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Determination of bisphosphonate drugs in pharmaceutical dosage formulations by ion chromatography with indirect UV detection*

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Abstract: Application of ion chromatography (IC) to the analysis of non-chromophoric bisphosphonate drugs in pharmaceutical dosage formulations is described. The method is based on the use of single-column ion chromatography in conjunction with indirect UV detection that obviates the need for tedious chemical derivatization procedures. Diluted drug samples are chromatographed directly on a Waters IC–Pak HR anion-exchange column with dilute nitric acid (1.6–12 mM) as the mobile phase which exhibits a UV absorption maximum near 220 nm. Analyte detection is monitored by measuring the decrease in absorption of the mobile phase. The IC method has been validated and shown to be precise, accurate, specific and rugged for routine assay. Application of the method to the determination of alendronate sodium tablets, etidronate disodium injectable (which requires an eluent pH control for chromatographic resolution of active drug from chloride ions) and clodronate disodium injectable is presented. The performances of the Waters IC–Pak HR and several equivalent columns are also discussed.

Keywords: *Ion chromatography; indirect UV detection; bisphosphonate drugs.*

Introduction

Alendronate sodium, etidronate disodium and clodronate disodium belong to the bisphosphonate class of drugs (see Fig. 1 for molecular structures) which are generally targeted for the treatment of a variety of bone diseases such as hypercalcaemia of malignancy, Paget's disease and osteoporosis [1, 2]. Development of a chromatographic assay for this class of compound is challenging owing to the lack of a chromophore for conventional UV or fluorescence detection. The method presented in this study provides a direct measurement of the bisphosphonate drugs in pharmaceutical dosage forms based on the use of single-column ion chromatography with indirect UV detection (IC-indirect UV method) which monitors the decrease in UV absorption of the nitric acid eluent (negative signal). The method does not require pre-column or post-column derivatization procedures which are necessary when UV or fluorescence detection is applied.

Several chromatographic methods dealing with the bisphosphonates and their analogous compounds have been reported in the literature [3–11]. For example, a reversed-phase HPLC method utilizing pre-column derivatization of the primary amine of alendronate with 9-fluorenylmethyl chloroformate (FMOC) for UV detection has been reported from this laboratory for the determination of alendronate [3]. Recently, Kosonen *et al.* have described an ion chromatographic method with post-column derivatization with iron(III) nitrate for the determination of disodium clodronate [11]. All these procedures involve either pre-column derivatization techniques that usually require extensive and tedious sample preparations or post-column reactions where complicated and specialized equipment is generally necessary.

Our objective was to simplify the assay procedure by eliminating the derivatization reactions for these non-chromophoric bisphosphonate compounds. There are few liquid chromatographic methods with *direct* detection

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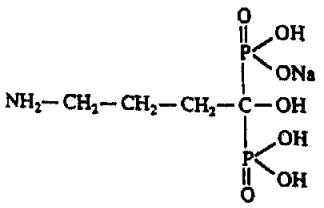
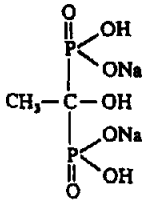


Alendronate Sodium



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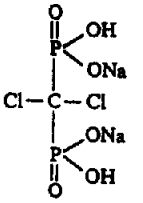

Clodronate Disodium

Note: All the compounds are presented as an anhydrous form.

Figure 1
Molecular structures of the bisphosphonate drugs.

(without derivatization) for bisphosphonate drugs published in the literature. For example, Chester *et al.* has reported an ion exchange chromatographic method with an on-line flame photometric detection for dichloromethylene diphosphonate [12], Forbes *et al.* described the use of an inductively coupled plasma (ICP) detector for specific phosphorus detection for etidronate disodium [13], and Tsai *et al.* described the use of an ion chromatographic method with conductivity detection for alendronate [14]. These detection devices are generally not available in the routine pharmaceutical analysis laboratories. We have previously reported a short communication describing an IC-indirect UV method for the direct quantitation of etidronate disodium tablets [15] which are generally assayed by a titration method with thoriumcyclohexylenedinitrilotetraacetic acid complex specified in the United States Pharmacopeia [16]. This work reports the assay of etidronate disodium formulated in i.v. solution with emphasis on the effect of eluent pH on chromatographic resolution of the two co-eluting peaks (etidronate and chloride ions). In addition, the ease of assay procedure of the IC-indirect method has prompted us to extend the application of this method to the analysis of *other* bisphosphonate drugs in order to demonstrate its potential applicability for the entire class of non-chromophoric bisphosphonate drugs present in various dosage forms.

The method described in this study is based on the use of single-column (Waters IC–Pak HR anion exchange column) ion chromatography because of the highly ionic nature of the bisphosphonate group. Nitric acid (exhibits a UV absorption maximum near 220 nm) was selected as the mobile phase eluent which not only properly maintains the charge state of the compounds but also facilitates the indirect UV detection. The indirect UV detection monitors the decrease in UV absorbance of the nitric acid eluent due to the replacement of the nitrate anions by the bisphosphonate drugs eluted into the detector cell. This approach does not require the tedious chemical derivatization procedures.

This paper presents the development and validation results of the IC-indirect UV method for the quantitative analysis of alendronate sodium tablets, etidronate disodium and clodronate disodium in experimental i.v. formulations (in saline solution). Typical validation studies included injection precision, linearity, specificity, recovery, method precision and ruggedness. The performances of several equivalent columns; namely, Waters IC-Pak, Dionex AS7 and AS4A, and Meta-Chem HEMA 1000Q columns are also discussed.

Experimental

Chemicals and reagents

Alendronate sodium (MK-0217, 4-amino-1-hydroxybutane-1,1-bisphosphonic acid monosodium trihydrate salt, $C_4H_{12}NO_7P_2Na{\cdot}3H_2O$, MW = 325.1) of pharmaceutical grade was manufactured by Merck Research Laboratories (Rahway, NJ, USA). Etidronate disodium (1-hydroxyethane-1,1-bisphosphonic acid disodium salt, $C_2H_6O_7P_2Na_2$, MW = 249.99) reference standard was purchased from USP (United States Pharmaco-

peia). Clodronate disodium (1,1-dichloromethane-1,1-bisphosphonic acid disodium tetrahydrate salt, $CH_3Cl_2O_6P_2Na_2 \cdot 4H_2O$, MW = 361.9) was received from Istituto Gentili (Pisa, Italy). Nitric acid (OPTIMA grade) and potassium nitrate (reagent grade) were purchased from Fisher Scientific (Philadelphia, PA, USA). All solvents and reagents were used as received without further purification. Deionized water with at least 18 M-Ohm purified by Milli-Q system was used for mobile phase, sample and standard preparations.

Equipment and assay conditions

Most of the development and validation work was performed on a Dionex 4500i inert chromatographic system equipped with a Spectra–Physics (SP) 100 variable wavelength UV detector. Stainless steel tubing systems such as Hewlett–Packard (HP) 1090 system and SP 8800 were also compared in order to establish ruggedness. A Waters IC–Pak HR anion exchange column (6 μm particle size, 75 mm × 4.6 mm i.d.) was used. The assay conditions for the three drugs are listed in Table 1. The UV detection was set at the wavelengths listed in Table 1 depending on the mobile phase concentrations used (i.e. a longer wavelength was used for higher mobile phase concentrations to avoid a saturated background absorbance which might result in a detector overrange). The polarity of the detector output was reversed. All the analyses were carried out at ambient temperature.

Standard and sample preparations

The standard solution was prepared by dissolving an appropriate amount of reference standard in water to yield the desired assay concentration for each drug (see Table 1). Tablet samples of alendronate sodium were dispersed and stirred in an appropriate volume of water for 30 min to yield a sample solution equivalent to the assay concentration. A portion of the resulting solution was filtered through a Millipore 0.22 μm filter unit and transferred to an HPLC vial for analysis. Etidronate disodium and clodronate disodium i.v. solutions were diluted with water to yield an assay concentration of 0.4 mg ml^{-1} and the resulting solutions were transferred to the HPLC vials for analysis.

Assay procedure

Generally, the system (including column) was first equilibrated with the mobile phase by injecting the standard solution until reproducible injections were observed (about three injections) prior to the sample analysis. Standard and sample solutions were injected directly.

Results and Discussion

Chromatography and indirect UV detection

Alendronate sodium. Under the optimized IC conditions specified in Table 1, alendronate sodium was eluted from the Waters IC-Pak HR column as alendronate anions at a retention time of *ca.* 10 min, as shown in Fig. 2. The selection of 1.6 mM HNO_3 (pH *ca.* 2.5) as an eluent 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. Increasing the nitrate (eluent) concentration resulted in a shorter retention of the compound. The authors have reported previously this chromatographic condition coupled with inverse conductivity detection for the assay of alendronate [14]. Since the conductivity detector is not as popular as the UV detector, we developed the indirect UV detection (see below) by maintaining the identical chromatography for alendronate. We found that the indirect UV detection is more rugged and easier for routine applications.

Table 1
Summary of experimental conditions* for the assay of the bisphosphonate drugs

Compound	Mobile phase	Flow rate (ml min^{-1})	Assay concentration (mg ml^{-1})	Injection volume (μl)	Detection wavelength (nm)
Alendronate	1.6 mM HNO_3	0.5	0.05	25	235
Etidronate	40:60 (v/v) HNO_3–KNO_3 (7.2 mM)	0.8	0.4	50	240
Clodronate	12 mM HNO_3	1.0	0.4	50	245

*The conditions were developed for the Waters IC–Pak HR column performed at ambient temperature.

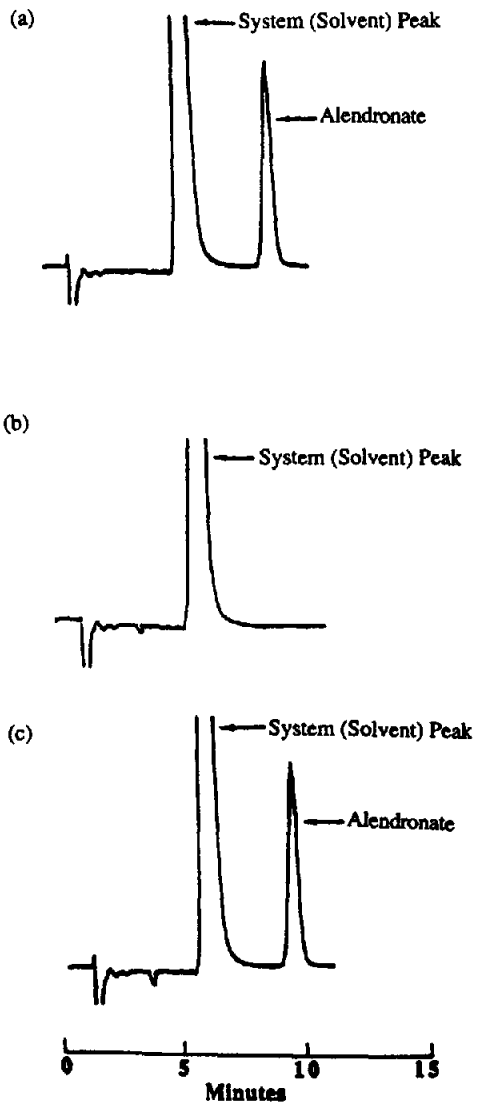

Figure 2
Typical chromatograms of (a) an alendronate sodium standard, (b) a tablet placebo and (c) an alendronate tablet sample.

The indirect UV detection monitored the decrease in UV absorbance of the nitric acid eluent, which exhibited a UV absorption maximum near 220 nm, due to the replacement of the nitrate anion by the eluting analyte. A typical chromatogram shown in Fig. 2 for a 0.05 mg ml^{-1} alendronate standard solution essentially reflected the negative (decreasing) signal detected with inverse polarity. The usable wavelength range was found to be 230 nm–245 nm. Wavelengths above (shorter than) 230 nm resulted in a strong background absorbance rendering a detector over-range. Longer wavelengths yielded a weaker absorption thereby a less sensitive detection signal. A wavelength of 235 nm was selected for alendronate because it provided suitable sensitivity as well as reasonable ease of baseline equilibration (230 nm required longer equilibration time to obtain a stable baseline).

Two equivalent columns were identified which were capable of providing similar chromatographic results as the Waters IC–Pak HR column; namely, the Dionex OmniPac PAX-100 analytical column and the Meta-Chem HEMA 1000Q ion-exchange column. The OmniPac column could generate a sharper peak shape with a shorter retention time than the Waters column. However, it was found that a relatively longer equilibration time was required for the OmniPac column for reproducible results [14]. The HEMA column was also found to be applicable. This column generated a relatively broader peak shape and a poorer resolution (between alendronate and the system peak) than the Waters column.

Etidronate disodium. Etidronate disodium, which does not contain a derivatizable primary amine group, possesses a higher charge state (molecule as a whole) than alendronate. It is generally true that the greater the charge state of the analyte, the later it elutes owing to its higher affinity for the anion-exchange resin. Thus, it requires a 'stronger' eluent (e.g. 7.2 mM nitric acid) to elute etidronate than alendronate on the Waters IC–Pak HR column. We have successfully demonstrated the application of the IC-indirect UV approach for the direct determination of etidronate disodium in tablet dosage form [15]. Using a Waters IC–Pak HR column with 7.2 mM nitric acid eluent, etidronate disodium was eluted as an ionic species (etidronate), and detected by indirect UV 240 nm wavelength, at a retention time of *ca.* 6.3 min without assay interference from the tablet excipients. However, when the same conditions were used to assay the drug formulated in normal saline solution an assay interference from the chloride anions was observed as shown in Fig. 3(a). Under the stated conditions, chloride co-eluted with etidronate and its concentration was about twice as much as the drug (0.4 mg ml^{-1}) rendering the quantitation of the active drug very difficult. Thus, chromatographic modification was necessary for the assay of etidronate disodium in i.v. solution.

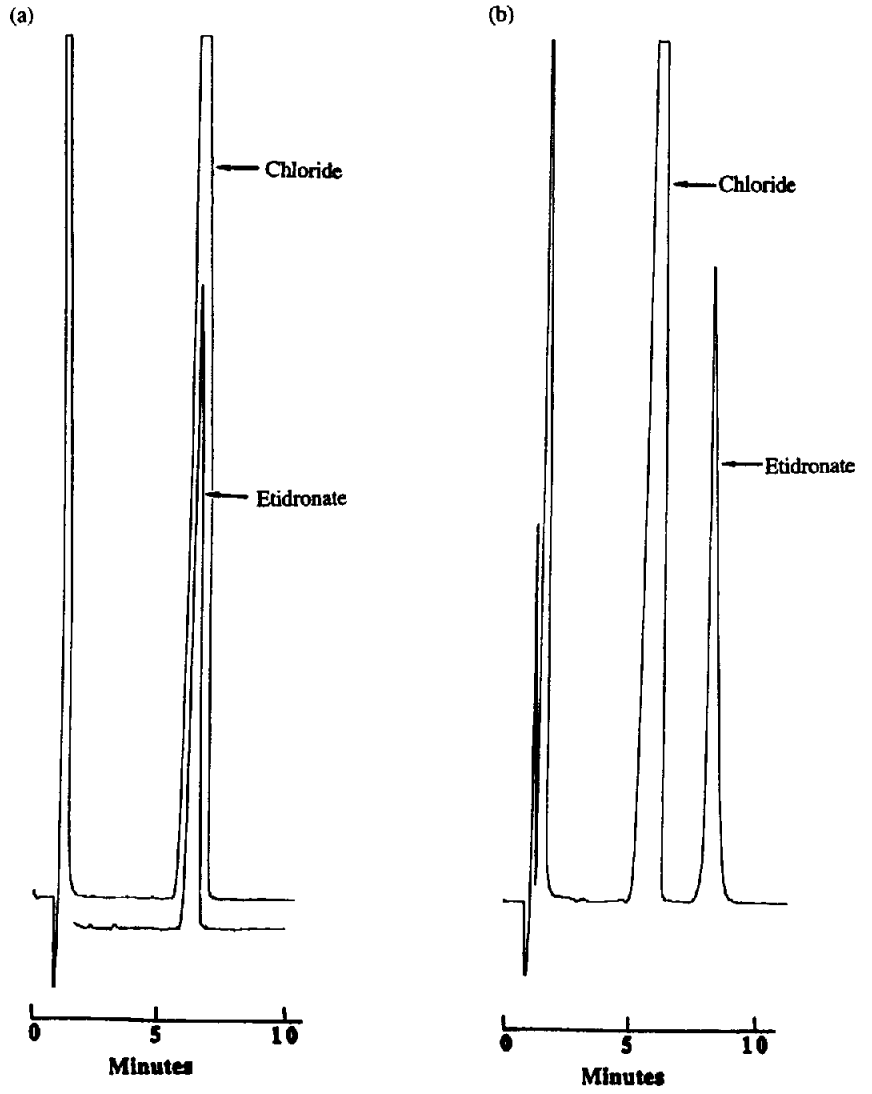

Figure 3
Chromatograms of an etidronate disodium present in normal saline solution assayed by the IC-indirect UV method using a mobile phase of (a) 7.2 mM HNO_3, pH 2.2 where the chloride ions co-elute with etidronate and (b) a mixture of 40:60 (v/v), HNO_3 (7.2 mM)–KNO_3 (7.2 mM), pH 2.7 yielding an excellent resolution between the chloride and etidronate.

It is important to note that the drug possesses multiple ionic properties (second pK_a is about 2.9) and that pH of the eluent can affect the ionic state (and hence the elution) of the drug; i.e. the higher the pH of the eluent, the longer the retention time of the drug. Contrarily, chloride is purely ionic and its elution will not be susceptible to the eluent pH change if the eluent (nitrate) concentration is maintained constant. On the basis of this property, the two co-eluting peaks (chloride and etidronate) could be well resolved (separation factor = 1.33) by slightly increasing the pH of the nitric acid eluent as shown in Fig. 3(b). It was achieved by mixing 7.2 mM nitric acid (pH = 2.2) with a potassium nitrate solution (equal molarity, 7.2 mM) at a ratio of 40:60 (final pH = 2.7) to keep the nitrate concentration unchanged so that the elution of chloride remained nearly constant. This assay condition was optimized and used for drug in i.v. solutions. For informational purposes, the feasible range of mobile phase composition of nitric acid–potassium nitrate was found to be between 60:40 (separation factor = 1.03) and 20:80 (separation factor = 1.63). The amount of potassium nitrate added (synonymous with change in eluent pH) played an important role in the resolution of chloride and etidronate. This observation exemplarily illustrates the

effect of eluent pH modulation on the ion-chromatographic behaviour of an ionizable compound.

Several equivalent columns were investigated for etidronate. The Dionex AS7 and AS4A columns, which exhibit lower anion exchange capacity than the Waters IC–Pak HR column, could provide suitable separation using 1.92 mM nitric acid as an eluent. This column requires a longer equilibration time to obtain reproducible results. The MetaChem HEMA 1000Q column (10 μm particle size, 250 × 4.6 mm i.d.) was also suitable using 9.6 mM HNO_3 as an eluent but it generated a broader peak shape than the Waters IC–Pak HR column. A similar Waters column (IC–Pak, 5 cm long with 10 μm particle size) was also found to be reproducible and suitable for the assay. This column yielded a shorter retention time and a slightly broader peak shape than the IC–Pak HR column under the same conditions.

Clodronate disodium. Similar to etidronate disodium, clodronate disodium also does not contain a derivatizable primary amine group. This compound possesses stronger affinity to the anion exchanger than etidronate disodium under the same elution conditions possibly related to the two chloride substituents (electron withdrawing group) on the bisphosphonate moiety. In order to obtain a reasonably short retention, a 12 mM nitric acid was selected for use on a Waters IC–Pak HR column. Figure 4 illustrates a typical chromatogram of a drug sample prepared in normal saline solution. In this case, the chloride peak does not interfere with the drug of interest and the sample can be readily determined without laborious sample preparation or complicated chromatography. Kosonen has recently reported an ion chromatographic method (using a Dionex IonPac AS7 column) coupled with a post-column derivatization with an iron(III) nitrate solution for the UV detection of clodronate disodium bulk material and oral dosage forms [11]. It is felt that our approach of indirect UV detection is more time saving since no derivatization reaction is required.

Equivalent columns described for etidronate were also investigated for clodronate with similar conclusions.

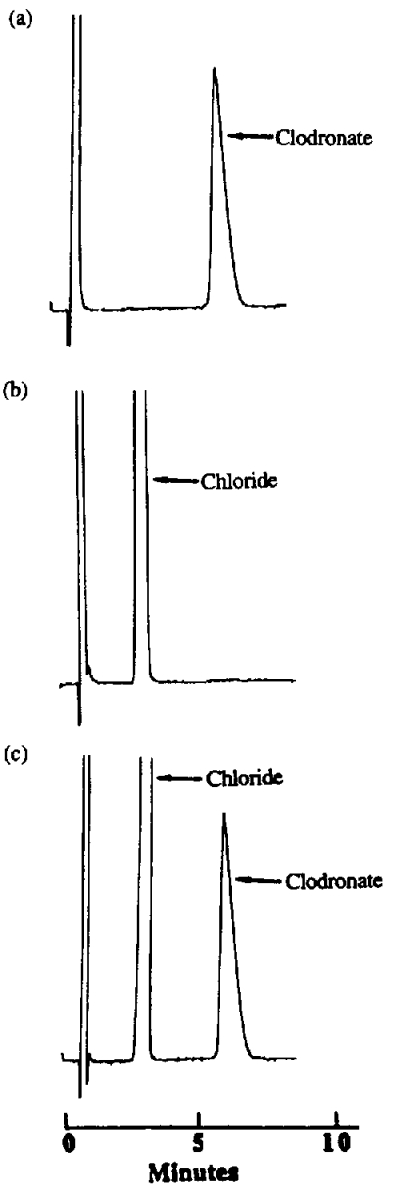

Figure 4
Typical assay results of (a) a clodronate disodium standard, (b) a saline solution (placebo) and (c) a clodronate i.v. solution.

Assay validation

Injection precision. Injection precision was performed by making 10 replicate injections of the standard solutions of the corresponding drugs dissolved in water. All three assays showed satisfactory results. For alendronate (0.05 mg ml^{-1}, 25 μl injection), RSD = 0.64% by peak height and RSD = 1.28% by peak area were obtained. The peak height measurements showed a better precision in many instances, therefore, it was utilized for the assay. For etidronate (0.4 mg ml^{-1}, 50 μl injection), the RSD by peak area was 0.76% and by peak height was 0.86%. Peak area and peak height measurements showed comparable

precision. For clodronate (0.4 mg ml^{-1}, 50 μl injection), the RSD by peak area was 0.36% (peak height showed comparable results).

Linearity. The detector responses were found to be linear for the three assays. The assay for alendronate showed a correlation coefficient, $R^2 = 0.999$ for the concentration range of 40–160% of the method concentration. An inconsequential non-zero intercept was observed in some cases in the linearity plot which was found to be insignificant to the accuracy of the measurement at the method concentration of 0.05 mg ml^{-1} (i.e. intercept/signal counts at assay concentration <2%). For etidronate and clodronate, a correlation coefficient of 1.0 for the concentration range of 20–200% of the method concentration was obtained in both cases. Peak height measurements showed slightly less linear data, therefore, peak area was selected for the assay for these two drugs.

Limit of detection. Because of the similar detection mechanism (replacement of nitrate ions) for all the three drugs, the limits of detection (LOD) for these bisphosphonates exhibited no significant difference. Under the method conditions for each drug, LOD was found to be about 1.0×10^{-3} mg ml^{-1} at a signal-to-noise ratio, S/N = 4. This concentration represented a 0.25% LOD relative to the nominal assay concentration (0.4 mg ml^{-1}) for etidronate and clodronate and a 2% LOD for alendronate which required a lower assay concentration (0.05 mg ml^{-1}) due to the low formulation potency. The existing method conditions suffer from detecting a level of 0.1%, which is a common goal to detect impurities or degradation products, of the parent compound present in the formulation. The detection signal can be extended to a more sensitive degree by slightly modifying the injection volume and detection wavelength.

Recovery. The recovery experiment was performed by spiking aliquots of a stock solution of the drug into a placebo (tablet or normal saline) in duplicate followed by an appropriate dilution to yield amounts of drug equivalent to 50%, 75%, 100%, 125% and 150% of the potencies of formulations. Table 2 summarizes the recovery results for the three drugs prepared in tablet form or i.v. solution. Recoveries for alendronate, etidronate and clodronate from placebos were all satisfactory. It is thus reasonable to conclude that the IC-indirect UV method is accurate without assay discrepancy, which might incur from different detector responses between the standard and sample solutions, for the three bisphosphonate drugs formulated in tablets or i.v. solutions.

Method specificity. For alendronate, method specificity utilizing the Waters IC–Pak HR column was demonstrated by the separation of the drug from its amino-hydroxyl-propyl analogue (relative retention time, RRT, of 1.16) and its thermal decomposition degradates obtained by melting alendronate at 260°C (three late eluting peaks at RRT = 1.55, 1.84 and 3.23). The degradation products induced by melting were generated under unrealistic high temperature condition for the purpose of illustrating method specificity only. Identification of these thermal decomposition products was not pursued. The method was also specific against the tablet placebo formulations (Fig. 2b). No bias was evidenced in each case under the described chromatographic conditions. For etidronate and clodronate, the i.v. placebo does not interfere with the drug chromatographically as shown in Figs 3 and 4. It is thus concluded that the IC-indirect UV method in this study is specific for the assay of drugs in dosage forms.

Method precision. Method precision for the three assays was determined by analysing 10

Table 2
Recovery data for alendronate, etidronate and clodronate from spiked placebo

Compound	Dose potency	Recovery* (%)	RSD† (%)
Alendronate	2.5 mg tablet	100.4	1.8
Etidronate	10 mg ml^{-1} i.v.	100.1	1.9
Clodronate	10 mg ml^{-1} i.v.	100.6	2.0

*Average recovery of 50, 75, 100, 125 and 150% concentration levels of the dose potency in duplicate.
†$n = 10$.

Table 3
Method precision data for the bisphosphonate drugs in tablet form or i.v. solution assayed by two analysts with the IC-indirect UV method

Compound	Analyst I		Analyst II	
	Claim* (%)	RSD (%)	Claim (%)	RSD† (%)
Alendronate (2.5 mg tablet)	99.3	1.5	100.0	1.9
Etidronate (10 mg ml^{-1} i.v.)	100.3	0.6	100.9	0.4
Clodronate (10 mg ml^{-1} i.v.)	100.8	0.9		

*The % claim reported is an average of 10 replicate samples.
†$n = 10$.

replicate samples (tablets or i.v. solutions) by two analysts. The results, which were generated with both Dionex LC (Analyst I) and HP 1090 LC (Analyst II) systems for establishing method ruggedness, are summarized in Table 3. Satisfactory precision data for alendronate tablets, etidronate i.v. solutions and clodronate i.v. solutions were obtained by both analysts indicating excellent method precision.

Method ruggedness testing. The data listed in Table 3 also indicated the ruggedness of the IC-indirect UV method which could be reproduced independently by two individual analysts using two different instruments with similar results. The ruggedness testing was more intensively investigated for the routine assays by addressing the column-to-column reproducibility. A batch of five columns was evaluated for the three assays by injection precision and linearity experiments by two analysts using different instruments. Results were reproducible. It was also found that no retention time drift was observed for at least 50 continuous injections. The column-to-column variation was found to be negligible under these IC conditions. Furthermore, the lifetime of the column was found to be acceptable after at least 500 injections. Based on this information, it was concluded that the method was rugged for the routine assay of these three bisphosphonate drugs in the said dosage forms since no special instrument or procedure was required.

Conclusions

The IC-indirect UV method using a Waters IC–Pak HR column has been validated and shown to be precise, accurate, specific, rugged and suitable for the assay of alendronate sodium, etidronate disodium and clodronate disodium in tablets or i.v. formulation. Several equivalent columns such as the Dionex AS7, AS4A, the MetaChem HEMA 1000Q, and the Waters IC–Pak have been evaluated. The use of nitric acid as an eluent can facilitate the chromatography (without interference from the sample excipients) and the indirect UV detection for the samples tested in this study. One exception is that chloride anions (saline solution) co-eluted with the etidronate peak rendering the quantitation of the active drug difficult. The two co-eluting peaks can be well resolved by slightly increasing the pH of the nitric acid eluent with a potassium nitrate solution (equal molarity as the nitric acid) to keep the nitrate concentration constant. This novel chromatographic method can offer a direct measurement of the active drugs without the need for the tedious chemical derivatization procedures. Thus, it is relatively simple and time-saving. From our perspective, the ease of assay procedure should be beneficial for the analysis of the bisphosphonate class of drugs other than the compounds discussed in this study, especially for similar compounds without a derivatizable functional group.

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