Determination of alendronate in pharmaceutical dosage formulations by ion chromatography with conductivity detection[☆]

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ABSTRACT

A method was developed and validated for the direct determination in pharmaceutical dosage formulations of alendronate, a non-chromophoric compound. It is based on the use of single-column ion chromatography with conductivity detection that obviates the need for the tedious chemical derivatization procedures that are required for UV and fluorescence detection. Diluted samples of 0.05 mg/ml were chromatographed directly on a Waters IC-Pak HR anion-exchange column or a Dionex OmniPac PAX-100 column with dilute nitric acid as the mobile phase followed by conductivity detection. The method was validated and shown to be precise, accurate and specific for the assay of alendronate in intravenous (i.v.) solution and tablet formulations. The ruggedness of the assay was studied by generating data from four different instruments. Also established was the equivalence between this method and a previously reported high-performance liquid chromatographic method with 9-fluorenylmethyl chloroformate derivatization and UV detection. Application of the method to the determination of alendronate in i.v. and tablet formulations is presented and the performances of the Waters IC-Pak HR and Dionex OmniPac columns are discussed.

INTRODUCTION

Alendronate (Fig. 1) is the monosodium trihydrate salt of 4-amino-1-hydroxybutane-1,1-bisphosphonic acid and belongs to the bisphosphonate class of drugs. The drug has important therapeutic indications in the treatment of a variety of bone diseases such as hypercalcemia of malignancy, Paget's disease and osteoporosis [1,2]. Development of an assay for this class of compounds is challenging owing to the lack of a chromophore for conventional UV or fluorescence detection. The method described here is capable of the direct measurement of alendronate in pharmaceutical dosage forms based on the use of single-column (non-suppressed) ion chromatography with conductivity detection (IC–CON method). The method obviates the need for

the chemical derivatization procedures that are required when UV or fluorescence detection is applied.

Three reversed-phase high-performance liquid chromatographic (HPLC) methods involving chemical derivatization have been described for the determination of alendronate. An HPLC method utilizing precolumn derivatization of the primary amine with 9-fluorenylmethyl chloroformate
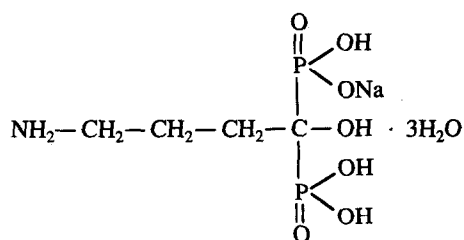

Fig. 1. Structure of alendronate.

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(FMOC) for UV detection has been reported previously from our laboratory [3]. Kwong *et al.* [4] employed ion-pair chromatography and postcolumn derivatization with *o*-phthalaldehyde (OPA) for fluorescence detection. Both methods were developed for the assay of alendronate in pharmaceutical formulations. Another HPLC procedure was published by Kline *et al.* [5] for the determination of alendronate in urine by automated precolumn derivatization with 2,3-naphthalenedicarboxyaldehyde (NDA) for fluorescence detection [5]. Several HPLC methods dealing with compounds analogous to alendronate have also been reported. For example, Flesch and Hauffe [6] used precolumn derivatization with fluorescamine for fluorescence detection for the determination of Pamidronate disodium (a propyl analogue of alendronate); Daley-Yates *et al.* [7] described the use of ion chromatography, postcolumn oxidation to orthophosphate followed by conversion to a phosphomolybdate complex for the determination of the propyl analogue and several related compounds. All these procedures require either precolumn derivatization techniques that usually require extensive and tedious sample preparation or by postcolumn reactions where complicated and specialized equipment is generally necessary.

Our objective was to simplify the assay procedure by eliminating the derivatization procedure for this non-chromophoric compound. Few chromatographic methods with direct detection for bisphosphonates or related compounds have been published. Chester *et al.* [8] reported an ion-exchange chromatographic method with on-line flame photometric detection for dichloromethylene diphosphonate and Forbes *et al.* [9] described the use of an inductively coupled plasma (ICP) detector for specific phosphorus detection for Etidronate (1-hydroxyethane-1,1-bisphosphonate) disodium. These detection devices are generally not available in pharmaceutical analysis laboratories. Den Hartigh *et al.* [10] presented results obtained using ion-exchange HPLC with conductivity detection for the determination of phosphonates in pharmaceutical preparations.

This paper reports the development, validation and application of the IC–CON method for the direct determination of alendronate in intravenous (i.v.) and tablet formulations. Typical validation

studies were carried out, including injection precision, linearity, specificity, recovery and method precision. Assay ruggedness was additionally addressed by generating data from four commonly used chromatographic systems. Also established was the equivalence of the IC–CON and FMOC methods by direct comparison of the analytical results. The performances of two different columns used in this work, namely Waters IC-Pak HR and Dionex OmniPac, are also discussed.

EXPERIMENTAL

Chemicals and reagents

Alendronate (MK-0217, $C_4H_{12}NO_7P_2Na \cdot 3H_2O$; mol. wt. 325.1) of pharmaceutical grade manufactured within Merck Sharp & Dohme Research Labs. (Rahway, NJ, USA) was used as an analytical standard. All solvents and reagents were used as received. Nitric acid (OPTIMA grade), acetonitrile (HPLC grade) sodium citrate, citric acid and sodium chloride (analytical-reagent grade) were purchased from Fisher Scientific (Philadelphia, PA, USA). Deionized water of at least $18M\Omega$ purified with a Milli-Q system (Millipore, Bedford, MA, USA) was used for mobile phase, sample and standard preparations.

Equipment

Most of the development and validation work was performed on a Dionex (Sunnyvale, CA, USA) Model 4500i inert chromatographic system equipped with a Dionex pulsed electrochemical detector (conductivity mode) and a Spectra-Physics (San Jose, CA, USA) Model 8880 autosampler. Stainless-steel systems such as a Hewlett-Packard (Avondale, PA, USA) Model 1090 system connected with a Milton Roy (Riviera Beach, FL, USA) conductivity detector, Perkin-Elmer (Norwalk, CT, USA) Series 4 with Milton Roy detector and Spectra-Physics Model 8800 with Milton Roy detector were also investigated in order to establish the ruggedness of the method. A Waters (Milford, MA, USA)IC-Pak HR anion-exchange column (6 μm particle size, 75 mm × 4.6 mm I.D.) and a Dionex OmniPac PAX-100 column (8 μm particle size, 250 mm × 4 mm I.D.) were used. Mobile phases of 1.6 mM nitric acid and 1.76 mM nitric acid + 20% acetonitrile were delivered at a flow-rate of 0.5 ml/min

for the IC-Pak HR and OmniPac columns, respectively. Analyses were carried out at ambient temperature with 25-μl injections of 0.05 mg/ml alendronate. Detection by conductivity was set at negative polarity with an output range of 50 μS for the Dionex detector and 1 μS for the Milton Roy detector.

Standard solution preparation

For the assay of i.v. solutions, a standard solution was prepared by dissolving 32 mg of alendronate (equivalent to 25 mg of free acid) in 500 ml of a placebo-equivalent diluent (205.8 mg of sodium citrate + 57.6 mg of citric acid + 98.2 mg of sodium chloride dissolved in 1000 ml of water) in order to maintain an identical ionic strength between the standard and i.v. sample solutions. For the tablet assay, water was used as a diluent for preparing the standard. The standard concentration was 0.05 mg/ml in both instances.

Sample solution preparation

The i.v. solution (2.5 mg/ml) was diluted appropriately with water to yield a concentration of 0.05 mg/ml and transferred to an HPLC vial for analysis. A tablet (2.5 mg) was dispersed and sonicated in an appropriate volume of water for 5 min and diluted to 0.05 mg/ml. The resulting solution was filtered through a Millipore 0.22-μm filter unit and transferred to an HPLC vial for analysis.

Assay procedure

Generally, the system was first equilibrated with the mobile phase by injecting a standard solution until reproducible results were observed (about three injections) prior to the i.v. or tablet assay. Standard and sample solutions were injected directly.

RESULTS AND DISCUSSION

Chromatography

The concept of ion chromatography with conductivity detection has allowed the determination of ionic and non-chromophoric compounds such as alendronate with minimum sample preparation. The single-column ion chromatographic method for anion determination was introduced by Gjerde and co-workers [11,12]. By carefully choosing the

anion exchanger and eluent for alendronate, the separation column can be directly coupled to a conductivity detector for direct detection. Under the IC–CON conditions described under Experimental, alendronate is eluted and directly detected by the use of an eluent of 1.6 mM nitric acid (pH *ca.* 2.5) as shown in Fig. 2a for the Waters IC-Pak HR column. The selection of dilute nitric acid as a mobile phase was appropriate for pH maintenance to produce predominantly monovalent ($-$1) charge state of alendronate so that a reasonably short retention (or ion-exchange process) was achieved. It is generally true that the greater the charge state of the sample, the later it elutes owing to its higher affinity for the anion-exchange resin. Because of the high background conductance of the nitric acid mobile phase (mainly because of H$^+$), the solute (alendronate) produces a decreasing or negative signal (indirect chromatographic signal, see Fig. 2) owing to its uptake of H$^+$. Similar results have been described by
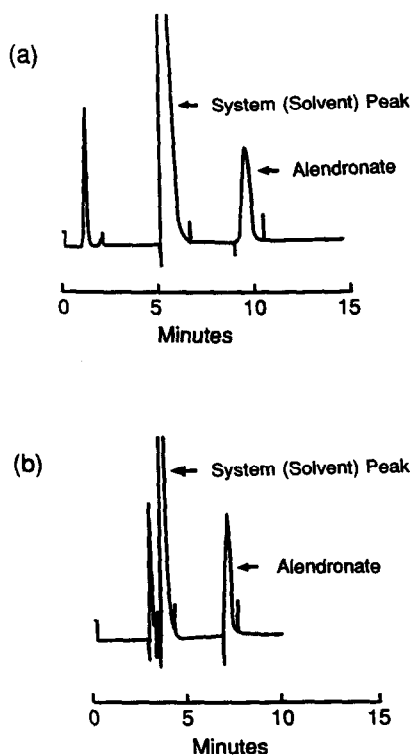

(a)

Fig. 2. Typical chromatograms of 0.05 mg/ml alendronate standard solution in water obtained with the IC–CON method on (a) the IC-Pak HR column and (b) the OmniPac column.

Gjerde and co-workers [13,14]. The greater negative deflection observed prior to the alendronate peak, a system peak, was produced from the sample water diluent [15]. This deflection is typically noted in non-suppressed ion chromatography [16] and was attenuated by the concentration of the cation present in the sample, *i.e.*, a higher cation concentration yielded a lower system peak. The use of a cation suppressor (Dionex) to reduce the H^+ concentration of the eluent and hence the background conductance for this method was attempted. This attempt was not successful owing to the simultaneous suppression of the alendronate signal, possibly related to the reduction of the H^+ concentration which was considered critical for the generation of the alendronate signal.

Fig. 2a was generated using a Waters IC-Pak HR anion-exchange column packed with trimethylammonium-functionalized polymethacrylate resin. As indicated, this column produced a relatively broad alendronate peak. Recently, a series of multi-phase columns capable of performing both reversed-phase and ion-exchange HPLC were introduced by Dionex. Experiments demonstrated that an OmniPac PAX-100 column (a highly cross-linked styrene–divinylbenzene polymeric microporous substrate with the surface functionalized by a quaternary ammonium base) with 20% of acetonitrile as an organic modifier yielded a typical chromatogram for alendronate as shown in Fig. 2b. This column has the advantage of yielding a shorter retention with a better peak shape for alendronate than the IC-PAK HR column. The performances of these two columns were preliminarily validated in terms of injection precision, linearity and method specificity for practical applications.

Validation

Injection precision for ten replicate injections of a 0.05 mg/ml standard solution (dissolved in an i.v. placebo-equivalent diluent for i.v. assay or dissolved in water for tablet assay) was satisfactory [relative standard deviation (R.S.D.) < 1% by peak height] for both the IC-Pak HR and OmniPac columns. The peak-area measurements showed higher R.S.D. values (*ca.* 2%), probably owing to the variation of area integrations with such a high background conductance. Peak-height measurements were therefore adopted to determine alendronate in this method.

The detector responses to a range of 40–160% of the assay concentration (in both i.v. and water media) was determined to be linear with $R^2 > 0.999$ for both columns. In some instances, a non-zero intercept was observed in the linearity plot, especially when peak-area measurements were used. This non-zero intercept was attributed to the background conductance drift during the experimental runs. It was less pronounced when peak-height measurements were used and had an inconsequential effect on accuracy at the assay concentration.

Method specificity utilizing the OmniPac column was demonstrated by the separation of alendronate from its aminohydroxypropyl analogue (relative retention time, t_R, = 1.16) and its thermal decomposition products obtained by melting alendronate at 260°C by differential scanning calorimetry (DSC) (two late-eluting peaks at t_R = 1.23 and 1.59). The degradation products induced by DSC melting were generated under unrealistic high temperature conditions for the purpose of illustrating method specificity only. Identification of these thermal decomposition products was not pursued. The method was also specific against components of the i.v. and tablet placebo formulations (see below). No bias was evident in each instance under the described chromatographic conditions. The validation was extended to the recovery and method precision for 2.5 mg/ml i.v. and 2.5 mg tablet formulations.

Analysis of formulations

Intravenous solution. Fig. 3 illustrates typical chromatograms obtained with the IC–CON method using the IC-Pak HR column for (a) and i.v. placebo containing citrate buffer in saline solution and (b) alendronate in an i.v. formulation. It is apparent that alendronate could be resolved from components in the i.v. placebo without any interference. The OmniPac column could not be utilized for this sample because of an interfering i.v. background, probably due to column overload owing to the amount of citrate present in the formulation. It is important to note that there was a slight difference (*ca.* 0.1 min) in the retention times (and hence peak heights) between the standard dissolved in water and the i.v. sample solution. This difference resulted in an experimental error of 2–3% when i.v. samples were analyzed against the standard solution in water. This error could be circumvented by
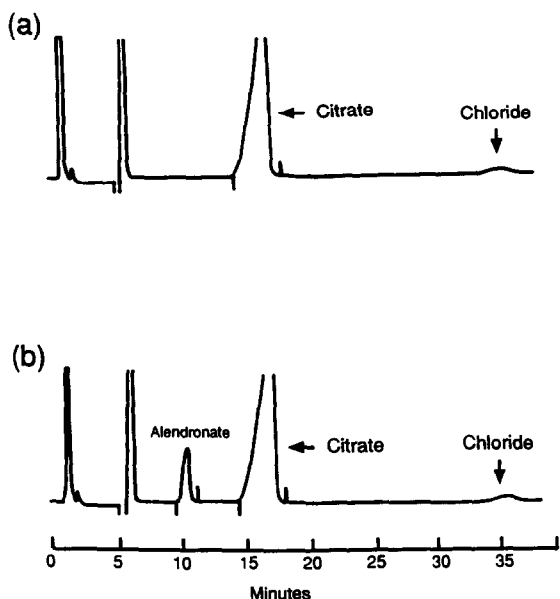
Fig. 3. Chromatograms of (a) diluted i.v. pacebo and (b) diluted i.v. sample solution obtained with the IC-Pak HR column.

matching the standard with an i.v. placebo-equivalent diluent (see Experimental) so that identical responses were observed for i.v. smaple and the "modified" standard solutions owing to their equivalent ionic strength. More accurate results could therefore be obtained. The validation data for i.v. solutions were therefore generated based on the selection of a matched standard solution.

Recovery studies were performed by spiking aliquots of a stock solution (0.1 mg/ml) of alendronate into an i.v. placebo in duplicate at 80%, 90%, 100%, 110% and 120% levels of potency followed

by appropriate dilution. The results were satisfactory with a mean recovery of 100.2% and R.S.D. = 1.4% ($n = 10$).

The method precision was determined by analyzing ten replicate samples. Table I summarizes the mean assay results for ten samples generated by two individual analysts. For comparison purposes, the results calculated against standard in water and standard in placebo-equivalent solution are also presented in Table I. Excellent precisions were attained with R.S.D. < 1% in all instances. It is obvious that data calculated with respect to the matched standard solution yield relatively more accurate results. This observation confirms the significance and necessity for the selection of a matched standard solution for i.v. sample analysis. Also listed in Table I are the data obtained by our previously published FMOC method for establishing equivalence (see below for discussion).

Tablet formulation. For the tablet formulation, both columns demonstrated satisfactory specificity, as shown in Fig. 4, where alendronate could be separated from the placebo excipients. It is pertinent to note that the assay for tablets provides more time saving than the i.v. assay. The later is complicated by the late-eluting ionic species of citrate and chloride present in the formulation. The OmniPac column yields a better peak shape and shorter retention time than the IC-Pak HR column and is therefore more suitable for the assay of tablet forms. The validation and analytical data for 2.5 mg tablets were collected on both columns for comparison.

Recovery experiments for the tablets were performed using similar experimental conditions to those described for the i.v. solution. Results of these experiments for duplicate samples prepared at 80%,

TABLE I

METHOD PRECISION DATA AND COMPARISON BETWEEN IC–CON AND FMOC METHODS

IC–CON[a]				FMOC method[a]	
Standard in placebo		Standard in H$_{2O}$			
Analyst I	Analyst II	Analyst I	Analyst II	Analyst I	Analyst II
2.52 mg/ml, 100.8% (R.S.D. 0.87%)	2.52 mg/ml, 100.7% (R.S.D. 0.42%)	2.42 mg/ml, 96.8% (R.S.D. 1.06%)	2.44 mg/ml, 97.5% (R.S.D. 0.35%)	2.54 mg/ml, 101.5% (R.S.D. 0.10%)	2.54 mg/ml, 101.6% (R.S.D. 1.04%)

[a] Data are given as mg/ml found with % of label claim for 2.5 mg/ml i.v. solutions, with R.S.D. ($n = 10$) in parentheses.
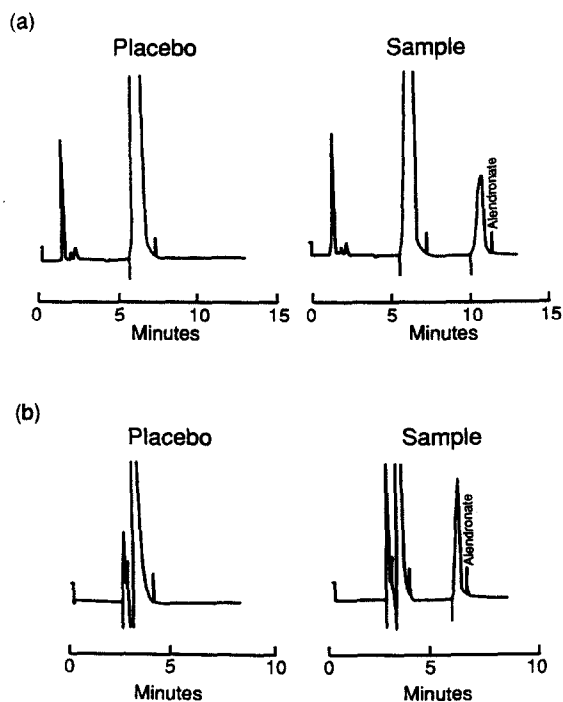
(a)

(b)

Fig. 4. IC–CON analysis of alendronate tablet formulations including placebo and sample performed on (a) the IC-Pak HR column and (b) the OmniPac column.

TABLE II

CONTENT UNIFORMITY DATA FOR 2.5 mg TABLETS ASSAYED BY THE IC–CON AND FMOC METHODS

IC–CON method[a]		FMOC method[a]
OmniPac column	IC-Pak HR column	
2.47 mg, 98.9% (R.S.D. 0.75%)	2.47 mg, 98.7% (R.S.D. 1.80%)	2.45 mg, 97.8% (R.S.D. 0.69%)

[a] Data are given as mg found with % of label claim, with R.S.D. (n = 10) in parentheses.

that the IC–CON method is precise, accurate, specific and suitable for the assay of alendronate in the two formulations. The limit of quantification (LOQ) was also determined in order to evaluate the possible application for the measurement of drug dissolution rates. An LOQ of 0.005 mg/ml was found to be achievable with an R.S.D. of 2.9% (the limit of detection was 0.002 mg/ml with signal-to-noise ratio = 4). Although the method demonstrates an advantage in ease of sample preparation, further evaluation of the method ruggedness and method equivalence between the IC–CON and FMOC methods was conducted as discussed below.

Method ruggedness and equivalence testing

Ruggedness testing was primarily demonstrated via data (Table I) showing that the IC–CON method could be reproduced by two individual analysts, indicating that the method is operator independent. The assay ruggedness was more intensively addressed by performing similar experiments on three chromatographic systems equipped with stainless-steel tubing (see Experimental), as the Dionex inert LC system (PEEK-based flow path) was considered to be a specialized instrument. Table III summarizes data reproducibility which substantiates the performances of the four LC systems. These systems could generate a smooth baseline, comparable injection precision (R.S.D. < 1.5%, n = 10), good linearity (R^2 > 0.999) and satisfactory assay results for ten i.v. samples. Consequently, it is reasonable to conclude that the method is rugged as a specialized instrument is not required. However, it is worth noting that the Spectra-Physics Model 8800 + Model 8880 autosampler exhibits an

90%, 100%, 110% and 120% levels of potency were satisfactory with a mean recovery of 99.8% (R.S.D. 0.95%) and 100.1% (R.S.D. 0.76%) for the OmniPac and IC-Pak HR columns, respectively. All the data were calculated using a standard solution in water. Unlike the i.v. solution, the tablet formulation does not contain ionic components such as chloride and citrate anions. Hence, the choice of water as the the standard diluent for simplicity can be justified without affecting the accuracy of the assay.

Table II summarizes the uniformity in composition of ten tablets assayed using both columns for establishing the method precision. Satisfactory analytical results and precision (R.S.D. < 2%) were obtained by the IC–CON method and were consistent with those of the FMOC method. The R.S.D. (n = 10) reported for the tablet formulation represents the sum of the variations from the assay and the manufacture of the dosage form.

Based on the above validation and analytical results for i.v. and tablet dosage forms, it is concluded

TABLE III

PERFORMANCES OF DIFFERENT INSTRUMENTS FOR RUGGEDNESS TESTING

HPLC system + autosampler[a]	Baseline	Injection precision (R.S.D., %)[b]	Linearity (R^2)	I.v. sample analysis[b]
Dionex + SP 8880	Smooth	0.85	1.000	100.7% of label (R.S.D. 0.43%)
SP 8800 + SP 8880	Smooth	0.98	0.999	100.9% of label (R.S.D. 0.91%)
HP 1090	Smooth	0.95	1.000	100.7% of label (R.S.D. 0.89%)
PE	Smooth	1.38	0.999	Not determined

[a] Detectors used are specified under Experimental. SP = Spectra-Physics; HP = Hewlett-Packard; PE = Perkin-Elmer.
[b] $n = 10$.

erratic baseline and unacceptable analytical results (about 2–8% error) when the system is switched from reversed-phase HPLC to ion chromatography. The problem could be eliminated by bypassing the mixer (which probably contains unknown contaminants) and passivating the flow path with 0.05 M nitric acid overnight, and also replacing the Vespel rotor seal in the autosampler with a Tefzel rotor seal. The exact reason for the erratic ion chromatography was not pursued. After these extensive cleaning and equilibration procedures, the Spectra-Physics system performed equally well, as indicated in Table III.

The data shown in Tables I (i.v. sample analysis) and II (tablet sample analysis) obtained with both the IC–CON and FMOC methods attest to the equivalence between these two methods. Degraded i.v. samples (e.g., stressed at 80°C for 16 weeks) were also analyzed by both methods with consistent results (94.1% of theory by the IC–CON method and 93.1% of theory by the FMOC method). These data additionally support the method equivalence. In order further to demonstrate the equivalence, sixteen lots of i.v. samples were assayed by the IC–CON method and the data compared with the available FMOC results. The data obtained with the two methods do not show a significant difference and yield similar variabilities from the label concentration of 2.5 mg/ml. The IC–CON mean result was 2.51 mg/ml (R.S.D. = 1.51%) and the FMOC mean result was 2.52 mg/ml (R.S.D. = 1.62%). The difference of 0.01 mg/ml was not statistically significant (t-test, $P = 0.33$). Hence these re-

sults strongly support the fact that the two methods are essentially equivalent.

CONCLUSIONS

A novel IC–CON method using a Waters IC-Pak HR column has been validated and shown to be precise, accurate, specific and suitable for the assay of alendronate in i.v. solution. It is also applicable to the assay of tablets using an OmniPac or IC-Pak HR column. The former yields a sharper peak shape and shorter retention time. The method is rugged based on evidence that four different instrumental systems can perform equally well, and that equivalent results can be achieved independently by different operators using similar instrumentation. The IC–CON method has been demonstrated to be equivalent to the current FMOC method. This newly developed method can offer a direct measurement of alendronate in various dosage forms without the need for the derivatization procedures that are required with UV–VIS and fluorescence detection and hence is relatively simple. The ease of sample preparation has led us to investigate a wide range of applications of this methodology, especially for similar compounds without a derivatizable amino group such as Etidronate, where FMOC derivatization is not possible. Finally, a similar approach with indirect UV absorption detection at 230 nm due to the decrease in nitrate concentration is under investigation.

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