Research Article

STABILITY-INDICATING RP-HPLC METHOD FOR DETERMINATION OF ZOLEDRONIC ACID AND THEIR DEGRADATION PRODUCTS IN ACTIVE PHARMACEUTICAL INGREDIENT AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

The objective of current study was to develop a validated specific stability indicating reversed-phase liquid chromatographic method for the quantitative determination of zoledronic acid in pure sample and pharmaceutical dosage forms in the presence of degradation products. Forced degradation studies were performed on pure sample of zoledronic acid as per ICH prescribed stress conditions using acid, base, oxidative, thermal stress and photolytic degradation to show the stability indicating power of the method. Significant degradation was observed during oxidative stress and the degradation product formed was identified by HPLC, and no degradation was observed in other stress conditions. The chromatographic method was optimized using the samples generated from forced degradation studies and the spiked solution. The method showed a linear response for concentrations in the range of 100-600 µg/mL using Methanol: Acetonitrile: Buffer solution (Dissolve 0.02 M potassium di-hydrogen orthophosphate in water. Adjust pH of solution to 5.5 with orthophosphoric acid) in the ratio (25:5:70) as the mobile phase with detection at 220 nm and a flow rate of 1.2 mL/min and retention time 6.2 min. The value of correlation coefficient, slope and intercept were, 0.9989, 204.57 and 1178.1, respectively. The method was carried out as per ICH requirements validated for precision, recovery, ruggedness and robustness. The developed HPLC method was found to be suitable to check the quality of pure sample of zoledronic acid at the time of batch release and also during its stability studies.

Keywords: Zoledronic acid, HPLC, Forced degradation studies.

INTRODUCTION

Zoledronic acid is chemically designated as (1-hydroxy-2-imidazol-1-yl-1-phosphono-ethyl)phosphonic acid is used to prevent cancers such as multiple myeloma and prostate cancer, as well as for treating osteoporosis[1, 2, 3] **(figure: 1)**. It can also be used to treat hypercalcemia of malignancy and can be helpful for treating pain from bone metastases. An annual dose of zoledronic acid may also prevent recurring fractures in patients with a previous hip fracture[4, 5]. Literature survey reveals that HPLC, RPLC for determination of content uniformity and simultaneous estimation of zoledronic acid is reported[6, 7], but there is no stability indicating reverse phase high-performance liquid chromatography (RP-HPLC) method for the determination of zoledronic acid from its tablets, as its Pharmaceutical dosage form.

The principal objective of this study was, therefore, to develop a new, simple, economical, selective, precise, reproducible, and stability-indicating high-performance liquid chromatographic (HPLC) method with a wide linear range and good sensitivity for assay of Zoledronic acid in the pure drug and in tablet form using UV detection. In the method proposed the mobile phase was used directly for dilution of the formulation after filtration, and then further used for analysis. Direct use of the mobile phase as diluent for formulations in quantitative analysis minimizes errors that occur during tedious extraction procedures. The method was validated in accordance with International Conference on Harmonization (ICH) guidelines[8, 9].

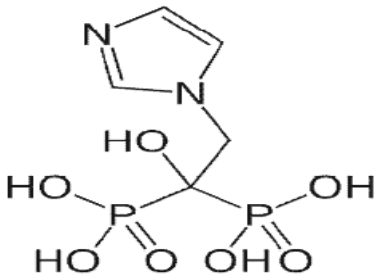

Figure 1: Chemical structure of Zoledronic acid

MATERIALS AND METHODS

Materials

Zolendronic acid was supplied by Sun Pharma and ZOBOLE tablets were procured from the market. Methanol, Acetonitrile, Potassium dihydrogen orthophosphate buffer and Orthophosphoric acid LR Grade were purchased from RFCL Ltd., New Delhi, India. High purity water was prepared by using Millipore Milli-Q plus water purification system.

Instrument used

The HPLC used was a shimazdu RP- HPLC LC-20AT series with SPD-20A UV photodiode array detector and LCsolution software, Japan was used for all the experiments. The column used was XTerra® RP18, 250 x

4.6 mm, 5 µ (water, Ireland) and Luna C8 (Octylsilane), 250 x 4.6 mm, 5 µ (Phenomenax, USA). Thermal Stability studies were performed in a dry air oven (Thermo labs, India). Micrositer syringer- 50 µL (Hamilton Company, USA).

METHODOLOGY

i. Chromatographic conditions

Chromatographic separation was achieved at ambient temperature on a reversed phase column using a mobile-phase consisting of a mixture of Methanol: Acetonitrile: Buffer solution (Dissolve 0.02 M potassium dihydrogen orthophosphate in water. Adjust pH of solution to 5.5 ± 0.05 with orthophosphoric acid) in the ratio (25:5:70). The mobile phase so prepared was filtered through 0.22 µm nylon membrane filter and degassed by sonication. Flow rate of 1.2 mL / min was maintained. Detection was carried out at 220 nm. The injection volume was 20 µL for assay and degradation level.

ii. Standard preparation

100 mg of Zoledronic acid working standard was accurately weighed and transferred to a 100 mL volumetric flask. Solution was sonicated and diluted up to the mark with mobile phase.

iii. Sample preparation

Injection sample equivalent to 50 mg of Zoledronic acid was transferred to a 100 mL volumetric flask. About 60 mL of mobile phase was added and the solution was sonicated for 15 min and make up to the mark with mobile phase. The resulting solution was filtered through 0.22µm nylon membrane filter. The solution was mixed well and centrifuged at 2500 rpm for 10 min.

METHOD VALIDATION

i. Linearity

The linearity of response for Zoledronic acid assay method was determined by preparing and injecting solutions with concentrations of about 100, 200, 300, 400, 500 and 600µg/ml of Zoledronic acid.

ii. Precision

Precision was measured in terms of repeatability of application and measurement. Repeatability of standard application was carried out using six replicates of the same standard concentration (300 µg / mL for standard application). Repeatability of sample measurement was carried out in six different sample preparations from same homogenous blend of marketed sample (300 µg / mL for sample application). It showed very low % relative standard deviation (% RSD) of peak area of Zoledronic acid.

iii. Accuracy

Accuracy (Recovery) study was performed by spiking 30, 50 and 70% of Zoledronic acid working standard to a preanalysed sample. The preanalysed sample was weighed in such a way that final concentration is half or 50% of the sample preparation before spiking. The percentage sum level of preanalysed sample and spiked amount of drug should be 80, 100 and 120% of simulated dosages nominal or target concentration of sample preparation. The accuracy of the analytical method was established in duplicate across its range according to the assay procedure.

$$\textbf{\% Recovery} = \frac{\%Amount\,\mathrm{Re}\,cov\,ered}{\%SumLevel} \times 100$$

iv. Ruggedness and robustness of the method

Method robustness and ruggedness was determined by analysing same sample at normal operating conditions and also by changing some operating analytical conditions such as column make, mobile phase composition, flow rate, instrument and analyst. The robustness and ruggedness of the method was established as the % deviation from mean assay value obtain from precision study is less than ±2.0%.

FORCED DEGRADATION STUDIES

i. Preparation of acid and based- induced degradation product

Injection sample equivalent to 100 mg of Zoledronic acid was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking. To it 5 mL of 1 N HCl was added and 5 mL of 1 N NaOH were added separately. The sample was heated on a boiling water bath for 30 min, cooled to room temperature and diluted to volume with mobile phase, mixed well. The acidic forced degradation and the alkaline forced degradation was performed in dark in order to exclude the possible degradative effect of light. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC system.

ii. Preparation of hydrogen peroxide - induced degradation product

Injection sample equivalent to 100 mg of Zoledronic acid was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking. To it 5 mL of 3.0% H_2O_2 was added. The sample was heated on a boiling water bath for 30 min, cooled to room temperature and diluted to volume with mobile phase, mixed well. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC system.

iii. Photo-degradation and Thermal degradation product

Injection sample equivalent to 100 mg of Zoledronic acid (previously kept in UV light for 24 hr) was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking

and diluted up to the mark with mobile phase. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC system.

Injection sample equivalent to 100 mg of Zoledronic acid was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking. The sample was heated on a boiling water bath for 30 min, cooled to room temperature and diluted to volume with mobile phase, mixed well. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC. The specificity degradation study data for the determination of Zoledronic acid and its degradants in pharmaceutical dosage form is given in **Table: 6.** The no stress treatment sample (as control) has been evaluated relative to the standard concentration where as rest of the stressed condition samples (**Figures: 2 to 4)** is evaluated relative to the control sample with respect to the % assay and % degradation. The percentage degradation results are calculated by area normalization method.

RESULTS AND DISCUSSION

Method of development

The chromatographic conditions were optimized with a view to develop a stability- indicating assay method. Two different columns were tried as under chromatographic conditions namely, XTerra® RP18, 250 x 4.6 mm, 5 µ (water, Ireland) and Luna C_8 (Octylsilane), 250 x 4.6 mm, 5 µ (Phenomenax, USA). Luna C8 gave good peak shape but a lower retention with low peak purity. XTerra® RP18 column had given a good peak shape with response at affordable retention time with peak purity of Zoledronic acid on higher side. The chromatographic conditions finally comprised of a mobile-phase in the ratio of Methanol: Acetonitrile: Buffer solution (Dissolve 0.02 M potassium dihydrogen orthophosphate in water. Adjust pH of solution to 5.5 ± 0.05 with ortho-phosphoric acid) in the ratio (25:5:70) at a flow rate of 1.2 mL / min using XTerra® RP18 column; 250 x 4.6 mm; 5 µ (G. L. Sciences, Japan) at 220 nm.

Validation of the method

i. Linearity

These results indicate that the response is linear over the range of 100, 200, 300, 400, 500 and 600µg/ml of Zoledronic acid with coefficient of regression, R^2, value as 0.9989 as shown in **table: 1**. The value of correlation coefficient, slope and intercept were, 0.9989, 204.57 and 1178.1, respectively.

Table 1: Regression characteristics of the proposed HPLC method

S. No	Drug	Zoledronic Acid
1.	Range (µg/ml)	100-600µg/ml
2.	Detection wave length	220nm
3.	Mean **'R^2'** value	0.9989
4.	Slope (m)	204.57
5.	Intercept (c)	1178.1
6.	Run time	10min
7.	Retention Time (min)	6.2
8.	Theoretical Plates (N)	9438
9.	Tailing Factor	1.02

ii. Precision

The %RSD for repeatability of sample preparation is 0.561%. This shows that precision of the method is satisfactory as % relative standard deviation is not more than ± 2.0%. **Table: 2** represent the precision of method.

Table 2: Method precision of Zoledronic Acid

Sample Preparation	% Assay Zoledronic Acid	% Deviation From Mean Assay value Zoledronic Acid
1	99.76	0.35
2	100.09	0.68
3	98.76	-0.65
4	99.26	-0.15
5	98.66	-0.75
6	99.94	0.53
Mean	99.41	0.01
±SD	0.558	
%RSD	0.561	

iii. Ruggedness and robustness of the method

Method robustness and ruggedness was determined by analysing same sample at normal operating conditions and also by changing some operating analytical conditions such as column make, mobile phase composition, flow rate, instrument and analyst The deliberate aforementioned changes in parameters alters the result of Zoledronic acid 0.01% to method precision study, which is not a significant change. The robustness and ruggedness of the method is established as the % deviation from mean assay value obtain from precision study is less than ±2.0%. **Table: 3** represent the ruggedness and robustness of the method.

iv. Accuracy

The accuracy of the method was established by recovery studies. Results indicate that the mean% recovery of Zoledronic acid ranges from 101.17% to 101.39% as shown in **table: 4.** The recovery of Zoledronic acid by proposed method is satisfactory as % relative standard deviation is not more than ± 2.0% and mean recovery between 99.0 - 102.0%.

Table 3: Ruggedness and robustness of Zoledronic Acid

Parameter	Normal (Original)	Changed conditions
Column make	X Terra ® RP18 column; 250 x 4.6 mm; 5 µ	Luna C_8 (Octylsilane), 250 x 4.6 mm; 5 µ
Flow rate	1.2 mL/min	1 mL/min
Mobile phase Composition	Methanol: Acetonitrile: Buffer (0.02M KH_2PO_4), Adjust pH of 5.5 (25:5:70)	Methanol: Acetonitrile: Buffer (0.02M KH_2PO_4), pH of 5.5 (23:10:67)
Pump	Jasco PU-2080 plus series	Shimazdu LC-20AT
Detector	Jasco UV-2075 plus series	Shimazdu UV-VIS detector
Analyst	Kalyan.B	Narasimha.V
% assay of Zoledronic Acid	99.41%	99.43%
% deviation from mean assay value obtained in method precision studies for Zoledronic Acid : 0.01%		

v. Stability- indicating property

The chromatogram of no stress treatment sample (as control) showed no additional peak (**Figure: 2 & 3**). The retention time (RT) of standard and sample were 6.2 min and 6.15 min respectively. The chromatogram of acid degraded sample showed no additional peaks. The chromatogram of alkali degraded sample showed no additional peaks. The chromatogram of hydrogen peroxide degraded sample showed additional peak at RT of 4.98 min as shown in **Figure: 4**. The chromatogram of UV degraded sample showed no additional peaks. The chromatogram of thermal degraded sample showed no additional peak. In each forced degradation sample were additional peaks were observed, the response of the drug was changing from the initial control sample the values were depicted in **Table: 5 & 6**. This indicates that the drug is not susceptible to acid-base hydrolysis degradation, UV degradation and thermal degradation but susceptible to oxidation. The lower RT of the degraded component indicated that they were more polar than the analyte itself.

Figure 2: The simple chromatogram of standard Zoledronic Acid.

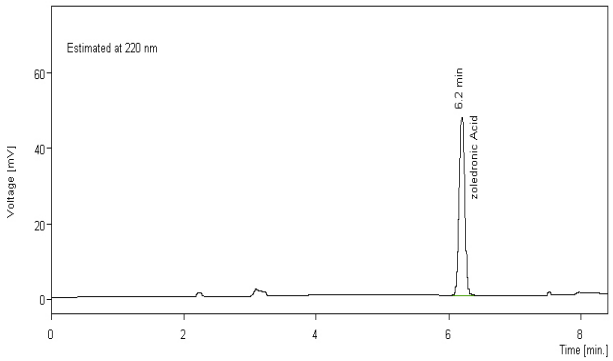

Figure 3: The simple chromatogram of test Zoledronic Acid.

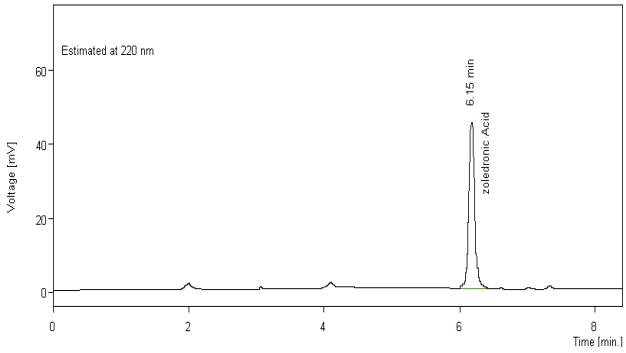

Figure 4: The simple chromatogram of Hydrogen Peroxide degraded sample

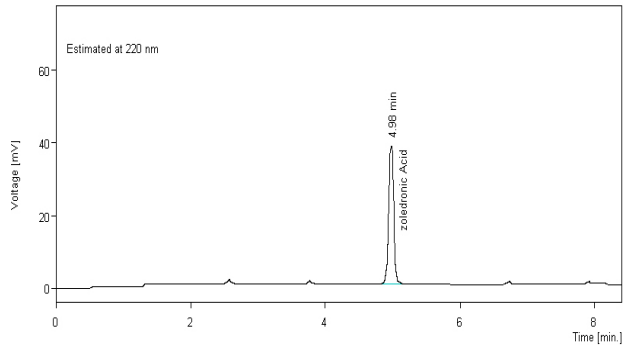

Table 4: Recovery (Accuracy) studies of Zoledronic Acid

Sample preparation	% simulated dosage normal	% sum level	% amount recovered	% recovery	Mean % recovery
Preanalysed sample				99.41	
1	80	80.56	81.28	100.89	101.39
2	80	79.88	81.39	101.89	
1	100	100.65	101.52	100.86	101.17
2	100	100.21	101.70	101.49	
1	120	120.24	121.85	101.34	101.36
2	120	120.04	121.71	101.39	

Table 5: Stressed study data of Zoledronic Acid

S. No	Condition	% assay Zoledronic Acid	Retention time of drug (min)	% Degradation
1	No stress treatment	99.41	6.15	Nil
2	Acid	-	-	--
3	Alkali	-	-	--
4	H_2O_2	98.65	4.98	0.08
5	UV	-	-	--
6	Thermal	-	-	--

Table 6: Summary of forced degradation results

S. No	Stress condition	Time	% Assay of active substance	Mass balance (% assay + % degradation products)	Remarks
1	Acid degradation (1 N HCl)	1/2 hr	-	-	-
2	Alkali degradation (1N NaOH)	1/2 hr	-	-	-
3	H_2O_2 degradation (3%)	1/2 hr	98.65	98.7	Mild degradation formed
4	UV degradation	24 hr	-	-	-
5	Thermal degradation (60 ^{0}C)	1/2 hr	-	-	-

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