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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF LAMIVUDINE AND DOLUTEGRAVIR BY RP-HPLC METHOD

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ABSTRACT

A simple, precise, accurate, efficient and reproducible, isocratic Reverse Phase- High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Lamivudine and Dolutegravir in bulk and tablet dosage form. Lamivudine and Dolutegravir were separated using a Shimadzu column the mobile phase contained a mixture of Methanol and 0.1% TFA (TriFluoro Acetic acid),(65:35 v/v). The flow rate was set to 1.0 ml/min. with the response measured at 266nm. The retention time of Lamivudine and Dolutegravir was found to be 2.769 min. and 7.496 min. respectively with a resolution of 11.429. Linearity was established for Lamivudine and Dolutegravir in the range of 12-60µg/ml.and 03-10µg/ml. with correlation coefficient of 0.9997 and 0.9993. The percentage recovery of Lamivudine and Dolutegravir was found to be 99.97%, and 99.93% respectively. Validation parameters such as specificity, linearity, precision, accuracy, robustness, limit of detection(LOD), limit of quantification (LOQ) was evaluated for the method according to the International Conference on Harmonization (ICH) Q2 (R1) guidelines.

Keywords: Lamivudine, Dolutegravir, RP-HPLC, Methanol, Trifluoro acetic acid

1. INTRODUCTION

Lamivudine (LAM) is (3TC, Epivir) is an antiviral drug that reduces the amount of HIV in the body. Anti-HIV drugs such as lamivudine slow down or prevent damage to the immune system, and reduce the risk of developing AIDS-related illnesses. Lamivudine is also active against hepatitis B virus (HBV). Lamivudine (Epivir-HBV) is used to treat hepatitis B infection. Lamivudine is in a class of medications called nucleoside reverse transcriptase inhibitors (NRTIs). It works by decreasing the amount of HIV and hepatitis B in the blood **[1]**. Lamivudine is chemically 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one. Lamivudine is a nucleoside reverse transcriptase inhibitor and works by blocking the HIV reverse transcriptase and hepatitis B virus polymerase. Dolutegravir is a human immunodeficiency virus (HIV) integrase inhibitor, the third in this class of agents that target the viral integrase. Dolutegravir is used only in combination with other antiretroviral agents in the treatment of HIV infection, and it has had limited use. Dolutegravir is associated with a low rate of serum aminotransferase elevations during therapy, but has not been linked to instances of acute, clinically apparent liver injury. Dolutegravir is chemically (4R,12aS)-N-(2,4-Difluorobenzyl)-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2H-pyrido[1',2':4,5]pyrazino[2,1-b][1,3]oxazine-9-carboxamide. It works by decreasing the amount of HIV in your blood and increasing the number of immune cells that help fight infections in your body. Dolutegravir is used with other HIV medications to help control HIV infection. It helps to decrease the amount of HIV in your body so your immune system can work better. This lowers your chance of getting HIV complications (such as new infections, cancer) and improves your quality of life **[1]**.

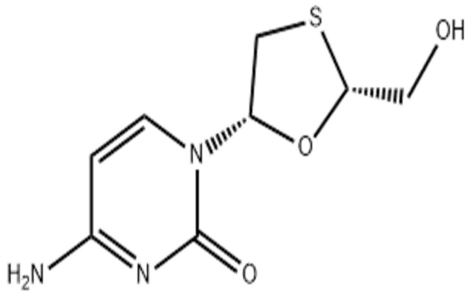

Figure 1: Lamivudine

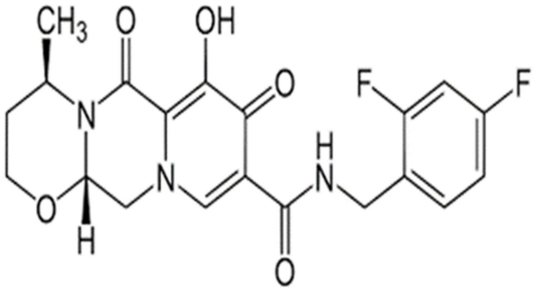

Figure 2: Dolutegravir

2. METHOD DEVELOPMENT:

2.1. Chemicals:

Lamivudine and Dolutegravir in Pharmaceutical formulations were procured from Sigma Aldrich. Dovato tablet was used as sample. Reagents and solvents such as Acetonitrile, Methanol, 0.1% TFA (TriFluoro Acetic acid) and HPLC water of analytical grade were used.

2.2 Instrumentation: Equipment used was Shimadzu 2030 LC Prominence with PDA detector. pH meter was manufactured by LABINDIA, Analytical balance manufactured by ESSAE.

2.3 Preparation of 0.1% TFA: 0.1 ml. of conc. of TFA was pipette out into a clean and dried 100.0ml. volumetric flask and volume was made up to the mark. The solution was filtered through 0.45µ membrane filter by using vacuum filtration apparatus.

2.4 Mobile phase: Methanol and0.1% TFA in HPLC water grade were used in the proportion of 65:35 v/v as mobile phase.

2.5 Solvent or Diluent: Acetonitrile and HPLC water were used in the ratio of 50:50v/v.

3. METHOD DEVELOPMENT

Lamivudine and Dolutegravir was analyzed in a Shimadzu Column for the chromatographic separation. The mobile phase was composed of Methanol and 0.1% TFA (65:35v/v).The solution was filtered through 0.45µm membrane filter under vacuum filtration and pumped at ambient temperature, at a flow rate of 1.0 ml/min with UV detection wavelength at 266 nm. Injection volume was 20µl.The run time was 11min. and the retention time of Lamivudine and Dolutegravir was found to be 2.769 min. and 7.496 min. with a resolution of 11.429.

3.1 Preparation of standard stock solutions of Lamivudine and Dolutegravir: Standard stock solutions of Lamivudine and Dolutegravir were prepared by dissolving 10mg. of Lamivudine and Dolutegravir in 10ml. of Acetonitrile and HPLC water (50:50 v/v) solution into a clean dry volumetric flask to get the concentration of 1000µg/ml. of Lamivudine and Dolutegravir.

3.2 Preparation of sample solution: 20 tablets (each contains 300mg of Lamivudine and 50 mg. of Dolutegravir) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Weight equivalent to one tablet powder of Lamivudine and Dolutegravir was dissolved in sufficient mobile phase. After that filtered the solution using 0.45µm membrane filter under vacuum filtration and sonicated for 5min and dilute to 100ml with mobile phase.

4. RESULTS AND DISCUSSION

The proposed method was validated according to ICH Q_2 (R1) guidelines.

4.1 System suitability test: System suitability test parameters were checked by repetitively injecting the drug solution at the concentration level of 36µg/ml and 06µg/ml for Lamivudine and Dolutegravir to check the reproducibility of the system. System suitability parameters like Retention time, Number of theoretical plate (N), Tailing factor, Resolution of repetitive injections were studied. No. of theoretical plate count, tailing factor, Resolution are reported in **Table 1**.

4.2 Linearity: A series of standards of Lamivudine and Dolutegravir were prepared over a range of 12-60µg/ml. and 03-10µg/ml. respectively from the stock solution and calibration graph was obtained by plotting peak area versus concentration of Lamivudine and Dolutegravir. The chromatograms were shown in **Figures 3, 4, 5, 6** and the results were given in **Table 2**.

4.3 Precision:

System Precision Peak Area and Method Precision Peak Area: Precision is the measure of closeness of the data values to each other for a number of measurements under the same analytical conditions. Precision of the test method was determined by six replicates (n=6) solutions were prepared and each solution was injected in duplicate under the same conditions and mean value of peak area response for each solution were considered. The results were given in **Table 3**.

4.4 Intermediate Precision/Ruggedness: The intermediate precision of the method was evaluated by performing precision on different lab by different analyst and different days. The standard preparation concentrations of 36µg/ml. of Lamivudine and 06µg/ml. of Dolutegravir was injected six times in to the HPLC and the %RSD for the area of 6 replicate injections was calculated. The results were given in **Table 4**.

4.5 Accuracy: The accuracy of the method was determined by calculating recovery of Lamivudine Dolutegravir at 50%, 100%, 150% was added to a pre quantified sample solution and injected in to the HPLC system. The mean percentage recovery of Lamivudine and Dolutegravir at each level was calculated and given in **Table 5 and 6**.

4.6 LOD and LOQ: LOD is the detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. LOQ is The quantification limit of an individual

analytical procedure is the lowest amount of analyte in a sample which can be quantitively determined with suitable precision and accuracy.LOD and LOQ were calculated as 3.3XSD/S and 10XSD/S respectively as per ICH guidelines, Where SD is the standard deviation of the response (Y-intercept) and S is the slope of the calibration curve. The LOD is the smallest concentration of analyte that gives a measurable response (Signal to noise ratio of 3). The LOD of Lamivudine and Dolutegravir was found to be 36µg/ml and 09µg/ml. The LOQ is the smallest concentration of analyte that gives a response that can be accurately quantified (Signal to noise ratio of 10). The LOQ of Lamivudine and Dolutegravir as found to be 12µg/ml and 03µg/ml. The LOD of Lamivudine and Dolutegravir value is 2.792 and 7.465. The LOQ Lamivudine and Dolutegravir value is 2.795 and 7.477. The LOD and LOQ of Lamivudine and Dolutegravir as shown in **Figure 7 and 8**.

4.7 Robustness: As part of robustness, deliberate change in the flow rate and the mobile phase proportion was made to evaluate the impact on the method. The theoretical plates for Lamivudine and Dolutegravir were found to be 636 and 3883 respectively. The tailing factor for Lamivudine and Dolutegravir were found to be 0.862 and 1.423. The results reveal that the method is robust. The results were given in **Table 7, 8**.

4.8 Assay: 20 tablets (each contains 300mg of Lamivudine and 50mg of Dolutegravir) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Weight equivalent to one tablet powder of Lamivudine and Dolutegravir was dissolved in sufficient mobile phase. After that filtered the solution using 0.45µm membrane filter under vacuum filtration and sonicated for 5min and dilute to 100ml with mobile phase. Peak area of both standard and test was determined. The percent of assay was calculated from the peak area of standard and sample. The percent of assay was calculated by using the following formula. The results were shown in the **Table 9**.

$$\frac{\text{Samplearea}}{\text{Standardarea}} \text{x} \frac{\text{Standard dilution factor}}{\text{Standard weight}} \text{x} \frac{\text{Sample weight}}{\text{Sample dilution factor}} \text{x} \frac{\text{Average weight}}{\text{Labledclaim}} \text{x} \frac{\text{Potency}}{100} \text{x } 100$$

Table 1: System suitability test

Parameter	Lamivudine	Dolutegravir
Retention time	2.782	7.451
Number of Theoretical Plate(USP)	2783	4635
Tailing factor	0.874	1.264
Resolution	NA	10.952

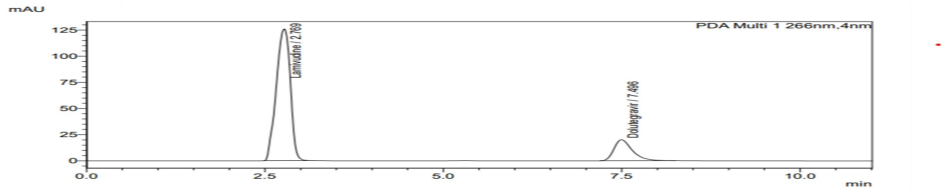

Figure 3: Standard chromatogram

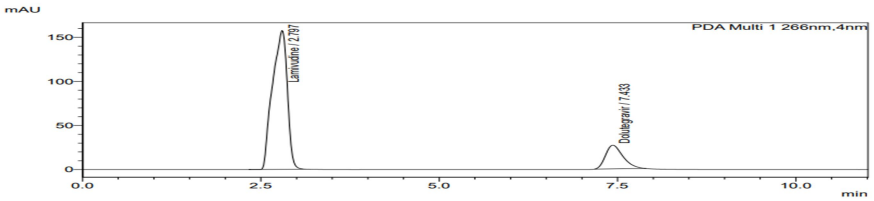

Figure 4: Sample chromatogram

Table 2: Linearity of Lamivudine and Dolutegravir

Linearity of Lamivudine		Linearity of Dolutegravir	
Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area
00	00	00	00
12	547302	03	170270
24	1134912	04	232530
36	1723020	06	355813
48	2274322	08	355813
60	2809820	10	576725

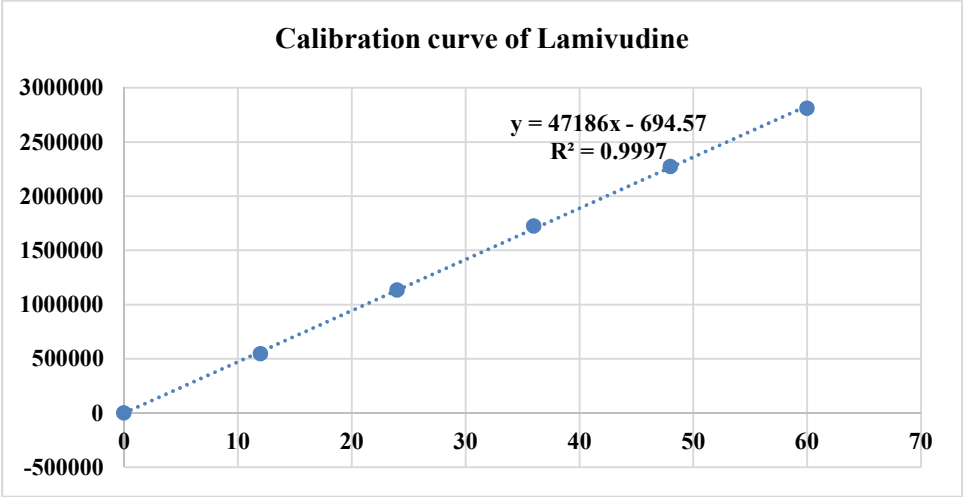

Figure 5: Linearity of Lamivudine

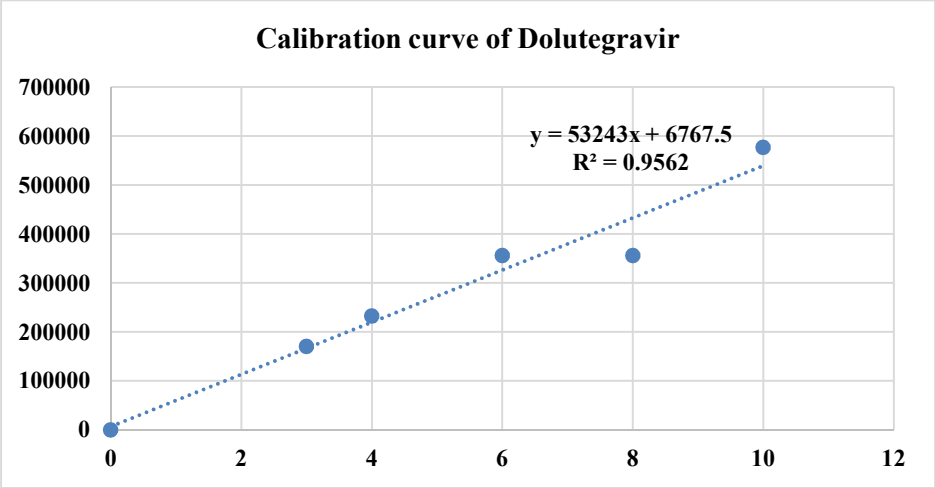

Figure 6: Linearity of Dolutegravir

Table 3: System Precision Peak Area and Method Precision Peak Area

Injection number	System Precision Peak Area		Method Precision Peak Area	
	Lamivudine	Dolutegravir	Lamivudine	Dolutegravir
01.	1740700	323405	1840712	325435
02.	1743526	320273	1843535	323285
03.	1741960	322131	1841965	324145
04.	1729272	322067	1829276	324078
05.	1742272	325686	1842278	327698
06.	1748307	325540	1848325	327658
AVG.	1741006.167	323184	1841015.167	325383.1667
STDEV	6323.737263	2130.258169	6327.402292	1906.558514
%RSD	0.36	0.66	0.343690938	0.585942578

Acceptance criteria: The %RSD for the peak area of six standard injections should not be more than 2.0%.

Table 4: Intermediate precision Peak Area:

Injection number	Intermediate Precision Peak Area	
	Lamivudine	Dolutegravir
01.	1740712	323435
02.	1743535	320285
03.	1741965	322145
04.	1729276	322078
05.	1742278	325698
06.	1748325	325558
AVG.	1741015.167	323199.8333
STDEV	6327.402292	2131.878366
%RSD	0.363431773	0.659616171

Acceptance criteria: The %RSD for the peak area of six standard injections should not be more than 2.0%

Table 5: Accuracy of Lamivudine

Recovery level	Accuracy of Lamivudine				Average percent recovery
	Standard area	Sample Peak Area	%Recovery	%Mean recovery	
50%	879444	1741006	100.88	100.83	100.40%
	878456	1741006	100.83		
	878296	1741006	100.73		
100%	1751006	1741006	100.49	100.3	
	1762006	1741006	101.11		
	1731006	1741006	99.30		
150%	2611006	1741006	101.03	101.08	
	2610123	1741006	101.08		
	2611023	1741006	101.13		

Table 6: Accuracy of Dolutegravir

Recovery level	Accuracy of Dolutegravir				Average percent recovery
	Standard Peak Area	Sample Peak Area	% Recovery	% Mean recovery	
50%	161125	323184	99.57	99.96	100.17%
	162054	323184	100.20		
	161956	323184	100.11		
100%	322965	323184	99.85	99.9	
	323269	323184	99.93		
	323350	323184	99.92		
150%	485265	323184	101.15	100.67	
	481250	323184	100.40		
	481520	323184	100.47		

Acceptance criteria: The % Mean recovery should be within 99.00-101.00%

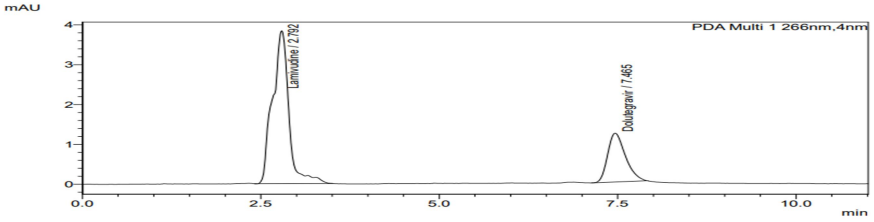

Figure 7: LOD of Lamivudine and Dolutegravir

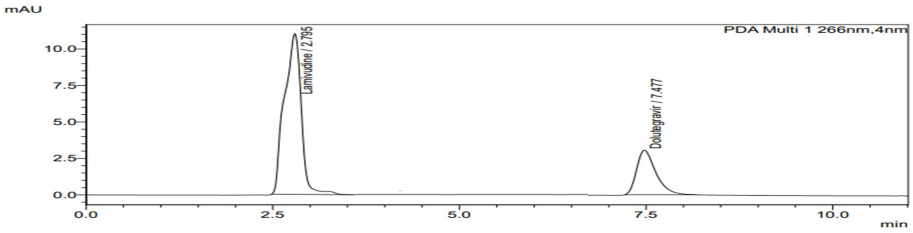

Figure 8: LOQ of Lamivudine and Dolutegravir

Table 7: Robustness: Change in flow rate

Drug	Change in flow (ml/min)	Retention time	Robustness		
			Average peak area	STDEV	%RSD
Lamivudine	0.8	3.456	2156859	2822.77	0.130874
	1.2	2.334	1456433	3524.22	0.241976
Dolutegravir	0.8	9.258	398249	834.386	0.209514
	1.2	6.262	268813	16.97056	0.006313

Table 8: Robustness:Change in mobile phase:

Drug	Change in mobile phase	Retention time	Robustness		
			Average peak area	STDEV	%RSD
Lamivudine	60:40 v/v	2.830	1764118	2114.249	0.119847
	70:30 v/v	2.754	1743841	2820.649	0.161749
Dolutegravir	60:40 v/v	10.462	293320.5	628.6179	0.214311
	70:30 v/v	5.730	362289.5	842.1642	0.232456

Acceptance criteria: The %RSD for the peak area by changing flow rate and mobile phase proportion should not be more than 2.0%

Table 9: Assay of Lamivudine and Dolutegravir

S.No.	Drug name	Labelled claim (mg)	%Assay
01.	Lamivudine	300	101.11
02.	Dolutegravir	50	99.93

Acceptance criteria: The % Assay should be within 99.00-101.00%.

4.9 Abbreviations: RP-HPLC: Reverse Phase High Performance Layer Chromatography; µg: Microgram; mL: Milliliter; mg: Milligram; g: Gram; RT: Retention Time; A: Area; NTP (USP): Number of Theoretical Plate; HETP(USP): Height Equivalent to a Theoretical Plate; TF: Tailing Factor; R(USP): Resolution; nm: Nanometer; µL: Microliter; LC: Layer Chromatography; °C: Degree Centigrade.

5. CONCLUSION

The present developed Gradient RP-HPLC method was found to be specific, simple, accurate and rapid for the determination of Lamivudineand Dolutegravir in bulk and tablet dosage form. A simple Gradient mode of development was done and there were no interactions with standard and sample with mobile phase. Retention times of the Lamivudineand Dolutegravir were 2.769min and7.496 min. Accuracy was achieved for the Lamivudineand Dolutegravir with 100.40% and 100.17% and correlation coefficient of 0.9997 and 0.9993. The percentage purity of Lamivudine and Dolutegravir is 99.97 and 99.93. Hence the proposed method can be adopted for the routine analysis for quality control in any quality control and testing laboratory.

ACKNOWLEDGEMENT

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REFERENCES

[1] S.S.Dhanwate, A.D. Pachauri, P.D Ghod, K.R Khandelwal, 2019. "Development and validation of analytical method for the estimation of Lamivudine and Dolutegravir sodium in dosage form", Journal of Pharmaceutical Sciences and Research. 11(8): 2886-2890.

[2] Ramreddy Godey and Sowjanya G 2020."An effective stability indicating RP-HPLC method for simultaneous estimation of Dolutegravir and Lamivudine in bulk and their tablet dosage form", Future Journal of Pharmaceutical Sciences; 6(1): 1-9, DOI: 10.1186/s43094-020-00026-0.

[3] Khaleel Noorbasha and Sharmila Nurbhasha, 2020. "A new validated stability-indicating RP-HPLC method for simultaneous quantification of

Dolutegravir and Lamivudine in bulk and pharmaceutical dosage form", Future Journal of Pharmaceutical Sciences, 6: 39, 1-10, DOI: 10.1186/s43094-020-00060-y.

[4] Sapna M Rathod, Paresh U Patel, 2020. "Development and Validation of RP – HPLC Method for Estimation of Lamivudine and Dolutegravir Sodium in Synthetic Mixture", Research Journal of Pharmacy and Technology, (2020); 13(6): 2864-2868, DOI: 10.5958/0974-360X.2020.00510.7.

[5] Padmavathi Sakinala, Naga Sharmila J, Sireesha M and Pujitha G, 2020. "HPLC Method Development and Validation of Lamivudine, Dolutegravir and Tenfovir in Human Plasma", Journal of Global Trends in Pharmaceutical Sciences, 2020; 11 (2): 7808 – 7817.

[6] Narottam Pal, Avanapu Srinivasa Rao and Pigilli Ravikumar, 2016. "Simultaneous HPLC Method Development and Validation for Estimation of Lamivudine, Abacavir and Dolutegravir in Combined Dosage Form with their Stability Studies", Asian Journal of Chemistry, 28 (2): 273-276, DOI: 10.14233/ajchem.2016.19116.

[7] Kalpana Nekkala, V. Shanmukha kumar D. and Ramachandran, 2017. "Development and Validation for the Simultaneous Estimation of Lamivudine, Tenofovir Disproxil and Dolutegravir in Drug Product by RP-HPLC", Journal of Pharmaceutical Sciences and Research, 9(9): 1505-1510.

[8] Rajkumar Prava, Ganapathy Seru, Vamsi Krishna Pujala and Surendra Babu Lagu, 2017. "RP-HPLC method development and validation for the simultaneous determination of Lamivudine, Abacavir and Dolutegravir in pharmaceutical dosage forms", World Journal of Pharmaceutical Sciences, 5(5): 168-181.