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Analysis of pamidronate disodium in pharmaceutical dosage forms by ion chromatography

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Abstract

Pamidronate disodium, an amino bisphosphonate, is a potent new inhibitor of osteoclastic bone resorption. A simple assay method using ion chromatography has been developed for this compound and its dosage forms. The chromatographic system consists of an hydroxyethyl methacrylate polymer column with quaternary amine functionalities, nitrate anion eluent and a refractive index detector. The active ingredient was separated from possible impurities such as phosphate and phosphite ions, and β-alanine. The different dosage forms analyzed include ampul solutions, lyophilized powders, tablets and capsules. Analytical and chromatographic criteria were met exceedingly well for the analysis of the active ingredient and the different dosage forms.

1. Introduction

Pamidronate disodium pentahydrate or disodium-3-amino-1-hydroxy-propylidene-1,1-bisphosphonate pentahydrate (APD, Aredia™, Ciba-Geigy, Suffern, NY, USA) (Fig. 1), belonging to a group of chemical compounds known as bisphosphonates, is a potent inhibitor of osteoclastic bone resorption [1]. It has been used clinically for the treatment of tumor-related hypercalcemia [2,3] and Paget's disease [4].

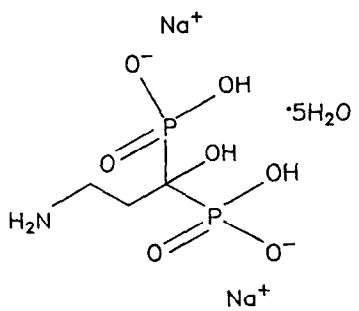

Fig. 1. Structure of APD.

APD has pK_a values ranging from 1.7 to 11.5 [5], thus it exists as an ionic specie over a broad pH range. The molecule has no detectable chromophore, making development of a chromatographic method a challenging task. The current methods that have been used in our laboratory are reversed-phase HPLC with pre-column derivatization with fluorescamine and isotachophoresis (ITP) for dosage and stability controls. The HPLC method requires a derivatization step to introduce a chromophore for analytical detection and the ITP technique needs specialized equipment. Both methods are slow and are sensitive to indefinite number of variables. Published analytical methods for bisphosphonates have similar requirements of derivatization pro-

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cedure and/or specialized instrumentation [6–10].

A simple method using ion chromatography to assay APD active ingredient and its pharmaceutical dosage forms which include injectables, capsules and tablets, has been developed. It is carried out with commercially available and conventional HPLC equipment. Samples are dissolved in water, filtered and appropriate amounts are injected onto the column. Analytical and chromatographic criteria are met well for both the active ingredient and its dosage forms.

2. Experimental

2.1. Instrumentation, reagents and samples

Instrumentation and reagents

Waters (USA) HPLC equipment included a Model 510 pump, a 710B automatic sampler, a 720 system controller, a 410 differential refractometer and a column heating chamber. An Alltech (USA) Universal Anion column was used, packed with polyhydroxyethyl methacrylate-based macroporous polymer with quaternary amine functionalities (270001) and phthalate counterion. An Alltech guard cartridge (38106) with a prefilter was connected before the column. The mobile phase was 5 m*M* potassium nitrate adjusted to pH 3.5 with nitric acid. It was filtered through a 0.45-μm membrane filter and degassed before use. The flow-rate used was 1.2 ml/min. The column and the detector temperatures were maintained at 35°C throughout the run. The detector was set at a sensitivity of 256 and adjusted accordingly depending on the amount of sample injected.

Sample and standard preparation

The samples analyzed were the APD active ingredient and the different dosage forms of APD consisting of lyophilized powders, enteric coated pellets, enteric coated tablets and ampul solutions.

For the assay, solid samples were accurately weighed, dissolved and diluted with water to obtain test solutions with APD concentration of 3.0 mg/ml (anhydrous basis). Ampul solutions, 1 mg/ml (anhydrous basis), were used as is.

For the chromatographic evaluation and method validation, samples tested include the active ingredient and the different dosage forms, the active ingredient spiked with theoretical impurities and the active ingredient spiked into placebos of the different dosage forms.

Standard test solutions were prepared from the APD active ingredient reference standard. Required amounts were accurately weighed into a volumetric flask, dissolved and diluted to volume with water to contain either 3.0 mg/ml or 1.0 mg/ml APD (anhydrous basis) depending upon the sample being analyzed.

2.2. Procedure

The column was equilibrated with the mobile phase and replicate 20-μl injections of APD standard solution were made onto the column. The peak responses were recorded using an on-line computer (HP9153C). When the relative standard deviation of five injections of the standard solution was not more than 2.0%, and the tailing factor [11] of the APD peak was not more than 2.0, analysis was started.

A 20-μl volume of sample or standard test solutions was injected onto the column and the chromatograms were recorded and analyzed using an on-line computer.

3. Results and discussion

The primary goal was to develop a simple and reliable chromatographic assay method which can analyze APD in the different pharmaceutical dosage forms, free from placebo interference, and completely resolved from the synthetic impurities/degradation products such as β-alanine, phosphate and phosphite ions.

Initial work involved evaluation of several reversed-phase and ion-exchange columns in different modes of separations such as ion pairing, ion exchange and ion exclusion. Shown in Fig. 2a was the most promising result from these

initial experiments. Separation of APD, phosphate and phosphite was obtained using a system which consists of a polymethacrylate strong-anion exchange column, a 2 m*M* nitric acid eluent and a post-column detection set-up with ferric nitrate as the reactant. With this chromatographic system however, APD was not baseline resolved from the phosphate ion. Optimization of the system to completely resolve APD from the phosphate ion ($R_s \geq 1.5$) by varying the pH, ionic concentration and addition of organic modifier, was unsuccessful. Finally, replacement of the polymethacrylate column with a polyhydroxymethacrylate column (also with quaternary amine functionalities), and using 5 m*M* nitrate eluent at pH 3.5 with a refractive index detector, complete separation of APD, phosphate and phosphite was achieved as shown in Fig. 2b. The resolution of APD and phosphate was significantly better with $R_s \simeq 1.9$ and the other chromatographic figures of merit are all reasonably good (see Table 1). The improvement in selectivity with this system has been attributed to an interaction occurring between the analytes and the hydroxyethyl moiety of the polymer packing.

3.1. Nitrate eluent concentration

Anion eluents such as acetate, chloride and citrate were evaluated along with the nitrate anion. The best separation of APD, phosphate and phosphite was obtained with the nitrate anion as eluent. A linear response of the capacity factor of APD, phosphate and phosphite was obtained as the nitrate eluent was increased from *ca.* 1 to 10 m*M* (Fig. 3).

For the assay method, 5 m*M* nitrate eluent concentration was selected for speed, excellent separation and peak profiles, and free from interference.

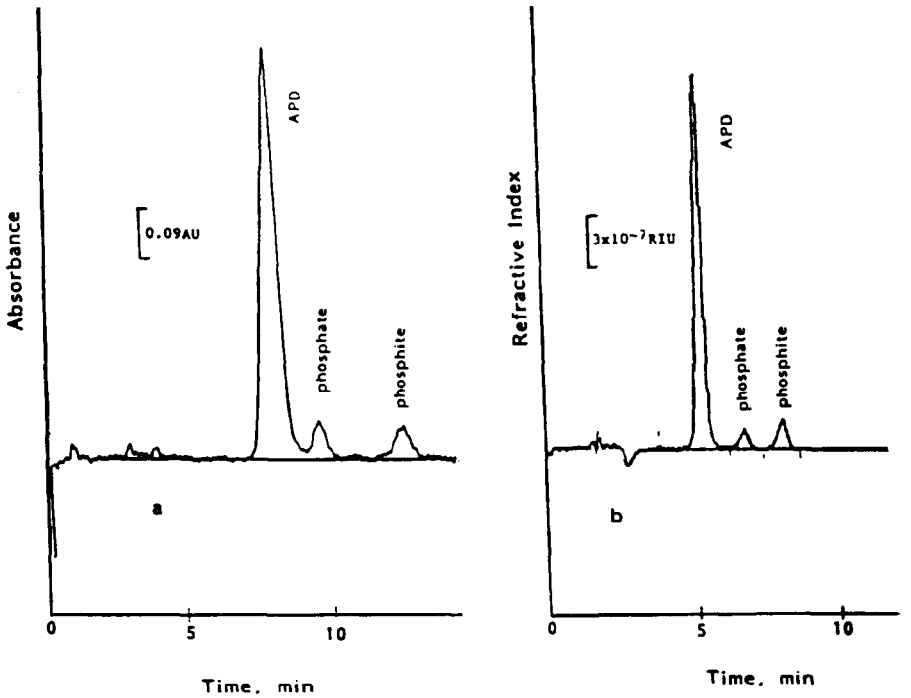

Fig. 2. Separation of APD, phosphate and phosphite on (a) methacrylate strong anion-exchange column (Super-sep Anion) with 2 m*M* HNO_3 eluent and UV detection at 300 nm after post-column reaction with $Fe(NO_3)_3$ and (b) hydroxyethyl methacrylate strong anion-exchange column (Universal Anion) with 5 m*M* KNO_3 eluent adjusted to pH 3.5 with nitric acid and refractive index detection.

Table 1
Chromatographic figures of merit

	APD	Phosphate	Phosphite
System precision			
R.S.D., %	0.25	2.20	1.58
Capacity factor, k'	1.94	2.75	3.50
S.D.	0.01	0.01	0.01
Tailing factor, T	1.0	0.98	0.95
S.D.	0.08	0.06	0.06
Resolution, R_s	1.9(a)	1.9(b)	–
S.D.	0.08	0.11	–

The system precision, as R.S.D. (%), was obtained from peak area response of six injections of standard solution containing APD, phosphate and phosphite. The capacity factor, k' (using water as the unretained peak), the tailing factor, T and the resolution, R_s between APD and phosphate (a), and between phosphate and phosphite (b), were determined according to ref. 11. Standard deviation (S.D.) was calculated for each figure of merit, with $n = 6$.

3.2. Eluent pH

The effect of eluent pH on the capacity factors, k', of APD, phosphate and phosphite at fixed eluent concentration of 5 mM nitrate, are shown in Fig. 4. Between pH 3 and 6, the k' of phosphate and phosphite remain essentially constant. A decrease below pH 3 was observed for phosphate and an increase above pH 6 was observed for both phosphate and phosphite. For APD k' increased with increasing pH with apparent changes in slopes of k' at several pH regions.

Since both the nitrate eluent and the quaternary ammonium groups (of the column) should be essentially ionized within the pH range studied (between 2.5 and 9.0), the retention behavior of APD, phosphate and phosphite at a specified pH, must be influenced primarily by their ionization constants. APD has pK values of 1.7, 2.7, 6.3, 10.8 and 11.5, with a zwitterion noted between pH 2 to 3 [5]. Orthophosphoric acid has pK values of 2.12, 7.21 and 12.67, and phophorous acid has values of 2.00 and 6.59 [12]. Between pH 3 and 6, the k' values of phosphate and phosphite were virtually unchanged indicating the predominance of one solute species, most likely the singly charged anion. Below pH 3, the

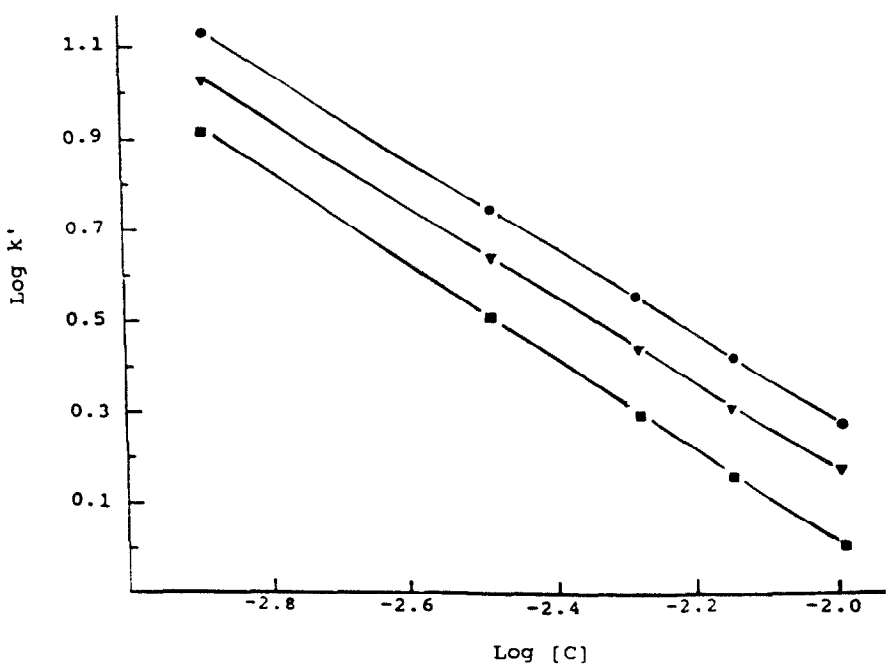

Fig. 3. Effect of eluent concentration, C: log k' *versus* log [C] for APD (■), phosphate (▼) and phosphite (●).

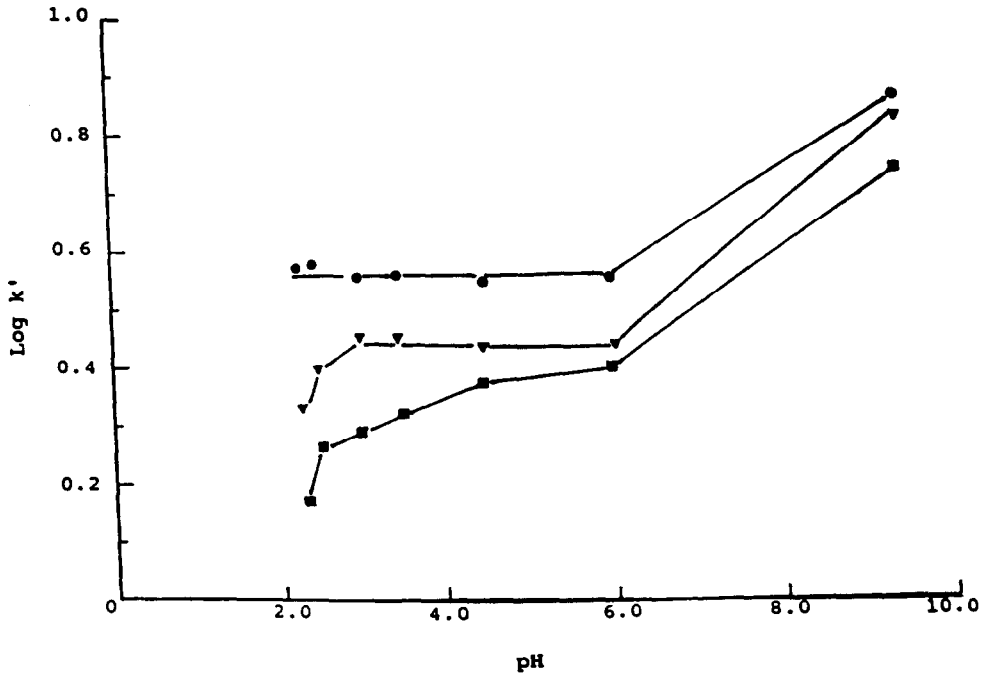

Fig. 4. Effect of pH: log k' *versus* pH for APD (■), phosphate (▼) and phosphite (●).

influence of the neutral species was apparent from a decrease in k', specifically for the phosphate. Above pH 6, the influence of the doubly charged species was similarly apparent from a drastic increase of k' for both phosphate and phosphite. For APD, k' increased with increasing pH and the slope of k' appeared to change at several pH regions. The different ionic species seem to exert influence on the retention behavior of APD, with one species predominating within certain pH range. At pH 3.5, which was the pH selected for the assay method, APD behaved as

Table 2
Analysis of APD in pharmaceutical products

Product (lot No.)	n	Claim potency	Found ± S.D.
Active ingredient (800388)	6	100.0%	99.6% ± 0.4
Ampul solutions (12/322/1)	6	5 mg/5 ml	4.9 mg/5 ml ± 0.04
Lyophilized powder (14/012/1)	6	30 mg	30.0 mg ± 0.2
Enteric-coated pellets in capsule (14/628/1)	6[a]	75 mg	75.8 mg ± 0.6
Enteric-coated tablets (PR 375-94)	10	150 mg	150.2 mg ± 0.9

[a] Two sets of samples of the enteric-coated pellets in capsule (3 and 3) were run on different days.

a singly charged anion. Presumably, at pH 3.5 the different ionic species including the zwitterion produced an effective net charge approaching minus one.

The choice of pH 3.5 for the assay method was made for the same reason as the choice of eluent concentration, *i.e.*, for speed, excellent separation and peak profiles and free from interference. Furthermore at pH 3.5, buffer was not necessary since a half unit change in pH from 3.5 did not significantly affect the chromatography nor the analytical results of APD, phosphate and phosphite.

3.3. Analytical application

The assay values obtained for the active ingredient and the different dosage formulations of APD are shown in Table 2, with typical chromatograms displayed in Fig. 5. A minimum of six samples of the different dosage forms were analyzed. The precision of the data and the

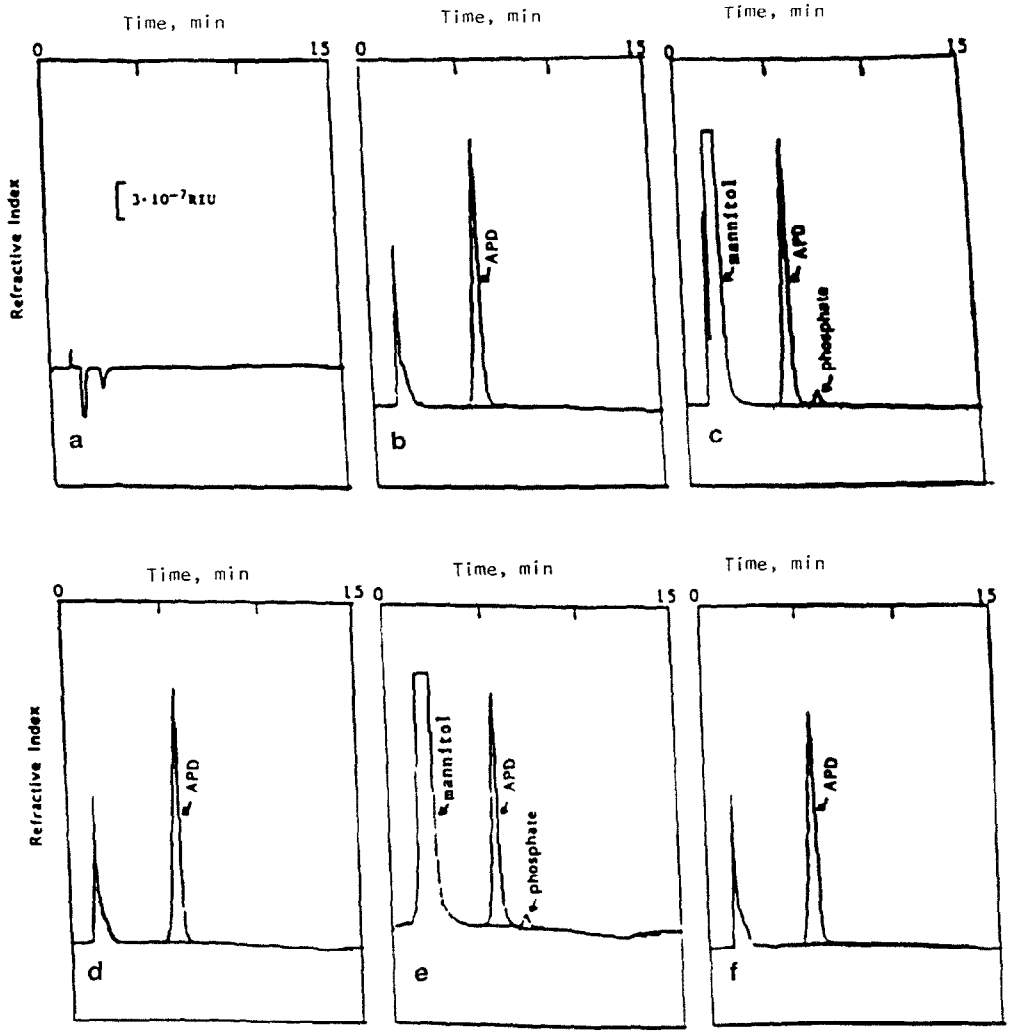

Fig. 5. Typical chromatogram of (a) water blank, (b) active ingredient, (c) lyophilized powder, (d) enteric coated tablet, (e) ampul solution and (f) enteric coated pellets in capsule. RIU = Refractive index units

agreement between the claim potency and the amount found were excellent.

The method was validated for the APD active ingredient and its dosage forms, using the analytical criteria of precision, accuracy and linearity.

Potential by-products and the formulation components of the different dosage forms which include isotonic and pH adjustors, binders, antifoaming and film-forming agents, anti-adherents and opacifiers did not interfere with the assay procedure for APD.

At least six columns from the same manufacturer have been tested so far and all have shown comparable results. On-line filter and a pre-column were always used to prolong column life.

4. Conclusions

A simple analytical method for APD, an amino bisphosphonate, has been developed using anion-exchange HPLC. It is carried out with commercially available and conventional HPLC equipment. The chromatographic system consists of a macroporous polymer column with quaternary amine functionalities, nitrate anion eluent and refractive index detector. APD active ingredient and the different dosage forms have been analyzed with excellent analytical and chromatographic results.

5. Acknowledgements

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