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ORIGINAL ARTICLE

# A simple RP-HPLC method for related substances of zoledronic acid in pharmaceutical products

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Zoledronic acid;
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UV-detector;
Zoledronic acid recovery studies

**Abstract** A novel, selective and sensitive reverse phase-high performance liquid chromatography (RP-HPLC) method has been developed for the validated estimation of imidazol-1-yl-acetic acid in zoledronic acid formulations. The separation was achieved on a 5 μ C18 column (250 × 4.6 mm) using a mobile phase that consists of the buffer (4.5 g of di-potassium hydrogen phosphate anhydrous and 2.0 g of tetra butyl ammonium hydrogen sulphate (TBAHS) in 1000 mL of water) and methanol in the ratio of 900:100 v/v. The flow rate was maintained at 1.0 mL $\text{min}^{-1}$. The detection of the constituents was done at 215 nm using a UV detector. The retention times of imidazol-1-yl-acetic acid and zoledronic acid were 7.2 and 10.2 min respectively. Recovery studies were satisfactory and the correlation coefficient, 0.999 indicates linearity of the method within the limits. The developed method can be applicable for regular qualitative analysis.

## 1. Introduction

Zoledronic acid is a nitrogen containing bisphosphonic acid, widely used to prevent or treat osteoporosis in post-menopausal women, in men or women who have taken glucocorticoids (a type of corticosteroid medication that may cause osteoporosis), paget's disease of the bone (a condition in which the bones are soft and weak, and may be deformed, painful or easily breakable), high levels of calcium in the blood may occur with certain types of cancer, due to its high potential and inhibits osteoclastic bone resorption (Adami et al., 2002; Amanat et al., 2007; Andrew et al., 2008; Aparicio et al., 1998; Berenson, 2005; Black et al., 2007; Body et al., 1999; Boutsen et al., 2001; Conte and Guarneri, 2004).

The literature survey reveals that HPLC and RP-HPLC for determination of zoledronic acid are reported (Jiang et al., 2004; Mallikarjuna Rao et al., 2005; Praveen Kumar and Sreeramulu, 2011; Srinivasan Raghu Nandan et al., 2009; Jiang et al., 2005; Zhang et al., 2004; Matuszewski et al., 2011). But, a few are (Praveen Kumar and Sreeramulu, 2011; Srinivasan Raghu Nandan et al., 2009; Zhang et al., 2004) about stability indicating reverse phase high-performance liquid chromatography (RP-HPLC) methods for the determination of zoledronic acid in pharmaceutical injections/tablets. Even for though those who are suffering from interference of

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impurities/intermediates when forced to stress conditions the method becomes less sensitive with low selectivity. Therefore, the development of a new economical, selective and sensitive RP-HPLC method for zoledronic acid and its related substances is intended.

The main objective of this study was to develop a novel, simple, economical, selective, sensitive and stable method indicating the use of reverse phase-high performance liquid chromatography (RP-HPLC) method for the assay of zoledronic acid and its related substances, imidazol-1-yl-acetic acid (Fig. 1) present in pharmaceutical products using a UV detector. It was included in the performance of stress/influenced factors, such as acid or base hydrolysis, hydrogen peroxide oxidation, light and heat on zoledronic acid and its related substances, inimidazol-1-yl-acetic acid determination. This paper also deals with the validity of the proposed method for the accurate estimation of zoledronic acid and related substances in pharmaceutical samples. The mobile phase was used as diluent for the preparation of working solutions, which minimizes errors that occur in quantitative separation techniques. This proposed method was successfully applied for regular analysis.

## 2. Materials and methods

### 2.1. Reagents

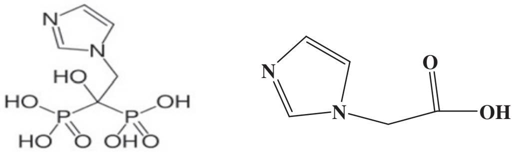
Dipotassium hydrogen phosphate anhydrous (Merck, GR Grade), tetra butyl ammonium hydrogen sulphate (TBAHS) (Merck, German, Catalogue. No. 8.18858.0100/synthesis grade), methanol (HPLC grade), water (HPLC grade), phosphoric acid 85% pure (Merck grade) was used in the present study. All other chemicals used were of analytical reagent grade, otherwise stated.

### 2.2. Chromatographic system conditions

Kromosil 5 μ C18 column ($250 \times 4.6$ mm) at 323 K oven temperature for 45 min (for blank, sample preparation), and 15 min (for standard preparation) run time with 1.0 mL min$^{-1}$ flow rate was used for the separation of impurities in zoledronic acid or related substances and detected at 215 nm using a UV detector. 15 μL of sample was used for the injection into the column. Water and mobile phases were used as diluents.

### 2.3. Preparation of mobile phase

Accurately weighed 4.5 g of dipotassium hydrogen phosphate anhydrous and 2.0 g of tetra butyl ammonium hydrogen sulphate in a 1000 mL glass beaker, added 1000 mL of water, and sonicated for 10 min on occasional stirring with a glass rod. Well mixed buffer and methanol in the ratio of 900:100 v/v was used as the mobile phase.



Zoledronic acid Imidazol-1-yl-acetic acid

**Figure 1** Chemical structure of zoledronic acid and impurity (Imidazol-1-yl-acetic acid).

### 2.4. Impurity (20 μg mL$^{-1}$) and standard (32 μg mL$^{-1}$) stock solution preparation

Accurately weighed and transferred 2.0 mg of imidazol-1-yl-acetic acid impurity into a 100 mL volumetric flask, dissolved 70 mL of the mobile phase and made up to the mark with mobile phase and used as impurity stock.

Weighed accurately about 21.4 mg of zoledronic acid monohydrate (equivalent to 20.0 mg of zoledronic acid) into a 25 mL volumetric flask, added about 20 mL of mobile phase, sonicated to dissolve for about 15 min with occasional shaking and diluted to volume with mobile phase and mixed well, which is used as a stock standard. Pipette out 1.0 mL of the above solution into a 25 mL volumetric flask and dilute to volume with mobile phase.

### 2.5. Standard and test solution preparation

Pipette out 2.0 mL each of the above standard stock solution and impurity stock solution into a 25 mL volumetric flask and made up with diluents for making a standard solution.

Opened five vials (each vial contains zoledronic acid: 4 mg 5 mL$^{-1}$). Pooled the content of each individual vial into a clean and dried 50 mL volumetric flask or into a dried 25 mL stoppered test tube and mixed well with diluents. This solution was used as test solution for further studies.

### 2.6. System suitability

The relative standard deviation for zoledronic acid or imidazol-1-yl-acetic acid peak area and peak area of six replicate injections should not be more than 10.0%; The tailing factor for zoledronic acid and imidazol-1-yl-acetic acid peaks should not be more than 2.0; The column efficiency (theoretical plates) for zoledronic acid and imidazol-1-yl-acetic acid peaks that should be not less than 2000 is necessary for good system suitability. For good system suitability to determine imidazole-1-yl-acetic acid less than 10% RSD, less than tailing factor and more than 2000 theoretical plates were maintained.

The presence of imidazol-1-yl-acetic acid content in the pharmaceutical samples was calculated using the following formula.

*% Of imidazol-1-yl-acetic acid*:

$$\frac{A \times W_{\mathrm{i}} \times 2 \times P_{\mathrm{i}}}{S_{\mathrm{i}} \times 100 \times 25 \times 0.8}$$

*% Of any unknown impurity*:

$$\frac{B \times \mathrm{Ws} \times 1 \times 2 \times P_{\mathrm{s}}}{\mathrm{Ss} \times 25 \times 25 \times 25 \times 0.8}$$

where, $A$ = peak area of imidazol-1-yl-acetic acid for test preparation; $B$ = peak area of any impurity other than blank, placebo, imidazol-1-yl-acetic acid and zoledronic acid for test preparation, $W_{\mathrm{i}}$ = weight of imidazol-1-yl-acetic acid impurity taken in mg for standard preparation; Ws = Weight of zoledronic acid monohydrate working standard taken in mg for

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