CHAPTER 3
RESEARCH ENVISAGED &
PLAN OF WORK
3. Research Envisaged and Plan of Work

3.1. Research envisaged

As discussed earlier, the ICH states that the conduct of stress testing on a drug substance is essential to determine its inherent stability characteristics under a variety of conditions, to identify degradation products formed under such conditions and to establish the degradation pathway of the drug. It is stated that the testing should include the effect of temperature, humidity (where appropriate), oxidation, photolysis and susceptibility to hydrolysis.

The drugs selected in the proposed study are nelfinavir mesylate, ritonavir, linezolid and lacosamide. For nelfinavir mesylate various high-performance liquid chromatographic (HPLC) methods are reported for its investigation in biological fluids. There exist a few reports on stability-indicating methods of the drug by using HPLC and high-performance thin layer chromatography (HPTLC). The drug was also reported for its simultaneous determination with other HIV protease inhibitors and non-nucleoside reverse transcriptase inhibitors by using HPLC, ion-pair HPLC, HPLC–MS, LC–MS–MS. But the characterization of degradation products of nelfinavir mesylate was lacking in the literatures. For ritonavir, there exist reports on simultaneous assay of ritonavir and other anti-HIV drugs in biological fluids by HPLC, capillary electrophoresis. The drug was also explored for its metabolic studies by using LC-MS. There exists a report on forced degradation studies of ritonavir by using LC-MS, but nature of HPLC method was not stability indicating and also a sample containing all the degradation products was not injected into HPLC. Literature search for linezolid revealed that, there exist a few reports on stability indicating assay methods of linezolid by using HPLC, high-performance thin layer chromatography (HPTLC) and thin layer chromatography (TLC). The drug was also reported for its metabolite identification and characterization studies. The drug was also explored for its pharmacokinetics and toxicological studies as well as isolation and characterization of its process related impurities. For lacosamide, there exists a report on HPLC method for the assay in biological fluids.

Literature search revealed that none of these methods reports the characterization of formed degradation products or the mass fragmentation pattern of the drug except ritonavir, which is
reported for the characterization of its eight degradation products while the stability indicating assay method (SIAM) was lacking. In this current study stress studies ritonavir was systematically explored to characterize nine degradation products along with the development of SIAM.

The investigations were carried out under the following heads:

- LC studies for the separation of drugs and their degradation products.
- MS\(^n\) and MS/TOF studies to determine the complete mass fragmentation profile of the above mentioned drugs.
- Stress degradation studies to determine their inherent stability.
- Identification of degradation products and development of validated SIAMs.
- Characterizations of degradation products through LC-MS studies and to propose the degradation pathway for the drug wherever possible.
3.2. Plan of work

Plan of work is divided into two parts, viz., Part A and Part B.

Part A:

It involves mass spectral studies on pure drugs by using mass spectrometry/time of flight mass analyzer (MS/TOF) to determine accurate mass of drugs as well as drug fragments. Moreover Part A also involves determination of mass fragmentation pattern of drugs under investigation. To achieve this objective a multi-stage mass spectrometric (MS\textasciitilde{n}) studies were carried out. MS\textasciitilde{n} studies on drugs helps to identify the origin of the parent ions as well as daughter ions generated from the drugs. On a whole, all these mass spectral studies helps to establish the fragmentation pattern of the drugs, which will be further required to compare to the fragments of the drug with that of the fragments of degradation products.

Part B:

It involves samples preparation for stress degradation studies and optimization of stress conditions to achieve sufficient degradation (10-15%). HPLC method optimization and development begins with pure drugs sample analysis and then subsequently on stressed samples, and finally mixture of stressed samples. The developed HPLC methods were validated with respect to the parameters viz., linearity, precision, accuracy, specificity and selectivity. The developed LC method was transferred to LC-MS/TOF analysis for the determination of molecular ion fragments of the degradation products as well as their daughter ion fragments. An MS compatible buffer system was used for LC-MS/TOF analysis. The degradation products were characterized and the structures were assigned to them by interpretation of LC-MS/TOF data. Finally mechanism of formation of degradation products and the degradation pathway of the drug was established.
Chapter 3. Research envisaged and plan of work

Part A

MS-TOF Parameter optimization (a)

MS\textsuperscript{n} studies

Mass fragmentation pattern of drug (b)

Part B

Solution/solid state stress study

HPLC method development

Transfer method to LC-MS/TOF (with the help of a)

Compare fragmentation pattern of degradation products with drug (part a & b)

Isolation of degradation products (if necessary) for reference standard

Structural elucidation by MS studies

Fig. 3.1. Block diagram showing plan of proposed research work.