Spectrophotometric Determination of Alendronate Sodium by using Sodium-1,2-Naphthoquinone-4-Sulphonate

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ABSTRACT

A simple, precise and accurate spectrophotometric method was developed for analysis of the osteoporosis drug alendronate sodium (ALS). The method is based on reaction of the drug with sodium-1,2-naphthoquinone-4-sulphonate (NQS) in presence of alkali to form a brown colored complex giving absorption maximum at 525 nm. The drug obeyed Beer’s law in the range of 5-70 µg/ml with a correlation coefficient of 0.999. The LOD and LOQ values are 1.7 µg/ml and 5.0 µg/ml, respectively. The average recoveries for recovery study were found to be in the range of 99.37%-100.46%. The R.S.D. values for intraday and inter-day precision were found to be 0.48 and 0.62, respectively. The optimized assay conditions were applied successfully for determination of ALS in pharmaceutical dosage forms. No interference was observed from the excipients present in the dosage form. The method is statistically validated as per the ICH requirements.

KEYWORDS: Alendronate; bisphosphonates; ICH; spectrophotometry; sodium-1,2-naphthoquinone-4-sulphonate; chromogenic reagent.

Introduction

Alendronate sodium (ALS) is used in treatment of osteoporosis (Prinsloo and Hosking, 2006). Chemically it is sodium [4-amino-1-hydroxy-1-(hydroxy-oxido-phosphoryl)-butyl]phosphonic acid trihydrate (Vachal et al., 2006). The bisphosphonates do not possess any chromophoric group in their chemical structure, which are responsible for giving spectra in UV-visible region. So the drug cannot be directly assessed by dissolving in solvents using UV spectrophotometry. So derivatization of the drug with chromogenic reagents or fluorogenic reagents can be carried out for spectrophotometric determinations. Many methods have been reported so far for analysis of the drug by various analytical techniques. The reported methods include spectrophotometric estimation by using o-phtalaldehyde at basic pH (Aldeeba and Hamdan, 2004), ion-pair complex formation with Cu(II) ions (Koba et al., 2008). Spectrofluorimetric method include by conjugating the drug with Rhodamine B sulphonyl group and its subsequent determination (Jeong et al., 2011). Other methods include electrophoretic methods (Pruthiwasan and Suntoornsuk et al., 2010; Svidritskii et al., 2010), potentiometry (de Haro Moreno et al., 2004), HPLC methods (Sibei fi et al., 2003; Yun and Kwon et al., 2006; Tsai et al., 1992; Kang et al., 2006; Chen et al., 2010; Meng et al., 2010; Liu et al., 2008; Hu et al., 2009; Yun et al., 2006)

Sodium-1,2-naphthoquinone-4-sulphonate (NQS) is a chromogenic reagent which has been used previously for determination of pharmaceuticals containing primary amino group (Ashraf et al., 2009; Li et al., 2007; Hasani et al., 2007; Li and Yang, 2007; Darwish, 2005; Xu et al., 2004). But as per literature review, no method has been reported so far for determination of ALS by using NQS. So a successful attempt was made to develop and validate a simple, precise and accurate spectrophotometric method for determination of ALS in bulk drug and pharmaceutical dosage forms.

Materials and Methods

Instrumentation

A Shimadzu 1800 UV Visible Double beam Spectrophotometer (Shimadzu, Tokyo, Japan) with 10 mm matched cuvettes, compatible with UV Probe 2.1 software was used. A high precision analytical balance, Model-GR-202 (AND Instrument India Pvt. Ltd., Gurgaon, India) of sensitivity 0.1 mg was used to weigh the chemicals and reagents. A water bath (Thermolab, India) with a thermostat was used for heating the drug solutions.

Chemicals and Reagents

All the chemicals used were of analytical grade. The drug alendronate sodium (ALS, purity 99.8%) was received from Novartis Healthcare Ltd., Hyderabad, India. Purity of the drug was assured by determining the melting point. Sodium-1,2-naphthoquinone-4-sulphonate (NQS;
97%; Aldrich Chemical Co., St.Louis, MO,USA), and sodium hydroxide (S.D. Fine Chem Ltd., Mumbai, India) were used. Water purified by TKA Water Purification System, Germany was used for preparing reagent solutions. The marketed formulation Denfos® tablets from Dr.Reddy's Laboratories Ltd., India (containing 70 mg of ALS) was procured from the market.

**Preparation of Drug and Reagent Solutions**

(a) Standard solution of the drug: An accurately weighed amount (25 mg) of ALS was taken in a 25 ml volumetric flask and was dissolved in around 15 ml of water, finally filled up to the mark by water to get a stock solution of 1mg/ml. Suitable aliquots from this solution were taken and further diluted to obtain the working solutions.

(b) Sample solution of the drug: Twenty tablets were weighed and finely powdered. Powder equivalent to 25 mg of ALS was weighed accurately and transferred into a 25 ml volumetric flask.15 ml of water was added and the contents were ultrasonicated for 15 minutes, finally the volume was made up to the mark with water. The contents were filtered and from the filtered solution, further dilutions were made to obtain suitable sample solutions for analysis.

(c) 0.5% (w/v) NQS solution: A 0.5% (w/v) solution of NQS was prepared by dissolving accurately weighed 250 mg of NQS in 50 ml of water using a 50 ml volumetric flask. The solution was protected from light during use as the chemical is photo-sensitive.

(d) 0.01M NaOH solution: A 0.01M NaOH solution was prepared by dissolving 0.4 g of NaOH in 1000 ml of water in a 1000 ml volumetric flask.

**Procedures**

(a) Construction of calibration curve: Into a series of 10 ml volumetric flasks appropriate aliquots of the standard solution was taken to finally produce a concentration range of 5-70 µg/ml. To each volumetric flask 1 ml each of 0.5% (w/v) NQS reagent and 0.01M NaOH were added. The contents were shaken for a while and were placed in a water bath maintained at 50°C±2°C for 10 minutes. Then the solutions were allowed to cool at room temperature for a while and finally diluted up to the mark with water. Absorbance of the resulting brown colored solutions was measured at 525 nm (Figure 1.).

(b) Application to marketed formulation: Suitable aliquots of the sample solutions were transferred into separate volumetric flasks and were analyzed by following the procedure described in (a).

(c) Determination of stoichiometric ratio of the reaction: To determine the stoichiometric ratio of the reaction Job’s method of continuous variation (Job, 1964) was followed. Equimolar (0.01M) solutions of ALS and NQS were prepared. To a series of 10 ml volumetric flasks the solutions of ALS and NQS were made up in different compositions of 0:10, 1:9... 9:1, 10:0; along with 1 ml of 0.01 M NaOH. Furthermore, the solutions were subjected to standard procedure.

(d) Method Validation: The developed method was validated statistically as per the requirements of ICH (Guideline on Validation of Analytical Procedures: Text and Methodology Q2-R1,2005) for accuracy, precision (intra-day and inter-day), LOD and LOQ. Sandell’s sensitivity was also calculated. To check the accuracy of the proposed method, recovery studies were carried out at 80%,100% and 120% of the test concentration. The recovery study was performed three times at each level. The amounts of ALS present in the sample were calculated using the calibration curve. The precision (intra-day and inter-day) of the method was ascertained separately from the response obtained by actual determination of six replicates of a fixed amount of drug. The percent RSD values for precision were calculated. LOD = 3.3 δ / S and LOQ = 10 δ / S; where δ is the standard deviation of the intercept and S is the slope. Sandell’s sensitivity was calculated as the minimum concentration of drug required to produce an absorbance of 0.001.
Results and Discussion

The present work describes a novel, simple, precise and accurate spectrophotometric method for determination of ALS by reaction of – NH$_2$ group of ALS with a chromogenic reagent NQS under alkaline conditions. A brown colored complex was developed after the completion of the reaction. The method was further optimized by optimizing the various reaction parameters. The effect of NQS concentration on the absorbance was studied. When the NQS concentration increased the absorbance of the reaction product also increased up to a certain concentration level. The maximum absorbance was attained at NQS concentration of 0.5% (w/v), and higher concentrations up to 1% had no remarkable effect on absorbance values (Figure 2). Basing on above result the concentration of 0.5% was selected as the optimum concentration of NQS.

The effect of alkalinity on the reaction was determined. The 0.01 M NaOH was selected for carrying out the reaction, as the study revealed that 0.01 M NaOH provided optimum results than compared to others. This may be probably due to the property of primary amino group of ALS, that allows nucleophilic substitution reaction in alkaline conditions. But at higher alkalinity, there was no significant change in the absorbance of the reaction product (Figure 3).

The effect of reaction time on the reaction was checked by allowing the reaction to proceed for various time ranges. It was noticed that the reaction achieved completion within 10 minutes, providing longer reaction times than 10 minutes was having no significant effect on the reaction (Figure 4). So, the reaction time of 10 minutes was used.

The effect of temperature was also studied by carrying out the experiments at different temperatures on a thermostatically controlled water bath. Results revealed that at 50°C±2°C the reaction product shows maximum absorption value and the absorbance decreased at higher temperatures (Figure 5). So further, the experiments were carried out at 50°C±2°C.

For diluting the reaction product, water was used as no solubility problems were noticed upon using it as the solvent. Water is relatively inexpensive and all the reagents and drug solutions used are soluble in water. Because of these advantages, water was selected as the diluting solvent.

The stability of the color developed by using the optimum conditions was studied checking its absorption intensity at different time intervals. It was found that the absorbance of the colored complex remained stable for 2.5 hour (Figure 6), which makes the method suitable for analysis of large number of samples.

The stoichiometry of the reaction was determined by using the Job's continuous variation method, which revealed a 1:1 ratio for drug ALS and NQS reagent. Basing on this ratio, the reaction pathway was suggested to be as shown in Figure 7.

The method was found to be linear over a concentration range of 5-70 µg/ml at 525 nm. The linear regression results and optical characteristics of ALS are given in Table 1. The % RSD values for intra-day and inter-day precision were well below 2%, which indicates high levels of precision of the method. The results for validation studies are summarized in Table 2. The results for analysis of marketed formulations are given in Table 3. The good recovery values indicated high levels of accuracy of the method. The commonly used excipients were not interfering in the determination of the drug. This justifies the suitability of the method for routine application for estimation of ALS.
Fig. 3. Effect of NaOH concentration on reaction of ALS and NQS. ALS (40 µg/ml), NQS (0.5%, w/v): 1 ml, NaOH: 1 ml, Temperature: Ambient, Reaction Time: 10 min.

Fig. 4. Effect of reaction time on reaction of ALS and NQS. ALS (40 µg/ml), NQS (0.5%, w/v): 1 ml, NaOH (0.01M): 1 ml, Temperature: Ambient.

Fig. 5. Effect of temperature on reaction of ALS and NQS. ALS (40 µg/ml), NQS (0.5%, w/v): 1 ml, NaOH (0.01M): 1 ml, Reaction time: 10 min.
**TABLE 1**

Optical parameters of the assay method.

<table>
<thead>
<tr>
<th>Method parameters</th>
<th>Obtained values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength(nm)</td>
<td>525</td>
</tr>
<tr>
<td>Linear range(µg/ml)</td>
<td>5-70</td>
</tr>
<tr>
<td>Regression equation (Y=ax+b)</td>
<td>0.007 x + 0.001</td>
</tr>
<tr>
<td>Sandell’s sensitivity</td>
<td>0.13</td>
</tr>
<tr>
<td>Molar Extinction Coefficient</td>
<td>$2.504 \times 10^3$</td>
</tr>
</tbody>
</table>

% Range of error
- 0.05 confidence limits: ±0.0880
- 0.01 confidence limits: ±0.1158
- Correlation coefficient: 0.999

*Y=ax+b; where Y is absorbance, a is slope, x is the concentration and b is the intercept*

**TABLE 2**

Validation parameters.

<table>
<thead>
<tr>
<th>Recovery Type</th>
<th>Drug(µg/ml)</th>
<th>Recovery%, % ± S.D.</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Added</td>
<td>Found</td>
</tr>
<tr>
<td>80 %</td>
<td>8</td>
<td>7.95</td>
</tr>
<tr>
<td>100 %</td>
<td>10</td>
<td>10.04</td>
</tr>
<tr>
<td>120 %</td>
<td>12</td>
<td>11.95</td>
</tr>
<tr>
<td></td>
<td>99.37 ± 1.1</td>
<td>100.46 ± 0.81</td>
</tr>
<tr>
<td></td>
<td>99.58 ± 0.72</td>
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</table>

**Precision**

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>R.S.D. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraday(n=6)</td>
<td>30</td>
</tr>
<tr>
<td>Interday(n=6)</td>
<td>30</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>1.7</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>5.0</td>
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</table>

*Average of three determinations*
TABLE 3

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Labeled Amount (mg)</th>
<th>Recovery % ± S.D.</th>
<th>R.S.D (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablet</td>
<td>70</td>
<td>100.36 ± 0.23</td>
<td>0.23</td>
</tr>
</tbody>
</table>

* Average of three determinations

Conclusion

A validated spectrophotometric method was developed for ALS using the chromogenic reagent sodium-1,2-naphthoquinone-4-sulphonate in alkaline medium. The method is simple, accurate, precise, and can estimate the drug as per ICH requirements. Hence, it can be used for routine analysis of ALS in bulk samples and for assay of pharmaceutical formulations.

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References


