SUMMARY AND CONCLUSION

The requirement of stability testing data has been recommended by the USFDA and ICH guidance to understand the influence of various environmental factors on the quality of a drug substance and drug product with time. Selection of suitable formulation and package as well as providing proper storage conditions and shelf life, which is essential for regulatory documentation, depends upon the stability of molecule. A stability-indicating method is defined as an analytical method that accurately quantifies the active ingredients without interference from degradation products, process impurities, excipients, or other potential impurities. It is also required that analytical method should be validated to demonstrate that impurity unique to the new drug substances do not interfere with or are separated from specified or unspecified degradation products in the drug product.

7.1 Stability Indicating HPLC Method Development and Validation for the Simultaneous Determination of Lafutidine & Domperidone Maleate in its Dosage Forms [Method I and II]

Two simple, precise and accurate RP–HPLC methods were developed for the quantitative estimation of Lafutidine and Domperidone in bulk and marketed formulations without any interference from the excipients and degradation products. Method I was developed using Buffer (0.1% v/v OPA in water): acetonitrile in the ratio of 65:35 in isocratic mode (Zodiac C18 column, flow rate of 1ml/ min, 215 nm using PDA detector). With run time of 10 minutes, the retention times were found to be 4.92 min and 8.32 for Lafutidine and Domperidone respectively in this method. The two marketed formulations (Lafukem– D and Lafutax–D) were analyzed by this method and the mean % assay for Lafutidine and Domperidone in Lafutax – D was found to be 100.7 % and 101.1% respectively. The mean % assay for Lafutidine and Domperidone in Lafukem –D was found to be 99.1 % and 102.0% respectively. The method was further validated as per ICH guidelines. The method was specific for the analysis of the drugs as there was no interference at retention time of Lafutidine and Domperidone. Precision of the method was evaluated in terms of system precision (% RSD 1.622% and 1.141% for Lafutidine & Domeperidone respectively), method precision (% RSD 0.45 % and 0.48 % for Lafutidine & Domeperidone respectively). The % RSD in intraday precision for Lafutidine (3, 6, 9 µg/ml) was found to be 0.419, 1.39, 1.34% and
for Domperidone (9, 18, 27 µg/ml) was found to be 0.645, 1.56, 0.204% respectively. In interday precision % RSD for Lafutidine (3, 6, 9 µg/ml) was found to be 0.77, 1.12, 1.17% and for Domperidone (9, 18, 27 µg/ml) was found to be 0.406, 1.57, 0.139% respectively. All the % RSD in precision study was found to well within the acceptable limit (not more than 2). The accuracy of the assay method was evaluated by recovery study in triplicate at 100 % level and the mean % recovery was found to be 99.46 % & 99.99% for Lafutidine and Domperidone respectively. The method was found linear in the concentration range of 3 – 9 µg/ml for Lafutidine ($r^2= 0.999$) and 9 – 27 µg/ml for Domeperidone ($r^2= 0.999$). No significant difference was found in the % Assay of both drugs before and after storing for 24 hrs in refrigerator and room temperature. This confirms the stability of the drugs in solutions. The LOD and LOQ were found to be 0.151µg/ml and 0.458µg/ml for Lafutidine and 0.661µg/ml and 2.01µg/ml respectively for Domperidone. Robustness of the method was evaluated by making deliberate changes in the flow rate and % RSD for the system suitability parameters was found within the acceptance criteria. Stability indicating capacity of assay method evaluated by subjecting the sample to various forced degradation conditions and subsequent recording their chromatograms. Lafutidine showed 16.4%, 16.3%, 17.5%, 16.6%, 17.5% & 17.4 %, 16.1%, 15.9% degradation and Domperidone showed 18%, 17.9%, 18%, 18.5%, 17.5% ,16.4%, 16.6%, 14.7% degradation in acidic, alkaline, peroxide, reduction, thermal, photolytic, humidity and water hydrolysis conditions respectively. The homogeneity of the analytes peak purity plots confirms the stability indicating capacity of the developed method.

Though the method I was stability indicating assay method for the estimation of Lafutidine and Domperidone and satisfactory in separating the main analytes without any interference of degradation products but it could not show any peak for degradation products in chromatograms. Therefore with the objective to separate degradation products from the main analytes a method II was developed. In method II separation was carried out by using gradient mode (Inertsil C18 250 x 4.6 mm, 5µm, flow rate of 1ml/ min, 217 nm using PDA detector). The run time of the method was 60 minutes, the retention times were found to be 19.9 min and 36.4 min for Lafutidine and Domperidone respectively. The second method showed presence of additional peaks along with analytes peaks in the chromatogram which proves the separation ability of this method. The two marketed formulations (Lafukem– D and Lafutax–D) were
analyzed by this method and the mean % assay for Lafutidine and Domperidone in Lafutax – D was found to be 99.33 % and 100.27% respectively. The mean % assay for Lafutidine and Domperidone in Lafü kem–D was found to be 100.6 % and 99.94 % respectively. Further the method was validated as per ICH guidelines. The method was specific for the analysis of the drugs as there was no interference at retention time of Lafutidine and Domperidone. Precision of the method was evaluated in terms of system precision (% RSD 1.046 % and 0.567 % for Lafutidine & Domeperidone respectively), method precision (% RSD 0.64 % and 0.67 % for Lafutidine & Domeperidone respectively). The % RSD in intraday precision for Lafutidine (7.5, 10, 12.5 µg/ml) was found to be 0.14, 0.48, 0.47% and for Domperidone (22.5, 30, 37.5 µg/ml) was found to be 0.06, 0.43, 0.02% respectively. In interday precision % RSD for Lafutidine (7.5, 10, 12.5 µg/ml) was found to be 0.26, 0.451, 0.49% and for Domperidone (22.5, 30, 37.5 µg/ml) was found to be 0.24, 0.09, 0.09 % respectively. All % RSD in precision study was found to well within the acceptable limit (not more than 2). The accuracy of the assay method was evaluated by recovery study at 80%, 100, 120% levels in triplicates and the mean % recovery was found to be 99.27 % & 99.43% at 80 % level, 99.30 % & 99.47% at 80 % level and 99.27% & 98.69% at 120 % level for Lafutidine and Domperidone respectively. The method was found linear in the concentration range of 0.1–15 µg/ml for Lafutidine ($r^2=0.9998$) and 0.3 – 45 µg/ml for Domeperidone ($r^2=0.9986$). No significant difference was found in the % Assay of both drugs before and after storing for 24 hrs in refrigerator and room temperature. This confirms the stability of the drugs in solutions. The LOD and LOQ were found to be 0.2952µg/ml and 0.849 µg/ml for Lafutidine and 0.146 µg/ml and 0.589 µg/ml respectively for Domperidone. Robustness of the method was evaluated by making deliberate changes in the flow rate and in column temperature and % RSD for the system suitability parameters was found within the acceptance criteria. Stability indicating capacity of assay method was evaluated by subjecting the standard drug alone, mixture of standard drugs and their formulation to the stress conditions. The degradant products in individual drug, standard mixture and formulation were compared and it was found that degradation product in formulation were same as that in individual drugs. From the same retention time it is clear that same degradation products are formed in the formulation and in pure drugs under identical stress conditions. Lafutidine showed 23.31 %, 21.26%, 24.62%, 4.9 %, 3.29 %, 22.28 %, 2.59 %, 2.0 %
degradation and Domperidone showed 23.72 %, 19.77 %, 20.25 %, 27.53 %, 1.14 %, 8.96 %, 0.87 %, 0.60 %, degradation in acidic, alkaline, peroxide, reduction, thermal, photolytic, humidity and water hydrolysis conditions respectively. The homogeneity of the analytes peak purity plots confirms the stability indicating capacity of the developed method.

Hence it can be concluded that two accurate, precise and robust stability indicating assay methods for the estimation of Lafutidine and Domperidone in their formulations were developed. In addition, method II may be applied for the estimation of related substances along with main analytes.

7.2 Stability Indicating HPLC Method Development and Validation for the Simultaneous Determination of Darunavir Ethanolate and Ritonavir in its Dosage Forms. [Method I and II]

Two simple, sensitive, precise and accurate RP–HPLC methods were developed for the quantitative estimation of Darunavir ethanolate and Ritonavir in bulk and their formulations without any interference from the excipients and degradents. Method I was developed using Buffer (0.1% v/v OPA in water): acetonitrile in the ratio of 70:30 in isocratic mode (Zodiac C18 column, flow rate of 1 ml/ min, 208 nm using PDA detector). With run time of 8 minutes, the retention times were found to be 2.71 min and 5.35 for Darunavir ethanolate and Ritonavir respectively in this method. In house bilayer tablet formulation was developed (Darunavir ethanolate 400 mg + Ritonavir 50 mg) and analyzed by this method. The mean % assay for Darunavir ethanolate and Ritonavir in developed formulation was found to be 100.9 % & 99.2 % respectively.

The method was further validated as per ICH guidelines. The method was specific for the analysis of the drugs as there was no interference at retention time of Darunavir ethanolate and Ritonavir. Precision of the method was evaluated in terms of system precision (% RSD 1.347 % and 0.983 % for Darunavir ethanolate and Ritonavir respectively), method precision (% RSD 0.18 % and 0.76 % for Darunavir ethanolate and Ritonavir respectively). The % RSD in intraday precision for Darunavir ethanolate (320,400, 480 µg/ml) was found to be 0.08, 0.46, 0.06 % and for Ritonavir (40, 50, 60 µg/ml) was found to be 1.12, 1.24, 1.26 % respectively. In interday precision % RSD for Darunavir ethanolate (320,400, 480 µg/ml) was found to be 0.17, 0.02, 0.10 % and for Ritonavir (40, 50, 60 µg/ml) was found to be 0.32, 0.52, 0.44 % respectively. All %
RSD in precision study was found to well within the acceptable limit (not more than 2). The accuracy of the assay method was evaluated by recovery study at 50%, 100, 150% levels in triplicates and the mean % recovery was found to be was found to be 98.88 % & 99.60% at 50 % level, 99.24 % & 100.82% at 100 % level, 99.23 % & 100.30 at 150 % level for Darunavir ethanolate and Ritonavir respectively. The method was found linear in the concentration range of 200 – 600 µg/ml for Darunavir ($r^2= 0.999$) and 25 – 75 µg/ml for Ritonavir ($r^2= 0.999$). No significant difference was found in the % Assay of both drugs before and after storing for 24 hrs in refrigerator and room temperature. This confirms the stability of the drugs in solutions. The LOD and LOQ were found to be 0.556µg/ml and 1.68µg/ml for Darunavir and 0.00081µg/ml and 0.00247 µg/ml respectively for Ritonavir. Robustness of the method was evaluated by making deliberate changes in the flow rate and % RSD for the system suitability parameters was found within the acceptance criteria. Stability indicating capacity of assay method evaluated by subjecting the sample to various forced degradation conditions and subsequent recording their chromatograms. In forced degradation study Darunavir showed around 20.0 %, 21.9 %, 22.0 % 24.2 %, 25 %, 24.2 %, 22.2 %, 21.1 % degradation and Ritonavir showed 21.4 %, 23.9 %, 22.62% and 21.6 %, 21.5 %, 24.4 %, 23.9 %, 24.9 % degradation in acidic, alkaline, peroxide, reduction, thermal, photolytic, humidity and water hydrolysis conditions respectively. The homogeneity of the analytes peak purity plots confirms the stability indicating capacity of the developed method.

Though the first method was stability indicating assay method for the estimation of Darunavir ethanolate and Ritonavir and satisfactory in separating the main analytes without any interference of degradation products but could not show any degradation products in chromatograms. Therefore with the objective to separate degradation products from the main analytes a second method was developed. In second method separation was carried out by using gradient mode (Zodiac C18 column, flow rate of 1ml/ min, 208 nm using PDA detector). The run time of the method was 60 minutes, the retention times were found to be 25.8 min and 41.6 min for Darunavir ethanolate and Ritonavir respectively. The second method showed presence of additional peaks along with analytes peaks in the chromatogram which proves the separation ability of this method. The mean % assay for Darunavir ethanolate and Ritonavir in developed formulation was found to be 99.9 % & 99.6 % respectively. Further the method was
validated as per ICH guidelines. The method was specific for the analysis of the drugs as there was no interference at retention time of Darunavir ethanolate and Ritonavir. The homogeneity of the peaks confirms that the method is specific for their determination. Precision of the method was evaluated in terms of system precision (% RSD 0.002 % and 0.011 % for Darunavir ethanolate and Ritonavir respectively), method precision (% RSD 0.65 % and 0.77 % for Darunavir ethanolate and Ritonavir respectively). The % RSD in intraday precision for Darunavir ethanolate (640, 800, 960 µg/ml) was found to be 0.037, 0.008, 0.004 % and for Ritonavir (80, 100, 120 µg/ml) was found to be 0.03, 0.03, 0.02 % respectively. In interday precision % RSD for Darunavir ethanolate (640, 800, 960 µg/ml) was found to be 0.017, 0.002, 0.0026% and for Ritonavir (80, 100, 120 µg/ml) was found to be 0.03, 0.08, 0.31% respectively. All % RSD in precision study was found to be well within the acceptable limit (not more than 2). The accuracy of the assay method was evaluated by recovery study at 50%, 100, 150% levels in triplicates and the mean % recovery was found to be 98.88 % & 99.4% at 50 % level, 99.70 % & 100.6 % at 100 % level and 100.0 % & 99.7 % at 150 % level for Darunavir ethanolate and Ritonavir respectively. The method was found linear in the concentration range of 400 – 1200 µg/ml for Darunavir (r²= 1.00) and 50 – 150 µg/ml for Ritonavir (r²= 0.9999). No significant difference was found in the % Assay of both drugs before and after storing for 24 hrs in refrigerator and room temperature. This confirms the stability of the drugs in solutions. The LOD and LOQ were found to be 0.8 µg/ml and 2.3 µg/ml for Darunavir and 0.2 µg/ml and 0.5 µg/ml respectively for Ritonavir. Robustness of the method was evaluated by making deliberate changes in the flow rate and % RSD for the system suitability parameters was found within the acceptance criteria. Stability indicating capacity of assay method was evaluated by subjecting the standard drug alone, mixture of standard drugs and their formulation to the stress conditions. The degradant products in individual drug, standard mixture and formulation were compared and it was found that degradation product in formulation were same as that in individual drugs. From the same retention time it is clear that same degradation products are formed in the formulation and in pure drugs under identical stress conditions. In forced degradation study Darunavir showed around 25.73 %, 26.92 %, 19.23 %, 11.44 %, 19.69%, 0.3 %, 22.53 % and 19.59 % degradation and Ritonavir showed 24.67 %, 20.59 %, 21.80%, 23.58 %, 24.36 %, 6.92 %, 23.77 % 20.03 % degradation in acidic, alkaline, peroxide, reduction, thermal, photolytic, humidity and
water hydrolysis conditions respectively. The homogeneity of the analytes peak purity plots confirms the stability indicating capacity of the developed method.

Hence it can be concluded that two accurate, precise, sensitive, reproducible and robust stability indicating assay methods for the estimation of Darunavir ethanolate and Ritonavir in their formulations were developed. In addition, while optimization of chromatographic conditions for method II it was observed that the method II also have the capacity to show the peaks of related substances present in it. Therefore it can be concluded that the method may be applied for the estimation of related substances along with main analytes.

7.3 Stability Indicating HPLC Method Development and Validation for the Simultaneous Determination of Azithromycin and Ofloxacin in its Dosage Forms

A simple, sensitive, precise, accurate and reproducible RP–HPLC method was developed for the quantitative estimation of Azithromycin and Ofloxacin in bulk and their formulations without any interference from the excipients and degradant products. Method was developed using buffer (0.02 M potassium dihydrogenphosphate): methanol: acetonitrile (pH 3.2 adjusted with orthophosphoric acid) in the ratio of 65:25:10 in isocratic mode (Hypersil keystone RP C18 column, flow rate of 1.2 ml/min, 285 nm). With run time of 25 minutes, the retention times were found to be 5.01 min and 9.73 min for Ofloxacin and Azithromycin respectively in this method. The marketed tablet formulation (Zithium–O) was analyzed by this method and the mean % assay for Azithromycin and Ofloxacin in Zithium –O was found to be 99.74% and 99.02% respectively. Validation of the developed was carried out as per ICH guidelines. The method was specific for the analysis of the drugs as there was no interference at retention time of Azithromycin and Ofloxacin. Precision of the method was evaluated in terms of system precision (% RSD 0.263 and 0.92 % for Azithromycin and Ofloxacin respectively), method precision (% RSD 0.794 % and 0.856 % for Azithromycin and Ofloxacin respectively). The % RSD for Azithromycin (10, 20, 30 µg/ml) and Ofloxacin (8, 16, 24 µg/ml) was found to be 0435, 0.86, 1.02, 0.507, 0.97 & 0.40 respectively in intraday study and 0.349, 0.4, 0.62, 0.1, 0.518 and 0.308 respectively in interday study. All % RSD in precision study was found to well within the acceptable limit (not more than 2). The accuracy study of the developed assay method was performed by the recovery of the added standards of Azithromycin and
Ofloxacin at three different levels that are 80%, 100%, 120% levels of the labeled claim in triplicates and the mean % recovery was found to be 98.81 % & 99.73% at 80 % level, 99.89% & 99.73% at 100 % level, 99.85 % & 99.86 at 120 % level for Azithromycin and Ofloxacin respectively. All % RSD in recovery study was found to well within the acceptable limit (not more than 2% and % assay deviation not more than 2%). The method was found linear in the concentration range of 4 – 40 μg/ml of Ofloxacin ($r^2=0.997$) and 5 – 50 μg/ml of Azithromycin ($r^2=0.998$). No significant difference was found in the % Assay of both drugs before and after storing for 24 hrs in refrigerator and room temperature. This confirms the stability of the drugs in solutions. The LOD and LOQ were found to be 0.00062μg/ml and 0.00185μg/ml for Azithromycin and 0.00636μg/ml and 0.00210μg/ml respectively for Ofloxacin. Robustness of the method was evaluated by making small but deliberate changes in the pH of the mobile phase, temperature of the column and flow rate. In these conditions % RSD for the system suitability parameters was found in the range of 0.0006 – 1.08 % which are well within the acceptance criteria(% RSD Should not be more than 2%). This indicates the robustness of the developed method. The % assay for both Azithromycin and Ofloxacin was calculated at same level in triplicate by the different analyst to prove the ruggedness of the developed method. The average % assay for Azithromycin was found to be 99.76 % and for Ofloxacin was 99.60 % with % RSD 0.234 and 0.268 % respectively. % RSD was found to be well within acceptable limit. Stability indicating capacity of assay method was evaluated by exposing the standard drug alone, mixture of standard drugs and their formulation to the various stress conditions. The degradant products in individual drug, standard mixture and formulation were compared and it was found that degradation product in formulation were same as that in individual drugs. From the same retention time it is clear that same degradation products are formed in the formulation and in pure drugs under identical stress conditions. In forced degradation study Azithromycin was completely degraded in 1N HCl and four degradants products were appeared at 0.87(68.88%), 1.91(5.32%), 2.4(6.78%) and 3.7 min (18.90%), while Azithromycin did not show any degradation when exposed to the stress conditions such as 0.1 N NaOH, 30% hydrogen peroxide solution, 10% sodium bisulphate and photolytic condition, temperature. This indicates that Azithromycin is sensitive to the acid hydrolysis. On the other hand, Ofloxacin showed 50.01 % degradation in acidic condition where degradant product appeared at
around 4.34 min. In alkaline degradation Ofloxacin showed one degradation product peak at 4.23 min and % degradation was found to be 25.01 %. Ofloxacin showed 19.82 % and 16.4% degradation in peroxide & photolytic degradation respectively, but the peak of degradant product was not detected by the detector. The degradation can be confirmed from the reduced % assay (80.41% in peroxide & 83.81% w/w in photolytic degradation). Ofloxacin did not show any degradation in sodium bisulphate. In thermal degradation study Ofloxacin showed 29.13 % degradation which appeared at around 4.19 min. The homogeneity of the analytes peak purity plots confirms the stability indicating capacity of the developed method.

Hence it can be concluded that a simple, accurate, precise, sensitive, reproducible and robust stability indicating assay methods for the estimation of Azithromycin and Ofloxacin in their formulations were developed.

7.4 Stability Indicating HPLC Method Development and Validation for the Simultaneous Determination of Cefpodoxime Proxetil & Ofloxacin in its Dosage Forms

A simple, sensitive, precise, accurate, robust and reproducible RP‒HPLC method was developed for the quantitative estimation of Cefpodoxime Proxetil and Ofloxacin in bulk and their formulations without any interference from the excipients and degradants. Method was developed using the mobile phase consisting of buffer (20 mM potassium dihydrogenphosphate): methanol: acetonitrile (pH 3.0 adjusted with orthophosphoric acid) in the ratio of 50:30:20 in isocratic mode (Hypersil keystone RP C18 column, flow rate of 1.2 ml/ min, 235 nm). With run time of 25 min Cefpodoxime Proxetil showed two peaks at 13.11 and 14.12 min which are due to R and S isomers respectively present in the recimic mixture and Ofloxacin was showed a peak at 5.01 min. The marketed tablet formulation (Zedocef –O) was analyzed by this method and the mean % assay for Cefpodoxime Proxetil and Ofloxacin in Zedocef –O was found to be 99.8 % & 99.2 % respectively. The % RSD for Cefpodoxime Proxetil and Ofloxacin was found to be 0.0708 & 0.596 respectively which was found well within the acceptable limit. Validation of the developed was carried out as per ICH guidelines. The method was specific for the analysis of the drugs as there was no interference at retention time of Cefpodoxime Proxetil and Ofloxacin. Precision of the method was evaluated in terms of system precision (% RSD 0.3351% and 1.1005 % for
Cefpodoxime Proxetil and Ofloxacin respectively), method precision (% RSD 1.33% and 0.64 % for Cefpodoxime Proxetil and Ofloxacin). The % RSD for Cefpodoxime Proxetil and Ofloxacin (6, 8, 10 µg/ml for both drugs) was found to be 0.552, 0.93, 0.83 & 0.508, 0.88, 0.40 respectively in intraday study and 0.671, 1.10, 0.81, 0.1, 0.74 and 1.0 respectively in interday study. All % RSD in precision study was found to well within the acceptable limit (not more than 2). The accuracy study of the developed assay method was performed by the recovery of the added standards of Cefpodoxime Proxetil and Ofloxacin at three different levels that are 80%, 100%, 120% levels of the labeled claim in triplicates. The mean % recovery was found to be 99.58 % & 99.64 % at 80 % level, 99.75% & 99.53% at 100 % level, 99.78 % & 99.78 at 120 % level for Cefpodoxime Proxetil and Ofloxacin respectively. All % RSD in recovery study was found to well within the acceptable limit (not more than 2% and % assay deviation not more than 2%). The method was found linear in the concentration range of 4 – 20 µg/ml of Cefpodoxime Proxetil ($r^2 = 0.999$) and 10–50 µg/ml of Ofloxacin ($r^2 = 0.997$). No significant difference was found in the % Assay of both drugs before and after storing for 24 hrs in refrigerator and room temperature. This confirms the stability of the drugs in solutions. The LOD and LOQ were found to be 0.00085µg/ml and 0.000282µg/ml for Cefpodoxime Proxetil and 0.00635µg/ml and 0.00209µg/ml respectively for Ofloxacin. Robustness of the method was evaluated by making small but deliberate changes in the pH of the mobile phase, temperature of the column and flow rate. In these conditions % RSD for the system suitability parameters was found in the range of 0.0006 – 0.74 % which are well within the acceptance criteria (% RSD Should not be more than 2%). This indicates the robustness of the developed method. The % assay for both Cefpodoxime Proxetil and Ofloxacin was calculated at same level in triplicate by the different analyst to prove the ruggedness of the developed method. The average % assay for Cefpodoxime Proxetil was found to be 99.63% and for Ofloxacin was 99.43% with % RSD 0.581 and 0.46 % respectively. % RSD was found to be well within acceptable limit.

Stability indicating capacity and specificity of assay method was evaluated by exposing the standard drug alone, mixture of standard drugs and their formulation to the various stress conditions. The degradant products in individual drug, standard mixture and formulation were compared and it was found that degradation product in formulation were same as that in individual drugs. From the same retention time it is clear that same
Conclusion

Degradation products are formed in the formulation and in pure drugs under identical stress conditions. In forced degradation study Cefpodoxime Proxetil showed around 91.36% degradation in 0.1N HCl and 91.62% in 0.1 NaOH. While Cefpodoxime Proxetil did not show any degradation when exposed the stress conditions such as hydrogen peroxide (30% hydrogen peroxide) solution, 10% sodium bisulphate and photolytic condition. In thermal degradation study Cefpodoxime Proxetil showed 24.67% degradation. On other hand, Ofloxacin showed 50.02%, 25.02% degradation in 0.1N HCl and 0.1 N NaOH. Ofloxacin also showed 19.80%, 26.82% and 16.44% degradation when treated with 30% H₂O₂, thermal and in photolytic degradation condition. Ofloxacin did not show any degradation in sodium bisulphate. The homogeneity of the analytes peak purity plots confirms the stability indicating capacity of the developed method.

Hence it can be concluded that a simple, accurate, precise, sensitive, reproducible and robust stability indicating assay methods for the estimation of Cefpodoxime Proxetil and Ofloxacin in their formulations were developed and validated.

7.5 Stability Indicating HPLC Method Development and Validation for the Simultaneous Determination of Cefpodoxime Proxetil & Levofloxacin in its Dosage Forms

A simple, sensitive, precise, accurate, robust and reproducible RP–HPLC method was developed for the quantitative estimation of Cefpodoxime Proxetil and Levofloxacin in bulk and their formulations without any interference from the excipients and degradation products. Method was developed using the mobile phase consisting of buffer (10 mM potassium dihydrogenphosphate): methanol: acetonitrile (pH 3.2 adjusted with orthophosphoric acid) in the ratio of 60:30:10 in isocratic mode (Hypersil keystone RP C18 column, flow rate of 1.2 ml/ min, 230 nm). With run time of 25 min Cefpodoxime Proxetil showed two peaks at 13.21 and 14.20 min which are due to R and S isomers respectively present in the recimic mixture and Levofloxacin was showed a peak at 4.91 min. The marketed tablet formulation (Glevopod) was analyzed by this method and the mean % assay for Cefpodoxime Proxetil and Levofloxacin in Glevopod was found to be 99.82% & 99.28% respectively. The % RSD for Cefpodoxime Proxetil and Levofloxacin was found to be 0.0836 & 0.6101% respectively which was found well within the acceptable limit. Validation of the developed was carried out as per ICH
guidelines. The method was specific for the analysis of the drugs as there was no interference at retention time of Cefpodoxime Proxetil and Levofloxacin. Precision of the method was evaluated in terms of system precision (% RSD 0.3114% and 1.372% for Cefpodoxime Proxetil and Levofloxacin respectively), method precision (% RSD 1.098% and 0.805% for Cefpodoxime Proxetil and Levofloxacin). The % RSD for Cefpodoxime Proxetil (7.5, 10, 12 µg/ml) and Levofloxacin (10, 12.5, 15µg/ml) was found to be 0.923%, 0.490%, 0.626% & 0.744%, 0.542%, 0.711% respectively in intraday study and 0.806%, 0.630%, 0.354%, 0.703%, 0.153% and 0.629% respectively in interday study. All % RSD in precision study was found to well within the acceptable limit (not more than 2). The accuracy study of the developed assay method was performed by the recovery of the added standards of Cefpodoxime Proxetil and Levofloxacin at three different levels that are 80%, 100%, 120% levels of the labeled claim in triplicates. The mean % recovery was found to be 99.67% & 99.69% at 80% level, 99.65% & 99.75% at 100% level, 99.69% & 99.65% at 120% level for Cefpodoxime Proxetil and Levofloxacin respectively. All % RSD in recovery study was found well within the acceptable limit (not more than 2% and % assay deviation not more than 2%). The method was found linear in the concentration range of 2 – 24 µg/ml of Cefpodoxime Proxetil ($r^2 = 0.999$) and 2.5–30 µg/ml of Levofloxacin ($r^2 = 0.999$). No significant difference was found in the % Assay of both drugs before and after storing for 24 hrs in refrigerator and room temperature. This confirms the stability of the drugs in solutions. The LOD and LOQ were found to be 0.0064 µg/ml and 0.00211 µg/ml respectively for Cefpodoxime Proxetil and 0.0011µg/ml and 0.0003µg/ml respectively for Levofloxacin. Robustness of the method was evaluated by making small but deliberate changes in the pH of the mobile phase, temperature of the column and flow rate. In these conditions % RSD for the system suitability parameters was found in the range of 0.0013 – 1.90% which are well within the acceptance criteria (% RSD Should not be more than 2%). This indicates the robustness of the developed method. The % assay for both Cefpodoxime Proxetil and Levofloxacin was calculated at same level in triplicate by the different analyst to prove the ruggedness of the developed method. The average % assay for Cefpodoxime Proxetil was found to be 99.81% and for Levofloxacin was 99.39% with % RSD 0.282 and 0.329% respectively. % RSD was found to be well within acceptable limit.
Stability indicating capacity and specificity of assay method was evaluated by exposing the standard drug alone, mixture of standard drugs and their formulation to the various stress conditions. The degradant products in individual drug, standard mixture and formulation were compared and it was found that degradation product in formulation were same as that in individual drugs. From the same retention time it is clear that same degradation products are formed in the formulation and in pure drugs under identical stress conditions. In forced degradation study Cefpodoxime Proxetil with respect to control showed around 91.19% degradation in 0.1N HCl and 91.45 % in 0.1 NaOH. While Cefpodoxime Proxetil did not show any degradation when exposed the stress conditions such as hydrogen peroxide (30% hydrogen peroxide) solution, 10% sodium bisulphate and photolytic condition. In thermal degradation study Cefpodoxime Proxetil showed 24.57 % degradation. On other hand, Levofloxacin with respect to control showed 49.02 %, 24.35% degradation in 0.1N HCl and 0.1 N NaOH. Levofloxacin also showed 19.71 %, 29.75 % and 19.35 % degradation when treated with 30% H2O2, kept in oven at 60 ºC for 24 hrs and in photolytic condition. Levofloxacin did not show any degradation when treated with sodium bisulphate. The homogeneity of the analytes peak purity plots confirms the stability indicating capacity of the developed method.

Hence it can be concluded that a simple, accurate, precise, sensitive, reproducible and robust stability indicating assay methods for the estimation of Cefpodoxime Proxetil and Levofloxacin in their formulations were developed and validated.