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Short Communication

Determination of etidronate disodium tablets by ion chromatography with indirect UV detection

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Introduction

Etidronate disodium belongs to the bisphosphonate class of drugs which have important therapeutic indications in the treatment of a variety of bone diseases such as hypercalcemia of malignancy, Paget's disease and osteoporosis [1, 2]. The molecular structure of etidronate disodium is illustrated in Fig. 1. Development of a chromatographic assay for this compound is challenging due to the lack of a detectable chromophore for conventional UV or fluorescence detection. The current assay method specified in the United States Pharmacopeia (USP) is based on titration with thorium–cyclohexylenedinitrilotetraacetic acid complex [3].

A very limited number of chromatographic methods for the direct quantitative analysis of etidronate disodium have been reported in the literature. A gas chromatographic method with flame ionization detection has been applied to the assay of etidronate disodium [4]. A method using high-performance ion chromatography in conjunction with a post-column reaction with molybdenum–ascorbate has been reported for some bisphosphonic acids [5]. There are few liquid chromatographic methods with direct detection for bisphosphonate drugs published in the literature. Chester *et al.* have reported an ion-exchange chromatographic method with an on-line flame photometric detection for dichloromethylene diphosphonate [6], Forbes *et al.* described the use of an inductively coupled plasma (ICP) detector for specific phosphorus detection for etidronate disodium [7], and Tsai *et al.* described the use of an ion chromatographic method with conductivity detection for alendronate [8]. These detection devices are generally not popular in the routine pharmaceutical analysis laboratories.

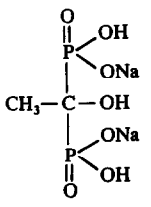

Figure 1
Molecular structure of etidronate disodium.

The method described here is capable of the direct quantitation of etidronate disodium tablets based on the use of single-column ion chromatography with indirect UV detection (IC–indirect UV method) which monitors the decreasing (negative) signals of the eluent (dilute nitric acid) and obviates the need for the tedious chemical derivatization procedures. A similar approach has been reported using post-column indirect fluorescence detection with aluminum–morin reagent for the assay of some phosphorus oxo acids including etidronate disodium [9]. The developed IC–indirect UV method is more rugged and easier to apply for the routine quantitative assay of etidronate disodium tablets.

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This paper presents the development and validation of the IC–indirect UV method for the assay of etidronate disodium tablets. Typical validation studies included injection precision, linearity, specificity, recovery, method precision and ruggedness. The suitability of applying the IC–indirect method for the assay of this compound and other related drugs is also discussed.

Experimental

Chemicals and reagents

Etidronate disodium (1-hydroxyethane-1,1-bisphosphonic acid disodium salt, $C_2H_6Na_2O_7P_2$, MW = 249.99) reference standard was purchased from the USP. All solvents and reagents were used as received without further purification. Nitric acid (OPTIMA grade) was purchased from Fisher Scientific. Deionized water with at least 18 M Ω-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 the ruggedness testing. A Waters IC-Pak HR anion-exchange column (6 μm particle size, 75 mm × 4.6 mm i.d.) was used. A mobile phase of 7.2 mM nitric acid was delivered at a flow rate of 0.8 ml min^{-1}. The analysis was carried out at ambient temperature with 50 μl injection of 0.4 mg etidronate disodium ml^{-1} in water as the method concentration. The UV detection was set at 240 nm with inverse polarity of the detector output.

Standard and sample preparations

The standard solution was prepared by dissolving 40 mg of etidronate disodium USP reference standard in 100 ml of water. Tablets of 400 mg potency were dispersed and stirred in an appropriate volume (content uniformity: one tablet in 100 ml, composite assay: 10 tablets in 1000 ml) of water for 30 min to yield a stock sample solution. A 10 ml volume of this stock solution was further diluted to 100 ml with water and a portion of 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 (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 samples analysis. Standard and sample solutions were injected directly.

Results and Discussion

Chromatography and assay optimization

IC conditions and indirect UV detection. Under the optimized IC conditions using a Waters IC-Pak HR column, etidronate disodium was eluted as an ionic species (etidronate) at a retention time of *ca* 6.3 min as shown in Fig. 2. An unknown peak was also observed at *ca* 3.3 min with 0.7% and <0.1% (by peak area) present in the standard and sample, respectively. The IC conditions using an eluent of 7.2 mM HNO_3 was found to be suitable to provide a reasonably short retention (or ion-exchange process) for etidronate which was present predominantly as a divalent (−2) charge species. It was also found that such an eluent facilitates the indirect UV detection (see

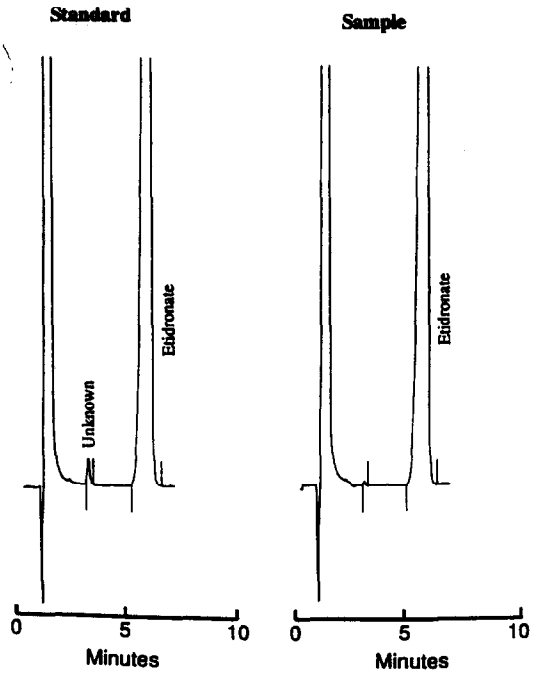

Figure 2
Typical chromatograms of etidronate disodium standard and sample.

below). Increasing the nitrate (eluent) concentration resulted in a shorter retention of the compound.

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 anions by etidronate eluted into the detector cell. The chromatograms shown in Fig. 2 essentially reflected the negative (decreasing) signal with an inverse polarity of the detector output. The usable wavelength range was 230–245 nm. Wavelengths above (shorter than) 230 nm resulted in a strong background absorbance rendering a detector overrange. Longer wavelength yielded a weaker absorption thereby a less sensitive detection signal. A wavelength of 240 nm was selected which provided suitable sensitivity as well as a reasonable ease of baseline equilibration (235 nm required longer equilibration time to obtain a stable baseline).

Equivalent columns. Several equivalent columns were identified to be capable of providing similar results to the Waters IC-Pak HR column. 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 HNO_3 as an eluent. The reproducibility of these columns was not satisfactory owing to the retention time drift (*ca* 20%) observed in some cases. 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 (*ca* 2 min) and a slightly broader peak shape than the IC-Pak HR column under the same conditions.

Selection of diluent. Water was selected as the standard and sample diluent for simplicity. The selection of water as a diluent for the assay of tablets was justified by the accuracy of the recovery data (see 'Validation' below).

Validation

Injection precision. Injection precision was performed by making replicate (n = 10) 50 μl injections of a 0.4 mg ml^{-1} standard solution. Results were satisfactory with RSD = 0.76% by peak area and RSD = 0.86% by peak height. Peak area and peak height measurements showed comparable precision.

Linearity. The detector responses to a range of 50–150% of the method concentration of 0.4 mg ml^{-1} were determined to be linear with R^2 = 1.000 for the peak area vs concentration plot. The peak height vs concentration plot was less linear with R^2 = 0.996. Peak area measurements were therefore utilized to determine etidronate disodium in this method. The linear dynamic range was also extended to 0.066–0.66 mg ml^{-1} for dissolution testing. A correlation coefficient of 0.999 was obtained.

Limit of detection. Limit of detection (LOD) was established at a level of 1×10^{-3} mg ml^{-1} with S/N = 4. The LOD can be extended to a more sensitive degree by slightly modifying the method conditions such as increasing injection volume and shorter detection wavelength.

Method specificity. Limited experimentation was performed to demonstrate the specificity due to the lack of information regarding the formulation (a placebo tablet was not available). According to the literature, etidronate disodium tablets contain magnesium stearate, microcrystalline cellulose and starch as excipients. No assay interference from these excipients was evident indicating that the method was specific against components of the tablet formulation.

Recovery. Because of the unavailability of the placebo for the normal spiking experiment, the recovery was demonstrated by the standard addition experiment to ensure the absence of assay bias due to the tablet matrix effects. It was performed by adding 80, 100 or 120% of the standard solution into an aliquot of the stock sample solution (composite assay) followed by an appropriate dilution. An aliquot of the same stock sample solution without spiking with the drug was used as a control for calculation. Results were satisfactory with an average recovery of 100.5%. Thus, it is reasonable to conclude that the assay is accurate without bias when water was used as a diluent.

Method precision. Method precision was

determined by analysing the content uniformity of 10 replicate tablets. Satisfactory uniformity with 2.1% RSD was obtained yet the contents were higher than expected (107.7% claim which was still within acceptable range as required by USP). For comparison purpose, the composite assays (n = 10 tablets) in duplicate were performed with results of 110.1% claim and 109.0% claim. The data of composite assays agreed well with the content uniformity results.

Method ruggedness testing. The ruggedness testing was investigated based on the performance of different instruments and the column reproducibility. The method could be successfully reproduced using SP 8800 LC and HP 1090 LC systems for the routine assay by different analysts with similar results. The success in dissolution testing using the identical IC–indirect UV conditions with another SP 8800 LC system also suggested that the method is rugged. Perhaps the most critical issue was the column reproducibility in terms of retention time drift and column-to-column variation. A batch of four columns was evaluated by continuous injections with no retention time drift observed for at least 50 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 etidronate disodium tablets 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 etidronate disodium tablets. This novel chromatographic method can offer a direct measurement of the active drug without the need for the tedious chemical derivatization procedures. Thus, it is relatively simple and time-saving. The ease of assay procedure has prompted us to apply this method for the analysis of the bisphosphonate class of drugs such as alendronate, clodronate and pamidronate which will be reported separately.

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