6. SUMMARY AND CONCLUSION.

Quality of any Active Pharmaceutical Ingredient (API) or drug product for patient compliance is directly depending on stability of drug. The ICH drug stability test guideline Q1A (R2) requires that analysis of stability samples should be done through the use of validated Stability indicating assay methods (SIAMs) 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. A proactive approach in developing a SIAMs involve forced degradation at the early stages of development with the key degradation samples used in the method development process.

The present research work focuses on the development of novel stability-indicating analytical methods using High performance liquid chromatography (HPLC) and High performance thin layer chromatography (HPTLC) for some drugs like (gemifloxacin mesylate-ambroxol hydrochloride, lamivudine-abacavir sulphate, tenofovir disoproxil fumarate- lamivudine, and cefixime trihydrate-moxifloxacin hydrochloride ). These drugs are used as multicomponent combined drug products for thesis work.

The work also includes validation of the developed analytical methods as per ICH requirements and demonstrates the suitability of developed methods to assess the stability of drug products. Further developed stability indicating HPLC method is applied to the study of accelerated aging condition with respect to temperature and humidity, and influence of photolytic effect on formulations and its effect on dissolution rate and release profiles.

Stress degradation or forced degradation studies are performed as per ICH drug stability test guideline Q1A (R2). Stress studies are performed as acid degradation, base degradation, photo degradation, thermal degradation and degradation by oxidation. For all combinations stress samples are run in developed stability indicating analytical method which is capable of discriminating between the major active (intact)
pharmaceutical ingredients (API) from any degradation (decomposition) product(s) formed under stress degradation studies.

The scheme of work was divided into VI parts and has been elaborated in Chapter 2. Initially an extensive literature survey was done followed by selection of drugs and combined drug products. The drug profile and review of literature for combined drug products is reported in Chapter 3.

The active pharmaceutical ingredients were identified and characterized as per official texts or manufacturers standards and were found to comply with the standards.

The drugs and combination drug products selected i.e gemifloxacin mesylate-ambroxol hydrochloride, lamivudine-abacavir sulphate, tenofovir disoproxil fumarate disoproxil fumarate- lamivudine, and cefixime trihydrate-moxifloxacin hydrochloride. were obtained as gift samples the sources are reported in Chapter 4. The research work was divided in four sections for the four combination drug products selected.

Section I: Gemifloxacin mesylate and ambroxol hydrochloride Combination (G – CIN – A Tablet); Novel stability indicating HPLC method for combination of GEM and AMB is developed successfully. In this study the HPLC method conditions for separation of drugs and its degradation products were optimized during method development phase. The influencing factors were investigated and the best results achieved was for the mobile phase comprising of 0.02M potassium dihydrogen orthophosphate buffer: Acetonitrile (70:30 v/v) with 1% Triethylamine (2 ml/100 ml mobile phase) and its pH adjusted to 5.5 with ortho phosphoric acid and detection was executed on PDA detector at 244 nm. The retention time (RT) of GEM and AMB was found to be 2.34 ± 0.2 and 4.21± 0.2 min respectively. This indicates good resolution between the two drugs and their degradation products (RRT). The method is extensively validated according to ICH guidelines. The results of analysis shows well separated and good quality peaks for the drug of interest since such result at the outset provide better confidence because of the unknown nature of the products formed during stressing.
Developed stability indicating HPLC method is applied to dissolution stability studies of G – CIN – A SR Tablet, with respect to Accelerated aging conditions on the formulations. Dissolution studies were performed using USP Type II apparatus for period of 12 h. first two hours in 0.1N HCl (Acid stage), then continued in phosphate buffer pH 6.8 (Buffer stage). After 10 h. both the drugs shows complete dissolution profile. Exposure of tablets to ICH accelerated stability condition 40°C ± 2°C/75% RH ± 5% for 6 months and photolytic studies in an open dish, resulted in rapid increases in tablet hardness, and changes in water content upon storage leads to decrease in dissolution rate. The tablet matrix appears to rapidly absorb atmospheric moisture, which has a negative influence on the stability of drug product as demonstrated by tablet weight gain and changes in dissolution curves as compared to fresh marketed formulation.

New stability indicating HPTLC method is also developed successfully for combination of GEM and AMB from its tablet dosage form. In this study HPTLC method condition for separation of drugs and its degradation products are optimized during method development phase. The optimal composition of the mobile phase for analysis was determined as n – butanol: methanol: water: ammonia in the ratio of (6:3:1:0.1 v/v/v/v). Gemifloxacin spots shows fluorescence and are detected at longer wavelength 366 nm while Ambroxol spots are detected at shorter wavelength 254 nm UV light. R_f value of GEM was found to be 0.02 ± 0.01 and AMB shows R_f of 0.55 ± 0.01. This indicates good resolution between the two drugs and their degradation products (RRT). The method is extensively validated according to ICH guidelines. The results of analysis shows well separated and good quality peaks for the drugs of interest.

Stress degradation studies were carried out following the condition prescribed in the parent drug stability testing guideline (Q1A) issued by ICH. The inherent stability of the drug substance, its degradation products under the various stress condition has been established. Both the drugs were found to be more liable to decompositions in acid, base, oxidative and photo degradation. Mild degradation is observed in thermal studies while both the drugs are found to be stable in neutral condition. Percent degradation and mass balance is calculated for both the drugs in HPLC and HPTLC methods respectively. Both stability indicating HPLC and HPTLC methods developed are simple, accurate, precise,
rugged, robust and found to be specific as well as selective for drugs analysis in presence of degradation products arising in different stress conditions.

Section – II. Lamivudine and abacavir sulphate Combination (ABAMUNE – L Tablet); Novel stability indicating HPLC method for combination of LAM and ABAC is developed successfully. In this study HPLC method condition for separation of drugs and its degradation products are optimized during method development phase. Investigated the influencing factors, the best results are achieved in mobile phase as methanol: water (50:50 v/v) and detection was executed on PDA detector at 260 nm. The retention time (RT) of LAM and ABAC was found to be 5.49 ± 0.2 and 3.31 ± 0.2 min respectively. This indicates good resolution between the two drugs and their degradation products (RRT). The method is extensively validated according to ICH guidelines. The results of analysis shows well separated and good quality peaks for the drug of interest in presence of degradation products formed during stressing.

Developed stability indicating HPLC method is applied to dissolution stability studies of ABAMUNE – L Tablet, with respect to Accelerated aging conditions on the formulations. Dissolution studies were performed using USP Type II apparatus for period of 45 min, in 0.01N HCl. Both the drugs shows complete dissolution profile within 30 min. Exposure of tablets to ICH accelerated stability condition 40°C ± 2°C/75% RH ± 5% for 6 months and photolytic studies in an open dish, resulted in rapid increases in tablet hardness, and changes in water content upon storage leads to decrease in dissolution rate. The tablet matrix appears to rapidly absorb atmospheric moisture, as demonstrated by tablet weight gain, this shows changes in dissolution curves as compared to fresh marketed formulation indicating influence on drug product stability.

New stability indicating HPTLC method is also developed successfully for combination of LAM and ABAC from its tablet dosage form. In this study HPTLC method condition for separation of drugs and its degradation products are optimized during method development phase. The optimal composition of the mobile phase for analysis was determined as methanol: chloroform in the ratio of 1: 9 v/v. R_f value of LAM was found to be 0.18 ± 0.01 and ABAC shows R_f value of 0.49 ± 0.01. LAM and ABAC spots are detected at shorter wavelength 254 nm UV light. This indicates good
resolution between the two drugs and their degradation products (RRT). The method is extensively validated according to ICH guidelines. The results of analysis shows well separated and good quality peaks for the drugs of interest presence of degradation products formed during stressing.

Stress degradation studies were carried out according to Q1A ICH guidelines. The inherent stability of the drug substance, its degradation products under the various stress condition has been established. Both the drugs were found to be more liable to decompositions in acid, base, and oxidative. While both the drugs were found to be stable in thermal, neutral and photo degradation studies. Percent degradation and mass balance is calculated for both the drugs in HPLC and HPTLC methods respectively. Both stability indicating HPLC and HPTLC methods developed are simple, accurate, precise, rugged, robust and found to be specific as well as selective for drugs analysis in presence of degradation products arising in different stress conditions.

Section – III. Lamivudine and Tenofovir disoproxil fumarate Combination (TENVIR – L Tablet); Novel stability indicating HPLC method for combination of LAM and TEN is developed successfully. In this study HPLC method condition for separation of drugs and its degradation products are optimized during method development phase. Investigated the influencing factors, the best results are achieved in mobile phase as methanol: water (80:20 v/v) and detection was executed on PDA detector at 269 nm. The retention time of LAM and TEN was found to be 5.26 ± 0.2 and 9.41 ± 0.2 min respectively. This indicates good resolution between the two drugs and their degradation products (RRT). The method is extensively validated according to ICH guidelines. The results of analysis shows well separated and good quality peaks for the drug of interest in presence of degradation products formed during stressing.

Developed stability indicating HPLC method is applied to dissolution stability studies of TENVIR– L Tablet, with respect to Accelerated aging conditions on the formulations. Dissolution studies were performed using USP Type II apparatus for period of 45 min, in 0.1N HCl at 50 RPM. Both the drugs shows complete dissolution profile within 30 min.
Exposure of tablets to ICH accelerated stability condition $40^\circ C \pm 2^\circ C/75\% RH \pm 5\%$ for 6 months and photolytic studies in an open dish, resulted in rapid increases in tablet hardness, and changes in water content upon storage leads to decrease in dissolution rate. The tablet matrix appears to rapidly absorb atmospheric moisture, as demonstrated by tablet weight gain, this shows changes in dissolution curves as compared to fresh marketed formulation indicating influence on drug product stability.

New stability indicating HPTLC method is also developed successfully for combination of LAM and TEN from its tablet dosage form. In this study HPTLC method condition for separation of drugs and its degradation products are optimized during method development phase. The optimal composition of the mobile phase for analysis was determined as chloroform: methanol in the ratio of 8:2 v/v. $R_f$ value of LAM was found to be $0.05 \pm 0.01$ and TEN shows $R_f$ of $0.34 \pm 0.01$. LAM and TEN spots are detected at shorter wavelength 254 nm UV light. This indicates good resolution between the two drugs and their degradation products (RRT). The method is extensively validated according to ICH guidelines. The results of analysis shows well separated and good quality peaks for the drugs of interest presence of degradation products formed during stressing.

Stress degradation studies were carried out according to Q1A ICH guidelines. The inherent stability of the drug substance, its degradation products under the various stress condition has been established. Both the drugs were found to be more liable to decompositions in acid, base, oxidative and photo degradation. While both the drugs are found to be stable in thermal and neutral degradation studies. Percent degradation and mass balance is calculated for both the drugs in HPLC and HPTLC methods respectively. Both stability indicating HPLC and HPTLC methods developed are simple, accurate, precise, rugged, robust and found to be specific as well as selective for drugs analysis in presence of degradation products arising in different stress conditions.

Section – IV. Moxifloxacin Hydrochloride and Cefixime Trihydrate Combination (ALEXIM – M Tablet); Novel stability indicating HPLC method for combination Moxifloxacin Hydrochloride and Cefixime Trihydrate is developed successfully. In this
study HPLC method condition for separation of drugs and its degradation products are optimized during method development phase. Investigated the influencing factors, the best results are achieved in mobile phase as 0.01M potassium dihydrogen orthophosphate buffer: methanol (40:60 v/v) with 1% Triethylamine (2 ml / 100 ml mobile phase) and its pH adjusted to 3.5 with Glacial acetic acid and detection was executed on PDA detector at 293 nm. The retention time (RT) of MOXI and CEFI was found to be 4.24 ± 0.2 and 2.32 ± 0.2 min respectively. This indicates good resolution between the two drugs and their degradation products (RRT). The method is extensively validated according to ICH guidelines. The results of analysis shows well separated and good quality peaks for the drug of interest in presence of degradation products formed during stressing.

Developed stability indicating HPLC method is applied to dissolution stability studies of ALEXIM– M Tablet, with respect to Accelerated aging conditions on the formulations. Dissolution studies were performed using USP Type II apparatus for period of 45 min, in 0.1N HCl at 50 RPM. Both the drugs shows complete dissolution profile within 30 min. Exposure of tablets to ICH accelerated stability condition 40°C ± 2°C/75% RH ± 5% for 6 months and photolytic studies in an open dish, resulted in rapid increases in tablet hardness, and changes in water content upon storage leads to decrease in dissolution rate. The tablet matrix appears to rapidly absorb atmospheric moisture, as demonstrated by tablet weight gain, this shows changes in dissolution curves as compared to fresh marketed formulation indicating influence on drug product stability.

New stability indicating HPTLC method is also developed successfully for combination of Lamivudine and Abacavir Sulphate from its tablet dosage form. In this study HPTLC method condition for separation of drugs and its degradation products are optimized during method development phase. The optimal composition of the mobile phase for analysis was determined as n-Propanol: methanol: water: ammonia in the ratio of 4:3:2:0.1 (v/v/v/v). R_f value of MOXI was found to be 0.13 ± 0.01 and CEFI shows R_f of 0.70 ± 0.01. MOXI spots shows fluorescence and are detected at longer wavelength 366 nm while CEFI spots are detected at shorter wavelength 254 nm UV light. This indicates good resolution between the two drugs and their degradation products (RRT). The method is extensively validated according to ICH guidelines. The results of analysis
shows well separated and good quality peaks for the drugs of interest presence of degradation products formed during stressing.

Stress degradation studies were carried out according to Q1A ICH guidelines. The inherent stability of the drug substance, its degradation products under the various stress condition has been established. Both the drugs were found to be more liable to decompositions in acid, base, oxidative and photo degradation. While both the drugs are found to be stable in thermal and neutral degradation studies. Percent degradation and mass balance is calculated for both the drugs in HPLC and HPTLC methods respectively. Both stability indicating HPLC and HPTLC methods developed are simple, accurate, precise, rugged, robust and found to be specific as well as selective for drugs analysis in presence of degradation products arising in different stress conditions.

During this research work it was critically observed that working on HPTLC is simpler, easier and rapid than HPLC for stability indicating method development. The most time consuming step in HPLC is the method set up procedure especially in stability indicating ones. It is found that HPTLC is a low cost and more convenient method compared to HPLC and can be utilized in order to provide as useful data as HPLC. The reason is based on the low solvent volume and almost cheap stationary phases compared to HPLC. Any spot in HPTLC can be detected via PDA or fluorescent detector qualitatively and quantitatively. Also working in concentration at nanogram level in HPTLC provides more prominent ground to work in stability indicating studies for drugs in presence of degradation products during different stress degradation studies.

Finally it may be concluded that all the novel stability-indicating analytical methods developed and validated herein using High performance liquid chromatography (HPLC) and High performance thin layer chromatography (HPTLC) for various combined drug products gemifloxacin mesylate-ambroxol hydrochloride, lamivudine-abacavir sulphate, tenofovir disoproxil fumarate disoproxil fumarate- lamivudine, and cefixime trihydrate-moxifloxacin hydrochloride, demonstrate suitability to assess the stability of drug products. The developed stability indicating HPLC method showed potential for application to study the effect of accelerated aging conditions such as thermal degradation, photo-degradation, on dissolution rate and release profiles on stability of...
combined drug products. This study examined the effect of accelerated-aging conditions on the performance of various drug products. Although storage conditions affected the dissolution behavior of all tablet formulations and suggest likely implications for drug bioavailability. Nevertheless, the potential impact of these results on the in vivo bioavailability would require further investigation, but it could be anticipated that this attribute would be affected.

Further the developed methods can be successfully implemented during the quality monitoring and also be used in the assessment of quality during storage and stability probe of the drug products.

6.1 Future Prospects:

- All combination products can be further studied for stability indicating LC-MS method development.

- Developed methods for all the above mentioned combination products can be studied further for their application to biodegradation studies (metabolic degradation).