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## Spectrophotometric determination of alendronate in pharmaceutical formulations via complex formation with Fe (II) ions

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### ABSTRACT

The formation of a complex between alendronate sodium, a non-chromophoric bisphosphonate, and iron (II) chloride in aqueous solution at pH = 6 was studied. The stoichiometric ratio of alendronate to Fe (II) ions was determined. Linear and non linear regression methods were used to estimate the complexation constant (K). A 1:1 stoichiometric ratio was observed for the binary complex between Fe (II) and alendronate with a formation constant of  $770 \pm 10 \text{ M}^{-1}$ .L. Subsequently, a spectrophotometric method was developed for the determination of alendronate in the concentration range 3.25-325 $\mu\text{g/mL}$ , the recovery value was  $97\% \pm 3$  and the limit of detection was 1 $\mu\text{g/mL}$ . The method was validated for the direct determination of alendronate in tablet dosage formulation.

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### KEYWORDS

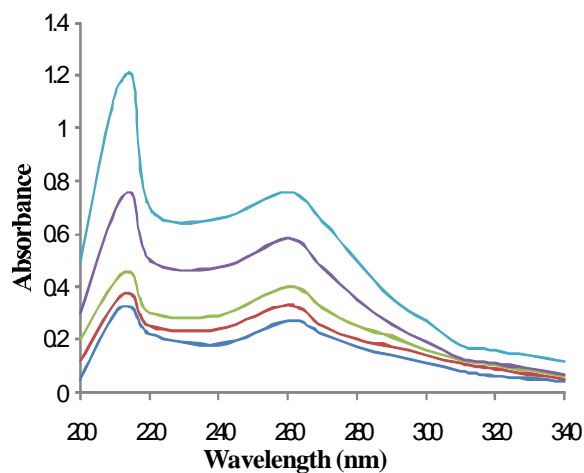
Complex formation;  
Stoichiometric ratio;  
Internal standard addition method;  
Alendronate sodium quantification;  
Iron (II);  
Spectrophotometer;  
Benesi-Hildebrand;  
Scatchard methods.

### INTRODUCTION

Alendronate sodium (AS), which binds to bone surfaces and inhibits osteoclast-mediated bone resorption, is a nitrogen containing bisphosphonate. As with many other bisphosphonates, direct chromatographic AS analysis is complex due to the lack of a suitable UV chromophore for high performance liquid chromatography (HPLC) analysis with spectrophotometric detection<sup>[1-3]</sup>. Most of the analytical methods for determination of bisphosphonates require specialized equipment or involve a derivatization step in order to introduce a chromophore into the molecule. AS contains a primary amine group which can be derivatized with number of reagents in order to form new compound with

chromophoric properties, thus facilitating HPLC analysis<sup>[3]</sup>. In both, in vitro and in vivo studies reported in the literature, HPLC was used for AS quantification<sup>[1,2,4]</sup>.

A reversed phase HPLC method utilizing pre-column derivatization of the primary amine group of AS with 9-fluorenylmethyl chloroformate (FMOC) for UV detection has been described in the literature for the determination of AS in tablets and capsules<sup>[2]</sup>. After derivatization, excess of reagent had to be extracted with methylene chloride and an aliquot of the aqueous portion was assayed on reversed phase HPLC. Ion chromatography with indirect UV detection has also been mentioned for the assay of AS<sup>[4]</sup>. In addition, ion chromatography with post-column derivatization<sup>[5]</sup> and an ion exchange HPLC method

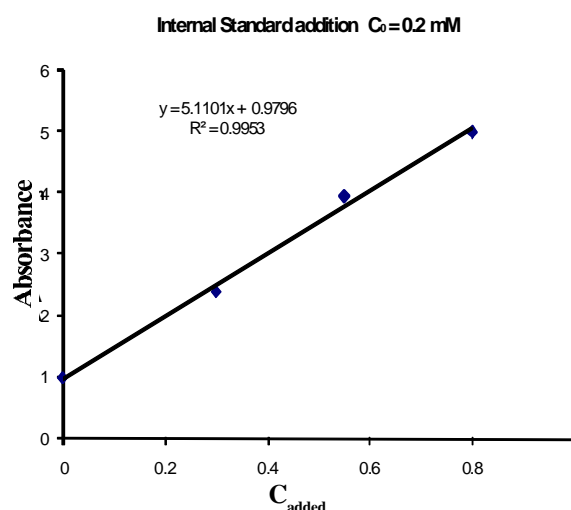


**Figure 1 :** UV-Vis spectra of AS/iron (II) complex at five different concentrations of AS :1mM, 0.75mM, 0.5mM, 0.2mM and 0.1mM (from the top to the bottom)

with conductivity detection<sup>[6]</sup> have been developed and used for the quantitative determination of bisphosphonates in pharmaceutical dosage forms such as tablets and solutions.

A study was carried out to examine the formation of a chromophoric complex between AS and copper (II) ion<sup>[4]</sup>. Another study examined the formation of a complex between AS and Fe (III) ions by UV spectrophotometry<sup>[7]</sup>. Spectrophotometric determination of AS in pharmaceutical formulations via complex formation with Fe (III) ions was found to be simple and non-sensitive. Methods like inductivity coupled plasma and anodic stripping voltammetry have also been reported for the analysis of AS in tablets<sup>[8,9]</sup>. Therefore, despite the numerous methods described in the literature, a reliable and simple method is still needed to determine the amount of AS in pharmaceutical formulations.

The objective of this work was to develop a simple, rapid, sensitive and non expensive method to quantitatively determine AS concentration in tablet formulation. The method was based on the complexation of AS by iron (II) ions. Contrarily to the complexation by iron (III) our method showed higher an accuracy and a greater advantage related to the limit of detection, than that involving AS and Fe (III) described by Kuljanin et al<sup>[7]</sup>. Quantification of AS has been obtained by using the internal standard addition method and the UV spectra of the AS-Fe (II) complex evolution.



**Figure 2 :** Calibration curve for the internal standard addition method

## MATERIALS AND METHODS

### Instruments

The UV absorption measurements were performed on a Shimadzu UV- 1650 PC, (Shimadzu, Kototo, Japan) with 10mm quartz cells used for spectrophotometric measurements. The pH values were measured using a Mettler Toledo pH-meter (Columbus, OH, USA). All measurements were performed at room temperature immediately after mixing the different solutions.

### Reagents

Working standard alendronate sodium trihydrate was purchased from Sigma-Aldrich GmbH (Steinheim, Germany) and ferrous chloride tetrahydrate was received from Merck, (Darmsdadt, Germany). Water purified by a Millipore Milli-Q system (Billerica, MA, USA) was used for the preparation of all solutions.

### Solutions

An iron (II) chloride standard solution (1000ppm) was prepared by dissolving ferrous chloride tetrahydrate (0.1g) in 100mL of acidified (pH=6) water.

A freshly prepared 1000ppm AS solution in water was used as the stock solution. This solution was stable for at least 10 days when stored in the dark at 4-8°C.

The calibration curve was built using a series of five standard solutions prepared by appropriate dilution of the stock solutions. The concentration of AS in these standard solutions varied in the range of 1 to 200ppm

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**TABLE 1 : Composition of different AS and iron (II) chloride mixtures**

Volume of AS solution (5ppm) in mL	Volume of iron(II) solution (5ppm) in mL	Volume of water in mL
0.1	0.2	4.7
0.2	0.2	4.6
0.5	0.2	4.3
0.7	0.2	4.1
1	0.2	3.8

( $\mu\text{g/mL}$ ).

### Procedure

The standard AS solutions were mixed with ferrous chloride solution and the absorbance of the resulting complex was measured immediately after mixing at 215 and 270nm.

The stoichiometry of AS/iron (II) complex was analyzed by the Scatchard and Benesi-Hildebrand plots<sup>[10,11]</sup>. Five mixtures of AS and iron (II) chloride were prepared according to the ratios summarized in TABLE 1.

### Stoichiometry of the AS/iron(II) complex

According to Scatchard's method (equation 1), we assume that the complex of AS with iron (II) is formed in a 1:1 ratio.

$$(A - A_1)/[AS]_1 = (A_\infty - A_1)K_1 - (A - A_1)K_1 \quad (1)$$

Where  $A_1$  denotes the absorbance intensity of the complex AS/iron (II) when the concentration of the AS is minimal;  $A_\infty$  denotes the absorbance intensity when all the iron (II) molecules are essentially complexed with AS;  $A$  is the observed absorbance at each AS concentration tested;  $K_1$  is the association constant and  $[AS]$  is the AS concentration tested. For 1:1 complex, a plot of  $(A - A_1)/[AS]_1$  versus  $(A - A_1)$  should give a straight line.

When a Benesi-Hildebrand plot of  $1/(A - A_1)$  versus  $1/[AS]$  is built (equation 2), a straight line is obtained. When the plot of  $1/(A - A_0)$  versus  $1/[AS]^2$  is considered, a downward concave curvature is obtained, confirming that the stoichiometry of the AS/iron (II) complex is not 1:2.

$$1/(A - A_1) = 1/(A_\infty - A_1)K_1[AS]_1 + 1/(A_\infty - A_1) \quad (2)$$

### Association constant of the inclusion complex

Once the stoichiometry of the complex is known,

**TABLE 2 : Composition of different AS and iron (II) chloride mixtures used to build the internal standard curve**

Volume (mL) of 2mM AS solution	Volume (mL) of iron(II) solution (1mM)	Volume (mL) of water
0.5	1	3.5
1	1	3
1.5	1	2.5
2	1	2
2.5	1	1.5

the association constant can also be calculated by applying the previously described methods. According to the first method, the slope of the straight line gives the association constant. In the Benesi and Hildebrand's method, the association constant is determined by dividing the intercept by the slope of the straight line obtained in the double reciprocal plot.

Non-linear regression method was also used to confirm the results obtained with the two methods described above. The non linear regression method is determined by equation 3):

$$\Delta I = K_1 H_0 \Delta I_{\max} / (1 + K_1 H_0) \quad (3)$$

Where  $\Delta I = (A - A_1)$  is the guest-induced absorbance intensity and is equal to  $\Delta I_{\max} = (A_\infty - A_1)$  when every host exists as inclusion complex.  $\Delta I_{\max}$  was obtained from the double reciprocal plot (Eq. (1)).  $H_0$  is the initial concentration of host (iron (II)). The formation constant ( $K_1$ ) was estimated by fitting equation (3) to the data obtained.

### Internal standard curve addition

The samples, described in TABLE 2, were prepared to build the internal standard curve.

### Pharmaceutical tablet solution preparation for spectrophotometric analysis

A portion of finely powdered tablets equivalent to 10mg of AS was accurately weighed and dissolved in 50mL of acidified water pH = 6. After sonication for 10 minutes, the sample was filtered using Whatman filter paper, and transferred to a 100mL volumetric flask.

### Sample fortification

Different volumes of AS solutions (1mM) were added to equal volume of AS sample solutions pro-

TABLE 3 : Composition of AS and iron (II) chloride mixtures in sample solutions

Volume of pharmaceutical tablet solution in mL	Volume of (1mM)AS solution in mL	Volume of (1mM) iron (II) solution in mL	Volume of water in mL
0.5	0	0.5	4
0.5	1	0.5	3
0.5	2	0.5	2
0.5	3	0.5	1
0.5	4	0.5	0

duced from pharmaceutical tablets, as summarized in TABLE 3.

### Calibration curves and recovery

Linearity and detection limits were established with working standard solutions including AS concentrations ranging from 3.25 µg/mL to 325 µg/mL. Recovery experiments were performed by standard addition method: 65 µg/mL of AS was added to samples and percentage of recovery (R%) was calculated as follows:

$$R\% = [(Cr-Cf)/Cr] \times 100$$

Cr = Real concentration of AS in the fortified samples;  
Cf = Concentration of AS obtained by the internal standard addition curve.

## RESULTS AND DISCUSSION

### Spectrophotometric method

Absorption spectra in the 200-350nm range were obtained for the AS/iron (II) complex. Figure 1 shows the absorption spectra of different mixtures. The iron (II) solution was fixed at 0.2mM and the concentrations of AS were varied from 0.01mM to 1mM. The absorption intensity of the AS/Iron (II) complex increased as the concentrations of AS increased.

Our study showed that AS forms a 1:1 complex with iron (II) and the association constant was estimated to be  $770 \text{ M}^{-1} \text{ L} \pm 10$ . Alendronate showed no absorbance over the entire range of wavelength.

### Regression curve of complex and the AS concentrations

A linear relationship between the absorbance (at

TABLE 4 : Validation parameters of the spectrophotometric method

Method	Spectrophotometer
Standard linearity	
Intercept	-0.32
Slope	0.66
R <sup>2</sup>	0.99
Range (µg/mL)	6-330
Relative standard deviation(RSD), Precision n=5	3%
Mean (µg/mL)	65µg/mL
Mean recovery (%) ± standard deviation	96.0 ± 4
Detection limit (µg/mL)	1

270nm) of the complex and the AS concentration was established over the examined concentration range (3.25-325 µg mL<sup>-1</sup>). The average regression equation ( $n=5$ ) was calculated by the method of least squares and found to be  $y = 0.6669x + 0.2139$ .

The relative standard deviation for the slope was 1.4% and the average correlation coefficient R<sup>2</sup> was equal to 0.9914.

The precision of the method was evaluated with relative standard deviations (RSDs) of AS determination in five samples. RSDs were about 3%. The limit of detection of the method was 1 µg/mL as defined by a signal-to-noise ratio of 3:1 [12].

### Spectrophotometric method for quantification of AS using the internal standard addition model

A spectrophotometric method using the internal standard addition was examined to determine AS concentrations present in pharmaceutical samples. A calibration curve was described by the following equation:

$$A^* = aC + b,$$

which is equivalent to

$$A^* = (A_0^*/C_0) \times C_{\text{add}} + A_0^*,$$

where  $A^* = (A/A_0)$  normalized absorbance intensity (arbitrary values) is equal to the ratio of the absorbance intensity after adding the internal standard A to the absorbance intensity before adding the internal standard ( $A_0$ ),  $C_0$ : solute concentration to be estimated.  $C_0$  is determined by the negative intercept of the curve with the abscissa axis [13,14],  $A_0^*$ : normalized absorbance intensity of the starting solution and  $C_{\text{add}}$ : known added concentration.

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The plot of  $A^*$  vs.  $C_{\text{add}}$  is shown in figure 2. The internal standard used in this method was the AS present in the solution before the incorporation of any additional amount. Figure 2 represents the internal standard addition method that allows determining the AS concentration and the recovery percentage.

The average recovery of five samples spiked with AS (as described in TABLE 2) was estimated to be about  $97\% \pm 3$ . TABLE 4 summarizes the validation parameters of the method.

### Spectrophotometric method for quantification of AS in a pharmaceutical tablet sample using the internal standard addition

UV spectra of the solutions described in TABLE 3 were obtained and the evaluation of those spectra was used to build the internal standard curve. The average AS content determined with this method was  $61.8 \pm 4 \mu\text{g/mL}$  ( $n = 5$ ).

### CONCLUSION

A spectrophotometric method for the determination of alendronate in tablet formulation was developed. The results confirmed the suitability of the proposed method for the analysis of alendronate. Also, the method showed higher accuracy and greater advantage related to the limit of detection, than the one involving AS and Fe (III) described by Kuljanin et al.<sup>[7]</sup>. Furthermore, since this method is rapid and not expensive in terms of laboratory equipments, it can be used for routine analyses and applied on bulk powder, or capsules.

### REFERENCES

- [1] P.Ptáček, J.Klíma, J.Macek; *J.Pharm.Sci.*, **28**, 183-192 (2003).
- [2] J.D.De Marco, S.E.Biffar, D.G.Reed, M.A.Brooks; *J.Pharm.Biomed.Anal.*, **7(12)**, 1719-1727 (1989).
- [3] D.Ostovic, C.Stelmach, B.Hulshizer; *Pharm.Res.*, **10(3)**, 470-472 (1993).
- [4] D.G.Reed, G.P.Martin, J.M.Konieczny, M.A.Brooks; *J.Pharm.Biomed.Anal.*, **13**, 1055-1058 (1995).
- [5] J.P.Kosonen; *J.Pharm.Biomed.Anal.*, **10**, 881-887 (1992).
- [6] J.Den Hartigh, R.Langebroek, P.Vermeij; *J.Pharm.Biomed.Anal.*, **11**, 977-983 (1993).
- [7] J.Kuljanin, I.Jankovic, J.Nedeljkovic, D.Prstojevic, V.Marinkovic; *J.Pharm.Biomed.Anal.*, **28**, 1215-1220 (2002).
- [8] D.G.Reed, G.P.Martin, J.M.Konieczny, M.A.Brooks; *J.Pharm.Biomed.Anal.*, **13**, 1055-1058 (1995).
- [9] O.Abdel Razak, Belal, S.F.Bedair, R.S.Haggag; *Desferrioxamine Mesylate and Lisinopril, Talanta.*, **59**, 1061-1069 (2003).
- [10] H.A.Benesi, J.H.Hildebrand; *J.Am.Soc.*, **71**, 2703-2707 (1949).
- [11] K.A.Connors; 'Binding Constants: the Measurements of Molecular Complex Stability', Wiley, New York, (1987).
- [12] D.MacDougall, W.B.Crummett; *Anal.Chem.*, **52**, 2242-2249 (1980).
- [13] B.Muel, G.Lacroix; *Bulletin de la société chimique de France*, **11**, 2139 (1960).
- [14] J.Rima, M.Lamotte, J.J.Dubien; *Anal.Chem.*, **54**, 1059-1064 (1982).