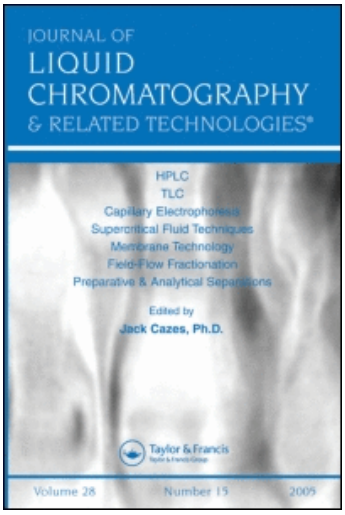

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Quantification of Sodium Alendronate by LC Anion Exchange Using In Line Complexation

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Abstract: A fast and sensitive high performance chromatographic method for quantification of sodium alendronate was developed and afterward implemented for tablet content and dissolution test. Sodium alendronate reference standard and samples of two different commercial tablets were used. The analysis was performed using an anion exchange column at 30°C and a mixture of nitric acid 1.5 mM and copper II nitrate 0.5 mM (50:50, v/v) as the mobile phase. The analysis run time was 15 minutes and the detection was measured at 240 nm. The limit of detection was approximately 0.3 $\mu g\,mL^{-1}$ and the limit of quantification was 0.9 $\mu g\,mL^{-1}$. The within day and between day precision were 0.64 and 1.52%, respectively. The accuracy results showed a recovery range from 99.87 to 101.07%. The validated method was applied to the tablets content and tablet dissolution assays successfully.

Keywords: Anion exchange, Dissolution test, LC, Sodium alendronate, Tablets

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INTRODUCTION

Sodium alendronate [4-amino-1-hydroxybutane bisphosphonic acid] is an amino bisphosphonate compound (Figure 1) used for the treatment of several bone diseases such as osteoporosis, hypercalcemia, and Paget's disease.[1,2]

The LC-analysis of sodium alendronate by UV detection is rather difficult because of the small absorption within the wavelength range of 220–700 nm normally used in analytical tests. Besides that, it is a zwitterionic drug with four pKa values, 2.72, 8.73, 10.5, and 11.6, which leads to a complex basic or acidic behavior of the substance.[3] It can therefore be analyzed at either end of the pH range, but the resolution of its mixtures of neutral and charged forms remains troublesome. Several methods intended for the bisphosphonates assay have been reported in the literature, most of them using reverse phase (RPC) and ion exchange (IEC) high performance liquid chromatography.[3] One study that used RPC for the analysis of sodium alendronate comprised the precolumn derivatization with 9-fluorenylmethyl chloroformate and UV detection.[4] The derivatization with 2,3-naphtalene dicarboxyaldehyde along with fluorescence detection,[4] as well as IEC with *o*-phthalaldehyde and mercaptoethanol derivatization, was also proposed.[5] In this context, the IEC is probably the main chromatographic method for analysis of bisphosphonate at present. The detection of sodium alendronate includes refractive index,[6] conductivity,[7,8] indirect UV[9] detection with nitric acid as the mobile phase, in-line complexation with copper II nitrate and UV detection.[10] Other non-common methods as capillary electrophoresis, inductively coupled plasma mass spectrometry,[11] and isotachophoresis[12] have also been reported for bisphosphonates analysis.

The United States Pharmacopoeia method[13] involves a precolumn derivatization with 9-fluorenylmethyl chloroformate. In the routine analysis, however, it become time consuming and quite intricate because of

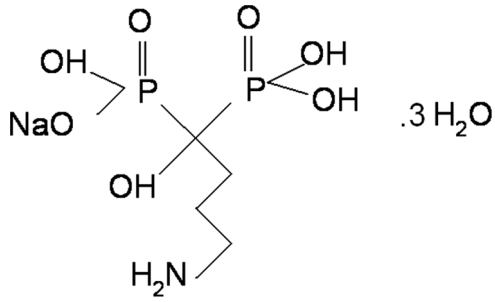

Figure 1. Chemical structure of sodium alendronate trihydrate.

the relatively high number of steps (samples derivatization, centrifugation, and needing of various reagents) to be executed. A more simple method by UV detection proposed earlier[10] seems to be more practicable for the routine analysis purpose.

The present work aims the development and validation of an ion exchange method for the quantification of sodium alendronate using in-line complexation with copper II nitrate, as well as its application on the content assay and dissolution test of alendronate containing tablets.

EXPERIMENTAL

Materials

Sodium alendronate trihydrate reference standard was purchased from United States Pharmacopoeia (Rockville, EUA, lot GOD288) and sodium alendronate trihydrate pharmaceutical grade was purchased from Farmoquímica (São Paulo, Brasil). Copper(II) nitrate trihydrate, nitric acid, sodium hydroxide, chloridric acid, and hydrogen peroxide 10% solution were purchased from Merck (Darmstadt, Germany). All reagents used were analytical grade. Ultrapure water (Millipore Milli-Q system) was used in all analyses.

Alendronate Tablets

Product A – labeled to contain 10 mg of the drug and the following excipients: microcrystalline cellulose, lactose, croscarmellose sodium, and magnesium stearate.

Product B – labeled to contain 10 mg of the drug and the following excipients: microcrystaline cellulose, spray dried lactose, corn starch, magnesium stearate, and colloidal silicon dioxide.

Preparation of Standard Solutions

The stock solutions of sodium alendronate trihydrate and reference standard were prepared by dissolving both compounds separately in 100.0 mL of water at a final concentration of 180 μg mL^{-1}.

Chromatographic System

The LC system consisted of two pumps, dual UV detector operating at 240 nm, autosampler, vacuum degasser, column oven, and data processor

TotalChrom software, all from Perkin Elmer Series 200 LC. The analysis was performed on a Hamilton PRP $\times 100$ (5 μm, 100×4.1 mm i.d.) anion exchange column operating at 30°C. The injection volume was 100 μL and the run time 15 min. The mobile phase consisted of a mixture of nitric acid 1.5 mM and copper II nitrate 0,5 mM (50:50, v/v). The eluent was filtered, degassed, and pumped at a flow rate of 1.0 mL min^{-1}. The detection wavelength was set at 240 nm.

Validation Method

The method was validated according to the International Conference on Harmonization and AOAC International guidelines for validation of analytical methods.[14,15] The specificity was evaluated after analyses of the sodium alendronate solution and also of solutions containing the tablet excipients dissolved in water without the drug.

The linearity of method was evaluated from least square regression analysis of the peak area obtained by the calibration curve. The calibration curve was prepared by diluting 18.0 mg of the reference standard in a 100.0 mL volumetric flask with water. This solution was sonicated for 15 minutes and aliquots were transferred to 50.0 mL volumetric flasks to final concentrations of 6.0, 15.0, 30.0, 60.0, 90.0, 120.0, 150.0, and 180.0 μg mL^{-1}. Each solution was prepared in triplicate, filtered through a 0.45 mm membrane filter (Millipore, HVHP), and injected three times in the chromatographic system. The same procedure was applied to the sodium alendronate working solution. Systematic errors were evaluated on the confidence limits of slope and intercept. The residues colinearity was assessed by Durbin-Watson and standardized residues tests.

The limit of detection and limit of quantification were determined on the basis of the regression standard deviation of the calculated slope. The repeatability (intra-day) and intermediate precision (inter-day) were determined at a concentration range of 50 to 150% of sodium alendronate tablet content. Five analysis of each working solution were realized and the results were expressed as the relative standard deviation (RSD) percent. Accuracy was determined by the recovery of the reference standard from three tablet working solutions at the concentration of 50.0; 75.0; 100.0; 125.0, and 150.0 μg mL^{-1} and appropriately spiked with sodium alendronate. Each solution was injected five times and the results expressed as the mean percentage recovery.

Content Quantification of Formulations A and B

Ten tablets of each formulation were crushed and samples containing the equivalent of 10.0 mg of sodium alendronate were exactly weighted,

dissolved in 100.0 mL of water, sonicated for 15 min, and filtered through a 0.45 mm membrane filter. Each result expresses the mean area peak of at least three replicates calculated as sodium alendronate.

Dissolution Assay

The tablet dissolution assay was carried out on a Pharma-Test model PTWS III (Hainburg, Germany). Six tablets were placed separately into each vessel containing 900 mL of dissolution medium (water). The paddle rotation was set up at 50 rpm and the temperature was kept at $37 \pm 0.5°C$ throughout the experiment. Samples of 10 mL were withdrawn from each vessel at 3, 7, 10, 15, 20, and 30 min, filtered through a 0.45 mm membrane filter. The sodium alendronate content was determined by the LC method previously validated. Each result expresses the mean value of three replicates.

RESULTS AND DISCUSSION

The specificity test realized with a solution containing the tablet excipients of formulations A and B and a mixture of each one with the working solution of sodim alendronate (120 μg mL^{-1}) showed that the components do not interfere neither with the elution of the reference standard nor with sodium alendronate. A representative chromatogram of both solutions is shown in Figure 2.

The linearity parameter is satisfactory at the concentration range of 6.0–180.0 μg mL^{-1} (Table 1). The residues analysis showed no colinearity tendency by the Durbin-Watson test ($d = 2.19$; $dw_U = 1.20$, and $dw_L = 1.41$) and no standardized residue exceeded the limit value of 2.0.[16] The limit of detection was approximately 0.3 μg mL^{-1} and the limit of quantification was 0.9 μg mL^{-1}.

The intra-day and inter-day precision values of the LC method are presented in Table 2. The RSD values were less than 0.60 and 1.52 for intra-day and inter-day tests, respectively (Table 2).

The data showed that the developed method presented a good precision, since the results obtained for the sodium alendronate tablets were smaller than 1.6%.

The results from the accuracy showed a mean recovery of 99.87% to 101.07% for a spiked solution in the concentration range of 50–150 μg mL^{-1} of sodium alendronate. The maximum coefficient of variation in these analyses were 0.80% (Table 3).

In general, the proposed method complies with the current validation requirements.[14,15] For the development of the methodology, the nitric acid and cooper nitrate ratio in the mobile phase was modified. As a

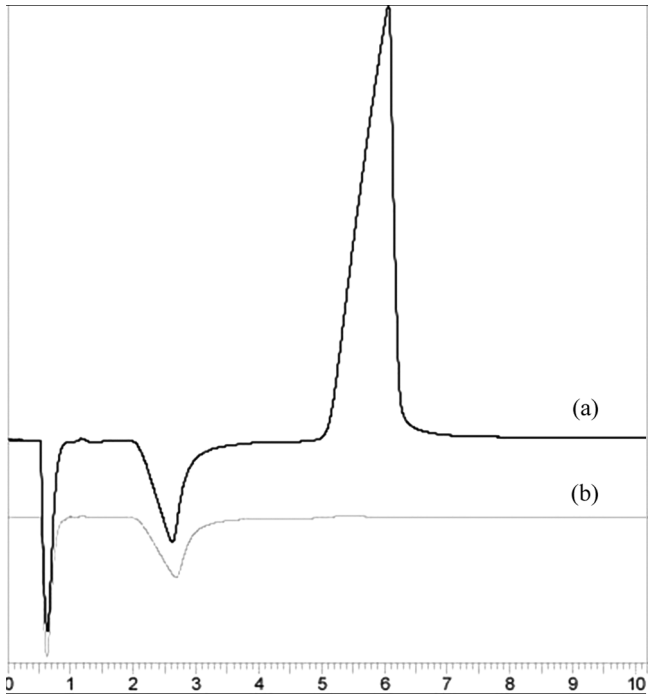

Figure 2. Representative LC-chromatogram obtained from the specificity test showing the mixture of sodium alendronate and excipients (a) and the tablet excipients alone (b).

result, the sodium alendronate peak area was expanded and the linearity was improved, avoiding deviation from linearity and poor accuracy due to smaller peak areas near the noise drift of the baseline. These results are similar to those observed for olpadronate and pamidronate.[10]

The retention time could be influenced by varying the nitric acid concentration or the copper II nitrate concentration. The increase in the concentration of nitric acid in the mobile phase showed an increase in the retention time and in the sodium alendronate peak area, which is

Table 1. Sensibility and linearity results of the LC-method validation

Regression Equation	$y = 41714x - 53696$
R^2 value	0.9994
$F_{calc.}$	106781.7^{**} $F_{(0.05;\ 6,20)} = 2.60$
Durbin-Watson test	2.19
Limit of detection ($\mu g\ mL^{-1}$)	0.3
Limit of quantification ($\mu g\ mL^{-1}$)	0.9

Table 2. Intra and inter-day precision results of the LC-method validation

Known concentration ($\mu g\,mL^{-1}$)	Concentration found (%)	RSD (%)
Intra-day precision ($n = 25$)		
50.0	98.46	0.53
75.0	99.24	0.58
100.0	99.88	0.15
125.0	102.24	0.17
150.0	101.30	0.25
Inter-day precision ($n = 75$)		
50.0	99.51	0.91
75.0	99.46	1.52
100.0	99.49	1.07
125.0	100.82	1.24
150.0	100.83	0.46

probably as a consequence of a higher ionic strength. Varying the copper II nitrate concentration should change eluting strength and formation of the copper complex in the aqueous eluent. The increase in the concentration of copper II nitrate led to higher peak areas and peak height, and a considerable decrease of the sodium alendronate retention time, because the formation of the copper II biphosphonate complex needs an excess of copper to assure the complete complexation. A feasible explication can be associated to the unsaturation condition of the stationary phase and a reduction of the column ion change capacity.[10]

Method Application

The results from the application of the developed method to the dissolution test assay of two different tablet formulations are shown in Figure 3.

Table 3. Accuracy results for the assay method

Spiked concentration ($\mu g\ mL^{-1}$)	Average recovery (%)	RSD (%)
Sodium alendronate		
50	100.14	0.31
75	101.07	0.11
100	100.30	0.20
125	99.87	0.07
150	100.37	0.80

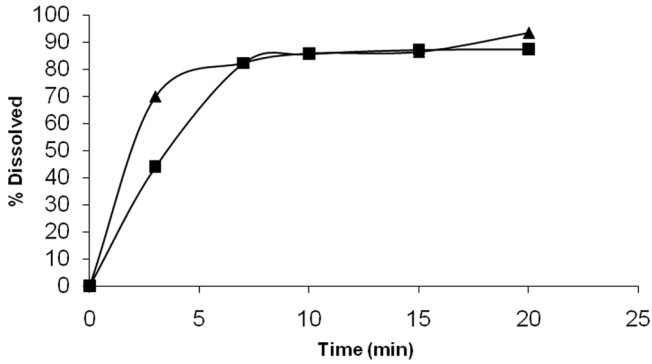

Figure 3. Results obtained for dissolution tests of tablet formulations A (■) and B (▲).

The content analysis of formulations A and B yield 10.89 mg and 10.55 mg of sodium alendronate, corresponding to 8.31 mg and 8.05 mg of alendronic acid, respectively. These values represent 108.9% and 105.5% of the declared content of sodium alendronate, respectively. Note, however, that tablet formulation A released sodium alendronate faster than formulation B, but both formulations became equivalent after about 7 min. This fact showed that the two formulations are immediate release products and do not retain the drug.

CONCLUSION

The validation results support the application of a sensitive, rapid, and specific anion exchange LC method for the assay of sodium alendronate. The validated method was able to determine accurately and specifically sodium alendronate in tablets, using a simply methodology that can be implemented in routine assays. For this purpose, the use of UV detection and in-line complexation appears to be a reliable and easier alternative.

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