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Short communication

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

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Abstract

The formation of the complex between alendronate, non-chromophoric bisphosphonate drug important for the treatment of a variety of bone diseases, and iron(III) chloride in perchloric acid solution was studied. The stoichiometric ratio of alendronate to Fe(III) ions in the chromophoric complex was determined to be 1:1. The conditional stability constant was $\log K'_{ave} = 4.50$ (SD = 0.15), indicating that the Fe(III)–alendronate complex is a complex of medium stability. The optimum conditions for this reaction were ascertained and a spectrophotometric method was developed for the determination of alendronate in the concentration range 8.1–162.5 μg ml^{-1}, the detection limit being 2 μg ml^{-1}. The method was validated for the direct determination of alendronate in tablet dosage formulations.

Keywords: Complex formation; Alendronate; Iron(III) chloride; Spectrophotometry

1. Introduction

The bisphosphonates, long-lived synthetic analogs of pyrophosphates, have been investigated over the past two decades for the treatment of various bone diseases and calcium metabolism [1]. The parent compound, etidronate, was first used in multicentered trials for treatment of primary osteoporosis. The recently approved drug alendronate is a more potent agent than etidronate in producing a greater increase in bone density. This agent is currently the bisphosphonate of choice for clinical use in treatment of osteoporosis, Paget's disease, primary hyperparathyroidism, hypercalcemia of malignancy and metastatic bone disease [2–4].

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The trihydrate of alendronate sodium having highly ionic character do not possess an appreciable chromophore, hence the determination by ordinary spectrophotometric method is not possible. Methods for its determination in pharmaceutical formulations are based on liquid chromatography–mass spectrometry [5], ion chromatography with indirect UV detection [6,7] or conductivity detection [8], capillary electrophoresis [9] and inductively coupled plasma [10]. Quantification of alendronate in biological fluids was performed by HPLC with fluorescence and electrochemical detection [11]. Formation of chromophoric complex between alendronate and copper(II) ions in acidic media was applied to analytical method development [9,12].

The present paper describes a simple and reproducible assay for the determination of alendronate in dosage forms. The method is based on the formation of a chromophoric complex of the drug with Fe(III) ions. Its advantages over already existing methods are rapidness, simplicity and inexpensiveness.

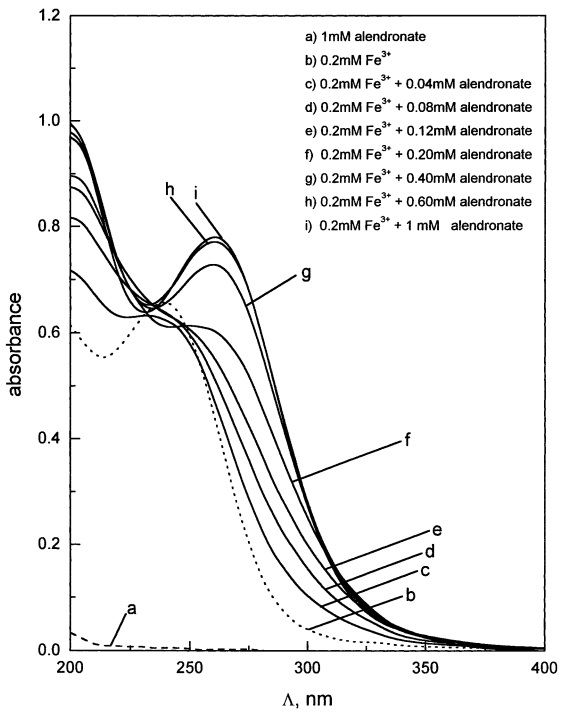

Fig. 1. Absorption spectra of alendronate (a), iron(III) chloride (b), and alendronate–Fe^{3+} complex (c-i) in 0.2 M perchloric acid.

2. Experimental

2.1. Instruments

Perkin–Elmer Lambda 5 and 15 UV–Vis spectrophotometer with 10 or 20 mm quartz cells were used for spectrophotometric measurements.

2.2. Materials

Working standard alendronate sodium trihydrate (Merck Sharp & Dohme International, USA), Alendronat tablets (Zdravlje, Leskovac) containing 13.6 mg of alendronate sodium trihydrate and lactose, maize starch and magnesium stearate as excipient, ferric chloride hexahydrate (Merck) and perchloric acid (Merck) were used. Water purified by a Millipore Milli-Q system was used for the preparation of all solutions.

2.3. Solutions

An iron(III) chloride standard solution (5 mM) was prepared by dissolving ferric chloride hexahydrate in 2 M perchloric acid (17.5 ml of 11.5 M perchloric acid was diluted with 50 ml water, 0.135 g of ferric chloride hexahydrate was added and the solution was diluted to volume of 100 ml with water).

A freshly prepared 5 mM alendronate solution in 2 M perchloric acid was used as the stock solution. This solution was stable for at least 10 days when stored in the dark at 4–8 °C. More dilute solutions were obtained by appropriate dilution.

For the calibration graph a series of five standard solutions was prepared by dilution of corresponding stock solution to obtain the concentration range of 8.1–162.5 μg ml^{-1} alendronate in perchloric acid solution.

For an assay a tablet containing 10 mg of alendronic acid was extracted with ferric chloride

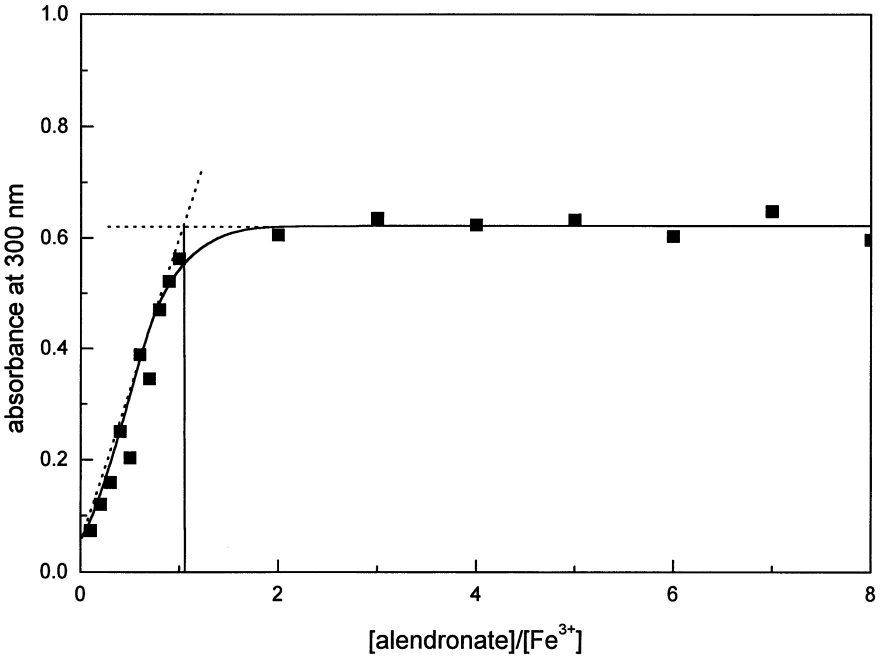

Fig. 2. Molar ratio method. $[Fe^{3+}] = 4 \times 10^{-4}$ M; [alendronate] $= 4 \times 10^{-5}$–3.2×10^{-3} M in 0.2 M perchloric acid.

solution, the final concentration being approximately 0.11 mg ml^{-1} alendronate.

Dissolution test examinations were performed according to Ph. Eur. Ed. 1997 using Apparatus II (with paddle) during 60 min [13].

2.4. Procedure

The standard solutions or sample solutions of alendronate were mixed with ferric chloride solution and the absorbance of the complex was measured at 290, 300 and 310 nm immediately after mixing. All measurements were performed at room temperature against a reagent blank (perchloric acid solution) in the wavelength range where the absorption of metal ions is low.

The experiments determining the composition of the complex by Job's method [14] were conducted by using 0.6 mM alendronate and 0.6 mM $FeCl_3$ in perchloric acid solutions. Nine mixtures of alendronate and iron(III) chloride were prepared. The volumes of alendronate solution used varied from 9 to 1 ml and those of iron(III) chloride solution from 1 to 9 ml; total volume was always 10 ml.

3. Results and discussion

3.1. Absorption spectra and stoichiometry of the Fe(III)–alendronate complex

The complex formation between Fe(III) ions and alendronate was investigated in the acidity range from 0.05 to 2 M $HClO_4$. Highly acidic medium was necessary in order to avoid the hydrolysis of Fe(III) ions [15]. The absorption spectra of the Fe(III)–alendronate complex recorded in the wavelength range from 200 to 400 nm are shown in Fig. 1 (curves c–i). For comparison, the absorption spectra of the reacting species (alendronate and Fe(III) ions) are also given in Fig. 1 (curves a and b, respectively). It can be noticed that alendronate does not absorb in the spectral region mentioned above, while Fe(III) ions and the complex have their absorption maxima at 238 and 260 nm, respectively. The absorption spectra of the complex achieved immediately after mixing remained unchanged for at least 90 min. The presence of clear isosbestic point at 235 nm indicated that only one type of the Fe(III)–alen-

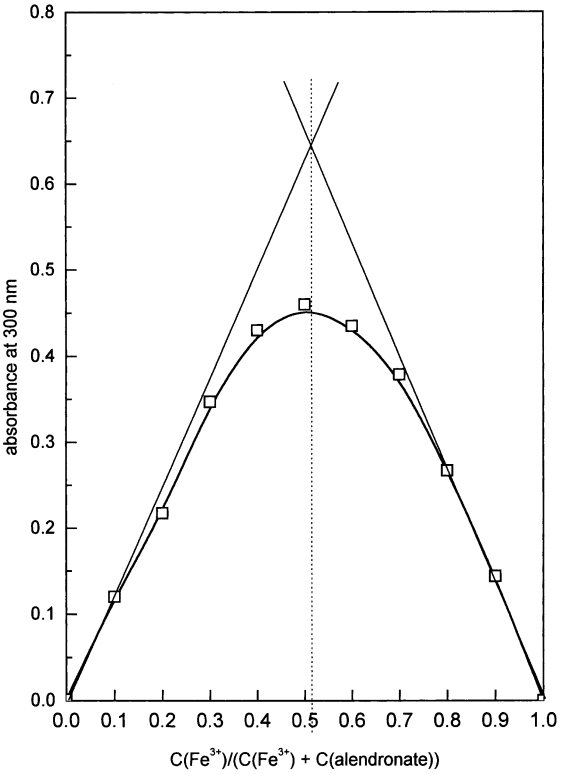

Fig. 3. Job's curve of equimolar solutions for alendronate–Fe^{3+} complex in 0.2 M perchloric acid; $[Fe^{3+}] + [alendronate] = 6 \times 10^{-4}$ M.

dronate complex is formed. The absorption spectra of the complex were followed as a function of pH ($c(HClO_4) = 0.05–2$ M). The shape and the position of the absorption maximum of the Fe(III)–alendronate complex were not affected by the change in the acidity.

The stoichiometry of the Fe(III)–alendronate complex was determined at different acidities by continuous variation of alendronate concentration, the concentration of Fe(III) ions being constant. The plot obtained by the molar ratio method [16] indicated that Fe(III) ions and alendronate form complex in molar ratio of 1:1 (Fig. 2). It can be noticed that the threefold molar excess of the drug was required to obtain the saturation of the absorbance of Fe(III)–alendronate complex.

Additionally, the stoichiometric ratio between Fe(III) ions and alendronate in the complex at different acidities was checked by Job's method of continous variation [14]. The Job's plot (Fig. 3) reached a maximum value at a mole fraction of 0.5, which confirmed that molar ratio between Fe(III) ions and alendronate in the complex is 1:1.

The conditional stability constant (K') of the Fe(III)–alendronate complex was calculated according to the methods of Sommer [17] and Asmus [18] using data from Job's plot for equimolar solutions. The comparison of the conditional stability constants obtained using two different methods is presented in Table 1. It can be noticed that the log K' values obtained by Sommer's and Asmus's methods are in good agreement. The value of conditional stability constant indicates that the Fe(III)–alendronate complex is a complex of medium stability.

Table 1
Determination of the conditional stability constant (K') of the alendronate–Fe^{3+} complex

$N = 6$	$c(HClO_4)$ (M)	log K'	log K'_{ave}	SD[a]	RSD (%)[b]
Asmus's method	0.2	4.54	4.56	0.136	3.0
	1.0	4.41			
	2.0	4.74			
Sommer's method	0.2	4.50	4.44	0.142	3.2
	1.0	4.57			
	2.0	4.24			

Conditions: $\lambda = 300$ nm, $t = 25$ °C.
[a] Standard deviation.
[b] Relative standard deviation.

Table 2
Determination of alendronate in the bulk drug and in Alendronat tablets

Sample ($n = 10$)	Taken (mg)	Found (mg)	RSD (%)	Recovery (%)	Dissolution (%)
Laboratory mixture[a]	10	9.76	1.9660	98.46–100.88	–
Alendronat tablets	10	9.58	2.5601	–	84.56

[a] Alendronate + excipient.

3.2. Quantitative determinations of alendronate in pharmaceutical formulations

The possibility to use complex formation between Fe(III) ions and alendronate for quantitative determination of alendronate in pharmaceutical formulations was tested for specificity, linearity, LOD/LOQ values, repeatability, accuracy (recovery), stability and ruggedness.

The specificity of the method was checked by observing if there was any interference of the tablet excipient in the Fe(III)–alendronate complex formation. Spectrophotometric measurements showed that placebo sample did not have any absorption under described experimental conditions. However, as a non-separative method it is not specific in relation to degradation products/alendronate related compounds and impurities, hence it cannot be used as a stability-indicating method.

Beer's low was verified in the entire investigated acidity range. A linear relationship between the absorbance and the concentration of alendronate was obtained over the concentration range 8.1–162.5 μg ml^{-1}. For example, in 0.05 M $HClO_4$ solution the regression equation was $y = 7.1331x - 0.0826$ ($SD_{slope} = 0.1255$, $SD_{intercept} = 0.0162$) with correlation coefficient (r) of 0.9991, indicating good linearity. The limit of detection (LOD) was 2 μg ml^{-1} of alendronate defined as the concentration that gives rise to a signal that is three times the noise of the method. Limit of quantition (LOQ) was 7 μg ml^{-1}, accepted to be 10 times the noise signal.

The precision of the proposed spectrophotometric method was accessed by analyzing laboratory mixtures (alendronate and excipient) and Alendronat tablets containing known quantity of drug. The results are presented in Table 2, as the mean value of 10 determinations. It is evident that developed method is of satisfactory repeatability, since the relative standard deviations (RSD) were 1.97 and 2.56% for laboratory mixture and Alendronat tablets, respectively. The results of the recovery of alendronate from laboratory mixtures are also presented in Table 2. The recovery values varied from 98.4 to 100.9% indicating that the developed method is quite efficient. Dissolution test results were within permitted declared limits (minimun 80%).

The stability of the complex was confirmed by measuring the absorbance six times within 2 h with the RSD = 3.12%.

The ruggedness of the method was studied by measuring the absorbance for different perchloric acid solutions (0.05–2 M) and analytical wavelengths (290–310 nm). It was concluded that the sensitivity of the method increased with the decrease of perchloric acid concentration and the decrease in wavelength.

4. Conclusions

The spectrophotometric method described was found to be simple and sensitive and therefore could be applied for the determination of alendronate in the bulk drug and in Alendronat tablets. The results obtained confirm the suitability of the proposed method for the precise analysis of alendronate. Since this method is rapid, simple and no expensive laboratory technique is needed, it can be used for routine analyses.

However, as a non-separative method its main disadvantage is the inability to differentiate degradation products/alendronate related compounds. Hence, the proposed method cannot be useful in stability-testing and determination of impurities.

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