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Research Article

**DEVELOPMENT, EVALUATION AND STABILITY OF  
ZOLEDRONIC ACID I.V INJECTION BY LYOPHILIZATION  
TECHNIQUE****Rajesh Akki<sup>1\*</sup>, M. Gayatri Ramya<sup>2</sup>, M. Hymavathi<sup>1</sup>, P. Dinesh Kumar<sup>1</sup>**

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2. University college of Pharmaceutical Sciences, Acharya Nagarjuna University,  
Nagarjuna Nagar- 522 510.**Abstract:**

The objective of the study is to develop a stable lyophilized formulation of Zoledronic acid for injection for better stability and for long term storage. The lyophilized product of all the formulations (F1- F6) prepared were an appearance of white to white Lyophilized cake. mannitol was used with water for injection. The filled vials were loaded into lyophilizer and lyophilized them as per cycle. Different composition of additives was used and the different pH concentrations of 5.7 to 6.7 were adjusted with sodium citrate were tried to formulate the formulation. The pH of all the formulations is in the range of 5.4-6.5. The related substances in formulations not exceeded the limit of 0.5%. The assay values of formulations (F1-F6) were in the range of 92% – 104 %. The formulations (F1- F6) show a water content range of 0.93% to 1.6 %. The results concluded that the formulation F4 is the optimized and the best formulation. Zoledronic acid was developed as lyophilised formulation for better stability. The obtained results suggested that a stable formulation for drug Zoledronic acid was developed which was comparable to reference listed product.

**Keywords:** Lyophilisation, mannitol, Zoledronic acid.**Corresponding Author:****Rajesh akki,**

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**INTRODUCTION:**

Zoledronic acid is an nitrogen containing bisphosphonates and, like the other molecules of this class, binds to hydroxyapatite in the bone mineral matrix and strongly inhibits bone resorption. The ability of bisphosphonates to persist in bone matrix and to reduce osteoclast activity depends on their affinity for the bone matrix and potency of the inhibition of farnesyl pyrophosphate[1]. Zoledronic acid has the highest affinity for bone, followed by alendronate, ibandronate, risedronate, etidronate, and clodronate and it also alters mineral-surface properties, allowing greater adsorption. These properties are believed to contribute to its prolonged action [2]. The bioavailability of the BPs is very low when they are administered orally, but this problem is avoided by intravenous administration, such as with the yearly formulation of zoledronic acid. Orally administered BPs have shown approximately 1% bioavailability, whereas intravenous formulations have shown 100% bioavailability. Like the other BPs, zoledronic acid is eliminated rapidly in the urine, and studies of its endogenous metabolism have shown that it does not inhibit human cytochrome activity in vitro, in particular the p450 enzyme, or undergo biotransformation in vivo, indicating that it is not extensively metabolized[3-5]. After a single intravenous dose of zoledronate, bone turnover marker reduction reaches up to 80% after 1 month and persists over the following 12 months[6-8]. This is due to the high potency and affinity of zoledronic acid for hydroxyapatite and the 100% bioavailability afforded by the intravenous infusion[9-10]. Zoledronic acid is 1-hydroxy-2-(1H-imidazole-1-yl) ethane-1,1-diyl]bis(phosphonicacid). Molecular formula of Zoledronic acid is C<sub>18</sub>H<sub>24</sub>N<sub>2</sub>

. Injections are sterile solutions, emulsions or suspensions. These are prepared by dissolving, emulsifying or suspending active substances and excipients in water, in non-aqueous vehicle or mixture of both. Injections are clear, free from particles and emulsions do not show any phase

separation. Freeze-drying, or lyophilization, is in simple terms a dehydration technique. The aspect of the freeze-drying process that makes it different from other dehydration techniques is that dehydration takes place while the product is in a frozen state under a vacuum. These conditions stabilize the product, minimizing the effects of oxidation and other degradation processes. Freeze-drying has become an accepted method of processing heat sensitive products that require long term storage at temperatures above freezing. Freeze-drying works by freezing the material and then reducing the surrounding pressure and adding enough heat to allow the frozen water in the material to sublime directly from the solid phase to gas. Lyophilization or freeze-drying is often used to stabilize various pharmaceutical products, including virus vaccines, protein and peptide formulations, and liposome and small chemical drugs susceptible to physical and chemical degradation when stored as a ready-to-use solution. The lyophilization process consists of three stages they are Freezing (Solidification), Primary drying (Ice sublimation), Secondary Drying (Desorption of Unfrozen Water). Secondary drying parameters are based on the quantity and nature of the residual water in the product, the absorption, adsorption and desorption processes. Tigecycline in liquid injection was found to be unstable. The major objective of this experiment is to formulate the tigecycline injection by lyophilization technique for better stability and for long term storage. In lyophilized preparations, Mannitol is used as a carrier to produce a stiff, homogeneous cake that improves the appearance of the lyophilized plug in a vial [11-12].

**MATERIALS AND METHODS**

Zoledronic acid procured from Natco Pharma Ltd, Hyderabad; Mannitol and Sodium citrate were supplied by Roquette, Germany.

Methods: Zoledronic acid drug is an active ingredient, Mannitol were used as lyophilization aid, water for injection as a vehicle for solubility and concentrated Sodium citrate for pH adjustment

**Table 1: Formulation of Zoledronic acid Injection**

S.No	Ingredients	F1	F2	F3	F4	F5	F6
1	Zoledronic acid drug	1gm	1gm	1gm	1gm	1gm	1gm
2	Mannitol	50gm	57.5gm	56.5gm	55gm	55.5gm	57gm
3	Sodium citrate	5gm	5.5gm	6.5gm	6gm	5gm	7gm
4	Water For Injection	q.s to 500 ml	q.s to 500 ml	q.s to 500 ml	q.s to 500 ml	q.s to 500 ml	q.s to 500 ml

**Method of Preparation:**

WFI is freshly collected at 70°C to 80°C in 1 liter glass vessel then cool to 30°C to 35°C. 1gm of Zoledronic acid drug was slowly added to the above WFI with continuous stirring. Mannitol was added to the above solution with continuous stirring for 8 min. Check clarity of solution for homogeneous mixing. Make up the volume to 500 ml with WFI. Adjust the volume on wt/ml. Withdraw 5 ml sample for analysis as per current approved in process specification ASSAY- (1.96 - 2.04) mg/ml WEIGHT/ml – 1.04 g/ml sodium citrate was added to the above solution and check the pH of solution 5.7 to 6.7 record pH. The solution is filtered through 0.22µ PVDF membrane filter (BACTERIA RETENTIVE FILTER). 2ml solution is filled into 6ml tubular Type-1 flint vials are half stoppered with 13mm lyophilized stoppers. The filled vials are located into the lyophilizer and the vials are lyophilized as per cycle (Set the shelf temperature, 55, prior to cycle starts). The samples are collected at the end of lyophilization cycle. The samples are withdrawn under the nitrogen atmospheric conditions after the atmospheric pressure is reached these vials are stoppered and sealed. The vials are unloaded from the lyophilizer and sealed by using flip of aluminium seals. The vials are stored at room temperature.

**Assay:** HPLC equipped with UV detector. Column: Inertsil-3v (250\*4.6mm) Flow rate: 0.8ml/min Wavelength: 215nm Column temp: 30°C Injection volume: 100µL Run time: 30 min for peak identification and 60 min for diluent, placebo and sample preparation.

**Stability studies:** Accelerated stability study was conducted for the optimised batch under various temperature and humidity conditions. The water content, assay and pH were determined and compared with standard conditions.

**RESULTS AND DISCUSSION:**

**Table 2: Determination of solubility of Zoledronic acid drug**

No of Trails	Description	pH	Assay
Formula 1	Turbid solution	6.62	97.5%
Formula 2	Turbid solution	6.65	94.5%
Formula 3	Turbid solution	6.76	103.56%
Formula 4	Clear solution	6.56	100.5%
Formula 5	Turbid solution	6.80	95.8%
Formula 6	Clear solution	6.75	104.4%

Zoledronic acid drug is freely soluble in NaOH and sparingly soluble in water. Bisphosphonate drug is poorly soluble in methanol and ethanol. From experimental data, it is proved that Zoledronic acid drug is sparingly soluble in NaOH and very slightly soluble at different concentrations of sodium citrate and mannitol. The pH and assay of Zoledronic acid drug in different concentrations of sodium citrate and mannitol are represented in the above table.

F1 is performed with the drug Zoledronic acid drug, mannitol, sodium citrate and water for injection. As the WFI content in the formulation is more, the moisture content in the lyophilized product is more so the assay of the finished product is less. So the next formulation is planned with different concentration of sodium citrate and mannitol. In F2 formulation the solubility of the drug in formula 2 is less but more compared to formula 1. So the assay is increased compared to F1. The moisture content is decreased as the percentage of WFI decreased in F2 compared to F1. So the further formulation is planned with different concentration of sodium citrate and mannitol. In F3 formulation the solubility of the drug is more compared to the solubility in F2. So there is an increase in the assay of formulation compared to F1 and F2. The percentage of WFI added in the formulation is less when compared to F1 and F2. So the water content in the final product was also low. To get even better results trials are planned with the increased different concentration of sodium citrate and mannitol. In F4 formulation, the drug Zoledronic acid drug gets completely solubilized in different concentrations of sodium citrate and mannitol. So there is a 100% assay for the final lyophilized product. For a lyophilized powder the related substance and water content plays critical role. The particulate count is also less compared with other formulations to reconfirming the above statement, further formulation is planned with increased different concentration of sodium citrate and mannitol. In F5 formulation, the drug Zoledronic acid drug completely soluble in different concentrations of sodium citrate and mannitol. The assay is increased because of decreasing hydrolysis as the water percentage has decreased in the formulation. The percentage of water content of final product has also decreased. In F6 formulation, the drug Zoledronic acid drug is completely soluble in different concentrations of sodium citrate and mannitol. This has resulted in an increase in the assay of the formulation and the water content of the lyophilized vials is also decreased.

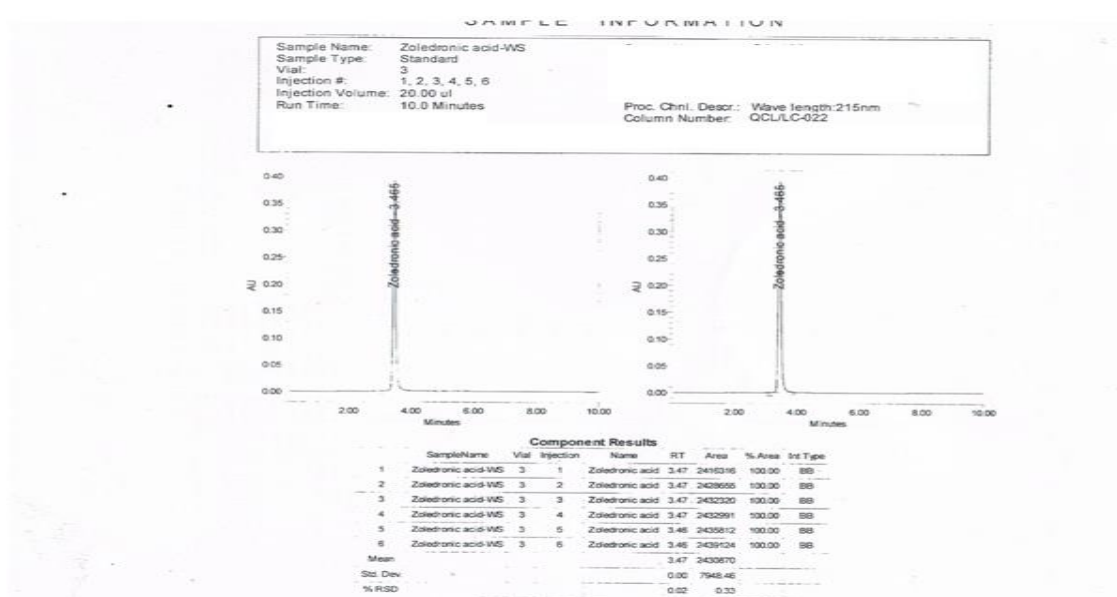
**Table 3: Evaluation Test for Zoledronic Injection**

S.No	Test	F1	F2	F3	F4	F5	F6
1	Description	White lyophilized cake	White lyophilized cake	White lyophilized cake	White lyophilized cake	White lyophilized cake	White lyophilized cake
2	pH	5.4	6.5	6.28	6.37	6.47	6.39
3	Related substance	0.6%	0.4%	0.30%	0.3%	0.5%	0.25%
4	Assay	92.4%	94.4%	96.3%	100.1%	104.3%	101.2%
5	Water content	1.201	0.935	1.631	0.937	1.103	1.199
6	Sub visible particle ≥10µm ≥25µm	1413.0 19.8	3637.8 31.8	2604.0 75.0	1937.8 28	1195.6 24	4252.8 72.0

**Stability studies:**

The accelerated stability studies were carried out as per ICH guidelines at  $40^{\circ}\text{C}\pm 2^{\circ}\text{C}/75\%\text{RH}\pm 5\%\text{RH}$ . The optimized lyophilized formulation under stability studies were analyzed at a time interval of one month up to three months. The formulations were analyzed for all the evaluation parameters such as color of the product, reconstitution time, pH, assay, particulate matter. In the 1<sup>st</sup> month stability analysis, the color of the product was same as the initial product and no color change was observed. The entire product was reconstituted with 5ml of special diluent and the reconstitution time noted was 0.02secs. The pH of all the solution was found to be 6.24 and no significance difference was observed compared to initial product. Assay was carried out by HPLC and was found to be within the I.P limits. The

particulate matters were found to be  $1326(\leq 10\mu\text{m})$  and  $23(\leq 25\mu\text{m})$ . These data were within I.P specification and hence, the product was stable after one month. In second month and third month stability analysis, the appearance of the product was same as the initial product and no color change was observed. All the evaluation parameters were found to be within limits as given. From the above result it was concluded the product was stable under accelerated stability study condition which qualifies batch-4 to have an expected shelf-life of 2years. From the above discussions, it is evident that finally batch-4 could be optimized as the target lyophilized product of Zoledronic acid IV injection with better stability than marketed formulations. Hence an objective of developing a stable and extended shelf-life lyophilized Zoledronic acid IV injection was achieved.

**Fig1: Stability studies of F4 at  $40^{\circ}\text{C}\pm 2^{\circ}\text{C}/60\%\text{RH}\pm 5\%\text{RH}$**

**Table 4: Comparative Evaluation Results of all the Formulations**

Evaluation Parameters	Innovator	F1	F2	F3	F4	F5	F6
Description	White lyophilized cake	White lyophilized cake	White lyophilized cake	White lyophilized cake	White lyophilized cake	White lyophilized cake	White lyophilized cake
pH	5.32	5.4	6.5	6.28	6.37	6.47	6.39
Related Substance	0.5%	0.6%	0.4%	0.3%	0.3%	0.5%	0.25%
Assay	108.5%	92.4%	94.4%	96.3%	100.1%	104.3%	101.2%
Water Content	2.5	1.201	0.935	1.631	0.937	1.103	1.199
Sub-Visible Particle							
≥10µm	6000	1413	3637	2604	781	1195	4252
≥25µm	600	19	31	75	10	24	72

The results of all the 6 formulations were tabulated and concluded that the formulation F4 was the optimized formulation with the best evaluation parameters.

**Table 5: Comparative analytical profile among reference listed drug Zoledronic acid (90hr and 58hrs) and Zoledronic acid drug for injection F4.**

Evaluation parameters	Reference listed (RLD) at 90hr	Reference listed (RLD) at 58hr	Formulation F4 at 40hr
Description	White lyophilized cake	White lyophilized cake	White lyophilized cake
pH	6.87	6.95	6.37
Related substance	0.48%	0.54%	0.3%
Assay	97.5%	98.4%	100.1%
Water content	1.40	1.45	0.937
Sub-visible particle			
≥10µm	1035	1565	781
≥25µm	15	25	10

**CONCLUSION:**

From the above results it is concluded that the formulation F4 is optimized and the best formulation. The evaluation parameters like assay, related substance, particulate matter and water content for the formulation F4 are within the limits. The comparative results between the RLD and Zoledronic acid drug F4 formulation have shown that the RLD which are comparatively less in efficiency than the Zoledronic acid drug for injection F4 formulation which was carried out using 40hrs lyophilization cycle period. From the accelerated stability studies it is concluded that the Zoledronic acid drug for injection should be stored at room temperature (25°C) and should not be subjected to high temperature.

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