

Artemether

HPLC Method has been validated for Assay of Artemether (Specification No: ADL/FP/050) and method involves analysis of Artemether using HPLC column, Zorbax C18 250 x 4.6mm, 5 μ m or equivalent. Validation parameters such as specificity, precision, linearity, accuracy, intermediate precision, robustness and stability of analytical sample solution were studied.

Specificity and system suitability:

System suitability has been studied using six replicate injections of Artemether working standard and relative standard deviation of peak areas for six replicate injections were calculated and the results are presented in **Table No. 1.01**

System precision: System precision was carried using six replicate injections of working standard of Artemether and to calculate % RSD of peak area of six replicate injections the observations are given in **Table No 1.02**

Method precision:

Method precision study was carried by using six replicate injections of working standard of Artemether and relative standard deviation of peak area of six replicate injections determined and the observations are given in **Table No.1.03**

Linearity: To calculate the correlation of coefficient (r) Linearity study was carried using five different concentration levels 5000 ppm, 8000 ppm, 10000 ppm, 12000 ppm and 15000 ppm. of Artemether and the observations are given in **Table No. 1.04.**

Accuracy: Accuracy of the method has been studied using three different concentration levels 7000 ppm, 10000ppm and 13000 ppm of Artemether in presence of fixed concentration of impurities. The results obtained are presented in **Table No1.050, 1.051, 1.052, 1.053 and 1.054.**

Intermediate precision: Intermediate precision of the method was studied for analysis of Artemether sample on different instruments, by different researcher on different day and the data is presented in **Table No.1.060 and 1.061**

Robustness: Robustness of the method was studied by varying the flow rate, pH of buffer and mobile phase .The findings are depicted in **Table No. 1.070,1.071, 1.072, 1.073, 1.074 ,1.075, 1.076, 1.077, 1.078, 1.0790, 1.0791, 1.0792 and 1.0793**

Stability of analytical sample Solution: Analytical sample solution stability of Artemether was carried for different time interval for initial (0 hrs), 6 hours,12 hours 18 hours and 24 hrs for standard and sample solution and the findings are given in **Table No.1.080,1.081, 1.082, 1.083, 1.084, 1.085, 1.086, 1.087, 1.088, 1.0890, 1.0891 and 1.0892 .**

Limit of detection and Limit of quantification:

LOD and LOQ method can be used for cleaning validation. In the present study the findings are given in **Table .No. 1.090**

Table No 1.01: % RSD for System Suitably.

Working Standard	Wt. of Standard (mg)	Area
Injection-1	250.09	4152.16
Injection-2	--	4152.02
Injection-3	--	4155.72
Injection-4	--	4150.38
Injection-5	--	4153.39
Injection-6	--	4154.95
AVG	--	4153.10
SD	--	1.99
%RSD	--	0.04

Table No 1.02: % RSD for System Precision.

Working Standard	Wt. of Standard (mg)	Area
Injection-1	250.10	4171.09
Injection-2	--	4168.80
Injection-3	--	4170.04
Injection-4	--	4166.80
Injection-5	--	4166.11
Injection-6	--	4166.83
AVG	--	4168.28
SD	--	2.01
%RSD	--	0.05

Table No 1.03: Method Precision.

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
1	250.91	4201.21	99.86	99.98
2	250.85	4199.11	99.84	99.96
3	250.61	4177.94	99.43	99.55
4	250.53	4174.28	99.37	99.49
5	250.10	4171.83	99.48	99.60
6	250.03	4171.93	99.51	99.63
AVG	--	4182.72	99.58	99.70
SD	--	13.71	0.21	0.21
%RSD	--	0.33	0.21	0.21

Table No 1.04: Linearity

Injection No.	Level-1 (5000 ppm)	Level-2 (8000 ppm)	Level-3 (10000 ppm)	Level-4 (12000 ppm)	Level-5 (15000 ppm)
1	2081.25	3293.92	4124.73	4950.73	6201.30
2	2078.63	3294.60	4128.09	4952.67	6199.71
3	2080.80	3295.98	4126.74	4950.54	6197.87
AVG	2080.23	3294.83	4126.52	4951.31	6199.63
SD	1.40	1.05	1.69	1.18	1.72
%RSD	0.07	0.03	0.04	0.02	0.03
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Table No 1.050 Accuracy. (Sample)

Accuracy Levels	Wt. of sample (mg)	Artemisinin Imp. (500 ppm)	Dihydroartemisinin Imp. (500 ppm)	Alpha-Artemether imp (500 ppm)	Dilution with diluent
Level-1	W1 350.41	1.5 ml	1.5 ml	1.5 ml	50 ml
	W2 350.52	1.5 ml	1.5 ml	1.5 ml	50 ml
	W3 350.35	1.5 ml	1.5 ml	1.5 ml	50 ml
Level-2	W1 497.03	1.5 ml	1.5 ml	1.5 ml	50 ml
	W2 497.55	1.5 ml	1.5 ml	1.5 ml	50 ml
	W3 498.01	1.5 ml	1.5 ml	1.5 ml	50 ml
Level-3	W1 649.74	1.5 ml	1.5 ml	1.5 ml	50 ml
	W2 649.78	1.5 ml	1.5 ml	1.5 ml	50 ml
	W3 649.75	1.5 ml	1.5 ml	1.5 ml	50 ml

Table No 1.051 Accuracy (Standard)

Working Standard	Wt. of Standard (mg)	Area
Injection-1	500.86	4177.98
Injection-2	--	4179.98
Injection-3	--	4178.52

Injection-4	--	4183.29
Injection-5	--	4178.88
Injection-6	--	4180.54
AVG	--	4179.87
SD	--	1.93
%RSD	--	0.05

Table No 1.052 Accuracy Level-1

Accuracy Level	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
Level-1	W1 350.41	2909.39	98.89	99.01
	W2 350.52	2913.32	99.00	99.11
	W3 350.35	2908.04	98.86	98.98
	AVG	2910.25	98.92	99.04
	SD	2.74	0.07	0.07
	%RSD	0.06	0.07	0.07

Table No 1.053 Accuracy Level-2

Accuracy Level	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
Level-2	W1 497.03	4098.23	98.21	98.33
	W2 497.55	4099.35	98.13	98.25
	W3 498.01	4109.50	98.29	98.40
	AVG	4102.36	98.21	98.33
	SD	6.21	0.08	0.08
	%RSD	0.15	0.08	0.08

Table No 1.054 Accuracy Level-3

Accuracy Level	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
Level-3	W1 649.74	5397.99	98.95	99.07
	W2 649.78	5401.25	99.01	99.13
	W3 649.75	5427.23	99.49	99.61
	AVG	5408.82	99.15	99.27
	SD	16.02	0.29	0.29
	%RSD	0.30	0.30	0.30

Table No 1.060: Intermediate Precision: Standard

Working Standard	Wt. of Standard (mg)	Area
Injection-1	250.25	4092.26
Injection-2	--	4089.62
Injection-3	--	4085.39
Injection-4	--	4086.77
Injection-5	--	4080.15
Injection-6	--	4078.22
AVG	--	4085.40
SD	--	5.40
%RSD	--	0.13

Table No 1.061: Intermediate Precision: Samples

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried
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				basis)
1	250.91	4076.69	99.93	99.05
2	250.85	4075.03	98.91	99.03
3	250.61	4086.91	99.29	99.41
4	250.12	4085.98	99.47	99.59
5	250.23	4088.54	99.48	99.60
6	250.89	4086.76	99.18	99.30
AVG	--	4083.32	99.21	99.33
SD	--	5.86	0.25	0.25
%RSD	--	0.14	0.25	0.25

Table No 1.070: Robustness:

Sr. No.	Parameters	Standard parameter	Changes studied	
1	Flow rate	1.5 ml/min.	1.4 ml/min.	1.6 ml/min.
2	Mobile phase composition	ACN :Water 620 : 380	ACN:Water 600 : 400	ACN:Water 640 : 360
3	Column temperature	25°C	20°C	30°C

Table No 1.071: Robustness. (Standard). :

Flow rate 1.6 ml/min.

Working Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	250.73	10.00	3916.70
Injection-2	--	10.02	3910.07
Injection-3	--	10.02	3909.15

Injection-4	--	10.02	3910.22
Injection-5	--	10.03	3912.22
Injection-6	--	10.02	3906.60
AVG	--	10.02	3910.83
SD	--	0.01	3.41
%RSD	--	0.10	0.09

Table No 1.072: Robustness. (Samples)

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	250.12	10.03	3904.57	99.48	99.60
2	252.45	10.02	3984.69	100.59	100.71
3	250.95	10.02	3950.74	100.33	100.45
4	250.10	10.02	3890.90	99.14	99.26
5	250.05	10.02	3892.30	99.20	99.32
6	250.97	10.02	3905.18	99.16	99.28
AVG	--	10.02	3921.40	99.65	99.77
SD	--	0.00	37.92	0.64	0.64
%RSD	--	0.04	0.97	0.64	0.65

Table No 1.073: Robustness. (Standard). :**Flow rate 1.4 ml/min.**

Working Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	250.23	11.44	4467.57
Injection-2	--	11.44	4460.84
Injection-3	--	11.45	4462.52
Injection-4	--	11.45	4458.42
Injection-5	--	11.45	4461.55
Injection-6	--	11.45	4464.72
AVG	--	11.45	4462.60
SD	--	0.01	3.19
%RSD	--	0.05	0.07

Table No 1.074: Robustness. (Samples)

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	250.14	11.46	4470.47	99.61	99.73
2	250.65	11.46	4459.08	99.16	99.27
3	250.14	11.47	4465.19	99.49	99.61
4	250.47	11.46	4458.84	99.22	99.34
5	250.58	11.46	4473.49	99.50	99.62
6	250.21	11.45	4450.40	99.14	99.26
AVG	--	11.46	4462.91	99.35	99.47
SD	--	0.01	8.51	0.21	0.21
%RSD	--	0.06	0.19	0.21	0.21

Table No 1.075: Robustness. (Standard). :**Acetonitrile: Water (600: 400)**

Working Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	250.38	12.45	4210.82
Injection-2	--	12.45	4210.56

Injection-3	--	12.45	4201.41
Injection-4	--	12.45	4198.65
Injection-5	--	12.45	4204.43
Injection-6	--	12.46	4210.82
AVG	--	12.45	4206.12
SD	--	0.00	5.38
%RSD	--	0.03	0.13

Table No 1.076: Robustness. (Samples)

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	250.02	12.45	4191.54	99.20	99.32
2	250.29	12.45	4188.43	99.02	99.14
3	250.41	12.45	4196.49	99.16	99.28
4	248.67	12.46	4176.00	99.37	99.49
5	250.11	12.46	4190.62	99.14	99.26
6	249.82	12.46	4183.17	99.08	99.20
AVG	--	12.46	4187.71	99.16	99.28
SD	--	0.01	7.19	0.12	0.12
%RSD	--	0.04	0.17	0.12	0.12

Table No 1.077: Robustness. (Standard). :

Acetonitrile: Water (640: 360)

Working Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	250.89	9.84	4199.06
Injection-2	--	9.84	4202.96
Injection-3	--	9.84	4195.79
Injection-4	--	9.83	4202.32

Injection-5	--	9.84	4202.25
Injection-6	--	9.83	4202.65
AVG	--	9.84	4200.84
SD	--	0.01	2.85
%RSD	--	0.05	0.07

Table No 1.078: Robustness. (Samples)

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	249.36	9.84	4170.19	99.28	99.40
2	248.64	9.83	4166.60	99.48	99.60
3	250.08	9.83	4173.37	99.07	99.19
4	248.25	9.83	4147.62	99.18	99.30
5	247.46	9.83	4138.49	99.28	99.40
6	248.82	9.83	4155.52	99.15	99.26
AVG	--	9.83	4158.63	99.24	99.36
SD	--	0.00	13.79	0.14	0.14
%RSD	--	0.04	0.33	0.14	0.14

Table No 1.079: Robustness. (Standard). :

Column temperature 20°C

Working Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	250.41	11.02	4152.71
Injection-2	--	11.02	4148.21
Injection-3	--	11.03	4150.91
Injection-4	--	11.04	4149.25
Injection-5	--	11.04	4152.89
Injection-6	--	11.05	4154.80
AVG	--	11.03	4151.46

SD	--	0.01	2.47
%RSD	--	0.11	0.06

Table No 1.0791: Robustness. (Samples)

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	250.12	11.07	4145.23	99.37	99.49
2	250.56	11.09	4153.78	99.40	99.52
3	250.43	11.10	4158.64	99.56	99.68
4	250.05	11.10	4147.19	99.44	99.56
5	250.23	11.10	4160.36	99.68	99.80
6	250.34	11.11	4145.46	99.28	99.40
AVG	--	11.10	4151.78	99.46	99.58
SD	--	0.01	6.76	0.15	0.15
%RSD	--	0.12	0.16	0.15	0.15

Table No 1.0792: Robustness. (Standard). :

Column temperature :30°C

Working Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	250.98	10.25	4269.57
Injection-2	--	10.26	4259.86
Injection-3	--	10.25	4257.56
Injection-4	--	10.26	4252.95
Injection-5	--	10.26	4253.63
Injection-6	--	10.26	4256.65
AVG	--	10.26	4258.37
SD	--	0.01	6.05

%RSD	--	0.05	0.14
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Table No 1.0793: Robustness. (Samples)

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	249.46	10.27	4211.95	98.92	99.03
2	250.65	10.27	4241.25	99.13	99.25
3	249.45	10.27	4217.50	99.05	99.17
4	245.52	10.28	4112.32	98.12	98.24
5	250.85	10.27	4261.91	99.53	99.65
6	249.64	10.28	4215.24	98.92	99.04
AVG	--	10.27	4210.03	98.95	99.07
SD	--	0.01	51.58	0.46	0.46
%RSD	--	0.05	1.23	0.47	0.47

Table No 1.080: Solution stability (Standard.). Time Period: 0 hr

Working Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	250.58	10.92	4186.48
Injection-2	--	10.93	4179.13
Injection-3	--	10.92	4175.26
Injection-4	--	10.92	4177.18
Injection-5	--	10.92	4177.82
Injection-6	--	10.92	4178.55
AVG	--	10.92	4179.07
SD	--	0.00	3.87
%RSD	--	0.04	0.09

Table No 1.081: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	250.01	10.94	4178.72	99.62	99.74

Table No 1.082: Solution stability (Standard.). Time Period: 5.0 hr

Working Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	250.85	10.94	4168.45
Injection-2	--	10.95	4164.44
Injection-3	--	10.95	4165.15
Injection-4	--	10.95	4156.01
Injection-5	--	10.95	4164.29
Injection-6	--	10.95	4132.14
AVG	--	10.95	4158.41
SD	--	0.00	13.51
%RSD	--	0.04	0.32

Table No 1.083: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1.	250.01	10.95	4136.04	99.20	99.32

Table No 1.084: Solution stability (Standard.). Time Period: 10.0 hr

Working Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	250.99	10.95	4183.34
Injection-2	--	10.92	4190.39
Injection-3	--	10.92	4196.44
Injection-4	--	10.94	4196.75
Injection-5	--	10.91	4200.58
Injection-6	--	10.92	4204.03
AVG	--	10.93	4195.26
SD	--	0.02	7.41
%RSD	--	0.14	0.18

Table No 1.085: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	250.01	10.92	4159.66	98.94	99.06

Table No 1.086: Solution stability (Standard.). Time Period: 15.0 hr

Working Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	250.75	10.91	4188.17
Injection-2	--	10.91	4190.66
Injection-3	--	10.91	4190.66
Injection-4	--	10.92	4189.77
Injection-5	--	10.91	4192.04
Injection-6	--	10.92	4197.05
AVG	--	10.91	4191.39
SD	--	0.01	3.05
%RSD	--	0.05	0.07

Table No 1.087: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	250.01	10.95	4158.51	98.91	99.03

Table No 1.088: Solution stability (Standard.). Time Period: 20.0 hr

Working Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	250.23	10.92	4185.89
Injection-2	--	10.92	4183.33
Injection-3	--	10.92	4181.82
Injection-4	--	10.92	4184.77
Injection-5	--	10.92	4184.74
Injection-6	--	10.92	4183.92
AVG	--	10.92	4184.08
SD	--	0.00	1.41
%RSD	--	0.00	0.03

Table No 1.0890: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	250.01	10.92	4159.10	98.89	99.01

Table No 1.0891: Solution stability (Standard.). Time Period: 25.0 hr

Working Standard	Wt. of Standard (mg)	Retention Time	Area
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Injection-1	248.00	10.92	4157.04
Injection-2	--	10.92	4120.86
Injection-3	--	10.92	4139.75
Injection-4	--	10.92	4141.43
Injection-5	--	10.92	4156.32
Injection-6	--	10.92	4163.13
AVG	--	10.92	4146.42
SD	--	0.00	15.57
%RSD	--	0.00	0.38

Table No 1.0892: Solution stability (Sample.).

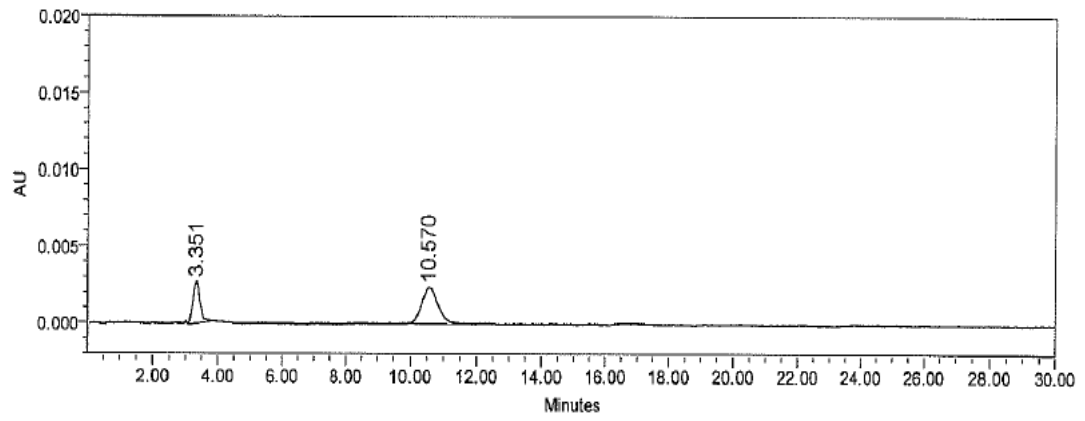
Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	250.01	10.92	4161.47	98.96	99.08

Table No 1.090: LOD/LOQ:

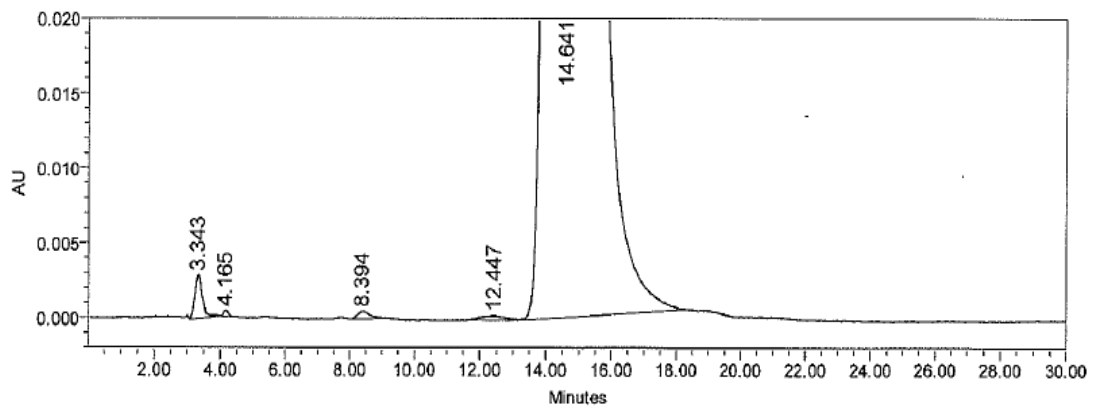
Levels	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8

Conc. in ppm	25 ppm	12.5 ppm	10 ppm	7.5 ppm	5.0 ppm	2.5ppm	1.0 ppm	0.75ppm
Injection - 1	10.85	5.42	4.52	3.61	2.19	1.31	0.70	0.60
Injection - 2	10.96	5.49	4.57	3.59	2.17	1.24	0.77	0.59
Injection - 3	10.95	5.48	4.54	3.63	2.10	1.22	0.75	0.69
Injection - 4	10.96	5.51	4.53	3.87	2.05	1.33	0.87	0.61
Injection - 5	10.95	5.52	4.47	3.54	2.06	1.16	0.91	0.65
Injection - 6	10.83	5.47	4.46	3.73	2.02	1.04	1.07	0.41
AVG	10.92	5.48	4.52	3.66	2.10	1.22	0.85	0.59
SD	0.06	0.04	0.04	0.12	0.07	0.11	0.14	0.10
%RSD	0.55	0.65	0.94	3.27	3.27	8.74	15.95	16.30

Artemether Standard



Artemether Sample



Flucytosine

HPLC Method has been validated for Assay of Flucytosine (Specification No: ADL/TS/FP/095) and method involves analysis of Flucytosine using HPLC column, Phenomenex Luna 5 μ NH₂ 100A 250 x 4.6 mm, 5 μ m.

Validation parameters such as specificity, precision, linearity, accuracy, limit of detection, limit of quantification, intermediate precision, robustness and stability of analytical sample solution were studied.

Specificity:

System suitability has been studied using six replicate injections of Zoledronic acid working standard and relative standard deviation of peak areas for six replicate injections were calculated and the results are presented in **Table No 2.01**

System precision:

System precision was carried using six replicate injections of working standard of Flucytosine and % RSD of peak area of six replicate injections the observation are given in **Table No. 2.02.**

Linearity:

Linearity study was carried by using five different concentration levels 25 ppm, 40 ppm, 50 ppm, 60 ppm and 75 ppm the results are presented in **Table No. 2.03.**

Accuracy:

Accuracy has been studied using three levels 35 ppm, 50 ppm and 65 ppm of Flucytosine. The observation made during the study is given in **Table No. 2.040, 2.041, 2.042, and 2.043.**

Intermediate precision: Intermediate precision of the method was carried by analysis of Flucytosine sample on different instruments; by different analyst on different day the results are shown in **Table No. 2.050 and 2.051** for % RSD of assay values and for deciding whether the method is rugged.

Robustness:

Robustness of the method was studied by varying the flow rate, pH of buffer and mobile phase. The findings are depicted in **Table No 2.060, 2.061, 2.062, 2.063, 2.064, 2.065, 2.066, 2.067, 2.068, 2.0690, 2.0691, 2.0692, and 2.0693.**

Stability of analytical sample Solution:

Analytical sample solution stability of Flucytosine was carried for different time interval for initial (0 hrs), 6 hours, 12 hours, 18 hours and 24 hrs for standard and sample solution and the findings are given in **Table No.2.070, 2.071, 2.072, 2.073, 2.074, 2.075, 2.076, 2.077, 2.078 and 2.079. Limit of detection and Limit of quantification:**

Limit of detection and Limit of quantification were determined by using different concentration of Flucytosine from Level 1 to Level 5 with varying concentration from 0.1 ppm to 0.005 ppm. The findings are given in **Table No 2.080.**

Table No 2.01: % RSD for System Suitably.

Working Standard	Wt. of Standard (mg)	Area
Injection-1	48.12 mg	1883.00
Injection-2	--	1880.88
Injection-3	--	1881.90
Injection-4	--	1884.62
Injection-5	--	1882.32
Injection-6	--	1882.69
AVG	--	1882.57
SD	--	1.25
%RSD	--	0.07

Table No2.02: % RSD for System Precision.

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
1	49.00	1915.17	99.58	99.93
2	49.69	1937.86	99.36	99.71
3	50.00	1949.59	99.34	99.70
4	53.89	2107.06	99.61	99.97
5	49.12	1909.46	99.04	99.39
6	48.15	1877.31	99.33	99.69
AVG	--	--	99.37	99.73
SD	--	--	0.21	0.21
%RSD	--	--	0.21	0.21

Table No 2.03: Linearity

Injection No.	Level-1 (25 ppm)	Level-2 (40 ppm)	Level-3 (50 ppm)	Level-4 (60 ppm)	Level-5 (75 ppm)
1	919.01	1466.89	1830.76	2210.76	2756.92
2	919.93	1466.37	1830.92	2212.81	2757.38
3	920.10	1466.82	1831.76	2210.17	2759.70
AVG	919.68	1466.69	1831.15	2211.25	2758.00
SD	0.59	0.28	0.54	1.39	1.49
%RSD	0.06	0.02	0.03	0.06	0.05
--	--	--	--	Correlation(r)	1.000

Table No 2.040 Accuracy. (Standard.)

Working Standard	Wt. of Standard (mg)	Area
Injection-1	50.49 mg	1833.14
Injection-2	--	1831.02

Injection-3	--	1834.89	
Injection-4	--	1836.75	
Injection-5	--	1838.90	
Injection-6	--	1832.19	
AVG	--	1834.48	
SD	--	2.96	
%RSD	--	0.26	0.16

Table No 2.041 Accuracy Level-1

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
Sample-1	35.72	1295.96	99.53	99.89
Sample-2	35.51	1289.31	99.60	99.96
Sample-3	35.49	1287.88	99.55	99.91
AVG	--	--	--	--
SD	--	--	--	--
%RSD	--	--	--	--

Table No 2.042 Accuracy Level-2

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
Sample-1	50.19	1823.12	99.64	100.00
Sample-2	50.61	1836.13	99.52	99.88
Sample-3	50.21	1822.07	99.55	99.91
AVG	--	--	--	--
SD	--	--	--	--

%RSD	--	--	--	--
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Table No 2.043 Accuracy Level-3

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
Sample-1	66.25	2404.37	99.56	99.92
Sample-2	66.29	2406.36	99.58	99.94
Sample-3	65.51	2377.21	99.54	99.90
AVG	--	--	--	--
SD	--	--	--	--
%RSD	--	--	--	--

Table No 2.050: Intermediate system precision – (Standard)

Working Standard	Wt. of Standard (mg)	Area
Injection-1	49.12	1931342.00
Injection-2	--	1934304.00
Injection-3	--	1929631.00
Injection-4	--	1933176.00
Injection-5	--	1933807.00
Injection-6	--	1930941.00

AVG	--	1932200.17
SD	--	1837.54
%RSD	--	0.10

Table No 2.051: Intermediate system precision – (Samples)

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
1	49.45	1948339.00	99.83	100.19
2	50.00	1970930.00	99.88	100.24
3	50.35	1979507.00	99.62	99.98
4	53.29	2099614.00	99.83	100.19
5	49.12	1930371.00	99.58	99.94
6	48.59	1913075.00	99.76	100.12
AVG	--	--	99.75	100.11
SD	--	--	0.13	0.13
%RSD	--	--	0.13	0.13

--	--	--	--	--

Table No 2.060: Robustness:

Sr. No.	Parameters	Standard parameter	Changes studied	
1	Flow rate	1.0 ml/min.	0.9 ml/min.	1.1 ml/min.
2	PH of Buffer	2.5	2.4	2.6
3	Mobile phase composition	Buffer : ACN 200 : 800	Buffer : ACN 180 : 820	Buffer : ACN 220 : 780

Table No 2.061: Robustness. (Standard). :

Flow rate 0.9 ml/min.

Working Standard	Wt. of Standard (mg)	Area	
Injection-1	49.95	2195.77	
Injection-2	--	2193.15	
Injection-3	--	2192.69	
Injection-4	--	2196.38	
Injection-5	--	2196.22	
Injection-6	--	2201.95	
AVG	--	2196.03	
SD	--	3.31	
%RSD	--	0.06	0.15

Table No 2.062: Robustness. (Samples)

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
1	50.19	2198.77	99.32	99.68
2	52.95	2322.59	99.44	99.80
3	51.15	2242.49	99.39	99.75
4	50.02	2193.72	99.43	99.79
5	49.49	2170.73	99.44	99.80
6	49.35	2162.07	99.32	99.68
AVG	--	--	99.39	99.75
SD	--	--	0.06	0.06
%RSD	--	--	0.06	0.06

Table No 2.063: Robustness. (Standard). :

Flow rate 1.1 ml/min.

Working Standard	Wt. of Standard (mg)	Area
Injection-1	49.85	1847.82
Injection-2	--	1848.44
Injection-3	--	1856.69
Injection-4	--	1852.55
Injection-5	--	1854.54
Injection-6	--	1858.36
AVG	--	1853.07
SD	--	4.30
%RSD	--	0.23

Table No 2.064: Robustness. (Samples)

Sample No.	Wt. of sample	Area	Assay (as such basis)	Assay (on dried basis)
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	(mg)				
1	49.61	1839.67	99.43	99.79	
2	52.89	1962.81	99.50	99.86	
3	48.55	1801.92	99.51	99.87	
4	49.91	1851.40	99.46	99.82	
5	52.05	1931.42	99.49	99.85	
6	47.85	1773.83	99.40	99.76	
AVG	--	--	99.47	99.83	
SD	--	--	0.05	0.05	
%RSD	--	0.20	--	0.05	0.05

Table No 2.065: Robustness. (Standard). :

pH of Buffer = 2.4

Working Standard	Wt. of Standard (mg)	Area
Injection-1	53.95	2103.83
Injection-2	--	2104.21
Injection-3	--	2112.00
Injection-4	--	2107.02
Injection-5	--	2111.29
Injection-6	--	2107.14
AVG	--	2107.58
SD	--	3.44
%RSD	--	0.16

Table No 2.066: Robustness. (Samples)

Sample No.	Wt. of sample	Retention time	Area	Assay (as such basis)	Assay (on dried
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	(mg)				basis)
1	48.40	9.10	1883.28	99.28	99.63
2	48.25	9.12	1877.42	99.27	99.63
3	49.24	9.10	1918.18	99.39	99.75
4	50.78	9.11	1978.43	99.40	99.76
5	50.42	9.10	1961.50	99.26	99.61
6	48.44	9.10	1884.43	99.25	99.61
AVG	--	9.11	--	99.31	99.67
SD	--	0.01	--	0.07	0.07
%RSD	--	0.09	--	0.07	0.07

Table No 2.067: Robustness. (Standard). :

pH of Buffer = 2.6

Working Standard	Wt. of Standard (mg)	Area
Injection-1	53.85	2108.74
Injection-2	--	2111.58
Injection-3	--	2110.51
Injection-4	--	2115.59
Injection-5	--	2116.73
Injection-6	--	2119.14
AVG	--	2113.72
SD	--	4.04
%RSD	--	0.19

Table No 2.068: Robustness. (Samples)

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
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1	48.35	1890.32	99.28	99.63
2	48.12	1881.33	99.28	99.63
3	49.85	1950.17	99.34	99.70
4	50.50	1976.03	99.36	99.72
5	50.12	1965.17	99.56	99.92
6	49.35	1930.41	99.33	99.69
AVG	--	--	99.36	99.71
SD	--	--	0.11	0.11
%RSD	--	--	0.11	0.11

Table No 2.069: Robustness. (Standard): Mobile phase composition.(Buffer: ACN=180:820)

Working Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	48.85	9.28	2125.97
Injection-2	--	9.29	2123.06
Injection-3	--	9.30	2123.16
Injection-4	--	9.30	2125.34
Injection-5	--	9.30	2125.62
Injection-6	--	9.30	2125.73
AVG	--	9.30	2124.81
SD	--	0.01	1.34
%RSD	--	0.09	0.06

Table No 2.0691: Robustness. (Samples)

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
1	50.55	2190.14	99.28	99.64

2	50.15	2171.28	99.21	99.57
3	50.55	2187.27	99.15	99.51
4	48.01	2075.56	99.06	99.42
5	51.85	2249.30	99.40	99.76
6	50.01	2162.62	99.09	99.45
AVG	--	--	99.20	99.56
SD	--	--	0.13	0.13
%RSD	--	--	0.13	0.13

**Table No 2.0692: Robustness. (Standard). : Mobile phase composition
(Buffer: ACN=220:780)**

Working Standard	Wt. of Standard (mg)	Area
Injection-1	53.69	2126.21
Injection-2	--	2127.75
Injection-3	--	2125.27
Injection-4	--	217.56
Injection-5	--	2128.84
Injection-6	--	2131.58
AVG	--	2127.87
SD	--	2.21
%RSD	--	0.10

Table No 2.0693: Robustness. (Samples)

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
1	48.35	1909.03	99.30	99.65
2	48.05	1898.48	99.36	99.72
3	50.38	1990.98	99.39	99.74
4	47.72	1884.83	99.33	99.69
5	47.05	1853.93	99.05	99.45

6	50.39	1990.67	99.35	99.71
AVG	--	--	99.30	99.66
SD	--	--	0.11	0.11
%RSD	--	--	0.11	0.11

Table No 2.070: Solution stability (Standard.). Time Period: 0 hr

Working Standard	Wt. of Standard (mg)	Area
Injection-1	49.35	1882.81
Injection-2	--	1883.87
Injection-3	--	1882.17
Injection-4	--	1883.08
Injection-5	--	1881.32
Injection-6	--	1885.75
AVG	--	1883.17
SD	--	1.53
%RSD	--	0.08

Table No 2.071: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	50.05	8.07	1912.05	99.78	100.14

Table No 2.072: Solution stability (Standard.). Time Period: 6.0 hr

Working Standard	Wt. of Standard (mg)	Area
Injection-1	50.32	1915.09
Injection-2	--	1916.45
Injection-3	--	1920.27
Injection-4	--	1920.32
Injection-5	--	1916.99
Injection-6	--	1918.06
AVG	--	1917.86
SD	--	2.11
%RSD	--	0.11

Table No 2.073: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1.	50.05	8.08	1909.40	99.77	100.13

Table No 2.074: Solution stability (Standard.).**Time Period: 12. 0 hr**

Working Standard	Wt. of Standard (mg)	Area
Injection-1	50.27	1925.28
Injection-2	--	1928.39
Injection-3	--	1930.48
Injection-4	--	1933.08
Injection-5	--	1938.72
Injection-6	--	1934.42
AVG	--	1931.73
SD	--	4.74
%RSD	--	0.25

Table No 2.075: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	50.05	8.08	1923.69	99.69	100.05

Table No 2.076: Solution stability (Standard.).**Time Period: 18. 0 hr**

Working Standard	Wt. of Standard (mg)	Area
Injection-1	49.52	1904.86
Injection-2	--	1904.66
Injection-3	--	1901.16
Injection-4	--	1901.98
Injection-5	--	1904.05
Injection-6	--	1906.63
AVG	--	1903.89
SD	--	2.01
%RSD	--	0.11

Table No 2.077: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	50.05	8.07	1923.77	99.64	100.00

Table No 2.078: Solution stability (Standard.). Time Period: 24.0 hr

Working Standard	Wt. of Standard (mg)	Area	
Injection-1	49.49	1905.70	
Injection-2	--	1911.32	
Injection-3	--	1912.61	
Injection-4	--	1911.68	
Injection-5	--	1909.84	
Injection-6	--	1909.91	
AVG	--	1910.18	
SD	--	0.01	2.44
%RSD	--	0.07	0.13

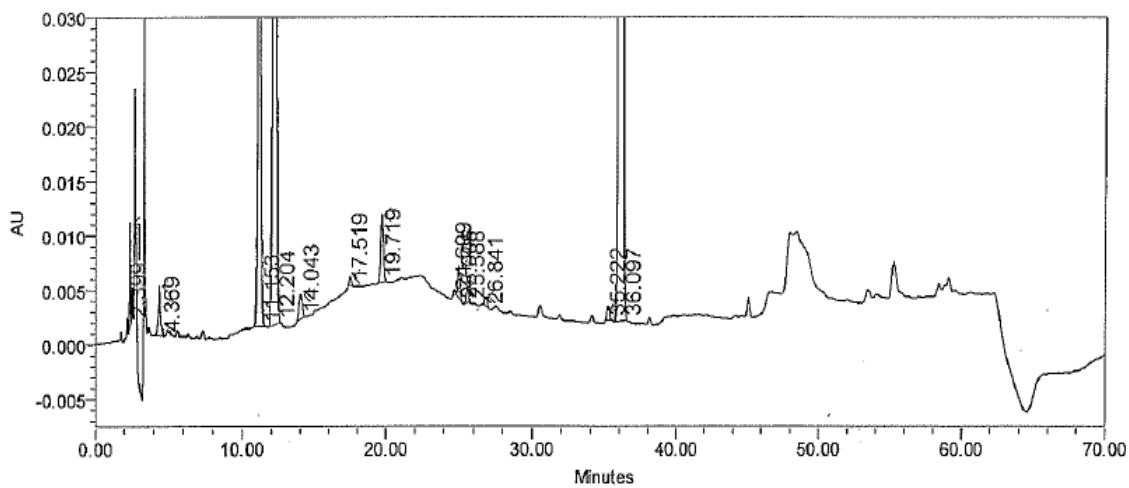
Table No 2.079: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	50.05	8.08	1930.28	99.59	99.95

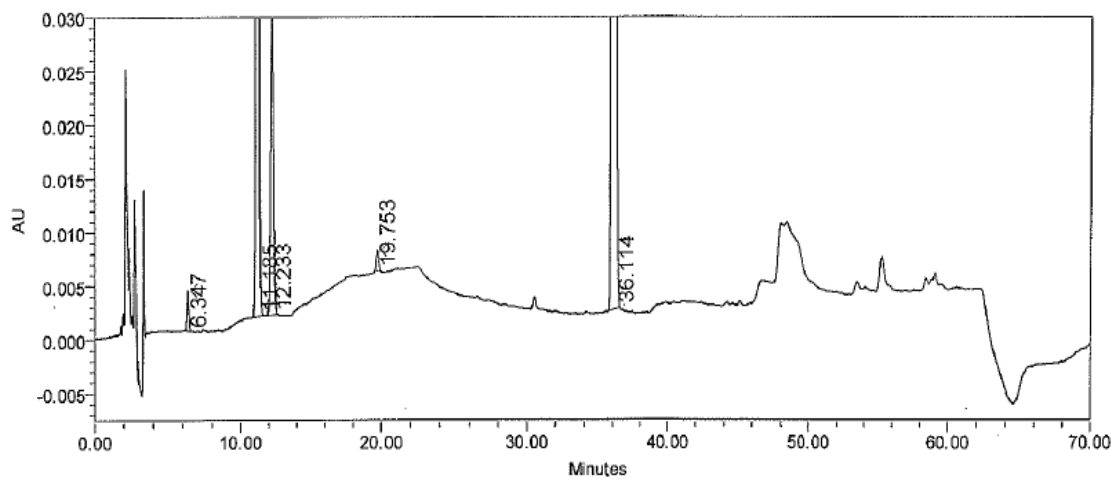
Table No 2.080: LOD/LOQ:

Sample	Level – 1	Level – 2	Level – 3	Level – 4	Level - 5
ppm	0.1 ppm	0.05 ppm	0.025 ppm	0.01 ppm	0.005 ppm
	Area	Area	Area	Area	Area
Injection - 1	4.67	1.75	0.98	0.41	Not detected
Injection - 2	4.70	1.69	1.08	0.43	Not detected
Injection - 3	4.71	1.64	0.86	0.56	Not detected
Injection - 4	4.55	1.77	0.99	0.42	Not detected
Injection - 5	4.62	1.91	0.87	0.70	Not detected
Injection - 6	4.99	1.92	0.86	0.56	Not detected
Average	4.71	1.78	0.94	0.51	--
SD	0.15	0.11	0.09	0.11	--
%RSD	3.20	6.42	9.68	22.30	--

Flucytosine Standard



Flucytosine Sample



Divalproex sodium

HPLC Method has been validated for Assay of Divalproex sodium (Specification No: ADL/TS/FP/056) and method involves analysis of Divalproex sodium using HPLC column, Phenyl Hypersil 150 x 4.6mm 5 μ m or equivalent. Validation parameters such as specificity, precision, linearity, accuracy, limit of detection, limit of quantification, intermediate precision, robustness and stability of analytical sample solution were studied.

Specificity:

All the components are well resolved from each other. Resolution between Divalproex sodium and N-Valeric acid is 15.31.

System suitability was studied by carrying six replicate injections of Zoledronic acid working standard and relative standard deviation of peak areas and the results are presented in

Table No 3.01

System precision and Method precision:

System precision was carried using six replicate injections of working standard of Divalproex sodium and % RSD of peak area of six replicate injections the observation are given in **Table No 3.02**

Method precision study was carried by using six replicate injections of working standard of Divalproex sodium and relative standard deviation of peak area of six replicate injections determined and the observations are given in **Table No 3.03**

Linearity: Linearity:

To calculate the correlation of coefficient (r) Linearity study of Divalproex sodium was carried using five different concentration levels 2500 ppm, 4000 ppm, 5000 ppm, 6000 ppm and 7500 ppm and the observations are made are presented in **Table No.3.04.**

Accuracy: Accuracy was determined by using three different concentration levels 3500 ppm, 5000 ppm and 6500 ppm of Divalproex sodium in presence of fixed concentration of impurities and the findings are depicted in **Table No.3.050, 3.051, 3.052 and 3.053**

Intermediate precision:

Intermediate precision of the method was studied for analysis of Divalproex sodium sample on different instruments, by different researcher on different day and the data is given in **Table No. 3.060 and 3.061.**

Robustness: Robustness of the method was carried by slight variation in flow rate, pH of phosphate buffer solution and mobile phase composition. The observation for different condition is given in **Table No.3.070, 3.071,3.072, 3.074, 3.075, 3.076, 3.077, 3.078, 3.0790, 3.0791, 3.0792 and 3.0793.**

Stability of analytical sample Solution:

Analytical sample solution stability of Divalproex sodium was carried in different conditions, time period. The findings are given in **Table No 3.080, 3.081, 3.082,3.083, 3.084, 3.085, 3.086, 3.087, 3.088 and 3.089**

Limit of detection and Limit of quantification: LOD and LOQ method can be used for cleaning validation. In the present study the findings are given in **Table .No 3.090**

Table No 3.01: % RSD for System Suitably.

Standard	Wt. of Standard (mg)	Area
Injection-1	248.52	3335.09
Injection-2	--	3335.25
Injection-3	--	3335.76
Injection-4	--	3334.51
Injection-5	--	3334.21
Injection-6	--	3336.14
AVG	--	3335.16
SD	--	0.67
%RSD	--	0.02

Table No 3.02: % RSD for System Precision.

Standard	Wt. of Standard (mg)	Area
Injection-1	248.74	3337.18
Injection-2	--	3335.29
Injection-3	--	3333.59
Injection-4	--	3335.44
Injection-5	--	3334.33
Injection-6	--	3332.47
AVG	--	3334.72
SD	--	1.64
%RSD	--	0.05

Table No 3.03: Method Precision. (Samples)

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
1	250.89	3359.33	99.62	99.68

2	250.86	3362.87	99.73	99.80
3	250.98	3357.64	99.53	99.60
4	250.93	3360.94	99.65	99.72
5	254.08	3404.30	99.68	99.75
6	250.91	3364.70	99.77	99.84
AVG	--	--	99.66	99.73
SD	--	--	0.09	0.09
%RSD	--	--	0.09	0.09

Table No 3.04: Linearity

Injection No.	Level-1 (2500 ppm)	Level-2 (4000 ppm)	Level-3 (5000 ppm)	Level-4 (6000 ppm)	Level-5 (7500 ppm)
1	1384.35	2273.90	2725.92	3257.82	4055.06
2	1382.77	2277.30	2724.14	3258.35	4047.72

Injection No.	Level-1 (2500 ppm)	Level-2 (4000 ppm)	Level-3 (5000 ppm)	Level-4 (6000 ppm)	Level-5 (7500 ppm)
3	1384.31	2276.87	2723.19	3257.55	4052.04
AVG	1383.81	2276.02	2724.42	3257.91	4051.61
SD	0.90	1.85	1.39	0.41	3.69
%RSD	0.07	0.08	0.05	0.01	0.09
--	--	--	--	Correlation(r)	0.9993

Table No 3.050: Accuracy. (Standard)

Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	250.98	6.91	3376.21
Injection-2	--	6.91	3376.23
Injection-3	--	6.91	3378.91
Injection-4	--	6.91	3377.15
Injection-5	--	6.91	3378.27
Injection-6	--	6.91	3375.70
AVG	--	6.91	3377.08
SD	--	0.00	1.28
%RSD	--	0.00	0.04

Table No 3.051 Accuracy Level-1

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
Sample-1	175.00	2359.41	99.94	100.01
Sample-2	175.19	2356.38	99.70	99.77
Sample-3	174.74	2356.57	99.97	100.04

AVG	--	--	99.87	99.94
SD	--	--	0.15	0.15
%RSD	--	--	0.15	0.15

Table No 3.052 Accuracy Level-2

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
Sample-1	250.74	3374.17	99.75	99.82
Sample-2	250.07	3359.57	99.58	99.65
Sample-3	250.04	3357.88	99.55	99.62
AVG	--	--	99.63	99.70
SD	--	--	0.11	0.11
%RSD	--	--	0.11	0.11

Table No 3.053 Accuracy Level-3

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
Sample-1	326.09	4383.17	99.64	99.71
Sample-2	324.96	4365.76	99.59	99.66
Sample-3	325.29	4369.36	99.57	99.64

AVG	--	--	99.60	99.67
SD	--	--	0.04	0.04
%RSD	--	--	0.04	0.04

Table No 3.060: Intermediate Precision: Standard

Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	250.81	6.80	3363.66
Injection-2	--	6.80	3363.41
Injection-3	--	6.80	3362.81
Injection-4	--	6.80	3361.55
Injection-5	--	6.80	3363.41
Injection-6	--	6.80	3357.43
AVG	--	6.80	3362.05
SD	--	0.00	2.39
%RSD	--	0.00	0.07

Table No 3.061: Intermediate Precision: Samples

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
Sample-1	250.18	3349.42	99.62	99.69
Sample-2	250.24	3351.08	98.64	99.71
Sample-3	250.17	3352.43	99.71	99.78
Sample-4	250.27	3352.37	99.67	99.74
Sample-5	250.14	3349.60	99.64	99.71
Sample-6	250.30	3351.60	99.63	99.70

AVG	--	--	99.65	99.72
SD	--	--	0.03	0.03
% RSD	--	--	0.03	0.03

Table No3.070: Robustness:

Sr. No.	Parameters	Standard parameter	Changes studied	
1	Flow rate	1.0 ml/min.	0.9 ml/min.	1.1 ml/min.
2	pH of mobile phase	3.00	2.90	3.10
3	Column temperature	35°C	30°C	40°C

Table No 3.071: Robustness. (Standard). :

Flow rate 0.9 ml/min.

Standard	Wt. of Standard (mg)	Area
Injection-1	250.61	3750.12
Injection-2	--	3753.33
Injection-3	--	3752.05
Injection-4	--	3749.55
Injection-5	--	3744.78
Injection-6	--	3744.65
AVG	--	3749.08
SD	--	3.64
%RSD	--	0.10

Table No3.072: Robustness: (Sample)

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
Sample-1	250.53	3739.49	99.52	99.59
Sample-2	250.47	3741.02	99.58	99.65
Sample-3	250.39	3738.60	99.55	99.62
Sample-4	250.55	3741.36	99.56	99.63
Sample-5	250.51	3741.16	99.57	99.64
Sample-6	250.29	3742.62	99.70	99.77
AVG	--	--	99.58	99.65
SD	--	--	0.06	0.06
%RSD	--	--	0.06	0.06

Table No 3.073: Robustness. (Standard). :**Flow rate 1.1 ml/min.**

Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	250.57	6.25	3068.79
Injection-2	--	6.24	3064.21
Injection-3	--	6.25	3068.13
Injection-4	--	6.25	3066.56
Injection-5	--	6.25	3067.88
Injection-6	--	6.25	3065.00
AVG	--	6.25	3066.76
SD	--	0.00	1.84
%RSD	--	0.07	0.06

Table No3.074: Robustness: (Samples)

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
Sample-1	250.51	3062.12	99.61	99.68
Sample-2	250.46	3061.33	99.61	99.68
Sample-3	250.37	3063.76	99.72	99.79
Sample-4	250.29	3058.90	99.60	99.67
Sample-5	250.53	3061.22	99.58	99.65
Sample-6	250.47	3063.12	99.66	99.73
AVG	--	--	99.63	99.70
SD	--	--	0.05	0.05
%RSD	--	--	0.05	0.05

Table No 3.075: Robustness. (Standard). :**pH of buffer: 2.9**

Standard	Wt. of Standard (mg)	Area
Injection-1	250.53	3362.49
Injection-2	--	3361.83
Injection-3	--	3360.89
Injection-4	--	3361.19
Injection-5	--	3356.72
Injection-6	--	3358.86
AVG	--	3360.33
SD	--	2.16
%RSD	--	0.06

Table No3.076: Robustness: (Samples)

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
Sample-1	250.20	3354.69	99.70	99.77
Sample-2	250.24	3354.97	99.70	99.77
Sample-3	250.29	3358.30	99.78	99.85
Sample-4	250.14	3347.33	99.51	99.58
Sample-5	250.33	3354.08	99.63	99.70
Sample-6	249.62	3357.60	99.62	99.69
AVG	--	--	99.66	99.73
SD	--	--	0.09	0.09
%RSD	--	--	0.09	0.09

Table No 3.077: Robustness. (Standard). :

pH of buffer: 3.1

Standard	Wt. of Standard (mg)	Area
Injection-1	250.95	3989.51
Injection-2	--	3996.44
Injection-3	--	3990.15
Injection-4	--	3990.75
Injection-5	--	3989.62
Injection-6	--	3985.54
AVG	--	3990.34
SD	--	3.51
%RSD	--	0.09

Table No3.078: Robustness: (Sample)

Sample	Wt. of sample	Area	Assay	Assay
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No.	(mg)		(as such basis)	(on dried basis)
Sample-1	250.33	3979.46	99.71	99.78
Sample-2	250.14	3972.33	99.61	99.68
Sample-3	250.43	3977.61	99.63	99.70
Sample-4	250.11	3970.26	99.57	99.64
Sample-5	250.19	3971.90	99.58	99.65
Sample-6	250.09	3970.77	99.59	99.66
AVG	--	--	99.62	99.69
SD	--	--	0.05	0.05
%RSD	--	--	0.05	0.05

Table No 3.079: Robustness. (Standard). Column oven temperature = 30°C

Standard	Wt. of Standard (mg)	Area
Injection-1	250.01	3367.48
Injection-2	--	3367.89
Injection-3	--	3365.66
Injection-4	--	3367.05
Injection-5	--	3366.73
Injection-6	--	3364.35
AVG	--	3366.53
SD	--	1.31
%RSD	--	0.04

Table No3.0791: Robustness: (Samples)

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)	
Sample-1	251.58	3386.42	99.70	99.77	
Sample-2	251.89	3393.03	99.77	99.84	
Sample-3	251.91	3392.20	99.74	99.81	
Sample-4	251.89	3389.85	99.68	99.75	
Sample-5	251.69	3387.02	99.68	99.75	
Sample-6	251.83	3389.43	99.69	99.76	
AVG	--	--	99.71	99.78	
SD	--	--	0.04	0.04	
%RSD	--	0.15	--	0.04	0.04

Table No 3.0792: Robustness. (Standard). Column oven temperature = 30°C

Standard	Wt. of Standard (mg)	Area
Injection-1	249.05	3361.81
Injection-2	--	3363.07
Injection-3	--	3361.74
Injection-4	--	3361.64
Injection-5	--	3361.76
Injection-6	--	3358.94
AVG	--	3361.49
SD	--	1.36
%RSD	--	0.04

Table No3.0793: Robustness: (Samples)

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
Sample-1	250.76	3382.50	99.68	99.75

Sample-2	250.91	3385.58	99.71	99.78
Sample-3	250.98	3390.35	99.82	99.89
Sample-4	250.86	3385.39	99.72	99.79
Sample-5	250.69	3381.53	99.68	99.75
Sample-6	250.97	3387.17	99.73	99.80
AVG	--	--	99.72	99.79
SD	--	--	0.05	0.05
%RSD	--	--	0.05	0.05

Table No 3.080: Solution stability (Standard.). Time Period: 0 hr

Standard	Wt. of Standard (mg)	Area
Injection-1	250.49	3376.56
Injection-2	--	3373.73
Injection-3	--	3374.11
Injection-4	--	3374.63
Injection-5	--	3374.27
Injection-6	--	3374.27
AVG	--	3371.42
SD	--	1.65
%RSD	--	0.05

Table No 3.081: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
Sample-1	250.45	6.94	3373.04	99.72	99.79

Table No 3.082: Solution stability (Standard.). Time Period: 6.0 hr

Standard	Wt. of Standard (mg)	Area
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Injection-1	250.29	3376.24
Injection-2	--	3374.40
Injection-3	--	3374.20
Injection-4	--	3374.38
Injection-5	--	3375.07
Injection-6	--	3373.94
AVG	--	3374.71
SD	--	0.84
%RSD	--	0.02

Table No 3.083: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
Sample-1	250.45	6.94	3375.94	99.71	99.78

Table No 3.084: Solution stability (Standard.). Time Period: 12.0 hr

Standard	Wt. of Standard (mg)	Area
Injection-1	249.32	3363.34
Injection-2	--	3362.43
Injection-3	--	3364.18
Injection-4	--	3363.25
Injection-5	--	3362.28
Injection-6	--	3361.86
AVG	--	3362.89
SD	--	0.85
%RSD	--	0.03

Table No 3.085: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
Sample-1	250.45	6.95	3376.41	99.69	99.76

Table No 3.086: Solution stability (Standard.). Time Period: 18.0 hr

Standard	Wt. of Standard (mg)	Area
Injection-1	249.69	3363.96
Injection-2	--	3363.13
Injection-3	--	3360.99
Injection-4	--	3366.17
Injection-5	--	3365.80
Injection-6	--	3361.29
AVG	--	3363.56
SD	--	2.19
%RSD	--	0.07

Table No 3.087: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
Sample-1	250.45	6.95	3370.84	99.65	99.72

Table No 3.088: Solution stability (Standard). Time Period: 24. 0 hr

Standard	Wt. of Standard (mg)	Area
Injection-1	249.39	3360.27
Injection-2	--	3359.71
Injection-3	--	3359.19
Injection-4	--	3362.10
Injection-5	--	3357.47
Injection-6	--	3357.77
AVG	--	3359.42
SD	--	1.71
%RSD	--	0.05

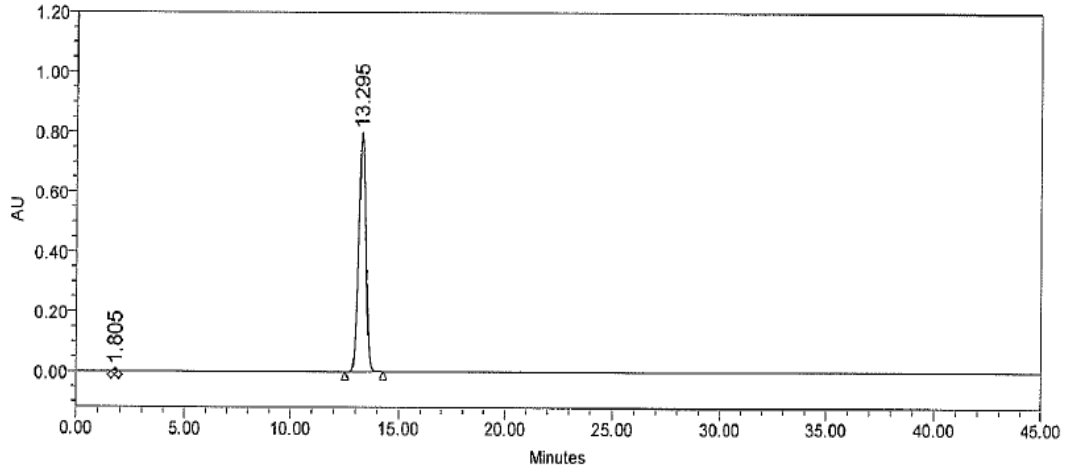
Table No 3.089: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
Sample-1	250.45	6.96	3371.29	99.67	99.74

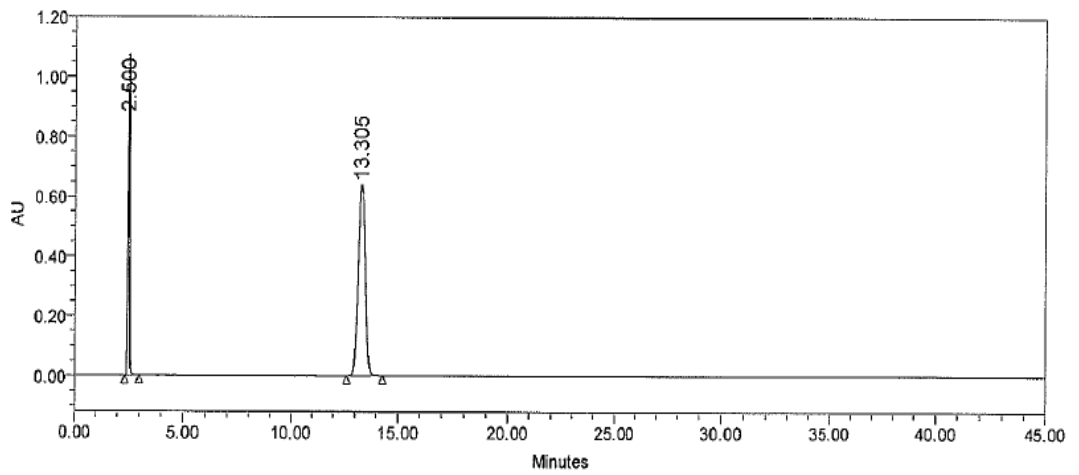
Table No. 3.090 LOD/LOQ

Sample	Level – 1	Level – 2	Level – 3	Level – 4	Level – 5	Level – 5
Conc. in ppm	50 ppm	10 ppm	5 ppm	2.5 ppm	1.0 ppm	0.25 ppm
	Area	Area	Area	Area	Area	Area
Injection - 1	34.00	7.15	3.58	1.55	0.43	Not detected
Injection - 2	34.39	6.97	3.24	1.67	0.50	Not detected
Injection - 3	34.03	6.32	3.24	1.54	0.40	Not detected
Injection - 4	33.90	6.96	3.21	1.86	0.47	Not detected
Injection - 5	34.07	6.70	3.36	1.60	0.79	Not detected
Injection - 6	34.08	6.64	3.40	1.43	0.34	Not detected
AVG	34.08	6.79	3.34	1.61	0.49	--
SD	0.17	0.30	0.14	0.15	0.16	--
%RSD	0.49	4.38	4.20	9.10	32.34	--

Divalproex sodium



Divalproex sodium Sample



Meclizine hydrochloride

HPLC Method has been validated for Assay of Meclizine hydrochloride (Specification No: ADL/TS/FP/093A) and method involves analysis of Meclizine hydrochloride using HPLC column, Waters X-bridge phenyl 250 x 4.6 mm, 5 μ m. Validation parameters such as specificity, precision, linearity, accuracy, limit of detection, limit of Quantification, intermediate precision, robustness and stability of analytical sample solution were studied.

Specificity:

All the components are well resolved from each other. Resolution between peak due to Meclizine and 4-Meclizine imp. is 2.06.

System suitability:

System suitability has been studied using six replicate injections of Zoledronic acid working standard and relative standard deviation of peak areas for six replicate injections were calculated and the results are presented in **Table No 4.01**

Method precision: To evaluate the relative standard deviation of peak area

Method precision study was carried by using six replicate injections of working standard of Meclizine hydrochloride and relative standard deviation of peak area of six replicate injections determined and the observations are given in **Table No. 4.02.**

Linearity: To calculate the correlation of coefficient (r) Linearity study was carried using five different concentration levels 125 ppm, 200 ppm, 250 ppm, 300 ppm and 375 ppm of Meclizine hydrochloride and the observations are given in **Table No. 4.03.**

Accuracy:

Accuracy was determined by using three different concentration levels 175 ppm, 250 ppm and 325 ppm of Meclizine hydrochloride in presence of fixed concentration of impurities and the findings are depicted in **Table No. 4.040, 4.041, 4.042 and 4.043**

Intermediate precision:

Intermediate precision of the method has been studied by analysis of six sample preparations of Meclizine hydrochloride separately by different analyst using different instrument and on different day and the consolidated data is given in **Table No 4.050 and 4.051.**

Robustness:

Robustness of the method was carried by slight variation in flow rate, pH of phosphate buffer solution and mobile phase composition. The observation for different condition are given in **Table No. 4.060, 4.061, 4.062, 4.063, 4.064, 4.065, 4.066, 4.068 and 4.069.**

Stability of analytical sample Solution:

Analytical sample solution stability of Meclizine hydrochloride was carried in different conditions, time period for 24 hours. The findings are given in **Table No 4.070, 4.071, 4.072, 4.073, 4.074, 4.075, 4.076, 4.077, 4.078 and 4.079.**

Limit of detection and Limit of quantification:

LOD and LOQ method can be used for cleaning validation. In the present study the findings are given in **Table .No. 4.08**

Table No 4.01: % RSD for System Suitably.

Working Standard	Wt. of Standard (mg)	Area
Injection-1	25.29 mg	11885.60
Injection-2	--	11890.10
Injection-3	--	11876.50
Injection-4	--	11882.40
Injection-5	--	11868.70
Injection-6	--	11876.00
AVG	--	11879.88
SD	--	7.68
%RSD	--	0.06

Table No 4.02: Method Precision.(Sample)

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
1	25.06	11648.80	95.65	99.54
2	25.01	11620.80	95.61	99.50
3	24.95	11589.40	95.58	99.47
4	25.08	11662.60	95.69	99.58
5	25.75	11968.60	95.64	99.53
6	24.96	11593.00	95.57	99.46
AVG	--	--	95.62	99.51
SD	--	--	0.04	0.05
%RSD	--	--	0.05	0.05

Table No 4.03: Linearity

Injection No.	Level-1 (125 ppm)	Level-2 (200 ppm)	Level-3 (250 ppm)	Level-4 (300 ppm)	Level-5 (375 ppm)
1	5496.24	8599.37	10894.10	12768.00	16099.80
2	5499.97	8590.34	10899.70	12893.80	16127.50
3	5502.34	8606.63	10889.20	12875.20	16135.70
AVG	5499.52	8598.78	10894.33	12845.67	16121.00
SD	3.08	8.16	5.25	67.90	18.81
%RSD	0.06	0.09	0.05	0.53	0.12
--	--	--	--	Correlation(r)	1.000

Table No 4.040 Accuracy. (Standard)

Working Standard	Wt. of Standard (mg)	Area
Injection-1	25.75 mg	11881.90
Injection-2	--	11881.00
Injection-3	--	11919.20
Injection-4	--	11907.20
Injection-5	--	11909.30
Injection-6	--	11928.00
AVG	--	11904.43
SD	--	19.30
%RSD	--	0.16

Table No 4.041 Accuracy Level-1

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
Sample-1	17.59	8049.84	95.68	99.58
Sample-2	16.81	7666.87	95.36	99.24
Sample-3	18.05	8290.19	96.03	99.94

AVG	--	--	--	--
SD	--	--	--	--
%RSD	--	--	--	--

Table No 4.042 Accuracy Level-2

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
Sample-1	25.02	11456.40	95.74	99.63
Sample-2	25.01	11446.40	95.69	99.58
Sample-3	25.15	11505.80	95.65	99.54
AVG	--	--	--	--
SD	--	--	--	--
%RSD	--	--	--	--

Table No 4.043 Accuracy Level-3

Sample No.	Wt. of sample (mg)	Area	Assay (as such basis)	Assay (on dried basis)
Sample-1	32.61	14878.60	95.40	99.28
Sample-2	32.65	14921.90	95.56	99.44
Sample-3	32.63	14915.00	95.57	99.46

AVG	--	--	--	--	
SD	--	--	--	--	
%RSD	--	0.00	--	--	--

Table No 4.050: Intermediate Precision: Standard

Working Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	25.01	10.61	10500154.00
Injection-2	--	10.61	10509628.00
Injection-3	--	10.60	10500217.00
Injection-4	--	10.60	10500103.00
Injection-5	--	10.61	10509909.00
Injection-6	--	10.61	10511842.00
AVG	--	10.61	10505308.83
SD	--	0.01	5693.83
%RSD	--	0.05	0.05

Table No 4.051: Intermediate Precision: – Samples

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	25.27	10.61	10509774.00	95.71	99.60
2	25.31	10.61	10511791.00	95.57	99.46
3	25.22	10.61	10490796.00	95.72	99.62
4	25.18	10.61	10470694.00	95.69	99.58
5	25.18	10.60	10468665.00	95.67	99.57

6	25.17	10.60	10453450.00	95.57	99.46
AVG	--	10.61	--	95.66	99.55
SD	--	0.01	--	0.07	0.07
%RSD	--	0.05	--	0.07	0.07

Table No 4.060: Robustness (Standard) :

Flow rate 1.3 ml/min.

Working Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	25.35	11.51	11349.70
Injection-2	--	11.51	11350.60
Injection-3	--	11.51	11347.90
Injection-4	--	11.51	11354.30
Injection-5	--	11.51	11337.10
Injection-6	--	11.51	11341.20
AVG	--	11.51	11346.80
SD	--	0.00	6.41
%RSD	--	0.00	0.06

Table No 4.061: Robustness (Sample)

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	25.27	11.51	11193.40	95.66	99.55
2	25.16	11.51	11143.90	95.65	99.54
3	25.17	11.51	11138.90	95.57	99.46
4	25.26	11.51	11181.80	95.59	99.48
5	25.47	11.51	11274.50	95.59	99.48

6	25.55	11.51	11308.10	95.58	99.47
AVG	--	11.51	--	95.61	99.50
SD	--	0.00	--	0.04	0.04
%RSD	--	0.00	--	0.04	0.04

Table No 4.062: Robustness (Standard) :

Flow rate 1.5 ml/min.

Working Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	25.12	9.97	9833.12
Injection-2	--	9.98	9832.34
Injection-3	--	9.98	9829.69
Injection-4	--	9.98	9836.30
Injection-5	--	9.98	9840.61
Injection-6	--	9.99	9830.59
AVG	--	9.98	9833.78
SD	--	0.01	4.06
%RSD	--	0.06	0.04

Table No 4.063: Robustness (Sample)

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	25.16	10.00	9750.05	95.68	99.58
2	25.08	9.99	9706.16	95.56	99.45
3	25.15	10.00	9744.09	95.66	99.56
4	25.26	10.00	9780.09	95.60	99.49
5	25.36	10.00	9815.53	95.57	99.46

6	25.47	10.00	9866.98	95.65	99.55
AVG	--	10.00	--	95.62	99.51
SD	--	0.00	--	0.05	0.06
%RSD	--	0.04	--	0.06	0.06

Table No 4.064: Robustness (Standard)

pH of Buffer = 6.4

Working Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	25.18	10.26	10426.20
Injection-2	--	10.27	10423.00
Injection-3	--	10.28	10415.40
Injection-4	--	10.29	10400.50
Injection-5	--	10.29	10404.60
Injection-6	--	10.30	10407.20
AVG	--	10.28	10412.82
SD	--	0.01	10.39
%RSD	--	0.14	0.10

Table No 4.065: Robustness (Sample)

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	25.60	10.30	10470.20	95.60	99.49
2	25.59	10.30	10472.60	95.66	99.55
3	24.68	10.31	10094.10	95.60	99.49
4	25.57	10.30	10455.50	95.58	99.46
5	25.47	10.30	10421.40	95.64	99.53
6	25.44	10.30	10404.20	95.59	99.48
AVG	--	10.30	--	95.61	99.50
SD	--	0.00	--	0.03	0.03

%RSD	--	0.04	--	0.03	0.03
------	----	------	----	------	------

Table No 4.066: Robustness (Standard)

pH of Buffer = 6.6

Working Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	25.15	11.94	12246.10
Injection-2	--	11.96	12249.10
Injection-3	--	11.97	12252.30
Injection-4	--	11.96	12237.00
Injection-5	--	11.97	12244.30
Injection-6	--	11.98	12248.90
AVG	--	11.96	12246.28
SD	--	0.01	5.31
%RSD	--	0.11	0.04

Table No 4.067: Robustness (Sample)

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	25.61	11.99	12330.60	95.58	99.47
2	25.38	11.99	12228.30	95.64	99.54
3	25.63	11.99	12348.40	95.64	99.53
4	25.76	11.97	12413.30	95.66	99.55
5	25.57	11.97	12320.40	95.65	99.54
6	25.74	11.97	12394.40	95.59	99.48
AVG	--	11.98	--	95.63	99.52
SD	--	0.01	--	0.03	0.04
%RSD	--	0.09	--	0.04	0.04

Table No 4.068: Robustness (Standard)

M. P. composition (Water: ACN: Sulphate buffer: Phosphate buffer, 230:770:50:50)

Working Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	25.21	8.91	10508.70
Injection-2	--	8.92	10511.80
Injection-3	--	8.92	10526.60
Injection-4	--	8.92	10524.50
Injection-5	--	8.92	10529.90
Injection-6	--	8.92	10532.10
AVG	--	8.92	10522.27
SD	--	0.00	9.72
%RSD	--	0.05	0.09

Table No 4.069: Robustness (Sample)

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	25.35	8.91	10464.50	95.60	99.49
2	25.25	8.91	10421.20	95.58	99.47
3	25.49	8.91	10515.80	95.54	99.43
4	25.51	8.92	10515.60	95.46	99.35
5	25.53	8.92	10524.80	95.47	99.36
6	25.61	8.92	10581.10	95.68	99.58
AVG	--	8.92	--	95.56	99.44
SD	--	0.01	--	0.08	0.09
%RSD	--	0.06	--	0.09	0.09

Table No 4.070: Solution stability (Standard.). Time Period: 0 hr

Working Standard	Wt. of Standard (mg)	Retention Time	Area
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Injection-1	25.22	11.40	10575.90
Injection-2	--	11.40	10579.50
Injection-3	--	11.40	10570.80
Injection-4	--	11.40	10574.40
Injection-5	--	11.40	10569.90
Injection-6	--	11.40	10574.10
AVG	--	11.40	10574.10
SD	--	0.00	3.49
%RSD	--	0.00	0.03

Table No 4.071: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	25.32	10.62	11188.70	95.62	99.51

Table No 4.072: Solution stability (Standard.). Time Period: 6.0 hr

Working Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	25.19	10.61	11219.20
Injection-2	--	10.61	11220.10
Injection-3	--	10.62	11231.00
Injection-4	--	10.62	11257.90
Injection-5	--	10.62	11259.20
Injection-6	--	10.62	11262.50
AVG	--	10.62	11241.65
SD	--	0.01	20.44
%RSD	--	0.05	0.18

Table No 4.073: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1.	25.32	10.61	11178.00	95.62	99.51

Table No 4.074: Solution stability (Standard.). Time Period: 12.0 hr

Working Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	25.17	10.62	11217.90
Injection-2	--	10.61	11244.80
Injection-3	--	10.61	11252.80
Injection-4	--	10.61	11255.20
Injection-5	--	10.61	11253.10
Injection-6	--	10.61	11249.70
AVG	--	10.61	11245.58
SD	--	0.00	14.04
%RSD	--	0.04	0.12

Table No 4.075: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	25.32	10.61	11191.40	95.62	99.52

Table No 4.076: Solution stability (Standard.). Time Period: 18.0 hr

Working Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	25.13	10.62	11265.20
Injection-2	--	10.62	11262.70
Injection-3	--	10.62	11259.60
Injection-4	--	10.63	11266.90
Injection-5	--	10.63	11260.40
Injection-6	--	10.63	11263.80
AVG	--	10.63	11263.10
SD	--	0.01	2.79
%RSD	--	0.05	0.02

Table No 4.077: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	25.32	10.63	11223.30	95.60	99.49

Table No 4.078: Solution stability (Standard.) Time Period: 24.0 hr

Working Standard	Wt. of Standard (mg)	Retention Time	Area
Injection-1	25.24	10.65	11297.20
Injection-2	--	10.65	11303.20
Injection-3	--	10.65	11306.80
Injection-4	--	10.65	11309.00
Injection-5	--	10.65	11314.90
Injection-6	--	10.65	11314.20
AVG	--	10.65	11307.55
SD	--	0.00	6.74
%RSD	--	0.00	0.06

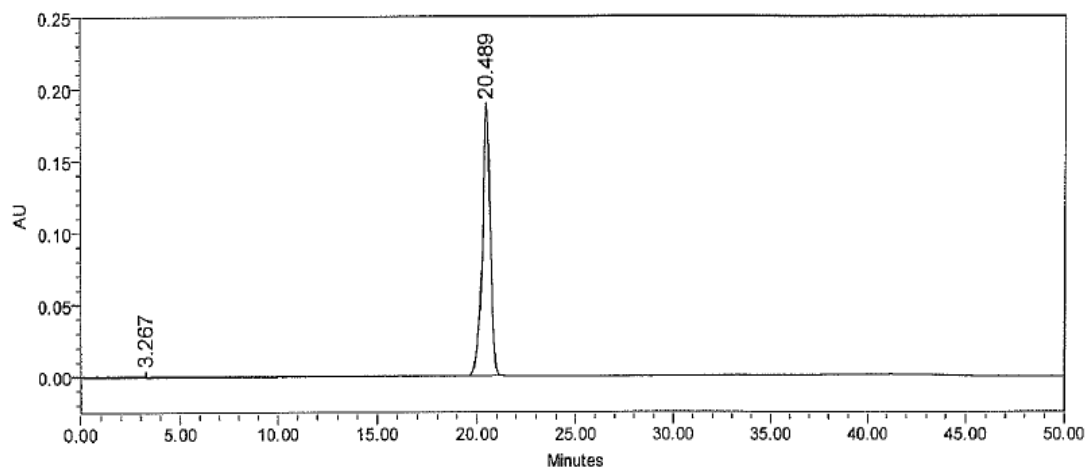
Table No 4.079: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time	Area	Assay (as such basis)	Assay (on dried basis)
1	25.32	10.65	11222.50	95.63	99.52

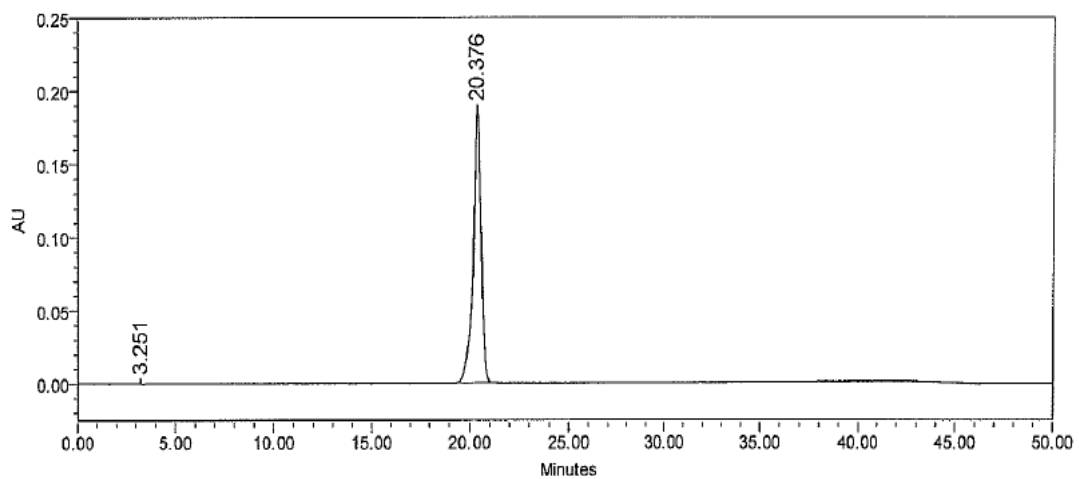
Table No.4.08LOD/LOQ:

Sample	Level – 1	Level – 2	Level – 3	Level – 4	Level - 5	Level - 6
ppm	0.125 ppm	0.06 ppm	0.03 ppm	0.015 ppm	0.0075 ppm	0.004 ppm
	Area	Area	Area	Area	Area	Area
Injection - 1	8.35	3.32	1.87	0.75	0.44	Not detected
Injection - 2	8.25	3.52	1.86	0.82	0.52	Not detected
Injection - 3	8.39	3.64	1.66	0.76	0.49	Not detected
Injection - 4	8.34	3.49	1.90	0.82	0.67	Not detected
Injection - 5	8.33	3.55	1.91	0.77	0.66	Not detected
Injection - 6	8.15	3.75	1.78	0.89	0.55	Not detected
Average	8.30	3.55	1.83	0.80	0.56	--
SD	0.09	0.15	0.09	0.05	0.09	--
%RSD	1.05	4.09	5.17	6.57	16.70	--

Meclizine Hydrochloride standard



Meclizine Hydrochloride Sample



Zoledronic acid

The HPLC Method for determination of assay in Zoledronic acid has been validated for parameters Such as system suitability, precision, limit of detection, limit of quantification, linearity, accuracy, intermediate precision, robustness and stability of analytical sample solution.

System suitability and System Precision:

System suitability has been studied using six replicate injections of Zoledronic acid working standard and relative standard deviation of peak areas for six replicate injections were calculated and the results are presented in **Table No 5.01 and 5.02** .

Precision:

Method precision has been studied by analysis of six sample preparations of Zoledronic acid and relative standard deviation of assay values found to be 0.03% and are given in **Table No. 5.03**.

Linearity: Linearity of the method has been studied using eight different concentration levels 2.0 ppm (LOQ level = x), 4.0 ppm (2x), 10 ppm (5x), 500 ppm, 800 ppm, 1000 ppm, 1200 ppm and 1500 ppm. Correlation coefficient (r) for Zoledronic acid is 1.000 indicating linear response in the concentration range studied. The results obtained are depicted in **Table No. 5.04**

Accuracy:

Accuracy of the method has been studied using three different concentration levels 800 ppm, 1000 ppm and 1200 ppm of Zoledronic acid in presence of fixed concentration of impurities. The results obtained are presented in **Table No. 5.050, 5.051, 5.052 and 5.053**.

Intermediate precision: Intermediate precision of the method has been studied by analysis of six sample preparations of Zoledronic acid by different analyst using different instrument and on different day and are presented in **Table No. 5.06 and 5.061 ..**

Robustness:

Robustness of the method has been studied by slight variation in flow rate, pH of mobile phase and column temperature. The results obtained in the present finding are given in **Table No. 5.070, 5.071, 5.072, 5.073, 5.074, 5.075, 5.076, 5.077, 5.078, 5.0790, 5.091, 5.0792 and 5.093**

Stability of analytical sample Solution:

Stability of analytical sample solution for Zoledronic acid has been studied for 24 hours .The findings of the work carried are given in tabular form in **Table No. 5.080, 5.081, 5.082, 5.083, 5.084, 5.085, 5.086, 5.087 5.088 and 5.089 .**

Limit of detection and limit of quantification: LOD and LOQ method can be used for cleaning validation. In the present study the findings are given in **Table No 5.090**

Table No 5.01: % RSD for System Suitably.

Working Standard	Wt. of standard (mg)	Area
Injection-1	25.00	15069.20
Injection-2	--	15080.10
Injection-3	--	15100.50
Injection-4	--	15095.10
Injection-5	--	15109.50
Injection-6	--	15101.00
AVG	--	15092.57
SD	--	15.02
%RSD	--	0.10

Table No 5.02: % RSD for System Precision.

Working Standard	Wt. of standard (mg)	Area
Injection-1	24.95	15148.90
Injection-2	--	15188.70
Injection-3	--	15185.20
Injection-4	--	15208.20
Injection-5	--	15215.80
Injection-6	--	15210.70
AVG	--	15192.92
SD	--	24.85

%RSD	--	0.16
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Table No 5.03: Method Precision.

Sample No.	Wt. of sample (mg)	Area	Assay (%) (as such basis)	Assay (%) (on dried basis)
1	25.16	15221.80	99.57	99.82
2	25.14	15211.40	99.58	99.83
3	25.23	15274.00	99.64	99.89
4	25.15	15212.90	99.55	99.80
5	24.98	15118.30	99.61	99.86
6	24.96	15105.00	99.60	99.85
AVG	--	--	99.59	99.84
SD	--	--	0.03	0.03
%RSD	--	--	0.03	0.03

Table No 5.04: Linearity

Injecti on No.	Level-1 2.0 ppm Area	Level- 2 4.0 ppm Area	Level-3 10 ppm Area	Level-4 500 ppm Area	Level-5 800 ppm Area	Level-6 1000 ppm Area	Level-7 1200 ppm Area	Level-8 1500 ppm Area
1	9.11	19.81	64.53	7114.46	11867.30	14800.80	17874.00	22109.10
2	9.33	19.55	63.67	7107.58	11830.20	14865.50	17878.60	22119.50
3	9.77	18.28	64.47	7135.05	11860.60	14879.10	17861.30	22137.30
AVG	9.40	19.21	64.22	7119.03	11852.70	14848.47	17871.30	22121.97
SD	0.34	0.82	0.48	14.29	19.77	41.84	8.96	14.26
%RSD	3.57	4.26	0.75	0.20	0.17	0.28	0.05	0.06
Correlation coefficient (r) = 1.000								

Table No 5.050: Accuracy.(Standard)

Working Standard	Wt. of standard (mg)	Retention Time(min.)	Area
Injection-1	25.09	6.91	15156.00
Injection-2	--	6.92	15103.80
Injection-3	--	6.91	15119.90
Injection-4	--	6.92	15114.10
Injection-5	--	6.92	15098.60
Injection-6	--	6.92	15097.60
AVG	--	6.92	15115.00
SD	--	0.01	21.92
%RSD	--	0.07	0.15

Table No 5.051: Accuracy Level-1.

Sample No.	Wt. of sample (mg)	Retention Time (min.)	Area	Assay (%) (as such basis)	Assay (%) (on dried basis)
Sample-1	19.92	6.89	11830.40	98.80	99.05
Sample-2	20.14	6.90	11983.20	98.98	99.23
Sample-3	20.04	6.90	11911.70	98.88	99.13
AVG	--	6.90	--	--	--
SD	--	0.01	--	--	--
%RSD	--	0.08	--	--	--

Table No 5.052: Accuracy Level-2 .

Sample No.	Wt. of sample (mg)	Retention Time (min.)	Area	Assay (%) (as such basis)	Assay (%) (on dried basis)
Sample-1	24.71	6.92	14697.20	98.95	99.20
Sample-2	24.88	6.92	14783.40	98.85	99.10
Sample-3	24.55	6.92	14597.80	98.92	99.17
AVG	--	6.92	--	--	--
SD	--	0.00	--	--	--
%RSD	--	0.00	--	--	--

Table No 5.053: Accuracy Level-3

Sample No.	Wt. of sample (mg)	Retention Time (min.)	Area	Assay (%) (as such basis)	Assay (%) (on dried basis)
Sample-1	30.79	6.96	18449.80	99.68	99.93
Sample-2	30.65	6.96	18362.00	99.66	99.91
Sample-3	30.27	6.96	18123.30	99.60	99.85
AVG	--	6.96	--	--	--
SD	--	0.00	--	--	--
%RSD	--	0.00	--	--	--

Table No 5.06: Intermediate Precision: (Standard)

Working Standard	Wt. of standard (mg)	Retention Time (min.)	Area
Injection-1	25.01	7.47	14917337.00
Injection-2	--	7.47	14906594.00
Injection-3	--	7.47	14968079.00
Injection-4	--	7.48	15007264.00
Injection-5	--	7.47	15022647.00
Injection-6	--	7.47	14979561.00
AVG	--	7.47	14966913.67
SD	--	0.00	46887.05
%RSD	--	0.05	0.31

Table No 5.061: Intermediate Precision: (Sample)

Sample No.	Wt. of sample (mg)	Retention time (min.)	Area	Assay (%) (as such basis)	Assay (%) (on dried basis)
1	25.11	7.47	14934665.00	99.61	99.86
2	25.15	7.47	14960124.00	99.62	99.87
3	25.18	7.48	14968623.00	99.55	99.80
4	25.17	7.48	14974581.00	99.63	99.88
5	25.21	7.48	14988387.00	99.57	99.82
6	25.23	7.48	15004582.00	99.60	99.85
AVG	--	7.48	--	99.60	99.85
SD	--	0.01	--	0.03	0.03
%RSD	--	0.07	--	0.03	0.03

Table No 5.070: Robustness

Sr. No.	Parameters	Standard parameter	Changes studied	
1	Flow rate	1.0 ml/min.	0.9 ml/min.	1.1 ml/min.
2	pH of mobile phase	3.50	3.40	3.60
3	Column temperature	35°C	30°C	40°C

Table No 5.071: Robustness. (Standard)

Flow rate 0.9 ml/min

Working Standard	Wt. of standard (mg)	Retention Time (min.)	Area
Injection-1	24.74	7.79	16906.50
Injection-2	--	7.79	16738.40

Injection-3	--	7.79	16746.50
Injection-4	--	7.79	16747.60
Injection-5	--	7.79	16748.70
Injection-6	--	7.79	16796.50
AVG	--	7.79	16780.70
SD	--	0.00	65.04
%RSD	--	0.00	0.39

Table No 5.072: Robustness. (Sample)

Sample No.	Wt. of sample (mg)	Retention time (min.)	Area	Assay (%) (as such basis)	Assay (%) (on dried basis)
1	25.16	7.79	16961.40	99.61	99.86
2	24.88	7.79	16777.60	99.64	99.89
3	25.13	7.79	16934.50	99.57	99.82
4	25.05	7.79	16890.90	99.63	99.88
5	25.03	7.79	16868.10	99.57	99.82
6	25.07	7.80	16892.80	99.56	99.81
AVG	--	7.79	--	99.60	99.85
SD	--	0.00	--	0.03	0.03
%RSD	--	0.05	--	0.03	0.03

Table No 5.073: Robustness. (Standard)

Flow rate 1.1 ml/min

Working Standard	Wt. of standard (mg)	Retention Time (min.)	Area
Injection-1	24.98	6.37	13610.00
Injection-2	--	6.37	13615.00

Injection-3	--	6.37	13622.90
Injection-4	--	6.37	13634.70
Injection-5	--	6.37	13626.00
Injection-6	--	6.37	13632.10
AVG	--	6.37	13623.45
SD	--	0.00	9.60
%RSD	--	0.00	0.07

Table No 5.074: Robustness. (Sample)

Sample No.	Wt. of sample (mg)	Retention time (min.)	Area	Assay (%) (as such basis)	Assay (%) (on dried basis)
1	25.12	6.37	13618.20	99.62	99.87
2	25.22	6.36	13676.60	99.65	99.90
3	25.21	6.37	13665.60	99.61	99.86
4	25.18	6.37	13645.40	99.58	99.83
5	25.26	6.36	13639.10	99.62	99.87
6	25.28	6.37	13697.80	99.57	99.82
AVG	--	6.37	--	99.61	99.86
SD	--	0.01	--	0.03	0.03
%RSD	--	0.08	--	0.03	0.03

Table No 5.075: Robustness. (Standard)

Mobile phase pH = 3.40

Working Standard	Wt. of standard (mg)	Retention Time (min.)	Area
Injection-1	24.79	8.13	14875.00
Injection-2	--	8.13	14880.90
Injection-3	--	8.13	14924.70
Injection-4	--	8.13	14922.70

Injection-5	--	8.13	14943.80
Injection-6	--	8.13	14949.30
AVG	--	8.13	14916.07
SD	--	0.00	31.35
%RSD	--	0.00	0.21

Table No 5.076: Robustness. (Sample)

Sample No.	Wt. of sample (mg)	Retention time (min.)	Area	Assay (%) (as such basis)	Assay (%) (on dried basis)
1	25.82	8.14	15444.70	99.63	99.88
2	25.05	8.14	14982.60	99.62	99.87
3	25.55	8.14	15285.30	99.65	99.90
4	25.07	8.14	14991.70	99.60	99.85
5	25.43	8.14	15212.10	99.64	99.89
6	25.48	8.14	15238.20	99.61	99.86
AVG	--	8.14	--	99.63	99.88
SD	--	0.00	--	0.02	0.02
%RSD	--	0.00	--	0.02	0.02

Table No 5.077: Robustness. (Standard)

Mobile phase pH = 3.60

Working Standard	Wt. of standard (mg)	Retention Time (min.)	Area
Injection-1	24.85	6.59	15124.50
Injection-2	--	6.59	15291.50
Injection-3	--	6.59	15285.80
Injection-4	--	6.59	15231.10
Injection-5	--	6.59	15205.70
Injection-6	--	6.59	15207.80
AVG	--	6.59	15224.40

SD	--	0.00	61.52
%RSD	--	0.00	0.40

Table No 5.078: Robustness. (Sample)

Sample No.	Wt. of sample (mg)	Retention time (min.)	Area	Assay (%) (as such basis)	Assay (%) (on dried basis)
1	25.16	6.59	15315.60	99.58	99.83
2	25.47	6.58	15509.50	99.61	99.86
3	25.31	6.58	15410.10	99.60	99.85
4	25.22	6.59	15361.00	99.64	99.89
5	25.26	6.59	15374.70	99.57	99.82
6	25.48	6.58	15516.70	99.62	99.87
AVG	--	6.59	--	99.60	99.85
SD	--	0.01	--	0.03	0.03
%RSD	--	0.08	--	0.03	0.03

Table No 5.079: Robustness. (Standard)

Column temperature = 30°C

Working Standard	Wt. of standard (mg)	Retention Time (min.)	Area
Injection-1	25.02	7.01	15034.50
Injection-2	--	7.00	15010.40
Injection-3	--	7.00	15028.50
Injection-4	--	7.00	14969.40
Injection-5	--	7.00	14955.70
Injection-6	--	7.00	14957.10
AVG	--	7.00	14992.60
SD	--	0.00	36.11

%RSD	--	0.06	0.24
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Table No 5.0791: Robustness. (Sample)

Sample No.	Wt. of sample (mg)	Retention time (min.)	Area	Assay (%) (as such basis)	Assay (%) (on dried basis)
1	25.15	7.00	14981.40	99.63	99.88
2	25.21	7.00	15018.90	99.64	99.89
3	25.17	7.00	14988.60	99.60	99.85
4	25.16	7.00	14978.80	99.57	99.82
5	25.06	7.00	14926.80	99.62	99.87
6	25.22	6.99	15018.00	99.59	99.84
AVG	--	7.00	--	99.61	99.86
SD	--	0.00	--	0.03	0.03
%RSD	--	0.06	--	0.03	0.03

Table No 5.0792: Robustness. (Standard)

Column temperature = 40°C

Working Standard	Wt. of standard (mg)	Retention Time (min.)	Area
Injection-1	24.96	7.07	14848.50
Injection-2	--	7.08	15323.30
Injection-3	--	7.07	14868.40
Injection-4	--	7.07	14879.20
Injection-5	--	7.07	14896.20
Injection-6	--	7.07	14909.30
AVG	--	7.07	14954.15
SD	--	0.00	182.08
%RSD	--	0.06	1.22

Table No 5.0793: Robustness. (Sample)

Sample No.	Wt. of sample (mg)	Retention time (min.)	Area	Assay (%) (as such basis)	Assay (%) (on dried basis)
1	25.06	7.07	14920.90	99.60	99.85
2	25.03	7.07	14907.20	99.63	99.88
3	25.04	7.08	14907.60	99.59	99.84
4	25.10	7.07	14947.40	99.62	99.87
5	25.04	7.07	14915.50	99.64	99.89
6	25.01	7.08	14893.40	99.61	99.86
AVG	--	7.07	--	99.61	99.86
SD	--	0.01	--	0.02	0.02
%RSD	--	0.07	--	0.02	0.02

Table No 5.080: Solution stability (Standard.). Time Period: 0 hr

Working Standard	Wt. of standard (mg)	Retention Time (min.)	Area
Injection-1	24.99	7.32	14893.50
Injection-2	--	7.32	14938.50
Injection-3	--	7.32	14915.80
Injection-4	--	7.32	14870.30
Injection-5	--	7.32	14868.80
Injection-6	--	7.32	14953.00
AVG	--	7.32	14906.65
SD	--	0.00	35.14
%RSD	--	0.00	0.24

Table No 5.081: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time (min.)	Area	Assay (%) (as such basis)	Assay (%) (on dried basis)
1	25.15	7.33	14908.00	99.59	99.84

Table No 5.082: Solution stability (Standard.). Time Period: 6.0 hr

Working Standard	Wt. of standard (mg)	Retention Time (min.)	Area
Injection-1	25.16	7.32	15109.30
Injection-2	--	7.32	15102.30
Injection-3	--	7.32	15053.10
Injection-4	--	7.32	15138.40
Injection-5	--	7.32	15054.70
Injection-6	--	7.31	15039.80
AVG	--	7.32	15082.93
SD	--	0.00	39.23

%RSD	--	0.06	0.26
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Table No 5.083: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time (min.)	Area	Assay (%) (as such basis)	Assay (%) (on dried basis)
1	25.15	7.32	14983.10	99.60	99.85

Table No 5.084: Solution stability (Standard.). Time Period: 12.0 hr

Working Standard	Wt. of standard (mg)	Retention Time (min.)	Area
Injection-1	25.23	7.32	15261.60
Injection-2	--	7.32	15216.00
Injection-3	--	7.32	15254.70
Injection-4	--	7.32	15218.40
Injection-5	--	7.32	15233.50
Injection-6	--	7.32	15294.60
AVG	--	7.32	15246.47
SD	--	0.00	29.99
%RSD	--	0.00	0.20

Table No 5.085: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time (min.)	Area	Assay (%) (as such basis)	Assay (%) (on dried basis)
1	25.15	7.31	15097.70	99.56	99.81

Table No 5.086: Solution stability (Standard.). Time Period: 18.0 hr

Working Standard	Wt. of standard (mg)	Retention Time (min.)	Area
Injection-1	25.10	7.33	15282.90
Injection-2	--	7.33	15096.90
Injection-3	--	7.34	15068.50
Injection-4	--	7.34	15100.60
Injection-5	--	7.33	14996.70
Injection-6	--	7.33	15003.00
AVG	--	7.33	15091.43
SD	--	0.01	104.01
%RSD	--	0.07	0.69

Table No 5.087: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time (min.)	Area	Assay (%) (as such basis)	Assay (%) (on dried basis)
1	25.15	7.33	15024.20	99.58	99.82

Table No 5.088: Solution stability (Standard.). Time Period: 24.0 hr

Working Standard	Wt. of standard (mg)	Retention Time (min.)	Area
Injection-1	24.72	7.33	14935.80
Injection-2	--	7.33	14832.80
Injection-3	--	7.33	14866.40
Injection-4	--	7.33	14836.70
Injection-5	--	7.33	14833.60
Injection-6	--	7.33	14826.30
AVG	--	7.33	14855.27
SD	--	0.00	41.87
%RSD	--	0.00	0.28

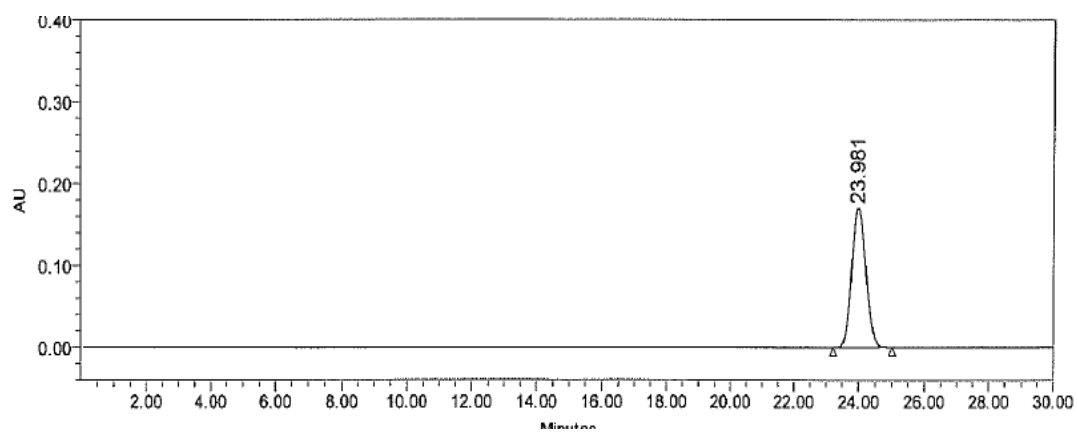
Table No 5.089: Solution stability (Sample.).

Sample No.	Wt. of sample (mg)	Retention time (min.)	Area	Assay (%) (as such basis)	Assay (%) (on dried basis)
1	25.15	7.33	15020.70	99.60	99.85

Table No 5.09: Solution stability (Standard) LOD/LOQ

Injection No.	Level – 1	Level – 2	Level – 3	Level – 4	Level – 5
Conc. in ppm ---->	3.0 ppm	2.0 ppm	1.0 ppm	0.5 ppm	0.25 ppm
	Area	Area	Area	Area	Area
Injection - 1	9.87	3.97	1.99	0.81	Not detected
Injection - 2	10.21	3.61	2.09	1.15	Not detected
Injection - 3	9.55	3.89	1.89	1.21	Not detected
Injection - 4	10.47	3.49	1.84	0.82	Not detected
Injection - 5	10.28	4.00	2.21	1.15	Not detected
Injection - 6	9.67	3.96	2.57	1.12	Not detected
AVG	10.01	3.82	2.10	1.04	--
SD	0.37	0.22	0.27	0.18	--
%RSD	3.66	5.64	12.73	17.18	--

Zoledronic acid Standard



Zoledronic acid Sample

