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Simultaneous RP-HPLC and UV Spectroscopic Method Development and Validation for Estimation of Ibandronate Sodium in Bulk and Pharmaceutical Dosage Form

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The present study describes a simple, accurate, precise and cost effective UV-Vis Spectroscopic and RP-HPLC method for the estimation of Ibandronate sodium (IBN). The determination of Ibandronate sodium (IBN) was performed by both UV and RP-HPLC method using 215 nm as the determination wavelength. The drug was dissolved in NaOH solution (0.1N NaOH) for estimation in UV and in distilled water for the estimation in RP-HPLC using mobile phase 0.01 M Sodium dihydrogen phosphate (NaH_2PO_4): Acetonitrile (80:20), pH being adjusted to 3.3 with 10% *ortho*-phosphoric acid. A linear response was observed in the range of 10-50 μg ml^{-1} (R^2 = 0.9981) for UV-Spectroscopy, whereas for RP-HPLC the linear response was observed in the range of 20-70 μg ml^{-1} (R^2 = 0.9965). The limits of quantitation (LOQ) were estimated as 0.1 μg ml^{-1} and 0.05 μg ml^{-1}, respectively for UV and RP-HPLC respectively. The recoveries of IBN from the marketed formulation were found to be within 100 ± 2% by both the methods. These methods were then effectively applied for the estimation of Boniva (tablet) and the results were obtained according to nominal content. The statistical analysis revealed that there is no significant difference (p > 0.05) between UV and HPLC methods regarding validation parameters and assay content.

Keywords: Ibandronate sodium (IBN), UV-Vis spectroscopy, RP-HPLC, Calibration curve, LOD

INTRODUCTION

Ibandronate sodium [(1-hydroxy-3-(methyl pentyl amino) propylidene 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 quantification of impurities and assay of ibandronate sodium, a few analytical methods have been reported so far [3-5]. For example, indirect fluorescence detection was used in a high performance ion exchange chromatographic method; which proceeded *via* formation of a non-fluorescent Al^{3+}-ibandronate complex after post-column addition of fluorescent Al^{3+}-morin reagent. In another report, Ibandronate was determined by high performance ion exchange chromatography with UV detection at 240 nm after complex formation with Cu^{2+} ion [4]. Furthermore, Ibandronate and related impurities (phosphate, phosphite) were determined by capillary zone electrophoretic method within the direct detection at 254 nm [5], The limit of detection (LOD) values reported for phosphate, phosphite and ibandronate were 5 μg ml^{-1}, 3 μg ml^{-1} and 176 μg ml^{-1}, linearity ranges were 92-460 μg ml^{-1}, 24-384 μg ml^{-1} and 352-1760 μg ml^{-1} [6]. Stability indicating ion chromatography method for the simultaneous determination of Ibandronate sodium drug substance and its impurities has also been demonstrated [7]. Other methods are also available such as quantification with HPLC-PDA detector

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[8] and HPLC with anion column [9]. However, all these methods are costly and need sample resource to determine a single drug like Ibandronate. In addition, no such method is available with HPLC using UV detector which would have been more cost, resource effective and simpler to operate. Hence, the aim of this study was to develop a simple, sensitive, and precise method by both UV-Vis spectrophotometry and HPLC-UV for the first time to estimate Ibandronate sodium in tablet dosage form.

MATERIAL AND METHODS

Reagents and Chemicals

Ibandronate sodium (IBN) (Fig. 1) was obtained from Natco Pharmaceuticals, Hyderabad. Solution of NaOH (0.1 N NaOH, Analytical Grade) was used for UV method development and validation, whereas sodium dihydrogen phosphate (NaH_2PO_4) made phosphate buffer has been used for HPLC. All Analytical Grade (AR) solvents were purchased from Merck (Mumbai, India) while HPLC grade water and acetonitrile were procured from Sigma Aldrich (Aldrich, USA).

Instrumentation

The instruments used for the present study were UV-Vis double beam spectrophotometer (model Shimadzu, Kyoto, Japan 1800) with 1cm matched pair quartz cell. The data acquisition was made with UV-Prove Version 2.3. HPLC (Waters, USA) system was composed of a 515 HPLC Pump solvent delivery module, having manual rheodyne injector with a 20-µl fixed loop and having a Waters 2489 UV-Vis detector. The separation has been done by using a waters Nova-Pak® C8G column (Column Length: 150 mm × 3.9 mm i.d.; 4 µm; particle size) at an ambient temperature. The data acquisition was made with Empower 2 Software Build 2154® (Waters Corporation).

Methods

Preparation of Stock and Working Standard Solution of Ibandronate Sodium (IBN). Stock solution (100 µg ml^{-1}) of the drug was prepared by sonicating the drug with the suitable solvent (0.1 N NaOH for UV and H_2O for HPLC). A working concentration of 50 µg ml^{-1} was prepared by appropriate dilution of the stock solution.

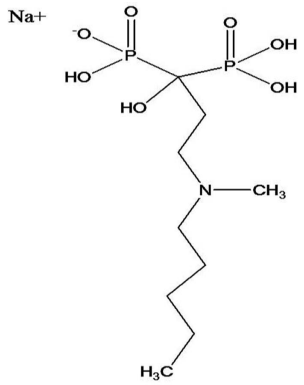

Fig. 1. Chemical structure of ibandronate sodium.

High Performance Liquid Chromatography (HPLC). High-Performance Liquid Chromatography (Isocratic, Waters, USA) system was composed of a pump (model no 515), solvent delivery module having manual rheodyne injector with a 20-µl fixed loop and having a UV-Vis detector (model no 2489). Analytes were separated by using Waters Nova-Pak C8 Column (50 mm × 3.9 mm, particle size 4 µm) at an ambient temperature. The data acquisition was made with Software (M-power, Waters, USA). The mobile phase consisted of 0.01M NaH_2PO_4: Acetonitrile (80:20, v/v); pH was adjusted to 3.5 with 10% *ortho*-phosphoric acid and the flow rate was set at 0.5 ml min^{-1}.

Method Development for UV and HPLC

Determination of λ_{max}. A working sample solution (50 µg ml^{-1}) in 0.1 N NaOH solution was prepared by suitable solvent addition, dilution and subsequently scanned with UV-Vis spectrophotometer in the range 400-200 nm against 0.1 N NaOH solution as blank.

Preparation of calibration curve for UV and HPLC. From the stock solution (100 µg ml^{-1}) in 0.1 N NaOH, further dilutions were made to produce series concentrations such as 10 µg ml^{-1}, 15 µg ml^{-1}, 20 µg ml^{-1}, 30 µg ml^{-1}, 40 µg ml^{-1} and 50 µg ml^{-1}, 60 µg ml^{-1} and 70 µg $ml^{-1}$and the absorbance was noted at a wavelength of 215 nm. The construction of calibration curve showed a straight line with a coefficient of determination (r^2) of 0.9981 for UV.

For HPLC the above stock solution (100 µg ml^{-1}) was diluted in water to produce concentrations from 20-50 µg ml^{-1} and was injected into the column with UV detector set

at 215 nm. The construction of calibration curve showed a straight line with a coefficient of determination (r^2) of 0.9965 for HPLC.

Method Validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.

The proposed method was validation according to the ICH guidelines by UV and HPLC using parameters like Linearity, Accuracy, Precision, Robustness, Ruggedness, Limit of detection (LOD) and Limit of quantitation (LOQ) (ICH, Methodology, 19th May 1997, and ICH, Q2 (R1), Step. 4 2005).

Linearity

The linearity was tested for both UV and HPLC over the concentration range of 10, 15, 20, 30, 40 and 50 μg ml^{-1} and 20, 30, 40, 50, 60 and 70 μg ml^{-1}, respectively for IBN. The calibration curve was constructed and evaluated by its coefficient of determination (r^2). The calibration plot for UV (concentration of IBN versus Absorbance of IBN at 215 nm) and HPLC (concentration of IBN versus the Peak Area of IBN at 215 nm) was generated by replicate analysis (n = 6) at all concentration levels and a linear relationship was evaluated using the least square method by Microsoft Excel® program. For minimum error with precise, concise and accurate data, six different concentrations were being taken which gave a wide range of linearity. The coefficient of determination (r^2) for IBN was 0.9981 for UV and 0.9965 for HPLC.

Accuracy

Accuracy of the method was determined by replicate analysis of three sets of samples spiked with three different levels of IBN at level 80%, 100% and 120% and comparing the difference between the spiked value (theoretical value) and that actually found value by both UV and HPLC methods.

Precision

The precision of the method based on within-day repeatability was determined by replicate analysis of three sets of samples spiked with three different concentrations of IBN 10, 30 and 50 μg ml^{-1} for UV and 20, 40 and 70 μg ml^{-1} for HPLC, respectively. The reproducibility (day-to-day variation) of the method was validated using the same concentration range as described above, but only a single determination of each concentration was made in three different days by both the methods. Relative standard deviation (R.S.D.) was calculated from the ratios of standard deviation (S.D.) to the mean and expressed as percentage for both the methods.

Specificity

Specificity study was performed by analyzing standard solution in the presence of an excipient to find out is there any interference of excipients in %recovery of IBN. Amount of IBN was spiked with 50%, 100% and 150% of excipient (magnesium stearate) and the samples were analyzed for IBN recovery by UV-Vis spectrophotometer and HPLC.

Limit of Detection and Limit of Quantitation

The limit of detection of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The limit of quantitation of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices. A limit of detection (LOD) and a limit of quantitation (LOQ) were established based on the calibration curve parameters, according to the formula:

LOD = 3.3*S. D./Slope

LOQ = 10*S. D./Slope

The limit of quantitation (LOQ) of the assay procedure for both the methods were determined from the lowest concentration of IBN (spiked sample) that produced a peak height three times the baseline noise at a sensitivity of 0.005 AUFS (absorbance unit full scale) for HPLC and absorbance which is three times the base line noise at

absorbance maxima (λ_{max}) for UV-Spectroscopy.

Statistical Analysis

ONE way ANOVA (Analysis of variance) was performed to compare the output parameters between UV and HPLC methods. Considering the two methods mutually exclusive, independent sample t-test was performed. First, F-test was carried out to characterize whether the output parameters had been evolved from the same population or not. If found from the same population (F-test p value > 0.05), Student's t-test with equal variance was performed; otherwise Student's t-test with unequal variance was executed. The result was considered significant if the result lies within 95% confidence interval (*i.e.* $p < 0.05$).

RESULT AND DISCUSSION

Determination of λ_{max} and Retention Time

The λ_{max} was found from UV scanning of the sample solution and was exhibited as 215 nm (Fig. 2). Using this as detection wavelength, the retention time of the compound was found as 2.08 min (Fig. 3).

Calibration Curve

The correlation coefficient, r^2, slope and intercept were estimated 0.9981, 0.0058 and 0.6173 by UV method and 0.9965, 1.298.8543 and 668173.0571 by HPLC with absorbance maxima set at 215 nm. Linear regression of data from the calibration curve indicated a linear response over the concentration range by both the methods. The curves can therefore be used for determination of Ibandronate sodium in pharmaceutical dosage form.

ANALYTICAL METHOD VALIDATION

Linearity

The coefficient of determination (r^2) for IBN were 0.9981 and 0.9965, respectively for UV and HPLC methods

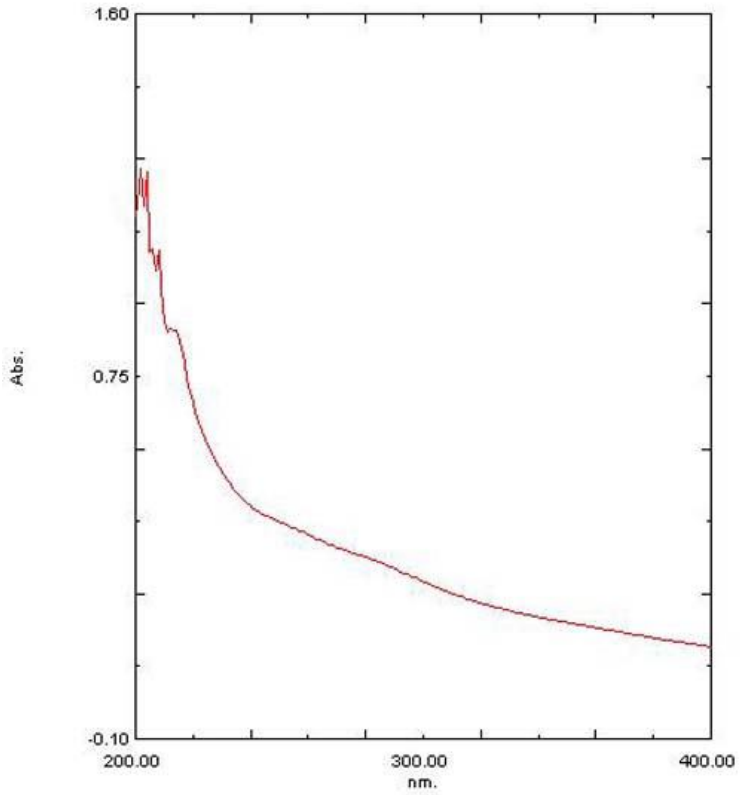

Fig. 2. UV Spectra of ibandronate sodium.

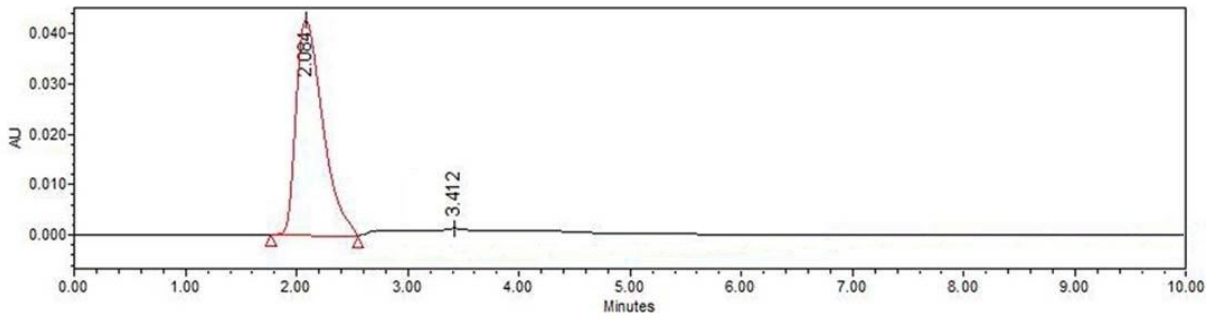

Fig. 3. Chromatogram of Ibandronate sodium.

Table 1a. Linear Regression Equations Generated from Validation of Ibandronate Sodium: Slope, Intercept and Coefficient of Determination for UV Spectrophotometry

Analyte		
Ibandronate sodium		
Conc. ($\mu g\ ml^{-1}$)	Peak area (mV s)	
10	0.681	Slope: 0.0058
15	0.701	Intercept: 0.6173
20	0.734	r^2: 0.9981
30	0.787	
40	0.851	
50	0.911	

Table 1b. Linear Regression Equations Generated from Validation of Ibandronate Sodium: Slope, Intercept and Coefficient of Determination for RP-HPLC

Analyte		
Ibandronate sodium		
Conc. ($\mu g\ ml^{-1}$)	Peak area (mV s)	
20	695753	Slope: 1298.8543
30	705483	Intercept: 668173.0571
40	718597	r^2: 0.9965
50	734177	
60	747181	
70	758538	

(Tables 1a and 1b)

Precision

The relative standard deviation was found to be < 2.0% for both UV and HPLC methods (Tables 3a and Table 3b). The interday and intraday precisions have been expressed as %RSD (Tables 4a, 4b, 5a and 5b).

Accuracy

The accuracy of both the methods was determined by preparing solutions of different concentrations that is 80%, 100% and 120% in which the amount of marketed formulation was kept constant (10 mg) and the amount of pure drug was varied that is 8 mg, 10 mg and 12 mg for 80%, 100% and 120%, respectively. The solutions were prepared in triplicates and the accuracy was indicated by the %recovery (Tables 6a and 6b). The mean %recoveries of IBN were found to be in the range 99.6667-101.73 for UV and 99.87-101.0667% for HPLC. From the results, it can be concluded that the accuracy of determination has been well within the acceptable limit.

Specificity

Specificity study was performed by analyzing standard solution in the presence of an excipient (magnesium stearate). 10 mg each IBN was spiked with 50% (5 mg),

Table 2. Spectral and Statistical Data for Determination of Ibandronate Sodium by Proposed UV & RP-HPLC Method

Parameters	Value for IBN in UV	Value for IBN in HPLC
Absorption maxima, λ_{max} (nm)	215 nm	215 nm
Linearity range (µg ml^{-1})	10-50	20-70
Coefficient of determination (r^2)	0.9981	0.9965
Correlation coefficient (r)	0.9974	0.9982
Regression equation (Y^a)	y = 0.005x + 0.617	1,298.8543x + 668,173.0571
Slope (b)	0.0058	1298.8543
Intercept (a)	0.6173	668173.0571
Limit of detection, LOD (µg ml^{-1})	0.0303	0.0151
Limit of quantitation, LOQ (µg ml^{-1})	0.1	0.05

[a]*Y* = mx + c, where *x* is the concentration (µg ml^{-1}).

Table 3a. Repeatability of IBN by UV-Spectrophotometry

Analyte				
	Ibandronate sodium			
Conc. (µg ml^{-1})	Conc. found at (µg ml^{-1})	Avg. (µg ml^{-1})	S.D.	RSD (%)[a]
30	28.22414			
30	29.08621			
30	28.74138			
30	27.87931	28.39655	0.404346	1.423927
30	27.87931			
30	28.22414			
30	28.39655			
30	28.74138			
30	28.39655			

[a]%RSD = SD/Mean *100.

Table 3b. Repeatability of IBN by RP-HPLC

Analyte				
	Ibandronate sodium			
Conc. ($\mu g\ ml^{-1}$)	Conc. found at ($\mu g\ ml^{-1}$)	Avg. ($\mu g\ ml^{-1}$)	S.D.	RSD (%)[a]
40	38.7279			
40	38.9527			
40	37.9441			
40	38.7964	38.56283	0.375113	0.972733
40	38.8988			
40	38.2829			
40	38.8811			
40	38.4754			
40	38.1058			

[a]%RSD = SD/Mean*100.

Table 4a. Inter-Day (n = 3) Precision by UV-Spectrophotometry

Analyte				
	Ibandronate sodium			
Conc. ($\mu g\ ml^{-1}$)	Conc. found in ($\mu g\ ml^{-1}$)	Avg. ($\mu g\ ml^{-1}$)	S.D.	RSD (%)[a]
10	10.8103	10.63793	0.172414	1.620746
10	10.6379			
10	10.4655			
30	27.8793	28.16667	0.263366	0.935029
30	28.2241			
30	28.3966			
50	50.4655	50.46552	0.172414	0.341647
50	50.2931			
50	50.6379			

[a]%RSD = SD/Mean*100.

Table 4b. Inter-Day (n = 3) Precision by RP-HPLC

Analyte				
	Ibandronate sodium			
Conc. ($\mu g\ ml^{-1}$)	Conc. found in ($\mu g\ ml^{-1}$)	Avg. ($\mu g\ ml^{-1}$)	S.D.	RSD (%)[a]
20	21.1817	21.23713	0.158354	0.745649
20	21.4157			
20	21.1139			
40	38.7279	38.54162	0.529476	1.373777
40	38.9527			
40	37.9441			
70	69.4912	68.89093	0.526159	0.763756
70	68.5095			
70	68.6720			

[a]%RSD = SD/Mean*100.

Table 5a. Intra-Day (n = 3) Precision by UV-Spectrophotometry

Analyte				
Ibandronate sodium				
Conc. (µg ml^{-1})	Conc. found in (µg ml^{-1})	Avg. (µg ml^{-1})	S.D.	RSD (%)
10	10.9827	11.15517	0.172414	1.545595
10	11.1551			
10	11.3275			
30	28.7413	28.16667	0.497716	1.767038
30	27.8793			
30	27.8793			
50	50.6379	50.46552	0.172414	0.341647
50	50.4655			
50	50.2931			

[a]%RSD = SD/Mean*100.

Table 5b. Intra-Day (n = 3) Precision by RP-HPLC

Analyte				
Ibandronate Sodium				
Conc. (µg ml^{-1})	Conc. found in (µg ml^{-1})	Avg. (µg ml^{-1})	S.D.	RSD (%)
20	21.3326	21.29719	0.035416	0.166293
20	21.2617			
20	21.2971			
40	37.9441	38.23416	0.268954	0.70344
40	38.2829			
40	38.4754			
70	68.5095	68.89709	0.337387	0.489697
70	69.0562			
70	69.1254			

[a]%RSD = SD/Mean*100.

Table 6a. Accuracy by UV-Spectrophotometry

Analyte	Added (%)	Constant amount added (µg ml^{-1})[a]	Amount added (µg ml^{-1})[b]	Total amount found (µg ml^{-1})[c]	Amount found (µg ml^{-1})[d]	Recovery (%)[e]	Average recovery (%)	RSD (%)
IBN	80	5	4	9.05	4.05	101.25	100.0833	1.0399
(µg ml^{-1})	80	5	4	8.99	3.99	99.75		
	80	5	4	8.97	3.97	99.25		
	100	5	5	10.17	5.17	103.4	101.7333	1.6328
	100	5	5	10.11	5.11	102.2		
	100	5	5	9.98	4.98	99.6		
	120	5	6	11.07	6.07	101.1667	99.66667	1.6402
	120	5	6	10.89	5.89	98.16667		
	120	5	6	10.98	5.98	99.66667		

[a]Preanalyzed sample found to be 3 µg ml^{-1}. [b]Pure drug added. [c]Total concentration found *i.e.* c = a + b. [d]Amount found *i.e.* d = c - a. [e]%Recovery of ibandronate sodium = Ibandronate sodium recovery (µg ml^{-1})/Ibandronate sodium input (µg ml^{-1})*100 OR d/b*100.

Table 6b. Accuracy by RP-HPLC

Analyte	Added (%)	Constant amount added (μg ml^{-1})[a]	Amount added (μg ml^{-1})[b]	Total amount found (μg ml^{-1})[c]	Amount found (μg ml^{-1})[d]	Recovery (%)[e]	Average recovery (%)	RSD (%)
IBN	80	5	4	8.96	3.96	99	99.8333	1.2352
(μg ml^{-1})	80	5	4	8.97	3.97	99.25		
	80	5	4	9.05	4.05	101.25		
	100	5	5	10.12	5.12	102.4	101.0667	1.6477
	100	5	5	9.96	4.96	99.2		
	100	5	5	10.08	5.08	101.6		
	120	5	6	11.11	6.11	101.8333	100.7778	1.2845
	120	5	6	11.07	6.07	101.1667		
	120	5	6	10.96	5.96	99.3333		

[a]Preanalyzed sample found to be 3 μg ml^{-1}. [b]Pure drug added. [c]Total concentration found *i.e.* c = a + b. [d]Amount found *i.e.* d = c - a. [e]%Recovery of ibandronate sodium = ibandronate sodium recovery (μg ml^{-1})/ ibandronate sodium input (μg ml^{-1})*100 OR d/b*100.

Table 7a. Specificity by UV-Spectrophotometry

Analyte	Added (%)	Excipient amount added (mg)	Conc. found (μg ml^{-1})[a]	Recovery (%)[b]	Avg. recovery (%)	S.D.	RSD (%)
Ibandronate	50	5	10.1	101			
sodium	50	5	10.01	100.1			
(10 mg)	50	5	9.89	98.9			
	100	10	10.01	100.1			
	100	10	9.91	99.1	100.311	0.8978	0.895
	100	10	10.01	100.1			
	150	15	10.09	100.9			
	150	15	10.11	101.1			
	150	15	10.15	101.5			

[a]The final dilution was made to 10 μg ml^{-1} and analyzed for %recovery. [b]%Recovery of ibandronate sodium = ibandronate sodium recovery (μg ml^{-1})/ibandronate sodium input (μg ml^{-1})*100.

Table 7b. Specificity by RP-HPLC

Analyte	Added (%)	Excipient amount added (mg)	Conc. found (μg ml^{-1})[a]	Recovery (%)[b]	Avg. recovery (%)	S.D.	RSD (%)
Ibandronate	50	5	9.96	99.6			
sodium	50	5	9.89	98.9			
(10 mg)	50	5	10.05	100.5			
	100	10	10.11	101.1			
	100	10	10.07	100.7	100.678	0.901	0.895
	100	10	10.09	100.9			
	150	15	10.13	101.3			
	150	15	10.16	101.6			
	150	15	10.15	101.5			

[a]The final dilution was made to 10 μg ml^{-1}and analyzed for %recovery. [b]%Recovery of ibandronate sodium = ibandronate sodium recovery (μg ml^{-1})/Ibandronate sodium input (μg ml^{-1})*100.

Table 8. Determination of Robustness for Ibandronate Sodium by UV-Spectrophotometry

Sample I.D.	Analytical condition	IBN input (mg)	IBN Rec. (mg)	IBN Sod. Rec. (%)a	Mean Rec. IBN (%)	S.D.	RSD (%)
Set-1	Solvent pH: 3.3 Ratio of solvent: 60:40 Temperature: 18	10	9.89	98.9			
Set-2	Solvent pH: 3.3 Ratio of solvent: 60:40 Temperature: 18	10	10.22	102.2	100.3	1.66	1.655
Set-3	Solvent pH: 3.3 Ratio of solvent: 60:40 Temperature: 18	10	9.85	98.5			
Set4	Solvent pH: 3.3 Ratio of solvent: 60:40 Temperature: 18	10	10.2	102			
Set-5	Solvent pH: 3.3 Ratio of solvent: 60:40 Temperature: 18	10	9.91	99.1			
Set-6	Solvent pH: 3.3 Ratio of solvent: 60:40 Temperature: 18	10	10.11	101.1			

[a]%Recovery of ibandronate sodium = ibandronate sodium recovery (mg)/ibandronate sodium input (mg)*100.

Table 8. Determination of Robustness for Ibandronate Sodium by RP-HPLC

Sample I.D.	Analytical condition	IBN input (mg)	IBN Rec. (mg)	Ibandronate sodium Rec. (%)a	Mean Rec. IBN (%)	S.D.	RSD (%)
Set-1	Flow rate: 0.52 ml min^{-1} Mobile phase pH: 3.3 Mobile phase ratio: 60:40 Column: C8 waters	10	9.98	99.8			
Set-2	Flow rate: 0.48 ml min^{-1} Mobile phase pH: 3.3 Mobile phase ratio: 60:40 Column: C8 waters	10	10.1	101	100.26	0.84	0.84
Set-3	Flow rate: 0.5 ml min^{-1} Mobile phase pH: 3.2 Mobile phase ratio: 60:40 Column: C8 waters	10	10.02	100.2			
Set-4	Flow rate: 0.5 ml min^{-1} Mobile phase pH: 3.4 Mobile phase ratio: 60:40 Column: C8 waters	10	10.12	101.2			
Set-5	Flow rate: 0.5 ml min^{-1} Mobile phase pH: 3.3 Mobile phase ratio: 58:42 Column: C8 waters	10	9.89	98.9			
Set-6	Flow rate: 0.5 ml min^{-1} Mobile phase pH: 3.3 Mobile phase ratio: 62:38 Column: C8 waters	10	10.05	100.5			

[a]%Recovery of ibandronate sodium = ibandronate sodium recovery (mg)/ibandronate sodium input (mg)*100.

100% (10 mg), and 150% (15 mg) of magnesium stearate and the samples were analyzed for IBN recovery by UV-Vis spectrophotometer and HPLC. Interference was found to be 98.9-101.5% of UV and 98.9-101.5% for HPLC, respectively which is again within the acceptable limit. Hence the excipients do not interfere with the estimation of drug. The detailed data has been provided in Tables 7a and 7b.

Limit of Detection and Limit of Quantitation

The limit of quantification (LOQ) for IBN has been estimated as 0.1 µg for UV-Vis Spectrophotometer and 0.05 µg for HPLC (Table 2).

Determination of Assay Content

Ten tablets of IBN having brand name Boniva (150 mg) (Genetech, USA) were triturated and the powder was diluted to obtain a concentration of 50 µg ml^{-1} of IBN for further analysis in UV-Spectrophotometry (Fig. 4) and in HPLC (Fig. 5). The results have been summarized in Tables 9a and 9b for UV and HPLC.

Statistical Analysis

The UV and HPLC methods are compared based on output parameters such as repeatability, Interday and Intraday precession, accuracy, specificity and assay content. For all of the cases *p*-value obtained is greater than 0.05

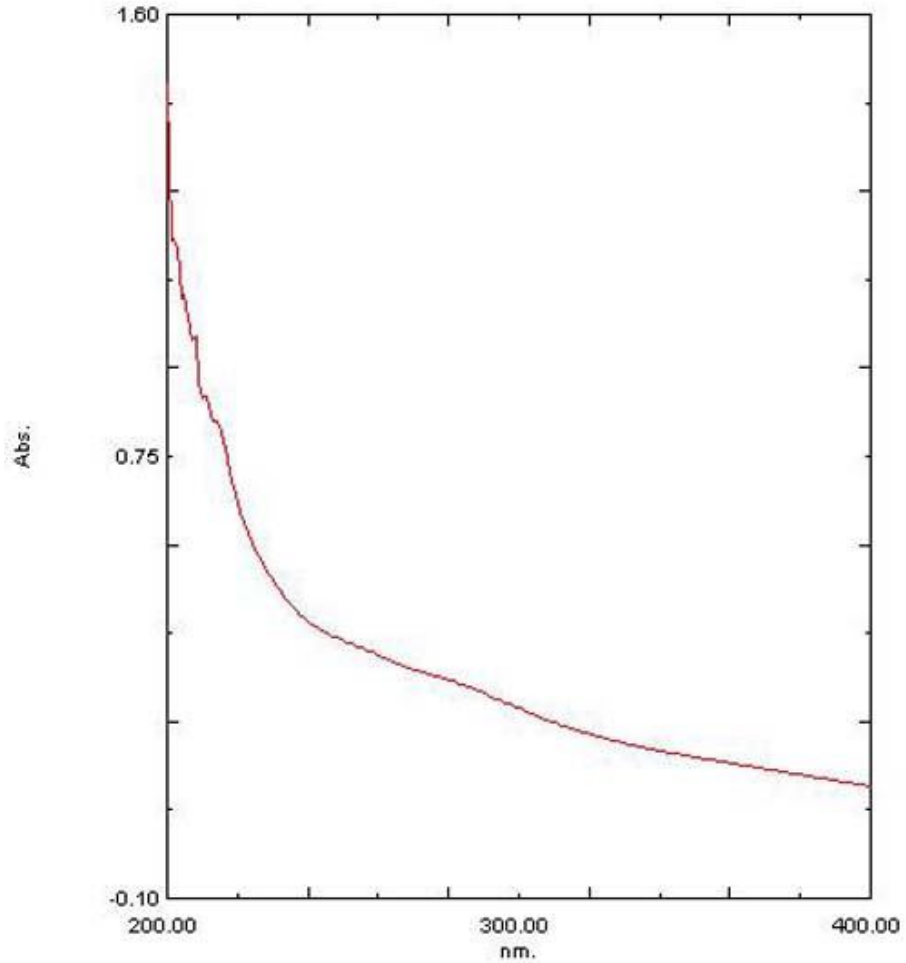

Fig. 4. UV spectra of ibandronate sodium tablet (Boniva) assay containing 50 µg ml^{-1} of ibandronate sodium.

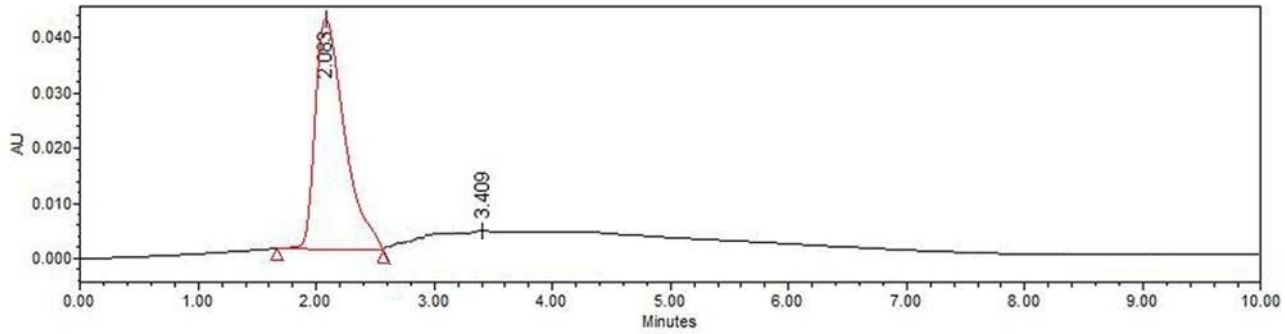

Fig. 5. Chromatogram of ibandronate sodium tablet (Boniva) assay containing 50 µg ml^{-1} of ibandronate sodium.

Table 9a. Determination of %Assay for Ibandronate Sodium (Boniva) by UV-Spectroscopy

Trade name	Drug	Label claim mg/tablet	Conc. taken (mg)	Conc. estimated (mg)	Mean conc. estimated (mg)	%Assay (w/w) ± a	RSD (%)[b]
Boniva	Ibandronate sodium	150mg	75	74.56	75.6967	100.9288	1.8783
			75	76.51			
			75	73.21			
			75	77.36			
			75	78.06			
			75	74.48			

[a]%Recovery of ibandronate sodium = ibandronate sodium recovery (mg)/ibandronate sodium input (mg) * 100 ± SD. [b]%RSD = SD/Mean*100.

Table 9b. Determination of %Assay for Ibandronate Sodium (Boniva) by RP-HPLC

Trade name	Drug	Label claim mg/tablet	Conc. taken (mg)	Conc. estimated (mg)	Mean conc. estimated (mg)	%Assay (w/w) ± a	RSD (%)[b]
Boniva	Ibandronate sodium	150 mg	75	75.36	75.7833	101.0444	1.3823
			75	76.07			
			75	74.48			
			75	76.89			
			75	74.89			
			75	77.01			

[a]%Recovery of ibandronate sodium = ibandronate sodium recovery (mg)/ibandronate sodium input (mg)* 100 ± SD. [b]%RSD = SD/Mean*100.

Table 10. System Suitability Parameters for Ibandronate Sodium by HPLC

S. No.	Parameters	Ibandronate sodium (min)
1	Retention time, Rt (min)	2.089
2	Theoretical plates (USP)	1649.7
3	HETP (mm)	0.09
4	Capacity factor (k)	1.611

Table 11. Statistical Analysis of the UV & RP-HPLC Methods

Name of the measured parameter	UV		HPLC		*p*-value by F-test	*p*-value by Student's t-test
Repeatability (Difference between theoretical and measured concentration)	Average (mg) 1.603	Stdev. (±) 0.0404	Average (mg) 1.437	Stdev. (±) 0.375	0.837	0.379
Inter day precision (Difference between theoretical and measured concentration)	0.979	0.670	1.268	0.411	0.094	0.286
Intra day precision (Difference between theoretical and measured concentration)	1.151	0.654	1.389	0.366	0.060	0.356
Accuracy (%Recovery)	100.494	1.635	100.559	1.343	0.296	0.928
Specificity (%Recovery)	100.311	0.898	100.677	0.901	0.496	0.400
%Assay (Amount found out of 75.00 mg)	75.700	1.896	75.783	1.048	0.109	0.924

(Table 10) which indicates there is no significant difference between output parameters of UV and HPLC methods.

CONCLUSIONS

Linearity was determined at different concentration IBN shows linearity in the concentration range of 10-50 μg ml^{-1} and 20-70 μg ml^{-1} of UV and HPLC, respectively. The percent recovery of the drug was within the rage 95-105% for UV and HPLC which indicates the method accuracy. The %RSD values for precision were < 2.0% for both the methods. Both the methods showed a positive response to all validation parameters. The result of the marketed formulation (Boniva) was found to be within 100.42 ± 0.03% for both UV and HPLC. The above proposed both the methods were found to be simple, rapid and sensitive. Also, the statistical analysis unraveled no significant difference between these two High Throughput Screening Methods that means both these methods are equally comparable to extract satisfactory output from the proposed drug estimation procedure. Therefore, validated UV spectrophotometric and HPLC method will play an important role for the determination of Ibandronate sodium in pharmaceutical dosage form.

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