Development and Validation for the Identification of Zoledronic acid Impurities by HPLC in Zoledronic Acid Injection

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ABSTRACT

A novel isocratic reverse-phase high-performance liquid chromatographic method for the estimation of Zoledronic acid in parenteral dosage form has been developed. This method is simple, rapid, selective, precise, and accurate. Inert Sustain Swift C18 (250 x 4.6 mm,5µ) column was used, and the separation was accomplished with a mobile phase of pH 2.80 triethylamine buffer and methanol (96:40 volume/volume). A constant 0.8 mL/min was recorded. The 220nm UV detector was used to detect zoledronic acid. Run time is 35 minutes at column temperatures of 30°C and sample temperatures of room temperature with an injection volume of 20µL. It was found that 5.37 min was the retention time of zoledronic acid. Validation of the procedure was performed in accordance with ICH standards. Accuracy, reproducibility, and consistency were all observed using the proposed procedure.

Keywords: Zoledronic acid, Liquid chromatography, Forced degradation, validation.

INTRODUCTION

"Zoledronic acid is assigned chemically as (1-hydroxy-2-imidazol-1-yl-phosphonoethyl) phosphonic acid monohydrate. It is a white crystalline powder"[1]. Bisphosphonic acid zoledronic acid inhibits bone resorption by osteoclasts. Bone resorption can be stopped by administering biphosphonates, which are hydrolytically stable pyrophosphate analogues that suppress osteoclast and, presumably, osteoblast activity. [2].

In the treatment of Paget's disease, tumor-induced hypercalcemia (TIH), and multiple myeloma[3], bisphosphonates have been shown to be effective as a therapeutic option for disorders of bone turnover. In addition to treating tumor-induced hypercalcemia and preventing bone metastases from cancer in general, this medication is currently in

development as a treatment for advanced breast cancer and localised prostate cancer that has spread to the bones[4].

"Molecular formula is $C_5H_{10}N_2O_7P_2$ and Molecular weight 272.090 g/mol[5]. The chemical structure of Zoledronic acid shown in Figure1".

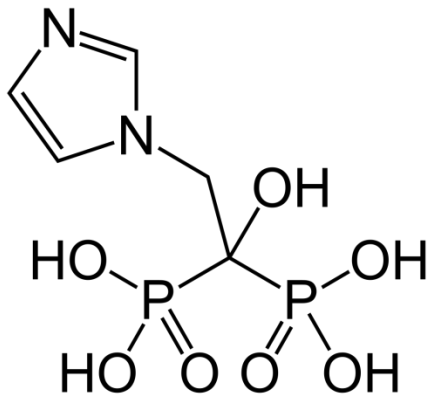

Figure 1: Chemical structure of Zoledronic acid

While there are a handful of HPLC methods[6] reported for the estimation of Zoledronic acid in pharmaceutical dosage forms[7], a review of the literature reveals that only a handful of these methods have been used to date.

This study details a straightforward RP-HPLC method for analysing Zoledronic acid in its parenteral dosage form in accordance with ICH guidelines[8].

MATERIALS & METHOD

Chemicals and Reagents

"Analytical-grade Triethylamine, Orthophosphoric acid, Methanol, Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide and water, reagents and chemicals were procured from Merck Chemicals. Mumbai, India".

Instrumentation

Waters HPLC model: 2695 with PDA, Ultrasonic bath Sonicator, pH Meter (Mettler Toledo) and Analytical Balance (Sartorious) were used in the present study.

Preparation of Buffer: Accurately transfer 2.0 mL of Triethylamine in 1000 mL of water, adjust the pH with orthophosphoric acid to 2.80 ± 0.05, filter and degas prior to use.

Preparation of Mobile phase: "Mix 960 mL of buffer, 40 mL of methanol and degas.The flow rate was 0.8mL/min. Zoledronic acid was detected using UV detector at the wavelength of 220nm. Column temperature 30°C and sample temperature ambient and injection volume 20µL, run time 35 minutes. Used mobile phase as diluent".

Preparation of Impurity Stock Solution:

Weigh and transfer around 2.0 mg of Imidazole RS and 2.0 mg of Imidazole-1-yl-ethanoic acid RS (RC-A) separately into a 50 mL volumetric flask containing 15 mL diluent dissolve and dilute to volume up to the mark with diluent.

Preparation of System Suitability Solution:

Weigh and transfer around 4 mg of ZA into a 5 mL volumetric flask containing 2.5 mL diluent dissolve and add 0.5 mL of each impurity stock solution to the volumetric flask, dissolve and dilute to volume up to the mark with diluent.

Reference standard solution for RS:

Dissolve and dilute to the appropriate concentration using the diluent, and then mix in about 4.0 mg of the ZA standard that has been weighed and transferred to a 5 mL volumetric flask containing 2.5mL diluent. To make a solution with a known concentration of 0.01 mg/ mL, put 0.5 mL of this solution into a 50 mL volumetric flask and dilute it to the appropriate volume with diluent until the mark is reached..

System Suitability and System Precision[8]

As per methodology, injected Blank (diluent) diluted standard for six times into HPLC system. Retention times of Imidazole, RC-A and Zoledronic acid are respectively 3.501, 4.661, and 5.415

Specificity

Blank, placebo and Impurity Interference:

Study Design:

As per the method, the Blank (diluent) solution and the placebo solution were put into the HPLC, and the interference between the blank and placebo peaks at the time of retention of Zoledronic acid and its impurities was checked. Each impurity was made and injected at the level specified, and interference was checked at each impurity retention time.

Preparation of the test solution:

As Such Zoledronic acid Injection (Label claim-0.8 mg/ mL) taken directly into a HPLC vial.

Analyzed six test preparations as per the methodology and determined the % RSD for total impurities from six sample Preparations.

Method Precision:

Analyzed six test preparations as per the methodology and determined the % RSD for total impurities from six sample Preparations.

Accuracy:

Design: As per methodology, injected Blank (diluent), and demonstrated by spiking impurities at 50 %, 100 %, and 150 % to the target concentration on sample and then injected accuracy solutions into HPLC. Calculated the system suitability parameters and % mean recovery.

Accuracy for Unknown Impurities:

As per methodology, injected Blank (diluent), and demonstrated by spiking standard solution to Blank at 50 %, 100 %, and 150% level to the target concentration and then injected accuracy solutions into HPLC. Calculated the system suitability parameters and % mean recovery.

Linearity:

Linearity for Zoledronic acid and its impurities was determined in the concentration range from LOQ, 50 %, 80 %, 100 %, 120 %, and 150 %, concentration levels.

LOD & LOQ:

As per methodology, injected Blank (diluent), System suitability solution for two times and reference standard for three times and then injected LOD & LOQ Solutions into HPLC.

RESULTS:

Table 1: System suitability

Parameter	Resolution Between Imidazole, RC-A	Resolution Between RC-A, Zoledronic acid	% RSD
System suitability	6.12	3.69	1.2
Acceptance Criteria	NLT 2.0	NLT 2.0	NMT 5.0

Table 2: System Precision

Injection Number	Zoledronic acid peak area	Acceptance criteria
01	106503	% RSD for the peak area of Zoledronic acid replicate injections of reference standard solution should be not more than 5.0.
02	105888	
03	106226	
04	107047	
05	106669	
06	106266	
Mean	106433	
S.D	401.33	
% RSD	0.38	

The above results reveal that the system meets the required system suitability and System Precision.

Specificity

Table 3: Blank Interference Data:

S.No	RT of Impurity RC-A	RT of Impurity RC-B	RT of Zoledronic acid
	Interference found (Yes/No)		
1	No	No	No

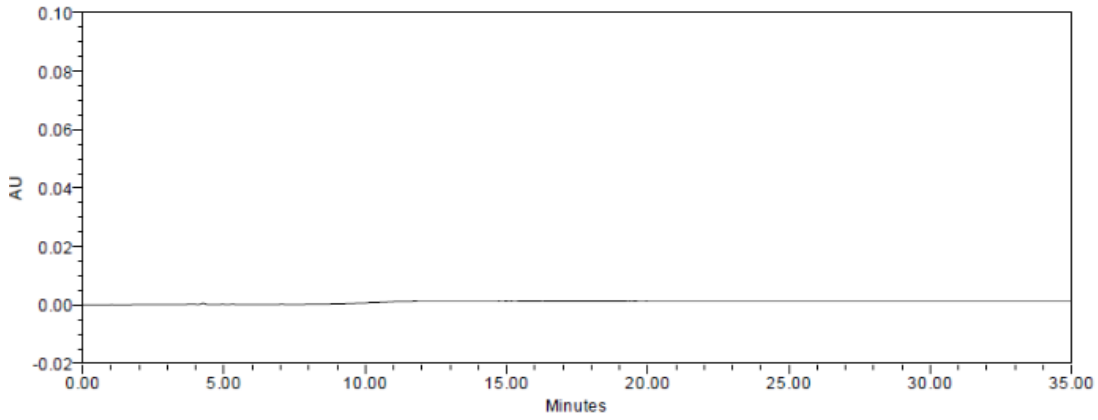

Figure 2: Typical Chromatogram of Blank

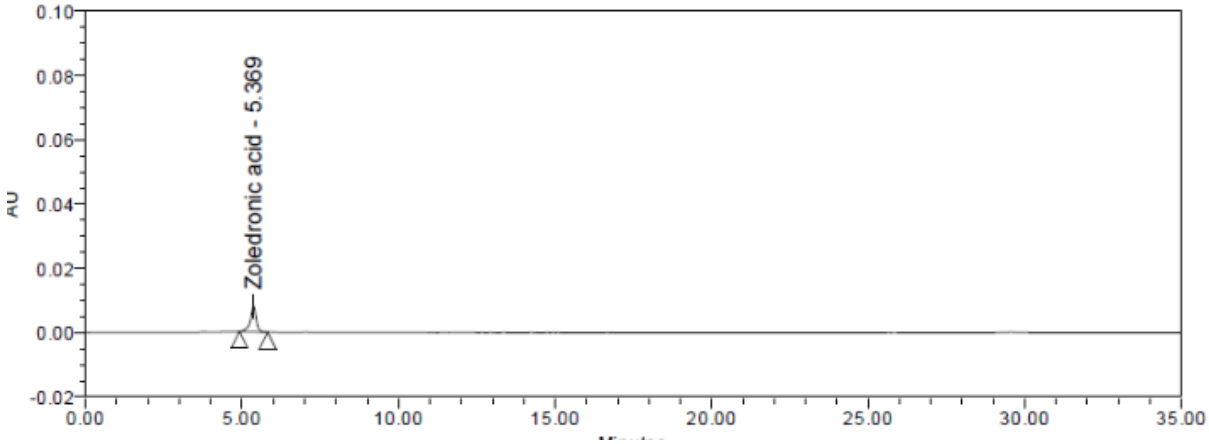

Figure 3: Typical Chromatogram of Diluted Standard

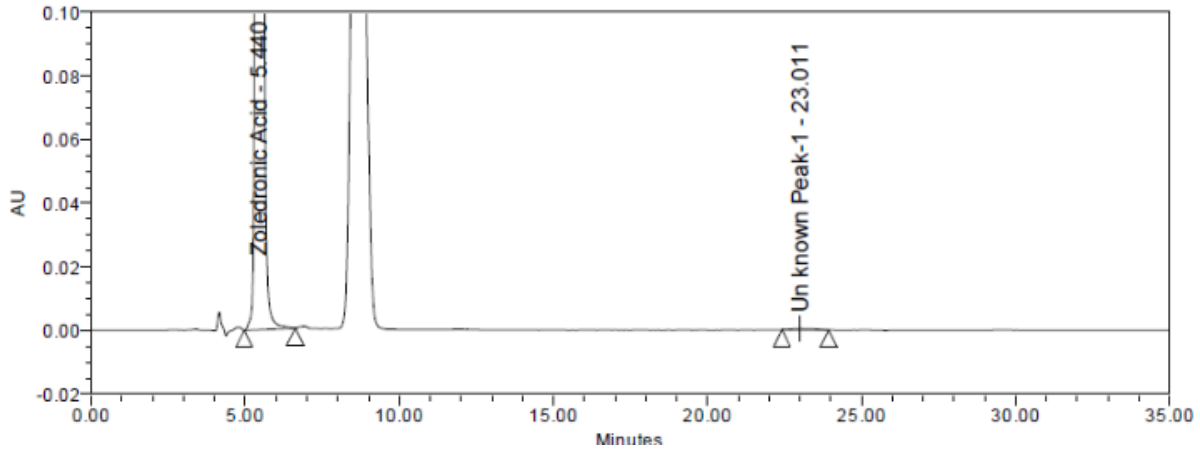

Figure 4: Typical chromatogram of as such sample

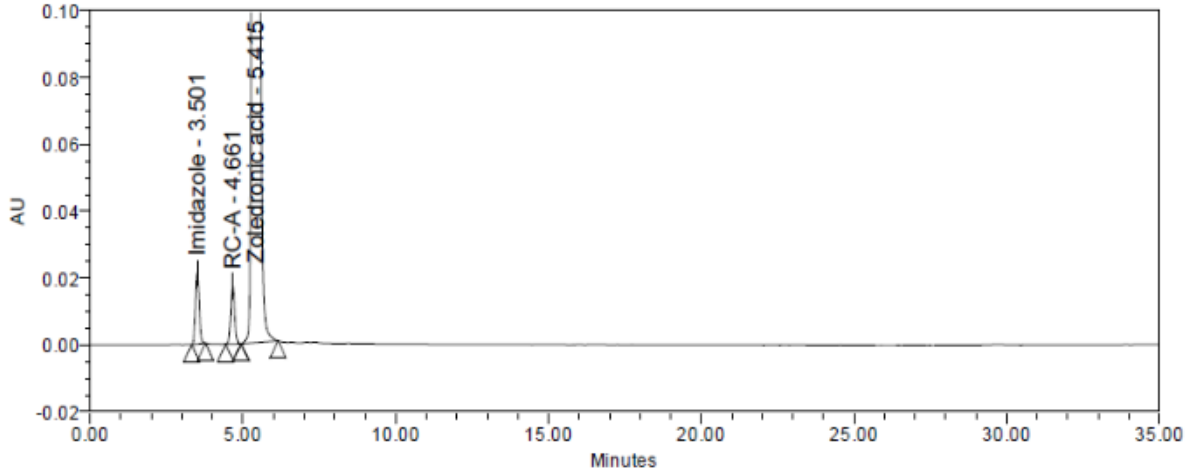

Figure 5: Typical chromatogram of SST

Table 4: System suitability

Parameter	Resolution Between Imidazole and RC-A	Resolution Between RC-A and Zoledronic acid	% RSD
System suitability	5.3	3.0	0.7
Acceptance Criteria	NLT 2.0	NLT 2.0	NMT 5.0

Method Precision:

Table: 5: Method precision Results:

Sample	Imidazole-RS (% w/w)	RC -A (% w/w)	Maximum Unknown Impurity (% w/w)	Total Impurities (% w/w)
01	1.066	1.089	0.116	2.331
02	1.067	1.090	0.123	2.340
03	1.065	1.091	0.125	2.342
04	1.065	1.089	0.118	2.334
05	1.067	1.091	0.117	2.337
06	1.068	1.093	0.123	2.346
Average	1.066	1.091	0.120	2.339
S.D	0.0011	0.0017	0.0037	0.0056
%RSD	0.1	0.1	3.1	0.2

The % Relative Standard Deviation of all impurities from six sample preparations should not be more than 10.0**.** The above results reveal that the method is precise

Accuracy

Table 6: System suitability

Parameter	**Resolution Between Imidazole and RC-A**	**Resolution Between RC-A and Zoledronic acid**	**% RSD**
System suitability	4.9	2.4	0.4
Acceptance Criteria	NLT 2.0	NLT 2.0	NMT 5.0

Table 7: Accuracy Imidazole RS (RC-B):

Sample No	**Spike level**	**Amount added (% w/w)**	**Amount found (% w/w)**	**% Recovery**	**Mean % recovery**	**% RSD**
1	50 %	0.268	0.292	109.0	108.3	0.7
2	50 %	0.268	0.292	109.0		
3	50 %	0.268	0.291	108.6		
4	50 %	0.268	0.287	107.1		
5	50 %	0.268	0.291	108.6		
6	50 %	0.268	0.288	107.5		
1	100 %	0.536	0.540	100.7	100.1	1.0
2	100 %	0.536	0.540	100.7		
3	100 %	0.536	0.530	98.9		

1	150 %	1.597	1.613	101.0	100.7	0.2
2	150 %	1.597	1612	100.9		
3	150 %	1.597	1.605	100.5		
4	150 %	1.597	1.612	100.9		
5	150 %	1.597	1.608	100.7		
6	150 %	1.597	1.603	100.4		

Table 8: Imidazole-1-yl-ethanoic acid RS (RC –A):

Sample No	**Spike level**	**Amount added (% w/w)**	**Amount found (% w/w)**	**% Recovery**	**Mean % recovery**	**% RSD**
1	50 %	0.293	0.338	115.2	112.4	1.7
2	50 %	0.293	0.330	111.9		
3	50 %	0.293	0.330	112.9		
4	50 %	0.293	0.330	112.6		
5	50 %	0.293	0.330	112.3		
6	50 %	0.293	0.320	109.4		
1	100 %	0.586	0.580	99.0	99.0	0.0
2	100 %	0.586	0.580	99.0		
3	100 %	0.586	0.580	99.0		
1	150 %	1.549	1.626	105	104.8	0.2
2	150 %	1.549	1.627	105		
3	150 %	1.549	1.620	104.6		
4	150 %	1.549	1.627	105.0		

5	150 %	1.549	1.623	104.8		
6	150 %	1.549	1.620	104.6		

Accuracy for Unknown Impurity:

Table 9: System suitability

Parameter	Resolution Between Imidazole and RC-A	Resolution Between RC-A and Zoledronic acid	% RSD
System suitability	5.3	3.0	0.7
Acceptance Criteria	NLT 2.0	NLT 2.0	NMT 5.0

Table 10: Unknown Impurities:

Sample No	Spike level	Amount added (% w/w)	Amount found (% w/w)	% Recovery	Mean % recovery	% RSD
1	50 %	0.3660	0.3070	83.9	85.6	3.4
2	50 %	0.3660	0.3190	87.2		
3	50 %	0.3660	0.3020	82.5		
4	50 %	0.3660	0.3300	90.2		
5	50 %	0.3660	0.3050	83.3		
6	50 %	0.3660	0.3170	86.6		
1	100 %	0.7320	0.6327	86.4	89.4	3.1
2	100 %	0.7320	0.6593	90.1		
3	100 %	0.7320	0.6721	91.8		
1	150 %	0.708	0.575	81.2	84.5	3.7
2	150 %	0.708	0.604	85.4		
3	150 %	0.708	0.588	83.0		
4	150 %	0.708	0.592	83.6		
5	150 %	0.708	0.640	90.3		
6	150 %	0.708	0.592	83.6		

Individual and mean % recovery value at 50%, 100% and 150% should be in between 80.0 % to 120.0 %**.** % RSD for each impurity should be not more than 10.0**.** The above results reveal that the method is accurate.

Linearity

Table 11: System suitability

Parameter	**Resolution Between Imidazole and RC-A**	**Resolution Between RC-A and Zoledronic acid**	**% RSD**
System suitability	5.3	3.0	0.7
Acceptance Criteria	NLT 2.0	NLT 2.0	NMT 5.0

Table 12: Linearity Results of Imidazole-1-yl-ethanoic acid RS (RC-A):

Level (%w/w)	**Concentration (%)**	**Area**
LOQ	0.031	10020
50 %	0.494	176643
80 %	0.790	288001
100 %	0.988	357494
120 %	1.185	433074
150 %	1.4481	541816
Correlation Coefficient	0.9997	
R^2	0.9994	
Slope	37297.0005	
Y-Intercept	-5642.618	
%Y-Intercept	1.6	

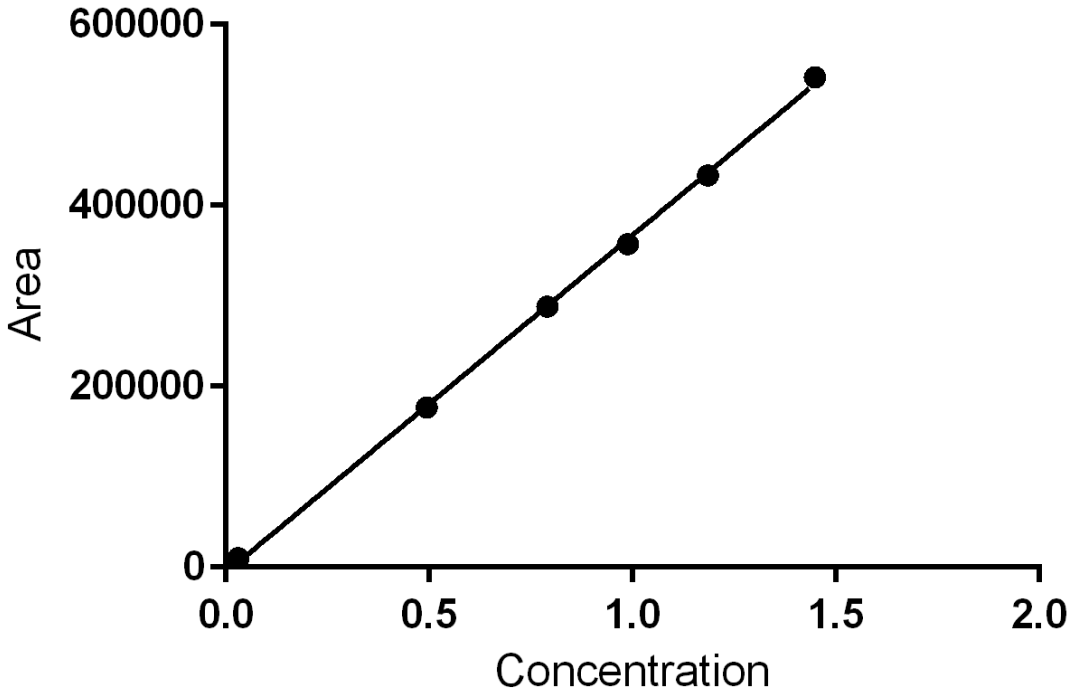

Figure 6: Linearity for Imidazole-1-yl-ethanoic acid RS (RC-A):

Table 13: Linearity Results of Imidazole-RS

Level (%w/w)	**Concentration (%)**	**Area**
LOQ	0.011	3053
50 %	0.507	206040
80 %	0.812	332398
100 %	1.014	410897
120 %	1.217	496281
150 %	1.522	619854
Correlation Coefficient	1.000	
R^2	1.000	
Slope	408081.8970	

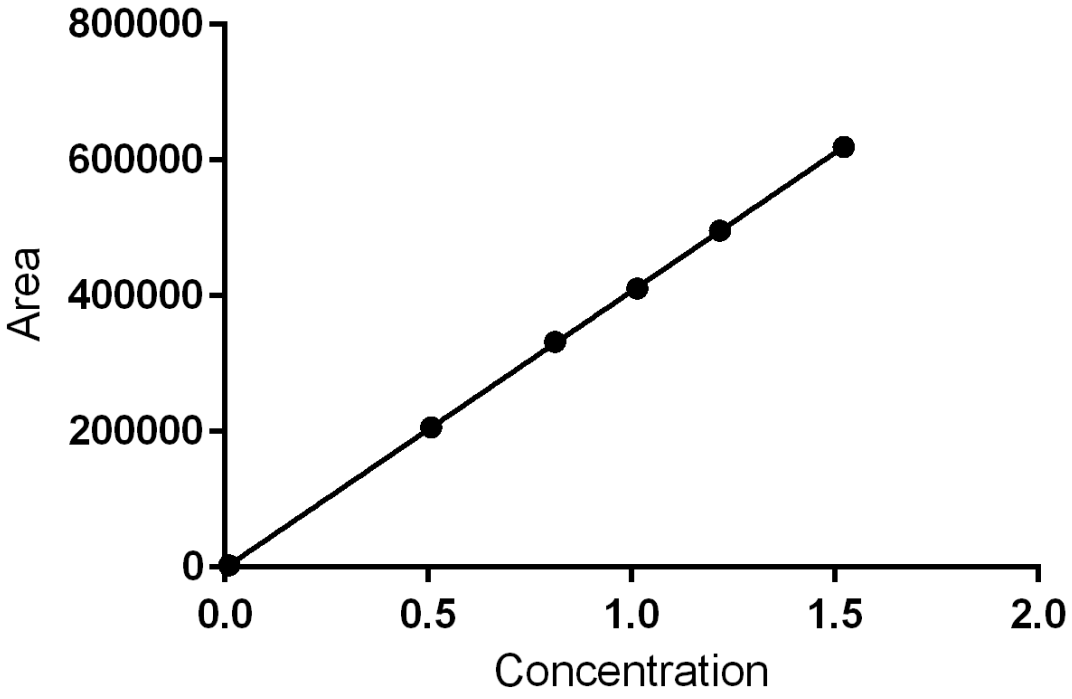

Figure 7: Linearity for Imidazole-RS

Table: 14. Linearity Results of Zoledronic Acid

Level (%w/w)	**Concentration (%)**	**Area**
LOQ	0.047	1623
50 %	0.256	17405
80 %	0.410	32996
100 %	0.512	42832
120 %	0.615	55390
150 %	0.768	70510
Correlation Coefficient	0.997	
R^2	0.9945	
Slope	97018.3769	
Y-Intercept	-5377.99	

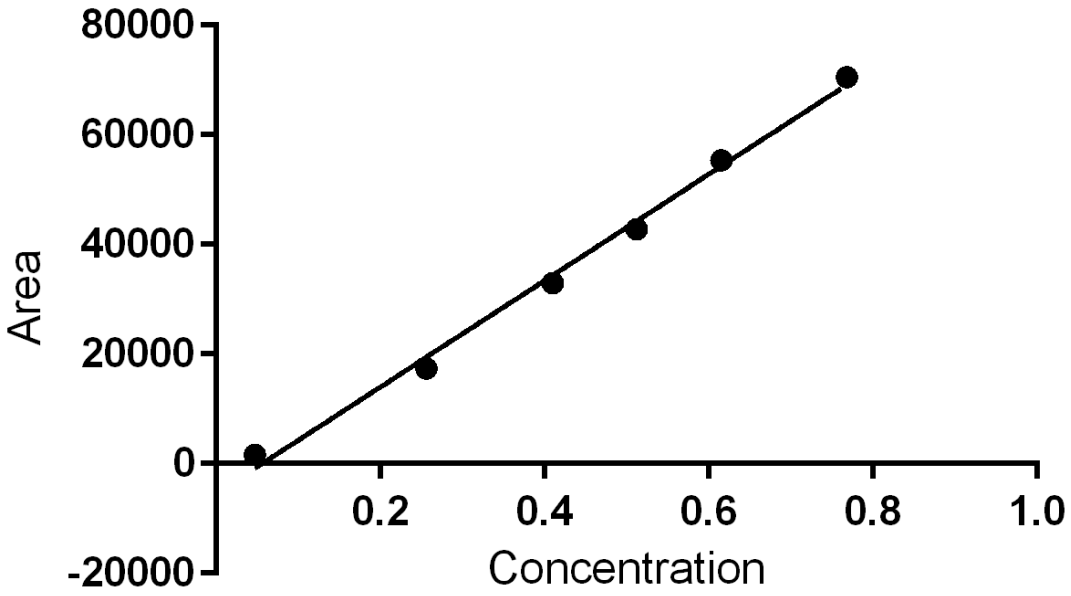

Figure 8: Linearity for Zoledronic Acid

The Correlation coefficient should be not less than 0.99. The above results reveal that the method is Linear from LOQ% to 150 % level.

LOD & LOQ:

Table 15: System suitability

Parameter	**Resolution Between Imidazole, RC-A**	**Resolution Between RC-A, Zoledronic acid**	**% RSD**
System suitability	5.7	3.3	2.2
Acceptance Criteria	NLT 2.0	NLT 2.0	NMT 5.0

Table 16: Limit of Detection and Limit of Quantification:

Name	**LOD (%)**	**LOQ (%)**	**LOD (S/N)**	**LOQ (S/N)**
Imidazole-1-yl-ethanoic acid RS (RC-A)	0.008	0.03	7	15
Imidazole-RS	0.004	0.01	3	10

Zoledronic acid	0.016	0.05	5	11

S/N ratio of LOQ should not be less than 10.0 and S/N ratio of LOD should not be less than 3.0

Table 17: Precision at LOQ for Imidazole-1-yl-ethanoic acid RS (RC-A):

Sample No.	RC-A Area	RC-A (%w/w)
1	7769	0.018
2	7855	0.019
3	7837	0.019
4	7862	0.019
5	8117	0.019
6	7896	0.019
Mean	7889.3	0.019
Standard deviation	119.1816	0.0003
%RSD	1.5	1.5

Table 18: Precision at LOQ for Imidazole-RS:

Sample No.	Imidazole-RS Area	Imidazole-RS (%w/w)
1	3840	0.008
2	3868	0.008
3	3877	0.008
4	3868	0.008
5	3968	0.009
6	3970	0.009
Mean	3898.5	0.008
Standard deviation	56.0133	0.0001
%RSD	1.4	1.4

Table 19: Precision at LOQ for Zoledronic acid:

Sample No.	Zoledronic acid Area	Zoledronic acid (%w/w)
1	6414	0.039
2	6429	0.039
3	6500	0.039
4	6388	0.039
5	6330	0.038
6	7411	0.045
Mean	6578.7	0.040
Standard deviation	411.5024	0.0025
%RSD	6.3	6.3

The % RSD of Zoledronic acid and its impurities obtained at LOQ level from six preparations should be not more than 15.0. The above results conclude that the obtained LOQ concentration is precise.

Table 20: Accuracy at LOQ Level for Imidazole-RS:

Sample No.	Imidazole-RS		
	% Added	**% Found**	**% Recovery**
1	0.009	0.008	88.9
2	0.009	0.008	93.2
3	0.009	0.008	93.6
Mean			91.9
S.D			2.6114
% RSD			2.8

Table 21: Accuracy at LOQ Level for Imidazole-1-yl-ethanoic acid RS (RC-A):

Sample No.	Imidazole-1-yl-ethanoic acid RS (RC-A)		
	% Added	**% Found**	**% Recovery**
1	0.02	0.018	90.0
2	0.02	0.019	95.0

3	0.02	0.019	95.0
Mean			93.3
S.D			2.8868
%RSD			3.1

Table 22: Accuracy at LOQ Level Zoledronic acid:

Sample No.	**Zoledronic acid**		
	% Added	**% Found**	**% Recovery**
1	0.042	0.039	92.9
2	0.042	0.039	92.9
3	0.042	0.039	92.9
Mean			92.9
S.D			0.000
%RSD			0.0

The Individual and mean recovery of Zoledronic acid and its impurities at Limit of Quantification should not be less than 80.0% and should not be more than 120.0%. The above results conclude that the obtained LOQ concentration is accurate.

Conclusion:

The present analytical method has been validated according to a defined procedure and found to be acceptable. It was found that the analytical approach is targeted, exact, linear, accurate, robust, and sturdy. The test preparation was only stable for 24 hours, but the usual solution was steady for 5 days. Up to day 5, there were no changes to the mobile phase. As a result, the current analytical approach has been validated as a stability indicator and is suitable for routine usage.

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