STABILITY INDICATING UPLC METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTIFICATION OF IBANDRONATE SODIUM AND CHARACTERISATION OF ITS DEGRADATION PRODUCTS

A Dissertation submitted to
THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY
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In partial fulfilment of the requirements for the award of the Degree of
MASTER OF PHARMACY
IN
PHARMACEUTICAL CHEMISTRY

Submitted by
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CERTIFICATE

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EXAMINERS

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LIST OF SYMBOLS AND ABBREVIATIONS

ABBREVIATION	EXPANSION
%	Percentage
µg	Microgram
µL	Microlitre
µm	Micrometer
ACN	Acetonitrile
cm	Centimeter
UPLC	Ultra Performance Liquid Chromatography
PDA	Photodiode Array
LOD	Limit of Detection
LOQ	Limit of Quantitation
mg	Milligram
ICH	International Conference on Harmonization
Hrs	Hours
LC-MS	Liquid Chromatography Mass Spectroscopy
IP	Indian Pharmacopoeia
nm	Nanometer
RSD	Relative standard deviation
r^2	Correlation coefficient
RP	Reversed phase
v/v	Volume by Volume
Σ	Sigma
λ	Lambda
min	Minute
SD	Standard deviation
m	Slope

ABBREVIATION	EXPANSION
c	Intercept
DL	Detection limit
QL	Quantitation limit
R_t	Retention time
k'	Capacity factor
µg/ mL	Microgram per milliliter
API	Active pharmaceutical ingredient
°C	Degree Celsius
g/ mol	Gram per mole
avg	Average
AR grade	Analytical reagent grade
g	Gram
IBT	Ibandronate sodium

CHAPTER 1
INTRODUCTION

1.1 MOTIVATION AND PROBLEM STATEMENT

The quality of a drug plays an important role in ensuring the safety and efficacy of the dosage forms. Quality assurance and control of pharmaceuticals and chemical formulations is essential for ensuring the availability of safe and effective drug formulations to consumers. Hence analysis of pure drug substances and their pharmaceutical dosage forms occupies a pivotal role in assessing the suitability to use in patients. The quality of the analytical data depends on the quality of the methods employed in generation of the data. Hence, development of rugged and robust analytical methods are very important for statutory certification of drugs and their formulations with the regulatory authorities.[1-5]

1.2CONCEPT OF ANALYTICAL CHEMISTRY

Analytical chemistry studies and uses instruments and methods to separate, identify and quantify the matter.[6-9] It also focusses on improvements in experimental design, chemometrics and the creation of new measurement tools having broad applications in forensic, medicine, science and engineering.

Analytical chemistry consists of classical, wet chemical methods and modern instrumental methods.

- Classical qualitative methods use separations such as precipitation, extraction and distillation.
- Classical quantitative methods use mass or volume changes to quantify amount.
- Instrumental methods separate samples using chromatography, electrophoresis or field flow fractionation.

The development of pharmaceuticals brought a revolution in human health. These pharmaceuticals would serve their purpose only if they are free

from impurities and administered in an appropriate amount. They may develop impurities at various stages of their development, transportation and storage which makes them risky to be administered, thus they must be detected and quantified.

In the field of pharmaceutical research, the analytical investigation of bulk drug materials, intermediates, drug formulations, impurities and degradation products and biological samples containing the drugs and their metabolites is very important. From the commencement of official pharmaceutical analysis, analytical assay methods were included in the compendial monographs with the aim to characterize the quality of bulk drug materials by setting limits of their active ingredient content. The assay methods in the monographs include titrimetry, spectrometry, chromatography, capillary electrophoresis and electro-analytical methods.

From the stages of drug development to marketing, analytical techniques play a great role in understanding the physical and chemical stability of the drug, selection and design of the dosage form, assessing the stability of the drug molecules, quantitation of the impurities and identification of these impurities, to evaluate the toxicity profiles to distinguish from the API, while assessing the content of drug in the marketed products.

1.3 IMPORTANCE OF NEWER ANALYTICAL METHODS

Newer analytical methods are developed for drugs or drug combination for the following reasons[10-15]

- Official compendia/ pharmacopoeia may not reveal an analytical procedure for the drugs or its combination.
- The literature search may not reveal an analytical procedure for the drug or its combination.
- Analytical methods may not be available for the drug combination due to interference caused by excipients.
- Analytical methods for the quantification of drug or drug combination

from biological fluids may not be available.

On the other hand, existing procedure may

- ✦ Require expensive instrument, reagent or solvents.
- ✦ Involve any extraction or separation steps which may be time consuming.
- ✦ Not rapid, reliable or sensitive.

The newer analytical methods developed find importance in various fields:

1. Research
2. Quality control department in industries
3. Approved testing laboratories
4. Biopharmaceutics and bioequivalence studies and clinical pharmacokinetics.

1.4 TYPES OF ANALYTICAL METHODS

➢Spectral methods

The spectral techniques measure electromagnetic radiation, which is either absorbed or emitted by sample. For example, UV-Visible spectroscopy, IR spectroscopy, NMR spectroscopy, ESR spectroscopy, Flame photometry, Flourimetry etc.

➢Electroanalytical methods

Electroanalytical methods involve the measurement of current, voltage or resistance.

For example, Potentiometry, Conductometry,Amperometry etc.

➢**Chromatographic methods**

There are various advanced chromatographic techniques, which are most reliable and widely used for estimation of multi component drugs in their formulations.

- GLC
- HPLC
- HPTLC

1.5 METHOD DEVELOPMENT

Chromatography is probably the most powerful analytical technique available to the modern chemist. Its power arises from its capacity to determine quantitatively many individual components present in a mixture by a single analytical procedure.[16-21]

Ultra-performance liquid chromatography (UPLC) is a chromatographic technique that can separate a mixture of compounds and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture.

1.5.1 Ultra-performance liquid chromatography (UPLC)

Assuring the safety of a new pharmaceutical compound or drug substance demands that new drug substance must meet the established purity standards. Due to easy availability of sub-standard drugs and medicines in the market it is crucial to assay the drugs in bulk and dosage forms. Hence development of rapid, simple and cost-effective analytical methods for the analysis of drugs is the need of the day in public interest.

Being decisive element for the quality of active pharmaceutical ingredients and formulations it is very important to develop efficient and accurately validated methods for pharmaceutical analysis as per guidelines. The developed method by the analyst should have sufficient controls and proper documentation to ensure the accuracy and reliability of the data.

New drug development requires that meaningful and reliable analytical data be produced at various stages of the development. The developed test methods by the laboratory analyst are used in the evaluation of product safety, dosage form's bioavailability, establishment of specifications for drug substance, intermediates and drug product, stability of the API / formulation, identification, quantification and qualification of impurities, and support to preclinical and clinical studies for product safety and efficacy.

1.5.2 Principle of UPLC

The underlying principle of UPLC is based on the van Deemter relationship which explains the correlation between flow rate and plate height. The van Deemter equation (i) shows that the flow range with the smaller particles is much greater in comparison with larger particles for good results.

$$H = A + \frac{B}{v} + Cv \qquad \text{(i)}$$

Where H represents height equivalent to the theoretical plate (HETP), A, B & C are the constants and v is the flow rate (linear velocity) of the carrier gas. The aim is to minimize HETP to improve column efficiency. The term A does not depend on velocity and indicates eddy mixing. It is smaller if the columns are filled with small and uniform sized particles. The term B denotes the tendency of natural diffusion of the particles. At high flow rates, this effect is smaller, so this term is divided by v. The term C represents the kinetic resistance to equilibrium during the process of separation. The kinetic resistance is the time lag involved in moving from the mobile phase to the stationary phase and back again. The higher the flow rate of the mobile phase, the more a molecule on the packing material inclines to lag behind molecules in the mobile phase. Thus, this term is inversely proportional to linear velocity. Consequently, it is likely to enhance the throughput, and without affecting the chromatographic performance, the separation can be speeded up. The emergence of UPLC has necessitated the improvement of existing instrumentation facility for LC, which takes the benefit of the separation performance (by decreasing dead volumes) and consistent pressures (about 500

to 1000 bars, compared with 170 to 350 bars in HPLC). Efficiency is proportionate to the length of the column and inversely proportional to the radius of the particles. Consequently, the column length can be reduced by the similar factor as the particle radius without affecting the resolution. The use of UPLC has helped in the detection of drug metabolites and enhancement of the quality of separation spectra.

Chemistry of small size particles

The chemistry of the particles used in this course of the method contributes the increased efficiency and potential to work at amplified linear velocity, thereby, providing both the speed and the resolution. Efficiency is one of the important separation parameters which plays a significant role in UPLC since it depends on the same selectivity and retentivity as HPLC. This may be understood with the help of the following basic resolution (Rs) equation (ii):

$$Rs = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k}{k+1}\right) \qquad \text{(ii)}$$

Where α represents the selectivity factor, N is efficiency and K denotes proportionality constant. According to this, resolution increases with increase in efficiency. Since efficiency (N) is inversely proportional to particle size (dp) equation (iii):

$$N \propto \frac{1}{dp} \qquad \text{(iii)}$$

As the size of the particle decreases by a factor of three, there is three times increase in the efficiency and in the resolution, there is an increase of the square root of three (nine times). So, there is an increase in efficiency with consequent increase in resolution and sensitivity as the particle size decreases. Similarly, efficiency (N) is directly proportional to the length of the column (L). So, the equation (iii) may be written as equation (iv) as follows:

$$N \propto \frac{L}{dp} \qquad \text{(iv)}$$

Therefore, the length of the column (L) may be reduced by the same

ratio as the size of the particle without losing the resolution.

Efficiency also has indirect relationship with the peak width (w) according to the equation (v):

$$N \alpha \frac{1}{w^2} \qquad \text{(v)}$$

This means that narrower the peaks are, easier is their separation from each other. Peak height (H) also has opposite relationship with peak width (w) according to equation (vi):

$$H \alpha \frac{1}{w} \qquad \text{(vi)}$$

Based on the above facts, in UPLC, by reducing the particle size ($1/3^{rd}$), the column length is reduced ($1/3^{rd}$), the flow rate is increased (3 times) and the separation is done faster ($1/9^{th}$ time) with maintaining the resolution. For this reason, short columns packed with small size particles (about 2 μm) are used with these systems, to quicken the separation with higher efficiency, while maintaining a tolerable loss of load.

Advantages of UPLC

It is more selective and sensitive with high resolution performance and faster resolving power. It also reduces process cycle time and assures end-product quality with reduced cost of operation and decreased run time. It increases sensitivity and provides quick analysis through the use of a novel column material of very small particle size. It decreases the consumption of solvent and increases sample throughput and also provides real-time analysis in step with manufacturing processes.[22-26]

Disadvantages of UPLC

A major disadvantage of UPLC is the higher back pressures compared to conventional HPLC which decreases the life of the columns. Increasing the column temperature reduces the back pressure problem in UPLC. Moreover, the particles of less than 2 μm are mostly non-regenerable and, therefore, have a narrow use.

1.5.3 Instrumentation of UPLC

The instrumentation of UPLC includes- sample injection, UPLC columns and detectors.

Sample Injection

The use of the injector is to add precisely measured, a small volume of solution containing the sample in the mobile phase. The injection must be done reproducibly and accurately. Conventional injection valves may be manual or programmed and to guard the column from extreme pressure instabilities, the injection process must be comparatively pulse-free. To reduce the potential band spreading, the swept volume of the device is desired to be minimal. A quick injection cycle time is required to fully avail the speed afforded by UPLC. To increase the sensitivity, low volume injections with minimal carryover are required. The volume of the sample in UPLC is usually 2-5 µL. Nowadays, direct injection approaches are utilized for the biological samples.

UPLC Column

Columns used for UPLC have been developed and manufactured by the following different companies:

1. Waters: Acquity UPLC columns and Vanguard Pre-columns have been produced.

2. Agilent technology provides highest performing columns that provide fast and reproducible results. These include Poroshell 120 columns, ZORBAX Rapid Resolution High definition columns, ZORBAX Eclipse plus columns and ZORBAX Rapid Reduction High Throughput columns.

3. AltechAssosciate.

4. Phenomenex provides Kinetex® Coreshell HPLC/UHPLC columns of high efficiency and performance.

Different types of columns being used in UPLC are packed with particles which are produced through different technologies. These are as

follows:

1. Charged Surface Hybrid [CSH] particle technology,
2. Ethylene Bridged Hybrid [BEH] particle technology,
3. High Strength Silica [HSS] particle technology and
4. Peptide Separation Technology (PST).

CSH Particle Technology

CSH Technology is the newest methodology in the development of hybrid materials which utilizes low-level surface charged particles for the enhancement of the selectivity and sharpness of the peaks. Hybrid based packing material approach provides sharp peaks specially for basic compounds under low pH with higher efficiency and chemical stability. CSH C_{18}, CSH Phenyl hexyl, and CSH Fluoro phenyl are the different types of CSH particles being widely used.

These columns have the advantage of exceptional peak shape, increased loading capacity (CSH C_{18}); complementary selectivity to straight chain alkyl phases (CSH-phenyl-hexyl); selectivity for positional isomers, halogenated and polar compounds (CSH-fluoro phenyl). The other advantages include- higher stability at a wide range of pH, improved batch to batch reproducibility and fast column equilibration after any change in the pH of the mobile phase.

Applications of CSH technology based columns include the analysis of basic compounds even in their ionized form. While analyzing the basic compounds under low pH and reversed phase conditions, poor peak shape and retention often result. Whereas, CSH Phenyl hexyl columns provide exceptional peak shape for basic drugs under acidic mobile phase conditions.

Solvent Delivery System

The solvent delivery system must perform reproducible high pressure pumping with a smooth and constant flow of solvents. UPLC systems routinely operate at 8000-15000 psi. The delivery system must also remunerate for a

variety of solvents used in isocratic, linear & nonlinear gradient elution and solvent compressibility for a wide range of pressures. The Acquity UPLC binary solvent manager has two solvent delivery modules operating in parallel for high pressure merging of two solvents in <140 µL internal system volume. The dissolved gases are removed by vacuum up to four eluents plus two wash solvents.

The Detector

The detector employed for the UPLC should be able to give a high sampling rate with narrow obtainable peaks (<1 s half-height peak width) and the dispersion of the peaks should be minimum so that the wastage of the separated solute is less on the column. The UPLC technique provides the sensitivity of separation two to three times more than the previous analytical method HPLC, which is also due to the method employed for the detection. The detectors employed in the UPLC are Acquity photodiode array (PDA) and Tunable Vis-UV (TUV) in which Teflon AF is used which provides an internally reflective surface and enhances the light transmission efficiency by eliminating the internal absorptions. These have path lengths 10 nun, acquisition rates 20 (PDA) and 40 (TUV) points, and total internal volume 500 nL. Mass spectrometric detection has also been used with UPLC.

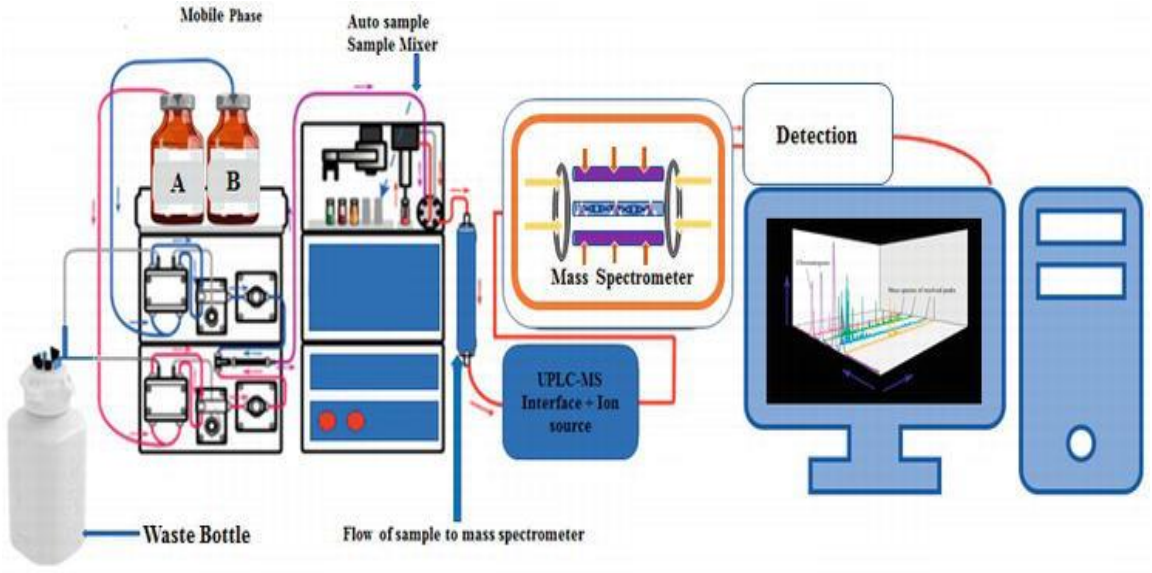


Figure 1.Diagramatic representation of UPLC Instrumentation

1.5.4 Applications of UPLC[27-32]

Determination of Pesticides in Groundwater

UPLC coupled with triple quadrupole tandem mass spectrometry (UPLC™-MS/MS) can be utilized to determine the trace level pesticides in groundwater in less time and speedy manner. The technique has enhanced the analysis speed, sensitivity, and resolution.

Improved Resolving Power in Peptide Maps

Peptide mapping is an essential technique for the characterization of proteins. Due to exceptionally reduced instrument and column dispersion, the analyzes of tryptic digest of phosphorylase by UPLC technology provides significantly improved resolution, peak capacity, and sensitivity compared to HPLC, allowing the detailed characterization of the protein.

Rapid Dose Formulation Analysis

Nowadays, the use of UPLC together with UV and MS detection has been widely utilized in pharmaceutical applications. Several commercial drug formulations were used as models to study the efficiency of separations with the change of flow rate. The efficiency was judged on the parameters of resolution, theoretical plates, column ruggedness, retention time, and peak area. For example, mefenamic acid and chloramphenicol separation was studied in dimethylacetamide/ polyethylene glycol-200 vehicle.

Analysis of Traditional Chinese Medicines (TCM)

The identification and quantification of components of TCM by chromatographic analysis is one of the major challenges. TCM is a complex matrix in which all the constituents play a specific role for the overall efficacy. Therefore, the analysis of all the constituents is synchronously necessary for the quality control. The new technique UPLC is used for the quality control of the TCM.

Multi-Residue Analysis of Pharmaceuticals in Waste Water

The water used in the pharmaceutical companies is found to have the traces of various cholesterol-lowering statin agents, anti-ulcer agents, antibiotics, beta-blockers, analgesics, anti-inflammatory agents, lipid regulating agents, psychiatric drugs, and histamine H2 receptor antagonists. UPLC coupled with Q-TOF-MS is used to confirm and screen these drugs in the samples of waste water treatment plant.

Identification of Metabolites

The identification and detection of all the possible metabolites of the candidate drugs for the discovery of new chemical entities is a very important step. For the identification of the metabolites, a high sample throughput is required to be maintained by the analysts to provide quick results to the medicinal chemists. UPLC-MS/MS is helpful in biomarker discovery as it meets tough analytical requirements and provides sensitivity, mass accuracy, dynamic range, and resolution.

In Manufacturing / Quality Assurance (QA) / Quality Control (QC)

Identification, quantification, purification, efficacy and safety are key parameters to be evaluated during manufacturing of a drug product and pharmaceutical dosage form. Material stability is also observed as a component of QA and QC. UPLC is used as an important tool in QA/QC laboratories for the quantitative and extremely regulated analysis.

Impurity Profiling

Impurity profiling should be efficient for consistent detection and separation of all the impurities present in the active compound. The drug development and formulation process demand accurate measurement/testing of low-level impurities present with the active pharmaceutical ingredients or the excipients or the raw materials used in the preparation of the final product. Thus, the presence of excipients in the sample makes the profiling difficult and with HPLC method, it takes longer time for analysis to achieve

sufficient resolution. Thus, the combination of UPLC with mass spectrometry has been useful for the documentation of drug and endogenous metabolites in the final product.

1.6 STABILITY INDICATING METHOD

- Today a majority of the drugs used are of synthetic origin. These are produced in bulk and used for their therapeutic effects in pharmaceutical formulations. These biologically active chemical substances are generally formulated into convenient dosage forms such as tablets, capsules, dry syrups, liquid orals, creams or ointments, parenterals, lotions, dusting powders, aerosols, metered dose inhalers and dry powder inhalers etc. These formulations deliver the drug substances in a stable, non-toxic and acceptable form, ensuring its bioavailability and therapeutic activity.

- In view of the wide variety of excipients used in formulating drugs for administration to patients, drug substances can undergo transformation by interacting with one or more components of the formulation. Formulated drugs can degrade due to acidic or basic environments created by the formulation matrix. Drugs can degrade due to exposure to temperature, humidity and light during manufacturing, transportation and storage during its shelf life. Due to this, it is essential to know the degradation pathways of the drugs in acidic, basic, neutral, oxidation conditions and their susceptibility to temperature and humidity and to formulate them in a manner in which they are stabilized and retain its quality throughout their shelf life.[33-38]

- As most drugs contain functional groups which can participate in reactions in some way or the other, it is essential that the analytical methods developed for estimation of the purity and impurities, are capable enough to separate all the desired and undesired components and devoid of any interferences from the formulation matrix. When analytical methods are able to precisely and accurately quantify without missing any impurities, without under estimation or over estimation and

detect all possible impurities and degradants which can form during stability studies, with adequate sensitivity and exactly reflect the quality of drug substances and drug products, those methods are called stability indicating methods.

- In recent times, the development of stability indicating assays has increased enormously, using the approach of stress testing as outlined in the International Conference on Harmonization (ICH) guideline $Q1AR_2$ and even this approach is being extended to drug combinations. This ICH guideline requires that stress testing on API and drug products should be carried out to establish their inherent stability characteristics, which should include the effect of temperature, humidity, light, oxidizing agents as well as susceptibility across a wide range of pH.[39-41]

- The knowledge gained from stress testing can be useful for (i) The development of stable formulation and appropriate packaging design (ii) Controlling manufacturing and processing parameters (iii) Identification and isolation of toxic degradants during API synthesis (iv) Recommendation of appropriate storage conditions and shelf-life determination and (v) Designing and interpreting environmental studies, as the degradation of the drug in the environment will often be similar to degradation observed during stress-testing studies.[43-45] It is also recommended that analysis of stability samples should be done through the use of a validated stability-indicating testing method.

1.6.1 Forced Degradation Study

The ICH guideline Q1A on Stability Testing of New Drug Substances and Products gives indications for the testing of parameters which may be susceptible to change during long storage and are likely to affect quality, safety and efficacy by validated stability indicating testing methods. It is mentioned that forced degradation studies or stress testing at temperatures in 10°C increments above the accelerated temperatures, extreme pH and under oxidative and photolytic conditions have to be carried out on the drug substance so as to set up the stability characteristics and degradation pathways to back up the

suitability of the proposed analytical procedures.

1.6.2 Objectives of forced degradation study

Forced degradation study provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light. Lack of drug substance or drug product stability may affect the purity, potency and safety of the drug product.[46-48]

- To establish the degradation pathways of drug substances and drug products
- To determine the intrinsic stability of a drug substance in formulation
- To provide information on drug substance / product characteristics
- Identification of potential degradants
- Establish a re-test period for the drug substance or shelf life of the drug product and recommended storage conditions
- To generate more stable formulations
- To differentiate degradation products that are related to drug products from those that are generated from non-drug products in a formulation
- Process development, design and optimization of manufacturing process
- To elucidate the structure of degradation products
- To understand the chemical properties of drug molecule
- Design of formulation (including selection of excipient for formulation)
- To generate a degradation profile similar to that of what would be observed in a formal stability study under ICH conditions
- Packaging development

- To solve the stability related problems
- Provide data to support clinical trials, registration submission or commercialization.

Forced degradation studies are used to generate product related variants and develop analytical methods to determine the degradation products formed during accelerated and long term stability studies. The degradation products obtained reveal the degradation pathwayof the drug and facilitate the development of stability indicating methods. The degradation products should be evaluated for potential hazard and hencearises the need for characterization and quantification.

Forced degradation of drug substances are usually conducted in solution and in solid state at temperature exceeding accelerated stability conditions (>40ºC). The degradation pathways include hydrolysis, oxidation, thermal, photolysis and polymerization. Hydrolysis is investigated in solution over a broad pH range and in the solid state by exposure of the drug to high relative humidity. Oxidation in solution can be investigated through control of exposure to molecular oxygen or by addition of oxidizing agents such as peroxides. The effects ofthermolysis are usually assessed in solid state by applying heat. Photolysis is investigated in solution or the solid state by irradiating samples with light of wavelengths in the range of 300-800nm. Polymerisation can be investigated by measuring the rate of degradation as a function of different initial drug concentrations in solution.

A degradation level of 10-15% is considered for validation of a chromatographic purity assay. The forced degradation conditions utilized for drug substances and finished dosage form is tabulated in **Table 1**.

Table 1. Conditions for Forced degradation study

Degradation type	Experimental conditions	Storage conditions	Sampling time
Hydrolysis	0.1N HCl	40ºC, 60ºC	1,3,5 days
	0.1 N NaOH	40ºC, 60ºC	1,3,5 days
	pH: 2,4,6,8	40ºC, 60ºC	1,3,5 days
Oxidative	3% H_2O_2	25ºC, 60ºC	1,3,5 days
	Peroxide control	25ºC, 60ºC	1,3,5 days
	Azobisisobutyronitrile (AIBN)	40ºC, 60ºC	1,3,5 days
Photolytic	Light, 1 X ICH	NA	1,3,5 days
	Light, 3X ICH	NA	1,3,5 days
	Light control	NA	1,3,5 days
Thermal	Heat environment	60ºC	1,3,5 days
	Heat environment	60ºC / 75%RH	1,3,5 days
	Heat environment	80ºC	1,3,5 days
	Heat environment	80ºC / 75%RH	1,3,5 days
	Heat control	Room temperature	1,3,5 days

1.7 METHOD VALIDATION

Analytical method validation is the process of demonstrating that an analytical method is reliable and adequate for its intended purpose. Reliable data for the release of clinical supplies, stability and setting shelf life can only be generated with appropriate validated methods.[49]

1.7.1 Validation Parameters

Accuracy

Accuracy is the measure of how close the experimental value is to the true value. The RSD of the replicates will provide the analysis variation or how

precise the test method is. The mean of the replicates, expressed as % label claim, indicates how accurate the test method is.

Detection Limit (LOD) and Quantitation Limit (LOQ)

Detection limit is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. Quantitation limit is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions.

Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration of analyte in the sample. The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Precision

Precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. ICH has defined precision to contain three components: repeatability, intermediate precision and reproducibility.

(a) Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

(b) Intermediate precision

Intermediate precision expresses within laboratory variations like different days, different analysts, different equipments, etc. Intermediate precision was previously known as part of ruggedness. The attribute evaluates the reliability of the method in a different environment other than that used during development of the method. The objective is to ensure that the method will provide the same results when similar samples are analyzed once the method development phase is over. Depending on time and resources, the method can be tested on multiple days, analysts, instruments, etc.

(c) Reproducibility

As defined by ICH, reproducibility expresses the precision between laboratories. Multiple laboratories are desirable but not always attainable because of the size of the firm.

Robustness

ICH defines robustness as a measure of the method's capability to remain unaffected by small, but deliberate variations in method parameters. Robustness can be partly assured by good system suitability specifications. Testing by varying some or all conditions, e.g., age of columns, column type, column temperature, pH of buffer in mobile phase, reagents, is normally performed.

Sample Solution Stability

Solution stability of the drug substance or drug product after preparation according to the test method should be evaluated according to the test method. This is of concern especially for drugs that can undergo degradation by hydrolysis, photolysis or adhesion to glassware.

Specificity/Selectivity

The analyte should have no interference from other extraneous components and be well resolved from them. A representative chromatogram or

profile should be generated and submitted to show that the extraneous peaks either by addition of known compounds or samples from stress testing are baseline resolved from the parent analyte.

System Suitability Specifications and Tests

The accuracy and precision of data collected begin with a well behaved chromatographic system. The system suitability specifications and tests are parameters that provide assistance in achieving this purpose.

Capacity factor (k')

The capacity factor is a measure of where the peak of interest is located with respect to the void volume. The peak should be well-resolved from other peaks and the void volume. Generally the value of k' is > 2.

Relative retention

Relative retention is a measure of the relative location of two peaks. This is not an essential parameter as long as the resolution (R) is stated.

Resolution (R)

R is a measure of how well the two peaks are separated. For reliable quantitation, well-separated peaks are essential. R of >2 is desirable.

Tailing factor (T)

The accuracy of quantitation decreases with increase in peak tailing. T of < 2 is desirable.

Theoretical plate number (N) and HETP

Theoretical plate number is a measure of column efficiency, i.e., how many peaks can be located per unit run-time of the chromatogram. HETP, the height equivalent to theoretical plate, measures the column efficiency per unit length (L) of the column. The theoretical plate number depends on elution time but in general should be > 2000.

1.8 IN-SILICO TOXICITY PREDICTION

The OSIRIS property explorer calculates on-the-fly various drug related properties whenever a structure is valid. Prediction results are valued and colour coded. Properties with high risks of undesired effects like mutagenicity or poor intestinal absorption are shown in red whereas a green colour indicates drug-conform behaviour.

CHAPTER 2
REVIEW OF LITERATURE

1. **Van Beek E *et al.*** (1999)[50] reported the specific involvement of these two enzymes, and also of those more upstream in the mevalonate pathway, in the mechanism of action of Bps and the production of mevalonate-derived intermediates of isoprenoid biosynthesis by HPLC, in response to bisphosphonates in crude homogenates of bovine brain. We specifically investigated the effects of the NBps, alendronate, risedronate, olpadronate, ibandronate and that of the non NBp clodronate on the formation of polyisoprenyl pyrophosphate Farnesyl pyrophosphate synthase is the molecular target of nitrogen-containing bisphosphonates.

2. **Barrett J *et al.*** (2004)[51] reported a clinical pharmacological and pharmacokinetic update of Ibandronate and the clinical efficacy of ibandronate at low doses is facilitating the development of robust intermittent oral and IV dosing regimens. These regimens are expected to provide greater convenience to the patient and potentially assist in improving long-term therapy adherence.

3. **Jiang Y *et al.*** (2005)[52] evaluated the determination of ibandronate and its degradation products by ion-pair RP LC with evaporative light-scattering detection and concluded that the newly developed method also enables direct measurement of ibandronate in different dosage forms without the need for derivatization. The method is also applicable as a simple quality-control method for ibandronate during production and storage. The result has led us to investigate a wide range of applications of this methodology, especially for quality control of other bisphosphonates.

4. **Endele R *et al.*** (2005)[53] reported the use of GC–MS also eliminates the need for a separate ELISA assay for urine samples. In the experimental setting, the quantification of bone ibandronate levels has led to greater understanding of the bone kinetics of ibandronate and the total dose concept,

thus supporting the use of intermittent dosing schedules in clinical trials.

5. **Delmas PDJ *et al.*** (2006)[54] reported one-year results from the dosing intravenous administration study. The findings of the DIVA study indicate that IV ibandronate injections administered every 2 months or every 3 months are at least as effective and similarly well tolerated as an established regimen of daily oral ibandronate, in postmenopausal women with osteoporosis. Intravenous administration of ibandronate is likely to be advantageous for patients who cannot tolerate oral bisphosphonates or have difficulty complying with oral treatment.

6. **Reginster JYM *et al.*** (2008)[55] reported the oral ibandronate is poorly absorbed from the GI tract, bioavailability is reduced when taken with food and, therefore, patients are required to fast before and after dosing. Once absorbed, ibandronate is either distributed and bound to the bone or excreted unchanged in the urine or faeces. No drug–drug interactions of clinical relevance have been reported.

7. **Huang YS *et al.*** (2011)[56] evaluated electrochemiluminescence detection method for the analysis of ibandronate in drug formulations and human urine by capillary electrophoresis and found that the feasibility of efficient and accurate determination of IBT in drug formulation by the new method was demonstrated. In couple with SPE pretreatment using magnetic Fe3O4@Al2O3 NPs as solid phase, the proposed method was successfully applied to the determination of IBT at sub-mM level in human urine. The novel magnetic SPE procedure developed for IBT in urine.

8. **Malpezzi L *et al.*** (2012)[57] evaluated Structural and thermal characterization of sodium ibandronate monohydrate and found that Crystalline NaIb monohydrate appears to undergo thermal dehydration and rehydration processes that were monitored by variable temperature X-ray powder diffraction analysis. Furthermore, thermogravimetric analysis shows a mass loss of 5% weight of the sample corresponding to a release of one water molecule per NaIb molecule.

9. **Jineetkumar B. Gawad*et al.*** (2012)[58] developed and validated RP-

HPLC assay method for the determination of ibandronate sodium in tablet dosage form and reported that the proposed method is more sensitive and the methods depend on the use of simple and cheap chemicals and techniques but provide sensitivity comparable to that achieved by a sophisticated and expensive technique like HPLC. Thus, they can be used as alternatives for rapid and routine determination of bulk sample and tablets.

10. **Rubén *et al.***(2013)[59] reported the practical and regulatory considerations for stability indicating methods for the assay of bulk drugs and drug formulations and reported that method validation and demonstration of suitability of the SIM for monitoring products actually formed and proves its stability indicating power.

11. **Ruiz-Medina A *et al.*** (2013)[60] evaluated Rapid fluorimetric quantitation of ibandronate by coupling quantum dots and multicommutated flow injection analysis and an automatic spectroscopic method for the analysis of IBT in pharmaceuticals has been reported. The method makes use of the quenching effect produced by the analyte over the fluorescence of MPA-capped CdTe QDs.

12. **Prajakta A *et al.*** (2013)[61] developed and validated UV Spectrophotometric Method for estimation of Ibandronate sodium in Pharmaceutical Formulation and reported that simple, accurate, precise, sensitive spectrometric method was done for the estimation of Ibandronate sodium in pharmaceutical formulation.

13. **Jineetkumar B. Gawad *et al.*** (2013)[62] developed and validated reverse phase HPLC dissolution method for determination of ibandronate sodium in tablet dosage form and reported that the developed stability indicating HPLC method has advantages over other analytical methods due to selectivity and better sensitivity.

14. **P. Bose *et al.*** (2014)[63] developed simultaneous RP-HPLC and UV Spectroscopic method and Validated the estimation of Ibandronate Sodium in Bulk and Pharmaceutical Dosage Form and reported that the result of the marketed formulation (Boniva) was found to be within

100.42 ± 0.03% for both UV and HPLC.

15. **Srikant Pimple *et al.*** (2014)[64] reported Formulation development and compatibility study of ibandronate sodium injection and reported that all the results obtained from stability studies were found to be well within the specified limits.

16. **Wahl O *et al.*** (2015)[65] evaluated the impurity profiling of ibandronate sodium by HPLC–CAD and found that the amount of drug estimated from tablet formulation were in precise with label claim. The method was statistically validated as per ICH guidelines and can be successively applied for analysis for tablet formulation. The proposed method is economical and sensitive for estimation of ibandronate sodium in pharmaceutical formulation.

17. **Demoro B *et al.*** (2018)[66] reported the Solution behavior and antiparasitic activity of Ibandronate and found that the ability of the obtained metal complexes of catalyzing the generation of free radical species in the parasite could be related to the observed anti-T. cruzi activity.

18. **Mabrouk M *et al.*** (2019)[67] evaluated Indirect spectrophotometric determination of ibandronate in pharmaceutical formulations via ligand exchange and found that the described method enables accurate and precise determination of ibandronate in the bulk drug and tablet dosage forms. Since the method is simple, rapid, inexpensive and needs only a spectrophotometer, it can efficiently be used for routine analyses in quality control laboratories.

19. **Mabrouk M *et al.***(2019)[68] reported the Online Post-column Indirect Detection for Determination of Ibandronate in Pharmaceutical Tablets by HPLC/DAD and reported that the validated LC-MS/MS method is suitable for analysis of IBN in pharmacokinetic studies.

20. **Kaufman TS *et al.*** (2019)[69] studied the Practical and regulatory considerations for stability-indicating methods for the assay of bulk drugs and drug formulations and found that not every degradation product found in the stress tests will be observed under natural degradation conditions, so, as suggested by the Q1A guideline,

simplified SIMs should be designed that allow separation of the pertinent degradation products from the main component, while still being capable of unveiling the effects of instability.

21. **Yılmaz DÇ *et al.*** (2019)[70] evaluated the Determination of Stability Constants of Ibandronate Complexes with Ca (II), Mg (II) and Sr (II).

22. **Moustapha ME *et al.*** (2020)[71] evaluated Solid phase extraction and LC-MS/MS quantification of ibandronate in human plasma.

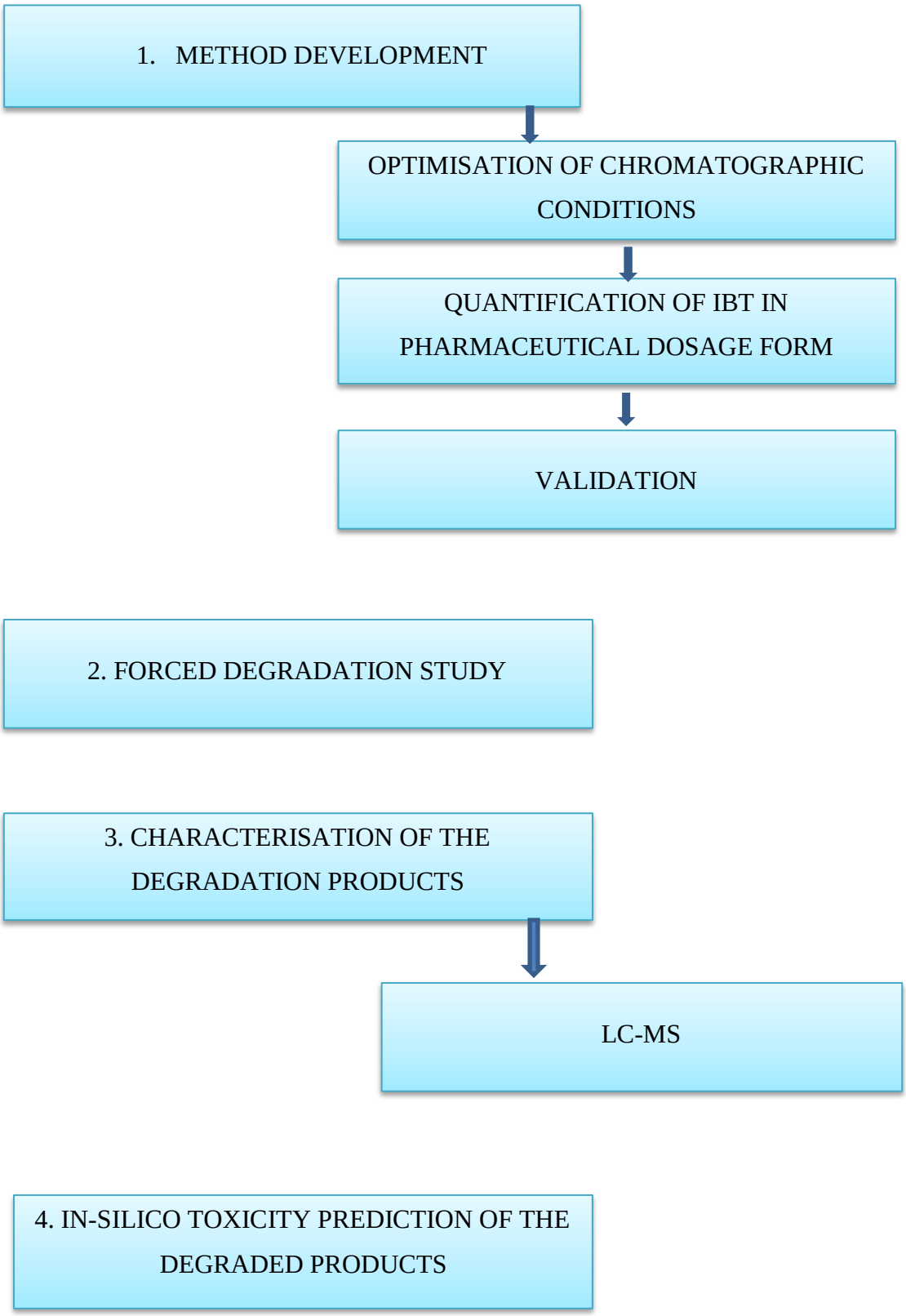
CHAPTER 3
AIM AND OBJECTIVE

3.1 NEED FOR METHOD DEVELOPMENT

Ibandronic acid is a bisphosphonate medication used in the prevention and treatment of osteoporosis and metastasis-associated skeletal fractures in people with cancer. It may also be used to treat hypercalcemia (elevated blood calcium levels). It is typically formulated as its sodium salt **ibandronate sodium**. The literature survey shows that no UPLC method has been developed for quantification of IBT in pharmaceutical formulation. Hence an attempt has been made to develop a Stability indicating UPLC method for the quantification of Ibandronate. The objectives of the study include the following:

- To develop Stability indicating UPLC method for the quantification of **ibandronate sodium** in bulk and pharmaceutical formulation
- To validate the developed method
- To prove the Stability indicating nature of the developed method
- Characterisation of the degradation product of **ibandronate sodium**
- In-Silico Toxicity prediction of the degradation product of **ibandronate sodium**

3.2 PLAN OF WORK



CHAPTER 4
DRUG PROFILE

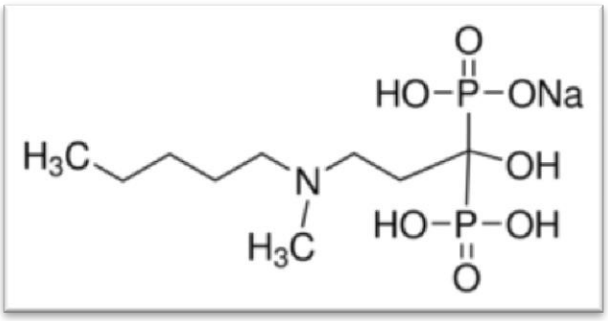
IBANDRONATE SODIUM

Molecular formula : $C_9H_{23}NNaO_7P_2$

Molecular weight : 359.21 g/mol

Chemical name : Sodium hydrate hydrogen {1-hydroxy-3-[methyl(pentyl)amino]-1-phosphonopropyl}phosphonate

Chemical structure :



Pharmacological action : Bisphosphonate which inhibits bone resorption via actions on osteoclast activity, leading to an indirect increase in bone density.

Dose : 150 mg PO every month

Indications : Indicated for treatment and prevention of osteoporosis in postmenopausal women; increases BMD and reduces vertebral fracture incidence.

Contraindication : Ibandronate is not indicated for use in women and lactating women.

Bioavailability : As with most bisphosphonates, ibandronate (pKa 2.0, 6.3, and 10.5) is poorly absorbed from the GI tract following oral administration.

Special precautions : Take at least 60 minutes before first food, drink or other medications; take with full glass of water, in upright position & remain upright for 60 minutes to minimize esophageal adverse events. Take with plain water only; juice or coffee can significantly reduce bioavailability. Do not chew or suck tablet.

Mechanism of action

Bisphosphonates (Bps) suppress osteoclastic bone resorption and are effective treatments of common skeletal disorders such as osteoporosis, metastatic bone disease and Paget's disease of bone. Their antiresorptive action involves inhibition of the function and survival of mature osteoclasts.[29] The action of ibandronate on bone tissue is based partly on its affinity for hydroxyapatite, which is part of the mineral matrix of bone. Nitrogen-containing bisphosphonates, such as ibandronate appear to act as analogues of isoprenoid diphosphate lipids, thereby inhibiting farnesyl pyrophosphate (FPP) synthase, an enzyme in the mevalonate pathway of cholesterol biosynthesis. Inhibition of this enzyme in osteoclasts prevents the biosynthesis of isoprenoid lipids (FPP and GGPP) that are essential for the post-translational farnesylation and geranylgeranylation of small GTPase signaling proteins. This activity inhibits osteoclast activity and reduces bone resorption and turnover. In postmenopausal women, it reduces the elevated rate of bone turnover, leading to, on average, a net gain in bone mass.

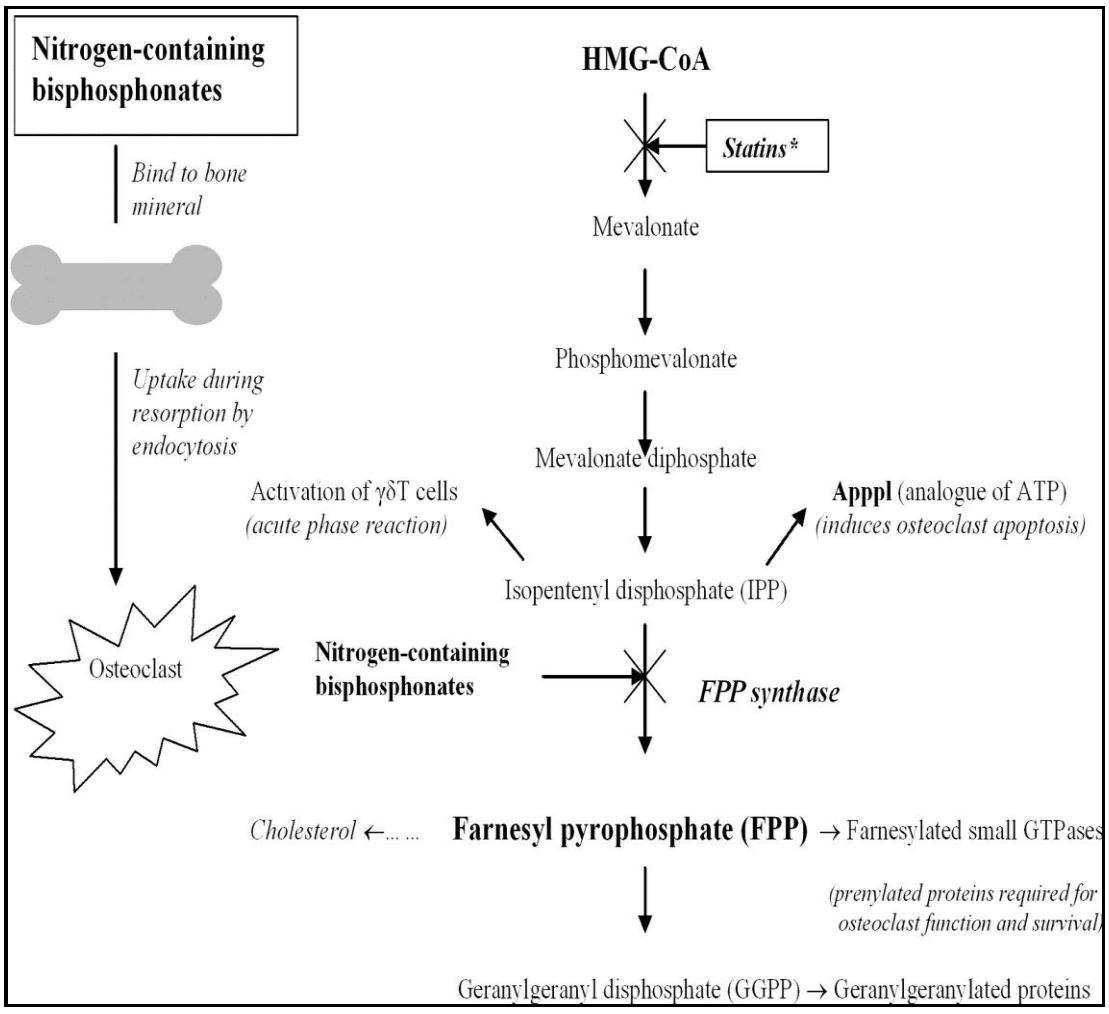


Figure 2. Mechanism of action of Ibandronate sodium

CHAPTER 5
MATERIALS AND METHODS

5.1 REFERENCE STANDARD USED

Ibandronate sodium standard was obtained as gift sample from Fleming Laboratories Limited., Hyderabad.

5.2 REAGENTS

All the solvents used for chromatographic study were of UPLC grade.

5.3 INSTRUMENTS

UPLC

- Instrument - TPDA detector
- Software - Chromquest
- Column - C18 (50mm) UPLC
- Pump - LC - 20 ATVP Series
- Injector - Autosampler
- Detector - UV Detector
- Syringe filter - PTFE, 0.45µm pore
- LC injection vials - 2mL with Teflon Coated Caps.

5.4 UPLC METHOD DEVELOPMENT

5.4.1 Chromatographic conditions

Chromatographic separation was achieved on Thermo Chromquest C_{18} column (50mm x 4.0mm ID, 2µm particle size) employing gradient elution using Ammonium formate buffer and Acetonitrile at pH 5.3 ± 0.05 (70:30)v/v as mobile phase. The mobile phase was filtered through membrane filter

(0.45µm) and sonicated for 30min prior to use. Separation was performed at at a flow rate of 0.4 mL/min and the retention time was 2.54 min. Detection was performed at a wavelength of 215 nm.

5.4.2 Standard solution and construction of calibration curve

Stock standard solution of ibandronate was prepared in water as a diluent.[30] Each solution was further diluted to give a series of working standards. About 50.42 mg of IBT standard was accurately weighed and transferred into a 100ml volumetric flask, dissolved and made upto volume with diluent. 5 ml of standard stock solution was pipetted out into a 50ml volumetric flask,. Thus the standard stock solution of IBT containing 50 µg/ml was prepared.

From the above stock solution, concentrations in the range of 40-120 µg/mL of IBT were obtained. The peak area for the different concentrations of IBT were recorded. The calibration curve was constructed between concentrations and respective peak area.

5.4.3 Analysis of formulation

For analysis of the tablet dosage form, ten tablets containing 50mg of Ibandronate sodium was weighed and the average weight was determined. The powder equivalent to the weight of 50 mg of Ibandronate sodium was transferred to a 100ml volumetric flask, dissolved and made upto the volume with diluent. 5 ml of the solution was pipetted out into a 50ml volumetric flask and made upto the volume using a diluent and shaken well. The identity of the compound was established by comparing the retention time of sample solution with that of standard solution and the amount of **IBT** was calculated.

5.5 VALIDATION OF THE PROPOSED METHOD

5.5.1 Linearity study

In order to prepare stock solution, 203 mg of Ibandronate sodium was accurately weighed, dissolved in diluent with sonication and diluted to 100 ml with the diluent. The mobile phase was filtered through 0.45-µm membrane

filter and delivered at 1ml/min for column equilibration; the baseline was monitored continuously during this process. The detection wavelength was 215 nm. The prepared dilutions were injected in series, peak area was calculated for each dilution, and concentration was plotted against peak area. The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is also termed as trueness. It was done by recovery study. Sample solutions were prepared with 100% in triplicate.

5.5.2 System Precision (Repeatability)

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision. Solutions of Ibandronate sodium were prepared as per test method and injected for 6 times. The mean SD and RSD were checked for precision. Intermediate precision (Ruggedness) Six samples were prepared by different analyst by using different column, different system on different day. The system suitability criteria were evaluated. % RSD of for above 6 preparations was calculated and the overall % RSD for above experiment results was also calculated.

5.5.3 Analytical solution stability

The stability of the drug in solution during analysis was determined by repeated analysis of standard and sample. The standard and sample were prepared and injected into UPLC at initial and different time intervals up to 24 hrs and cumulative % RSD for peak area was determined.

5.5.4 Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. Range to be inferred from the data of linearity, recovery and precision experiments. All data outside this range were regarded as invalid. Samples with concentrations higher

than the upper limit of quantification were diluted and re-assayed.[31]

5.5.5 Specificity and selectivity

Specificity is the ability to asses unequivocally the analyte in the presence of components which maybe expected to present. The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is the procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample. The method is quite selective. There was no other interfering peak around the retention time of Ibandronate Sodium also the baseline did not show any significant noise.

5.6 Forced Degradation Studies

5.6.1 Acid degradation studies

10 tablets were weighed. The average weight was determined. Sample powder equivalent to 100 mg of Ibandronate sodium was transferred in to a 100 ml volumetric flask. About 50 ml diluent was added and sonicated for 10mins. Added 5 ml of 5M HCl and kept at 80°C on water bath for 5 hrs. Then solution was allowed to cool at room temperature, 5 ml 5M of NaOH solution was added, for neutralization, and volume was made up to the mark with diluent and mixed properly. These solutions were centrifuged and subsequent solutions were collected. A 10 µl of these solutions were injected into LC, under optimized chromatographic conditions.

5.6.2 Alkali degradation studies

10 tablets were weighed. The average weight was determined. Sample powder equivalent to 100 mg of Ibandronate sodium was transferred in to a 100 ml volumetric flask. About 50 ml diluent was added and sonicated for 10mins. Added 5 ml of 5M NaOH and kept at 80°C on water bath for 5 hrs. Then solution was allowed to cool at room temperature, 5 ml of 5M HCl solution was

added, for neutralization, and volume was made up to the mark with diluent and mixed properly. These solutions were centrifuged and subsequent solutions were collected. A 10 µl of these solutions were injected into LC, under optimized chromatographic conditions.

5.6.3 Oxidation studies

Weighed and determined average weight of 10tablets. Weighed and transferred sample powder equivalent to 100 mg of Ibandronate sodium in to a 100 ml volumetric flask. About 50 ml diluent was added and sonicated for 10mins. 5 ml of 3% H_2O_2 was added and kept at 80°C for 5 hrs, equilibrated to room temperature and made up to volume with diluent and mixed. This solution was centrifuged and supernatant solution was collected. A 10 µl of this solution was injected into LC, under optimized chromatographic conditions.

5.6.4 Temperature stress studies/ Dry heat induced degradation

Crushed tablet content was heated at 105°C, for 24 hrs and allowed to cool to room temperature. Weighed and transferred sample powder equivalent to 100 mg of Ibandronate sodium in to a 100 ml volumetric flask, 75 ml of diluent was added and sonicated for 10mins and volume was made up to the mark with diluent and mixed. This solution was centrifuged and supernatant solution was collected. A 10 µl of this solution was injected into LC, under optimized chromatographic conditions.

5.6.5 Humidity degradation

Tablet was kept in 40°C/75% RH chamber for 24 hrs. Weighed and transferred sample powder equivalent to 100 mg of Ibandronate sodium in to a 100 ml volumetric flask, about 75 ml of diluent was added and sonicated for 10mins and made up to volume with diluent and mixed. This solution was centrifuged and supernatant solution was collected. A 10 µl of this solution was injected into LC, under optimized chromatographic conditions.

5.6.6 Photostability studies

Crushed tablet powder was kept in the photo stability chamber and exposed to, as per ICH guidelines (An overall illumination of not less 1.2

million lux hrs and an integrated near ultraviolet energy of not less than 200 watt hrs/sq m). Weighed and transferred sample powder equivalent to 100 mg of Ibandronate sodium in to a 100 ml volumetric flask, about 75 ml of diluent was added and sonicated for 10 mins and made up to volume with diluent and mixed. This solution was centrifuged and supernatant solution was collected. A 10 µl of this solution was injected into LC, under optimized chromatographic conditions.

5.7 CHARACTERIZATION OF DEGRADATION PRODUCT OF IBANDRONATE SODIUM

The degradation product obtained in the forced degradation study was targeted for its structural characterization. The degradation product was analysed by LC-MS. The mass spectrometer was run in positive ionization mode and negative ionization mode with turbo ion spray interface and mass to charge (m/z) ratio was recorded.

5.8 TOXICITY PREDICTION

Toxicity Prediction was done by OSIRIS Property explorer, the online software of Thomas Sander Actelion Pharmaceuticals Ltd, Switzerland.

CHAPTER 6
RESULTS AND DISCUSSION

The present work deals with the development of a new analytical method UPLC for the quantification of **Ibandronate sodium.** Only with proper validation, the data obtained can be reliable and trustworthy. Thus the developed method was validated as per ICH guidelines to prove the reliability of the method. The parameters which were validated are linearity, accuracy, precision, robustness and system suitability parameters. The validation acceptance criteria were met for the developed method.

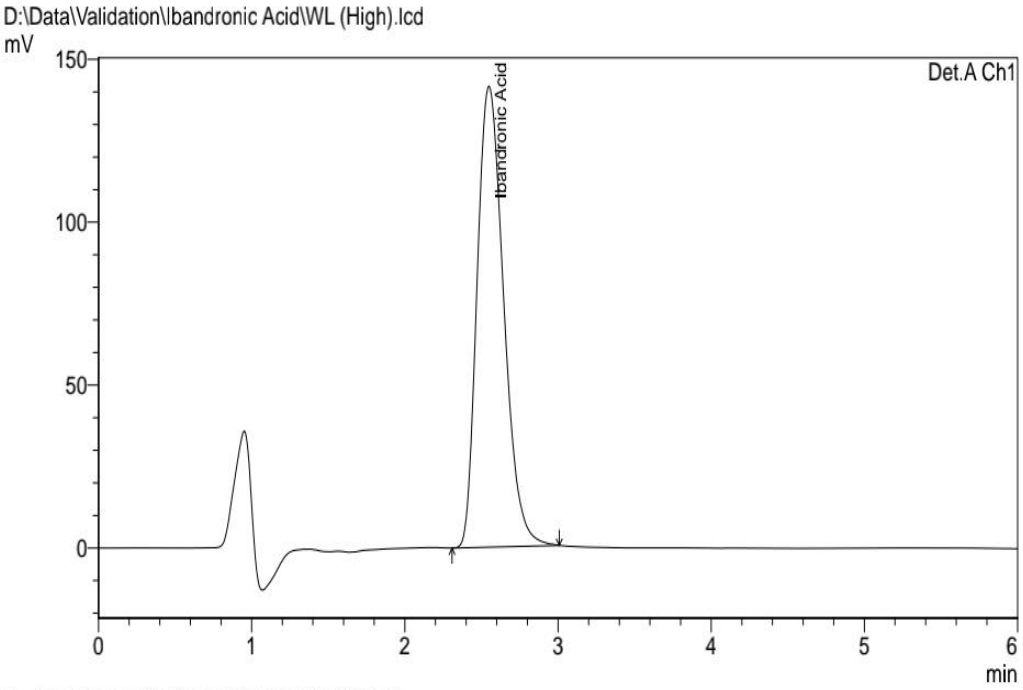
The linearity of an analytical method is based on its ability to elicit test results, that are directly or by a well-defined mathematical transformation proportional to the concentration of the analyte present in the sample within the given range. The developed method was found to be linear in the concentration range reported. The regression analysis was carried out to check the correlation coefficient, intercept and slope of the regression line, which estimates the degree of linearity.

Analytical methods need to be robust, so that they can be used routinely without problems and can be easily transferred for use in another laboratory if necessary. Robustness was tested by making small deliberate changes in the chromatographic conditions such as flow rate, variation in mobile phase, column, mobile phase consumption, etc., and measuring the peak areas. The percentage RSD should not be greater than 2. The percentage RSD obtained in the proposed method was found to be below 2, which was within the acceptance criteria.

Accuracy of the proposed method was confirmed by recovery studies which indicate that, the co-formulated substances do not interfere in the determination. The low values of standard deviation in the recovery data indicate the reproducibility of the proposed method.

6.1 DETERMINATION OF IBANDRONATE SODIUM BY UPLC METHOD

UPLC method has not been reported for quantitative analysis of IBT in pharmaceutical dosage form, hence an attempt was made to develop a new stability indicating UPLC method for the quantification of IBT. The UPLC separation was conducted on Thermo ChromquestC_{18} column as stationary phase using ammonium formate buffer : Acetonitrile in the ratio of 70:30v/v as the mobile phase. The detection of IBT was carried out at 215 nm. The chromatogram in **Figure 3.**shows the retention time of **Ibandronate sodium** as 2.54mins. The calibration curve was constructed between concentrations and respective peak area.



1 Ibandronate Sodium / Wavelength Change

PeakTable

Detector A Ch1 217nm

Name	Ret. Time	Area	Area %	Theoretical Plate#	Tailing Factor
Ibandronic Acid	2.54	1701778	100.00	4001	1.31
		1701778	100.00		

Figure 3. Chromatogram showing R_t of Ibandronate sodium

6.1.1 Method Validation

The validation of an analytical method verifies that the characteristics of the method satisfy the requirements of the application domain. The proposed method was validated in the light of ICH guidelines. The developed method was validated for linearity, accuracy, precision, repeatability, selectivity and specificity as per ICH guidelines. All the validation parameters were checked by replicate injections of the sample and standard solutions.

Linearity

Linearity was obeyed in the concentration range of 40-120 µg/ml. From the data obtained correlation coefficient, y-intercept and slope were calculated to provide mathematical estimates of the degree of linearity as shown in **Table 2**.

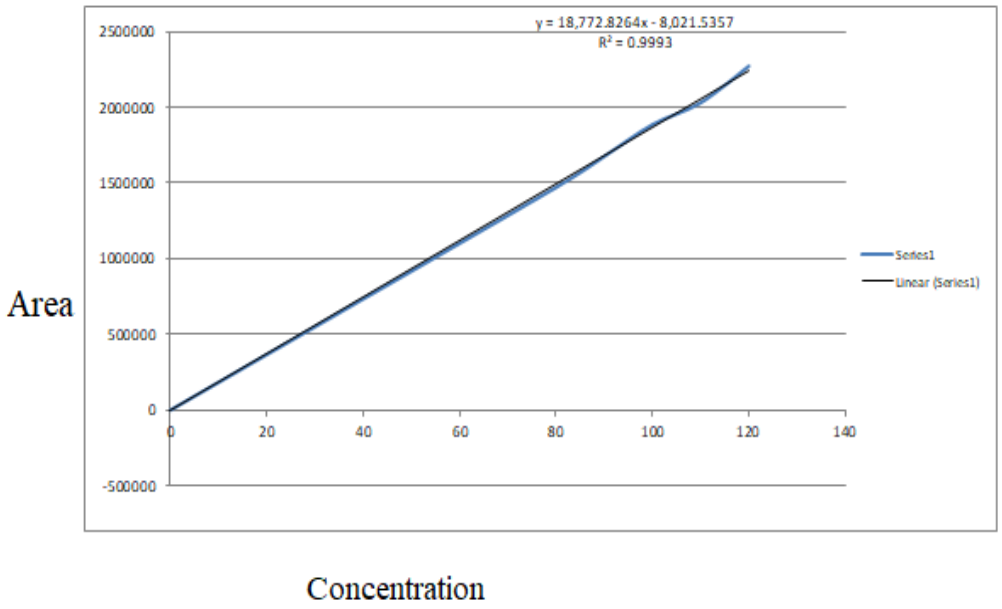


Figure 4. Linearity curve of IBT

Accuracy

Accuracy of the developed method was carried out by adding known amount of drug corresponding to three concentration levels 100%,110% and 120% of the label claim. The accuracy was expressed as the percentage of analyte recovered by the assay method. The results of percentage recovery are shown in **Table 3**.

Precision

Precision was studied by repeatability and intermediate precision studies. The results are reported in terms of relative percentage standard deviation (% RSD) as in **Table 4**.

Analysis of Formulation

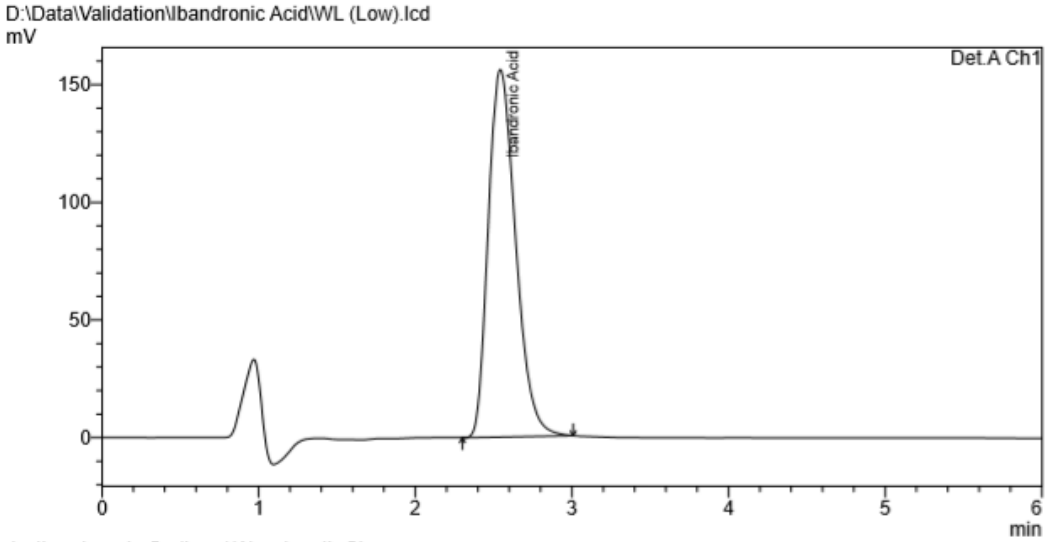
The proposed method was applied for the quantitation of IBT in its pharmaceutical dosage form. The results of analysis were in good agreement with the label claim as shown in **Table 5.**

Selectivity

The selectivity was checked by injecting the solution of the drug into the HPLC system. A sharp peak of IBT was obtained at the retention time of 2.54 minutes. It was observed that the excipients did not interfere with the retention time of the drug so the method developed is said to be selective.

Specificity

Specificity of the method was assessed by comparing the chromatogram obtained for the standard drug with the chromatogram obtained for tablet solution. The chromatogram of the formulation is shown in **Figure 5.** The retention time of standard drug and the drug in the sample solution were same, so the method is specific.



PeakTable

Detector A Ch1 213nm

Name	Ret. Time	Area	Area %	Theoretical Plate#	Tailing Factor
Ibandronic Acid	2.54	1901190	100.00	3977	1.33
		1901190	100.00		

Figure 5. Chromatogram showing R_t of IBT Formulation

System suitability parameters

The results of the system suitability parameters for the developed method are shown in **Table 8**.

Stability of analytical solution

Stability of sample solution was established by storage of the sample solution at 6°C for 48 hrs. Ibandronate sodium was re-analysed after 24 and 48 hrs time intervals and assay value was determined and compared against fresh sample. Sample solution does not show any appreciable change in assay value when stored at 6°C upto 48 hrs. The percentage label claim of ibandronate sodiumat 0, 24, 48 hrs were 99.9%, 100.2% and 100.1% respectively.

Table 2. Linear regression data for the Calibration curve*

	Calculations						
x	Injection volume (µl)	0	80	90	100	110	120
y	Area	0	1471564	1676478	1886070	2031240	2272932

Table 3. Accuracy study for the determination of**IBT***

Sample ID	Standard Area	Sample Area	mg / tab	Percentage
1	1844972.6	1803047	49.38	98.77
1	1844972.6	1989892	54.50	109.00
2	1844972.6	2170066	59.44	118.87
3	1844972.6	2363572	64.74	129.47

Table 4. Results of Precision study*

Sample ID	Standard Area	Sample Area	mg / tab	Percentage
1	1844972.6	1855010	50.16	100.32
2	1844972.6	1821480	49.54	99.08
3	1844972.6	1808124	49.28	98.56
4	1844972.6	1830989	49.66	99.31
5	1844972.6	1899814	50.90	101.80
6	1844972.6	1832735	49.78	99.57
			AVERAGE:	**99.77**
			SD:	**1.1**
			RSD:	**1.05**

Table 5. Analysis of the marketed formulation*

Ibandronic Acid	S.No.	Standard Area	Sample Area	mg/tab	% of Assay
	1	1844973	1863240	50.038	100.08

LIMIT : (90% to 110%)

Table 6. System suitability study

INJECTION ID	AREA
1	1886256
2	1818734
3	1832435
4	1855759
5	1831679
AVERAGE :	1844972.6
SD. :	26660
RSD :	1.45

6.2 STABILITY INDICATING UPLC METHOD FOR IBANDRONATE SODIUM

Stress studies were performed to evaluate the stability indicating properties and the specificity of the method. During the conversion of drugs into formulations a number of excipients are employed. Drugs undergo transformation by interacting with these excipients. Formulated drugs may undergo degradation due to the acidic or basic environment of the formulation matrix. Drugs can degrade due to exposure to environmental factors like temperature, light, humidity, etc., during manufacturing, transport and storage. Due to this, it is essential to know the degradation pathway of the drug in various environmental conditions. ICH guidelines require that stress testing on active pharmaceutical ingredient (API) and the drug products should be carried out to establish their inherent stability characteristics.

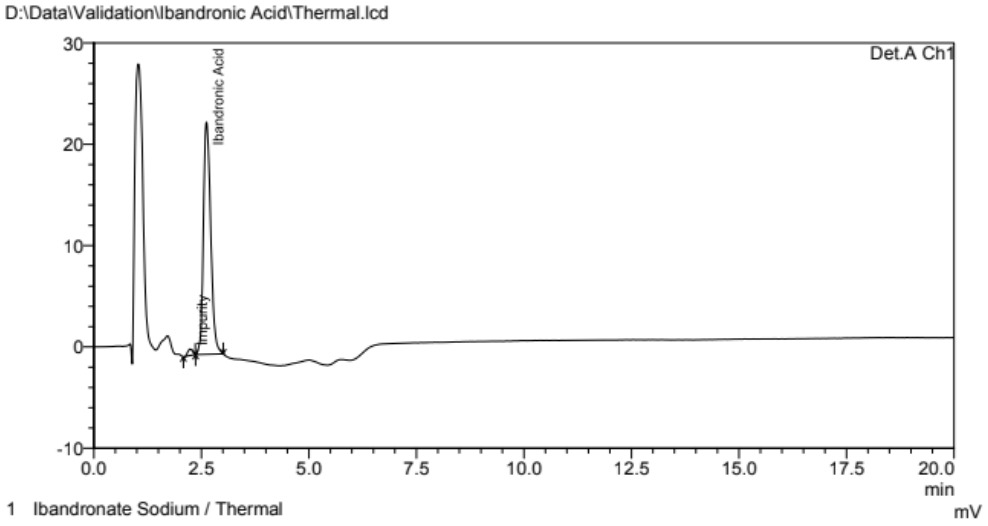
Stability indicating study is essential for the following reasons:

- Development of stable formulation
- Identification of toxic degradants
- Recommendation of appropriate storage conditions
- Elucidation of the possible degradation pathways.

Stability indicating method helps in understanding the quality of the drug, that varies with passage of time, when it is exposed to different environmental factors. A stability indicating assay should be able to measure the active ingredient without interference from degradation products.

Ibandronate sodiumwas subjected to acid and alkali hydrolysis, oxidation, photo degradation and thermal stress conditions. Stress testing of Ibandronate sodium under different conditions using ammonium formate buffer: acetonitrile (70:30%v/v) as the mobile phase solvent system suggested the degradation behaviour.

Initially 1M HCl was used at 60ºC for 1hr, no degradation was observed. The drug was found to be stable in 5M NaOH for 1hr. The drug was found to be labile in heat. The drug was found to be stable when treated with 5M HCl and humidity.



1 Ibandronate Sodium / Thermal

PeakTable

Detector A Ch1 215nm

Name	Ret. Time	Area	Area %	Theoretical Plate#	Tailing Factor
Impurity	2.24	4819	1.75	1742	1.08
Ibandronic Acid	2.61	270857	98.25	4134	1.29
		275677	100.00		

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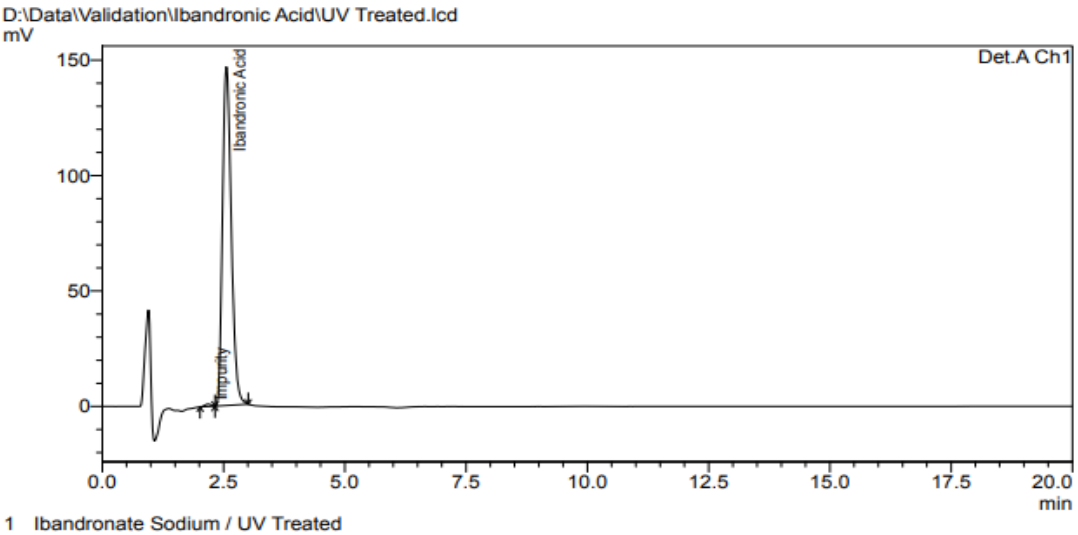
Figure 6. Thermal degradation chromatogram of IBT



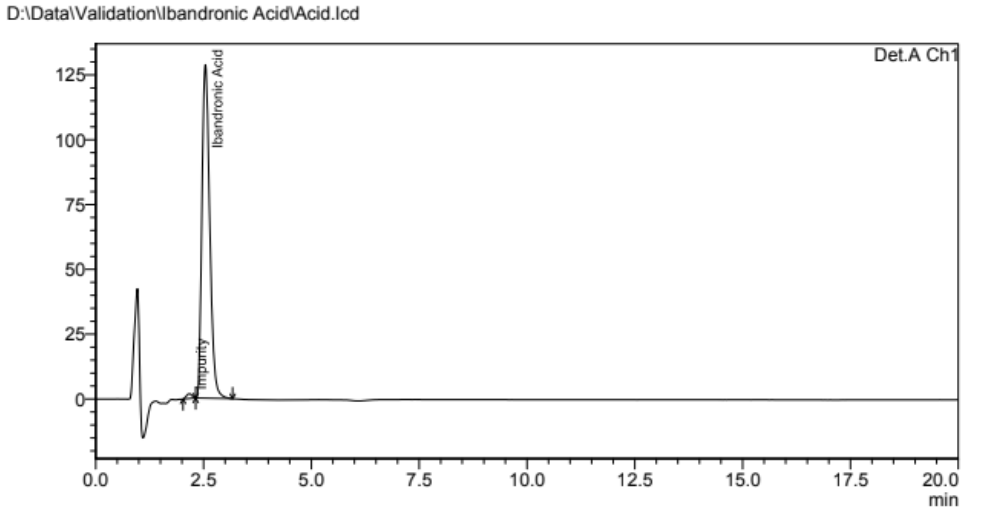
1 Ibandronate Sodium / UV Treated

PeakTable

Detector A Ch1 215nm

Name	Ret. Time	Area	Area %	Theoretical Plate#	Tailing Factor
Impurity	2.18	9834	0.53	1116	0.98
Ibandronic Acid	2.56	1845636	99.47	4012	1.31
		1855470	100.00		

Figure 7. Photolytic degradation chromatogram of IBT



1 Ibandronate Sodium / Acid

PeakTable

Detector A Ch1 215nm

Name	Ret. Time	Area	Area %	Theoretical Plate#	Tailing Factor
Impurity	2.16	16866	1.03	1143	1.06
Ibandronic Acid	2.54	1616658	98.97	4115	1.33
		1633524	100.00		

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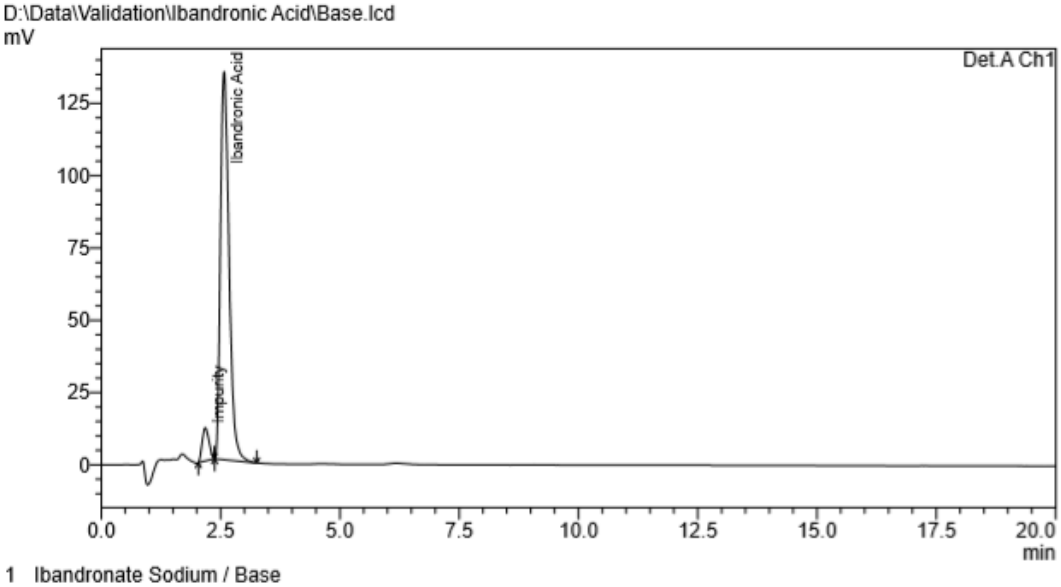
Figure 8. Acid degradation chromatogram of IBT



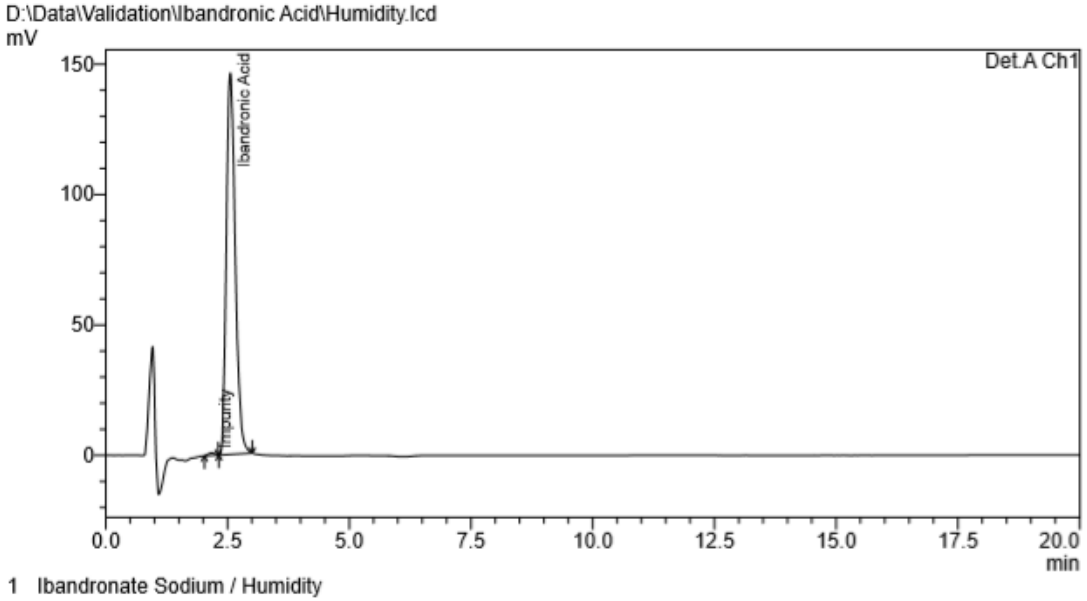
1 Ibandronate Sodium / Base

PeakTable

Detector A Ch1 215nm

Name	Ret. Time	Area	Area %	Theoretical Plate#	Tailing Factor
Impurity	2.17	113158	6.29	994	1.19
Ibandronic Acid	2.57	1685691	93.71	4130	1.42
		1798849	100.00		

Figure 9. Alkali degradation chromatogram of IBT



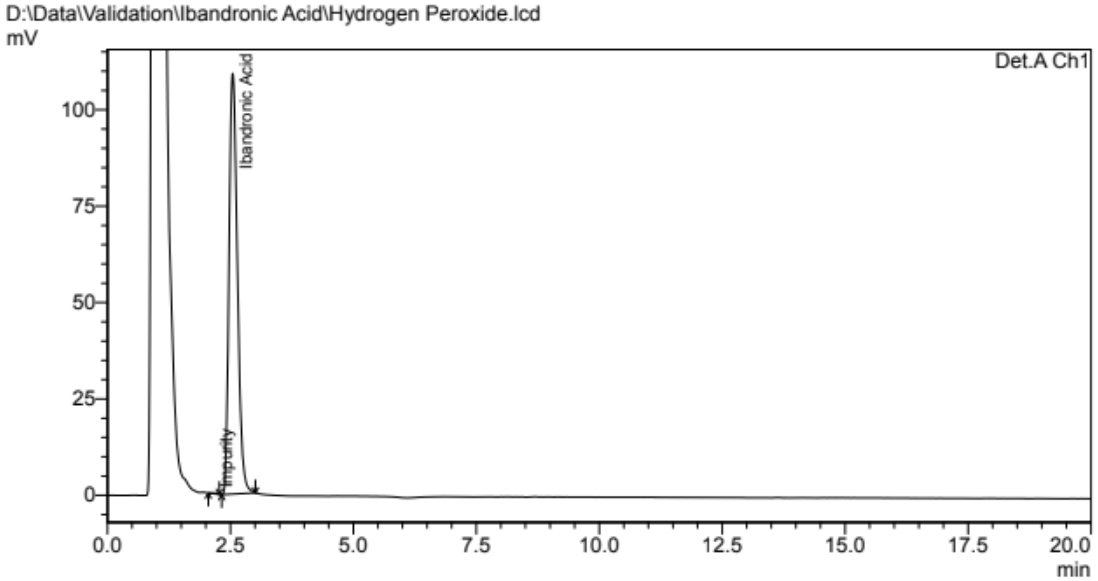
1 Ibandronate Sodium / Humidity

PeakTable

Detector A Ch1 215nm

Name	Ret. Time	Area	Area %	Theoretical Plate#	Tailing Factor
Impurity	2.17	9238	0.50	1138	1.00
Ibandronic Acid	2.55	1851425	99.50	4088	1.32
		1860662	100.00		

Figure 10. Humidity degradation chromatogram of IBT



1 Ibandronate Sodium / Hydrogen Peroxide

PeakTable

Detector A Ch1 215nm

Name	Ret. Time	Area	Area %	Theoretical Plate#	Tailing Factor
Impurity	2.14	1336	0.10	1761	1.43
Ibandronic Acid	2.54	1282589	99.90	4051	1.32
		1283925	100.00		

Figure 11. Oxidative degradation chromatogram of IBT

6.2.1 Characterisation of degradation product

The degradation product obtained in oxidative stress condition was further subjected to LC-MS study for characterization and structural elucidation. LC-MS spectrum was obtained is shown in the **Figure.13**

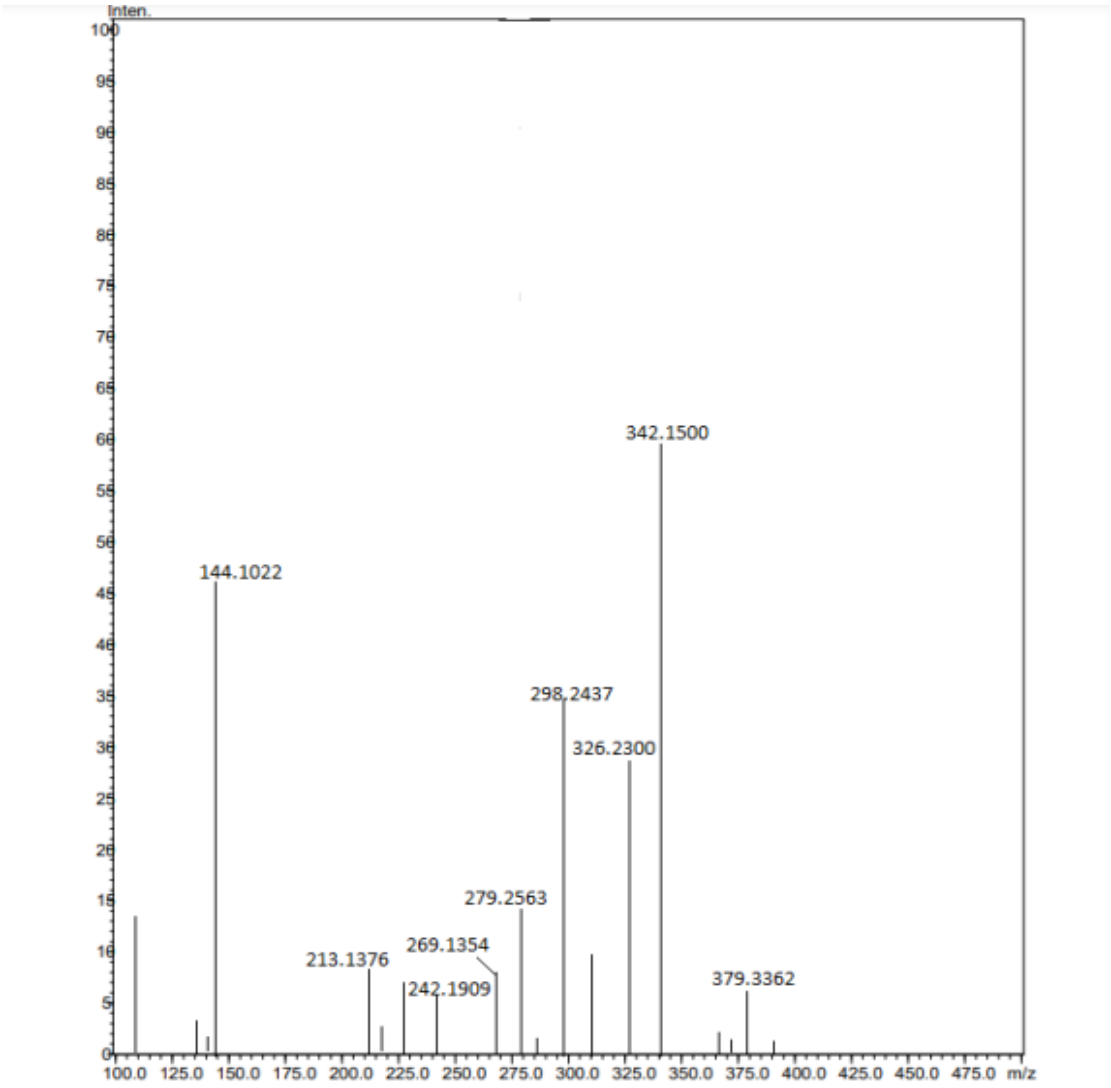
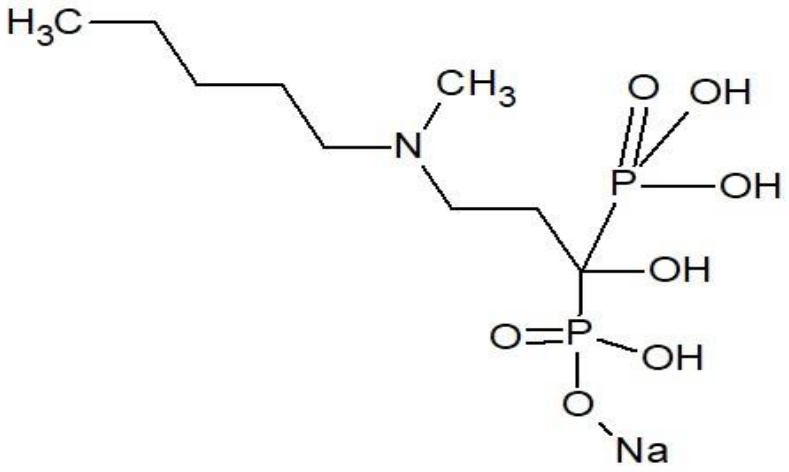
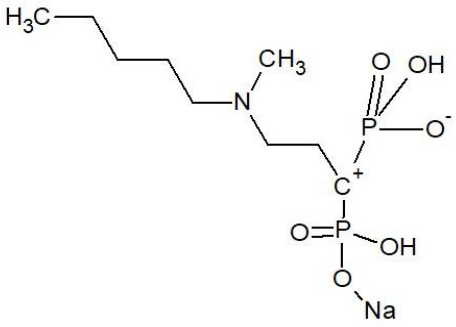


Figure.12 LC-MS Spectrum of degradation product

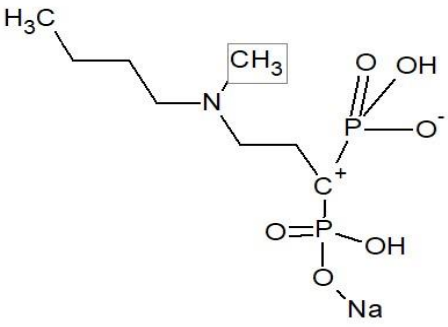
The m/z value of 342.1500 in the spectrum is the indicative of the formation of phosphonate ion and carbonium ion during thermal degradation. The fragmentation pathway is shown in **Figure 13.**



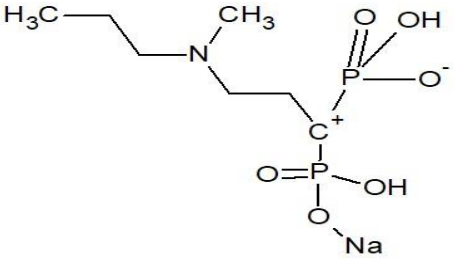
m/z 359.23

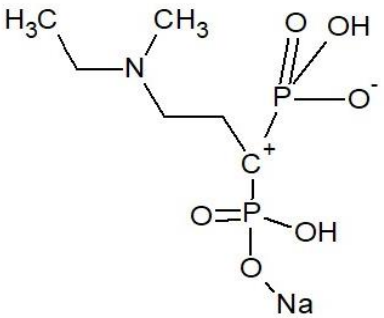


m/z 342.1500

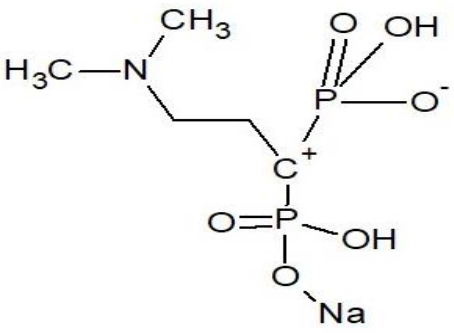


m/z 326.00

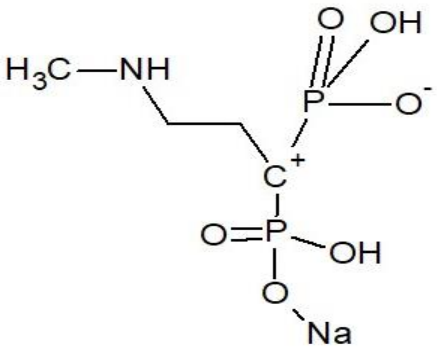




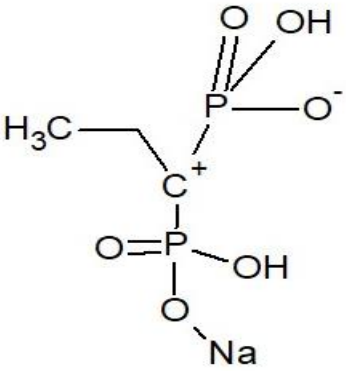
m/z 379.3362



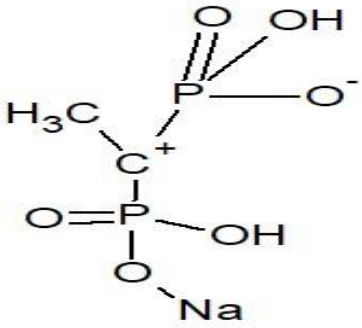
m/z 298.2437



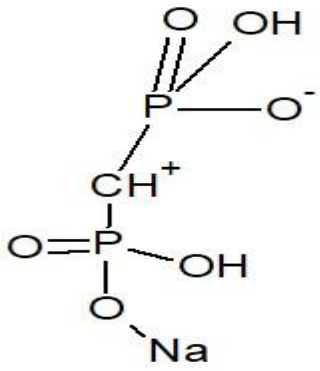
m/z 279.2563



m/z 269.1354



m/z 242.1909



m/z 213.1376

m/z 144.1022

Figure 13. Proposed fragmentation pathway of IBT

The results of LC-MS studies indicate the dehydration in Ibandronate sodium and the formation of phosphonate ion and carbonium ion in thermal degradation.

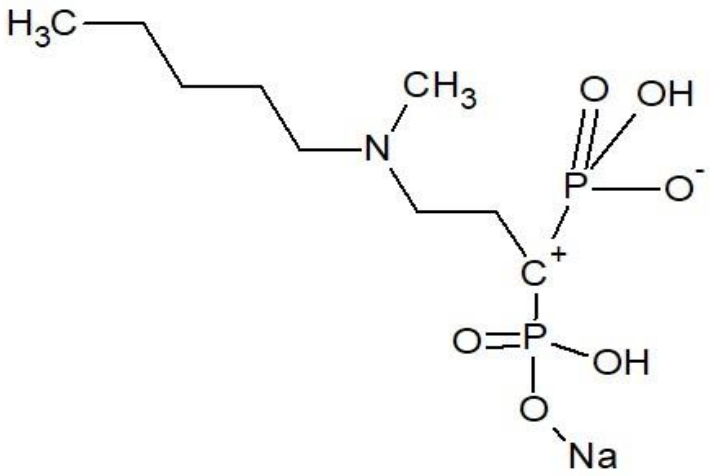
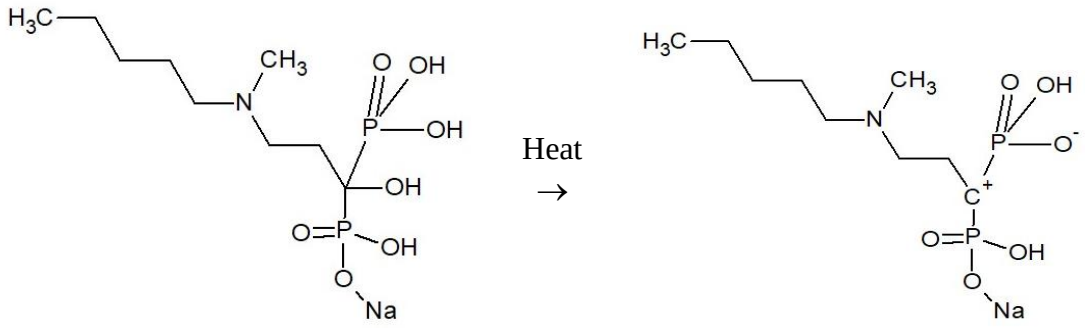


Figure 14. Proposed structure of IBT degradation product

The developed method could be conveniently used to quantify the drug in presence of its degradation product.



Mol Wt - 359 Mol Wt - 342

Figure 15. Probable degradation pathway of IBT

The developed method was found to be precise, accurate, reproducible, sensitive, specific and robust. The developed method was free from interferences due to other active ingredients and excipients present in the formulation and thus can be conveniently applied for the routine analysis of the selected drug in its pharmaceutical dosage form. The results of market sample analysis suggests the applicability,

reproducibility and utility of the method for the estimation of the drugs in quality control laboratories.

TOXICITY PREDICTION

The chemical structure was drawn in OS IRIS property explorer to show the biological properties of the compound. Properties like high risks of undesired effects like mutagenicity, tumorigenicity and reproductive effect are shown in red. Green colour indicates the drug conform behaviour. It was found that the degraded product is non-mutagenic, non-tumorigenic and has no irritability and reproductive effects as shown in Figure 16.

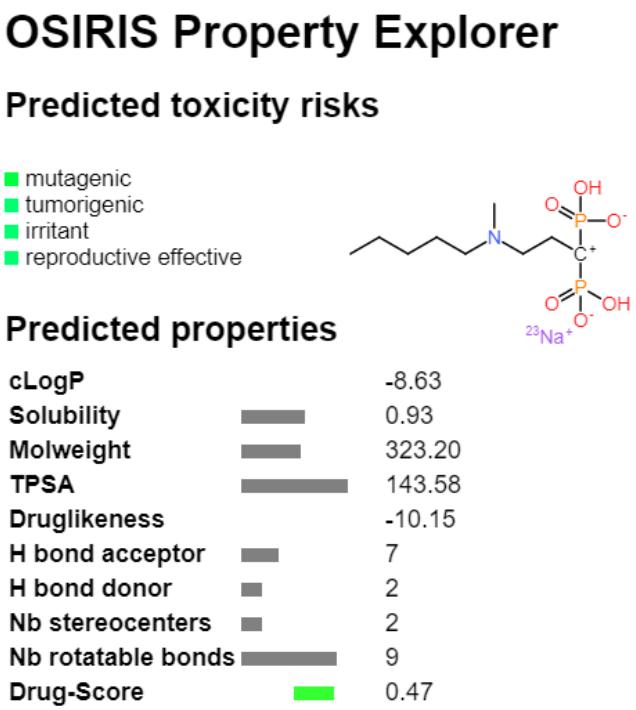


Figure 16. Toxicity prediction of degraded product of IBT

CHAPTER 7

SUMMARY AND CONCLUSION

In the present study a new stability indicating UPLC method has been developed and validated for the quantification of Ibandronate sodium. The developed method was validated as per ICH guidelines. The parameters which are validated are linearity, accuracy, precision, robustness and system suitability parameters.

The chromatographic conditions were optimized before the development of the chromatogram. The mobile phase consisted of a mixture of pH 5.3 ammonium formate buffer and acetonitrile (70:30 v/v) under under isocratic mode of elution. The system suitability parameters such as theoretical plates, tailing factor and peak symmetry were determined to check the validity of the developed UPLC method. The developed chromatographic method proved to be superior to most of the reported methods in terms of accuracy, precision and sensitivity. The data obtained was subjected to statistical analysis. The proposed method was successfully applied to the determination of the selected drug in its pharmaceutical dosage form.

The stability indicating UPLC method is useful to understand the degradation behaviour of Ibandronate sodium. Ibandronate sodium was subjected to forced degradation study. IBT was found to degrade in thermal degradation conditions and the degradation product was characterised by Mass spectrometry.

Toxicity of the degradation product was checked by using Osiris software. It was found that the degradation product is non-toxic. Prediction results are colour coded in which the red colour shows high risks with undesired effects like mutagenicity or poor intestinal absorption and green colour indicates drug-conform behaviour.

The proposed method is accurate, selective, sensitive and reproducible. The method is relatively free from any interference produced from common tablet excipients. Hence, the recommended procedure is well suited for the assay and evaluation of IBT in pharmaceutical quality control. The present work can be

extended for the quantification of the selected drug in bioavailability, bioequivalence, pharmacokinetics, in-vitro and in-vivo correlation studies.

A successful analyst must know what reactions are taking place during analysis and be able to understand and apply the theory upon which the method is dependent. The analyst must acquire skills of technique, patience, neatness and accuracy. Absolute integrity is demanded of every quantitative analyst. To become a successful analyst, one must realize that, analytical chemistry is not a simple routine procedure. Manipulative skill acquired by experience with the ability to follow directions under the supervision of a skilled analyst may enable one to carry out successfully certain analytical procedures.

CHAPTER 8
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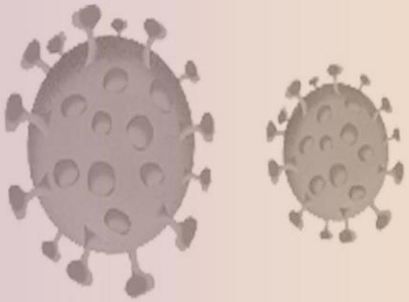

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Dedicated To The Almighty God


INTRODUCTION


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