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Research Article

New RP-HPLC Method for Simultaneous Estimation of Dolutegravir and Lamivudine in Its Pure and Pharmaceutical Dosage Form

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Abstract

A simple, rapid, accurate, precise and economical reverse phase high performance liquid chromatographic method is developed for simultaneous quantification of two anti-viral drugs, viz., Lamivudine and Dolutegravir. The separation of both the drugs was achieved on Inertsil ODS column (200×4.6mmid, 5µm particle size) column using an ortho phosphate buffer solution: Acetonitrile (20:80v/v). The flow rate was 1.0ml/min and detection was done at 230nm. The retention time of Dolutegravir and for Lamivudine was 3.417 mins and 4.392 mins respectively. Precision showed that % Relative standard deviation of Lamivudine and Dolutegravir was about 0.3 and 0.8 respectively. The percentage recoveries of both the drugs Lamivudine and Dolutegravir from the tablet formulation were 100.39% and 100.04% respectively. Linearity of Lamivudine and Dolutegravir was in the range of 50 to 250µg/ml and 25µg/ml is 12µg/ml respectively. Calibration curve showed good linearity and range. The Correlation Coefficient of Lamivudine and Dolutegravir were 0.999 each. And the results obtained for LOQ, LOD and Robustness were well within the acceptance criteria. The results of the analysis have been validated as per International conference on Harmonization (ICH) guidelines Q2B. Validation results indicated that method linearity, accuracy, precision, and ruggedness. The simple mobile phase composition makes this method cost effective, rapid, and non-tedious and can also be successfully employed for simultaneous estimation of both drugs in commercial products.

Keywords: Validation, HPLC, Lamivudine, Dolutegravir, Antiviral drugs.

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Contents

1. Introduction

Lamivudine is a synthetic nucleoside analogue and is phosphorylated intracellularly to its active 5'-triphosphate metabolite, lamivudine triphosphate (L-TP). This nucleoside analogue is incorporated into viral DNA by HIV reverse transcriptase and HBV polymerase, resulting in DNA chain termination. Dolutegravir is a HIV-1 intergrase inhibitor that blocks the strand transfer step of the integration of the viral genome into the host cell (INSTI). The effect of this drug has no homology in human host cells which gives it an excellent tolerability and minimal toxicity. Dolutegravir was developed by ViiV Healthcare and FDA approved on August 12, 2013. On November 21, 2017, dolutegravir, in combination with Lamivudine, was approved as part of the first complete treatment regimen with only two drugs for the treatment of adults with HIV-1 named Juluca.

2. Materials and Methds

Instrumentation: The instrument used was HPLC Alliance Waters model No. 2695 separation module. 2487 UV detector, Software- empower. The stationary phase used was Inertsil ODS (200x4.6mm,5µm). Weighing balance - BSA224SC, Sonicator (Enertech)-SE60US, PH meter Lab India model No. AD102U, UV/VIS spectrophotometer UV3000 Lab India Software-UV Win5

Materials and reagents

Dolutegravir, Lamivudine, KH2PO4, water, acetonitrile, orthophosphoric acid supplied by Hetero drugs Ltd & Merck chemicals used.

Method development

5 trials were made by changing different analytical columns and changing the run time and the mobile phase ratios and solvents finally, the mobile phase optimized mobile phase ratio was 0.1% OPA: Acetonitrile (80: 20), by using analytical column – Inertsil ODS, (200x 4.6mm, 5µm).

Chromatographic conditions

The chromatographic conditions were successfully developed for the separation of Lamivudine and dolutugiravir by using Inertsil ODS, (200x4.6mm, 5µm), flow rate was 1.5 ml/min, mobile phase ratio was 0.1% OPA: Acetonitrile (80:20), detection wavelength was 230nm.

3. Results and Discussion

Table 1: Area of different concentration of Lamivudine and Dolutegravir

S.No	Lamivudine		Dolutegravir	
	Concentration(µg/ml)	Area	Concentration(µg/ml)	Area
1	50	524876	25	380761
2	100	1059982	50	782401
3	150	1574201	75	1164038
4	200	2068062	100	1549472
5	250	2604868	125	1965315

Table 2: Analytical performance parameters of Lamivudine and Dolutegravir

Parameters	Lamivudine	Dolutegravir
Slope(m)	10336	15745
Intercept(c)	15979	12456
Correlation coefficient(R^2)	0.999	0.999

Table 3: Results of Precision for Lamivudine and Dolutegravir

Injection	Area for Lamivudine	Area for Dolutegravir
Injection-1	1610934	1228406
Injection-2	1609985	1223300
Injection-3	1619309	1213803
Injection-4	1608645	1201667
Injection-5	1610885	1228897
Injection-6	1618951	1220372
Average	1613118.2	1219407.5
Standard Deviation	4731.4	10327.1
%RSD	0.3	0.8

Table 4: Results of Intermediate precision for Lamivudine and Dolutegravir

Injection	Area for Dolutegravir	Area for Dolutegravir
Injection-1	1604507	1214125
Injection-2	1594158	1210517

Injection-3	1591505	1212127
Injection-4	1601953	1211539
Injection-5	1598025	1219177
Injection-6	1604821	1203992
Average	1599161.5	1211912.8
Standard Deviation	5538.0	4950.5
%RSD	0.3	0.4

Table 5: Accuracy (recovery) data for Lamivudine

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	809552.3	25	25.21	100.82	100.39
100%	1611682	50	50.18	99.36	
150%	2408440.7	75	74.99	99.98	

Table 6: Accuracy (recovery) data for Dolutegravir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	617877.7	12.5	12.59	100.75	100.04
100%	1224225.3	25	24.95	99.81	
150%	1831657.7	37.5	37.33	99.55	

Table 7: Results of LOQ

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Lamivudine	52	525	10.10
Dolutegravir	51	521	10.02

Table 8: Results for variation in flow for Dolutegravir

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	1.35	2630.90	1.45
2	1.5	2657.20	1.42
3	1.65	2611.73	1.35

Table 9: Results for variation in mobile phase composition for Lamivudine

S.No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2569.17	1.39
2	*Actual	2657.20	1.42
3	10%more	2526.40	1.38

Table 10: Results for variation in mobile phase composition for Dolutegravir

S.No.	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		USP Resolution	USP Plate Count	USP Tailing
1	10% less	3.48	3485.60	1.30
2	*Actual	3.52	3669.74	1.40
3	10%more	3.44	3416.12	1.34

Table 11: Results for Stability of Lamivudine and Dolutegravir

Sample Name	Lamivudine		Dolutegravir	
	Area	% Degraded	Area	% Degraded
Standard	1602702		1224118	
Acid	1583722	1.18	1207822	1.33
Base	1528333	4.64	1173832	4.11
Peroxide	1558673	2.75	1146223	6.36
Thermal	1492533	6.87	1196732	2.24
Photo	1509356	5.82	1127897	7.86

4. Conclusion

A new method was established for simultaneous estimation of Lamivudine and Dolutegravir by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Lamivudine and Dolutegravir by using C18 Inertsil ODS (200*4.6) 5umcolumn, flow rate was 1ml/min, mobile phase ratio was OPA (Orthophosphoric Acid) (0.1%) (80:20%v/v) CAN (detection wave length was 230 nm). The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, UV Detector 3000+Empower-softwareversion-2. The run times were found to be 3.401mins and 4.345mins. The % purity of Lamivudine and Dolutegravir was found to be 100.83% and 99.84% respectively. The system suitability parameters for Lamivudine and Dolutegravir such as theoretical plate sand tailing factor were found to be 3677.56, 1.5 and 4683.62, 1.04 the resolution was found to be 6.76. The estimation of Lamivudine and Dolutegravir was done by RP-HPLC. The assay of Lamivudine and Dolutegravir was performed with tablet sand the % assay was found to be 100.83 and 100.23 which shows that the method is useful for routine analysis. The linearity of Lamivudine and Dolutegravir was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.6 and 0.5 for Lamivudine and Dolutegravir which shows that the method is precise. The acceptance criteria of intermediate recision is RSD should benot more than 2.0% and the method show precision 0.6 and 0.2 for Lamivudine and Dolutegravir which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 98.0% 102.0%. The total recovery was found to be 100.40 % and 100.25% for Lamivudine and Dolutegravir. The validation of developed method shows that the accuracy is well Within the limit, which shows that the methodis capable of showing good accuracy and reproducibility. The acceptance criterion for LOD and LOQ is 3 and10. The LOD and LOQ for Lamivudine was found to be 2.98 and 10.00 and LOD and LOQ for Dolutegravir was found to be 3.00 and 9.98.. Compared to previous results the present method which was developed on Lamivudine and Dolutegravir there runtime is less with the less concentration used. We recommend this method for the routine analysis of these drugs.

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