In the present work, forced degradation study has been performed for the development of stability indicating assay method and for the identification and quantification of degradation products, to propose degradation pathways of degradation products. Degradation kinetics was performed and major degradation products were isolated. The present research work is summarized as:

## *Chapter 3: Development of Stability Indicating Assay Method of Clevidipine Butyrate*

***Section A**:* Stability indicating HPLC method was developed for determination of Clevidipine. Stress degradation was performed as per the guidelines prescribed by ICH. The degradation conditions were : 1 M HCl, 60ºC ,1 hr, for acid hydrolysis ; 0.01 M NaOH, 60ºC ,1 hr, for base hydrolysis; 3% $H_2O_2$, RT , 1 hr, for oxidative degradation ; 5382 Lux and 144UW/cm2,11 days for photolytic degradation; 80ºC 11days for thermal degradation . Four degradation products DP1, DP2, DP3 and DP4 were observed at Rt of 2.45, 6.74, 11.44 and 4.10 min respectively. Significant degradation was observed in alkaline condition while slight degradation was observed in acidic, oxidative and photolytic condition.

Separation of degradation products from Clevidipine was achieved on Thermo Hypersil BDS C-18 (250 x 4.6, 5 µ) column in an isocratic mode. Mobile phase composed of phosphate buffer 10 mM (pH 3.0) and acetonitrile in the ratio of 50: 50. The detection was performed at 239nm. The flow rate was kept at 1mL/min. The retention time of Clevidipine was found to be 10.65 min. The developed method was validated as per ICH guidelines. The method was linear in the concentration range of 20-120 µg/mL. LOD and LOQ were found to be 0.65 and 1.97 µg/mL. The percentage recoveries of Clevidipine in synthetic mixture were found to be in the range of 99.68-99.83%.

***Section B:*** Stability indicating HPTLC method was developed for determination of Clevidipine . Stress degradation was performed as per the guidelines prescribed by ICH. The degradation conditions were : 1 M HCl, 60ºC ,1 hr, for acid hydrolysis ; 0.01 M NaOH, 60ºC ,1 hr, for base hydrolysis; 3% $H_2O_2$, RT , 1 hr, for oxidative degradation ; 5382 Lux and 144UW/cm2,11 days for photolytic degradation; 80ºC 11days for thermal degradation .

Separation of degradation products from Clevidipine was achieved on pre-coated silica gel aluminum plate 60 $F_{254}$ (20 x 20 cm), 100µm thickness. Mobile phase used was toluene and ethyl acetate in the ratio of 8:2. Chromatographic chamber was saturated with mobile phase for 20 minutes. Development distance was 80 mm. Detection was performed at 370 nm. $R_f$ of Clevidipine was 0.49. Three degradation products were observed at $R_f$ of 0.01 (DP1) , 0.02 (DP2), 0.27 (DP3) respectively. Significant degradation was observed in alkaline condition while slight degradation was observed in acidic, oxidative and photolytic condition. The developed method was validated as per ICH guidelines. The developed method was linear in the concentration range of 1000-6000 ng/band. LOD and LOQ were found to be 403.22 and 1222.49 ng/band. The percentage recoveries of Clevidipine in synthetic mixture were found to be in the range of 99.03-99.57%.

### *Chapter 4: Development of Stability Indicating Assay Method of Acotiamide HCl Trihydrate*

***Section A**:* Stability indicating HPLC method was developed for determination of Acotiamide. Stress degradation was performed as per the guidelines prescribed by ICH. The degradation conditions were : 1 M HCl, 100ºC ,3 hrs, for acid hydrolysis ; 0.5 M NaOH, 100ºC ,3 hrs, for base hydrolysis; 6% $H_2O_2$, RT , 48 hrs, for oxidative degradation ; 5382 Lux and 144UW/cm2,11 days for photolytic degradation; 80ºC 11days for thermal degradation . Three degradation products DP1, DP2 and DP3 were observed at Rt of 3.59, 4.07, 5.12 respectively. Significant degradation was observed in alkaline condition while slight degradation was observed in acidic condition.

Separation of degradation products from Acotiamide was achieved on Thermo Hypersil BDS C-8 (250 x 4.6, 5 µ) column in an isocratic mode. Mobile phase composed of 0.1% Triethylamine in 0.2% formic acid (pH 3.0) and acetonitrile in the ratio of 70: 30. The detection was performed at 282 nm with column oven temperature of 40ºC. The flow rate was kept at 1mL/min. The retention time of Acotiamide was found to be 9.38 min. The developed method was validated as per ICH guidelines. The developed method was linear in the concentration range of 5-30 µg/mL. LOD and LOQ were found to be 0.36 and 1.13 µg/mL. The percentage recoveries of Acotiamide in formulation were found to be in the range of 98.92-99.35%.

***Section B***: The objective of this section was to isolate and characterize major degradation products of Acotiamide. Major degradation products DP2 and DP3 formed during alkaline hydrolysis were isolated by preparative HPLC. Isolation of degradation products from Acotiamide was performed on Phenomenex Luna C-8(2) column with 250x50 mm, 100 Å, 10µ in diameter. Detection was performed at 282 nm. Flow rate was maintained at 60mL/min. Samples were injected through Rheodyne 7725 injector valve. Mobile phase comprised of 0.1% triethylamine with 0.2% formic acid: acetonitrile in gradient mode. Degradation products DP2and DP3 were characterized by mass, NMR and IR techniques. Based on the spectral data DP2 was characterised as 2-hydroxy-4, 5-dimethoxy benzoic acid and DP3 as 2-[(2-hydroxy-4, 5-dimethoxybenzoyl) amino]-1, 3-thiazole-4-carboxylic acid.

***Section C*****:** Forced degradation study of Acotiamide was carried by LC-PDA and degradation products were identified by LC-MS. LC-PDA showed total 3 degradation products. One degradation product (DP3) was formed both in acidic and alkaline condition while two degradation products (DP1and DP2) were formed in alkaline conditions. Among 3 degradation products, 2 were isolated and characterized as above. Remaining degradation product DP1 was characterized by LC-MS and its fragmentation pathway was proposed. Based on the structures of degradation products, degradation pathway was proposed.

### *Chapter 5: Development of Stability Indicating Assay Method of Fimasartan Potassium*

***Section A:*** Stability indicating HPLC method was developed for determination of Fimasartan. Stress degradation was performed as per the guidelines prescribed by ICH. The degradation conditions were : 1 M HCl, 100ºC ,3 hrs, for acid hydrolysis ; 1 M NaOH, 100ºC ,3 hrs, for base hydrolysis; 0.9% $H_2O_2$, RT , 30 min , for oxidative degradation ; 5382 Lux and 144UW/cm$^2$,11 days for photolytic degradation; 80ºC 11days for thermal degradation Five degradation products DP1, DP2, DP3 , DP4 and DP5 were observed at Rt of 3.1, 3.9, 4.2,4.8 and 6.1 min respectively. Significant degradation was observed in oxidative condition while slight degradation was observed in acidic condition, photolytic condition.

Separation of degradation products from Fimasartan was achieved on Thermo Hypersil BDS C-18 (250 x 4.6, 5 µ) column in an isocratic mode. Mobile phase composed of 10mM phosphate buffer (pH 3.0) and acetonitrile in the ratio of 50: 50. The detection was performed at 262 nm. The flow rate was kept at 1mL/min. The retention time of Fimasartan was found to be 7.30 min. The developed method was validated as per ICH guidelines. The developed method was linear in the concentration range of 5-30 µg/mL. LOD and LOQ were found to be 1.54 and 4.67 µg/mL. The percentage recoveries of Fimasartan in synthetic mixture were found to be in the range of 98.75-100.80%.

***Section B:*** Degradation kinetics of Fimasartan was studied by HPLC under oxidative degradation. On the basis of degradation kinetics study, oxidative degradation followed first order kinetics. Based on this, half-life was calculated .Activation energy was found to be 16.71 KJ/mole.

***Section C*****:** Major degradation product DP4 in oxidative degradation was isolated by preparative HPLC. Isolation of degradation product from Fimasartan was performed on Daisogel-SP-100-10-ODS-P column with 250x50 mm, 100 Å, 10µ in diameter. Detection was performed at 262 nm. Flow rate was maintained at 55 mL/min. Samples were injected through Rheodyne 7725 injector valve. Mobile phase comprised of 0.1% formic acid: acetonitrile in the ratio of 45: 55. Degradation products DP4 was characterized by mass, NMR and IR techniques and mechanism of DP4 was proposed. Based on the spectral data, DP4 was characterised as 2-(1-((2’-(1H-tetrazol-5-yl)-[1,1’-biphenyl]-4-yl) methyl)-2-butyl-4-methyl-6-oxo-1,6-dihydropyrimidin-5-yl)-N,N-dimethylacetamide.

***Section D*****:** Forced degradation study of Fimasartan was carried by LC-PDA and degradation products were identified by LC-MS. LC-PDA showed total 5 degradation products. Two degradation products (DP2 and DP4) were formed both in alkaline and oxidative conditions while three degradation products (DP1, DP3 and DP5) were formed in alkaline conditions. Among 5 degradation products, one degradation product DP4 was isolated and characterized as above. Remaining degradation products were characterized on the basis of molecular mass. Degradation pathways in alkaline and oxidative condition were proposed.

***Chapter 6: Development of Stability Indicating Assay Method of Anagliptin***

***Section A:*** Stability indicating HPLC method was developed for determination of Anagliptin. Stress degradation was performed as per the guidelines prescribed by ICH. The degradation conditions were : 1 M HCl, 80ºC ,6 hrs, for acid hydrolysis ; 0.1 M NaOH, RT ,1 hr, for base hydrolysis; neutral hydrolysis ,80ºC,6 hrs; 0.3% $H_2O_2$, RT , 2 hrs, for oxidative degradation ; 5382 Lux and 144UW/cm2,11 days for photolytic degradation; 80ºC 11days for thermal degradation . Twelve different degradation products were observed under various stress conditions. Significant degradation was observed in alkaline and oxidative condition while slight degradation was observed in acidic condition, neutral hydrolytic, thermal and photolytic condition respectively.

Separation of degradation products from Anagliptin was achieved on Waters Symmetry C18 (150 x 4.6, 3.5 µ) column in gradient mode. Mobile phase composed of 10mM ammonium acetate buffer (pH 5.0), methanol and acetonitrile. The detection was performed at 247 nm with column oven temperature of 40ºC. The flow rate was kept at 1mL/min. The retention time of Anagliptin was found to be 22.43 min. The developed method was validated as per ICH guidelines. The developed method was linear in the concentration range of 10-120 µg/mL. LOD and LOQ were found to be 7.4 and 22.68 µg/mL. The percentage recoveries of Anagliptin in synthetic mixture were found to in the range of 98.70- 100.57%.

***Section B:*** Degradation kinetics of Anagliptin was studied by HPLC in alkaline and oxidative condition. Both alkaline and oxidative degradation followed first order kinetics. Based on this, degradation rate constant and half-life ($t_{1/2}$) were calculated.

***Section C*:** Two major degradation products formed DP1 in alkaline condition and DP2 in oxidative condition were isolated by preparative HPLC. Isolation of degradation products from Anagliptin was performed on Daisogel-SP-100-10-ODS-P column with 250x50 mm, 100 Å, 10µ in diameter. Detection was performed at 247 nm. Flow rate was maintained at 60 mL/min. Samples were injected through Rheodyne 7725 injector valve. Mobile phase comprised of 10 mM ammonium acetate buffer (pH 5): methanol: acetonitrile in gradient mode. Degradation products DP1 and DP2 were characterized by mass, NMR and IR techniques and mechanism of DP1 and DP2 were proposed. Based on the spectral data, DP1 was characterised as 1-[2-[1-

(2-methylpyrazolo [1, 5-alpha] pyrimidine-6-carboxamido}-methyla-proan-2-yl-amino) acetyl) pyrollidine-2-carboxamide and DP2 as N-[2-({2-[(2S)-2-cyanopyrrolidin-1-yl]-2-oxoethyl}amino)-2-methylpropyl]-2-methylpyrazolo-N-oxido -[1,5-a]pyrimidine-6-carboxamide.

***Section D***: Forced degradation study of Anagliptin was carried by LC-PDA and degradation products were identified by LC-MS. . Degradation products in alkaline and oxidative conditions were identified by LC-MS. LC-PDA showed total 7 degradation products. Two degradation products (DP1 and DP3) were formed both in alkaline and oxidative conditions while five degradation products (DP2, DP4, DP7) were formed in oxidative conditions. Among 7 degradation products, two degradation products DP1 and DP2 were isolated and characterized as above. Remaining degradation products were characterized on the basis of molecular mass. Degradation pathways in alkaline and oxidative condition were proposed.

***Chapter 7: Development of Stability Indicating Assay Method of Efonidipine HCl Ethanolate***

***Section A****: M*ethod development and validation of Efonidipine by HPLC method using QbD approach. Fractional factorial Design was used for preliminary screening of various factors. Factors such as concentration of ammonium acetate buffer, pH of buffer, % organic, flow rate and wavelength were and FFD model was constructed using 16 runs.

The results of FFD gave four prominent factors pH of buffer, concentration of buffer, flow rate, % organic. These factors were optimised by Box-Behnken design (BBD). The range of factors used for BBD were: pH (3-5), concentration of buffer (5-15mM), % organic (65 -75) , flow rate (0.8 -1.2 mL/min). 28 runs were designed and BBD model was constructed. The optimal conditions obtained from the BBD results were pH 5, concentration of buffer 10 mM, % organic 65, flow rate 1mL/min. Method was optimised on Thermo Hypersil BDS C-18 (250 x 4.6, 5 µ ).The retention time of Efonidipine was found to be 8.45 min. The method was validated as per ICH guidelines and was applied for estimation of EFO in marketed formulation. The developed method was linear in the concentration range of 20-120 µg/mL. LOD and

LOQ were found to be 0.23 and 0.75 µg/mL. The percentage recoveries of Efonidipine in formulation were found to be in the range of 99.88-100.16%.

***Section B****:* Stability indicating HPLC method was developed for determination of Efonidipine. Stress degradation was performed as per the guidelines prescribed by ICH. The degradation conditions were : 1 M HCl, 80ºC ,5 hrs, for acid hydrolysis ; 0.5 M NaOH, RT ,6 hrs, for base hydrolysis; 10% $H_2O_2$, RT , 24 hrs, for oxidative degradation ; 5382 Lux and 144UW/cm$^2$,11 days for photolytic degradation; 80ºC 11days for thermal degradation . Ten different degradation products were observed under various stress conditions. Significant degradation was observed in alkaline and photolytic condition.

Separation of degradation products from Efonidipine was achieved on Thermo Hypersil BDS C-18 (250 x 4.6, 5 µ) column in gradient mode. Mobile phase composed of 10mM ammonium acetate buffer (pH 5.8) and acetonitrile. The detection was performed at 254 nm. The flow rate was kept at 1mL/min. The retention time of Efonidipine was found to be 57.66 min. The developed method was validated as per ICH guidelines. The developed method was linear in the concentration range of 20-120 µg/mL. LOD and LOQ were found to be 0.41 and 1.24 µg/mL. The percentage recoveries of Efonidipine in marketed formulation were in the range of 99.77-100.16%.

***Section C***: Degradation kinetics of Efonidipine was studied by HPLC in alkaline condition. On the basis of this, alkaline degradation followed first order kinetics. Based on this, half-life was calculated .Activation energy was found to be 9.65 KJ/mole.

***Section D***: Three major degradation products DP1, DP3 and DP4 in alkaline condition were isolated by preparative HPLC. Isolation of degradation products from Efonidipine was performed on Phenomenex Luna column C 18 (250X 50 mm, 10µ). Detection was performed at 254 nm. Flow rate was maintained at 50 mL/min. Samples were injected through Rheodyne 7725 injector valve. Mobile phase comprised of 10 mM ammonium acetate buffer (pH 5) : acetonitrile in gradient mode. Degradation products DP1 , DP3 and DP4 were characterized by mass, NMR and IR techniques and mechanism of DP1, DP3 and DP4 were proposed. Based on the spectral data, DP1 was characterised as 3-2-(N-benzylanilino) ethyl 3-oxo-2, 2-

dimethylpropyl hydrogen 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridin-3-yl-3-phosphonate, DP3 was characterized as 2-(N-benzyl-N-phenyl amino) ethanol, DP4 was characterized as 3-methoxy-2,2-dimethylpropyl hydrogen 1,4-dihydro-2,6-dimethyl-5-methyloxycarbonyl-4-(3-nitro)phenylpyridin-3-yl-3-phosphonate.

***Section E:*** Forced degradation study of Efonidipine was carried by LC-PDA and degradation products were identified by LC-Q-ToF-MS. Degradation products in alkaline and oxidative conditions were identified by LC-Q-ToF-MS. LC-PDA showed total 10 degradation products. Six degradation products (DP1-DP6) were formed both in alkaline condition while four degradation products (DP7-DP10) were formed in photolytic condition. Among 10 degradation products, three degradation products DP1, DP3 and DP4 were isolated and characterized as above. Remaining degradation products were characterized on the basis of molecular mass and fragmentation pattern. Degradation pathways in alkaline and photolytic condition were proposed.

### *Chapter 8: Development of Stability Indicating Assay Method of Riociguat*

***Section A*:** Stability indicating HPLC method was developed for determination of Riociguat. Stress degradation was performed as per the guidelines prescribed by ICH. The degradation conditions were : 1 M HCl, 80ºC ,12 hrs, for acid hydrolysis ; 0.5 M NaOH, 60ºC ,3 hrs, for base hydrolysis; water, 80ºC ,12 hrs, for acid neutral hydrolysis , 10% $H_2O_2$, RT , 2 hrs, for oxidative degradation ; 5382 Lux and 144UW/cm2,11 days for photolytic degradation; 80ºC 11days for thermal degradation . Three degradation products DP1, DP2 and DP3 were observed at Rt of 11.4, 8.9 and 4.0 min respectively. Significant degradation was observed in alkaline condition and slight degradation was observed in oxidative condition.

Separation of degradation products from Riociguat was achieved on Waters Symmetry C18 (150 x 4.6, 3.5 µ) column in isocratic mode. Mobile phase composed of 10mM ammonium acetate buffer (pH 5.7) and acetonitrile in the ratio of 70:30. The detection was performed at 254 nm. The flow rate was kept at 1mL/min. The retention time of Riociguat was found to be 12.44 min. The developed method was validated as per ICH guidelines. The linearity was found in the concentration range of 5-160 µg/mL. LOD and LOQ were found to be 0.046 and 0.139 µg/mL. The percentage recoveries of Riociguat in marketed formulation were found in the range of 99.45-100.13%.

Robustness was evaluated by classical and QbD approach. Fractional factorial method DoE strategy was used to carry out robustness study. Four parameters selected were pH of buffer, % organic, flow rate, wavelength. Four critical quality attributes taken were retention time, area, tailing factor and number of the theoretical plates. 8 runs were designed and FFD model was constructed. Polynomial model was examined using lack of fit test, which indicated insignificant lack of fit value corresponding with higher p-value as compared to the model F-value. Graphical interpretation in the form of pareto charts, perturbation plots, 3-D response plot and contour plot showed the correlation of effect of factors on the response's retention time, area, tailing factor and theoretical plates of RIO.

***Section B:*** Degradation kinetics of Riociguat was studied by HPLC in alkaline condition. On the basis of this, alkaline degradation followed first order kinetics. Based on this, half-life was calculated .Activation energy was found to be 7.62 KJ/mole.

***Section C*****:** Three major degradation products DP1, DP2 in alkaline condition and DP3 in oxidative condition were isolated by preparative HPLC. Isolation of degradation products from Riociguat was performed on Promosil column (250X 50 mm, 10µ). Detection was performed at 254 nm. Flow rate was maintained at 55 mL/min. Samples were injected through Rheodyne 7725 injector valve. Mobile phase comprised of 10 mM ammonium acetate buffer (pH 5.7): acetonitrile in gradient mode. Degradation products were characterized by LC-HR-MS, NMR and IR techniques and mechanism of degradation products were proposed. Based on the spectral data, DP1 was characterised as 2-(1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b]pyridin-3yl)-N5-methylpyrimidine-4,5,6-triamine, DP2 as 2-(1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b]pyridin-3-yl)-6-amino-7-methyl-7H-purin-8(9H)-one and DP3 as methyl 2-(1-(2-fluorobenzyl)-1H-pyrazolo [3, 4-b] pyridin-3-yl)-4, 6-diaminopyrimidin-5-ylmethylcarbamate-N-oxide.

***Section D:*** Impurity profiling and degradation study of Riociguat was carried by LC-PDA and LC-HR-MS. Degradation products in alkaline and oxidative conditions were identified by LC-HR-MS. LC-PDA showed total 3 degradation products. Two degradation products (DP1and DP2) were formed in alkaline condition while one degradation product (DP3) was formed in oxidative condition. These degradation products were isolated and characterized as above. Degradation pathway in alkaline and oxidative condition was proposed.