CHAPTER 7
SUMMARY & CONCLUSIONS
7. Summary and Conclusions

7.1. Nelfinavir mesylate

The degradation behaviour of nelfinavir mesylate and its tablet formulation was explored by exposing it to ICH defined stress conditions. The drug degraded under acidic, basic, oxidative and photoacid stress, while it was found to be stable under all other stress conditions. HPLC analysis revealed the formation of three degradation products (DP I-III), all of which were found to be previously unknown. While DP-I was formed as a common hydrolytic degradation product under both acid and alkaline stress conditions. Two major isomeric DPs (DP-II and DP-III) were generated under oxidative and photoacid stress. The developed HPLC method was validated and the validation results were found to be well within the acceptance criteria. To resolve the structures of degradation products, mass fragmentation pattern of the drug was established with the help of MS/TOF and MS² studies. The degradation products were then characterized with the help of LC-MS/TOF data and comparison of the same to that for the drug. A complete degradation pathway of the drug was also established. The degradation products were identified as 3-hydroxy-N-((2R,3R)-3-hydroxy-1-(phenylthio)butan-2-yl)-2-methylbenzamide and (3S,4aS,8aS)-N-tert-butyl-2-((2R,3R)-2-hydroxy-3-(3-hydroxy-2-methylbenzamido)-4-(phenylsulfanyl)butyl)decahydroisoquinoline-3-carboxamide. The stability-indicating nature of the developed HPLC method (by using methanol and phosphate buffer in a gradient mode as mobile phase) for nelfinavir mesylate and its tablet formulation was investigated by chromatographic resolution of a mixture of stress reaction solutions, particularly those in which significant degradation was observed. It indicated that the method was able to separate the drug and all the major and minor degradation products. Hence, it can be stated that the method was found to be truly stability-indicating.

7.2. Linezolid

Degradation behaviour of linezolid and its tablet formulation was explored under stress degradation conditions prescribed by ICH guidelines. Three degradation products (DP-I, DP-II and DP-III) were formed during forced degradation study on linezolid. All the DPs
were separated in a single run by a gradient HPLC method and also the validation results were well within the acceptance criteria. DP-I and DP-II were the products of oxidative and acidic stress, respectively, while DP-III was formed as a common hydrolytic degradation product under both alkaline and neutral stress conditions. One of the major degradation product (DP-III) revealed that the drug was highly labile in alkaline condition. The degradation products were characterized with the help of LC-MS/TOF studies. It was found that all the DPs were hitherto unknown. The complete degradation pathway of the drug was also established. The study highlights the benefit of the use of ICH testing approach in establishment of complete degradation pathway of drugs. The degradation products were identified as \( N-(((S)-3-(3\text{-fluoro-4-morpholinophenyl})-2\text{-oxooxazolidin-5-yl})\text{methyl})\text{acetamide} \), \( (S)-5-((1\text{-hydroxyethylamino})\text{methyl})-3\text{-}(4\text{-}(2\text{-methoxyethylamino})\text{-3-fluorophenyl})\text{oxazolidin-2-one} \) and \( N-(((S)-3\text{-}(4\text{-}(2\text{-hydroxyethylamino})\text{-3-fluorophenyl})\text{-2-oxooxazolidin-5-yl})\text{methyl})\text{acetamide} \). The developed method was found to be rapid, sensitive and precise, and possess potential to separate the drug as well as all the major and minor degradation products, which proves its stability indicating nature.

### 7.3. Lacosamide

Lacosamide and its tablet formulation was subjected to hydrolytic (acid, alkaline and neutral), oxidative, thermal and photolytic stress conditions as advised in ICH Q1A(R2) guideline. The drug was found to degrade in acidic, basic and neutral stress while it was stable in oxidative, photolytic and thermal stress conditions. A total of two degradation products (DP) were formed, which were separated on a C-18 column employing a gradient HPLC method. LC method helped to separate drug as well as all the unknown degradation products (DP-I and DP-II). DP-II was formed as a major degradation product under alkaline stress, while the same also was generated under neutral stress conditions. Whereas the DP-I was the result of acid catalyzed hydrolysis. The drug was found to be stable under all other conditions including photoacid, photoneutral, photobase and solid state thermal stress conditions. A complete mass fragmentation pathway of the drug was first established with the help of multi-stage (MS\(^5\)) and MS/TOF accurate mass studies. Then stressed samples were subjected to LC–MS/TOF studies, which provided their fragmentation pattern and accurate masses. The mass spectral data were employed to characterize the DPs and assign
structures to them. The total information was also used to establish the degradation pathway of the drug. The degradation products were identified as \((R)-2\)-amino-\(N\)-benzyl-3-methoxypropanamide, and 3-benzyl-2-hydroxy-5-(methoxymethyl)-2-methylimidazolidin-4-one. The developed method was found to be rapid, sensitive and precise, and possess potential to separate the drug as well as all the major and minor degradation products, which proved to be stability indicating. Moreover, both the degradation products (DP-I and DP-II), were isolated by using glass column and tested for single dose acute toxicity study. The toxicity data revealed that both the DPs were found to be nontoxic.

7.4. Ritonavir

Degradation behaviour of ritonavir was explored by exposing it to ICH defined stress conditions. The developed LC method helped to separate drug as well as all the unknown degradation products (DP-I to DP-X). DP-I and DP-II were generated under both alkaline and neutral stress, while DP-III and DP-VIII were formed under both acid and neutral stress conditions. DP-IV, DP-V and DP-VII were the results of acid catalyzed hydrolysis, whereas DP-VI and DP-IX were exclusively alkali catalyzed degradation products. Among all the degradation products DP-X was formed as a common product under acid, alkaline and neutral stress conditions. The drug was found to be stable under photoacid, photoneutral, photobase and solid state thermal stress conditions. Fragmentation pattern of drug was established with the help of MS/TOF and MS\(^n\) studies. The degradation products were characterized with the help of LC-MS/TOF and MS\(^n\) studies. The DP-1 was not detected in positive ESI mode, probably due to its poor ionizability. The total information was also used to establish the degradation pathway of the drug. The degradation products were identified as 

\[(R,E)-1-((2\text{-isopropylthiazol-4-yl})\text{methyl})-1\text{-methyl}-3-((2-(1\text{-phenylpropan-2-ylamino})\text{vinyl})\text{urea}, \quad 1-((2\text{-isopropylthiazol-4-yl})\text{methyl})-1\text{-methyl}-3-((S)-3\text{-methyl}-1-((R)-1\text{-phenylpropan-2-ylamino})\text{butan-2-yl})\text{urea}, \quad (S)-N-((2S,4S,5S)-5\text{-amino}-4\text{-hydroxy}-1,6\text{-diphenylhexan-2-yl})-2-(3,3\text{-dimethylureido})-3\text{-methylbutanamide}, \quad \text{thiazol-5-ylmethyl (2S,3S,E)-5-((E)-2-(dimethylcarbamoylimino)-3\text{-methylbutanoylimino})-3\text{-hydroxy}-1,6\text{-diphenylhexan-2-ylcarbamate, (S)-N-((2S,4S,5S)-5\text{-amino}-4\text{-hydroxy}-1,6\text{-diphenylhexan-2-yl})-2-(3-((2\text{-isopropylthiazol-4-yl})\text{methyl})-3\text{-methylureido})-3\text{-methylbutanamide, thiazolidin-5-ylmethyl (2S,3S,5S)-3\text{-hydroxy}-5-((S)-3\text{-methyl}-2-(3-}\]
methylureido)butanamido)-1,6-diphenylhexan-2-ylcarbamate, thiazol-5-ylmethyl
(2S,3S,5S)-3-hydroxy-5-((R)-2-(3-((2-isopropylthiazol-4-yl)methyl)-3-methylureido)-3-
methylbutanamido)-1,6-diphenylhexan-2-ylcarbamate, (S)-N-((2S,4S,5S)-5-formamido-4-
hydroxy-1,6-diphenylhexan-2-yl)-2-(3-((2-isopropylthiazol-4-yl)methyl)-3-methylureido)-3-
methylbutanamide, methyl (2S,3S,5S)-3-hydroxy-5-((S)-2-(3-((2-isopropylthiazol-4-
yl)methyl)-3-methylureido)-3-methylbutanamido)-1,6-diphenylhexan-2-ylcarbamate.