Development and validation of stability indicating RP-HPLC method for quantitative estimation of Zoledronic acid in Zoledronic acid solution for infusion pharmaceutical dosage form

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ABSTRACT

A new, simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid chromatography assay method has been developed for estimation of Zoledronic acid in parenteral dosage form. The separation was achieved by using column Symmetry C18 (150 x 4.6 mm, 5µ) mobile phase consisted of pH 3.5 triethylamine buffer and methanol in the ratio of (90:10 volume/volume). The flow rate was 0.5mL/min. Zoledronic acid was detected using UV detector at the wavelength of 220 nm. Column temperature 40°C and sample temperature ambient and injection volume 20µL, run time 10 minutes. The retention time of Zoledronic acid was noted to be 3.8 min respectively. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible and consistent.

Keywords: Zoledronic acid, Liquid chromatography, Forced degradation and Validation.

1.0 Introduction

Zoledronic acid is assigned chemically as (1-hydroxy-2-imidazol-1-yl-phosphonoethyl) phosphonic acid monohydrate. It is a white crystalline powder. Zoledronic acid, a bisphosphonic acid, is an inhibitor of osteoclastic bone resorption. Biphosphonates are hydrolytically stable analogs of pyrophosphate, which inhibit bone resorption as a consequence of affecting osteoclast and likely osteoblast activity [1-3]. The therapeutic efficacy of bisphosphonates in disorders of bone turnover has been shown in the treatment of Paget's disease, tumor-induced hypercalcemia (TIH), and multiple myeloma. It is being formulated for the treatment of tumor induced hypercalcemia, bone metastases arising from any cancer, and for the prevention of bone metastases associated with advanced breast cancer and locally advanced prostate cancer [4].

Molecular formula is $C_5H_{10}N_2O_7P_2$ and Molecular weight 272.090 g/mol. The chemical structure of Zoledronic acid shown in (**Figure.1).**

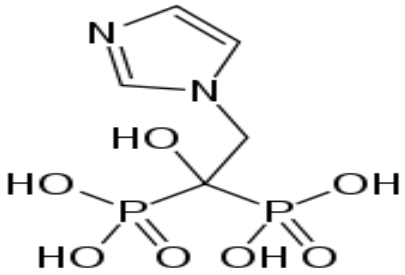

Figure.1 Chemical structure of Zoledronic acid

The literature survey reveals only few methods were reported till date a few HPLC [5-9] methods were reported for the estimation of Zoledronic acid in pharmaceutical dosage forms. Hence we tried to develop stability indicating HPLC method for Zoledronic acid. The present work describes a simple, stability indicating HPLC method for the determination of Zoledronic acid in Zoledronic acid in parenteral dosage form according to ICH guidelines [10-11].

2.0 Experimental

2.1. Chemicals and Reagents

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

2.2. Instrumentation

Waters HPLC model: 2690 & 2695 with PDA, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model)and Analytical Balance (Metller Toledo Model) were used in the present assay.

Preparation of pH 3.5 triethyl amine buffer solution:

Accurately transferred 2.0 mL of triethyl amine into 1000 mL of water and mixed well and pH was adjusted to 3.5 with diluted ortho phosphoric acid. Filtered through 0.45 µm membrane filter.

Preparation of mobile phase:

Prepared a mixture of pH 3.5 triethylamine buffer solution and methanol in the ratio of 90:10 (%volume/volume) mixed well and sonicate to degas.

Preparation of diluent:

HPLC grade water used as a diluent.

Preparation of standard solution:

Accurately weighed and transferred 50 mg of Zoledronic acid working standard into a 100 mL volumetric flask sonicated to dissolved the contents and made upto the volume with diluent.

Further diluted this solution 5 mL in to 50 mL volumetric flask and made up the volume with diluent and mixed well. (The concentration of the standard solution containing, Zoledronic acid 50 µg/mL).

Placebo solution preparation: (5mg / 100mL)

Injected as such placebo solution.

Preparation of sample solution-1: (5mg / 100mL)

Injected as such sample solution.

Preparation of sample solution-2: (5mg / 100mL)

Injected as such sample solution.

Preparation of pooled placebo solution: (4mg / 5mL)

Mixed the contents of 3 vials.

Preparation of placebo solution:

Transferred 3 mL of pooled placebo solution into a 50 mL volumetric flask, made the volume up to the mark with diluent and mixed well.

Preparation of pooled sample solution: (4mg / 5mL)

Mixed the contents of 3 vials.

Preparation of sample solution-1:

Transferred 3 mL of pooled sample solution into a 50 mL volumetric flask, made the volume up to the mark with diluent and mixed well.

Preparation of sample solution-2:

Transferred 3 mL of pooled sample solution into a 50 mL volumetric flask, made the volume up to the mark with diluent and mixed well.

Chromatographic conditions:

Chromatographic analysis was performed on Symmetry C18 (150 x 4.6 mm, 5µ) mobile phase consisted of pH 3.5 triethylamine buffer and methanol in the ratio of (90:10 volume/volume). The flow rate was 0.5mL/min, column oven temperature 40°C and sampler cooler temperature 25°C , the injection volume was 20 µL, and detection was performed at 220 nm using a photodiode array detector (PDA).

3.0 Method development:

UV-spectroscopic analysis of Zoledronic acid drug substance was showed that maximum UV absorbance (λmax) at 220 nm respectively.

To develop a suitable and robust LC method for the determination of Zoledronic acid, different mobile phase pH were employed to achieve the good peak shape and resolution between analyte

peak and placebo peaks. The method development was started with Symmetry C18 (150 x 4.6mm, 5µm), flow rate 0.5mL/min. Column oven temperature 40°C, sample cooler temperature 25°C, injection volume 20µL, UV detection 220nm and run time 10 minutes with pH 2.50 triethylamine buffer and methanol in the ratio of 90:10 volume/volume. Based on the chromatogram observation, it was concluded that analyte and placebo peaks were closely eluted. So need to separate the analyte and placebo peaks in sample solution.

For next trial to get the analyte peak shape by changing the mobile phase pH from 2.5 to 3.0 and keeping all other parameters are constant i.e. All other chromatographic conditions, standard preparation and sample preparation procedures were same as existing method. Based on the chromatogram observation, it was concluded that analyte and placebo peaks were closely eluted. So need to separate the analyte and placebo peaks in sample solution.

For next trial to get the analyte peak shape by changing the mobile phase pH changed from 3.0 to 3.5 and keeping all other parameters are constant i.e. All other chromatographic conditions, standard preparation and sample preparation procedures were same as existing method.

Based on the chromatogram observation, it was concluded that analyte and placebo peaks were well separated chromatogram of Zoledronic acid standard using the proposed method is shown in **Figure. 3** system suitability results of the method are presented in **Table.1.**

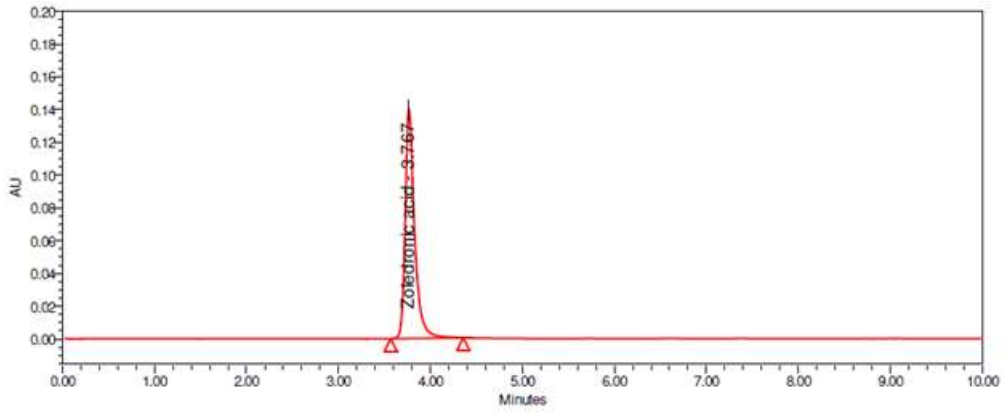

Figure. 3 Typical chromatogram of Zoledronic acid standard

4.0 Method validation:

The developed RP-HPLC method extensively validated for assay of Zoledronic acid in Zoledronic acid parenteral formulation using the following parameters.

4.1 Specificity & System suitability:

Blank and Placebo interference:

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the

blank and placebo chromatograms were recorded. Chromatogram of blank solution **Figure.4** showed no peak at the retention time of Zoledronic acid peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Zoledronic acid in Zoledronic acid parenteral formulation. Similarly chromatogram of placebo solution **Figure.5** showed no peaks at the retention time of Zoledronic acid peak. This indicates that the placebo used in sample preparation do not interfere in estimation of Zoledronic acid in Zoledronic acid parenteral formulation.

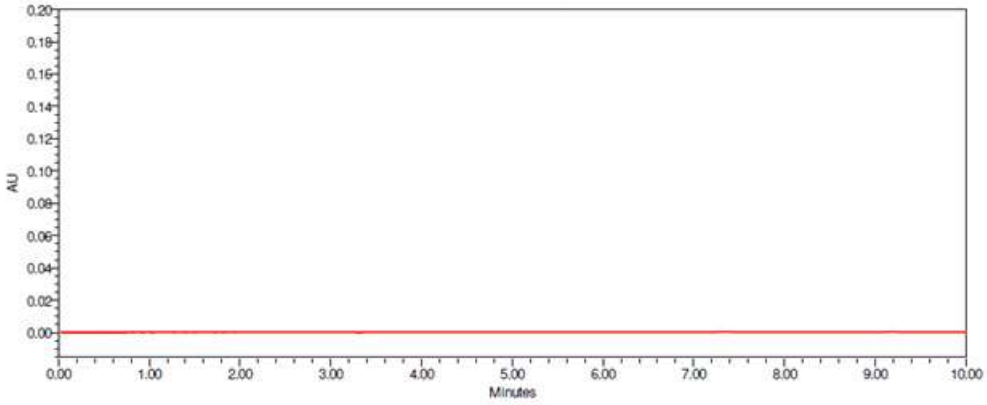

Figure.4 Typical chromatogram blank

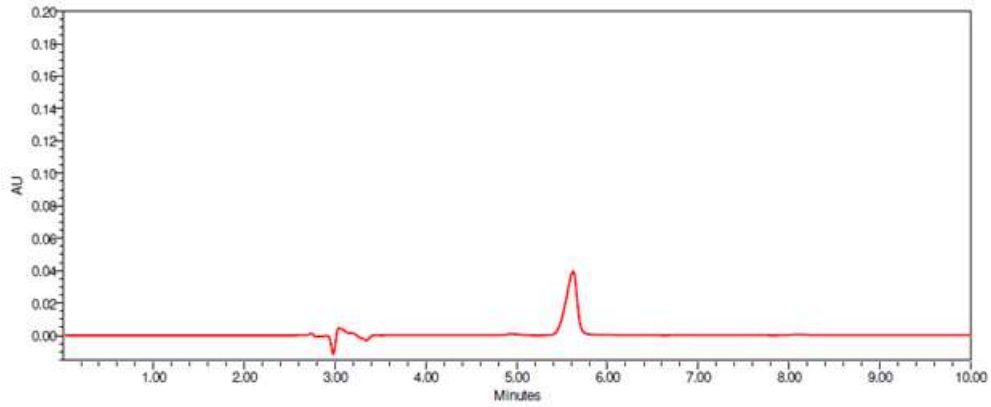

Figure. 5 Typical chromatogram placebo

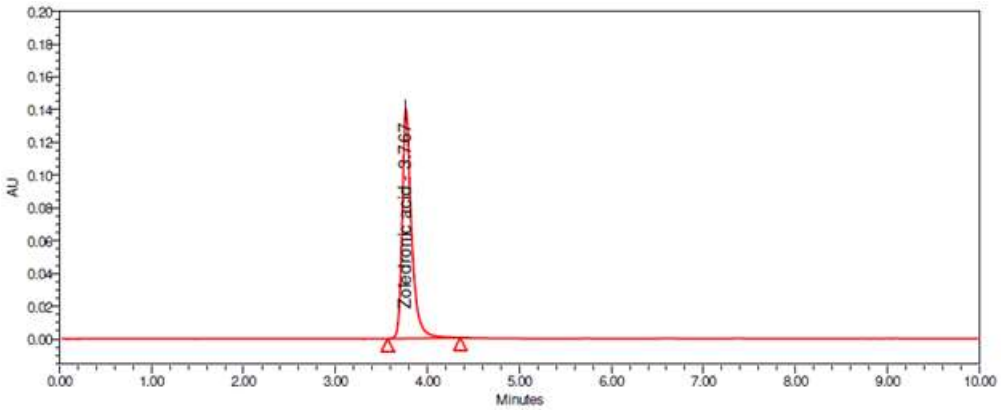

Figure. 6 Typical chromatogram of Zoledronic acid standard

Table.1 Specificity and System suitability results

S.No	Name	Retention Time (min)	Theoretical plates	Tailing factor
1	Blank	ND	NA	NA
2	Placebo solution	ND	NA	NA
3	Standard solution	3.75	7410	1.4
4	Sample solution	3.78	NA	NA

4.1.1 Force Degradation studies:

A study was conducted to demonstrate the effective separation of degradants/impurities from Zoledronic acid. Separate portions of sample and placebo solutions were exposed to the following stress conditions to induce degradation. Stressed and unstressed samples were injected into the HPLC system with photo diode array detector. Degradation study results were presented in **Table.2 & 3.**

Table. 2 Forced degradation results of Zoledronic acid (5mg/100 mL)

S.No.	Degradation	Assay (%)	Purity Angle	Purity Threshold	Peak Purity
01	Control sample	99.9	0.153	0.234	Pass
02	Acid degradation (0.05N HCl/0.5mL/BT/24hrs)	98.9	0.155	0.234	Pass
03	Base Degradation (0.5N NaOH/0.5mL/BT/24hrs)	99.3	0.114	0.232	Pass
04	Peroxide Degradation (3% H_2O_2/0.5 mL/BT/24hrs)	97.5	0.152	0.238	Pass
05	Thermal/60°C/24hrs	99.6	0.224	0.235	Pass

Table. 3 Forced degradation results of Zoledronic acid (4mg/5 mL)

S. No.	Degradation	Assay (%)	Purity Angle	Purity Threshold	Peak Purity
01	Control sample	101.3	0.158	0.232	Pass
02	Acid degradation (0.05N HCl/0.5mL/BT/24hrs)	99.8	0.194	0.233	Pass
03	Base Degradation (0.5N NaOH/0.5mL/BT/24hrs)	102.1	0.179	0.236	Pass
04	Peroxide Degradation (3% H_2O_2/0.5 mL/BT/24hrs)	97.8	0.177	0.240	Pass
05	Thermal/60°C/24hrs	100.5	0.152	0.235	Pass

Significant degradation was observed in oxidation (peroxide) stress condition. Hence it can be concluded that Zoledronic acid is sensitive to oxidation.

4.2 System precision:

The standard solution was prepared as per the test method, injected into the HPLC system for six times and evaluated the % RSD for the area responses. The data were shown in **Table.4.**

Table. 4 System precision data for Zoledronic acid

S.No.	No.of injections	Area of Zoledronic acid
1	Inj-1	989186
2	Inj-2	986741
3	Inj-3	987852
4	Inj-4	988325
5	Inj-5	989654
6	Inj-6	989753
	Average	**988585**
	SD	**1172.47**
	%RSD	**0.12**

- ❖ The relative standard deviation of six replicate standard solution results found to be within the specification limit i.e.0.12%.

4.3 Method precision:

The precision of test method was evaluated by doing assay for six samples of Zoledronic acid injection (5mg/100 mL and 4mg/5mL) as per test method. The content in mg and % label claim for Zoledronic acid for each of the test preparation was calculated. The average content of the six preparations and % RSD for the six observations were calculated. The data were shown in **Table.5 & 6**

Table.5 Method precision data for Zoledronic acid (5mg/100 mL)

No. of Preparations	% Assay
Preparation 1	100.21
Preparation 2	99.98
Preparation 3	100.02
Preparation 4	99.94
Preparation 5	99.81
Preparation 6	100.03
Average	**100.0**
SD	**0.1308**
%RSD	**0.1**

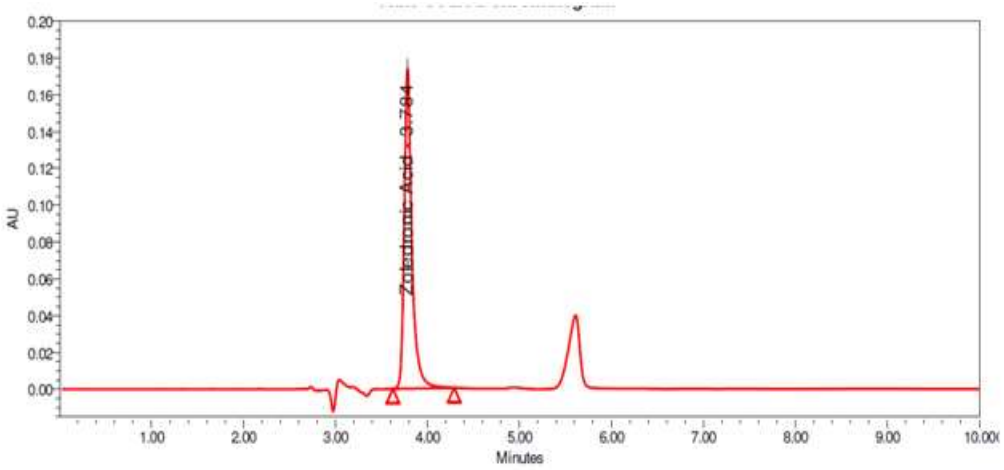

Figure. 6 Typical chromatogram of sample (5mg/100mL)

Table.6 Method precision data for Zoledronic acid (4mg/5 mL)

No. of Preparations	% Assay
Preparation 1	101.87
Preparation 2	102.04
Preparation 3	102.03
Preparation 4	101.79
Preparation 5	101.51
Preparation 6	101.86
Average	**101.9**
SD	**0.1940**
%RSD	**0.2**

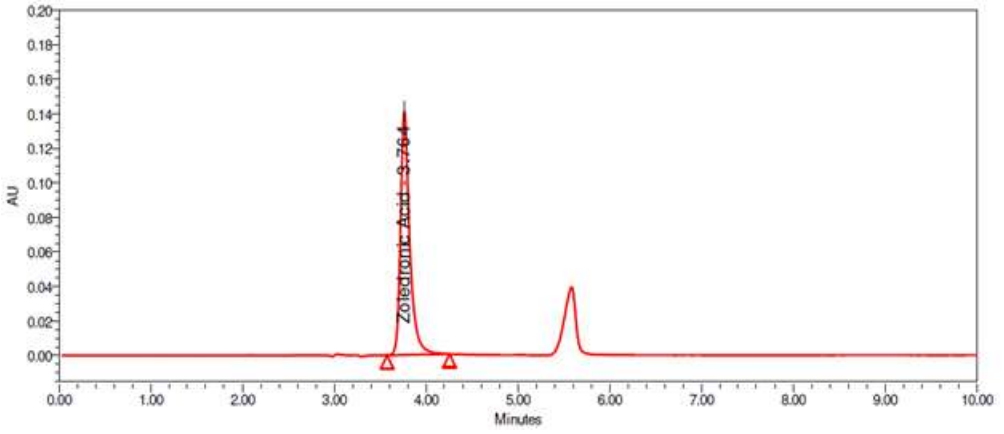

Figure. 7 Typical chromatogram of sample (4mg/5mL)

- Overall and individual % of Assay are complies as per test method specification.
- The relative standard deviation of six assay preparations is 0.1.
- The relative standard deviation of six assay preparations is 0.2.

4.4 Linearity of detector response

The linearity of an analytical method is its ability to obtain test results which has a definite mathematical relation to the concentration of analyte. The linearity of response for Zoledronic acid was determined in the range of 25% to 150 % (12.56-75.18 µg/ml for Zoledronic acid). The calibration curve of analytical method was assessed by plotting concentration versus peak area and represented graphically. The correlation coefficient [r] was found to be 0.9997. Therefore the HPLC method was found to be linear standard curve were calculated and given in **Figure.8** to demonstrate the linearity of the proposed method. From the data obtained which is given in **Table. 7** the method was found to be linear within the proposed range.

Table.7 Linearity studies for Zoledronic acid by proposed method

S.No	Linearity Level	Concentration (ppm)	Area response
1	Linearity at 25%	12.56	247758
2	Linearity at 50%	25.12	507904
3	Linearity at 75%	37.15	743274
4	Linearity at 100%	50.48	988758
5	Linearity at 125%	62.36	1236516
6	Linearity at 150%	75.18	1496662
Correlation coefficient (r)			0.9997
Intercept			2795.6012
Slope			19798.7377
100% Y-intercept			0.28

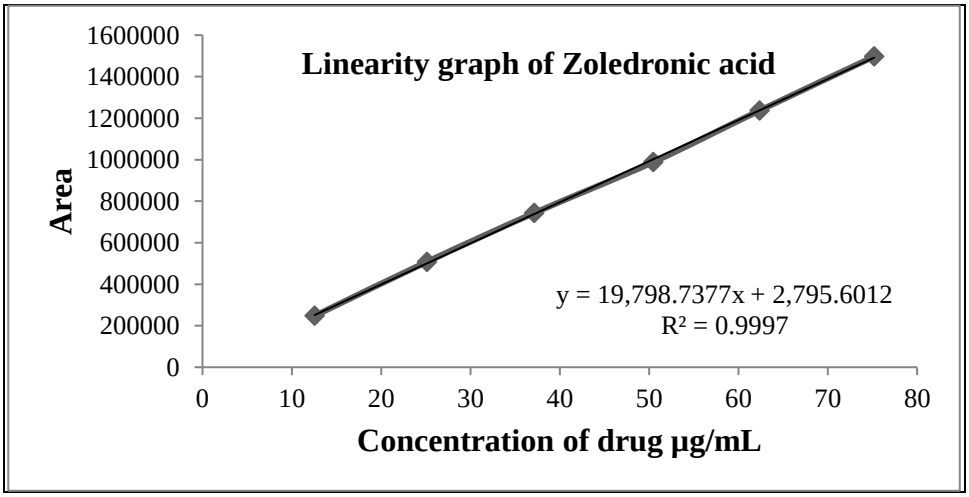

Figure.8 Calibration curve for Zoledronic acid

4.5 Accuracy

The accuracy of the test method was demonstrated by preparing recovery samples of Zoledronic acid at 50% to 150% of the target concentration level. The recovery samples were prepared in triplicate preparations on Zoledronic acid API spiked to placebo, analyzed as per the proposed method for each concentration level. The above samples were chromatographed and the percentage recovery of each sample was calculated for the amount added. Evaluated the precision of the recovery at each level by computing the Relative Standard Deviation of six preparations for 50% and 150% level recovery samples results. The data obtained which given in **Table.8** the method was found to be accurate.

Table.8 Recovery studies for Zoledronic acid by proposed method

Sample	Recovery of Zoledronic acid		
	% Recovery	Mean Recovery	% RSD
50% sample-1	99.5	99.0	0.79
50% sample-2	98.1		
50% sample-3	99.4		
100% sample-1	100.2	100.1	0.12
100% sample-2	100.0		
100% sample-3	100.0		
150% sample-1	99.6	100.4	0.80
150% Sample-2	100.5		
150% Sampe-3	101.2		

4.6 Solution stability of analytical solutions:

Solution stability standard and sample solutions was established at various conditions such as bench top at room temperature and in refrigerator 2-8°C. The stability of standard and sample solutions was determined by comparison of initial prepared standard and sample solutions with freshly prepared standard solutions.

Table.8 Results for solution stability of standard

Time Interval	Similarity factor	
	Room temperature	Refrigerator
Initial	NA	NA
24hrs	1.00	1.02

Table.9 Results for solution stability of sample at room temperature

Time Interval	%Assay (5mg/100mL)	% Assay difference	%Assay (4mg/5mL)	% Assay difference
Initial	100.0	NA	101.9	NA
24hrs	100.5	0.5	101.7	0.2

Table.10 Results for solution stability of sample in Refrigerator

Time Interval	%Assay (5mg/100mL)	% Assay difference	%Assay (4mg/5mL)	% Assay difference
Initial	100.0	NA	101.9	NA
24hrs	100.1	0.1	101.4	0.5

Standard and sample solutions are stable for 24 hours when stored at Room temperature and 2-8°C.

5.0 RESULTS & DISCUSSION

An RP-HPLC method for estimation of Zoledronic acid was developed and validated as per ICH guidelines. A simple, accurate and reproducible reverse phase HPLC method was developed for the estimation of Zoledronic acid in parenteral dosage form. The optimized method consists of mobile phase consisted of pH 3.5 triethylamine buffer and methanol in the ratio of (90:10 volume/volume) with Symmetry C18 (150 x 4.6 mm, 5µ)column. The retention time of Zoledronic acid was found to be 3.8 minutes. The developed method was validated as per ICH Q2A (R1) guideline. As there is no interference of blank and placebo at the retention time of Zoledronic acid. The proposed HPLC method was linear over the range of 12.56-75.18 µg/mL, the correlation coefficient was found to be 0.9997. Relative standard deviation for method precision 5mg/100mL and 4mg/5mL was found to be 0.1% & 0.2%.

The accuracy studies were shown as % recovery for Zoledronic acid 50% to150% level. The limit of % recovered shown is in the range of 98 and 102% and the results obtained were found to be within the limits. Hence the method was found to be accurate.

The solution stability of standard and samples are stable upto 24hrs on bench top and refrigerator (2-8°C).

6.0 CONCLUSION

The developed method was validated for various parameters as per ICH guidelines like accuracy, precision, linearity, specificity, system suitability, solution stability and robustness. The results obtained were within the acceptance criteria. So, it can be concluded that the developed method

is simple, precise, cost-effective, eco-friendly, safe and can be successfully employed for the routine analysis of Zoledronic acid in bulk and pharmaceutical dosage forms.

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Conflict of interests:

The authors claim that there is no conflict of interest.

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