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DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF DOLUTEGRAVIR AND LAMIVUDINE IN DRUG PRODUCT BY RP-HPLC

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ABSTRACT

Analytical method was developed for the estimation of Lamivudine and Dolutegravirin drug product by liquid chromatography. The chromatographic separation was achieved on C18 column (Inertsil ODS 3V 250*4.6mm) at ambient temperature. The separation achieved employing a mobile phase consists of 0.1%v/v TFA in water: ACN (30:70). The flow rate was 0.8ml/ minute and ultra violet detector at 260nm. The average retention time for Lamivudine and Dolutegravir found to be 2.373min and 4.558min. The proposed method was validated for selectivity, precision, linearity and accuracy. All validation parameters were within the acceptable range. The assay methods were found to be linear from 300-900µg/ml for Lamivudine 50-150µg/ml and Dolutegravir.

KEYWORDS

Lamivudine and Dolutegravir, Isocratic, HPLC, Inertsil ODS3V, C18, Formic acid, Acetonitrile, Methanol and Validation.

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INTRODUCTION

Lamivudine is a nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1) and hepatitis B (HBV). It is also used to treat chronic hepatitis B when other options are not possible. It is effective against both HIV-1 and HIV-2. It is typically used in combination with other antiretroviral such as zidovudine and abacavir. Lamivudine is taken by mouth as a liquid or tablet[1].

Lamivudine is chemically designated as 4-Amino-1-[(2*R*, 5*S*)-2-(hydroxymethyl)-1, 3-oxathiolan-5-yl]-1, 2-dihydropyrimidin-2-one. Its molecular formula is $C_8H_{11}N_3O_3S$, and its molecular weight is 229.26 g/mol.

Dolutegravir

Dolutegravir (DTG) is a medication used for the treatment of HIV infection. Dolutegravir is an integrase inhibitor. The drug is marketed as Tivicay[2] by Glaxo Smith Kline. Dolutegravir is approved for use in a broad population of HIV-infected patients. It can be used to treat HIV-infected adults who have never taken HIV therapy (treatment-naïve) and HIV-infected adults who have previously taken HIV therapy (treatment-experienced), including those who have been treated with other integrase strand transfer inhibitors.

Dolutegravir is chemically designated as 4-{[(2S, 4R)-1-(4-Biphenylyl)-5-ethoxy-4-methyl-5-oxo-2-pentanyl] amino}-4-oxobutanoic acid. Its molecular formula is C24H29NO5, and its molecular weight is 411.49 g/mol[3-4].

EXPERIMENTAL[5-10]

Equipments

The chromatographic technique performed on a waters 2695 with 2487 detector and Empower 2 software, reversed phase C18 column (Inertsil ODS 3V 250*4.6, 5µ) as stationary phase, Ultrasonic cleaner, Scaletech analytical balance, Vaccum micro filtration unit with 0.45µ membrane filter was used in the study.

Materials

Pharmaceutically pure sample of Lamivudine/Dolutegravir were obtained as gift samples from Fortune pharma training Institute, Sri Sai Nagar Colony, KPHB, Hyderabad, India.

HPLC-grade Methanol was from qualigens reagents Pvt ltd. Formic acid (AR grade) was from sd fine chem.

Chromatographic conditions

The sample separation was achieved on a (5 µ, 250 cm X 4.6 mm i.d.)Inertsil ODS 3V column, aided by mobile phase mixture of 0.1%v/v TFA in water: ACN (30:70). The flow rate was 0.8 ml/ minute and ultra violet detector at 260nm, that was filtered and degassed prior to use, Injection volume is 10µl and ambient temperatures.

Preparation of mobile phase

Buffer Preparation

Taken accurately 1ml of TFA in1000mL of water.

Mobile phase

Then added30volumes of buffer and 70 volumes of CAN mixed well and sonicated for 5 min.

Diluents:

Water: Acetonitrile (50:50 v\v).

Preparation of standard stock solution

A 300mg of pure Lamivudine and 50 mg of Dolutegravir were weighed and transferred to 50 ml of volumetric flask and dissolved in diluent. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution. From the above solution 1ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with diluent to give a solution containing 600µg/ml of Lamivudine and 100 µg/ml Dolutegravir.

Preparation of sample solution

Accurately weighed twenty tablets were ground to obtain fine powder equivalent to 300mg of Lamivudine and 50mg of Dolutegravir sample were weighed and transferred to 50 ml of volumetric flask and dissolved in diluent. The flask was shaken and volume was made up to mark with diluents to give a primary stock solution. From the above solution 1 ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with diluents to give a solution containing 600µg/ml of Lamivudine and 100 µg/ml Dolutegravir .

RESULTS AND DISCUSSION

Determination of Working Wavelength (λ max)

10 mg of the Lamivudine and Dolutegravir standard drug is taken in a 10 ml volumetric flask and dissolved in Diluent and volume made up to the mark, from this solution 0.1ml is pipette into 10 ml volumetric flask and made upto the mark with the Water to give a concentration of 10 µg/ml. The

above prepared solution is scanned in uv between 200-400 nm using Water as blank. The λ max was found to be 260nm.

After several initial trails with mixtures of methanol, water, ACN and buffer in various combinations and proportions, a trail with a mobile phase mixture of 0.1%v/v Formic acid in water: ACN (30:70). The flow rate was 0.8 ml/ minute brought sharp peaks. The chromatogram was shown in Figure No.1.

METHOD VALIDATION

Linearity

Linearity was studied by analyzing five standard solutions covering the range of 300-900µg/ml for Lamivudine and 50-150µg/ml and Dolutegravir. From the primary stock solution 0.5ml, 0.75ml, 1.0ml, 1.25ml, 1.5 ml of aliquots are pipette into 10 ml volumetric flasks and made up to the mark with the diluent to give a concentrations of 50µg /mL, 75µg/mL, 100µg/mL, 125µg/mL and 150µg/mL of Dolutegravir and 300µg/mL, 450µg/mL, 600µg/mL, 750µg/mL and 900µg/mL Lamivudine. Calibration curve with concentration verses peak areas was plotted by injecting the above prepared solutions and the obtained data were subjected to regression analysis using the least squares method.

Result

A linear relationship between peak areas versus concentrations was observed for Lamivudine and Dolutegravirin the range of 50% to 150% of nominal concentration. Correlation coefficient was 0.9991 and 0.9992 for both Lamivudine and Dolutegravir which prove that the method is linear in the range of 50% to 150%.

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (1) and (2), respectively.

$$LOD = 3.3\ \sigma/S \quad \ldots\ldots\ldots (1)$$

$$LOQ = 10\ \sigma/S \quad \ldots\ldots\ldots (2)$$

Where,

σ = the standard deviation of the response (STEYX)

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Method precision (repeatability)

The precision of the method was checked by repeated preparation (n=6) of 600µg/ml of Lamivudine and 100µg/ml Dolutegravir without changing the parameter of the proposed chromatographic method. And measure the peak areas and retention times.

Result

Results of variability were summarized in the above table. Percentage relative standard deviation (%RSD) was found to be less than 2.0% which proves that method is precise**.**

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of Lamivudine and Dolutegravir by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of Lamivudine and Dolutegravir. The percentage recovery results obtained are listed in Table No.6 and 7.

Result

Results of accuracy study are presented in the above table. The method is highly accurate**.**

Robustness

Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.2 ml/min. The results were shown in (Table No.8 and 9)

Result

The results of Robustness of the present method had shown that changes are not significant we can say that the method is Robust.

Ruggedness

The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts. The results were shown in Table No.10 and 11.

Result

The %RSD assay values between two analysts was calculated, this indicates the method was rugged.

Table No.1: Linearity data of Lamivudine

S.No	Level	Concentration (mg/mL)	Peak area
1	50%	0.3	1094895
2	75%	0.45	1575825
3	100%	0.6	1938291
4	125%	0.75	2372107
5	150%	0.9	2847386

Table No.2: Linearity data of Dolutegravir

S.No	Level	Concentration (mg/mL)	Peak area
1	50%	0.05	791517
2	75%	0.075	1146502
3	100%	0.1	1473223
4	125%	0.125	1821943
5	150%	0.15	2233794

Table No.3: LOD and LOQ values Calculated from calibration curve

S.No		Lamivudine mg	Dolutegravir mg
1	LOD	0.03	0.006
2	LOQ	0.11	0.018

Table No.4: Summary of peak areas for method precision of Lamivudine

S.No	Sample No	Retention time	Peak area	% Assay
1	1	2.372	1926157	100.1
2	2	2.371	1942639	99.5
3	3	2.371	1932993	99.6
4	4	2.370	1890193	98.6
5	5	2.371	1933047	98.8
6	6	2.370	1956298	99.4
7	Mean	2.371	1930221	99.4
8	%RSD	0.03	1.15	0.54

Table No.5: Summary of peak areas for method precision of Dolutegravir

S.No	Sample No	Retention time	Peak area	% Assay
1	1	4.572	1433404	100.2
2	2	4.571	1438312	99.7
3	3	4.571	1436765	99.4
4	4	4.572	1393554	99.2
5	5	4.573	1439888	99.6
6	6	4.574	1453754	99.4
7	Mean	4.572	1432613	99.6
8	%RSD	0.03	1.42	0.35

Table No.6: Recovery data of Lamivudine

LEVEL	S.No	%Recovery of Lamivudine	Average
50	1	99.5	99.4%
	2	99.3	
	3	99.4	
100	1	100.1	99.74%
	2	99.5	
	3	99.6	
150	1	99.3	99.8%
	2	100.1	
	3	100.0	

Table No.7: Recovery data of Dolutegravir

LEVEL	S.No	%Recovery of Dolutegravir	Average
50	1	99.1	99.2%
	2	98.9	
	3	99.6	
100	1	100.2	99.76%
	2	99.7	
	3	99.4	
150	1	100.0	100%
	2	100.2	
	3	99.9	

Table No.8: Results of Lamivudine

S.No	Parameter	Rt of Lamivudine	Theoretical plates	Asymmetry
1	Decreased flow rate (0.6ml/min)	2.697	4947	1.17
2	Increased flow rate (1.0ml/min)	2.115	4364	1.14
3	Wave Length 258nm	2.372	4722	1.18
4	262	2.371	4664	1.17

Table No.9: Results of Dolutegravir

S.No	Parameter	Rt of Dolutegravir	Theoretical plates	Asymmetry
1	Decreased flow rate (0.6ml/min)	5.210	10078	1.22
2	Increased flow rate (1.0ml/min)	4.074	8880	1.17
3	Wave Length 258nm	4.572	9498	1.20
4	262	4.571	9456	1.20

Table No.10: Results of Lamivudine

S.No			%Assay	%RSD
1	Analyst-1	LAMIVUDINE	100.1	0.38%
2	Analyst-2		99.8	

Table No.11: Results of Dolutegravir

S.No			%Assay	%RSD
1	Analyst-1	DOLUTEGRAVIR	100.2	0.32%
2	Analyst-2		99.7	

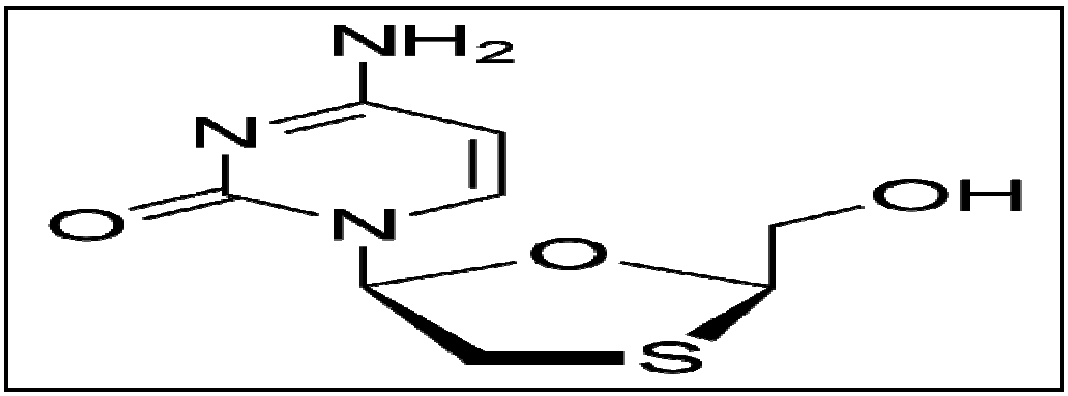
Table No.12: Summary of Lamivudine

S.No	PARAMETER	RESULT	ACCEPTENCE CRITERIA
1	System suitability Theoretical plates Asymmetry Retention time %RSD	 4728 1.17 2.372 1.35	 Not less than 3000 Not more than 2.0 Not more than 2.0
2	Specificity	Specific	Specific
3	Method precision(%RSD)	0.54	Not more than 2.0%
4	Linearity Correlation coefficient(r^2)	300-900 mcg/ml 0.9991	 Not less than 0.990
5	Accuracy (Mean % recovery) 50% 100% 150%	 99.4 99.7 99.8	 97 - 103%
6	Robustness	All the system suitability parameters are within the limits.	

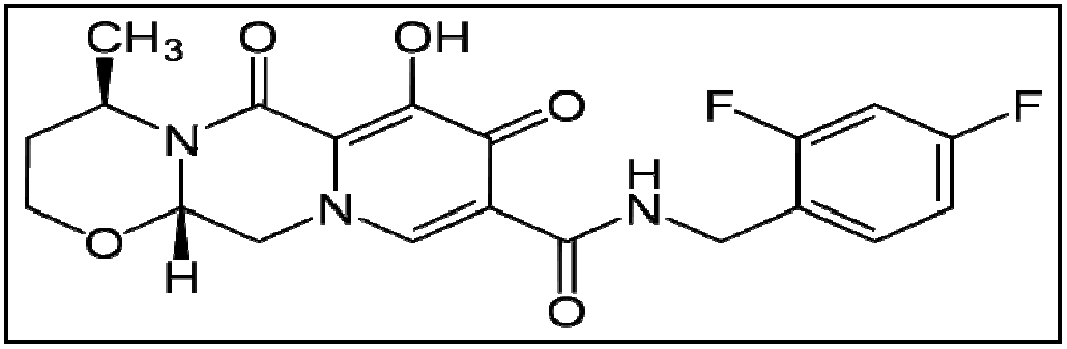
*RSD = Relative standard deviation

Table No.13: Summary of Dolutegravir

S.No	PARAMETER	RESULT	ACCEPTENCE CRITERIA
1	System suitability Theoretical plates Asymmetry Retention time %RSD	 9459 1.18 4.560 1.51	 Not less than 3000 Not more than 2.0 Not more than 2.0
2	Specificity	Specific	Specific
3	Method precision(%RSD)	0.35	Not more than 2.0%
4	Linearity Correlation coefficient(r^2)	50-150 mcg/ml 0.9992	 Not less than 0.990
5	Accuracy (Mean % recovery) 50% 100% 150%	 99.2 99.76 100.0	 97 - 103%
6	Robustness	All the system suitability parameters are within the limits.	



Structure of Lamivudine



Structure of Dolutegravir

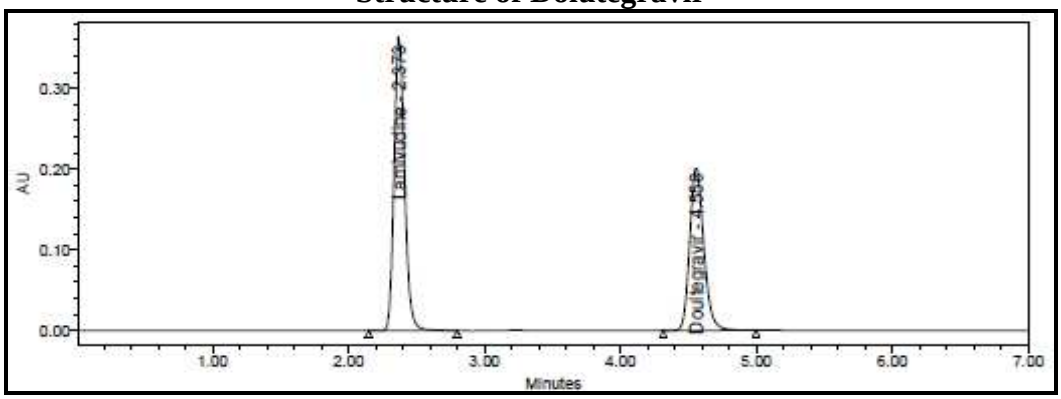


Figure No.1: Chromatogram of Lamivudine/Dolutegravir

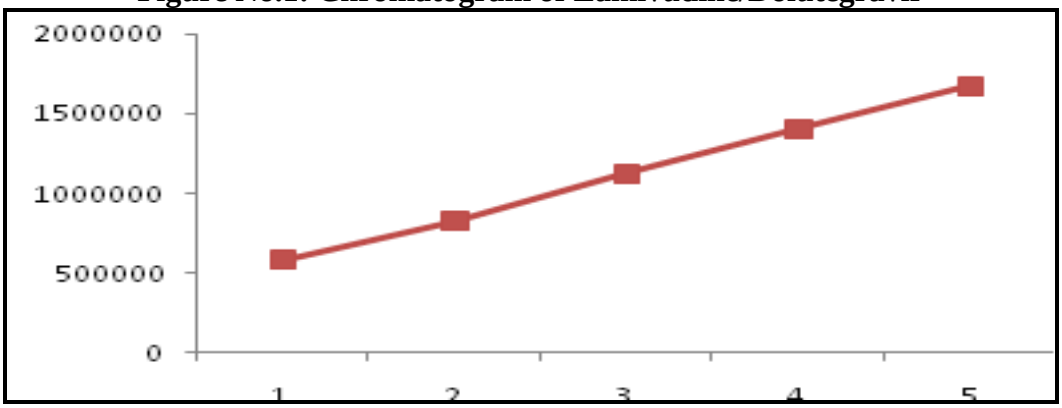


Figure No.2: Linearity (calibration) curve of Lamivudine

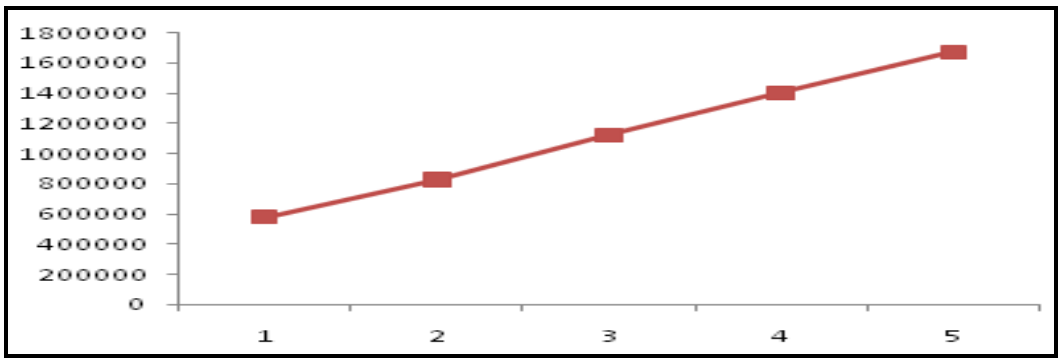


Figure No.3: Linearity (calibration) curve of Dolutegravir

CONCLUSION

This newly developed method for the simultaneous estimation of LAMIVUDINE and DOLUTEGRAVIR was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries and approved testing laboratories.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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