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Spectroscopic and HPLC methods for the determination of alendronate in tablets and urine

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Abstract

Two methods (spectrophotometric and HPLC) have been developed and validated for the analysis of alendronate sodium in tablet dosage form. Both methods depend on the ability of alendronate sodium to react with *o*-phthalaldehyde (OPA) at basic pH to produce a light-absorbing derivative. The derivative was found to possess absorption maximum at 330 nm where neither the derivatizing agent nor the analyte had any absorption. Thus, spectroscopic method was based on the derivatization-induced absorption of alendronate sodium at 333 nm. The HPLC method was based on separation of the formed derivative from other ingredients in tablets with detection at 333 nm. Both methods were satisfactory with regard to accuracy, prescion and linearity. Moreover, a HPLC method with fluorescence detection (HPLC-FD) was developed for the quantification of alendronate sodium in urine. The method was also based on the derivatization of alendronate with OPA, but fluorescence detection was employed. Linearity, recovery, selectivity, prescision and sensitivity were satisfactory for the proposed HPLC-FD method. Yet a new quantification limit (0.6 ng ml^{-1}) for alendronate in urine was achieved.

Keywords: Alendronate sodium; *o*-Phthalaldehyde; Bisphosphonates

1. Introduction

Alendronate sodium is an amino bisphosphonate compound which is used for the treatment of a variety of bone diseases including osteoporesis, Paget's disease and metastatic bone disease [1,2]. From the analytical point of view, it represents a challenging example because it contains no absorbing chromophore. However, several HPLC methods have been reported for its determination many of which relied on derivatization of alendronate using either precolumn [3–6] or post-column techniques [7,8]. Direct HPLC analysis of alendronate using refractive index detector [9], ion chromatography with conductivity detection [10] or indirect UV detection [11] have also been reported. Ion chromatography-MS techniques have employed in the characterization of alendronate sodium [12]. Alendronate sodium was determined in pharmaceutical dosage forms by HPLC after derivatization with 9-fluorenyl methylchloroformate (FMOC). Excess of reagent had to be extracted with methylene chloride and an aliquot of the aqueous portion is assayed on RP HPLC [3]. Methods like inductivity coupled plasma and anodic stripping voltammetry have also been reported for the analysis of alendronate sodium in tablets [13,14].

There has been only one spectrophotometric method for the analysis of alendronate in tablet form. The method was based on formation of complex between the drug and iron in perchloric acid [14]. Due to medium stability of the reported complex an excess of the drug is required if a reliable quantitative data was to be obtained. Therefore, it is desirable to have a robust and simple spectroscopic method for routine quality control of alendronate tablets.

Due to the extremely low plasma concentrations of alendronate [16], many pharmacokinetic studies relied on the determination alendronate concentrations in urine rather than plasma [5,6]. Determination of alendronate in urine by automated pre-column (RP-HPLC) derivatization with 2,3-naphthalene dicarboxyaldehyde with electrochemical

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or fluorescent detection was described [5]. The limit of quantification (LOQ) in urine was 2.5 and 1 ng ml^{-1} using electrochemical or fluorescence detector respectively. However, in that method [5] the total run time was significantly long (30 min). When FMOC was used to derivatize alendronate, a LOQ of 3.5 ng ml^{-1} was achieved with fluorescence detection [6]. In that method a gradient system was required and total run time for one sample was 21 min.

Therefore, it is still desired to develop a method which is reliable, robust and simple yet with a better sensitivity than previously reported. In this paper, we describe the development, optimization and validation of spectrophotometric and HPLC with diode array detection (HPL-DAD) procedure for the analyzes of alendronate in pharmaceutical tablets and a HPLC method with fluorescence detection (HPLC-FD) for its analysis in urine. Both methods are based on the ability of alendrnate to form an absorping and fluorescent derivative with *o*-phthalaldehyde (OPA).

2. Experimental

2.1. Reagents and materials

Alendronate monosodium trihydrate was kindly provided by Jordan–Swedish Company (JOSWE). OPA Was obtained from Research Organic (Cleveland), and stored AT 4 °C. 2-Mercaptoethanol (2ME) and methanol (HPLC grade) were obtained from BDH (Poole, UK). Ethylenediaminetetraacetic acid disodium salt dihydrate (Na_2EDTA) and tetrabutylammoniumperchlorate (TBAP) were obtained from Fluka (Ronkonkoma, NY, USA). Alendronate containing tablets (Fosmax® 10 mg) were purchased from the local market. All other material were obtained from Merck (Darmstadt, Germany).

2.2. Equipment

All UV spectrophotometric measurements were made using Cary-Varian UV Spectrophotometer. Emission scans were made using Spectrofluorometer SFM 25 (Kontron instrument). HPLC analysis were made with Shimadzu Class-VP HPLC system consisting of a SIL-10ADVP autosampler, a LC-10ADVP pump, a DGE-14A degasser, a SCL-10AVP controller, RF-10AXL fluorescence detector, and SPD-M10AVP diode array detector. Mettler Toledo MP200 pH meter (USA) was used for pH measurements.

2.3. Methods

2.3.1. Preparation of standard solutions of alendronate

Stock solutions of alendronate sodium were prepared by dissolving the equivalent of 100 mg of alendronate monosodium trihydrate in 100 ml of 0.05 M NaOH. Then different sets of working standards at different concentrations were prepared by appropriate dilution of the stock solution.

2.3.2. Preparation of the derivatizing reagent

A working solution of the derivatizing reagent was prepared by dissolving 50 mg of anhydrous OPA with 5 ml of 0.05 M NaOH, then 250 μl of 2ME solution was added and the volume completed to 50 ml with 0.05 M NaOH. The solution was freshly prepared for each experiment on daily basis and no signs of reagent deterioration were observed within that period of time.

2.3.3. Spectrophotometric method

2.3.3.1. Preparation of sample for spectrophotometric analysis of tablet. A portion of finely powdered tablets equivalent to 10 mg of alendronate was accurately weighed and dissolved volumetrically with 50 ml of 0.05 M NaOH. After sonication for 10 min, the sample was filtered using Whatman filter paper, and 10 ml of the filtrate were transferred to another 50 ml volumetric flask. Before completing the volume with 0.05 M NaOH, 4 ml of freshly prepared derivatizing reagent (OPA/2ME) solution (1 mg ml^{-1}) were added, and the absorbance of the resulting solution was measured after 60 min at 333 nm.

2.3.3.2. Optimization of the pH of the reaction. In order to find the optimum pH of the reaction, standard solutions of alendronate sodium (40 μg ml^{-1}) were prepared in borate buffer (pH = 10, 10.7, and 11) or NaOH (0.01, 0.03, 0.05, 0.1, 0.2, 0.5, and 1.0 M). The resulting solutions were scanned in range of 200–600 nm.

2.3.3.3. Stability study. Solutions of alendronate sodium (50 μg ml^{-1}) in 0.05 M NaOH were allowed to react with OPA in the presence of either 2ME or sodium sulfite. In each case, solutions were scanned and λ_{max} determined. The absorbance of each solution was recorded at the proper wavelengths over a period of 2 h.

2.3.3.4. Calibration curves. Aliquots (0.7, 1, 1.5, 2, 2.5, and 3 ml) of the standard alendronate solution (1 mg ml^{-1}) prepared as described above were transferred to a series of 50 ml volumetric flasks. Four milliliters of the derivatizing reagent (1 mg ml^{-1}) were added to each flask and the volume completed to 50 ml with 0.05 M NaOH. The absorbance values were measured after 60 min at λ_{max} (333 nm) and plotted against concentrations to construct calibration curves.

2.3.4. HPLC method for solid dosage form

2.3.4.1. Chromatographic conditions. Chromatographic separations and subsequent quantifications were carried out at room temperature using Hamilton reversed phase HPLC column (5 μm, 150 mm × 4.1 mm) which is based on rigid spherical styrene-divinylbenzene copolymer. Chro-

matograms were monitored by diode array detection (200–400 nm) and displayed at a single wavelength of 333 nm. An isocratic elution system was employed with a mobile phase (1 ml min^{-1}) consisting of a mixture of acetonitrile/phosphate buffer pH 9.6 (15:85) containing 3 mg% of tetrabutylammoniumperchlorate.

2.3.4.2. Preparation of calibration curves for HPLC analysis of alendronate. A solution of 1 μg ml^{-1} alendronate was prepared from stock solution (1 mg ml^{-1}). Aliquots (10, 20, 30, 40, 50, and 60 μl) of this solution were transferred to a small HPLC vial using micropipette. Sixty microliter of the OPA/2ME reagent were added to each vial and the volumes completed to 1 ml using 0.05 M NaOH. After 60 min; 50 μl of each solution was injected to the HPLC system using the autosampler. Chromatograms were monitored at λ_{max} (333 nm). The obtained peak areas were plotted against concentrations to construct a calibration curve.

2.3.4.3. Preparation of tablet for HPLC analysis. Sample preparation was carried out as previously mentioned for spectrophotometric analysis until the filtration step. After filtration, 200 μl of the filtrate were transferred to a small HPLC autosampler vial, 60 μl of OPA reagent was added and volume was completed to 1 ml with 0.05 M NaOH. 50 μl of the resulting solution were injected after 60 min into the HPLC system.

2.3.4.4. HPLC method for determination of alendronate in urine. The same chromatographic conditions which were employed in the previous section were applied here except that chromatograms were monitored by a fluorescence detector set at an excitation wavelength of 333 nm and emission of 455 nm.

2.3.4.5. Biological urine sample preparation. The extraction procedure was a modification of a previously reported method [6] so that a solid phase extraction step became not necessary. Five milliliters of urine were transferred to a centrifuge glass tube and spiked with the desired volume of aqueous solution of alendronate sodium (if required). Then 100 μl of 0.1 M $CaCl_2$, and 200 μl of 1 M NaOH were added and the sample was centrifuged for 3 min at 2000 × *g* and the supernatant was discarded. The white precipitate was completely dissolved in 0.5 ml of 0.2M acetic acid and diluted with 5 ml of water. The resulting solution was then re-precipitated by addition of 200 μl of 1 M sodium hydroxide and centrifuged for further 3 min at 2000 × *g* and the supernatant was discarded again. Finally, the resulting precipitate was dissolved in 0.8 ml of 0.2M acetic acid, and 0.4 ml of 0.1M ethylenediaminetetra acetic acid (EDTA) disodium salt. The 0.3 ml of sodium acetate were added. Two hundred microliter of the resulting solution was transferred to an HPLC vial, 60 μl of OPA/2ME reagent were added and the volume was completed to 1 ml with 0.05 M NaOH. From the resulting mixture 50 μl were injected after 60 min into the HPLC.

3. Results and discussion

3.1. Spectrophotometric method

Derivatization of alendronate with OPA plus 2ME, was employed for the detection and quantification of alendronate sodium. The reagent OPA/2ME was found to react readily with alendronate at ambient temperature (few seconds) in 0.05 M sodium hydroxide solution. According to the known reaction between OPA and amino compounds the primary amine group of alendronate is believed to react with the two aldehyde groups of OPA to yield isoindole derivative [17]. OPA is not fluorescent by itself as the other commonly used derivatizing reagents which eliminates the need for post-derivatization removal of excess reagent.

Absorption spectra in the range (200–600 nm) were obtained for the derivatized alendronate (Fig. 1a). The

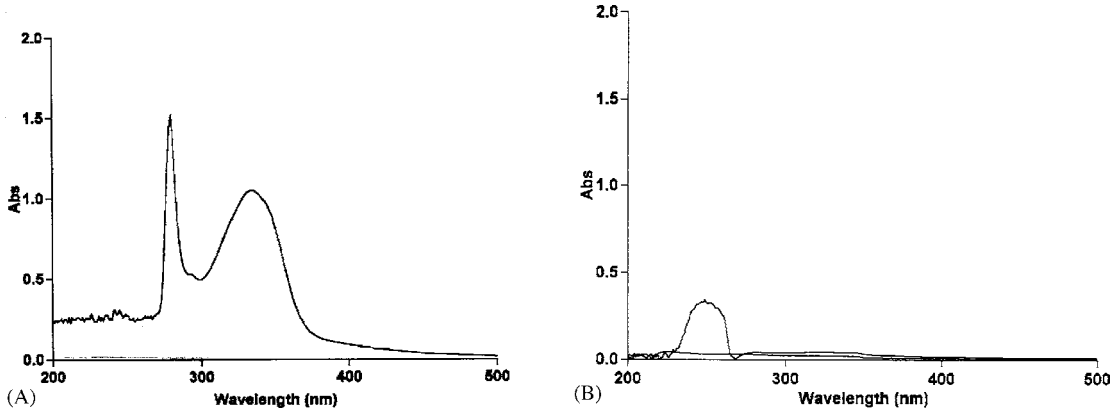

Fig. 1. (A) Absorption spectra of OPA/2ME-derivatized alendronate sodium in 0.05 M sodium hydroxide solution at alendronate concentrations of 200 μg ml^{-1}. (B) Absorption spectra of OPA/2ME reagent at concentration of 1 mg ml^{-1} (λ_{max} at 250 nm) compared to that of alendronate alone (no absorption).

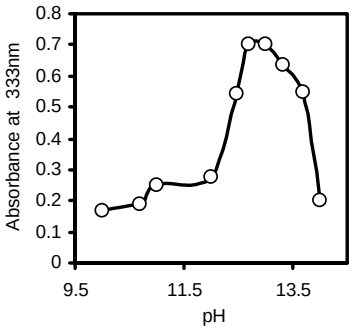

Fig. 2. Effect of pH on the derivatization of alendronate with OPA/2ME reagent.

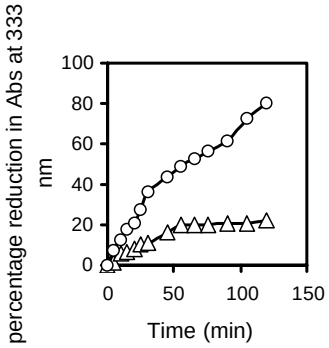

Fig. 3. Two hours stability study of OPA/2ME (△) and OPA/Na-sulfite (○) derivatized alendronate sodium plotted as percentage decrease in absorbance against time.

derivatized drug exhibited two absorption maxima (at 279 and 333 nm). Absorption spectra of alendronate alone and the reagent (OPA/2ME) were also obtained. Alendronate showed no absorbance at all for the entire range of wavelength. However, a broad absorption band was obtained for the derivatizing reagent at about 250 nm (Fig. 1b). As the peak of the reagent could interfere with the peak at 279 nm for the derivatized alendronate, the latter was not used for obtaining calibration curves and further measurements were made at 333 nm.

The molar ratio between alendronate and the derivatizing reagent (OPA/2ME) required to obtain maximum absorbance (sensitivity) and precision was also investigated at the following ratios: 1:0.5, 1:1, 1:2, 1:5, 1:6, 1:10. An equimolar ratio (1:1) was found to be enough to give maximum yield (as indicated by absorbance), but a ratio of 1:2 alendronate to reagent produced more reproducible results (relative standard deviation, R.S.D. = 0.5 compared to 2.2). Increasing the ratio of the derivatizing reagent to alendronate more than 2:1 showed no significant effect on the reaction yield.

It has been previously reported that derivatization reactions with OPA are pH-dependant [17] with optimum pH around 9 for derivatization of amino acids. A systematic study was performed to optimize the pH of the reaction. Initial attempts showed that no significant derivatization occurred below pH 10. A solution of alendronate (40 μg ml^{-1}) was derivatized under different pH values in the range 10–14. A plot of absorbance of each solution against pH is shown in Fig. 2. Maximum absorption was observed at pH value of 12.7 and 13. Therefore, it was decided to carry out further experiments at pH 12.7 (0.05 M NaOH) in order to obtain the highest sensitivity.

The observed need for a high basic media is probably related to stabilization of the free amine group of alendronate in non ionized form which is necessary for the reaction with the two aldehyde groups of *o*-phthalaldehyde to proceed rapidly at room temperature. The high pH value found essential for the optimum derivatization of alendronate sodium could be advantageous for the proposed method as it adds further selectivity to the analytical procedure. That is because the optimum pH for the derivatization of many endogenous amino compounds (e.g. amino acids) lies in the range between 9 and 9.5 [17]. Thus, at the high pH value used in the proposed method these contribute minimum to the overall derivatization reaction which enhances the selectivity of the method.

The instability of OPA derivatives has been reported and is a major drawback for OPA as a derivatizing reagent [17]. Stability of the obtained isoindole derivative was also shown to be dependent on the type of the nucleophile used. For the derivatization of amino acids, sodium sulfite was shown to be superior to 2ME [17]. In order to determine the optimum time of measurement, the absorbance of a solution of alendronate (50 μg ml^{-1}) derivatized with OPA/2ME and OPA/sodium sulfite was monitored over a period of 2 h. A plot of percentage reduction in absorbance against time is presented in Fig. 3. Although sodium sulfite has been reported to produce more stable derivative of OPA with amino acids [17], Fig. 3 showed that 2ME is better in this regard. More over the absorbitivity of the resulting derivative was higher in the case of 2ME compared to sodium sulfite. In the case of OPA with 2ME the maximum reduction of absorbance at 333 nm was almost constant at about 20% after 60 min. This observation could be explained as a result of the completion of the degradation reaction. Therefore, it was decided to choose 2ME as the nucleophile with measurements being taken after 60 min. Since the absorbance remained almost constant after 60 min, measurements after that time was expected to produce more robust method.

3.1.1. Linearity, precision, specificity, recovery, and LOQ

A linear relationship between the absorbance (at 333 nm) and the concentration of alendronate was established over the examined concentration range (14–60 μg ml^{-1}). The average regression equation ($n = 5$) was calculated by the method of least squares and found to be $y = 0.0142x - 0.024$. The relative standard deviation for the slope was 1.4% and the average correlation coefficient = 0.9987.

Within day and between days precision (repeatability) were examined at three concentration levels (low, medium,

Table 1
Within day and between days precisions for spectrophotometric analysis of alendronate sodium ($n = 8$)

Alendronate concentration ($\mu g\,ml^{-1}$)	Within day R.S.D. (%)	Between days R.S.D. (%)
14	1.6	1.69
40	0.5	0.66
60	0.37	0.48

and high). The obtained results expressed as relative standard deviations are summarized in Table 1. Accordingly the precision of the method was judged to be satisfactory. The specificity of the method was examined by subjecting the potential excipients of the tablets to the proposed analytical procedure and observing the response. Spectrophotometric measurements showed that a placebo sample (from JOSWE, Jordan) did not have any absorption under the described experimental conditions.

The recovery of the proposed spectrophotometric method was examined by analyzing laboratory mixtures (alendronate and excipients). It was evident that the proposed method had a satisfactory recovery, since the average percent recovery for laboratory mixture ($n = 10$) was 98.6% (R.S.D. = 0.74). LOQ taken as the concentration which produce absorbance value of 0.2 was 14 $\mu g\,ml^{-1}$ (R.S.D. = 1.6%). Overall the proposed method was shown to have very good linearity, precision, recovery, accuracy, and specificity. The method provides a simple alternative for the determination of alendronate in pharmaceutical tablet.

3.2. HPLC method

3.2.1. HPLC method for analysis of alendronate sodium in tablet (HPLC-DAD)

Reversed phase high performance liquid chromatography method was developed for the determination of alendronate in dosage form. The method was based on pre-column derivatization with OPA/2ME and UV–Vis detection at 333 nm. Diode array detector was used to aid peak purity assessment.

Using polymeric phase column that tolerates high pH value (enable direct injection) a satisfactory mobile phase composition was found to be 15% acetonitrile in phosphate buffer ($pH = 9.6$). Diode-array detection was employed for identification and examination of the purity of the peaks. Only one peak eluted in the chromatogram of a standard solution of alendronate derivatized with OPA (monitoring at 333 nm). Fig. 4 shows representative chromatograms for alendronate (Fosamax® tablets) and a blank solution subjected to the proposed method. Therefore, the peak appearing at the retention time of 4.8 min was confirmed to be that of the derivatized alendronate. Moreover, diode array scans that were obtained at different times of the peak (peak slicing) showed identical UV spectra which suggested that only one derivatization product was formed by this reaction.

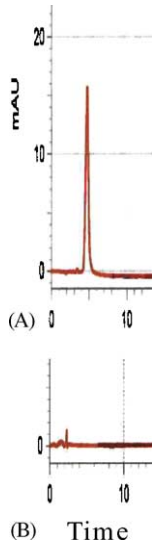

Fig. 4. Representative chromatograms for (A) alendronate sodium from a Fosamax® tablets derivatized with OPA/2ME reagent in 0.05 M NaOH and (B) the blank solution of derivatization reaction using a polymeric reversed phase column and monitored at 333 nm. Mobile phase consisted of acetonitrile: phosphate buffer pH 9.6 (15:85).

In attempts to improve the peak shape of alendronate, various concentrations of tetrabutylammonium perchlorate (TBAP) and tetrabutylammonium bromide (TBAB) as ion pairing agents were incorporated in mobile phases and chromatograms obtained. Although, both TBAP and TBAB improved peak shape and symmetry; TBAP was superior in this effect (Fig. 5). Asymmetry factor (AF) for the peak of derivatized alendronate improved from 2 to 1.1 and efficiency (N) from 98 to 537 as a result of adding TBAP to mobile phase. Therefore, TBAP was decided to be incorporated in the mobile phase (3 mg%). The positive effect of TBAP might be attributed to the formation of ion pairs between TBAP and alendronate which tends to minimize the interaction of alendronate with the residual polar groups on the column that represent a common reason of peak broadening and tailing [18].

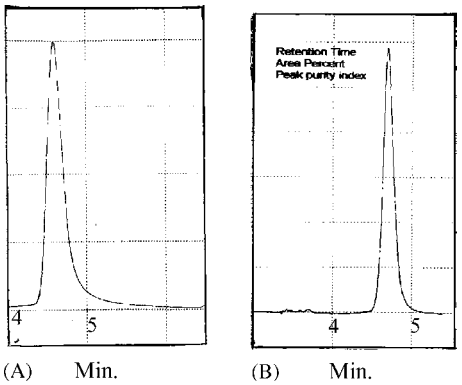

Fig. 5. Representative chromatograms of standard alendronate sodium obtained with the same mobile phase of Fig. 4. (A) No ion pairing agent was added. (B) The ion pairing agent TBAP was added (3 mg%).

Table 2
Within day and between days precisions for alendronate sodium analyzed by HPLC-DAD method ($n = 8$)

Alendronate concentration ($\mu g\,ml^{-1}$)	Within day R.S.D. (%)	Between days R.S.D. (%)
10	1.55	1.97
40	0.83	1.03
60	0.76	0.87

3.2.1.1. Linearity, precision, accuracy, specificity limit of detection (LOD), and LOQ. A linear relationship between the peak area (monitored at 333 nm) and the concentration of alendronate was established over the examined concentration range (10–60 $\mu g\,ml^{-1}$). The average regression equation ($n = 5$) was calculated by the method of linear regression and found to be $y = 8194.2x - 0.08$. The method was found to be linear with a good correlation coefficient (0.9932) over the examined concentration range.

The precision of the assay procedure as applied to alendronate raw material was determined at low (10 $\mu g\,ml^{-1}$), intermediate (40 $\mu g\,ml^{-1}$), and high (60 $\mu g\,ml^{-1}$) concentrations within the same day and over a period of 4 days. Results are summarized in Table 2. From results presented in Table 3 the inter and intraday pricisions of the method were judged to be satisfactory. The recovery of the proposed HPLC method was estimated by analyzing laboratory mixtures of standard alendronate and excipients at concentrations that are expected to be found in tablets. The obtained average recovery ($n = 5$) for the mixture was 98.8% (R.S.D. = 0.7) which indicated good recovery.

Specificity of the method was examined by two approaches: (1) through observing if there was any response of the placebo of the tablet and (2) by diode array peak slicing technique. When common tablet excipients (obtained from JOSWE, Jordan) was subjected to the proposed analytical procedure, no components eluting at the retention time of alendronate (4.8 min) were observed. Obtaining UV spectra of the eluting peak at various time points revealed exactly the same spectra which indicated peak purity. The limit of detection defined as the concentration that gives rise to a signal that is three times the noise of the baseline was 90 $ng\,ml^{-1}$ of alendronate. LOQ defined as the concentration that produce signal that is 10 times the noise in the base line was 0.3 $\mu g\,ml^{-1}$ (R.S.D. = 1.9).

3.2.1.2. Application to real samples and comparison with reference method. Commercially available tablets of alendronate sodium (Fosamax® 10 mg) were subjected to analysis by the two proposed methods (spectrophotometric and HPLC-DAD). The same batch of tablets was also analyzed by a reference spectrophotometric method [15]. Results of analysis (as percentage per label) for the three methods are summarized in Table 3. The results in Table 3 indicate that the three methods produce close results. The accuracy of the method can be inferred from the demonstrated lack of interference from commonly used tablet-additives (selectivity) as well as from the demonstrated precision of the methods [19]. However, the precision of the two proposed methods (experiments were repeated five times) appeared to be significantly higher than that obtained by the reference method.

3.2.2. HPLC method for the determination of alendronate in urine (HPLC-FD)

The same chromatographic conditions discussed earlier were employed for the determination of alendronate in urine samples. The only difference was in the detection, where fluorometric detection was employed using $\lambda_{exc} = 333$ nm and $\lambda_{em} = 445$ nm. The method was validated for the analysis of alendronate in urine samples.

3.2.2.1. Linearity, precision, accuracy, specificity, LOD and LOQ. The method was found to be linear over the examined concentration range (2–32 $ng\,ml^{-1}$, the estimated concentration after sample treatment). The average calibration equation ($n = 5$) when analyzed by linear regression method could be described by: $y = 54264x - 0.095$, with a correlation coefficient = 0.9956.

Five determinations of the spiked urine samples were made at three different concentrations levels (2, 14, and 32 $ng\,ml^{-1}$, the estimated concentration after sample treatment). The spiked urine samples were analyzed on the same day and over successive days. The obtained results with their relative standard deviations are summarized in Table 4.

Urine samples were spiked with alendronate sodium at three concentrations and treated in accordance with the described method. The amounts of recovered alendronate were determined and the process was repeated over three consecutive days (Table 5). Data presented in Table 5 indicate high recovery with reasonably high precision and consequently high accuracy [19].

Table 3
Percentage per label of Fosamax® 10 mg tablets analyzed by the three methods ($n = 5$)

	Proposed spectrophotometric method	Proposed HPLC-DAD method	Reference method [15]
Average percentage per label	99.5	99.2	99.7
R.S.D.	0.70	0.91	2.85

Table 4
Within day and between days precision for alendronate sodium-spiked urine samples ($n = 5$)

Estimated alendronate concentration after sample treatment ($ng\,ml^{-1}$)	Within day R.S.D. (%)	Between day R.S.D. (%)
2	8.3	8.6
14	3.9	4.1
32	1.8	1.9

Table 5
Recoveries of alendronate sodium (over 3 days) from urine samples at three concentration levels analyzed by HPLC-FD ($n = 5$)

Concentration expressed as that obtained after sample treatment ($ng\,ml^{-1}$)	Mean recovered concentration ($ng\,ml^{-1}$)	Recovery (%)	R.S.D. for recovery (%)
2	1.86	92.9	3.3
14	13.7	97.9	2.8
32	31.8	99.3	0.4

The specificity of the assay was assessed (in addition to peak purity assessment using DAD) by analyzing drug-free urine samples of five different healthy volunteers (males and females). Chromatograms obtained showed that blank urine samples did not have any other peaks under described experimental conditions, so no endogenous interference was encountered (Fig. 6). The LOD defined as the concentration that gives rise to a signal that is three times the average noise of the baseline was $0.2\,ng\,ml^{-1}$ of alendronate. Quantification limit was taken as $2\,ng\,ml^{-1}$ which is equivalent to $0.6\,ng\,ml^{-1}$ in the original urine sample.

The proposed method was applied to the determination of alendronate in urine samples from one volunteer who was administered 30 mg of alendronate daily as a single oral dose for 3 days. Urine collection started with the first dose and continued up to 24 h after the last dose. Representative chromatograms for the collected urine sample and a drug-free urine are presented in Fig. 6. The average concentration ($n = 5$) of alendronate in the collected sample was $29.7\,ng\,ml^{-1}$ with R.S.D. = 2.3% which indicated good precision.

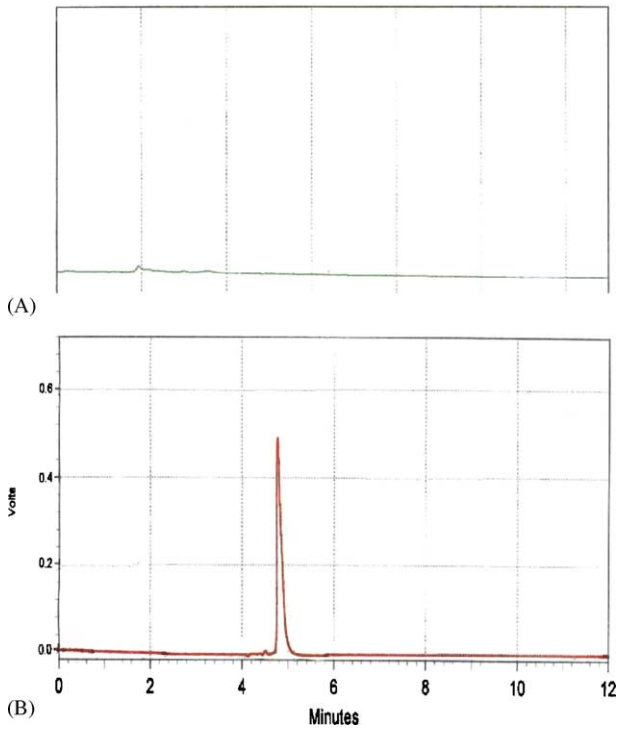

Fig. 6. (A) Representative chromatograms of drug-free urine and (B) a collected urine sample after 72 h of the start of alendronate administration.

In this method, alendronate was analyzed in urine samples based on the selective precipitation of alendronate by calcium ions in basic media, removal and subsequent dissolution by chelating calcium with excess EDTA. The idea of using calcium chloride for precipitation of alendronate, and probably most of other bisphosphonates, was not new and has been reported in many previously mentioned studies of bisphosphonate analysis in biological fluids. However, sample treatment was optimized and re-validated to be a simple, reliable, rapid, and less costly. This was done by the addition of EDTA that chelates and re-dissolves calcium.

Consequently farther purification of the sample with solid phase extraction cartridge (SPE) that was used in most previously reported methods, was not necessary. Recoveries using this sample-cleaning procedure were between 92 and 99% for alendronate sodium. The low quantification limit achieved by this method ($0.6\,ng\,ml^{-1}$ using 5 ml urine) makes this analytical method valuable to be employed in pharmacokinetic investigations of alendronate sodium in human (based on urinary excretion data).

4. Conclusion

Alendronate sodium can be determined in pharmaceutical tablets and urine based on reaction with OPA in the presence of 2ME. One of the most attractive features of this method is the simplicity of the preparation of derivatives. However, stability considerations impose a waiting period of 60 min before actual measurements. The proposed spectrophotometric method is based on the fact that only the derivatized drug has absorbance at the measurement wavelength of 333 nm. It is a simple and nonexpensive method which is suitable for routine quality control of alendronate in pharmaceutical tablets.

The proposed HPLC-DAD method provides a sensitive and reliable method for the determination of alendronate in tablets. For the determination of alendronate in urine, a more sensitive and selective detection technique was described based on fluorescence detection (HPLC-FD). HPLC-FD was found to be highly sensitive and could be used to detect low concentrations of alendronate usually found in such samples.

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