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Effect of Manganese Ions (Mn^{2+}) on Ninhydrin Colour Development by Alendronate for the Spectrophotometric Determination of Alendronate Sodium in Tablets

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ABSTRACT

The purpose of this study was to investigate the effect of Manganese ions (Mn^{2+}) on colour development of ninhydrin by alendronate for the spectrophotometric analysis of alendronate sodium in tablets. The analysis was based on the reaction of ninhydrin with the primary amino group in alendronate in the presence of 0.01 M sodium bicarbonate solution and microgram quantity of Mn^{2+} ions. The mixture was heated in a water bath at 95 ± 5°C for 35 min, and then cooled down at room temperature for 10 min. The absorbance of the purple-colour complex was measured against reagent blank at 568 nm. The method was found to be linear in the range of 2-12 µg/ml^{-1} and the correlation coefficient was 0.9997. Limit of quantification was found to be 1.4 µg/ml^{-1}. Mean percentage recovery was found to be 99.73 ± 1.33%. The validated method was used for the quantification of alendronate in tablets. Tablet excipients showed no interference with the analysis and percentage of labeled amount was found to be 100.07 ± 1.2%. The proposed spectrophotometric method is suitable for the routine analysis of alendronate in tablets.

Keywords: Alendronate sodium, Ninhydrin, Manganese ions, Spectrophotometry, Tablets

INTRODUCTION

Bisphosphonates are chemically stable derivatives of Inorganic Pyrophosphate (PPi) that consists of two phosphate groups linked by esterification. Bisphosphonates are potent anti-resorptive drugs. Alendronate sodium is the sodium salt of 4-amino-1-hydroxy-1-bisphosphonic acid trihydrate. Alendronate is widely used medicine for bone diseases. It is used for the treatment of osteoporosis and works by the inhibition of osteoclasts, the bone breakdown cells, which leads to reduced bone turnover, increased bone density, improved mineralization and reduced fracture risk [1]. Alendronate sodium was the first potent nitrogen-containing bisphosphonate approved by U.S. Food and Drug Administration (FDA).

Direct analysis of alendronate is challenging due to the lack of a chromophore in its structure. Several High Performance Liquid Chromatography (HPLC) methods have been reported for the quantitative analysis of alendronate, most of them relied on derivatization using either pre-column or post-column techniques [2-6]. Other reported methods for the determination of alendronate in tablets include ion chromatographic methods [7-9], electrochemical methods [10,11], liquid chromatography-mass spectrometry [12] and high resolution proton nuclear magnetic resonance spectroscopy [13].

Ninhydrin is a widely used reagent for the determination of amino acids. Addition of few microgram quantities of Manganese ions (Mn^{2+}) on ninhydrin colour development by amino acids was found to enhance sensitivity by increasing the intensity of the colour complex [14]. Therefore, the purpose of this study was to investigate the effect of Mn^{2+} on colour development of ninhydrin by alendronate for the spectrophotometric determination of alendronate sodium in tablets.

MATERIALS AND METHODS

Apparatus

Lambda 25 UV-vis Spectrometer (Perkin Elmer, Singapore) with UV WinLab Software was used for all measurements.

Chemicals and reagents

Alendronate sodium trihydrate (purity 99.5%) (Figure 1) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ninhydrin, sodium bicarbonate manganese (II) chloride and methanol (99.5%) were purchased from (Merck, Germany). Alendronate tablets (Apotex, Canada) were purchased from local pharmacy in Kuala Lumpur, Malaysia.

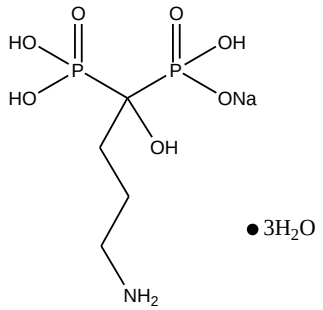

Figure 1: Chemical structure of alendronate sodium trihydrate

Preparation of standard stock solution

Alendronate stock solution (200 µg/ml^{-1}) was prepared by accurately weighing 20 mg of alendronate standard which was then transferred into 100 ml volumetric flask. Volume was completed to 100 ml with ultra-purified water. And solution was shaken well to obtain a homogenous solution.

Preparation of sample stock solution

One alendronate tablet (352.7 mg) containing 91.37 mg of alendronate sodium was grinded and mixed well. 270 mg of the powder was accurately weighed and dissolved in 50 ml ultra-purified water, shaken well and then filtered. The filtrate solution was transferred into 100 ml volumetric flaks and was made up to 100 ml with ultra-purified water to get a final concentration of 700 µg/ml^{-1}.

Preparation of derivatizing agent and other reagents

Ninhydrin solution (0.2%) was used as derivatizing agent. It was prepared by accurately weighing 200 mg of ninhydrin and dissolving it in 100 ml methanol. The solution was freshly prepared on daily basis. Manganese chloride solution (50 µg/ml^{-1}) was prepared by accurately weighing 12.5 mg of manganese chloride and dissolving it in 250 ml ultra-purified water. Different molarities of sodium bicarbonate solution (0.01 M, 0.05 M, 0.1 M, 0.5 M and 1M) were prepared by accurately weighing (84, 420, 840, 4200 and 8400 mg, respectively) of sodium bicarbonate salt and dissolving them in 100 ml ultra-purified water.

Preparation of reagent blank

Blank solution was prepared by measuring 2.5 ml of ninhydrin solution (0.2%), 0.5 ml of 0.01M sodium bicarbonate solution and 0.4 ml of manganese chloride solution (50 µg ml^{-1}) and transferring them into a test tube. The mixture was then heated in a water bath for 35 min, and then left to cool down for 10 min at room temperature. The solution was transferred into a 10 ml volumetric flask and ultra-purified water was added to final volume of 10 ml.

Optimization of reaction conditions

An investigation of the optimal reaction conditions of ninhydrin with alendronate was conducted. The investigated conditions included: heating temperature, heating time, ninhydrin volume, sodium bicarbonate concentration and volume, and amount of Mn^{2+}.

Construction of calibration curve

Two milliliter of alendronate stock solution (200 µg/ml^{-1}) was transferred to 10 ml test tube. 2.5 ml of 0.2% ninhydrin solution, 0.5 ml of 0.01 M sodium bicarbonate and 0.4 ml of 50 µg/ml^{-1} manganese chloride solutions were added. The mixture was mixed well and heated in water bath at 95 ± 5°C for 35 min, then cooled for 10 min at room temperature and transferred quantitatively into a 10 ml volumetric flask. The test tube was washed with few milliliters of water and the washings were added to the volumetric flask, then the volume was made up to 10 ml with ultra-purified water and shaken well. Aliquots of (0.5, 1, 1.5, 2, 2.5 and 3 ml) of the diluted solution were transferred into a series of 10 ml volumetric flasks. Volumes were completed to 10 ml with ultra-purified water and then shaken well to give final concentrations of 2, 4, 6, 8, 10 and 12 µg/ml^{-1}. The absorbance was measured at 568 nm against a reagent blank. Calibration curve of the concentration (µg/ml^{-1}) of alendronate sodium against absorbance was plotted. Regression equation, correlation coefficient, slope and intercept were calculated from the graph.

Analytical method validation

The proposed method was validated based on the International Conference on Harmonization (ICH) guidelines [15]. Linearity of the proposed method was determined using TQ analyst software by statistically calculating the correlation coefficient of the calibration curve. Sensitivity of the method was expressed in Limit of Detection (LOD) and Limit of Quantification (LOQ). LOD and LOQ were calculated from the formulas set by ICH:

$$\mathrm{LOD} = 3.3\ \frac{\mathrm{SD}}{\mathrm{S}}, \mathrm{LOQ} = 10\ \frac{\mathrm{SD}}{\mathrm{S}}$$

Where, SD is the standard deviation of y-intercept of the regression line, S is the slope of the calibration curve, 3.3 is signal-to-noise ratio for LOD and 10 is signal-to-noise ratio for LOQ.

Intra-day and inter-day precision studies were done by repeating analysis of the quality control standards (2, 6 and 12 µg/ml^{-1}) five times on the same day for intraday precision, and once for successive 5 days for interday precision. Results were presented as relative standard deviation (%RSD).

Accuracy of the proposed method was measured by performing recovery studies. 1 ml aliquots of the sample stock solution were added to three 10 ml test tubes. Aliquots (2.8, 3.5 and 4.2 ml) of alendronate sodium stock solution (200 µg/ml^{-1}) were added to each tube.

To each test tube, 2.5 ml of 0.2% ninhydrin, 0.5 ml of 0.01 M sodium bicarbonate and 0.4 ml of 50 µg/ml^{-1} of manganese chloride were added and shaken well. Tubes were heated at 95 ± 5°C for 35 min, and then cooled down for 10 min at room temperature. Each test tube was quantitatively transferred to 10 ml volumetric flask and each flask was diluted with ultra-purified water up to 10 ml and shaken well.

Absorbance was measured at 568 nm and the recovered amount of alendronate sodium was calculated using the regression equation, taking into account dilution factor. The results were presented as percentage recovery.

Stability of the coloured product

One and a half milliliter (1.5 ml) of alendronate stock solution (200 µg/ml^{-1}) and 1 ml of sample stock solution (700 µg/ml^{-1}) were transferred into two 10 ml test tube. 2.5 ml of 0.2% ninhydrin solution, 0.5 ml of 0.01 M sodium bicarbonate and 0.4 ml of 50 µg/ml^{-1} manganese chloride solutions were added to each tube. Both test tubes were shaken well and heated in water bath at 95 ± 5°C for 35 min, then cooled down for 10 min at room temperature and each tube was transferred into a 50 ml volumetric flask and ultra-purified water was added to get the final volume of 50 ml. After shaking, the solutions were divided into two 25 ml volumetric flasks. One volumetric flask was stored at room temperature (25°C) and the other was stored in the refrigerator (2-4°C). Absorbance was measured for each solution directly after preparation and after 2, 4, 6, 8, 10 and 12 h. And percentage difference of the absorbance was measured. All measurements were done in triplicates.

$$\% \text{ Difference} = \frac{\text{Measured absorbance at } \times \text{ Hour}}{\text{Initial absorbance value}} \times 100$$

Quantification of alendronate sodium in tablets

The validated proposed method was used for the quantification of alendronate content in tablets. 0.5 ml of sample stock solution (700 µg/ml^{-1}) was transferred to 10 ml test tubes. Then, 2.5 ml of 0.2% ninhydrin, 0.5 ml of 0.01 M sodium bicarbonate and 0.4 ml of manganese chloride solution (50 µg/ml^{-1}) were added. The mixture was shaken and heated in water bath at 95 ± 5°C for 35 min, then cooled down for 10 min at room temperature and transferred into a 10 ml volumetric flask. The tube was washed with few milliliters of water and the washings were added to the volumetric flask, then the volume was completed to 10 ml with ultra-purified water. Absorbance was then measured at 568 nm against reagent blank. The absorbance measurements were repeated six times. Concentration of alendronate was calculated from the regression equation, taking into account the dilution factor.

RESULTS

Method development and validation

The UV-vis absorption spectrum of the purple-colour complex produced by the reaction of ninhydrin with alendronate was recorded in the range from 200 to 800 nm against reagent blank. The maximum absorbance λ_{max} was found at 568 nm (Figure 2). The reaction between ninhydrin and alendronate is temperature dependent. The results show that the optimum colour intensity was achieved when the solutions were heated at 95°C for 35 min (Figure 3A and 3B). The effect of ninhydrin volume on colour intensity of 10 µg/ml^{-1} and 20 µg/ml^{-1} alendronate sodium concentrations is shown in Figure 4. Effect of sodium bicarbonate concentration on colour intensity and volume of 0.01 M sodium bicarbonate solution on colour intensity are shown in Figure 5A and 5B. The effect of the amount of manganese (II) chloride amount on colour intensity using the optimized experimental conditions is shown in Figure 6. The results of this study show that addition of 20 µg of Mn^{2+} (0.4 ml of 50 µg/ml^{-1} manganese chloride solution) gave the highest absorbance value.

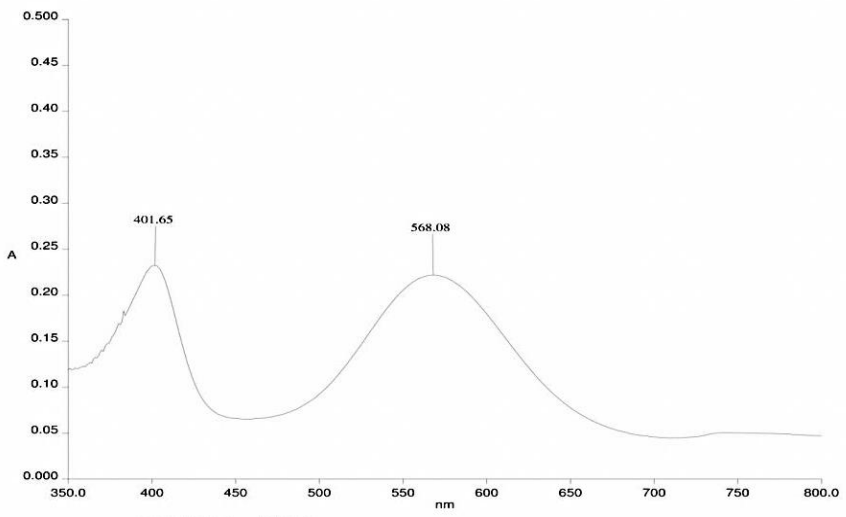

Figure 2: Absorption spectrum of purple-colour complex produced from the reaction of ninhydrin and alendronate (10 µg/ml^{-1})

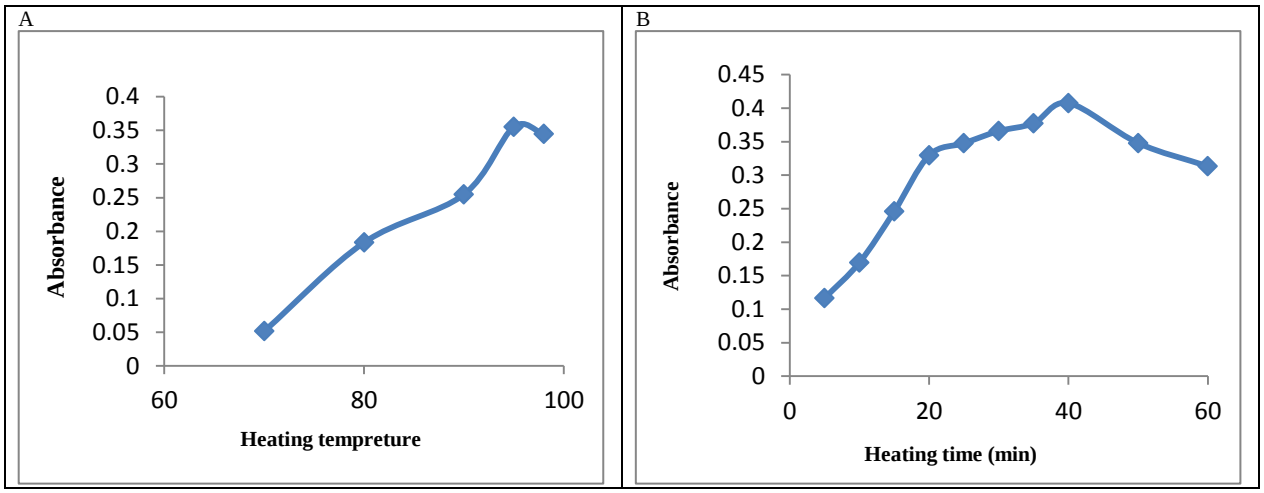

Figure 3: Effect of (A) heating temperature and (B) heating time on the colour intensity of ninhydrin and alendronate reaction

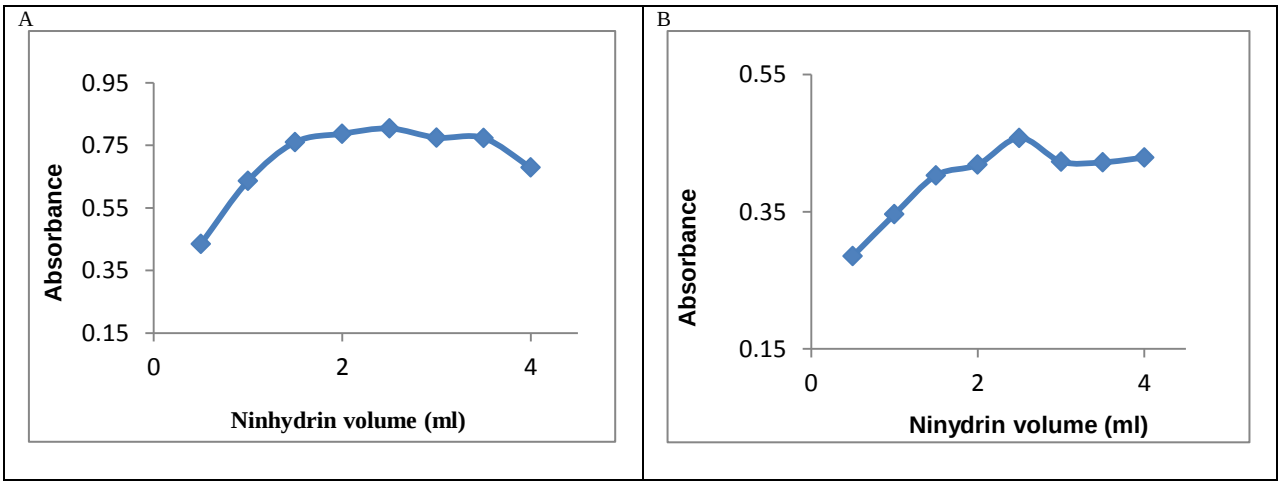

Figure 4: Effect of Ninhydrin volume on colour intensity (A) 10 µg ml alendronate sodium concentration and (B) 20 µg ml alendronate sodium concentration

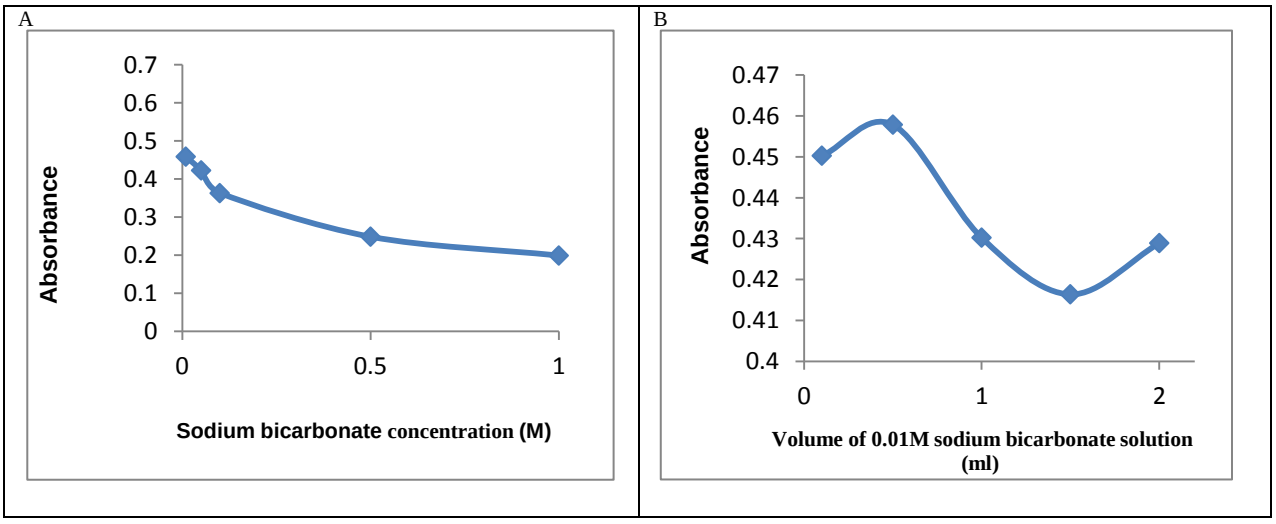

Figure 5: Effect of (A) sodium bicarbonate concentration on colour intensity and (B) the volume of 0.01 M sodium bicarbonate solution on colour intensity

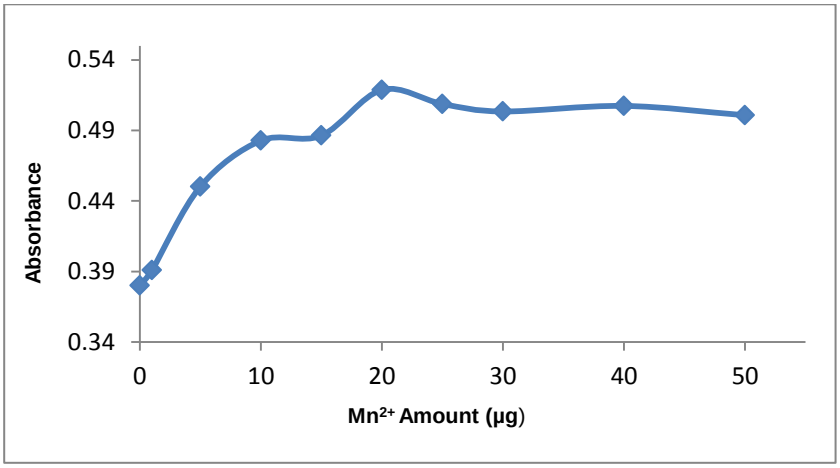

Figure 6: Effect of manganese chloride amount on colour intensity (using the optimized experimental conditions)

The method was validated based on ICH [15]. The calibration curve of alendronate sodium concentration against pruple-colour absorbance at 568 nm using the using the optimized experimental conditions showed a good linearity (r^2=0.9997) in the concentration range of 2-12 µg/ml^{-1} (Table 1). The LOD and LOQ values were 0.46 µg/ml and 1.39 µg/ml, respectively (Table 1). The calculated %RSD values for the interday and intraday precision are shown in Table 2. The mean recovery percentage values, close to 100% and low standard deviation values (SD<1.5) were obtained (Table 3).

Table 1: Parameters of calibration graph and sensitivity of the proposed method

Linear range		2–12 µg/ml^{-1}
regression equation		A=0.03977867–0.0555614*X
correlation coefficient (r^2)		0.9997
slope (S)		0.0398
Intercept (a)		0.0556
Sensitivity	Limit of Detection (LOD)	0.46 µg/ml
	Limit of Quantification (LOQ)	1.39 µg/ml

Table 2: Evaluation of precision of the proposed method

		2 µg/ml^{-1}	6 µg/ml^{-1}	12 µg/ml^{-1}
Intraday	1	2.03	6.05	11.94
	2	2.01	6.07	12.02
	3	2.02	6.01	12.03
	4	2.04	6.00	12.01
	5	1.99	6.01	11.96
	Mean	2.02	6.03	12.00
	SD*	0.02	0.02	0.04
	RSD (%)**	0.84	0.40	0.36
Interday	1	1.99	6.00	12.02
	2	1.96	6.04	11.94
	3	1.97	6.01	11.98
	4	2.00	6.02	11.91
	5	1.99	6.07	11.95
	Mean	1.99	6.03	11.96
	SD	0.02	0.03	0.04
	RSD (%)	0.87	0.43	0.35
*SD=The standard deviation, ***Relative standard deviation (%RSD)=(SD/Mean) × 100				

Table 3: Results of recovery study

Concentration of drug			Recovery %*	Mean recovery ± SD
Fixed (µg/ml^{-1})	spiked (µg/ml^{-1})	Recovered (µg/ml^{-1})		
70.00	56.00	125.70	99.58	99.18 ± 1.30
		126.17	100.25	
		124.41	97.73	
70.00	70.00	139.67	99.53	100.99 ± 1.27
		141.33	101.90	
		141.08	101.54	
70.00	84.00	153.03	98.61	99.00 ± 0.35 99.73 ± 1.33
		153.39	99.12	
		153.49	99.28	

$$*\text{Recovery } \% = \frac{\text{Recovered} - \text{Spiked}}{\text{Fixed}} \times 100$$

Quantification of alendronate sodium in tablets using the proposed method

Alendronate tablets containing 91.37 mg alendronate sodium which is the moral equivalent of 70 mg of the free alendronic acid were used for quantification. Absorbance values were measured for 6 alendronate tablets at 568 nm. And concentrations were calculated from the regression equation of the calibration curve, taking into consideration the dilution factor. Table 4 shows the results of quantification of the six alendronate tablets. The excipients used in the tablets showed no interference in the analysis. And the percentage of labeled amount was found to be very close to 100 with standard deviation less than 1.5 (SD<1.5).

Table 4: Results of quantification of Apo-Alendronate® tablets by the proposed UV-Vis spectrophotometric method

Sample	Absorbance	Alendronate labeled (mg)	Alendronate found (mg)	Percentage of labeled amount (%)*
1	0.4274	91.37	91.06	99.66
2	0.4253	91.37	90.57	99.12
3	0.4247	91.37	90.43	98.97
4	0.4291	91.37	91.46	100.10
5	0.4298	91.37	91.63	100.29
6	0.4377	91.37	93.48	102.31
				Mean ± SD=100.07 ± 1.2

$$^{*}\text{Percentage of labeled amount} = \frac{\text{Amount found}}{\text{Labeled amount}} \times 100$$

DISCUSSION

Ninhydrin (2,2-Dihydroxyindane-1,3-dione) reacts with primary and secondary amines to produce a purple color. The mechanism of this reaction consists of condensation step that leads to Schiff's base formation, then decarboxylation, hydrolysis and finally condensation with another molecule of ninhydrin to give the final coloured product. Similarly, the primary amine group in alendronate reacts with ninhydrin. The reaction takes place in weak alkaline medium (sodium bicarbonate or pyridine) and requires heating in a water bath in order to form the purple-colour complex that has maximum absorbance at 565-569 nm. Reaction of ninhydrin with alendronate in the presence of Mn^{2+} increased the intensity of the purple-colour complex compared to the same reaction conditions without the addition of manganese chloride. The results of this study showed that addition of Mn^{2+} ions increased the absorbance value, which is similar to the reported method [11,15,16].

The proposed method was validated based on ICH, 2005. The Calibration curve of alendronate sodium concentration showed a good linearity in the concentration range tested. The LOD and LOQ values showed that the proposed UV-Vis spectrophotometric method is sensitive to carry out quantitative analysis of alendronate sodium in tablets. %RSD values for the interday and intraday precision values were found to be small indicating good repeatability of the proposed method. The mean recovery percentage values indicated high accuracy of the proposed method. Stability of the coloured product was conducted and only 0.5% and 0.4% of the absorbance values were decreased when the solutions were stored at room temperature and in the refrigerator, respectively. The coloured product produced from the reaction of ninhydrin with alendronate tablets was found to be stable with 0.56% and 0.37% reduction in colour absorbance at room temperature and when refrigerated, respectively. These results show that the coloured product is stable for enough time that allows accurate analysis of alendronate.

Spectrophotometric methods for the determination of alendronate in bulk drug and tablets based on the reaction of the primary amino group of alendronate with ninhydrin to form a purple colour complex have been reported [11,16-18]. Addition of pyridine to similar reaction medium increases the colour intensity and the sensitivity of the reaction by formation of coordinate bond between pyridine and electron deficient carbon atom in the complex formed [16]. However, the reaction of alendronate with ninhydrin in the presence of pyridine imposed heating time 60 min compared to 20 min for similar reaction without pyridine. Pyridine is classified as moderate hazardous material by Hazardous Materials Identification System (HMIS). In the present method, manganese ions were added to the reaction medium instead of pyridine and the colour intensity increased with the heating time of 35 min. The result obtained for the quantification values of alendronate tablets was comparable to the reported method which utilized pyridine in the reaction medium [16]. Ninhydrin is cheaper derivatizing agent compared with other derivatizing reagents used in determination of alendronate, such as 7,7,7,8-tetracyanoquinodimethane, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone and 2,4-dinitrofluorobenzene.

CONCLUSION

Alendronate sodium is a widely used drug for the treatment of osteoporosis and Paget's disease. Its analysis is challenging due to the lack of chromophore in its chemical structure. Most of the reported methods in literature for the analysis of alendronate in tablets involved the use of expensive reagents, tedious sample and reagents preparations and/or sophisticated instrumentations. The present spectrophotometric method for the determination of alendronate in tablets was based on the reaction of ninhydrin with alendronate in the presence of Mn^{2+} ions to the reaction medium which increased the colour intensity and the sensitivity of the method in a reaction time of 35 min. Method validation results showed that the method was sensitive, precise and accurate with no interference from tablet excipients.

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REFERENCES

[1] M.T. Drake, B.L. Clarke, S. Khosla, *Mayo Clinic Proceedings*., **2008**, 83, 1032-1045.
[2] S.K. Al Deeb, I. Hamdan, S.M. Al Najjar, *Talanta*., **2004**, 64, 695-702.
[3] H.S. Kang, S.J. Hwang, J.S. Park, C.K. Kim, *J. Liq. Chromatogr. Relat. Technol*., **2006**, 29, 1589-600.
[4] L. Yongming, *Chinese Pharm. Affairs*., **2009**, 10, 974-977.
[5] T. Perez-Ruiz, C. Martinez-Lozano, M.D. Garcia-Martinez, *J. Chromatogr. A*., **2009**, 1216, 1312-1318.
[6] Z.Z. Yuan, L.N. You, H. Zha, *Chin. J. Spectrosc. Lab*., **2013**, 2, 757-762.
[7] C. Fernandes, R.S. Leite, F.M. Lancas, *J. Chromatogr. Sci*., **2007**, 45, 236-241.
[8] S.W. Su, Y.C. Liao, C.W. Whang, *J. Sep. Sci*., **2012**, 35, 681-687.
[9] J. Zirojevic, Z. Jovic, A. Djurdjevic, A. Ciric, P. Djurdjevic, *Acta Chromatogr*., **2015**, 27, 215-237.
[10] O.A. Razak, S.F. Belal, M.M. Bedair, R.S. Haggag, *Talanta*., **2003**, 59, 1061-1069.
[11] E.A. Taha, N.F. Youssef, *Chem. Pharm. Bull*., **2003**, 51, 1444-1447.
[12] A.S.Y. Wong, E.N.M. Ho, T.S.M. Wan, K.K.H. Lam, B.D. Stewart, *J. Chromatogr. B*., **2015**, 998, 1-7.
[13] B. Xie, A. Liu, X. Fang, Y. Chen, H. Zhong, *J. Pharm. Biomed. Anal*., **2014**, 93, 73-76.
[14] J.V. Singh, S.K. Khanna, G.B. Singh, *Anal. Biochem*., **1978**, 85, 581-585.
[15] International Conference on Harmonization (ICH), Guideline Q2 (R1), Validation of analytical procedures: Text and Methodology, Geneva, **2005**.
[16] N.A. Alarfaj, S.A.A. Razeq, F.N. AL-Qahtani, *Asian J. Chem*., **2011**, 23, 697-700.
[17] S. Sultana, S. Talegaonkar, G. Mittal, A. Bhatnagar, F. Ahmad, *Chromatographia*., **2010**, 72, 321-326.
[18] A. Raza, M. Zia-ul-Haq, *Int. J. Anal. Chem*., **2011**, 1-6.