%
% RSD of Area with Bkt Standard	0.45	NMT 5.0%

Sample Data:

Sample details	Ramipril Impurity E	Ramipril Impurity D	Total % Impurities
Centrifuged Sample	0.84	4.49	5.33
0.45 μ PVDF	0.85	4.50	5.35
0.45 μ Nylon	0.85	4.50	5.35
Whatman no.41	0.85	4.51	5.36

% Difference:

Sample details	Ramipril Impurity E	Ramipril Impurity D	Total % Impurities
Centrifuged Sample	NA	NA	NA
0.45 μ PVDF	0.01	0.01	0.02
0.45 μ Nylon	0.01	0.01	0.02
Whatman no.41	0.01	0.02	0.03

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 49 of 52

Conclusion:

1. The relative standard deviation for six replicate injections for Ramipril were 2.47% & Hydrochlorothiazide were 0.10%.
2. The difference of impurities results between filtered solution through different filters and centrifuged samples met acceptance criteria.

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 50 of 52

12. BATCH ANALYSIS RESULTS

Standard details:

Ramipril		
Injection No.	Retention Time	Peak Area
1	16.807	2297.079
2	16.764	2318.205
3	16.756	2219.142
4	16.829	2343.709
5	16.761	2299.695
6	16.749	2339.516
Average	16.778	2302.891
SD	0.0324	45.3870
%RSD	0.19	1.97

System suitability results for Ramipril		
% RSD of area	1.97	NMT 5.0%
% RSD of Area with Bkt Standard	1.75	NMT 5.0%

Hydrochlorothiazide		
Injection No.	Retention Time	Peak Area
1	6.324	4326.284
2	6.274	4346.335
3	6.332	4359.288
4	6.319	4381.489
5	6.331	4358.936
6	6.279	4347.931
Average	6.310	4353.377
SD	0.0263	18.2724
%RSD	0.42	0.42

System suitability results for Hydrochlorothiazide		
% RSD of area	0.42	NMT 5.0%
% RSD of Area with Bkt Standard	2.38	NMT 5.0%

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 51 of 52

Method precision sample details:**(Ramithiazide 5/12.5 mg Batch no.: GP181203)**

Sample	Ramipril Impurity E	Ramipril Impurity D	% Total Impurities
1	0.18	1.93	2.27
2	0.18	1.92	2.26
3	0.15	1.93	2.24
4	0.18	1.93	2.28
5	0.18	1.92	2.26
6	0.17	1.92	2.26
Average	0.17	1.93	2.26
SD	0.0100	0.0100	0.0100
%RSD	5.88	0.52	0.44
Limit	NMT 15 %		NMT 10 %

Method precision sample details:**(Ramithiazide 10/12.5 mg Batch no.: GP181206)**

Sample	Ramipril Impurity E	Ramipril Impurity D	% Total Impurities
1	0.15	1.37	1.52
2	0.14	1.36	1.50
3	0.15	1.35	1.50
4	0.14	1.37	1.51
5	0.15	1.36	1.51
6	0.14	1.35	1.49
Average	0.15	1.36	1.51
SD	0.0100	0.0100	0.0100
%RSD	6.67	0.74	0.66
Limit	NMT 15 %		NMT 10 %

13. REFERENCES

ICH guidelines

	ANALYTICAL METHOD VALIDATION REPORT FOR RELATED SUBSTANCES IN RAMITHIAZIDE TABLET 10/25 MG BY HPLC METHOD		
	Report Number	QbD-REP-592	Page No Page 52 of 52

14. REVISION HISTORY

Version	Date	Changes	Reason for change
00		Not Applicable	New

15. INCIDENTS

Sr. No.	Incidence No.	Incidence Details
1	N/A	N/A

16. OVERALL CONCLUSION

The results of the analytical method validation for Related substances in Ramithiazide Tablet 10/25 mg, adequately supports that the validated method is specific, precise, accurate, linear and robust and suitable for Related substances analysis in the subject drug product.