1.1. Overview of Drug Stability Studies

Everything made by human hands is known to deteriorate and pharmaceutical product is no exception to it. Degradation is a phenomenon where certain measurements of quality characteristics deteriorate over time. When a material is subjected to lifespan testing, we often turn our attention to degradation analysis and extrapolate the measurements to obtain life time information from the degradation data. Pharmaceutical products are routinely monitored for their stability over a period of time. When there is a change in the functional moiety of the drug, the various changes in the molecule, nature of impurities and shelf life determination comes under the purview of drug product stability.

Stability is defined as the capacity of a drug substance or a drug product to remain within the established specifications to maintain its identity, strength, quality, and purity throughout the retest or expiration dating periods. The evaluation of the stability of a drug or a drug product is a highly specialized and esoteric in nature. Stability is a critical attribute playing a crucial role in drug development process. Stability, particularly, chemical stability is one of the most important issues that impact the quality and safety of pharmaceuticals.

Pharmaceutical fraternity has witnessed increasing intervention by the regulatory agencies such as, the FDA that eventually stimulated the need for establishing standard approaches for global stability testing and consequent harmonization of regulatory guidelines.

1.1.1. Gamut of Stages in Drug Stability Studies

Pharmaceutical stability is evaluated and applied in different ways depending upon, whether it assesses a drug substance, formulation, or a packaged product. Stability studies encompass all the phases of drug development and drug discovery. In early stages, stability studies commences with the understanding of purity and stability profile of the API. It is
essential to subject the API to accelerated stress and establish the conditions which minimize the formation of forced degradants. Accelerated stability testing is considered as the “worst case” evaluation technique to unravel the nature of forced degradation products that may potentially appear after long-term storage conditions.

Next, the formulation development cycle involves addition of excipients or non-active ingredients to the API, so as to meet the intended performance criteria of the drug product. Addition of the excipients is essential for adding colour, controlling pH, moisture or oxygen content. Interaction of the excipients with one another or with the API is imperative to establish the excipient compatibility that consequently dictates the quality, safety and efficacy of the drug product reaching the outer domain of the health industry.

Expiry dating of the drug product must be determined on the actual packaged drug product, but the extrapolated stability data may provide product registration. Real time stability data needs to be established for actual expiration date for the drug product. After approval, stability studies are continued to support the commercialization of the drug product. Representative lots are placed at the stability stations for annual product monitoring. These are termed as post-approval studies which help in determining the changes in processing or packaging of the drug product.

1.1.2 Rationale for Concern with Stability of Drug and Drug products

Apart from the regulatory requirements, the fundamental reason for performing stability testing is for the well-being of the patient using the product. Safety and efficacy of the drug products are established during development through clinical studies. If the drug product stability profile changes beyond established acceptance criteria, the estimated safety and efficacy will no longer be applicable resulting in additional stress stability studies.
According to a recent report to the nation\textsuperscript{2} issued by Center for Drug Evaluation and Regulation CDER of the USFDA, 851 prescription drugs and 136 over-the-counter drugs were recalled in the fiscal year of 2007. The top five reasons for drug recalls were; cGMP deviations, lack of efficacy, stability data not supporting the expiration date, generic drug or new drug application discrepancies and dissolution failure. The CDER report to the Nation indicated that a drug product must be recalled owing to a problem occurring in the manufacture or distribution of the product that may represent a significant risk to public health. These problems usually, but not always occur in small batches of the drug product. This clearly spells out the importance of the drug stability studies.

Instability of the drug product result in potential adversaries such as, loss of API due to chemical degradation, microbiological status decline, loss of content uniformity, loss of package integrity, formation of toxic degradation products and unacceptable modifications of active moiety with functional relevance. The effectiveness of any drug product or a dosage form is a function of its ability to deliver the required amount of the active therapeutic moiety to the site of action for the intended length of time to achieve the purpose of therapy. This forms the major concern during stability studies. Apart from effective drug formulation, the delivery of the drug within the shelf life of the drug product is also dictated by the stability studies. Hence, the drug products like pre-filled syringes, spray bottles, powder inhalers, metered-dose inhalers needs to be monitored in terms of drug delivery consistency over the entire shelf life period.

The potency of any drug product is not expected to remain constant \textit{ad infinitum}; however, it is expected that the quality of the drug is maintained until it reaches the expiration date. The expiration date specifies the period within which the efficacy, safety and esthetics of the drug product can be assured; also known as the shelf life of the drug product. The formation
of toxic decomposition products within the shelf life\(^3\) of the drug product warrants for the reassignment of a different expiration date. This is an exercise to give a thorough idea about the therapeutic activity after deliberate changes in the structure, but may lead to undesirable changes in the physical attributes due to aging. Though the potency will be intact, such drug products will not appeal the patients. Hence, the health and pharmaceutical industries are concerned with the amount and nature of degradation products.

1.2. Stability- Indicating Method (SIM) - An Imperative Requirement

To determine the shelf life, one must measure two different aspects of the drug after it has been stressed. First, is to determine the significant loss in the amount of active ingredient. Second, is to determine the degradants that appears as a result of aging. This determination is difficult, as one has to anticipate the nature of degradants that might be present, and measure all of them at concentrations of more than 0.1% of the parent drug. This portion of the assay falls in the realm of trace analysis, and here comes the need to establish a stability-indicating analytical method for the drug and dosage forms.

The definition for stability-indicating methods in the draft guideline of 1998 reads as: “Validated quantitative analytical methods that can detect the changes with time in the chemical, physical, or microbiological properties of the drug substance and drug product, and that are specific so that the contents of active ingredient, degradation products, and other components of interest can be accurately measured without interference.” This statement implies that, stability indicating method is an analytical procedure capable of discriminating between the intact APIs from many degradation products formed under defined storage conditions. Further, it can accurately measure the active ingredients, without interference
from degradation products, process impurities, excipients, or other potential impurities, and can
detect changes with time in the pertinent properties of the drugs.\textsuperscript{4}

The requirement of SIM is listed in World Health Organization\textsuperscript{5} (WHO), European
Committee for Proprietary Medicinal Products\textsuperscript{6} and Canadian Therapeutic Products
Directorate’s guidelines\textsuperscript{7} on stability testing of well established or existing drug substances and
products. The United States Pharmacopoeia (USP) has a requirement listed under ‘Stability
Studies in Manufacturing’, which suggests that samples of the products should be assayed for
potency by the use of a stability-indicating assay\textsuperscript{8}. The requirement in such an explicit manner is
absent in other pharmacopoeias. Current ICH guideline on Good Manufacturing Practices for
API (Q7A), which is under adoption by WHO, also clearly mentions that the test procedures
used in stability testing should be validated and be stability-indicating.\textsuperscript{9}

1.3. Factors affecting stability of Drug and Drug Products

The preceding section elaborated on the importance and significance of stability of drugs
and drug products. Safety, quality and purity of a drug and drug product are the essential
attributes and form a fundamental basis for designing the stability studies. Pharmacopoeias are
traditionally considered to be the safeguards and guarantee for the quality of drugs. The FDA
emphasizes on the drug safety issue in terms of, efficacy of drug therapy determined by the side
effect profile of the drug.\textsuperscript{10} Quality and purity of the drug product is determined by the various
pathways of degradation such as, physical, chemical, microbiological and environmental
degradation.

Drug substances used as pharmaceuticals have diverse molecular structures and are,
therefore, susceptible to variable degradation pathways. Physical degradation emphasizes on the
physical state of the drug substance or the excipient affecting the stability of the drug product. Components of pharmaceuticals (drug substances and excipients) exist in various microscopic physical states with differing degrees of order ranging from, amorphous to crystalline and hydrated to solvated states. With time, the drug or the excipient may change from metastable to a more thermodynamically stable state. Changes in the physical state of the drug substance are assessed by DSC, DTA and X-ray diffraction analysis. 11

Physical degradation is generally considered in terms of, physicochemical degradation to design stability studies. Chemical degradation includes susceptibility of functional groups in the drug molecules to various reaction pathways that include, hydrolysis, dehydration, isomerization, racemization, elimination, oxidation, photodegradation, and complex interactions with excipients and other drugs. 12 If one could predict the chemical instability of a drug based on its molecular structure, it would help in designing the stability studies and identifying routes for formulating problematic drugs, at the earliest stages of drug development.

Most parenteral drug products come in contact with water, and even in solid dosage forms; moisture is present that can lead to hydrolytic degradation. Hydrolysis is a very common reaction pathway followed mainly in drug molecules that generally possess ester, ether or amide linkages. Lactones, lactams, spirans, carbozyclic acids, and other analogs are known to extensively undergo hydrolytic mechanism and processes. 13

Oxidative degradation is one of the most frequently occurring mechanisms of pharmaceutical degradation and stability and methods for stability evaluation have been widely researched. 14 Oxygen is abundant in the environment to which pharmaceuticals are exposed, either during processing or long-term storage conditions. Oxidative mechanism is known to follow any one of the three major pathways, viz, (i) autooxidation or radical-mediated involving
the reversal loss of electrons catalyzed by peculiar initiators like, azobisisobutyronitrile (AIBN) or with transition metals such as Fe(II) or Cu(II); (ii) peroxide-mediated, involving the exposure of drug products to dilute solutions of peroxide; (iii) photochemically induced, in which the there is a direct reaction of ground state oxygen with electronically excited state of drug molecule or from photosensitization of triplet oxygen to singlet oxygen and direct reaction with the drug molecule.

(i) Autooxidation

(ii) Peroxide-mediated oxidation

(iii) Photochemically induced oxidation

Photodegradation has been reported for a large number of drug substances. The mechanisms for these reactions are inherently complex and known to lead to loss in potency due to the formation of toxic photodegradation products. Light, generally sunlight may cause degradation of the drug or excipient molecule. In order to initiate a photolytic reaction, the energy from the light radiation must be absorbed by the molecules. If the energy absorbed is
sufficient to achieve the activation energy, then degradation of the molecule is possible. In certain cases, the molecules absorbing the light pass on their increased energy to other molecules which then degrade (photosensitization). Since the light energy may be converted to heat, photolysis is generally accompanied by a thermal reaction.

1.4. Regulatory Perspective in Stability Studies

Efforts were enunciated by the drug regulatory authorities and pharmaceutical industries in Europe, United States and Japan to establish harmonized regulation for testing and analysis during research and development of new drugs. These harmonized guidelines came to be known as the International Conference on Harmonization (ICH) which has been incorporated as law in the EU, Japan and in the US. Besides these, other countries are also using them as a de facto force of regulation.

The ICH guideline Q1A, exemplifies the core stability recommendations and testing of the features which are susceptible to change during storage, that are likely to influence the quality, safety and efficacy must be done by validated SIM. It is also mentioned that forced decomposition studies at temperatures in 10°C increments above the accelerated temperatures, extremes pH and under oxidative and photolytic conditions should be carried out on the drug substance, so as to establish the inherent stability characteristics and degradation pathways to support the suitability of the proposed analytical procedures.

The ICH Q1B, outlines the photostability testing should be an integral part of stress studies. Generally, an ICH dose of $1.2 \times 10^6$ and $2.4 \times 10^6$ lxh of fluorescent light and 200 Wh/m$^2$ UV light is recommended to estimate the photostability considerations of the given drug substance and products.
The ICH guideline Q3B emphasizes on providing documented evidence that analytical procedures are validated and suitable for the detection and quantitation of degradation products\textsuperscript{18}. The guideline gives a clear concept on the threshold levels of degradation products in drug substances, in terms of reporting, identification and quantitation thresholds.

The ICH guideline Q6A\textsuperscript{19} provides note for guidance on specifications and mentions the requirement of stability-indicating assays under Universal Tests/Criteria for drug substances and drug products. The similar requirement has been laid down in the ICH Q5C guideline on Stability Testing of Biotechnological/Biological Products.\textsuperscript{20} Such products have distinguishing characteristics and thus the document outlines the necessity of determining the changes in identity, purity and potency of the product.

As discussed earlier, the most important point in conducting stability studies are the storage conditions. They should simulate the conditions under which the drug substance or drug product is subjected; from the stage of manufacturing to the final application. Storage conditions are derived from real climatic conditions.

Most chemical reactions follow logarithmic functions and this characteristic is taken into consideration while defining stress testing conditions\textsuperscript{21}. The four major climate zones and associated conditions stipulated in current stability guidelines are summarized in Table. 1.1. Considering the global climatic zones, India has diverse climatic conditions across the country; the northern Himalayan mountain ranges, central and eastern fertile Indo-Gangetic plains, western Thar Desert to the southern Peninsula composed of Deccan plateau and hilly coastal ranges, and the Western and Eastern Ghats. Diverse climatic conditions were a point of focus to design the long-term stability testing by risk-based approach\textsuperscript{22} postulated by Zahn and co-workers.
Table 1.1: Climatic zones and associated storage conditions

<table>
<thead>
<tr>
<th>Climatic zones</th>
<th>Calculated Data</th>
<th>Derived Data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temp °C</td>
<td>MKT °C</td>
</tr>
<tr>
<td>Zone I-Temperate</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Zone II-Mediterranean subtropical</td>
<td>21.6</td>
<td>22</td>
</tr>
<tr>
<td>Zone III-Hot, dry</td>
<td>26.4</td>
<td>27.9</td>
</tr>
<tr>
<td>Zone IV-Hot, humid</td>
<td>26.7</td>
<td>27.4</td>
</tr>
</tbody>
</table>

This approach involves the use of mean daily temperatures and dew points to calculate the daily and monthly fluctuations in temperature, partial water vapour pressure, mean kinetic temperature and relative humidity. The safety margins for temperature and partial vapour pressure are calculated, taking into consideration the difference between measured meteorological parameters and stability testing conditions. Hence, long-term stability testing for a particular country or region is based on the classification of appropriate climatic zones.

1.5. Identification of forced degradation products

In recent times, an issue that is receiving maximum attention is regarding the evaluation, detection and characterization of impurities. The shift of focus from ‘purity’ to ‘impurity’ happened after a pharmaceutical product was recalled in 1965\(^{23}\), when the adverse drug reactions were traced to the presence of a low level impurity in some lots of drug substance. At that time, a thin-layer chromatographic test was developed to control and measure the specific impurity. This led to drafting of ICH guidelines that envisaged the role of impurities in the drug substances and drug products.
Pharmaceutical impurities observed in a drug substance or a drug product needs to be identified when their levels exceed regulatory thresholds (i.e., \(~0.1–0.2\%\), for majority of drug substances and drug products, according to their maximum daily doses), Identification of drug impurities at these low levels can be very challenging due to a number of factors. One of the frequently encountered challenges is the limited availability of appropriate product samples for analysis, in particular in the case of commercial products on stability programs. Even in cases, where the supply of the samples is abundant, isolation of impurities at such minute amounts can be extremely tedious, time consuming, and difficult.

Degradant and impurity profiles are critical to the safety and potency assessment of the drug products for clinical trials. Therefore, identification of degradants in drug formulations has been of increasing importance in drug product assessment. Since degradants are usually in very low levels along with interferences, it is difficult to isolate these species and to examine them subsequently by mass spectrometer and NMR to obtain structural information. On-line LC-MS and LC-MS/MS is an ideal choice for this purpose.\(^\text{24}\)

Identification of pharmaceutical impurities is a critical analytical activity in the drug development process that fully elucidates the chemical structures of unknown pharmaceutical impurities present in either, drug substances or drug products above a particular threshold. Knowledge of the chemical structure of an impurity is essential to assess its toxicological implications and gain an understanding of its formation mechanism. Knowledge of the formation mechanism is critical for improving the synthetic chemical processes and optimizing the drug formulation to reduce or eliminate the impurity.

The health industry and pharmaceutical professionals emphasize on identification and chemical origins of various impurities, particularly those derived from degradation of drugs. In
general, the guidelines expect reporting, detection and control of impurities below 0.1 % and recommend characterization of individual impurities above this threshold. The main question that arises here is to how one goes about structure identification of impurities present in minute quantities (≥ 0.1 %). The conventional way is hypothesizing the structure based on possible side reactions, synthesizing the possible compounds and spiking the same in the chromatographic method. An alternate approach is to isolate the component(s) of interest using, preparative techniques coupled with structure elucidation through spectral analyses, including IR, MS and NMR. In recent times, various analytical techniques have emerged as frontrunners such as, HPLC-DAD, LC-MS, LC-MS/MS, LC-NMR, GC-MS and NMR for the characterization of low level or sub-ppm level forced degradation products that cannot be separated or crystallized during preparative techniques.

1.6. Aims and Objectives

As described in the introductory section, though stability testing comes across as a routine procedure, if flawed at any stage of formulation cycle, can have irrevocable negative consequences on the health of the patient.

The role of the pharmaceutical scientist is not only to formulate the drug substances in their various forms and adjuvant therapy, but also to ensure that these preparations are stable up to and inclusive of administration to the patient. This can be ascertained by either researching stability data in the literature or reliable databases, or alternatively, the study has to be performed for the relevant drug or drug combinations. Hence, an integral aim of the study was to identify degradation products and postulate complete degradation pathway of the drug molecules.
The endeavour of the present study was to establish a validated stability-indicating method for Lamotrigine and Itopride (API) and identify the forced degradation products formed under variety of stress conditions using LC-MS technique.

The above aim was achieved by performing the following steps;

- Postulation of the degradation route for the drug molecules, viz, Lamotrigine and Itopride
- Performing all the modes of forced degradation viz., acid, alkali, neutral hydrolysis, oxidation, photolysis and thermal stress testing, based on the ICH guidelines.
- Establishing a stability-indicating method to separate analyte from the degradation products and amongst themselves.
- Validation of the stability-indicating methods, in accordance with the ICH guidelines.
- Identifying the degradation products by suitable instrumentation using LC-MS.

The investigation highlighted on the following targeted objectives;

- Suggest intrinsic stability of drugs in the given storage conditions and assess the modification of the labile groups to improve stability.
- Evaluate and convey the environmental impact on the drug.
- Suggest variables to improve the shelf lives of the drug in the prescribed storage conditions and provide guidelines on compound handling and storage.
1.7. Scope of the Thesis

The thesis is divided into seven chapters, viz,

**CHAPTER 1** describes an introductory concept of drug stability studies, regulatory and global concerns of stability, stability-indicating methods, and current challenges such as, identification of degradation products. The chapter concludes with a brief note on aims and objectives stated for the investigation.

**CHAPTER 2** deals with an extensive literature review performed on practice and understanding of the stability studies. It covers the literature review on the drug molecules, viz, Lamotrigine and itopride, selected for the study.

**CHAPTER 3** describes the instrumentation of HPLC and LC-MS utilized for the present investigation. This chapter also includes the general strategy designed for the investigation.

**CHAPTER 4** includes the complete theoretical aspects of chromatography. This chapter clearly spells out the various concepts and calculations used to study validation parameters and degradation kinetics of lamotrigine and itopride. Complete detail of the instrumentation such as HPLC and LC-MS/MS used for the present investigation, is described.

**CHAPTER 5** describes the experimental methodology for lamotrigine. The establishment of SIM, validation parameters, degradation kinetics and profiling of degradation products is elucidated with supportive data.

Statistical evaluation of the validation data is deduced and performed by the method of least squares using regression factors, slopes and intercepts and the statistical errors in the parameters. Identification of forced degradation products by LC-MS has been explained in detail with postulation profile studies.
CHAPTER 6 includes the experimental methodology for itopride. The establishment of SIM, validation, degradation kinetics and profiling studies has been described. Statistical evaluation of the validation data is deduced. Identification of forced degradation products by LC-MS has been explained in detail with supportive explanation of the postulation profiles.

CHAPTER 7 is dedicated to the summary of the investigation with supportive conclusions. Critical explanation regarding the challenges faced during the study is also put forth, paving way to the future prospects.

The exhaustive list of references has been presented.