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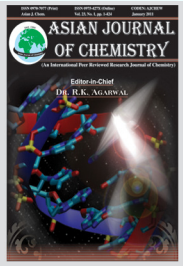

Spectrophotometric Determination of Alendronate Sodium in Bulk Drug and in Pharmaceutical Formulation

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A sensitive, simple, precise and low-cost spectrophotometric method for alendronate sodium (ALD) determination has been proposed. The procedure is based on the reaction of the primary amino group of alendronate sodium with ninhydrin reagent in a boiling water bath in presence of pyridine to yield a bluish-violet product measured at 565 nm. Linear calibration curve was obtained in the range 1-60 µg/mL, with minimum detectability of 0.1 µg/mL. The correlation coefficient was 0.9999 (n = 7). The different experimental parameters have been carefully studied. The proposed method has been successfully applied to the analysis of the studied drug in pure form and in commercial tablets. The average percentage recovery (n = 7) was 99.8 ± 0.35. The results were compared statistically with those obtained from a published method as revealed by t- and F- tests.

Key Words: Spectrophotometry, Alendronate sodium, Ninhydrin, Pyridine, Pharmaceutical analysis.

INTRODUCTION

Alendronate sodium (ALD) is the sodium salt of 4-amino-1-hydroxy-1,1-biphosphonic acid trihydrate. It is an aminobiphosphonate drug with potential utility in treatment of diseases characterized by abnormal bone turnover, such as metastatic bone disease, hypercalcemia of malignancy, Paget's disease, periodontal disease and osteoporosis. Unlike earlier bisphosphonate compounds (etidronate, clodronate and tiludronate), ALD contains a side-chain primary amino group, which imparts greater potency and specificity[1].

The main limitations for the development of analytical assays for the determination of ALD is the lack of chromophores in the molecule, as well as in many other biphosphate, makes the analytical methods development for this class of compounds challenging. The developed assays for the determination of ALD in pharmaceutical samples and biological fluids are mostly based on separation techniques such as ion chromatography (IC) using conductivity[2], indirect UV[3] or refractive index detection[4], capillary electrophoresis (CE)[5] and chromatographic methods[6-11]. These techniques are unattractive to routine analysis in terms of sophisticated instrumentation, high cost and requirement of labour-intensive sample preparation procedure and personal skills. An inductively coupled plasma (ICP)-based approach[12] also employs very expensive instrumentation with high operational cost. Other methods for ALD determination include: voltammetric[13], spectrophotometric[10,14-17], fluorimetric[17] and flow-injection chemiluminescence[18] methods. These methods are generally simple, require low-cost and widely available instrumentation. However, some of these methods are laborious and time-consuming as based on several steps or employ difficult-to-handle reagents or involve a derivatization step in order to introduce a chromophore into the molecule.

The aim of the present paper is to develop a new, simple, sensitive, reproducible and accurate-quantitative spectrophotometric method for the fast control analysis of alendronate sodium, based on condensation reaction of the drug with ninhydrin reagent in presence of pyridine to yield a coloured product measurable at 565 nm. The proposed method was applied successfully to determine ALD in pure form and in tablets.

EXPERIMENTAL

Ultrospec 2100 pro-88683 Biochrom UV-visible spectrometer (Cambridge, UK) with 10 mm quartz cells were used for spectrophotometric measurements.

Alendronate sodium trihydrate was kindly donated by Saudi Pharmaceutical Industries and Medical Appliances Corporation, Al Qassim Pharmaceutical Plant, Spimaco (Saudi Arabia) and was used without any purification. Standard aqueous stock solution containing 100 µg/mL ALD was prepared

weekly and kept refrigerated and protected from light. More dilute solutions were obtained by appropriate dilution. FOSAMAX tablets (Hertford Road, Hoddesdon, Merck Sharp and Dohme Limited, UK) were collected from local market. Each tablet labeled to contain 70 mg ALD (Batch No. NA55740).

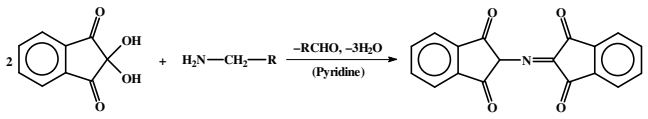
All solvents and chemicals used were of analytical-reagent grade and all the solutions were made in distilled water. The following reagents were used: ninhydrin(2,2-dihydroxyindon-1,3-dion) was obtained from Merck (DARMSTADT). Ninhydrin stock solution, 0.6 %, was prepared in distilled water. Pyridine was obtained from BDH Laboratory Supplies, England.

General procedure: Into 20 mL test tubes, aliquot volumes from the stock ALD solution corresponding to 1- 60 µg/mL were transferred. 0.1 mL of pyridine and 0.6 mL of 0.6 % ninhydrin solution were added, respectively to each tube. The tubes were placed in a boiling water bath for 1 h, then cooled and transferred quantitatively into a series of 10 mL volumetric flasks. Each test tube was washed with little water and the washings were added to the volumetric flasks, then completed to volume and mixed well. The absorbances at 565 nm were measured against a similarly prepared reagent blank. A calibration curve relating the absorbance *versus* drug concentrations in µg/mL was constructed; alternatively, the regression equation was derived.

Procedure for tablets: Ten tablets of the ALD-containing formulation (FOSAMAX) were weighed, ground and homogenized in a mortar and pestle. A weighed amount of the fine powder equivalent to 10 mg of ALD was dissolved in distilled water by sonication for 10 min. The solution was filtered into a 100 mL volumetric flask and the filtrate was diluted to volume with distilled water. This solution, labeled to contain 100 µg/mL, was analyzed using the above described procedure. Nominal content of tablets was calculated using the standard additions method.

RESULTS AND DISCUSSION

Ninhydrin, when heated with α-amino acids (which contains a primary aliphatic amino group), a bluish-violet coloured product is obtained[19,20] (**Scheme-I**).



Scheme-I

Alendronate sodium was found to react with ninhydrin in presence of pyridine in a boiling water bath to yield a blue violet coloured product that absorbed at 565 nm (Fig. 1).

Optimization of the reaction conditions: The optimum conditions should be selected so that the maximum sensitivity, largest linear range and low blank readings are obtained together with the maximum correlation coefficient and precision. In order to find these optimum conditions, the influence of the variables affecting the reaction, such as volume of pyridine, ninhydrin concentration, temperature and reaction time, were studied.

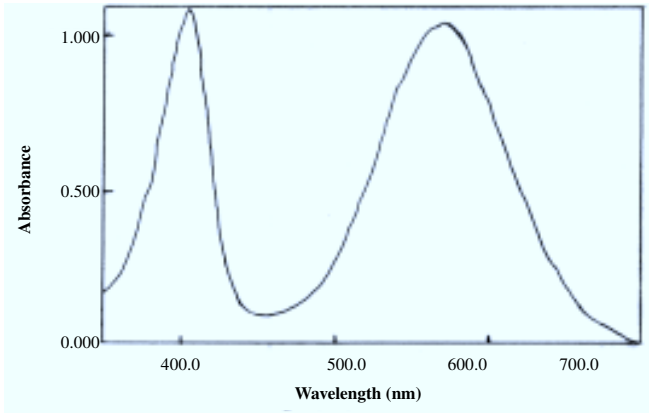


Fig. 1. Absorption spectrum of alendronate sodium (30 µg/mL)/ninhydrin (0.003 %) product in presence of pyridine

Effect of volume of pyridine: Pyridine is essential for the production and stability of the colour[21]. The role of pyridine is not described in the literature in hand. Its role is increasing colour stability and intensity. It may be due to the formation of coordinate bond between pyridine (unshared electron pair) and electron deficient carbon atom in the formed complex[22]. A volume of 1 mL of pyridine is optimal to produce maximum colour intensity. Below and above this volume the colour intensity at 565 nm decreases (Fig. 2).

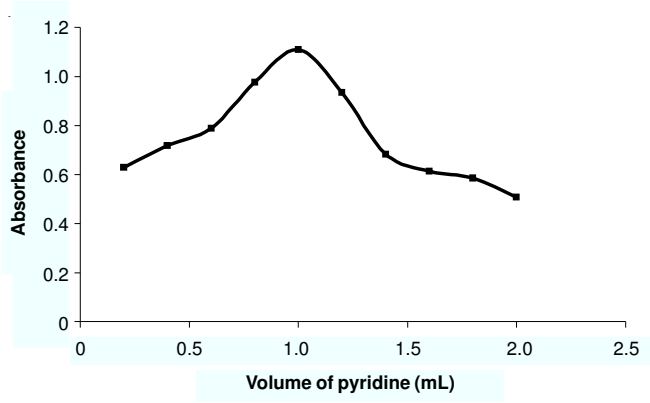


Fig. 2. Effect of pyridine volume on the absorbance of alendronate sodium (30 µg/mL)/ninhydrin product at 565 nm

Effect of ninhydrin concentration: The effect of ninhydrin concentration was investigated in the range 0.2-1.0 %. It was found that 0.6 mL of 0.6 % aqueous ninhydrin solution is optimal for maximum development of the violet colour for ALD (Figs. 3 and 4).

Effect of temperature and time: The reaction between ALD and ninhydrin is temperature dependent. The effect of the temperature on the formation of the reaction product was performed starting from 50 ºC for 1 h. Fig. 5 shows that 100 ºC is the suitable working temperature. The effect of heating time on the formation of the reaction product was studied at 100 ºC.

It was observed from Fig. 6 that heating in a boiling water bath for 1 h is optimum to attain maximum intensity of the violet colour below which lower absorbance was obtained. The developed colour was stable for 2 h.

Validation of the proposed method: Under the optimum experimental conditions prescribed, a linear relationship

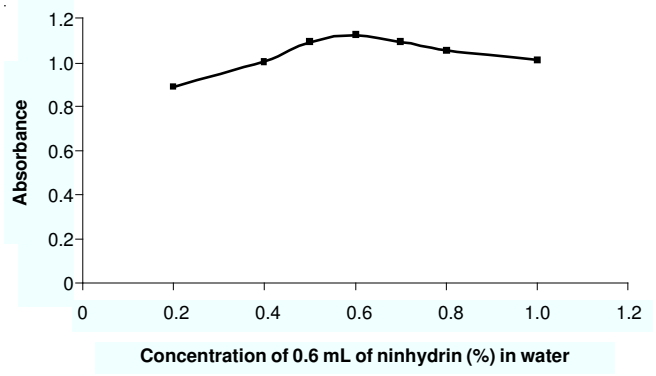


Fig. 3. Effect of ninhydrin concentration on the absorbance of alendronate sodium (30 µg/mL)/ninhydrin product in the presence of pyridine at 565 nm

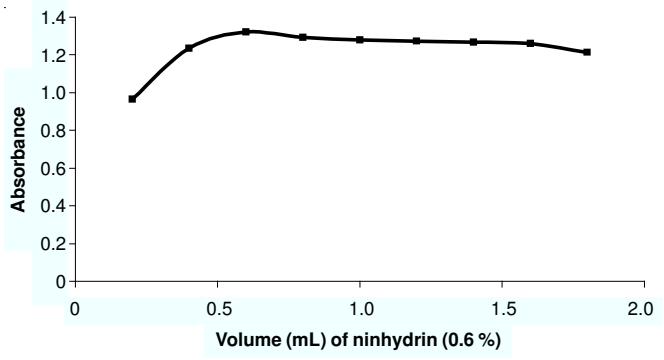


Fig. 4. Effect of volume of ninhydrin (0.6 %) on the absorbance of alendronate sodium (30 µg/mL)/ninhydrin product in the presence of pyridine at 565 nm

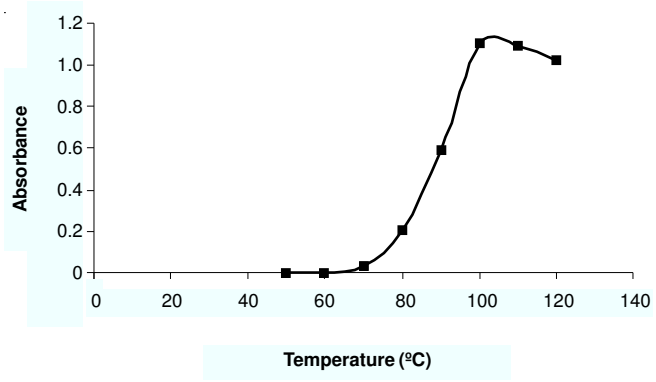


Fig. 5. Effect of heating on the absorbance of alendronate sodium (30 µg/mL)/ninhydrin product in the presence of pyridine at 565 nm

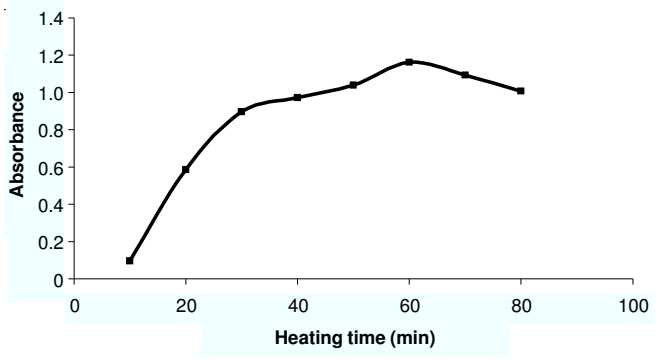


Fig. 6. Effect of heating time on the absorbance of alendronate sodium (30 µg/mL)/ninhydrin product in the presence of pyridine at 565 nm

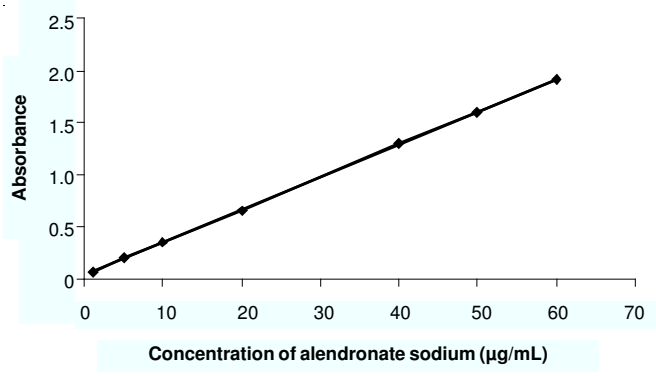


Fig. 7. Calibration curve of alendronate sodium/ninhydrin product at 565 nm

between the absorbance and concentration of ALD was obtained over the concentration range 1-60 µg/mL and was described by the regression equation: A = 0.0311C + 0.0441 with correlation coefficient (r) = 0.9999 for n = 7 indicating good linearity (Fig. 7). The limit of detection (LOD) was 0.1 µg/mL of ALD defined as the concentration that gives rise to a signal that is three times the noise of the method. Limit of quantitation (LOQ) was 0.5 µg/mL, accepted to be 10 times the noise signal.

TABLE-1
DETERMINATION OF ALENDRONATE SODIUM IN BULK POWDER AND TABLETS BY THE PROPOSED AND REPORTED METHOD

Drug form	Proposed method			Reported method[16]
	Taken (µg/mL)	Recovery (%)		
Bulk powder	1	100.0		
	5	102.0		
	10	100.0		
	20	99.0		
	40	101.3		
	50	99.6		
	60	100.0		
$\bar{X}$		100.3		100.1 (n = 4)
± SD		1.03		0.80
Variance $(SD)^2$		1.06		0.64
F-ratio			1.66 (8.94)*	
t-value			0.332 (2.262)*	
FOSAMAX tablets (70 mg alendronate sodium trihydrate/tablet)** B.N./NA55740	5	100.0		
	10	100.0		
	30	99.3		
	40	100.0		
$\bar{X}$		98.8***		99.2 (n = 5)
± SD		0.35		0.52
Variance $(SD)^2$		0.12		0.27
F-ratio			2.25 (9.12)*	
t-value			1.97 (2.365)*	

*Figures in parenthesis are the tabulated values of t and F at 95 % confidence limits. **Merck Sharp and Dohme Limited, UK. ***Standard addition method.

Good reproducibility was obtained upon application of the proposed procedure to different blind experiments of pure samples of the drug; the mean accuracy was 100.3 ± 1.03 (n = 7).

Analytical application: The proposed assay was applied to the determination of ALD in a commercially available pharmaceutical FOSAMAX which is labeled to contain 70 mg of the analyte per tablet. FOSAMAX was analyzed by the proposed procedure[16]. The results obtained using the standard additions method were statistically compared with those obtained by the published method. The data in Table-1 shows that the calculated t- and F-values are less than the tabulated ones, confirming accuracy and precision at 95 % confidence limits[23].

Conclusion

Alendronate sodium, an amino-biphosphonate drug used in the treatment of osteoporosis, reacts with ninhydrin reagent in the presence of pyridine to form a bluish-violet product, which can be used for the spectrophotometric determination of ALD. This product is stable for 2 h. The reaction was found to be temperature dependent, therefore heating in a boiling water bath is necessary for the reaction to complete. The method is simple and reasonably sensitive for the determination of microgram amounts of ALD. The wide range of linearity is also another advantage. The statistical analysis is in good agreement with those of the published method. The other advantages of the present method include low detection limit with high accuracy and precision compared to the published spectrophotometric method. The proposed method was applied to the analysis of FOSAMAX tablets and gave accurate results.

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