SUMMARY AND CONCLUSION

Presence of impurities and degradation products in drugs poses huge risk to patients health. This is even worsening effects if the drugs are anticancer categories as in such cases the chances of impurities and degradation products to be carcinogenic or genotoxic are very high. Hence to control the impurities and degradation products, various regulatory agencies put great emphasis on identification, characterisation and generation of specification limit for control of impurities and other degradation products which are possible to generate in excessive storage conditions.

In present work, three anticancer category drugs were chosen as alectinib, nelarabine and gimeracil. Stability indicating analytical methods was developed for each of these drugs for their estimation by HPLC in presence of their degradation products. To identify the degradation products, detailed forced degradation studies were carried out in the degradation conditions of acid, alkali, oxidative, heat, UV and light. The some major degradation products were isolated using preparative HPLC and then characterized them using various characterization instrument techniques such as NMR, FT-IR, LC-MS/MS and HRMS.

Isolation, identification and characterization of degradation products and degradation kinetic study of alectinib drug is covered under chapter-3 which was bifurcated into four sections namely Section-A, Section-B, Section-C and Section-D:

Section-A:

Section-A was again discussed in three subsections: Section 3.4.1: Analytical method development, Section-3.4.2: Analytical method validation and Section-3.4.3: Forced degradation study.

Section 3.4.1 covered the analytical method development trials details for estimation of alectinib in presence of its degradation products. Efforts were made and succeeded to develop MS compatible LC method so that same method can be utilised in structure elucidation by MS and MS/MS in the later section of the study. After taking various experimental trials, following conditions were finalized for LC method: Kromasil C18 (250 X 4.6) mm, i.d., 5µ (Make: Akzonobel) column was maintained at 50 °C temperature during analysis. The mobile phase-A was composed of 10mM ammonium formate buffer (the pH was adjusted to 7.00 with 0.25 % ammonia solution in water) and acetonitrile in the ratio of 95:05. The mobile phase-B was a mixture of 10mM ammonium formate buffer (the pH was

adjusted to 7.00 with 0.25 % ammonia solution in water) and acetonitrile in the ratio of 25:75. The gradient program was planned as a linear gradient program (Time/%B) which was set at 0.00/50, 25.0/100, 30.0/100, 30.1/50, 35.0/50. The injection volume and flow rate were set as 20µl and 1.5mL/min respectively. The appropriate response of alectinib and its degradation products were observed at a detection wavelength of 230nm. The chromatographic conditions in both HPLC and LC-MS/MS studies were identical as the conditions were compatible with LC-MS/MS instrument. Mass spectrometric conditions were set as: capillary voltage: 3.5kV, cone voltage: 15V and 30V, extractor voltage: 1.00 V, RF lens: 0.4V, source temperature: 110°C, desolvation temperature: 350°C, cone gas flow: 25 L/Hr, desolvation gas flow: 650 L/Hr, collision energy: 2.0eV.

Section 3.4.2 covered analytical method validation of developed LC method with parameters as per ICH guidelines such as linearity, sensitivity, accuracy, precision, robustness and specificity. The results of each of these parameters were obtained within the satisfactory limit hence the method was confirmed to be validated as per ICH guideline.

Section-3.4.3 covered forced degradation study under various degradation conditions. For alectinib, the drug was found to be degraded in oxidative stress conditions only. In oxidative degradation condition, alectinib was found to generate sixteen degradation products out of which initial four degradation products namely DP-1 to DP-4 were isolated and characterized using NMR, IR and LC-MS/MS spectra interpretation. The later DP-5 to DP-16 degradation products were identified in HRMS study and characterized using HRMS/MS spectra.

Section-B: covered degradation kinetic study of alectinib in oxidative degradation condition under varied conditions of time duration exposure. During this study, the degradation behavior of alectinib was noted and based on statistical values the order of degradation reaction was also identified as first order. Based on rate of reaction, rate constant, activation energy and half life has been calculated.

Section-C: covered the most crucial segment of the study and that is the isolation and structure elucidation of degradation products. The initial four degradation products DP-1 to DP-4 were isolated and run through NMR, FT-IR and LC-MS/MS instrument to elucidate their structures. The biggest challenge here was the isomeric nature of DP-1, DP-2 and DP-3 each of them had same molecular ion peak at 499.15, 499.26, 499.19 m/z respectively.

However, after rigorous thinking and interpretation of NMR, IR and LCMS/MS spectra, they were elucidated as N-oxide impurity, epoxide impurity, and N-hydroxy impurity respectively. DP-4 had different molecular ion peak at 501.27 m/z which was confirmed as amide impurity. Degradation products from DP-5 to DP-16 were characterized in detail based on the very accurate HRMS spectra and their fragmentation pattern observed in HRMS spectra. All of these degradation products were discussed in detail in the individual subsections in Section-C. To the best of our knowledge, the these degradation products have not been reported in literature.

Section-D covered the development and validation of method for quantitative estimation of alectinib by Q-NMR. Alectinib concentration can be easily estimated by this method using internal standard without requirement of alectinib primary standard. This method would be very useful for the scientist for easy estimation of alectinib content in the drug. This method was also validated and proved to be efficient as per ICH guideline.

Isolation, identification and characterization of degradation products and degradation kinetic study of nelarabine drug is covered under chapter-4 which was bifurcated into three sections namely Section-A, Section-B, and Section-C:

Section-A:

Section-A was again discussed in three subsections: Section 4.4.1: Analytical method development, Section-4.4.2: Analytical method validation and Section-4.4.3: Forced degradation study.

Section 4.4.1 covered the analytical method development trials details for estimation of alectinib in presence of its degradation products. Efforts were made and succeeded to develop MS compatible LC method so that same method can be utilised in structure elucidation by MS and MS/MS in the later section of the study. After taking various experimental trials, following conditions were finalized for LC method: Mobile phase-A is composed of 0.01% trifluoroacetic acid in water whereas mobile phase-B is the mixture of mobile phase-A and acetonitrile in the ratio of 10:90. Waters X-Bridge C18 (250X4.6), 3.5µ column was maintained at room temperature during analysis. The gradient program was planned as linear gradient (Time/%B) which was set at 0.00/00, 10.0/10, 35.0/10, 35.5/00,

50/00. The injection volume and flow rate were set as 20µl and 0.5mL/min respectively. The appropriate response of nelarabine and its degradation products were observed at a detection wavelength of 248nm. Mass spectrometric conditions were set as: capillary voltage: 3.5kV, cone voltage: 15V and 30V, extractor voltage: 1.00 V, RF lens: 0.4V, source temperature: 110°C, desolvation temperature: 350°C, cone gas flow: 25 L/Hr, desolvation gas flow: 650 L/Hr, collision energy: 2.0eV.

Section 4.4.2 covered analytical method validation of developed LC method with parameters as per ICH guidelines such as linearity, sensitivity, accuracy, precision, robustness and specificity. The results of each of these parameters were obtained within the satisfactory limit hence the method was confirmed to be validated as per ICH guideline.

Section-4.4.3 covered forced degradation study under various degradation conditions. For alectinib, the drug was found to be degraded in acidic, alkali and oxidative stress conditions. DP-1 and DP-2 were found to be generated in acidic and alkali degradation condition respectively. DP-3 to DP-9 were observed to be generated in oxidative degradation condition. Hence, nelarabine was found to generate nine degradation products out of which initial two degradation products namely DP-1 to DP-2 were isolated and characterized using NMR, IR and LC-MS/MS spectra interpretation. The later DP-3 to DP-9 degradation products were identified in HRMS study and characterized using HRMS/MS spectra.

Section-B: covered degradation kinetic study of nelarabine in acidic, alkali and oxidative degradation condition under varied conditions of time duration and temperature exposure. During this study, the degradation behavior of nelarabine was noted and based on statistical values the order of degradation reaction was also identified as zero order. Based on rate of reaction, rate constant, activation energy and half life has been calculated.

Section-C: covered the most important segment of the study and that is the isolation and structure elucidation of degradation products. The initial two degradation products DP-1 and DP-2 were isolated and run through NMR, FT-IR and LC-MS/MS instrument to elucidate their structures. The degradation products from DP-3 to DP-9 were characterized in detail

based on the very accurate HRMS spectra and their fragmentation pattern observed in HRMS spectra. All of these degradation products were discussed in detail in the individual subsections in Section-C. To the best of our knowledge, except DP-1, all the degradation products have not been reported in literature.

Isolation, identification and characterization of degradation products and degradation kinetic study of gimeracil drug is covered under chapter-5 which was bifurcated into two sections namely Section-A and Section-B:

Section-A: was again discussed in three subsections: Section 5.4.1: Analytical method development, Section-5.4.2: Analytical method validation and Section-5.4.3: Forced degradation study.

Section 5.4.1 covered the analytical method development trials details for estimation of alectinib in presence of its degradation products. Efforts were made but it seemed difficult to develop MS compatible LC method hence initially LC method was developed and later on MS method was separately developed during forced degradation study to identify the molecular mass of degradation product generated. After taking various experimental trials, following conditions were finalized for LC method: Waters X-Bridge C18 (250 X 4.6) mm, i.d., 3.5µ (Make: Waters) column was maintained at room temperature during analysis. Mobile phase-A was composed of 10mM solution of potassium dihydrogen orthophosphate with 10mL of triethylamine and 1.00 g of 1-octane sulphonic acid salt per liter of mobile phase and further adjusting the pH of this solution to 2.50 with orthophosphoric acid. Mobile phase-B was composed of acetonitrile only. Diluent was optimized as the mixture of mobile phase-A and acetonitrile in the ratio of 50:50. Wavelength for estimation of gimeracil was kept at 248nm and flow rate was maintained at 0.5mL/min. Injection volume was set at 10µl and the gradient was linear for 0 to 30 minutes starting %mobile phase-B from 0% to 30% at 30 minute. Last five minutes of gradient was kept at initial equibration phase for mobile phase-A and mobile phase-B

Section 5.4.2 covered analytical method validation of developed LC method with parameters as per ICH guidelines such as linearity, sensitivity, accuracy, precision, robustness and

specificity. The results of each of these parameters were obtained within the satisfactory limit hence the method was confirmed to be validated as per ICH guideline.

Section-5.4.3 covered forced degradation study under various degradation conditions.
Since MS compatible method was required for identification of degradation products in LCMS and HRMS, trials were taken to optimize the mobile phase of LC method which was developed in section 5.4.1. After some experimental trails following method for LCMS and HRMS was finalized: Mobile phase-A was composed of 0.05% formic acid solution in water and mobile phase-B was composed of acetonitrile only. Diluent was decided as water only. YMC Pack pro C18 (250x4.0)mm, 3µ column was taken for the LC-MS study for better peak shape. Wavelength, gradient, flow rate, injection volume and other parameters were kept same and LC method.
For gimeracil, the drug was found to be degraded in oxidative stress conditions only. However, in oxidative degradation condition, the main peak of gimeracil observed to be reduced to almost half but again the degradation products were not detected under the chromatographic conditions i.e. UV/PDA detector even if the run time was extended to 90 minutes. It was therefore planned to develop a LC method with MS detector. The degraded sample under oxidative degradation condition was run in HRMS instrument, total 14 degradation products peaks were detected in TIC chromatogram with exact MS and MS/MS spectra.

Section-B: covered the most important segment of the study and that is the isolation and structure elucidation of degradation products. All the degradation products from DP-1 to DP-14 were characterized in detail based on the very accurate HRMS spectra and their fragmentation pattern observed in HRMS spectra. All of these degradation products were discussed in detail in the individual subsections in Section-B. To the best of our knowledge, all the degradation products have not been reported in literature.