2.1. General Review on Stability Studies

As explained in Section 1.2, the regulatory guidelines mandated the need for establishing stability-indicating assay while assessing the drug stability. The practical steps for establishing the stability indicating method are elusive in the regulatory guidelines and pharmacopoeias. Hence, a literature search was undertaken replete with the publications on the development of SIMs of bulk drugs and pharmaceutical formulations.

The review of literature began with factual collection of a large number of methods reported over the past decades under the nomenclature ‘stability-indicating.’ On review, it was found that most of the reported methods fall short in meeting the current regulatory requirements. A general review\textsuperscript{25} was published as early as 1971, which described the general principles and methods developed till that period. Kingsford\textsuperscript{26} presented recommendations for designing drug stability trials to improve the methods in use. These included the choice of suitable stability-indicating assay, assay precision, temperature factors relevant to the application of the Arrhenius equation and examined the stability trials at room temperature. Mollica \textit{et.al} reviewed the stability studies in terms of the rates of the reaction, mechanisms of degradation concerning dosage forms, market product stability and regulatory considerations\textsuperscript{27}.

Boehlert\textsuperscript{28} discussed a variety of analytical methods used in the drug stability studies and presented the need for validation of these methods such as, evaluation of specificity, linearity, precision, accuracy, sensitivity and ruggedness. It elucidated the development of SIMs for five different drugs representing various challenging stability issues. Taylor and Shivji\textsuperscript{29} critically discussed the commonly adopted criteria for stability-indicating assay and merits for choosing a reactant or degradation product for monitoring decomposition. The examples for the application
of the initial rate method to simple to complex drug decomposition systems for determination of
degradation rate constants were also provided in the review.

Dong et al.\textsuperscript{30} presented a review emphasizing on the method development and drug
evaluation with reference to USP 22, 1990 validation guidelines and the use of modern LC
instrumentation with diode-array detection\textsuperscript{8}. Mehta et al. identified the issues that are critical in
the development of analytical methods in the stability testing of drugs in solution\textsuperscript{31}. It also
included a short review on the analytical techniques used in stability test with emphasis on the
requirements for the stability-indicating analytical methods and a brief discussion on reaction
kinetics. Subsequently, Ho and Chen\textsuperscript{32,33} reviewed stability-indicating high performance liquid
chromatography assay methods reported till 1997.

A compilation of stability-indicating assays (> 500) for various drugs was published in 1999
by Xu and Trissel\textsuperscript{34}. A recent publication by Carstensen and Rhodes\textsuperscript{35} provided general
discussion on HPLC method development and validation with emphasis on SIM.

A critical review\textsuperscript{36} on the stability-indicating methods has been presented. This review gave
clear elucidation of current practices followed and enlisted a comprehensive coverage on various
SIMs with incomplete stress testing in different dosage forms of drug substances. Actual steps,
procedures, pitfalls and challenges have been critically communicated in this report. This article
also described the challenging perspectives like, characterization of degradation products and
mass balance issues. Recently, a compiled book\textsuperscript{37} discussed the technical issues that impact the
stability programs encompassing pertinent topics on stability regulations, methodologies and best
practices adopted therein.

On a detailed review of various monographs, book chapters and research articles, it was
observed that most of the reported methods included titrimetric, spectrophotometric and
chromatographic techniques, as the stability-indicating method. If the objective of the analysis is to determine the drug of interest alone in the matrix of excipients, additives, degradation products, impurities, etc., titrimetric and spectrophotometric, especially derivative spectroscopy is a convenient cost-effective choice of instrumentation. However, these methods report lower sensitivity and specificity.

The nature of requirement of separation of multiple components during analysis of stability samples drove chromatographic methods to take precedence over the conventional methods of analysis. Thin layer chromatography (TLC), high performance thin layer chromatography (HPTLC), high performance liquid chromatography (HPLC), and gas chromatography (GC) had been elucidated for developing stability-indicating assay method. TLC is a reliable, fast and accurate method of quantitative analysis of drug samples. The shortcomings of TLC are now overcome by HPTLC. GC is stability-indicating, but not a versatile technique, as the drug substance may be nonvolatile or thermally unstable. Further, any attempt to increase the volatility of the drug and components by increasing the temperature may lead to degradation or racemization. Therefore, there are very few reports on the use of GC.\textsuperscript{38,39,40}

In comparison, HPLC has been widely employed. This technique has gained popularity in stability studies due to its high-resolution capacity, sensitivity and specificity. Non-volatile, thermally unstable or polar/ionic compounds can also be analyzed by this technique. Therefore, most of the stability-indicating methods have been established using HPLC. Other miscellaneous techniques include capillary electrophoresis\textsuperscript{41} (CE) with the advantages of high sensitivity, resolution and high efficiencies with minimal peak dispersion, UPLC\textsuperscript{42}, nuclear magnetic resonance (NMR), and hyphenated techniques like LC-MS, LC-MS/MS, LC-NMR\textsuperscript{43} and CE-MS.\textsuperscript{44,45}
2.2. Review on Drug Molecules

2.2.1. Lamotrigine

Lamotrigine, 3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine, is a new generation antiepileptic drug used in the treatment of epilepsy, bipolar disorder and pain syndromes. Lamotrigine is efficacious in treatment of epileptic seizures like, refractory partial, primary and secondary ionic seizures and seizures associated with Lennox-Gastaut syndrome.

The mechanism of drug action is unknown, but it is known that lamotrigine acts pre- and postsynaptically. Presynaptically, it inhibits the release of neurotransmitters like the, excitatory amino acids glutamate and aspartate, whereas postsynaptically, it diminishes the excitability of neurons like other anticonvulsive sodium channel blockers. Apart from inhibiting the sodium conductance, lamotrigine may reduce high-voltage activated calcium currents. Lamotrigine has been approved for clinical use in 1993. Its effectiveness has been investigated in comparison with placebo and with drugs like carbamazepine, phenytoin, valproic acid and topiramate.

Several HPLC methods for the determination of lamotrigine in biological fluids have been developed. Pertinent reports on determination of lamotrigine in presence of metabolites were surveyed and assessed. A liquid chromatographic method with diode array detection was developed for the analysis of lamotrigine and its metabolites like 2-N-glucuronide and 2-N-methylated in human plasma. Another determination of lamotrigine and three metabolites, namely, lamotrigine-2-N-glucuronide, lamotrigine-2-N-methyl and lamotrigine-2-N-oxide in human blood plasma was reported using LC-MS. Further, a recent report explained HPLC method for the analysis of lamotrigine and related substances in tablet formulations. However, this method employed a low UV detection of 210 nm and a higher column temperature of 40°C. Apart from HPLC, various analytical techniques have been employed for analysis of
lamotrigine including, HPTLC, capillary electrophoresis, gas chromatography with nitrogen phosphorus detector, electrochemical, immuno fluorometric assay and radioimmunoassay.

Lamotrigine is official in USP 32 with three impurities namely, 2,3-Dichlorobenzoic acid, process-related impurity, 3-Amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-5(4H)-one and N-[5-Amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-3-yl]-2,3-dichlorobenzamide. An analytical method has been developed for the detection of trace amounts of principal synthetic route indicative impurity, 3,5-diamo-no-6-(2,3-dichlorophenyl)-1,2,4-triazine (14W80) in lamotrigine. It reported that 14W80 was more hydrophobic than lamotrigine and hence eluted with a longer retention time, which was difficult for low level detection in HPLC. However, the procedure involved sample pre concentration by normal-phase HPLC and later quantified the impurity by subsequent on-line reversed-phase HPLC–thermospray mass spectrometry (TSP-MS) with single ion monitoring. This method enabled the detection of 14W80 in the 50–100 ppb range. The additional step of sample pre concentration was eliminated in a recent report which discussed use of LC-MS/MS in-source fragmentation with atmospheric pressure chemical ionization (APCI) followed by multiple reaction monitoring to analyze 14W80, chemically known as, (Z)-2-(2,3-dichlorophenyl)-2-(guanidinylimino)acetonitrile isobaric with lamotrigine.

The determination of lamotrigine in presence of its synthetic impurity, 2,3-dichlorobenzoic acid has also been reported. It discussed spectrophotometric, TLC densitometric and HPLC techniques and presented a comparative statistical picture. It also reported a novel complexation reaction between lamotrigine and p-chloroanilic acid.

A stability indicating method on the separation and quantification of process related impurities of lamotrigine was reported. The report involved the preparation of the drug and its
impurities in the laboratory, so the method actually described the in-house synthesized lamotrigine and the impurity profiling. It focused on the separation of process-related impurities of lamotrigine using harsh chromatographic conditions, such as strong buffer and trifluoroacetic acid, as the mobile phase can deteriorate the reverse-phase column. On a closer look at the chromatographic profiles, the impurities and lamotrigine were separated at an elution time of 14 min, such that lamotrigine peak was pushed towards a retention time of 2 min. The impurities were also programmed to elute at an early stage, hence, this method only focused on polar impurities during method development.

Recently, a stress degradation study of lamotrigine was published which reported limited stress testing. The method reported only acid, alkaline and thermal stress studies of lamotrigine, suggesting that the drug was only susceptible in acidic and alkaline stress conditions. On examining the chromatograms, it was observed that the method cannot be considered as a stability-indicating method, as the extraneous peaks merged with the drug peak, indicating a single product was formed under acid and alkaline conditions. Further, the paper stated that lamotrigine exhibited extensive lability in alkaline condition and degraded to about 98.2 %, however, the rate of the reaction was not statistically proven.

### 2.2.2. Itopride hydrochloride

Itopride hydrochloride is a novel gastroprokinetic drug which stimulates gastrointestinal motor activity through the synergistic effects of dopamine D2-receptor blockade and acetylcholine esterase inhibitors. Itopride is not official in any pharmacopoeia. Few analytical methods have been reported for the quantitative estimation of itopride. Mushiroda et al. have reported an HPLC method with fluorescence detection. The limitation of this method
is the use of high ratio of buffer in the mobile phase that can cause damage to the reverse phase column and the elution time of itopride is more than 10 min. Takahara et al. have reported an HPLC method for simultaneous determination of itopride (HSR-803) and its metabolites in human serum and urine using automated column switching.\(^7^3\)

Two sensitive and reproducible methods have been described for the quantitative determination of itopride hydrochloride in the presence of its degradation products\(^7^4\). It was observed that the method adopted to stress itopride were not in accordance with the ICH guidelines. The drug was exposed to high concentrations of acid, alkali and peroxide solutions that do not elucidate the real-time stability testing. The comparative picture of two stability-indicating methods, viz, HPLC and HPTLC was statistically explained. Though the method suggested the presence of impurities in form of extraneous peaks, the identification of impurities was remains elusive.

HPLC method for the quantitative determination of itopride hydrochloride in pharmaceutical dosage forms and purity evaluation in bulk drugs has been reported\(^7^5\). This report determined the drug in presence of known impurities namely, dimethyl amino ethyl benzyl amine (DEBA) and veratric acid chloride. The retention times of DEBA, itopride and veratric acid chloride were about 3.0, 13.5 and 28.5 min respectively, which was an undesirable resolution.

Quantitative estimation of itopride in pharmaceutical formulation and drug substance by normal phase HPTLC has been reported\(^7^6\). The densitometric analysis was performed in presence of diltiazem hydrochloride as an internal standard at 1230nm. The elucidation of impurity profiling or identification was yet elusive. Determination of itopride hydrochloride in human plasma using LC–MS/MS has been reported in the literature\(^7^7\). Recently, simultaneous determination of
itopride and domperidone in human plasma using LC-MS techniques was also reported. It communicated about the simultaneous quantitation of fixed dosage combination dosage form of itopride and domperidone with pantoprazole as an internal standard in human plasma using positive ESI-MS/MS. The method was later applied for studying the clinical pharmacokinetics in healthy human volunteers. Hyphenated techniques employed in both the reports did not mention about the forced degradation products and eventual identification.