Chapter 8

HPLC METHOD DEVELOPMENT AND VALIDATION FOR DOLUTEGRAVIR TENOFOVIR AND RILPIVIRINE

8.1 EXPERIMENTALS

8.1.1 Instruments Utilised

The Shimadzu-HPLC system LC-20-AT-system with LC-Solution and Peak chrom software with both PDA & UV detector. Stationary phase coloumn in reverse phase has been used C-18-Hypersil-BDS and Hypersil-ODS-250 x 4.6 mm, 5 micron size has been selected.

Systronics UV-visible spectrophotometer was used along with other Shimadzu UV 1800 spectrophotometer & Systronics UV for the wavelength maxima estimation. FTIR Spectrometer Shimadzu 8400 series has been utilised for identification of drugs standard samples. Melting point apparatus Labtronics was used for melting point determinations.

Wist Temperature Chamber was used for drying the drug samples and thermal degradation study. Ultra-sonicator Lab Branson ultrasonic's corporation was utilised. Digital pH meter labtronics was utilised. Photostability Test Chamber Sanwood SM-LHH-GSD-UV Series was utilised. Electronic analytical balance AUX-220 Shimadzu has been used. Borosil glass-wares volumetric flasks measuring cylinder pipettes of analytical were used. 0.22 and 0.45 µm nylon Millipore filters caps were used.

8.1.2 Materials and Reagents Utilised

The chemicals used working reference standard drugs Dolutegravir DOLU, Tenofovir TEN & Rilpivirine RILP drugs samples of sun-pharma, solisom & upcare pharma has been utilised. Acetonitrile, Methanol, potassium dihydrogen ortho phosphate, orthophosphoric acid, used analytical HPLC Merck grade. H_2O_2, HCl , NaOH analytical grade of Rankem used. Milli-Q pure water is utilized.

8.1.3 Identification of Standard Drug Samples

8.1.3.1 Melting Point Determination

The working standard drugs Dolutegravir DOLU, Tenofovir TEN & Rilpivirine RILP were identified by melting point determination. Melting point apparatus used was made of Labtronics™ Melting Point Apparatus. The melting points observed for the standard drug samples are shown in the Table 5.1.

Drug	Observed Melting Range	Standard Melting Range
DOLU	192.32-195.04 ^{0}C	190-193 ^{0}C
TEN	112-115 ^{0}C	113-115 ^{0}C
RILP	249.9 – 251.36 ^{0}C	248-251 ^{0}C

Table 5.1: Melting Points of DOLU, TEN & RILP

8.1.3.2 FTIR Spectral Determination for Identification Standard drug samples DOLU, TEN & RILP

The pure active pharmaceutical working standard drug substances DOLU, TEN & RILP were scanned between 400-4000cm^{-1} in FTIR Spectrometer Shimadzu 8400 series. The drug dry powder samples were made die pressed pellets with KBr and the FRIR spectra were determined shown in Fig 5.1 for DOLU, Fig 5.2 for TEN & Fig 5.3 for RILP. The principal IR peaks recorded and observed for the drugs are shown in Table 5.2, 5.3 & 5.4 for DOLU, TEN & RILP respectively.

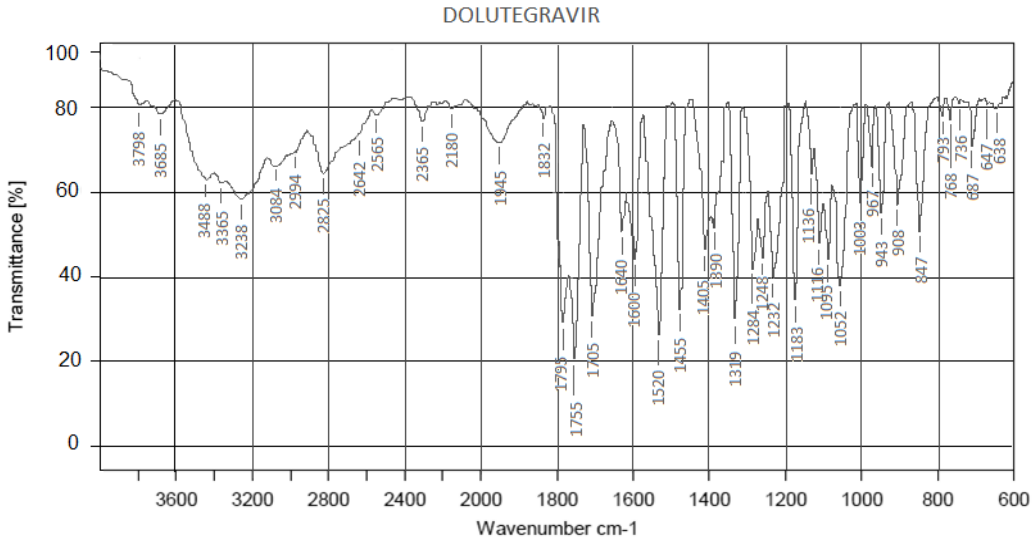


Figure 5.1: FTIR Spectra of Dolutegravir DOLU

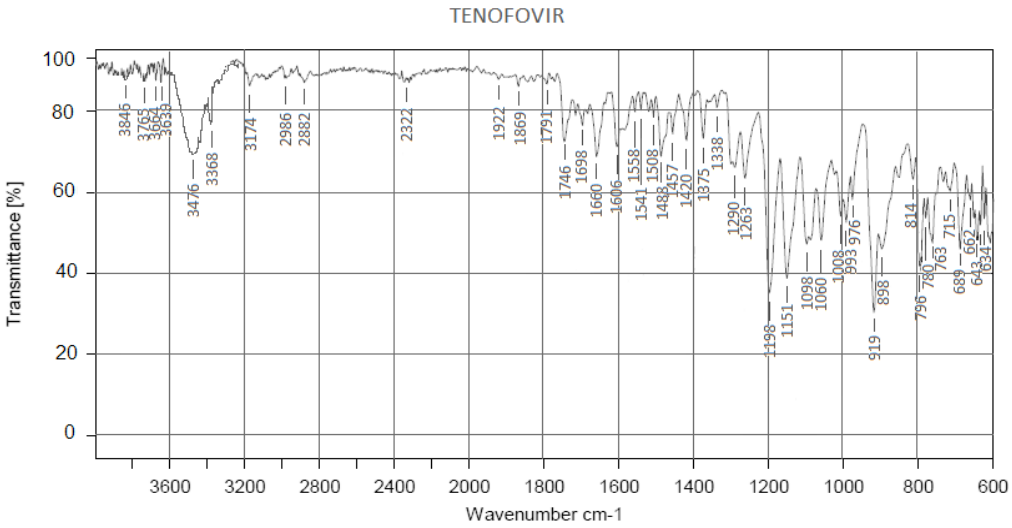


Figure 5.2: FTIR Spectra of Tenofovir TEN

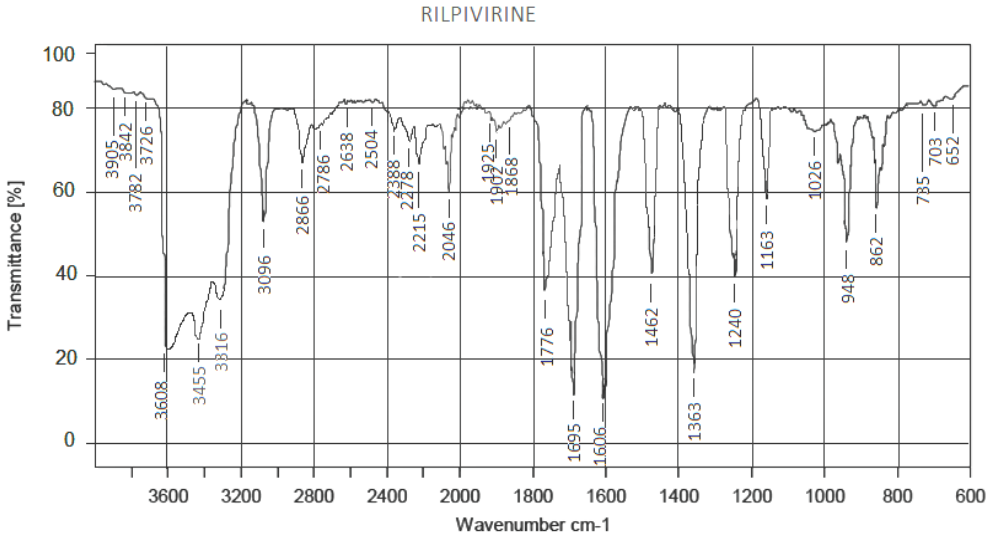


Figure 5.3: FTIR Spectra of Rilpivirine RILP

DOLUTEGRAVIR					
Energy (Cm^{-1})	**Band Assignment**	**Peak Intensity**	**Energy (Cm^{-1})**	**Band Assignment**	**Peak Intensity**
1647	C=C (Aromatic)	53.26	1462	C-H Aromatic	32.85
1400-1000	-CF	38.54 44.84 57.49	1500-1700	N-H (amine)	31.25 43.14
1800-1700	C=O ketone	20.65 31.25	3000-2800	C-H Methyl	64.29 71.03 68.46

				group	
1400- 1390-1310	-OH phenolic	31.25 44.84	3350-3310	N-H (2^0 Amine)	62.06
1749-1792 1275-1200	C-O (Ether)	29.86 43.58	1235-1268	C-N amine	40.98 44.24
1680- 1640-1630	C=O Amide	52.63 45.42	2000-1650	C-H Aromatic	74.23 78.35 31.25
1250-1020	C-N Amine	46.52 37.94	1462	C-H Aromatic	32.85

Table 5.2: FTIR Interpretation of Dolutegravir DOLU

TENOFOVIR					
Energy (Cm^{-1})	**Band Assignment**	**Peak Intensity**	**Energy (Cm^{-1})**	**Band Assignment**	**Peak Intensity**
1200-1100	C-O (S)	44.53 36.26 39.41	1320-1440 909-1000	P=O P-O	80.52 72.37 31.21 59.17 56.18
1690-1640	C=N	77.54 68.56	1350-1250	N-C=N	81.46 63.34
1210- 1163-1300	C-O	65.72 64.51	1250-1020	C-N Amine	56.71 47.23 63.46
1100-1260	C=O	63.24 37.62	1200-1350	N-C (3^0 Amine)	36.67 80.13
3100- 2900-2840	C-H Alkyl groups	92.54 91.49	3500-3100	N-H (1^0 Amine)	67.71 78.51 85.84

Table 5.3: FTIR Interpretation of Tenofovir TEN

RILPIVIRINE					
Energy (Cm^{-1})	**Band Assignment**	**Peak Intensity**	**Energy (Cm^{-1})**	**Band Assignment**	**Peak Intensity**
1235-1268	C-N (S)	40.04	200-1650	C-H Aromatic	58.72
1675-1665	C=C Alkene	41.86	1250-1020	C-N Amine	76.48
1690-1640	C=N imine	16.56	2240-2200	-CN Aromatic Nitrile	66.25
1462	C-H Aromatic	41.23	3000-2800	C-H Methyl group	56.42
1500-1700	N-H (amine)	38.34	890-950	C=N Aromatic	48.67
3350-3310	N-H (2^0 Amine)	18.62	1675-1665	C=C Alkene	41.86

Table 5.4: FTIR Interpretation of Rilpivirine RILP

8.1.4 Preparation of Solutions

8.1.4.1 Preparation of standard solutions of DOLU, TEN & RILP

The standard stock soln. individual drugs prepared in 50:50 Methanol : ACN solvent mixture. 10mg of DOLU, 30mg of TEN and 15mg of RILP were individually dissolved in solvent mixture and made upto 100ml with same solvent to give 100 µg/ml DOLU, 300 µg/ml TEN & 150 µg/ml RILP standard stock solution.

From the above stock solutions of individual drugs DOLU, TEN, RILP each, 10ml from each was taken individually and diluted upto 100ml in individual volumetric flasks to give DOLU 10 µg/ml , TEN 30 µg/ml , RILP 15 µg/ml individual drug standard Final solutions.

8.1.4.2 Preparation of Sample Solutions

Sample solution from tablets, DOLUVIR™ contains 50mg Dolutegravir, TENVIR™ contains 300mg Tenofovir, EDURANT™ contains 25mg of

Rilpivirine. Accurately the avg. wt. of each 10 tablets individually was done and crushed triturated, the individually taken tablet powder was taken weighing equiv alent wt of DOLU 50mg dissolved in 50ml of 50:50 Methanol : ACN, & made upto 50ml to give (stock soln D1 1000ug/ml), and TEN 300mg in 100ml same solvent mixture to give (stock soln T1 3000ug/ml), and RILP 25mg in 10ml solvent mixture to give (stock soln R1 2500ug/ml). The combined solution B was made by mixing aliquots of 10ml of D1, 10ml of T1 & 6ml of R1 stock solns taken in a common single flask 100ml flask & made up to 100ml with solvent to give combined solution B (DOLU:TEN:RILP 100:300:150 ug/ml). From this combined solution B, 1ml was taken and diluted to 10ml to give final solution C (DOLU:TEN:RILP 10:30:15 ug/ml)

8.1.4.3 Preparation of Optimized Mobile Phase

The mobile phase made by taking 65:15:20 ratio, 0.05M Phosphate buffer : ACN : Methanol of pH -3.5. The phosphate buffer was prepared by accurately weighing 6.8gm KH_2PO_4 (MW. 136) in 1000ml HPLC grade milli-Q system purified water. The pH adjusted by 1% OPA Ortho-phosphoric acid. After filtration it was sonicated and the 1% OPA was prepared by taking (1.176ml) of 85%w/v orthophosphoric acid (MW 98) in 100ml HPLC grade water.

8.1.5 Selection of Wavelength for Detection

The Final standard solns of DOLU 10 µg/ml , TEN 30 µg/ml & RILP 15 µg/ml scanned in 200 - 400 nm in UV-visible double beam spectrophotometer at a medium scanning speed. The overlain spectra shown in Fig. 5.4 of DOLU 10 µg/ml , TEN 30 µg/ml & RILP 15 µg/ml were taken in 50:50 Methanol : ACN and the 229.6nm wavelength was selected for estimation in the detection during the HPLC analysis.

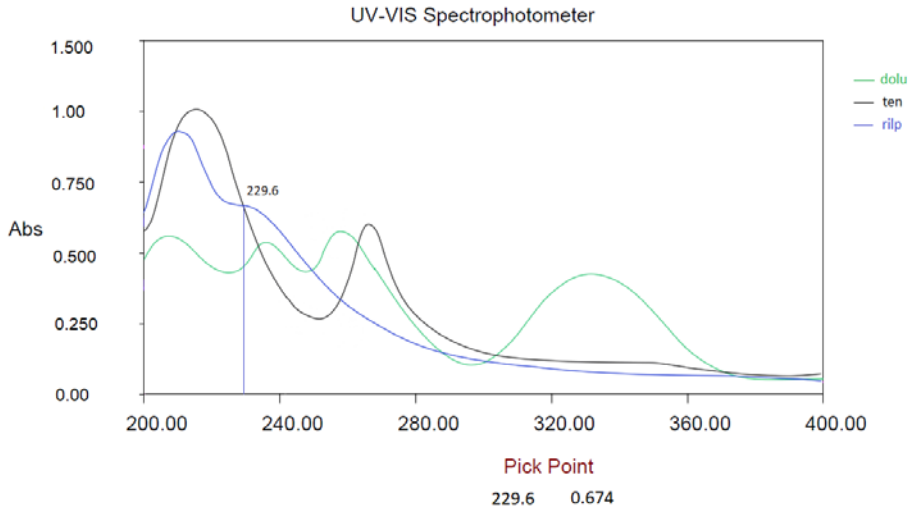


Figure 5.4: UV Spectra Overlay of DOLU, TEN & RILP

8.1.6 Selection and Optimization of Mobile phase

For the detection analysis of the DOLU, TEN & RILP drugs in the combined form in the working standard solutions by the HPLC method had been carried out in reverse phase by using polar solvents in mobile phase. The various trials with different mobile phase's has been carried out for the detection and seperation of the drugs was carried out shown in Table 5.5

Sr No	Mobile Phase	pH	Ratio (v/v)	Retention Time (min)			REMARK
				DOLU	TEN	RILP	
1	H_2O:MeOH	-	50:50	-	-	-	No peak detected
2	H_2O:MeOH	-	80:20	-	-	-	No peak detected
3	H_2O:MeOH	-	20 :80	-	-	-	No peak detected
4	ACN : Methanol	-	50:50	-	-	-	No peak detected

5	ACN : Methanol	-	80:20	-	-	-	No peak detected
6	ACN : Methanol	-	20:80	-	-	-	No peak detected
7	0.05 M Phosphate buffer:ACN	7	50:50	8.52	12.39	-	Peak Tailing No peak of RIL detected
8	0.05 M Phosphate buffer:ACN	7	20:80	14.49	-	-	Longer Run time, Tailing in peak No peak of TEN & RIL
9	0.05 M Phosphate buffer:ACN	6.5	80:20	8.15	8.76	8.89	Peak Merging & Tailing Not good separation
10	0.05 M Phosphate buffer:ACN	5	80:20	5.81	7.92	8.13	TEN+RIL peak merging
11	0.05 M Phosphate buffer:ACN	5	75:25	6.75	6.53	9.37	Peak Tailing Peak merging DOL+TEN
12	0.05 M Phosphate buffer:ACN	4	80:20	4.59	7.04	7.48	TEN+RIL peak merging Not good separation

13	0.05 M Phosphate buffer:ACN	3.5	80:20	3.16	5.52	6.06	TEN+RIL peak merging Not good separation
14	0.05 M Phosphate buffer : ACN : Methanol	7	80:10 :10	5.73	-	-	No peak of TEN, RILP detected
15	0.05 M Phosphate buffer : ACN : Methanol	6	50:25 :25	4.68	10.53	10.3 7	Tailing of peaks & Peak merging TEN+RIL
16	0.05 M Phosphate buffer : ACN : Methanol	5	60:20 :20	4.08	8.19	8.28	Peak merging TEN+RIL
17	0.05 M Phosphate buffer : ACN : Methanol	4.5	60:20 :20	3.24	6.83	7.14	Peak merging TEN+RIL Less Resolution
18	0.05 M Phosphate buffer : ACN : Methanol	4	60:20 :20	2.84	4.72	4.96	Peak merging TEN+RIL Less Resolution
19	**0.05 M Phosphate buffer : ACN: Methanol**	**3.5**	**65:15 :20**	**2.53**	**3.46**	**4.76**	**Good separation**

Table 5.5: Trials for Selection of Mobile Phase for DOLU, TEN & RILP

8.1.7 Optimized Chromatographic Conditions

Optimized chromatographic conditions for developed HLPC analytical method are shown below-

Parameters	Conditions
Stationary Phase Coloumn	C18 Hypersil BDS 250 x 4.6mm , 5 micron
Mobile phase	Phosphate buffer :ACN:Methanol: 65:15:20- pH- 3.5
Flow rate	1ml/minl
Injection volume	20ul
Temp	Ambient Lab Temperature
Detection Wavelength	229.6nm
Retention Times (min)	DOLU-2.53, TEN-3.46, RILP-4.76

Table 5.6: Optimized Chromatographic Conditions for DOLU, TEN & RILP

8.2 METHOD VALIDATION

8.2.1 Linearity (Calibration Curve)

The working standard and sample solutions of DOLU was 2.5, 5, 7.5, 10, 12.5, 15 & TEN was 7.5, 15, 22.5, 30, 37.5, 45ug/ml, while 3.75, 7.5, 11.25, 15, 18.75, 22.5ug/ml of RILP, for conc. range, linearity, validation parameters and same con. ranges were used for the stability forced degradation studies. The calibration curves has been generated by plotting graph of peak area vs conc. for the drugs, and the regression equations, correlation coefficient R^2 value and the, Limit of Detection (LOD) & Limit of Quantification (LOQ) had been calculated

8.2.2 Specificity and Selectivity

The selectivity and specificity parameters are utilised in selective detection particular analyte which are in the matrix or along with other substances without any interventions. 10ug/ml of DOLU & TEN 30ug/ml & RILP 15ug/ml were injected individually, and blank mobile phase as well as sample solutions from dosage form were compared to check the specificity & selectivity. Selectivity is a type of a qualitative determination of analytes, while the specificity is applied for both

qualitative as well as quantitative estimations. The developed method must be selective and highly specific for the analyte for which the method is intended to use, even in presence of impurities or any other degraded products, additives, excipients, reagents or other substances.

8.2.3 Accuracy (Recovery Studies)

Accuracy is one of the important validation parameter which describes the trueness-exactness of the test results in accordance with the true values. The accuracy studies has been performed by doing the drug recovery studies of deliberately added working standard drugs from the sample, n=3 samples taken for each drug DOLU, TEN, RILP at 50%, 100% & 150% had performed at each level to the pre-analysed samples. The amount of drug-substance added and amount of drug-substance recovered were calculated from the sample peak area and total peak area and the % Recovery had been calculated.

8.2.4 Precision

8.2.4.1 Repeatability (n=6)

The repeatability study has been performed by repeatedly n=6 sample standards injected 10ug/ml of DOLU & TEN 30ug/ml & RILP 15ug/ml and the area response of drugs was obtained and the %RSD had been calculated

8.2.4.2 Intraday Precision (n=3)

The intraday precision was performed by using the 2.5, 10, 15 ug/ml of DOLU & TEN was 7.5, 30, 45ug/ml, while 3.75, 15, 22.5 ug/ml for RILP was used, and the solutions were repeatedly injected analysed by HPLC three times on same day, obtained results calculated into the terms of %RSD.

8.2.4.3 Interday Precision (n=3)

The interday precision was performed by using the 2.5, 10, 15 ug/ml of DOLU & TEN was 7.5, 30, 45ug/ml, while 3.75, 15, 22.5 ug/ml for RILP was used, and the solutions were repeatedly injected analysed by HPLC three times in different days obtained results calculated into the terms of %RSD.

8.2.5 LOD and LOQ

The LOD Limit of Detection has been obtained from 5 set of the calibration curves performed in the linearity-range studies, the LOD is calculated as LOD = 3.3 x SD/Slope

LOQ Limit of Quantitation has been obtained from the same 5 set of the calibration curves performed as per the linearity-range studies, the LOD is calculated as LOD = 10 x SD/slope

8.3 APPLICATION OF DEVELOPED ANALYTICAL METHOD AS A ASSAY METHOD FOR MARKETED FORMULATION

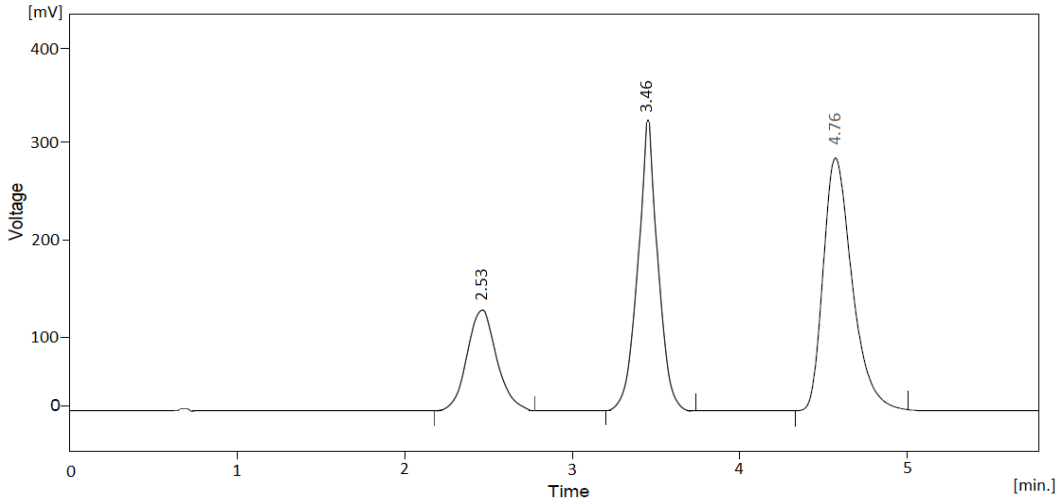
The developed analytical HPLC method is applied in the estimation-analysis of DOLU, TEN, RILP, in the tablets DOLUVIR™ contains 50mg Dolutegravir, TENVIR™ contains 300mg Tenofovir, EDURANT™ contains 25mg of Rilpiviri ne. Accurately the avg. wt. of each 10 tablets individually was done and crushed triturated, the individually taken tablet powder was taken weighing equiv alent wt of DOLU 50mg dissolved in 50ml of 50:50 Methanol : ACN, & made upto 50ml to give (stock soln D1 1000ug/ml), and TEN 300mg in 100ml same solvent mixture to give (stock soln T1 3000ug/ml), and RILP 25mg in 10ml solvent mixture to give (stock soln R1 2500ug/ml). The combined solution B was made by mixing aliquots of 10ml of D1, 10ml of T1 & 6ml of R1 stock solns taken in a common single flask 100ml flask & made up to 100ml with solvent to give combined solution B (DOLU:TEN:RILP 100:300:150 ug/ml). From this combined solution B, 1ml was taken and diluted to 10ml to give final solution C (DOLU:TEN:RILP 10:30:15 ug/ml), were prepared and analysed by HPLC and the % purity or % label claim was estimated by comparing the area & calculating from regression equation, for working standard drug and marketed formulation.

8.4 RESULTS & DISCUSSIONS

8.4.1 Method Development

The developed analytical HPLC method found to be reliable, accurate.,- precise for analysis and quality control testing for DOLU, TEN and RILP in pure form, in marketed tablet dosage form's. The method is advantageous as the low cost solvents are used, good resolution and seperation has been achieved, as well as the peak symmetry tailing factor are in greater acceptable limits. The isocratic mode adds the advantage of simplicity of the developed method. Method consists of the optimized mobile phase Phosphate buffer:ACN:Methanol (65:15:20) pH 3.5, flow rate 1ml / min , detection wavelength at 229.6nm. Excipients in the marketed formulation

does not affect in the resolution, separations as well do not have any interfering peaks. The average retention times were found to be DOLU-2.53, TEN-3.46 and RILP-4.76 minutes. The chromatogram of the drugs are shown below.



Result Table (Uncal - DOLU- TEN- RIL-standard) phosphate buffer pH - 3.5 : ACN : Methanol 65:15:20

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Peak Purity
1	2.531	1207.651	134.249	19.999	0.999
2	3.467	2379.594	323.458	39.406	0.998
3	4.762	2451.298	278.137	40.594	0.999
	Total	6038.543	735.844	100.000	

Column Performance Table (From - DOLU- TEN- RIL- standard)

	Reten. Time	Asymmetry [-]	Efficiency [th.pl]	Resolution [-]
1	2.531	1.416	5537	-
2	3.467	1.237	6972	1.985
3	4.762	1.314	7891	2.349

Figure 5.5: Chromatogram of Standard DOLU, TEN & RILP

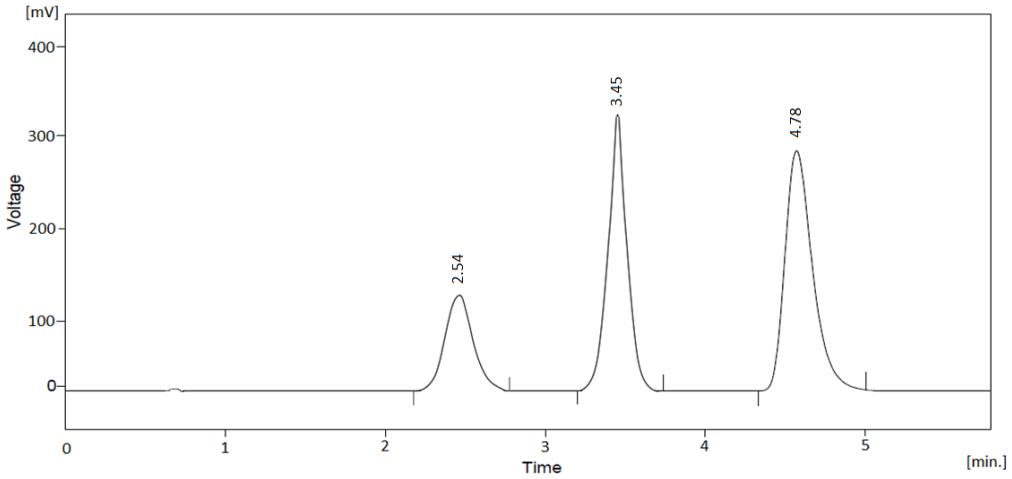


Figure 5.6: Chromatogram of Sample DOLU, TEN & RILP

8.4.2 Method Validation

8.4.2.1 Specificity

Developed method is specific and selective as the no other peaks of, mobile phase or any excipients impurities were interfering or overlapping in the chromatograms.

The method effectively analyses the drugs in pure form as well as in the marketed formulations with accuracy, and has reproducible results for individual drugs as well as for the combined formulation analysis.

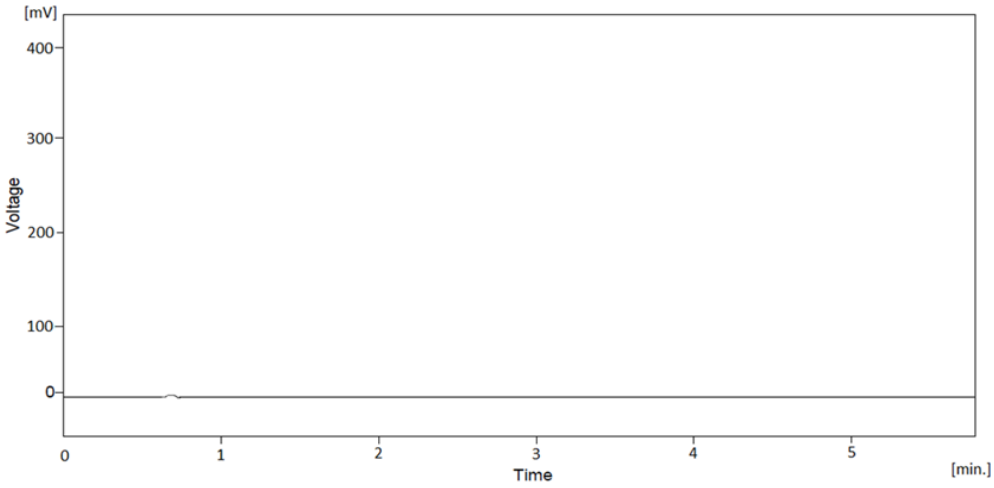


Figure 5.7: Blank Chromatogram

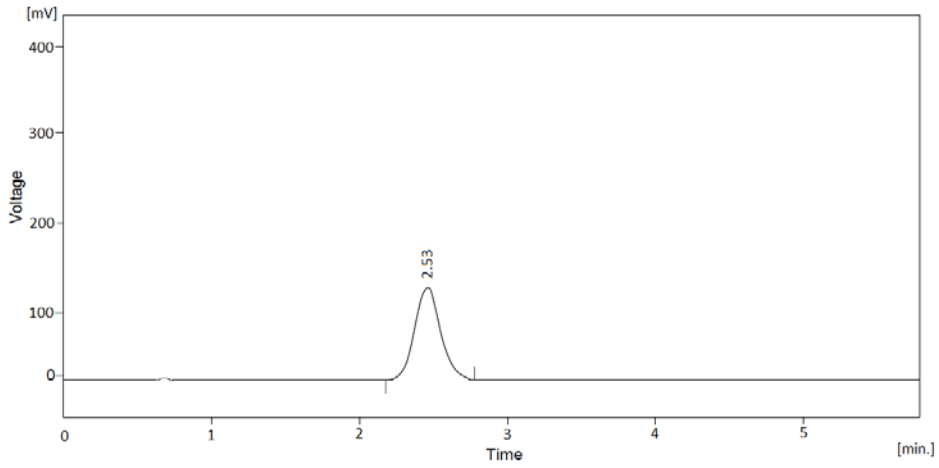


Figure 5.8: Chromatogram of DOLU

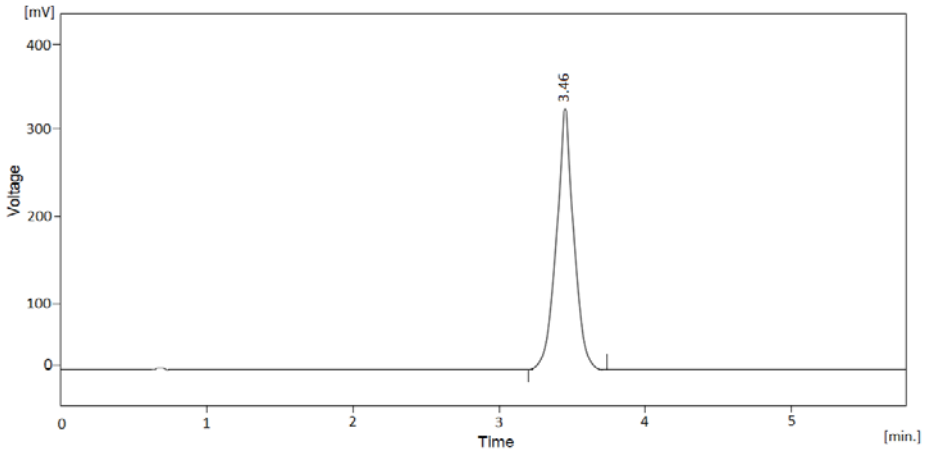


Figure 5.9: Chromatogram of TEN

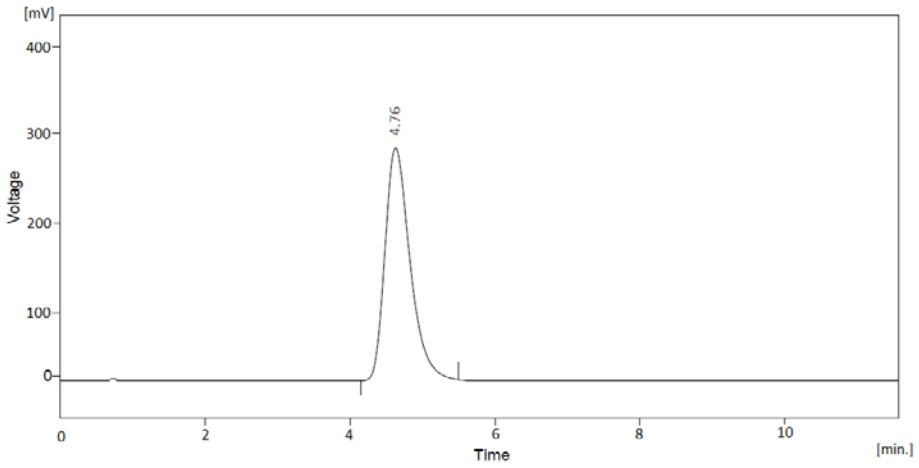


Figure 5.10: Chromatogram of RILP

8.4.2.2 Linearity and Range (n = 5)

Drugs LAM, TEN and DOR Linearity has been followed in a particular concentration ranges of 2.5-15ug/ml for DOLU, 7.5-45ug/ml for TEN and 3.75 - 22.5ug/ml for RILP. The linearity showing overlain chromatogram had been generated and the calibration curve been plotted of peak area vs conc. and straight line eqn. and correlation coefficient had been calculated.

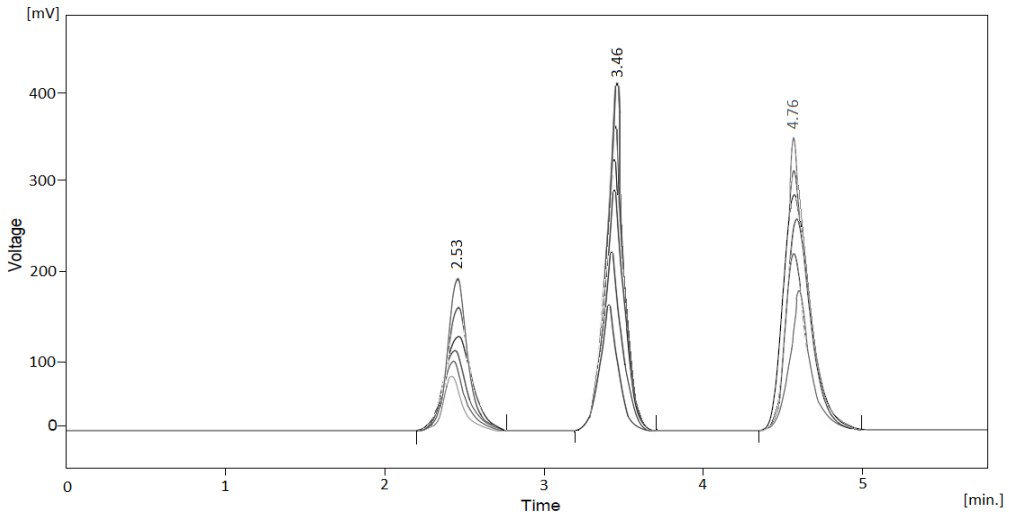


Figure 5.11: Overlain Chromatogram of Linearity for DOLU, TEN & RILP

(x) Conc. µg/ml	(y) Area
2.5	289.64
5	601.16
7.5	895.30
10	1207.65
12.5	1488.78
15	1815.29
STD ERROR	9.16
Slope	121.1
LOD	0.24
LOQ	0.75

Table 5.7: Linearity data of DOLU

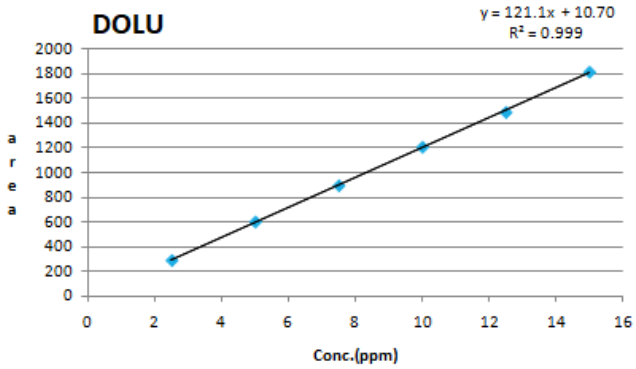


Figure 5.12: Calibration Curve for DOLU

(x) Conc. µg/ml	(y) Area
7.5	611.49
15	1138.25
22.5	1798.52
30	2379.59
37.5	2982.66
45	3559.18
STD ERROR	31.81
Slope	79.43
LOD	1.32
LOQ	4.00

Table 5.8: Linearity data of TEN

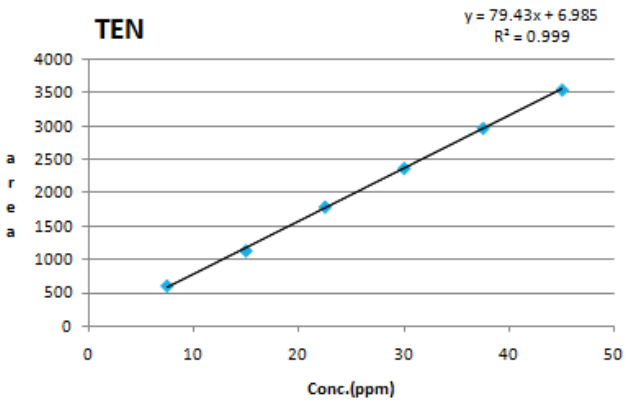


Figure 5.13: Calibration Curve for TEN

(x) Conc. µg/ml	(y) Area
3.75	618.76
7.5	1229.52
11.25	1844.80
15	2451.29
18.75	3082.39
22.5	3674.27
STD ERROR	6.92
Slope	163.3
LOD	0.14
LOQ	0.42

Table 5.9: Linearity data of RILP

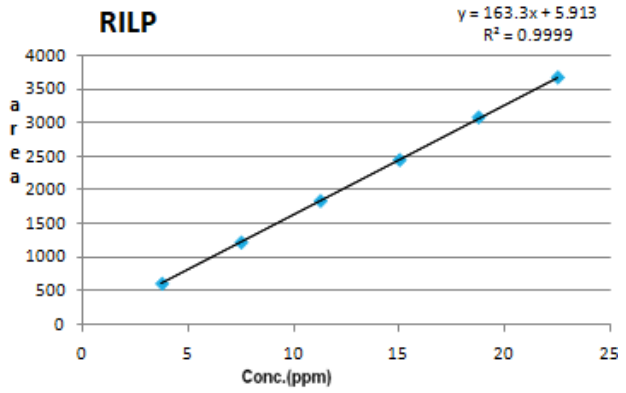


Figure 5.14: Calibration Curve for RILP

8.4.2.3 Accuracy (Recovery Studies) (n = 3)

The accuracy has been done by performing the recovery studies of the working standard drug from the pre-analysed sample of the drugs DOLU, TEN and RILP. The recovered drug from the samples has been calculated as % Recovery is been reported in the table below.

Drug	Amt of Sample Taken (µg)	% Amt of Std Added	Spiked Std Drug Amount (µg)	Spiked Amt Recovered Mean (µg)	% Recovery	% Mean Recovery
DOLU	10	50	5	4.97	99.52	98.98
	10	100	10	9.81	98.11	
	10	150	15	14.89	99.29	
TEN	30	50	15	14.94	99.65	99.38
	30	100	30	29.80	99.33	
	30	150	45	44.61	99.15	
RILP	15	50	7.5	7.53	100.42	100.34
	15	100	15	15.06	100.44	
	15	150	22.5	22.53	100.16	

Table 5.10: Accuracy Study of DOLU, TEN & RILP (n = 3)

8.4.2.4 Precision

8.4.2.4.1 Repeatability (n = 6)

The repeatability study of DOLU, TEN and RILP have been performed by multiple injections of the samples of the drugs (n = 6). The repeatability data for the DOLU, TEN and RILP is shown in the table below.

Conc. of DOLU (µg/ml)	Area	Conc. of TEN (µg/ml)	Area	Conc. of RILP (µg/ml)	Area
10	1207.65	30	2379.59	15	2451.29
	1211.85		2386.17		2456.72
	1216.72		2375.51		2453.52
	1221.94		2377.38		2459.86
	1213.45		2372.42		2461.43
	1217.63		2376.73		2455.58
Mean	1214.87	Mean	2377.96	Mean	2456.4
SD	4.98	SD	4.66	SD	3.80
% RSD	0.41	% RSD	0.19	% RSD	0.15

Table 5.11: Repeatability Study of DOLU, TEN & RILP (n = 6)

8.4.2.4.2 Intraday Precision (n = 3)

The Intraday precision for the DOLU, TEN & RILP has been performed by taking multiple injections (n = 3) in a same day at different 25, 100, 150 % Levels. The data for the intraday precision is shown in table below.

DOLU			TEN			RILP		
Conc. (µg/ml)	Mean area ± SD	% RSD	Conc. (µg/ml)	Mean area ± SD	% RSD	Conc. (µg/ml)	Mean area ± SD	% RSD
2.5	287.5 ± 2.8	0.99	7.5	611.4 ± 6.8	1.11	3.75	618.5 ± 3.9	0.64
10	1218.3 ± 6.1	0.50	30	2384.0 ± 4.1	0.17	15	2453.0 ± 4.5	0.18
15	1820.3 ± 4.4	0.24	45	3558.4 ± 5.7	0.16	22.5	3679.1 ± 5.7	0.15

Table 5.12: Intraday Precision of DOLU, TEN & RILP (n = 3)

8.4.2.4.3 Interday Precision (n = 3)

The Interday precision for the DOLU, TEN & RILP has been performed by taking multiple injections (n = 3) in different day at different 25, 100, 150 % Levels. The data for the intraday precision is shown in table below.

DOLU			TEN			RILP		
Conc. (µg/ml)	**Mean area ± SD**	**% RSD**	**Conc. (µg/ml)**	**Mean area ± SD**	**% RSD**	**Conc. (µg/ml)**	**Mean area ± SD**	**% RSD**
2.5	288.5 ± 4.3	1.49	7.5	613.1 ± 2.1	0.35	3.75	624.2 ± 6.5	1.0
10	1213.8 ± 5.7	0.47	30	2372.3 ± 6.8	0.28	15	2458.4 ± 7.7	0.31
15	1819.4 ± 4.6	0.26	45	3564.2 ± 4.8	0.13	22.5	3681.0 ± 5.9	0.16

Table 5.13: Interday Precision of DOLU, TEN & RILP (n = 3)

8.4.2.5 LOD and LOQ

It has been calculated from the n=5 samples from the calibration curve slope and standard deviation. The LOD value are found to be 0.24, 1.32, and 0.14 ug respectively for DOLU, TEN & RILP, and the LOQ values are found to be 0.75, 4.0 and 0.42 ug respectively for DOLU, TEN & RILP.

8.4.3 Application of the Developed Analytical Method to Formulation

The proposed analytical method been tested in assay analysis % Assay of the Label claim on the DOLUVIR™ contains 50mg Dolutegravir, TENVIR™ contains 300mg Tenofovir, EDURANT™ contains 25mg of Rilpivirine.

Analytical method successfully applied to the estimation of drugs in marketed product by comparing with the standard and the sample formulation. The assay result are shown in the table below.

	Serial no	Label claim (mg)	Result (mg)	% Label Claim	Avg % Assay	SD	% RSD
DOLU	1	50	49.23	98.46	98.01	0.52	0.53
	2	50	48.72	97.44			
	3	50	49.07	98.14			
TEN	1	300	297.70	99.23	99.24	0.13	0.13
	2	300	298.14	99.38			
	3	300	297.36	99.12			
RILP	1	25	24.81	99.24	98.40	0.26	0.26
	2	25	24.53	98.12			
	3	25	24.46	97.84			

Table 5.14: Assay of Formulations (n = 3)

8.4.4 Summary of Results

Sr No	**Parameters**	**Results**		
		DOLU	**TEN**	**RILP**
1	System Suitability:			
	Theoretical plates-	5537	6972	7891
	Tailing Factor-	1.41	1.23	1.31
	Retention time min-	2.53	3.46	4.76
2	Precision (%RSD)	0.50	0.17	0.18
3	Linearity (R^2)	0.9999	0.9999	0.9998
4	Accuracy (% Recovery)	98.98	99.38	100.34
5	LOD (ug/ml)	0.24	1.32	0.14
6	LOQ (ug/ml)	0.75	4.00	0.42
7	% Assay	98.01	99.24	98.10

8.5 CONCLUSIONS

The Analytical HPLC method for DOLU, TEN & RILP combinational drugs has been successfully developed and validated. The analytical method is optimized in testing, analysis of these selected drugs in individual as well in the combined forms and all validation parameters., are performed in the acceptance criteria as per ICH regulatory guideline. Method that has been developed., is been, optimized to analyse minimum conc. of drugs in-pure form and, in testing-analysing marketed formulation. The HPLC analytical method is applied in the estimation for dissolution profile studies of the combined tablet marketed dosage form. Accurate precise method developed., can be used for analysis of DOLU, TEN & RILP combination as well as individual in as Assay method and dissolution testing procedures in academics, research, analytical laboratories and pharmaceutical industries.