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Short communication

Stability indicating ion chromatography method for the simultaneous determination of ibandronate sodium drug substance and its impurities

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ABSTRACT

A simple and sensitive ion chromatography method has been developed for the simultaneous assay of ibandronate sodium drug substance and the determination of its impurities. The separation was achieved on Allsep™ anion column 150 mm × 4.6 mm, 7 μm particle diameter. The mobile phase consisted of 1% (v/v) aqueous formic acid and acetone 98:2% (v/v); flow rate 1.0 ml min^{-1} at ambient temperature. The analytes were monitored by conductometric detector. The drug substance was subjected to stress conditions of hydrolysis, oxidation, photolytic, thermal and humidity degradation. Considerable degradation was achieved only under oxidative conditions. Mass balance was demonstrated in all stress conditions. The method was validated for specificity, precision, linearity, solution stability and accuracy. The limits of detection (LOD) and limits of quantification (LOQ) for impurities were in the range of 0.36–0.80 μg ml^{-1} and 1.00–2.40 μg ml^{-1}, respectively. For ibandronate LOD was 38 μg ml^{-1} and LOQ was 113 μg ml^{-1}. The average recoveries for impurities and ibandronate were in the range of 99.0–103.1% and the method can be successfully applied for the routine analysis of ibandronate sodium drug substance.

1. Introduction

Ibandronate sodium (1-hydroxy-3-(methylpentylamino)propy lidene]bisphosphonic acid monosodium monohydrate) is the sodium salt of ibandronic acid, a synthetic nitrogen-containing bisphosphonate drug. This new third generation bisphosphonate is used to treat patients with bone disease like Paget's disease, malignant hypercalcemia and postmenopausal osteoporosis [1,2].

For the quantification of impurities and assay of ibandronate, few analytical methods have been reported in literature [3–6]. Indirect fluorescence detection was used in a high performance ion exchange chromatographic method based on the formation of the non-fluorescent Al^{3+}-ibandronate complex after post-column addition of the fluorescent Al^{3+}-morin reagent [3]. Ibandronate was determined by high performance ion exchange chromatography with UV detection at 240 nm after complex formation with Cu^{2+} ion [4]. Ibandronate and related impurities (phosphate, phosphite and 3-(*N*-methylpentylamino)propionic acid) were determined by capillary zone electrophoretic method with indirect detection at 254 nm [5], and by ion pair reversed phase high performance liquid chromatography (RPIC) with evaporative light scattering detection (ELSD) [6], respectively. The limit of detection (LOD) values reported for phosphate, phosphite, 3-(*N*-methylpentylamino)propionic acid and ibandronate were 5 μg ml^{-1}, 3 μg ml^{-1}, 10 μg ml^{-1} and 176 μg ml^{-1}, linearity ranges were 92–460 μg ml^{-1}, 24–384 μgml^{-1}, 23–372 μg ml^{-1} and 352–1760 μg ml^{-1} [6]. For a review see [7].

In the synthetic pathway of ibandronate sodium in APL research laboratory, methanesulfonic acid is a potential process impurity along with phosphate, phosphite and 3-(*N*-methylpentylamino)propionic acid. The aim of this study was to develop a simple, sensitive and direct ion chromatographic method with conductometric detection for the simultaneous determination of ibandronate and all the above listed potential process impurities together with methanesulfonate.

2. Experimental

2.1. Chemicals, reagents and samples

The standard and samples of ibandronate sodium drug substance and 3-(*N*-methylpentylamino)propionic acid, were procured from APL Research Centre (a division of Aurobindo Pharma Ltd., Hyderabad). Analytical reagent (AR grade) orthophosphoric acid, phosphorous acid, methanesulfonic acid, formic

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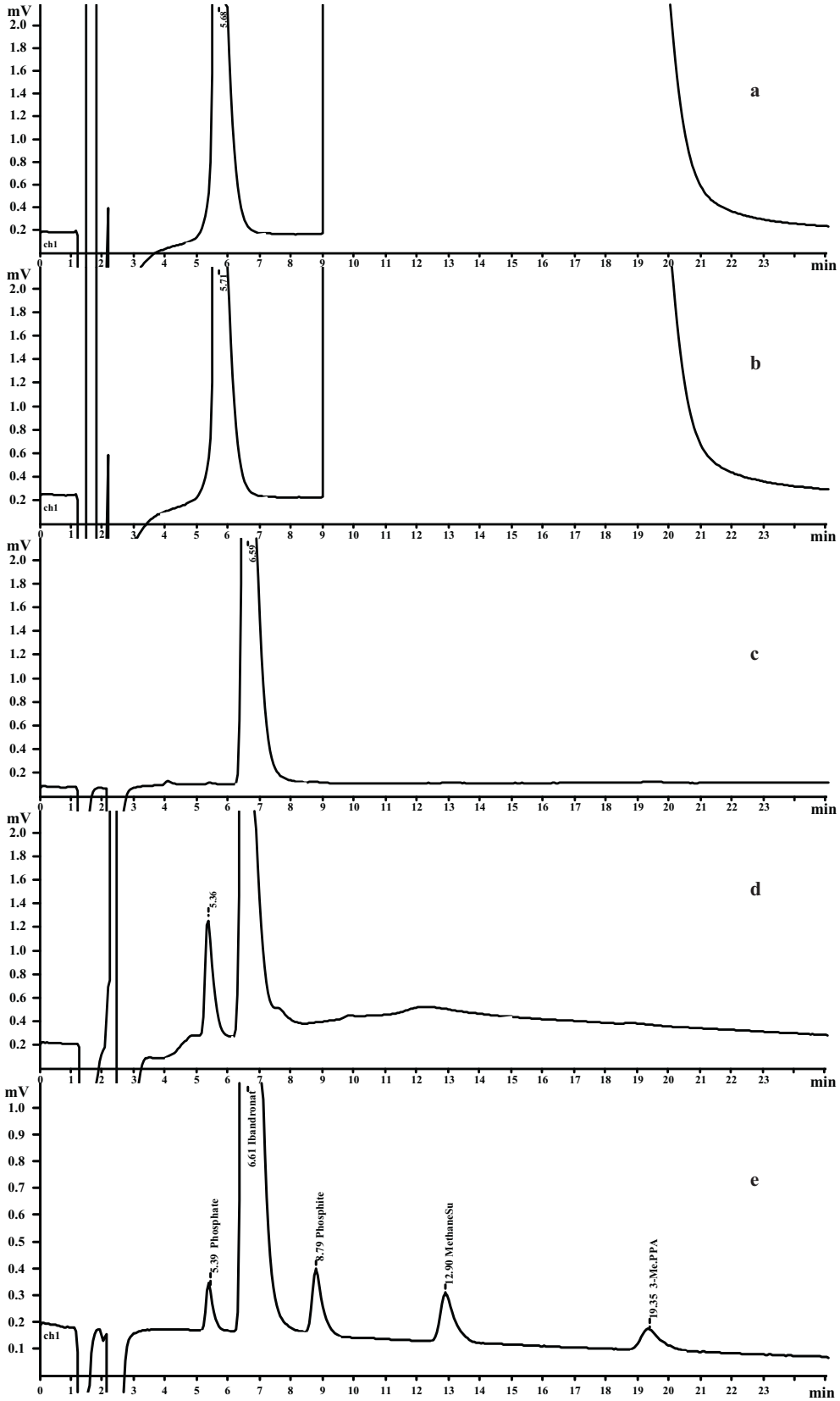

Fig. 1. Typical representative overlaid ion chromatograms of (a) acid hydrolysis, (b) alkaline hydrolysis, (c) photolytic and (d) oxidative conditions along with (e) standard.

Table 1
Evaluation of forced degradation studies.

Type of degradation	Degradation condition	Ibandronate assay (%w/w)	Degradation (%w/w)	Degradation of impurity (%w/w)
Sample as such	–	99.1	–	–
Acid degradation	5 M HCl/85 °C/120 min	99.1	Nil	Nil
	5 M HCl/85 °C/240 min	99.1	Nil	Nil
Alkaline	5 M NaOH/85 °C/120 min	99.0	0.1	Nil
degradation	5 M NaOH/85 °C/240 min	99.2	Nil	Nil
Peroxide	Peracetic acid/85 °C/60 min	95.1	4.0	5.5
degradation	Peracetic acid/85 °C/120 min	91.9	7.3	8.0
	Peracetic acid/85 °C/240 min	86.5	12.7	12.8
Thermal degradation	105 °C/120 h	98.8	0.3	Nil
Photolytic degradation	10 K Lux/120 h	99.0	0.1	Nil
Humidity degradation	90% RH/25 °C/120 h	99.0	0.1	Nil

acid, hydrochloric acid, sodium hydroxide, hydrogen peroxide, peracetic acid, sodium carbonate, sodium bicarbonate, potassium carbonate, phthalic acid, HPLC grade methanol, acetonitrile and acetone were procured from E Merck India. Highly purified water was obtained from Millipore purification system.

2.2. Ion chromatography

An ion chromatography system Metrohm 733 IC separation centre equipped with 732 conductometric detector, 709 IC pump, 762 IC interface and Metrohm 813 compact auto sampler with Metrohm IC net 2.3 data handling system was used. The mobile phase was a 98:2% (v/v) mixture of 1% (v/v) aqueous formic acid and acetone. The analysis was carried out on Allsep™ anion (Alltech Associates Inc.,) 150 mm × 4.6 mm, 7 μm particle diameter column at ambient temperature. The mobile phase was delivered in an isocratic mode at a flow rate of 1.0 ml min^{-1}. Detector scale range was 5 mS cm^{-1} and full scale was 50 μS cm^{-1}. The injection volume was 20 μl and run time was 25 min. Water was used as diluent. The retention times of the phosphate, ibandronate, phosphite, methanesulfonate and 3-(*N*-methylpentylamino)propionate peaks are at about 5.4, 6.6, 8.8, 12.9 and 19.4 min, respectively. The resolution between phosphate and ibandronate peaks and between ibandronate and phosphite peaks, respectively, was not <1.5. Relative standard deviation for the peak areas of the six replicate injections for each impurity peak is not more than 5.0% and that of ibandronate peak is not more than 1.0%.

2.3. Standard and sample solutions

2.3.1. Preparation of impurities stock solution

Accurately weigh and transfer 240 mg of ortho phosphoric acid, 210 mg of phosphorous acid, 205 mg of methanesulfonic acid and 201 mg of 3-(*N*-methylpentylamino)propionic acid into a 100 ml volumetric flask, dilute to volume with water and further dilute 5 ml of this solution to 100 ml with water.

2.3.2. Standard solution

Accurately weigh and transfer 100 mg of ibandronate sodium into a 50 ml volumetric flask, add 30 ml of water and dissolve by shaking, then add 5 ml of impurities stock solution, and make up to the volume with water. Filter through the 0.45 μ porous membrane.

2.3.3. Sample solution

Accurately weigh and transfer 100 mg of sample into a 50 ml volumetric flask, add 30 ml of water and dissolve by shaking and make up to the volume with water. Filter through the 0.45 μ porous membrane.

3. Results and discussion

3.1. Method development and optimization

As there is no chromophore present in ibandronate sodium, there was no possibility for UV or fluorescence detection and no suitable groups are present for derivatization. Ibandronate and its impurities are ionic; for this reason water was chosen as a diluent. Preliminary experiments were carried out based on the retention of phosphate and phosphite, which are discussed in many Metrohm ion chromatography applications, using Metrosep A Supp 5, Metrosep Anion Dual 2 and Metrosep Super-Sep columns. Using sodium carbonate, sodium bicarbonate, potassium carbonate and phthalic acid in the mobile phase and using acetonitrile, methanol and acetone as organic modifiers the analytes were not sufficiently separated. Separation was achieved on Allsep™ anion (Alltech Associates Inc.) 150 mm × 4.6 mm, 7 μm particle diameter column, with 1% (v/v) aqueous formic acid as mobile phase. Several trials were made using aqueous formic acid as mobile phase in concentrations ranging from 0.1% to 2.0% (v/v). In 0.1% (v/v) aqueous formic acid condition, ibandronate and phosphite peaks merged as well as late elution of analytes was observed. Satisfactory separation was achieved with 1.0% (v/v) aqueous formic acid with moderate conductivity and reasonable retention times of analytes. For better resolution between analytes, trials were performed with 1.0% (v/v) aqueous formic acid using methanol, acetonitrile and acetone

Table 2
Statistical data of linearity, LOD/LOQ for ibandronate and its impurities (six replicates).

Statistical parameters	3-(*N*-Methylpentylamino) propionic acid	Phosphate	Phosphite	Methanesulfonic acid	Ibandronate
Correlation coefficient	0.9986	0.9997	0.9996	0.9992	0.9999
Intercept	−0.285	0.161	0.174	0.369	−1.369
Residual standard on deviation response	0.090	0.031	0.069	0.101	0.769
Slope	0.383	0.281	0.537	0.573	0.070
Concentration range (μg ml^{-1})	2.0–15.0	2.0–15.0	2.0–15.0	2.0–15.0	380–2846
Limit of detection (μg ml^{-1})[a]	0.80	0.36	0.44	0.60	38
Limit of quantification (μg ml^{-1})[a]	2.40	1.00	1.40	1.81	113
Precision for limit of detection (%R.S.D)	9.5	22.9	13.4	16.9	6.5
Precision for limit of quantification (%R.S.D)	4.3	6.6	4.9	7.9	2.7

[a] Precised LOD and LOQ values.

Table 3a
Accuracy data of ibandronate impurities.

S. No.	3-(*N*-Methylpentylamino) propionic acid			Phosphate			Phosphite			Methanesulfonic acid		
	Level-I (50%)	Level-II (100%)	Level-III (150%)	Level-I (50%)	Level-II (100%)	Level-III (150%)	Level-I (50%)	Level-II (100%)	Level-III (150%)	Level-I (50%)	Level-II (100%)	Level-III (150%)
Added (%w/w)	0.251	0.501	0.749	0.251	0.501	0.749	0.250	0.501	0.748	0.252	0.503	0.752
Recovered (%w/w)	0.244	0.499	0.765	0.253	0.503	0.740	0.265	0.506	0.744	0.261	0.524	0.765
Recovery (%)	97.2	99.6	102.1	100.8	100.4	98.8	106.0	101.0	99.5	103.6	104.1	101.7
%RSD	3.3	1.6	2.9	0.8	1.6	1.5	3.8	4.7	2.7	3.4	3.8	1.6

Average of 3 replicates.

Table 3b
Accuracy data of ibandronate.

S. No.	Ibandronate		
	Amount added (mg)	Amount found (mg)	Recovery (%)
1	45.98	46.30	100.7
2	46.99	46.92	99.9
3	47.77	47.08	99.2
4	94.66	94.17	99.5
5	95.60	94.28	98.6
6	95.41	94.39	98.9
7	144.54	141.61	98.0
8	144.61	141.73	98.0
9	144.10	142.01	98.5
Average recovery			99.0
%RSD			0.9

as organic modifiers. With methanol and acetonitrile, broad peak shapes and poor base line were observed, while with acetone, peak shapes of analytes were improved. For better peak shapes and good resolution, several trials were performed with 1.0% (v/v) aqueous formic acid using acetone in 2%, 5%, 10% and 20% (v/v) concentrations. Satisfactory separation and good peak shapes were achieved within a reasonable time using a mobile phase of 98:2% (v/v) mixture of 1% (v/v) aqueous formic acid and acetone with a flow rate of 1.0 ml min^{-1}. The effect of column temperature on separation was studied at different temperatures ranging from 15 °C to 65 °C. Ambient temperature was found to be optimal from the point of view of both resolution and peak shape.

3.2. Method validation

The method was validated as per the ICH guidelines [8], in terms of specificity, forced degradation studies (stability indicating nature), limit of detection, limit of quantification, linearity, accuracy, precision and stability of sample solution.

3.2.1. Specificity

For specificity determination of the assay of ibandronate the possible interference of diluent and impurities, 3-(*N*-methylpentylamino)propionic acid, phosphate, phosphite and methanesulfonic acid was studied. It was found that their peaks did not interfere with the ibandronate peak and are well resolved. The method is also specific in the presence of common anions like fluoride, bromide, iodide, carbonate, bicarbonate, nitrate, nitrite, sulphate and sulphite. Chloride co-eluted with 3-(*N*-methylpentylamino)propionate; for this reason we used chloride-free water as diluent.

The stability indicating nature of the method was evaluated by performing forced degradation studies as per International Conference on Harmonization (ICH) hydrolytic, photolytic, thermal and oxidative stress testing [9]. The results are shown in Table 1. Ibandronate sodium was found stable at all conditions except in oxidative degradation. Drug substance peak was homogeneous and pure under the stress conditions; there was no interference observed for ibandronate peak from other peaks. In hydrogen peroxide degradation experiments interference was found at the retention time of phosphate peak. Further we degraded the samples in presence of peracetic acid instead of hydrogen peroxide for oxidative degradation condition. An overlaid chromatogram of acidic, alkaline hydrolysis, oxidative and photolytic conditions along with standard solution is shown in Fig. 1.

3.2.2. LOD and LOQ

For determining the limit of detection (LOD) and limit of quantification (LOQ), the method based on the residual standard

Table 4
Statistical data of precision experiment for ibandronate and its impurities.

	Ibandronate	3-(*N*-Methylpentylamino) propionic acid	Phosphate	Phosphite	Methanesulfonic acid
Repeatability (system precision) Area (mV s)					
1	118.142	3.724	2.515	5.279	5.310
2	120.341	3.650	2.642	5.355	5.866
3	120.494	3.660	2.622	5.342	5.632
4	120.825	3.666	2.753	5.370	5.701
5	120.180	3.630	2.617	5.159	5.753
6	120.352	3.995	2.686	5.125	5.786
Avg	120.056	3.721	2.639	5.272	5.675
SD	0.962	0.138	0.079	0.106	0.195
%RSD	0.8	3.7	3.0	2.0	3.4
Reproducibility (method precision) (%w/w)					
1	99.4	0.508	0.507	0.496	0.496
2	99.8	0.496	0.506	0.520	0.512
3	99.2	0.509	0.510	0.500	0.496
4	98.9	0.494	0.513	0.497	0.512
5	99.6	0.488	0.516	0.505	0.501
6	98.6	0.490	0.514	0.515	0.504
Avg	99.2	0.498	0.511	0.506	0.504
SD	0.44	0.009	0.004	0.010	0.007
%RSD	0.4	1.8	0.8	2.0	1.4
Reproducibility (ruggedness) (%w/w)					
1	99.0	0.497	0.483	0.503	0.502
2	98.9	0.514	0.487	0.493	0.491
3	99.2	0.508	0.484	0.495	0.505
4	98.9	0.505	0.485	0.498	0.504
5	99.4	0.512	0.484	0.504	0.501
6	99.4	0.509	0.498	0.492	0.503
Avg	99.1	0.508	0.487	0.498	0.501
SD	0.24	0.006	0.006	0.005	0.005
%RSD	0.2	1.2	1.2	1.0	1.0

deviation of a regression line and slope was adopted. Standard solution was injected into ion chromatograph from lower concentration to higher concentration. A plot of peak area (mV s) versus concentration ($\mu g\,ml^{-1}$) was drawn and LOD/LOQ values were calculated by residual standard on deviation response (SD) and slope (S) method using the formula 3.3 × SD/S for LOD and 10 × SD/S for LOQ (see Table 2).

3.2.3. Linearity

The linearity of conductometric detector response of ibandronate and its impurities at different concentrations were studied in the range 380–2846 $\mu g\,ml^{-1}$ for ibandronate and 2.0–15.0 $\mu g\,ml^{-1}$ for 3-(*N*-methylpentylamino)propionic acid, phosphate, phosphite and methanesulfonic acid. The data was subjected to statistical analysis using a linear-regression model. The statistical parameters slope, intercept, residual standard on deviation response and correlation coefficient values were calculated and shown in Table 2.

3.2.4. Accuracy

Accuracy of the method was determined by recovery experiments using standard addition technique. The recoveries were determined by spiking the impurities at three different levels ranging from 50% to 150% (with respect to 0.5% level) into ibandronate sodium drug substance. Similarly recovery experiment was carried out ranging from 50% to 150% of ibandronate (with respect to test concentration). The average recovery values were 99.6%, 100.0%, 102.2% and 103.1% for 3-(*N*-methylpentylamino)propionic acid, phosphate, phosphite and methanesulfonic acid respectively (three replicates). For ibandronate assay the average recovery was 99.0%. The results are shown in Tables 3a and 3b.

3.2.5. Precision

The precision was the study of the method using repeatability and reproducibility (ruggedness). The performance of the method was evaluated with replicate injections of standard and sample solutions. Standard solution was analyzed six times for checking the performance of the system under the chromatographic conditions on the day tested (System precision). Repeatability was the intra-day variation (Method precision) and the intermediate precision was the inter-day variation (Ruggedness). The repeatability and reproducibility of the method was studied by analyzing six sample solutions separately by adding impurities at known concentration levels. The ruggedness of the method was defined as the degree of reproducibility obtained by the analysis of the same sample (which is used in the Method precision) under a variety of conditions using different series of column, with different analyst on different day by preparing new standards and new mobile phase. The precision results are shown in Table 4.

3.2.6. Solution stability

The sample solution was prepared by the addition of impurities with known concentration level into ibandronate sodium drug substance. The stability of the solution was tested by recording and comparing the chromatograms freshly prepared and at different intervals up to 12 h at ambient temperature. The results indicate that the sample solution was stable for up to 12 h at ambient temperature.

3.2.7. Comparison with RPIC – ELSD method

The LOD values of the newly developed IC method were compared with the LOD values of the ion pair reversed phase high performance liquid chromatography (RPIC) with evaporative light scattering detection (ELSD) method [6]. The results show that the present IC method is more sensitive and easy to perform the anal-

ysis. In addition, methanesulfonic acid was quantified by using this methodology.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2010.09.026.

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