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A validated HPLC method for determination of ibandronate sodium residue in cleaning validation by RI detector

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ABSTRACT

A high performance liquid chromatography (HPLC) method has been developed and validated for the quantitative determination of trace amount of Ibandronate Sodium (IBN) residue in pharmaceutical equipment. A new, generic method is presented for detection and estimation of a potent compound IBN, with which it is possible to verify the cleaning process of equipment used in pharmaceutical manufacturing. The chromatographic separation was performed on an Allsep anion column with a particle size of 7µ (150X4.6) and 0.2% v/v formic acid adjusted to pH 3.2 as mobile phase at flow rate of 1.2mL/minutes. The refractive index detector has been used for the determination of the compound with very poor UV detection. Sensitivity of the method is found to be satisfactory (LOQ value of the IBN is 3 µg mL^{-1}) in RI detector for a compound with very poor UV absorbance and linear calibration curves were established over the concentration range 3–60 µg $mL^{-1.}$ The method validation results indicated that the method is specific, accurate, linear and reproducible. The HPLC method is suitable for cleaning control assay with good manufacturing practice (GMP) of pharmaceutical industry. . This method was also suitable for the assay determination of IBN in pharmaceutical dosage forms.

KEYWORDS

Ibandronate Sodium;
Residue;
Method Validation;
HPLC;
RI detector.

INTRODUCTION

Ibandronate sodium or Ibandronic acid is a nitrogen-containing highly potent bisphosphonate drug used in the prevention and treatment of osteoporosis[1]. Chemically it is designated as 3-(N-methyl-N-pentyl) amino-1-hydroxypropane-1,1diphosphonic acid, monosodium salt. From a chemical point of view, the pharmalogical function of the active compound is determined by P-C-P configuration, where two phosphate groups are covalently linked to a carbon atom as shown in Figure 1.

Ibandronate contains a tertiary amine group, which does not easily form chromophore derivatives that can be detected by UV light or fluoroscence emission[1]. Development of a chromatographic technique for this class of compound is a challenging task. Many analytical approaches has been published for the determina-

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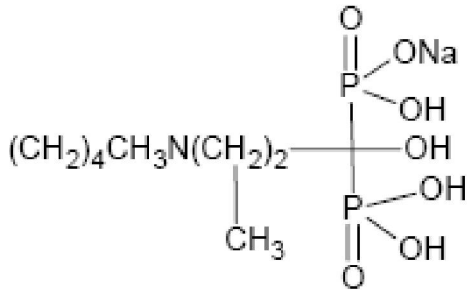

Figure 1 : Chemical Structure of Ibandronate

tion of biopospho nate by intorducing a chromophore fluorophore, or an elctrochemically active group into the molecules by pre and post-column derivatization, thus UV[2, 3] fluoroscence[4, 5], Refractive index (RI) detector[6] or evaporative light-scattering detection (ELSD)[7] respectively. The ELSD method reported for IBN and its impurites is very less sensitivity with high LOD(176 µg mL-1)[7]. There are some other methods available for direct detection are flame photometry[8], conductivity[9] and mass spectrometr[10]. A method based on ion exchange chromatography by using copper ion in mobile phase with UV detection has been reported for analysis of Ibandronate[11].

The pharmaceutical industry follows ethical rules and is bound to monitor strict control over GMP practices when manufacturing drug substance and drug product. The cleaning procedure is one of the most important task to avoid the cross contamination in subsequent batches. There should be always a sensitive analytical method to ensure proper cleaning of equipment used in manufacturing purpose. Analytical method used to determine residual or contaminants should be specific in nature and method should be validated before use[12-14]. Guideline recommend thin layer chromatography (TLC), UV-photometric, total organic analysis (TOC), conductivity, gas chromatography and conventional HPLC method for cleaning control or validation[15].

HPLC with UV detector is a very well established technique and commonly used in most of the analysis in pharmaceutical industry. HPLC with Refractive index detector (RI) is used for quantitative determination of drug substance and their impurities, where UV detection is not possible due to absence of chromophoric group. The RI detector is universal in the sense that for any given compound, a solvent probably found such that RI of analyte differ enough to permit detection. The satisfactory uses of other analytical technique have been reported for the assay and impurity profiling of Ibandronate, however there is no official method available for residue estimation. In our study, a simple HPLC method with RI detection technique was developed and validated for determination of Ibandronate Sodium residue in pharmaceutical manufacturing. The method was validated as per ICH guideline[16] and successfully applied for estimation of compound of interest routine quality control activity.

MATERIALS AND METHODS

Chemicals and reagents

IBN was obtained from Dr.Reddy's Laboratories, Hyderabd, India. Formic acid (HPLC grade), Amonia solution (Analytical Reagent grade), were from Merck (Darmstadt, Germany). Water was purified by a Millipore (Bedford, MA, USA) Milli-Q water-purification system and passed through a 0.22 µm membrane filter (Durapore; Millipore, Dublin, Ireland) before use.

A stock standard solution of IBN was prepared in Milli-Q water as diluent.

Instrumentation

HPLC analysis was performed with a Waters (Milford, MA, USA) alliance HPLC system equipped with a quaternary solvent manager, sample manager, column-heating compartment, and refractive index detector. This system was controlled by Waters Empower software. An Allsep anion column, 150 mm x 4.6mm, 7µm (Grace, Deerfield, USA) employed for chromatographic separation. All samples were centrifuged by Thermo Scientific multifuged machine. The specificity study was conducted by using stainless steel plate. 2.3 Standard and Sample Preparation:

The standard stock solution was prepared by dissolving an accurately weighed amount of drug substance in water, resulting in a concentration of 10 µg mL-1of Ibandronic acid. The test solution was prepared by taking swab with Johnson bud. The Johnson buds were soaked in water, sonicated for 10 minutes and decanted. The sampled surface was swabbed by using above Johnson bud from top to bottom and again from left to right. The wipes were placed in a test tube containing 10 ml of water and sonicated for 10 minutes to make

all particles soluble. Finally each solution was centrifuged at 3000RPM for 5 minutes in order to eliminate insoluble excipients. The supernatant liquid was used for chromatographic analysis.

Chromatography

The analytes were separated on an Allsep anion column (150 mm x 4.6 id, 7μm) at column oven temperature of 60°C with a isocratic run program at a flow-rate of 1.2 mL min^{-1}. The mobile phase was prepared by mixing 2.0 mL of formic acid in 1 Litter of purified water. The pH of mobile phase was adjusted to 3.2 with liquid ammonia solution. The mobile phase was filtered through a 0.22 μm Millipore filter, before use. The RI detection was performed at 50° C optical unit temperature with positive polarity and attenuation 500 X 10^3 nRIU. The sample injection volume was 100 μL.

Method validation

The method was validated for specificity, precision, accuracy, sensitivity, linearity, ruggedness and robustness as per the International Conference on Harmonization (ICH) guidelines[16].

Specificity

The surface of the pharmaceutical equipment consists of mostly stainless steel but there are some other materials used in equipments (Silicon, plexi-glass, textile etc.). However stainless steel is used in most of the manufacturing equipments. During the specificity study stainless steel surface were investigated.

A study was conducted to investigate the interference of surface by injecting swab of stainless steel surface. The placebo solution also injected to determine the interference of placebo peaks at the retention time of active drug peak.

Sensitivity

Sensitivity of the method was established with respect to Limit of detection (LOD) and limit of quantification (LOQ) for IBN. The LOD was determined by identifying the concentration where the signal to noise ratio is found to be about 3. The LOQ was determined by identifying the concentration where the signal to noise ratio is found to be about 10. Precision was verified by injecting six preparations at LOQ concentration. The relative standard deviation of area at LOQ concentration should not be more than 15% is used in cleaning validation.

LOD => S/N = 3 & LOQ => S/N = 10

Accuracy

To confirm the accuracy of the proposed method, recovery studies were carried out by standard addition technique. Samples were prepared in triplicate by spiking ibandronate sodium stock solution at limit concentration over a 4"X4" stainless steel plate. The samples were dried by blowing warm air. The stainless steel surface was swabbed and collected into a test tube containing 5 ml of water and 5ml water added after swabbing. Finally solution was sonicated for 10 minutes and centrifuged at 3000 RPM for 10 minutes. The solution were injected into HPLC.

Precision

The precision of test method was evaluated by using six swab sample preparation, spiking with Ibandronate Sodium solution to get the LOQ concentration of 3μg mL-1 and analyzed as per test method. Intermediate precision was also studied using different column and performing analysis on different day.

Linearity of detector response

A series of solutions of IBN in the concentration ranging from limit of quantification level (3 μg mL-1) to 300% (60 μg mL-1) of standard concentration were prepared and injected into the HPLC system.

Application of developed method

The method suitability was verified by analyzing swab of three different equipments used for finished product manufacturing. Swabbing was done at different location of equipments as defined procedure and solution were centrifuged and injected into HPLC system. This method was also implemented for the assay determination of IBN in pharmaceutical dosage forms.

RESULTS AND DISCUSSION

A reversed-phase chromatographic technique was developed to quantitate a non-chromophoric biophosphonate (IBN) residue in pharmaceutical equipment by using a Refractive index detector. The presence of non-aqueous solvents in the mobile phase, such

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as methanol and Acetonitrile, was studied. Since the solubility and sensitivity of the detection system was strongly reduced in the presence of methanol & Acetonitrile, only aqueous was chosen as mobile phase. Satisfactory peak shape was achieved at the mobile phase pH 3.2. The effect of formic acid concentration on analyte retention was studied. Formic acid is known to improve peak shape and resolution by reducing the analyte interaction with residual silanol groups at the chromatographic surface. Formic acid is expected to reduce the virtual polarity of the analyte in acidic media. Consequently the retention of the compound increases when the eluent contains formic acid. At low pH and high operating column temperature, hydrolysis of the siloxane bond can occur, stripping the bonded phase from the silica support. To avoid this, 2.0 mL of formic acid was used in mobile phase by adjusting pH to 3.2. Satisfactory peak shape was observed by use of formic acid as demonstrated in Figure 2C.

C8 and C18 column were first evaluated as stationary phase for the IBN analysis but none of these column works to retain IBN in column and peak shape was very poor. Because the compound has more than one ionizable group they had to retain on non polar stationary phases for examples C8 or C18, so it is necessary to use ion exchange chromatography or add ion pairing reagent to the mobile phase. Allsep anion column was adopted for the analysis because it provided a better separation of the analytes with good peak shape. Sensitivity of the method is found to be satisfactory in RI detector for a compound with very poor UV absorbance. Selectivity, sensitivity, resolution, and speed of chromatographic separation were optimized for the HPLC method. Although there are other techniques where sensitivity of IBN established in nanogram, however those techniques are more expensive and usually not available at all pharmaceutical manufacturing facilities. Chromatograms obtained from blank, IBN standard and Test solutions are shown in Figure 2A. Figure 2B, & Figure 2C respectively. The retention times were much more reproducible on an anion column and a mixture of formic acid and water mobile phase.

RI detector has been proved to be a promising tool for detection and estimation of IBN residue in pharmaceutical equipments. Use of anion stationary phase enabled optimization of HPLC for both peak selectivity and analysis speed. IBN is well retained and separated from placebo with good peak shape and resolution. No interfering peaks were observed in blank & placebo, indicating that signal suppression or enhancement by the product matrices was negligible. The organic solvent use has been avoided without compromising productivity and performance.

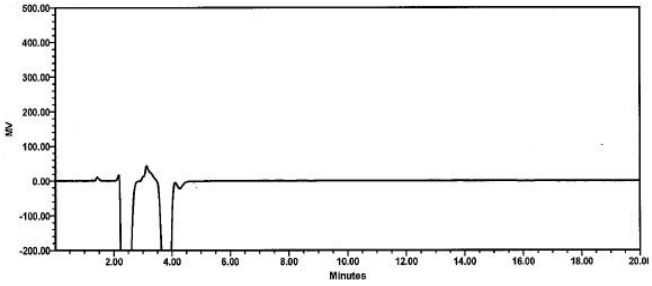

Figure 2A : Typical Chromatogram of Blank

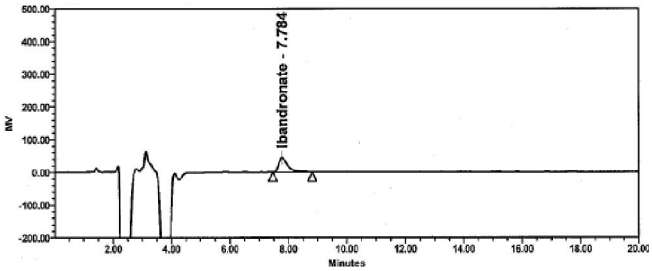

Figure 2B : Typical Chromatogram of Ibandronate Standard

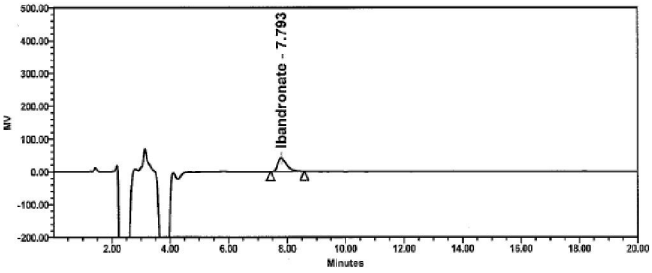

Figure 2C : Typical Chromatogram of Ibandronate Test

TABLE 1 : Results of System suitability

Parameter	Results
Tailing Factor of Ibandronic Acid peak	1.3
% RSD of Ibandronic Acid peak area from six replicate injection of standard preparation	1.2

RSD stands for relative standard deviation

TABLE 2 : Summarized Method Validation data

Parameter	Results
Specificity	No swab interference
Precision (% RSD)	7.7
Recovery(% mean)	101.9

RSD stands for relative standard deviation. Specificity, Precision & accuracy results from method validation.

TABLE 3 : LOD and LOQ of IBN in µg mL-1

Parameter	µg mL^{-1}
LOD	1
LOQ	3
Precission at LOQ (% RSD)	7.7

LOD stands for limit of detection & LOQ stands for limit of quantification.

TABLE 4 : Linear Regression data for calibration curve

Parameters (n=7)	Ibandronic Acid
Slope	9147.7
Y intercept	-15109.409
Standard error	5187.06
Correlation Coefficient	0.999

TABLE 5 : Cleaning validation data of manufacturing equipment

Equipment Name	Residue found in PPM
Fluid Bed Dryer	6.2
Blender	BLD
Mechanical Shifter	4.4
Compression Machine	3.5
Coating Machine	BLD

PPM stands for parts per million, BLD stands for below detection limits.

TABLE 6 : Percentage Assay of Ibandronate Sodium Tablet

Test Preparation	% Assay
Test preparation-1	99.2
Test preparation-2	98.6
Test preparation-3	99.5
Test preparation-4	101.2
Test preparation-5	99.1
Test preparation-6	99.0
Average	99.4
% RSD	0.92

RSD stands for relative standard deviation.

After satisfactory method development it was subjected to method validation as per ICH guideline[16]. The method was validated to demonstrate that it is suitable for its intended purpose by standard procedure to evaluate adequate validation characteristics. The result of system suitability parameter was found to be complying with acceptance criteria: relative standard deviation of replicate injection is not more than 2.0% and USP tailing factor is less than 2.0 as shown in TABLE 1. The result of specificity study ascertained the separation of placebo peak and there is no interference from surface of stainless steel. The % RSD of replicate determination was found to be <10% precision study, which indicates that the method is precise and the data of precision studies are shown in TABLE 2. The result obtained from the recovery study was found within the range of 90% to 110%, which indicates that method is accurate and data for the same are shown in TABLE 2. Sensitivity of the method was verified and the method was found to be linear and precise at limit of quantification and the data of LOD & LOQ studies are given in TABLE 3. The calibration curve of IBN was obtained by plotting the peak area versus concentration over the range of about 3-60 µg/mL and was found to be linear (r = 0.999). The data of regression analysis of the calibration curves are shown in TABLE 4. The applicability of the method was verified by the determination of IBN residue in manufacturing equipment and assay determination of IBN in pharmaceutical dosage forms. The result of residue analysis of all equipment and assay was found to be satisfactory, summarized results obtained are given in TABLE 5 and 6.

CONCLUSION

Although Liquid chromatography (LC) with UV detection is a versatile technique for the analysis of drug in complex matrices, such as biological or pharmaceuticals but the IBN quantification is difficult due to its poor ultra violet absorbency and presence of interfering substances. A number of analytical approaches have been previously described to determine IBN in biological materials and pharmaceutical preparation; however, this is the first study reporting a validated reversed phase method for quantification of IBN residue in cleaning validation. The simple HPLC method developed in this study makes it suitable for estimation of trace amount of residue without interference from excipients and other related substances present in the pharmaceutical matrices. The analytical performance and the result obtained from analysis of cleaning validation demonstrated that the method is reliable and sufficiently robust. In conclusion, the high sensitivity, good selectivity, accuracy and reproducibility of the HPLC method developed in this study makes it suitable for quality control analysis of IBN residue and assay of IBN in pharmaceutical prepara-

tion. The usage of organic solvent is totally avoided, which is one of the best solutions to the current Global Acetonitrile Shortage and will safeguard against future risk.

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