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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR QUANTITATIVE ANALYSIS OF ZOLEDRONIC ACID IN PURE AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Simple reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the estimation zoledronic acid in pure and pharmaceutical formulation. The separation was done on a 5µ c_{18}column (250×4.6mm) using a mobile phase that consist of the phosphate buffer(pH adjusted ortho phosphoric acid 3.5) and methanol in the ratio of 96:4 (v/v). The flow rate was maintained at 0.7 ml/min^{-} The detection was done at 210nm using a uv detector. The retention times of zoledronic acid is 7.8 respectively. The method was validation done according ICH parameters.

KEYWORD: Zoledronic acid, orthophosphoric acid, rp-hplc, column.

1. INTRODUCTION

Zoledronic acid, a bisphosphonic acid, is an inhibitor of osteocalstic bone resorption. Zoledronic acid is designated chemically as (1-hydroxyl-2- imidazol-1-yl-phosphonoethyl) phosphonic acid monohydrate. Zoledronic acid is a white crystalline powder. which inhibit bone resorption as a consequence of affecting osteoclast and probably osteoblast activity.

Zoledronic aicd is being developed for the treatment of tumor induced hypercalcemia. Literature survey a few methods for estimation of Zoledronic acid.[1-6]

2. MATERIALS AND METHODS

2.1 Standards and Chemical Used

Zoledronic acid was gift sample for Noverties company, Hydrabad. All the chemicals Methanol HPLC Grade, HPLC grade Water.

2.2 Instrumentation: HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Zoledronic acid.

2.3. Preparation of Mobile phase: The separation was achieved on a 5µ C18 column (250 X 4.6 mm) using mobile phase consist Mixture of buffer 4.5g g of Di-potassium hydrogen phosphate anhydrous and 2.0 g of tetra butyl ammonium hydrogen sulphate (TBAHS) in 1000 mL of water) pH adjusted 4.3 with ortho phosphoric acid and methanol in the ratio of 96:4 v/v. The flow rate was maintained at 0.7 mL min^{-1}.

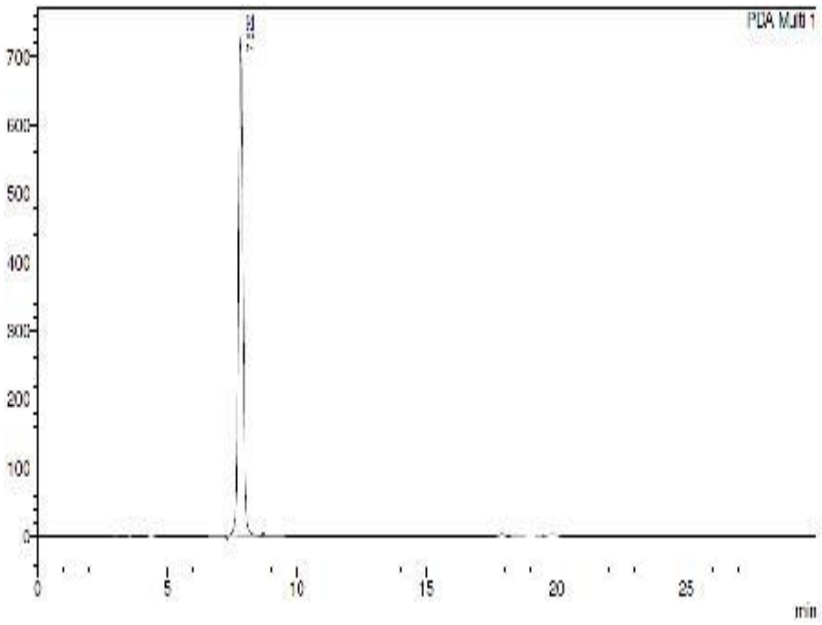
2.4. Preparation of standard stock solution: Accurately weigh and transfer approximately 100.0mg of Zoledronic acid into 100ml volumetric flask, and volume make up to the mark with diluent. (solution-01).

2.5. Preparation of standard solution: Accurately weigh and transfer approximately 100.0 mg of zoledronic aicd into 100 ml volumetric flask, and volume make up to the mark with diluent. From the above stock solution concentrations ranging from 0.2mg/ml to 0.6mg/ml were prepared.

2.6. Preparation of sample solution: Transfer contents of 10 vials into 60 ml volumetric flask by rinsing vials 2-3 times with mobile phase & sonicated and make up the volume with diluent. Take 10 ml of this solution into another 60 ml volumetric flask & make up to volume with diluent.

3. RESULT AND DISCUSSIONS

Injected standard preparation's (6repilcate injections) into chromatograph and recorded the system suitability parameters as per test procedure



Chromatogram for system suitability parameter

3.1 VALIDATION OF THE PROPOSED METHOD

As an integral part of analytical method development is validation. The proposed method was validated as per ICH guidelines.

3.1.1 Linearity

Linearity of an analytical procedure was validated the ability to elicit test results that were directly proportional to concentration of analyte in sample within a give range Linearity was directly established on zoledronic acid working Standard across specified range with minimum 5 concentrations that are within specified range A standard curve should be prepared over the range of approximately 0.2mg/ml to 0.7 mg/ml which is equivalent to 50% to 150% of the ZOLEDRONIC ACID working concentrations.

Table No 1: Linearity of ZOLEDRONIC ACID.

Level	Concentration (mg/ml)	Avg.Peak Area
50	0.2002	4254723
75	0.3002	7525577
100	0.4003	8777444
125	0.5004	10857807
150	0.7005	13107958

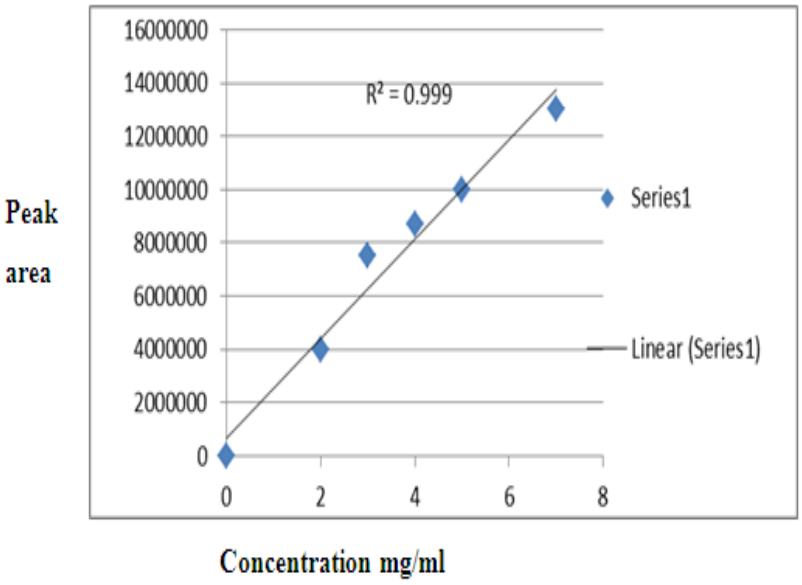


Fig no1: Calibration curve.

Table: 2 Optimized conditions.

Column:	**Zorbax C18(260x4.6),6µm**
Injection volume:	20~~µl~~
Run time:	30min
Mode:	Isocratic
Column temperature:	36°c
Flow rate:	0.7ml/min
Wave length:	210nm
Mobile phase:	96:4

3.1.2. Precision

Precision is the measure of the degree of repeatability of an analytical method under normal operation and is normally expressed as the percent relative standard deviation for a statistically significant number of samples.

Intermediate precision

The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. Intermediate precision expresses within laboratory variations: different days, different analysts, different equipment, different columns, etc. The procedure followed for assay method in method precision was repeated on two different days, by two analysts, using two different columns and using different HPLC systems. The results for the Intermediate precision are recorded in the table.

Analyst-1

TABLE.3

Name of parameter	Acceptance criteria	Result
% RSD for replicate injections of peak response of zoledronic acid from Standard preparation	NMT 2	0.43
Tailing factor	NMT 2	1.27
Plate count	NLT 2000	9723

ANALYST-2

TABLE. 4.

Name of parameter	Acceptance criteria	Results
% RSD for replicate injections of peak response of Zoledronic acid from standard preparation	NMT2	0.35
Tailing factor	NMT2	1.24
Plate count	NLT2000	9773

3.1.3. Repetability

System precision was performed by analyzing a sample solution of ZOLEDRONIC ACID at working concentration six times (Six replicate Injection). Table shows the % RSD of ZOLEDRONIC ACID peak areas and retention time of ZOLEDRONIC ACID peaks and the results obtained were in the acceptable range.

Table 5: Summary of Results For System Precision.

S.no	Retention time of zoledronic acid (min)	Peak area of zoledronic acid	Tailing factor for zoledronic acid	Theoritical plates
1	7.82	8738317	1.1	9732
2	7.82	8770227	1.1	9714
3	7.83	8780532	1.1	9771
4	7.82	8787112	1.1	9733
5	7.82	8793874	1.1	9724
6	7.82	8794141	1.1	9720
7	7.82	8794241	1.1	9721
AVG	7.82	8777377	1.1	9731
% RSD	0.03	0.24	0.43	0.17

3.1.4. Ruggedness

Method Ruggedness is defined as the reproducibility of results when the method is performed under actual use conditions. This includes different analysts, laboratories, columns, instruments, source of reagents, chemicals, solvents etc. Method ruggedness may not be

known when a method is first developed, but insight is obtained during subsequent use of that method.

3.1.5. LOD and LOQ solutions

The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value.

The limit of quantitation is the lowest amount of analyte in the sample that can be quantitatively determined with precision and accuracy. The quantification limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

TABLE 6.

Component	Concentration (mg/ml) around	Signal to noise ratio Around
LOD	0.0001770	3.74
LOQ	0.0005070	9.7

3.1.6. Accuracy

Accuracy must be calculated with respect to above prepared solution at the levels of 75%, 100% and 125% of the target concentration. The accuracy of the method was demonstrated through recovery experiment on 3 samples at concentration 75%, 100%, 125% of the actual concentration employed in the usual procedure. The actual concentration employed in the determination was 0.4mg/ml of Zoledronic acid. The accuracy of the method was determined by analyzing three solutions containing ZOLEDRONIC ACID at approximately 75%, 100% and 125% of the working concentration of Assay. Each solution was analyzed in triplicate. The Percentage recovery results obtained are listed in Table 7.

Table 7: Accuracy of ZOLEDRONIC ACID.

LEVEL	THEORITICAL CONCENTRATION (mg/ml)	MEASURED CONC. (mg/ml)	% RECOVERY	% RSD
75%	0.3000 0.3000 0.3000	0.3002 0.3002 0.3002	99.8 100.2 100.3	**0.27**
100%	0.4000 0.4000 0.4000	0.4003 0.4003 0.4003	99.7 100.0 99.8	**0.15**
125%	0.5000 0.5000 0.5000	0.5004 0.5004 0.5004	100.1 100.2 99.9	**0.15**

3.1.7 Robustness

The robustness of an analytical method is measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Table: 8 Robustness data.

Acceptance criteria	The % RSD for area response of Zoledronic acid obtained from five replicate injections of standard preparation should be NMT 2.0%	Tailing factor for Zoledronic acid peak from the standard preparation should be NMT2.0.	Theoretical plates of Zoledronic acid peak should be NLT 2000 in standard preparation.
Original condition	0.24%	1.1	9731
Decrease in flow	0.18%	1.23	9243
Increase in flow	0.23%	1.09	9822
Decrease in Ph	0.35%	1.07	9774
Increase in pH	0.28%	1.1	9757
Decrease in temperature	0.43%	1.05	9182
Increase in temperature	0.33%	1.35	9874

3.1.8. System suitability

If measurements are susceptible to variations in analytical conditions, these should be suitably controlled, or a precautionary statement should be included in the method.

Table 9: System suitability.

Time points	Area of zoledronic acid	of initial	Area % of unknown impurity	Area % of known impurity	
				AREA%	I.D
Initial	8288445	100.0	0.07	0.07	RC-B
24Hrs	8284572	100.0	0.07	0.07	RC-B
37Hrs	8283478	99.9	0.08	0.07	RC-B
48Hrs	8247952	99.5	0.09	0.07	RC-B

3.1.9. Assay of Zometa

Table: 10.

Drug	Mean Standard Area	Mean Sample area (mV.s)	% Assay	Amount present	Labeled amount
ZA	8666268	8669606	99.66	3.78 mg	4 mg

4. CONCLUSION

The proposed RP-HPLC method allows for accurate, precise and reliable measurement of Zoledronic acid in dosage form. The developed RP-HPLC method was found to be simple, rapid, selective, accurate and precise for the concurrent estimation of drug injectable form. The method was evaluated in best condition, linear relation including coefficient of correlation, robustness, accuracy, reproducibility and precision. The %RSD for all parameters was found to be less than two, which indicates the validity of method and assay results obtained by this method are in fair agreement. The developed method can be used for routine quantitative estimation of Zoledronic acid in pharmaceutical preparation.

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