CHEPTER 7

SUMMARY, CONCLUSION AND RECOMMENDATION

The work presented in this thesis deals with the HPLC/UPLC investigations of various impurities in erlotinib hydrochloride, raloxifene hydrochloride, fampridine tablet dosage forms and simultaneous quantification of impurities in the combined dosage forms of lamivudine and tenofovir disoproxil fumarate and triple combination dosage forms of emtricitabine, tenofovir and efavirenz. All the developed HPLC/UPLC methods are validated in terms of the specificity, linearity, accuracy, precision, robustness and ruggedness along with their solution stability studies as per the guidelines given in ICH.

7.1 Method Development and validation of a stability indicating HPLC method for the quantification of impurities in erlotinib hydrochloride dosage forms

A sensitive and selective stability indicating HPLC method was developed for the quantitative determination of ERL IMP-A and ERL IMP-B in erlotinib tablets. Satisfactory extraction of erlotinib and its impurities from tablet dosage forms found in the mixture of acetonitrile and water in 70:30 (% v/v), better peak shapes and reproducibility observed in Waters X- terra RP 18 150 mm × 4.6 mm, 3.5 µm column. Sodium di hydrogen orthophosphate buffer (pH 4.5) and acetonitrile were selected as mobile phase for the better peak shape. Flow rate of the mobile phase was maintained at 1.0 mL/min, column oven temperature was maintained at 40°C and sample cabin temperature was at 25°C. The injection volume was 10 µL for the quantification of ERL IMP-A and ERL IMP-B in the erlotinib drug product. The developed method is able to determine the ERL IMP-A and ERL IMP-B impurity at 0.07 µg/mL in the presence of high concentration of erlotinib drug substance by satisfying all the requirements of ICH guidelines and regulatory authorities. The results of various validation parameters along with their limits are included in Table 7.1.

Table 7.1: Summary of erlotinib tablets related substances method validation results

Parameter	Acceptance Criteria	Results
Specificity and forced degradation	**i. Specificity** • No blank and placebo interference should be observed at the RT of **e**rlotinib HCl and known impurities. • Erlotinib HCl and known impurities should elute at different RT's. • Peak purity should pass for erlotinib HCl and known impurities. **ii. Forced degradation** • Peak purity index for the main peak of the drug i.e. for raloxifene should pass. • Mass balance of should be in the range 95% to 105%	 • No blank and placebo interference was observed at the elution RT of Erlotinib HCl and known impurities. • Erlotinib HCl and known impurities were eluted at different RT's. • Peak purity was passed for Erlotinib HCl and known impurities. • Peak purity of erlotinib passed in all degradation conditions. • Mass balance observed in the range of 98.3% to 100.0%.
Limit of detection (LOD)	• S/N ratio about 3 or 2:1 for LOD solution.	• S/N ratio observed for erlotinib and its impurities was about 3 for LOD solution and the concentration was about 0.04 µg/mL.
Limit of Quantitation (LOQ)	• S/N ratio about 10:1 for LOQ solution. • %Recovery should be between 80 to 120% at LOQ level.	• S/N ratio observed for erlotinib and its impurities was about 3 for LOQ solution and the concentration was about 0.12 µg/mL. • %Recovery at LOQ level was 96.7 to 98.9, which is within acceptable range.

Linearity	• Correlation coefficient (r) for Erlotinib HCl and known impurities should not less than 0.99 from LOQ to 150% of the specification level.	• Correlation coefficient (r) observed for Erlotinib HCl is 0.999, ERL IMP-A is 0.999 and ERL IMP-B is 0.999 which is not less than 0.99 from LOQ to 150% of the specification level.
Accuracy	• Recovery should be in the range of 80 to 120% at any four levels between LOQ to 150% of the specification level. • %RSD should be not more than 10.0 at each level from LOQ to 150%.	• %Recovery was within the range of 80 to 120%. • %RSD was less than 10.0 at each level from LOQ to 150%.
Method precision	• %RSD of recovery obtained for both impurities from six spiked samples should not be more than 10.0.	• %RSD of recovery obtained for ERL IMP-A is 6.6 and ERL IMP-B is 7.3 from six spiked samples, which was within the acceptable range.
Intermediate precision	• %RSD of recovery obtained for both impurities from six spiked samples should be not more than 10.0	• %RSD of recovery obtained for ERL IMP-A is 7.3 and ERL IMP-B is 5.4from six spiked samples, which was within the acceptable range.
Robustness		
Flow rate , column oven temperature and mobile phase buffer pH variation	• System suitability criteria should pass for standard solution in all the robustness conditions.	• System suitability criteria were passed for standard solution in all the robustness conditions.

Solution stability and mobile phase stability	• Solution should be stable both at room temperature and cooler temperature (2-8°C) for spiked sample solution and standard solution up to 24 hrs. • Mobile phase should not be hazy.	• Solution was stable up to 24 hrs both at room temperature and cooler temperature (2-8°C) for spiked sample solution and standard solution. • Mobile phase was clear.

7.2 Development and validation of stability indicating RP-HPLC method for the determination of related substances in raloxifene hydrochloride tablets dosage forms

A simple, precise, accurate and rapid HPLC method was developed for the determination and quantification of 5 related substances in Raloxifene. Raloxifene is a non-polar molecule, which is slightly soluble in water. Satisfactory extraction of raloxifene and its impurities from tablet dosage forms found in the mixture of pH 4.5 buffer and acetonitrile in 50:50 (% v/v). Good separation and quantification of 5 related substances in raloxifene was achieved by selecting potassium di hydrogen phosphate buffer with pH 4.5 as mobile phase A and acetonitrile as mobile phase B, stationary phase used is Inertsil C_8 with the dimentions 150 x 4.6 mm ID, 3.5µm. The flow rate was 1.0 mL/min in liquid chromatography. The column oven temperature was maintained at 30°C, the sample cooler temperature was 25°C. The injection volume was 10 µL for the quantification of impurities in the raloxifene tablet dosage forms. The developed method is able to determine the impurities in raloxifene tablet dosage form below the specification level. The results of various validation parameters along with their limits are included in Table 7.2.

Table 7.2: Summary of raloxifene tablets related substances method validation results

Parameter	**Acceptance Criteria**	**Results**
Specificity and forced degradation	**i. Specificity** • No blank and placebo interference should be observed at the elution RT of Raloxifene HCl and known impurities. • Raloxifene and known impurities should elute at different RT's. • Peak purity should pass for Raloxifene HCl and known impurities.	 • No blank and placebo interference was observed at the elution RT of Raloxifene HCl and known impurities. • Raloxifene HCl and known impurities were eluted at different RT's. • Peak purity was passed for Raloxifene HCl and its known impurities.

	ii. Forced degradation • Peak purity index for the main peak of the drug i.e. for raloxifene should pass. • Mass balance of should be in the range 95% to 105%	 • Peak purity of raloxifene passed in all degradation conditions. • Mass balance observed in the range of 99.2% to 100.4%.
Limit of detection (LOD)	• S/N ratio about 3 or 2:1 for LOD solution.	• S/N ratio observed for raloxifene and its impurities was about 3 for LOD solution and the concentration was about 0.13 µg/mL.
Limit of Quantitaion (LOQ)	• S/N ratio about 10:1 for LOQ solution. • %Recovery should be between 80 to 120 at LOQ level.	• S/N ratio observed for raloxifene and its impurities was about 3 for LOQ solution and the concentration was about 0.4 µg/mL. • %Recovery at LOQ level was 96.7 to 98.9, which is within acceptable range.
Linearity	• Correlation coefficient (r) for Raloxifene HCl and its known impurities should not be less than 0.99 from LOQ to 150% of the specification level.	• Correlation coefficient (r) observed for RLF IMP-1 is 0.9997, RLF IMP-2 is 0.9999, RLF IMP-3 is 0.9995, RLF IMP-4 is 0.9999, RLF IMP-5 is 0.9996 and Raloxifene HCl is 0.9953. Values were not less than 0.99 in the range of LOQ to 150% specification level.
Accuracy	• Recovery should be in the range of 80 to 120% at any four levels between LOQ to 150% of the specification level.	• Recovery was within the range of 80 to 120% from LOQ to 150% of the specification level for known impurities.

	• %RSD should be not more than 10.0 at each level from LOQ to 150%.	• %RSD was 0.1 to 2.2 at each level from LOQ to 150% of the specification level for known impurities
Precision		
Method precision	• %RSD of recovery obtained for both impurities from six spiked samples should be not more than 10.0	• %RSD of recovery obtained for RLF IMP-1 is 1.64, RLF IMP-2 is 2.18, RLF IMP-3 is 2.27, RLF IMP-4 is 2.60 and RLF IMP-5 is 2.47 from six spiked samples which was within acceptable range.
Intermediate precision	• %RSD of recovery obtained for both impurities from six spiked samples should be not more than 10.0	• %RSD of recovery obtained for RLF IMP-1 is 2.65, RLF IMP-2 is 2.26, RLF IMP-3 is 1.34, RLF IMP-4 is 2.33 and RLF IMP-5 is 2.65 from six spiked samples which was within acceptable range.
Robustness		
Flow rate, column oven temperature and mobile buffer pH variation.	• Should meet all the system suitability criteria for standard solution in all the robustness conditions.	• System suitability criteria were passed for standard solution in all the robustness conditions.
Solution stability and mobile phase stability	• Solution should be stable at both room temperature and cooler temperature (2-8°C) for spiked sample solution and standard solution up to 24 hrs. • Mobile phase should not be hazy.	• Solution was stable up to 24 hrs at both room temperature and cooler temperature (2-8°C) for spiked sample solution and standard solution. • Mobile phase was clear.

7.3 Development and validation of a stability indicating HPLC method for the quantification of impurities in fampridine dosage forms

Stability indicating RP-HPLC method was developed for the determination of related substances in fampridine drug substances and drug product. Fampridine is soluble in water and other polar solvents such as alcohols, acetonitrile, and N,N-dimethyl formamide. The optimized buffer concentration and pH was 0.05 M potassium dihydrogen orthophosphate with pH 4.0. Stationary phase used in this work was inertsil C_8 150 x 4.6 mm ID, 5µm and wavelength was selected as 260 nm based on the UV-Vis spectra. The flow rate was maintained at 1.0 mL/min in liquid chromatography. The column oven temperature was maintained at 35°C. The injection volume was 10µL for the quantification of impurities in the fampridine tablet dosage form. The developed method is able to determine the impurities in the fampridine tablet dosage form at 0.03 µg/mL in the presence of high concentration of fampridine drug substance by satisfying all the requirements of ICH guidelines and regulatory authorities. The results of various validation parameters along with their limits are included in Table 7.3.

Table 7.3: Summary of fampridine tablets related substances method validation results

Parameter	**Acceptance Criteria**	**Results**
Specificity and forced degradation	**i. Specificity** • No blank and placebo interference should be observed at the elution RT of fampridine and known impurities. • Fampridine and known impurities should elute at different RTs. • Peak purity should pass for Fampridine and known impurities.	 • No blank and placebo interference was observed at the elution RT of Fampridine and known impurities. • Fampridine and known impurities were eluted at different RTs. • Peak purity was passed for Fampridine and its known impurities.

	ii. Forced degradation • Peak purity index for the main peak of the drug i.e. for fampridine should pass. • Mass balance of should be in the range 95% to 105%.	 • Peak purity of fampridine passed in all degradation conditions. • Mass balance observed in the range 98.5% to 99.9%.
Limit of detection (LOD) **Limit of Quantitaion (LOQ)**	• S/N ratio about 3 or 2:1 for LOD solution. • S/N ratio about 10:1 for LOQ solution. • %Recovery should be between 80 to 120% at LOQ level.	• S/N ratio observed for fampridine and its impurities was about 3 for LOD solution and the concentration was about 0.03 µg/mL. • S/N ratio observed for fampridine and its impurities was about 10 for LOQ solution and the concentration was about 0.1 µg/mL. • %Recovery at LOQ level was 97.4 to 100.9 for Isonicotinamide and N-Oxide impurities, which is within acceptable range.
Linearity	• Correlation coefficient (r) for Fampridine and its known impurities should not be less than 0.99 from LOQ to 150% of the specification level.	• Correlation coefficient (r) observed for Isonicotinamide is 0.9994, N-Oxide impurity is 0.9995 and Fampridine is 0.9995, were not less than 0.99 from LOQ to 150% of the specification level.
Accuracy	• Recovery should be in the range of 80 to 120% at any four levels in between LOQ to 150% of the specification level of impurities.	• Recovery was within the range of 80 to 120% from LOQ to 150% of the specification level for both impurities.

Precision		
Method precision	• %RSD of recovery obtained for both impurities from six spiked samples should be not more than 10.0.	• %RSD of recovery obtained for Isonicotinamide is 3.4 and N-Oxide impurity is 4.2 from six spiked samples, which was within acceptable range.
Intermediate precision	• %RSD of recovery obtained for both impurities from six spiked samples should be not more than 10.0.	• %RSD of recovery obtained for Isonicotinamide is 3.5 and N-Oxide impurity is 2.9 from six spiked samples, which was within acceptable range.
Robustness		
Flow rate, column oven temperature and mobile phase buffer pH variation.	• System suitability criteria should pass for standard solution in all the robustness conditions.	• System suitability criteria were passed for standard solution in all the robustness conditions.
Solution stability and mobile phase stability	• Solution should be stable at both room temperature and cooler temperature (2-8°C) for spiked sample solution and standard solution up to 24 hrs. • Mobile phase should not be hazy.	• Solution was stable up to 24 hrs at both room temperature and cooler temperature (2-8°C) for spiked sample solution and standard solution. • Mobile phase was clear.

7.4 Development and validation of a stability indicating HPLC method for the quantification of eleven impurities in lamivudine and tenofovir disoproxil fumarate.

A simple, precise, accurate and rapid method developed for the determination and quantification of eleven related impurities in lamivudine and tenofovir disoproxil fumarate tablet dosage forms. Lamivudine and tenofovir are polar molecules, which are soluble in water. Satisfactory extraction of lamivudine and tenofovir and its impurities from tablet dosage forms found in the mixture of ammonium acetate buffer in water with pH 5.0±0.05 and methanol in 80:20 (% v/v). Good separation of analytes achieved with the mobile phase composition of ammonium acetate buffer (0.02 M with pH 5.0) and methanol in 20:80 (%v/v). Good resolution, good peak shapes and reproducibility observed on X-terra RP18 (150 x 4.6 mm, 3.5 µm) column at the wavelength of 260 nm. The flow rate of the mobile phase was maintained at 1.0 mL/min in liquid chromatography. The column oven temperature was maintained at 35°C, the sample cooler temperature was 25°C. The injection volume was 10µL for the quantification of impurities in lamivudine and tenofovir tablet dosage forms. The developed method is able to determine the impurities in lamivudine and tenofovir tablet dosage forms at 0.3% (1.6 µg/mL) for Isopropyl impurity, 1.0% (5.0 µg/mL) for monoester impurity and 0.2% (1.0 µg/mL) for remaining all impurities by satisfying all the requirements of ICH guidelines and regulatory authorities. The results of various validation parameters along with their limits are included in Table 7.4.

Table 7.4: Summary of lamivudine and tenofovir disoproxil fumarate tablets related substances method validation results

Parameter	Acceptance Criteria	Results
Specificity and forced degradation	**i. Specificity** • No blank and placebo interference should be observed at the elution RT of Lamivudine, Tenofovir disoproxil fumarate and its known impurities. • Lamivudine, tenofovir disoproxil fumarate and its known impurities should elute at different RT's. • Peak purity should pass for Lamivudine, Tenofovir disoproxil fumarate and their known impurities. **ii. Forced degradation** • Peak purity index for the main peak of the drug i.e. for lamivudine and tenofovir should pass. • Mass balance should be in the range 95% to 105%	• No blank and placebo interference was observed at the elution RT of Lamivudine, Tenofovir disoproxil fumarate and its known impurities • Lamivudine, tenofovir disoproxil fumarate and its known impurities were eluted at different RT's. • Peak purity was passed for Lamivudine, Tenofovir disoproxil fumarate and their known impurities. • Peak purity of lamivudine and tenofovir disoproxil passed in all degradation conditions. • Mass balance observed in the range 98.1% to 101.2%.

Limit of detection (LOD) **Limit of Quantitation (LOQ)**	• S/N ratio about 3 or 2:1 for LOD solution. • S/N ratio about 10:1 for LOQ solution. • %Recovery should be between 80 to 120% at LOQ level.	• S/N ratio observed for lamivudine, tenofovir disoproxil and their impurities was about 3 for LOD solution and the concentration was about 0.05 µg/mL. • S/N ratio observed for lamivudine, tenofovir disoproxil and their impurities was about 3 for LOQ solution and the concentration was about 0.15 µg/mL. • %Recovery at LOQ level was 99.1 to 100.7 for Lamivudine and Tenofovir disoproxil fumarate known impurities which is within acceptable range.
Linearity	• Correlation coefficient (r) for Raloxifene HCl and its known impurities should not be less than 0.99 from LOQ to 150% of the specification level.	• Correlation coefficient (r) observed for Cytosine is 0.999, Uracil is 0.992, Acid impurity is 0.995, Sulfoxide impurity is 0.991, Adenine is 0.999, Tenofovir impurity is 0.998, Lamivudine is 0.995, Mono ester impurity is 0.996, Di ethyl impurity is 0.995, Isopropyl impurity is 0.993, Tenofovir disoproxil is 0.999, n-propyl impurity is 0.994 and Tenofovir

		dimer impurity is 0.996 were more than 0.99 from LOQ to 150% of the specification level.
Accuracy	• Recovery should be in the range of 80 to 120% from LOQ to 150% of the specification level for known impurities • %RSD should be not more than 10.0 at each level from LOQ to 150% for known impurities.	Recovery was within the range of 80 to 120% from LOQ to 150% of the specification level for Lamivudine and Tenofovir disoproxil fumarate known impurities • %RSD was between 0.1 to 2.2 at each level from LOQ to 150% of the specification level for known impurities.
Precision		
Method precision	• %RSD of recovery obtained for both impurities from six spiked samples should be not more than 10.0	• %RSD of recovery obtained for Cytosine is 7.3, Uracil is 8.4, Acid impurity is 4.5, Sulfoxide impurity is 5.1, Adenine is 4.3, Tenofovir impurity is 3.9, Mono ester impurity is 3.8, Di ethyl impurity is 5.9, Isopropyl impurity is 6.1, n-propyl impurity is 5.1 and Tenofovir dimer impurity is 8.9 from six spiked samples which was within acceptable range.

Intermediate precision	• %RSD of recovery obtained for both impurities from six spiked samples should be not more than 10.0.	• %RSD of recovery obtained for Cytosine is 7.1, Uracil is 7.3, Acid impurity is 5.6, Sulfoxide impurity is 5.7, Adenine is 6.3, Tenofovir impurity is 7.7, Mono ester impurity is 5.1, Di ethyl impurity is 5.9, Isopropyl impurity is 5.0, n-propyl impurity is 5.6 and Tenofovir dimer impurity is 7.9 from six spiked samples which was within acceptable range.
Robustness		
Flow, Oven temperature Mobile phase buffer pH variation.	• System suitability criteria should pass for standard solution in all the robustness conditions.	• System suitability criteria were passed for standard solution in all the robustness conditions.
Solution stability and mobile phase stability	• Solution should be stable at both room temperature and cooler temperature (2-8°C) for spiked sample solution and standard solution up to 24 hrs. • Mobile phase should not be hazy.	• Solution was stable up to 24 hrs at both room temperature and cooler temperature (2-8°C) for spiked sample solution and standard solution. • Mobile phase was clear.

7.5 RP-UPLC method for the simultaneous quantification of related substances in emtricitabine, tenofovir disoproxil fumarate and efavirenz pharmaceutical dosage forms

Simple, precise, accurate and rapid UPLC method developed for the determination and quantification of related substances in efavirenz, emtricitabine, and tenofovir disoproxil fumarate tablet dosage forms. Satisfactory extraction of emtricitabine, tenofovir disoproxil fumarate, efavirenz and their impurities from tablet dosage forms found in the mixture of buffer: MeOH in the ratio of 20:80 (%v/v).Good separation of analytes achieved with the mobile phase composition of pH 4.0 buffer (0.1% TEA, pH adjusted with dilute OPA) and acetonitrile in 20:80 (%v/v). Good resolution, good peak shapes and reproducibility observed on Waters BEH phenyl 2.1 X100 mm, 1.7 mm column at the wavelength of 265 nm. The flow rate of the mobile phase was maintained at 0.5 mL/min in liquid chromatography, the column oven temperature was maintained at 30°C, and sample cooler temperature was 25°C. The injection volume was 2µL for the quantification of impurities in combination of efavirenz, emtricitabine, and tenofovir disoproxil fumarate tablet dosage form. The developed method is able to determine the impurities in efavirenz, emtricitabine, and tenofovir disoproxil fumarate tablet dosage forms at 0.2% (4 µg/mL) of emtricitabine RC-02, 1.0%(30 µg/mL) of monoester, 0.3% (9.2 µg/mL) of isopropyl POC, 0.2% (6 µg/mL) of n-propyl POC, tenofovir mixed dimer, tenofovir dimer, 0.2% (12 µg/mL) of efavirenz RC-A and efavirenz RC-D. The results of various validation parameters along with their limits are included in Table 7.5.

Table 7.5: Summary of emtricitabine, tenofovir disoproxil fumarate and efavirenz tablets related substances method validation results

Parameter	Acceptance criteria	Results
Specificity and forced degradation	**i. Specificity**	
	• No blank and placebo interference should be observed at the elution RT of Efavirenz, Emtricitabine and Tenofovir disoproxil fumarate and their known impurities.	• No blank and placebo interference was observed at the elution RT of Efavirenz, Emtricitabine and Tenofovir disoproxil fumarate and their known impurities.
	• Efavirenz, Emtricitabine and Tenofovir disoproxil fumarate and their known impurities should elute at different RT's.	• Efavirenz, Emtricitabine and Tenofovir disoproxil fumarate and their known impurities were eluted at different RT's.
	• Peak purity should pass for Efavirenz, Emtricitabine and Tenofovir disoproxil fumarate and their known impurities	• Peak purity was passed for Efavirenz, Emtricitabine and Tenofovir disoproxil fumarate and their known impurities.
	ii. Forced degradation	
	• Peak purity for the main peaks of the drugs i.e. for Efavirenz, Emtricitabine and Tenofovir disoproxil should pass.	• Peak purity of Efavirenz, Emtricitabine and Tenofovir disoproxil passed in all degradation conditions.
	• Mass balance of should be in the range 95% to 105%.	• Mass balance observed in the range of 98.1% to 101.8%.

Limit of detection (LOD)	• S/N ratio about 3 or 2:1 for LOD solution.	• S/N ratio observed for Efavirenz, Emtricitabine and Tenofovir disoproxil and their impurities was about 3 for LOD solution and the concentration was about 0.05 µg/mL.
Limit of Quantitaion (LOQ)	• S/N ratio about 10:1 for LOQ solution. • %Recovery should be between 80 to 120% at LOQ level.	• S/N ratio observed for Efavirenz, Emtricitabine and Tenofovir disoproxil and their impurities was about 10 for LOQ solution and the concentration was about 0.15 µg/mL. • %Recovery at LOQ level was 99.1 to 100.7 for efavirenz, emtricitabine and tenofovir disoproxil fumarate known impurities which is within acceptable range.
Linearity	• Correlation coefficient (r) for efavirenz, emtricitabine and tenofovir disoproxil fumarate and their known impurities should not be less than 0.99 from LOQ to 150% of the specification level.	• Correlation coefficient (r) observed for Emtricitabine RC 02 is 0.991, Emtricitabine is 0.997, Mono ester is 0.999, Isopropyl POC is 0.998, Tenofovir Disoproxil is 0.996, n-Propyl POC is 0.998, Tenofovir mixed dimer is 0.999, Efavirenz RC-A is 0.993, Efavirenz is 0.991, Tenofovir dimer is 0.998 and efavirenz RC -D is 0.995 were more than 0.99 from LOQ to 150% of the specification level.
Accuracy	• Recovery should be in the range of 80 to 120% from LOQ to 150% of the specification level for known impurities.	Recovery was within the range of 80 to 120% from LOQ to 150% of the specification level for Efavirenz, Emtricitabine and Tenofovir disoproxil known impurities.

	• %RSD should be not more than 10.0 at each level from LOQ to 150% for known impurities.	• %RSD was between 0.06 to 0.76 at each level from LOQ to 150% of the specification level for known impurities.
Precision		
Method precision	• %RSD of recovery values obtained for all impurities from six spiked samples should not be more than 10.0	• %RSD of recovery obtained for Emtricitabine RC 02 is 8.6, Mono ester is 3.6, Isopropyl POC is 5.4,n-Propyl POC is 7.1, Tenofovir Mixed dimer is 7.9, Efavirenz RC-A is 6.7, Tenofovir dimer is 4.4 and Efavirenz RC -D is 5.6 from six spiked samples which was within acceptable range.
Intermediate precision	• %RSD of recovery obtained for all impurities from six spiked samples should not be more than 10.0	• %RSD of recovery obtained for Emtricitabine RC 02 is 6.3, Mono ester is 3.3, Isopropyl POC is 8.5,n-Propyl POC is 8.6, Tenofovir Mixed dimer is 8.6, Efavirenz RC-A is 8.1, Tenofovir dimer is 8.7, Efavirenz RC -A is 8.1 and Efavirenz RC -D is 8.4 from six spiked samples which was within acceptable range.

Robustness		
Flow rate, column oven temperature and mobile phase buffer pH variation.	• System suitability criteria should pass for standard solution at all the robustness conditions.	• System suitability criteria were passed for standard solution at all the robustness conditions.
Solution stability and mobile phase stability	• Solution should be stable at both room temperature and cooler temperature (2-8°C) for spiked sample solution and standard solution up to 24 hrs. • Mobile phase should not be hazy.	• Solution was stable up to 24 hrs at both room temperature and cooler temperature (2-8°C) for spiked sample solution and standard solution. • Mobile phase was clear

7.6 Conclusions and recommendation

All the HPLC/UPLC methods presented in this thesis are able to detect and quantify the possible impurities and degradants in the single dosage forms of erlotinib hydrochloride, raloxifene hydrochloride and fampridine, double combination dosage forms of lamivudine and tenofovir disoproxil fumarate and triple combination dosage forms of emtricitabine, tenofovir disoproxil fumarate and efavirenz drug substances for the their quality testing during the various storage conditions. These methods could be easily adapted by quality control labs of pharmaceutical industries and testing labs of government organizations for the monitoring of impurities in both active pharmaceutical ingredient and their dosage forms.