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Research Article

A fast and simple spectrofluorometric method for the determination of alendronate sodium in pharmaceuticals

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Abstract

Introduction: Alendronate sodium enhances bone formation and increases osteoblast proliferation and maturation and leads to the inhibition of osteoblast apoptosis. Therefore, a rapid and simple spectrofluorometric method has been developed and validated for the quantitative determination of it.

Methods: The procedure is based on the reaction of primary amino group of alendronate with *o*-phthalaldehyde (OPA) in sodium hydroxide solution.

Results: The calibration graph was linear over the concentration range of 0.0-2.4 μM and limit of detection and limit of quantification of the method was 8.89 and 29 nanomolar, respectively. The enthalpy and entropy of the reaction between alendronate sodium and OPA showed that the reaction is endothermic and entropy favored (ΔH = 154.08 kJ/mol; ΔS = 567.36 J/mol K) which indicates that OPA interaction with alendronate is increased at elevated temperature.

Conclusion: This simple method can be used as a practical technique for the analysis of alendronate in various samples.

Introduction

Alendronate sodium is a bone resorption inhibitor, which is used for the treatment of Pagets disease, postmenopausal osteoporosis, primary hyperparathyroidism, malignant hypercalcemia and metastatic bone diseases.[1-3] Bisphosphonates increase bone formation and enhance osteoblast proliferation and maturation and lead to inhibition of osteoblast apoptosis.[2]

Since alendronate sodium has no absorption chromophores or fluorophores and because of its polarionic nature and chelation properties, its determination represents a challenge.[3,4] Derivatization of these kinds of analytes with ninhydrin reagent, 2,3-naphthalene dicarboxylaldehyde, iron in perchloric acid or *o*-phthalaldehyde (OPA) has been used as a common technique for its spectroscopic analysis.[3-7] However, the poor stability of the derivatized product was a major disadvantage of these reported methods.[3] Thus, a method is required which could be simple, reliable and robust yet with a high sensitivity than the previously reported procedures.

Al Deeb *et al.* developed and validated a liquid chromatography (HPLC) method with diode array detection to determine alendronate sodium in pharmaceutical tablets and an HPLC method with fluorescence detection for its analysis in urine.[6] They reported the LOQ of 14 and 0.3 $\mu g ml^{-1}$ for the spectrophotometric and HPLC fluorometric procedures, respectively.

To the best of our knowledge, there is no report about the determination of alendronate sodium in pharmaceutical tablets using fluorimetric method. Therefore, the aim of the present study was the development, optimization and validation of a spectrofluorimetric procedure for the alendronate sodium analysis.

Materials and methods

Apparatus and reagents

All fluorescence measurements were carried out with a JASCO spectrofluorometer (FP6200; Tokyo, Japan). A 360 nm excitation wavelength was used for the fluorescence measurements and the emission spectra were recorded between 380 and 600 nm. The excitation and emission slits were both fixed at 10 nm, and the scan speed was 125 nm min^{-1}.

The monosodium alendronate trihydrate was kindly provided by Modava Company (Karaj, Iran). OPA Was obtained from Sigma-Aldrich (Co., Steinheim, Germany). 2-Mercaptoethanol (2ME) was purchased from Merck (Darmstadt, Germany).

Preparation of standard stock and test solutions

All solutions were prepared using double distilled water. The stock solution of alendronate was prepared by dis-

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solving 10 mg of alendronate sodium in 10 ml of 0.05 M NaOH and used within 2 days. A working solution of the derivatizing reagent was prepared by dissolving 10 mg of OPA with 2 ml of 0.05 M NaOH, then 50 µl of 2ME solution was added and the volume completed to 10 ml with 0.05M NaOH. The solution was freshly prepared for each experiment daily. The stock solution was stored at 4°C.
Aliquots (0.0, 0.4, 0.8, 1.2, 1.6, 2 and 2.4 µM) of this solution were transferred to a 2 ml vial. One hundred microlitter of the OPA/2ME reagent was added to each vial and the volumes completed to 2 ml using 0.05 M NaOH.[6]
One tablet of alendronate sodium was milled and an accurately weighed tablet power equivalent to 70 mg of alendronate sodium was transferred into 100 ml volumetric flask dissolved in 70 ml of 0.05 M NaOH and sonicated for 10 min and the solution was filtered using paper filter to obtain 1mg/ml solution.

Preparation of calibration curve

Standard stock solution was diluted with 0.05 M NaOH to obtain concentrations ranging from 0.4 to 2.4 µM. The emission intensity of these solutions was measured at 360-600 nm for an excitation wavelength of 360 nm (λmax470 nm). Using emission intensity at 470 nm, calibration curve was obtained by plotting graph between concentration and emission intensity.

Limit of detection (LOD), Limit of quantification (LOQ) and Linearity

The LOD and LOQ of alendronate sodium were determined using the standard deviation of five measurements of the blank response and the slope of the calibration line, as defined by ICH guidelines. Linearity was obtained between 0.4 and 2.4 µM concentrations.

Thermodynamic parameters

Thermodynamic parameters of the OPA-alendronate association were determined from measurements made at four different temperatures (283, 293, 303, and 310° K) for the OPA-alendronate complex in order to use the Van't Hoff equation.

Results

Fluorescence spectroscopy studies

Since luminescence was not observed for alendronate sodium solution, derivatization of alendronate with the OPA was carried out in 0.05 M NaOH (Fig. 1). Fig. 2 shows that the poor emission of OPA between 380 and 600 is highly increased by the presence of alendronate with a maximum wavelength of about 470 nm due to interaction of OPA with alendronate. The emission intensity increases by increasing the concentration of alendronate, which indicates that there is an interaction between OPA and it.

Analytical figures of merit

The analytical curve established in the range 0.4-2.4 µM provided a linear relationship between emission intensity at 470 nm and alendronate concentration, I= 3E+8C +0.372 with R of 0.995. The LOD and LOQ were determined according to the IUPAC recommendations: LOD = 3× (S_d/m) and LOQ = 10× (S_d/m), where "S_d" is the standard deviation of measurements of the blank (n = 5) and "m" is the slope of the linear dynamic range.[8] The LOD and LOQ of the method were determined to be 8.89 and 29 nanomolar, respectively, for the mentioned drug; so, better than those reported by Al Deeb *et al.* using also derivation with OPA.[6] In another study, Gupta *et al.* obtained an LOD of 0.25 ngml^{-1} for derivative formation of diazomethane with alendronate sodium using HPLC/electrospray–mass spectrometry.[2] Also Yun *et al.* used 9-fluorenylmethyl derivative for fluorescence detection and quantified the alendronate in human plasma using HPLC method and reported the LOQ of 1 ngml^{-19}. However, our method has some advantages as well as high sensitivity, simplicity and low cost compared to the other techniques such as HPLC and electrochemical technique and can be easily used for the alendronate determination.[2,3,5,9,10]
Recovery studies were made by spiking tablet samples with 70 mg of alendronate sodium, using the equation I:

$$\text{Recovery } \% = \frac{\textit{Drug amount found}}{\textit{Drug amount used}} \times 100 \qquad \text{Eq. (I)}$$

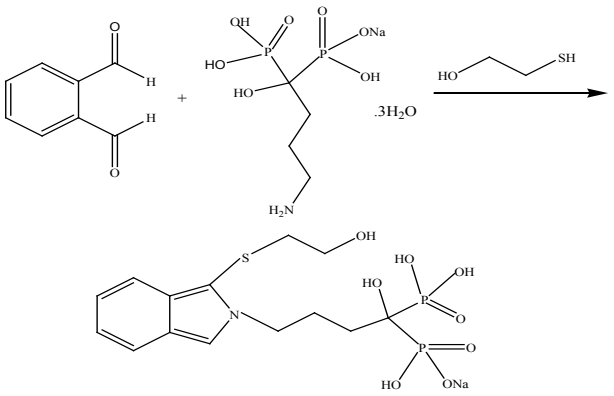

Fig. 1. Schematic illustration of chemical derivatisation of alendronate sodium with OPA

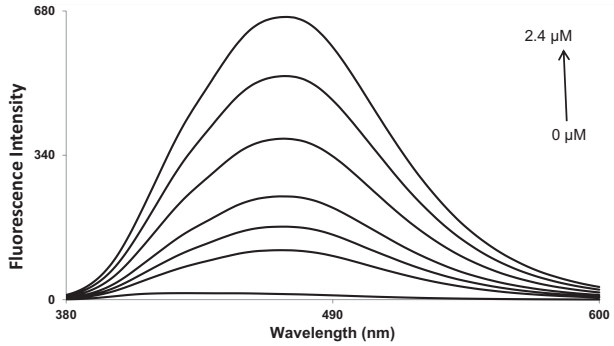

Fig. 2. Emission spectra of OPA in the presence of increasing sodium alendronate concentrations in 0.05 M NaOH ([alendronate sodium] = 0.0, 0.4, 0.8, 1.2, 1.6, 2.0 and 2.4 µM)

Table 1. Figures of merit of the fluorometric determination of alendronate in pharmaceuticals

Recovery percentage %	Determined amount (mg)	Label claim (mg)	R^2	Linear calibration
107.14	75	70	0.995	Y = 3E+08x + 0.372

Note: recovery studies were made by spiking tablet samples with an amount of 70 mg of alendronate sodium tablet.

Additionally the accuracy of the developed methodology was evaluated from the comparison of data found for a commercial formulation with those indicated in the pharmaceutical label (Table 1).

Equilibrium binding titration

The binding constant (K_f) and the binding stoichiometry (n) for the complex formation of OPA with alendronate has been determined using fluorescence titration data. It can be seen that the fluorescence intensity at 470 nm increases with increased alendronate concentration. In order to estimate K_f and n for the binding of OPA to alendronate from the following equation (Eq II), the change in fluorescence intensity at 470 nm was used:[11,12]

$$\log\frac{(F_0-F)}{F} = \log K_f + n\log[\text{Alendronate}] \qquad \text{Eq. (II)}$$

Here F_0 and F are the fluorescence intensities of the fluorophore in the absence and presence of different concentrations of CT-DNA, respectively. But in the case of enhanced emission intensity, since F_0<F, Equation II becomes:

$$\log\frac{(F-F_0)}{F} = \log K_f + n\log[\text{Alendronate}] \qquad \text{Eq. (III)}$$

The linear equations of $\log\frac{F-F_0}{F}$ versus log [Alendronate] at different temperatures are shown in Table 2. The values of K_f obviously show the remarkable high affinity of OPA for alendronate especially at temperatures 303 and 310° K.[12-14]

Thermodynamic studies

To have a better understanding of thermodynamics of the complexation, the contributions of enthalpy and entropy should be determined in the reaction between OPA and alendronate. Estimation of the formation constant for the OPA-alendronate complex at four different temperatures (283, 293, 303, and 310° K) allows determination of thermodynamic parameters of OPA-alendronate formation via Van't Hoff equation (Eq. IV):[15]

$$\ln K_f = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \qquad \text{Eq. (IV)}$$

By plotting $\ln K_f$ vs. 1/T (Fig. 3), ΔH (enthalpy change) and ΔS (entropy change) of complex formation were determined. Knowing these two values changes in Gibs, free energy (ΔG) was calculated from the following standard equation (Eq. V):[16]

$$\Delta G = \Delta H - T\Delta S \qquad \text{Eq. (V)}$$

The results are shown in Table 3. The ΔH and ΔS values of the OPA-alendronate complex were +154.08 kJ/mol and +567.36J/mol K, respectively. It seems that the reaction between OPA and alendronate is endothermic and entropy favored. From the thermodynamic data, it is quite clear that while complex formation is entropy favored, it is conversely enthalpy disfavored. Therefore, the formation of the complex results in a more ordered state and these are other evidences to indicate that OPA interaction with alendronate has been increased with temperature increase.[13]

Discussion

OPA is a generally utilized agent for the determination of amino acids and primary amines. It reacts with primary aliphatic amino groups of alendronate to attain the fluorescence emission properties.[1,2,4] Besides, the use of chemical derivatisation of alendronate with OPA not only improves the separation of the analyte but also after some optimization, it enhances the detection sensitivity.[2,5] Therefore, spectrofluorimetric method for quantifying alendronate sodium in pure and tablet has been developed and validated for the first time. The technique has advantages such as simplicity and low cost. It requires minimal amounts of samples and reagents/solvents, and is

Table 2. Linear equations of log $(F-F_0)/F$ versus log [alendronate], and K_f of OPA with alendronate at different temperatures

Temperature °K	Linear equation	K_f
283	Y = 0.285 X + 1.588	3.87×10^1
293	Y = 0.281 X + 1.607	4.05×10^1
303	Y = 0.537 X + 2.880	7.58×10^2
310	Y = 0.729 X + 4.044	1.11×10^4

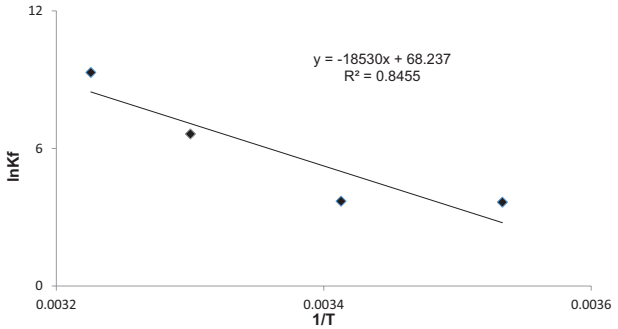

Fig. 3. Van't Hoff plot for OPA–alendronate complex

Table 3. Thermodynamic parameters for binding of OPA to sodium alendronate

T (°K)	$\ln K_f$ (M^{-1})	ΔS° (J mol^{-1} K^{-1})	ΔH° (kJ mol^{-1})	ΔG° (kJ mol^{-1})
283	3.656	567.363	154.085	-6.4784
293	3.700	567.363	154.085	-12.152
303	6.631	567.363	154.085	-17.825
310	9.311	567.363	154.085	-21.797

therefore environmentally friendly.

The proposed method has acceptable sensitivity for determination of alendronate sodium in pure form and tablets. The LOD and the LOQ was 8.9 and 29 nanomolar, respectively with this validated method in concentration range of 0.0-2.4 μM. Also good linearity has been attained between fluorescence intensity and alendronate sodium concentration. Furthermore, the determination of thermodynamic parameters showed that the OPA interaction with alendronate is endothermic and the best complexation temperature for analysis and linearity is 303° K. Thus, the spectrofluorimetric procedure can provide a very practical technique for the analysis of less sensitive analytes like alendronate sodium.

Conclusion

Alendronate sodium can be determined in pure form and pharmaceutical tablets based on reaction with OPA in the presence of 2ME. Advantages of proposed method are simplicity, accuracy and rapidness. The obtained results verified the suitability of this procedure for the precise analysis of alendronate sodium in quality control laboratories.

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Ethical issues

There is none to be declared.

Competing interests

The authors declare no conflict of interests.

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