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METHOD VALIDATION FOR ZOLEDRONIC ACID AND ITS RELATED COMPOUNDS IN LARGE AND SMALL VOLUME INJECTIONS BY RP CHROMATOGRAPHY WITH UV DETECTION

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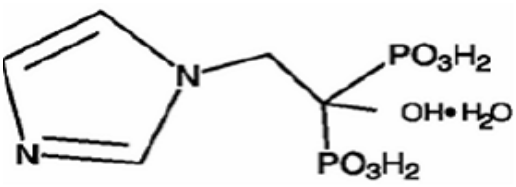
ABSTRACT
A validated, specific, stability indicating reversed-phase liquid chromatographic method has been developed for quantitative analysis of Zoledronic acid and its related substances in liquid injection. The degradation products was achieved on a Phenyl Hexyl (250x4.6mm,5µm) column with a mobile phase constituted of 1-octane sulphonic acid of sodium salt monohydrate, EDTA disodium salt, Perchloricacid and Ortho phosphoric acid, the detection was performed at 215nm.The retention times of Zoledronic acid, Imidazole-1-yl acetic acid and Imidazole standard are in minutes 5.94, 17.10 & 24.71 respectively. The method was linear with the concentration range from 0 to 300% with correlation and regression coefficient are above 0.998 for Zoledronic acid, Imidazole-1-yl acetic acid and Imidazole standard respectively. The method was validated in according to ICH guidelines USP with respect to accuracy, precision, specificity and can be used to determine related impurities and degradation products.

KEYWORDS: RP HPLC, UV&PDA detector, Zoledronic acid, Imidazole standard, Imidazole-1-yl-standard, validation, ICH guideline.

1. INTRODUCTION

1.1 Zoledronic acid: Zometa contains zoledronic acid, a bisphosphonic acid which is an inhibitor of osteoclastic bone resorption. Zoledronic acid is designated chemically as (1-Hydroxy-2-imidazol-1-yl-phosphonoethyl) phosphonic acid monohydrate and its structural formula.



Zoledronic acid is a white crystalline powder. Its molecular formula is $C_5H_{10}N_2O_7P_2 \bullet H_2O$ and its molar mass is 290.1g/Mol. Zoledronic acid is highly soluble in 0.1N sodium hydroxide solution, sparingly soluble in water and 0.1N hydrochloric acid, and practically insoluble in organic solvents. The pH of a 0.7% solution of zoledronic acid in water is approximately 2.0. Zoledronic acid is used to treat osteoporosis (condition in which the bones become thin and weak and break easily) in women who have undergone menopause ('change of life,' end of regular menstrual periods). Zoledronic acid is also used to treat osteoporosis in men, and to treat osteoporosis in men and women who are taking glucocorticoids (a type of corticosteroid medication that may cause osteoporosis). Zoledronic acid is also used to treat Paget's disease of bone (a condition in which the bones are soft and weak and may be deformed, painful, or easily broken). Zoledronic acid is used to treat high levels of calcium in the blood that may be caused by certain types of cancer. Zoledronic acid is also used along with cancer chemotherapy to treat bone damage caused by multiple myeloma [cancer that begins in the plasma cells (white blood cells that produce substances needed to fight infection)] or by cancer that began in another part of the body but has spread to the bones. Zoledronic acid is not cancer chemotherapy, and it will not slow or stop the spread of cancer. However, it can be used to treat bone disease in patients who have cancer. Zoledronic acid is in a class of medications called bisphosphonates. It works by slowing bone breakdown, increasing bone density (thickness),and decreasing the amount of calcium released from the bones into blood. [1]Srinivasan Raghu Nandan et al., A Regulatory Requirement – Validated, Specific, and Stability Indicating Analytical Method for Zoledronic Acid and Its

Related Impurities by Ion Pair Reversed Phase Liquid Chromatography.[2] Y.Jiang, X.Q. Zhang, Z.R.Xu, et al Analysis of Zoledronic acid and its Related Substances by Ion-Pair RP-LC Chromatographia.[3] B. Mallikarjuna Rao, M.K. Srinivasu, Ch. Prathima Rani, et al A validated stability indicating ion-pair RP-LC method for zoledronic acid.[4] Blessy M, Ruchi D. Patel, Prajesh N. Prajapati, et al Development of forced degradation and stability indicating studies of drugs—A review Journal of Pharmaceutical Analysis.[5] D.Durgasrinivas, P. Venkateswararao, K.V. Ramanjeyulu, et al presented Method Development and Validation for the assay of Zoledronic acid in pharmaceutical dosage form using High Performance Liquid Chromatography technique.

1.1.1 Points of Emphasis

Wash HPLC instrument lines (without column) with 25% Glacial Acetic acid in water 25ml of acetic acid to 100ml of water) for 10minutes with 3.00ml/min flow rate. Flush the HPLC instrument with HPLC grade water at flow of 3.00ml/min for about 20 minutes. Perform the column condition with mobile phase about 30 minutes. Use the same preparation of eluent for mobile phase & for diluent. Inject 20µL of Zoledronic acid injection sample as such (0.05mg/ml) for five times with the run time of 10 minutes for faster baseline stabilization.

2. MATERIALS AND METHODS

All the reagents were AR grade (Analytical Reagents) or HPLC grade unless stated otherwise. Milli Q water/HPLC Grade water (Commercial available) was used throughout the experiments. Perchloric acid(70.0%w/v) from Merck, Orthophoshoric acid(85%w/v), 1-octane sulphonic acid sodium salt monohydrate, EDTA disodium salt monohydrate were purchased from Rankem. The HPLC System was used Water Alliance with model no.2695/ Shimadzu LC2010CHT with PDA Detector, series of Compressed of degasser, Quatery pump, Auto sampler, Thermostated Column compartment, System was controlled through Empower software. The analytical column was used for this method is Phenyl Hexyl (250x4.6mm,5µm). Transferred 10 ml of Perchloric acid (70%) into 1000 ml of water and added 1.93ml of Orthophoshoric acid (85%) to it. Added 11.7 g of 1-Octane sulphonic acid Sodium salt monohydrate (50mM) and 37.2 mg of EDTA Disodium salt dehydrate (0.1mM) and sonicated to dissolved and mixed well. Mixed 970 volumes of buffer and 40 volumes of acetonitrile. Filter and degas. The Mobile phase was filtered through 0.45 µm, nylon membrane filter and degassed using vacuum before delivering into the system.The retention times of Zoledronic acid, Imidazole-1-yl acetic acid and Imidazole standard are 5.94, 17.10 & 24.71 respectively. Stock standard solutions were prepared by dissolving separately Zoledronic acid monohydrate (2.7mg), Imidazole acetic acid standard (5.0mg) and Imidazole standard (5.0mg) into separate volumetric flask and sonicated for 2 minutes. The stock solutions were sufficiently diluted with mobile phase to give 0.05 mg/ml solutions.

2.1 Test Solution Preparation for 4mg/5ml

Transferred 2.5 ml of test solution into 10ml flask and dilute to volume with Mobile Phase.

2.1.1 Test solution preparation for 4mg/100ml by evaporation technique

Take 2 test tubes. Poured 15ml &10 ml of sample solution in two test tubes. Flushed the sample solution with nitrogen gas at about 55ºC till 10ml of test solution evaporated up to about 2ml in each test tube. Transferred carefully the resulting whole content of 2 test tube into a 5ml volumetric flask. Rinsed both test tube with diluents and transferred into same 5 ml volumetric flask and diluted to volume with mobile phase. Filtered the sample solution through 0.45µ nylon filter discarded the first few ml and collected into HPLC vial.

2.1.2 Test solution preparation for 5mg/100ml by evaporation technique

Take 2 test tubes. Poured 15ml &10 ml of sample solution in two test tubes. Flushed the sample solution with nitrogen gas at about 55ºC till 10ml of test solution evaporated up to about 2ml in each test tube. Transferred carefully the resulting whole content of 2 test tube into a 5ml volumetric flask. Rinsed both test tube with diluents and transferred into same 5 ml volumetric flask and diluted to volume with mobile phase. Filtered the sample solution through 0.45µ nylon filter discarded the first few ml and collected into HPLC vial.The proposed method was validated according to the ICH guidelines.[12, 13] The method was validated for its linearity, range, accuracy, precision (repeatability and Intermediate), sensitivity and specificity. The standard calibration curves were prepared with 6 concentrations ranging from 0.0005-0.004µg/mL for Zoledronic acid,0.0002-0.0057µg/mL for Imidazole-1-yl acetic acid, 0.0002-0.0057 µg/mL for Imidazole into the HPLC system keeping injection volume constant. The 50µL Aliquots of each solution were injected under the operating of each solution were injected under the operating chromatographic condition described above and chromatogram was recorded. The typical chromatograms are shown in Fig.1 The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

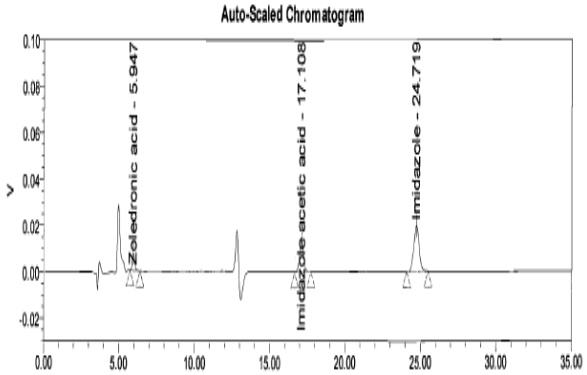


Fig. 1: Typical chromatogram of Zoledronic acid, Imidazole 1-yl acetic acid and Imidazole at 215nm.

The accuracy of the method was at three different levels, 15%, 30%, 50%, 100%, 200%, and 300% was determined by calculating recovery of Zoledronic acid, Imidazole-1-yl acetic acid and Imidazole by the standard method. The solutions were prepared in triplicates and the accuracy is expressed as % recovery. The precision of the instrument was checked by repeatedly injecting (n=6) solutions of Zoledronic acid, Imidazole-1-yl acetic acid, Imidazole (0.004µg/mL,0.0057 µg/mL and 0.0057 µg/mL respectively).The Intermediate precision of the method was determined on different day using column of different make of same dimensions. The standard solution were injected for six times and area was measured. The % RSD for all injections was within the specified limits. The limit of detection (LOD) and limit of Quantitation(LOQ) of the developed method were determined by injecting progressively low concentration of standard solution using the HPLC method. The limit of detections(LOD) and limit of Quantitation(LOQ) were calculated using the following equation as per ICH guide lines. LOD = 3.3 x □ /S and LOQ = 10 x □ /S.

3. RESULTS AND DISCUSSION

To optimize the RP-HPLC parameters, several mobile phases of different compositions were tried. A satisfactory separation, good peak symmetry and best resolution was obtained with a mobile phase consisting of 970 volumes of 10 ml of Perchloric acid (70%) into 1000 ml of water and added 1.93ml of Orthophoshoric acid (85%) to it. Added 11.7 g of 1-Octane sulphonic acid Sodium salt monohydrate (50mM) and 37.2 mg of EDTA Disodium salt dehydrate (0.1mM) and 40 volumes of Acetonitrile. Quantitation was achieved with UV detection at 215nm based on peak area. The retention time for Zoledronic acid, Imidazole-1-yl acetic acid and Imidazole 5.94, 17.10 & 24.71 respectively. Suitability of chromatographic system was monitored by calculating tailing/asymmetry factor and theoretical plates. The calibration graphs were linear for each drugs and the system adhered to Beer's law over the concentration range from 0.0127- 3.0587 for Zoledronic acid, 0.005-6.435 for Imidazole-1-yl acetic acid, 0.005-6.3975 for Imidazole respectively. Linearity was evaluated by precision analysis at high concentration 0.0030 µg/mL, 0.0064 µg/mL and 0.0063 µg/mL Zoledronic acid, Imidazole-1yl acetic acid and Imidazole. The chromatogram of Zoledronic acid, Imidazole-1yl acetic acid and Imidazole at 215 nm(Fig.2).

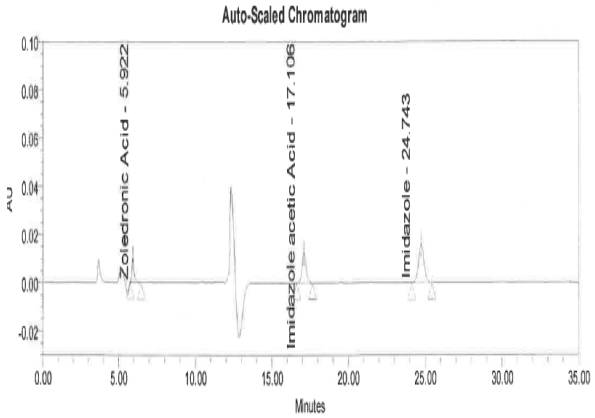


Fig. 2: Typical chromatogram of Zoledronic acid, Imidazole 1-yl acetic acid and Imidazole at 215nm.

LOD and LOQ were determined from standard deviation of Y-intercept of regression line and slope as per ICH guidelines. The results showed that, the method is sensitive for the determination of Zoledronic acid, Imidazole-1yl acetic acid and Imidazole. System suitability and validation parameters are given in **Table 1**.

Table. 1: validation and system suitability.

Parameter	Zoledronic acid	Imidazole-1-yl acetic acid	Imidazole
Linearity	0.0127-3.0587	0.005-6.435	0.005- 6.3975
Slope	48930.212	118743.417	229276.284
Intercept	1.07	–2.00	–1.57
Corr.coeff	0.998	1.000	1.000
R.Time(min)	5.855	18.053	26.341
Area	52853	115458	222734
LOD	0.015	0.045	0.100
LOQ	0.040	0.014	0.300

Accuracy was determined by calculating % recovery. The method was found to be accurate with %recovery 96.5%, 99.5% and 96.7% for Zoledronic acid, Imidizole-1yl acetic acid and Imidazole respectively. The high values indicates that method is accurate and are shown in **Table 2**.

Table. 2. Recovery study of Zoledronic acid injection.

% concentration	Amount added	Amount found	% recovery	Mean Recovery
Zoledronic acid				
50	0.10	0.0023	93.3	97.6
100	0.20	0.0051	102.6	
200	0.40	0.0103	106.0	
300	0.80	0.0173	88.4	
Imidazole-1yl-acetic acid				
15	0.025	0.0012	102.8	99.5
30	0.055	0.0028	103.7	
50	0.10	0.0048	96.0	
100	0.20	0.0106	100.7	
200	0.40	0.0200	100.3	
300	0.57	0.0265	93.5	
Imidazole				
15	0.025	0.0011	91.7	96.7
30	0.055	0.0026	96.3	
50	0.10	0.0050	100.7	
100	0.20	0.0094	100.7	
200	0.40	0.0195	98.3	
300	0.57	0.0262	92.3	

The precision of the assay was studied with respect to both repeatability and Intermediate precision. Repeatability was calculated from six replicate injections of freshly prepared Zoledronic acid test solution in the same equipment at a concentration value of 200µg/mL of Zoledronic acid on the same day. The experiment was repeated by freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak areas of the drugs were determined and precision was reported as %RSD which clearly indicates the method is precise shown in **Table.3**.

Table3. Method and Intermediate precision studies of zoledronic acid injection (5mg/100mL).

Precision	Imidazole acetic acid	Total Impurity
Method precision (5mg/100ml) Average area and %RSD	0.0351 and4.3%	0.0351 and4.3%
Intermediate precision (5mg/100ml) Average area and %RSD	0.0298 and 3.7	0.0298 and 3.7
Total %RSD	9.0	9.0

Specificity was performed to exclude the possibility of interference with excipients in the region of elution of Zoledronic acid. The specificity and selectivity of the method was tested under optimum conditions and the results of the tests proved that the components other than drug did not produce a detectable signal at the retention place of Zoledronic acid, Imidazole-1-yl acetic acid and Imidazole. The validated HPLC method was adopted for the Quantitation of Zoledronic acid, Imidazole-1-yl acetic acid and Imidazole in dosage form and the results of analysis are given in Table 4. The content of the pharmaceutical dosage form were found to be in the range of LOQ to 300% with RSD less Than 10% this indicates suitability of the method for routine analysis of Zoledronic acid in pharmaceutical dosage form.be simple, sensitive, accurate and precise. It was also proved to be convenient and effective for the determination of Zoledronic acid and its impurities in the pharmaceutical dosage formulations.

CONCLUSION

The proposed study describes a new RP-HPLC method using simple mobile phase for the estimation of Zoledronic acid and its impurities in pharmaceutical dosage formulations. The method was validated and found to dosage form. The percentage recovery shows that, the method is free from interference of the excipients used in formulation. Moreover, the lower solvent consumption along with the short analytical run time leads to cost effective chromatographic method.

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