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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF RILPIVIRINE AND DOLUTEGRAVIR IN BULK AND PHARMACEUTICAL DOSAGE FORMS BY RP-HPLC

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ABSTRACT

For the simultaneous evaluation of Dolutegravir and Rilpivirine in dosage form, a simple, precise, detailed technique has been optimized. The mobile phase comprising of water and methanol (60:40 v / v) ratio was injected into a column at a flow rate of 0.8 ml/min. Chromatogram was run through Discovery-C_{18} (4.6 x 150 mm, 5µm). Optimised wavelength selected at 260nm. Retention time of Dolutegravir and Rilpivirine were found to be 3.013 min and 2.241 min. %Recovery was obtained as 99.66% and 99.57% in that order. LOD, LOQ values obtained from regression equations of Dolutegravir and Rilpivirine were 0.48, 1.44 and 0.17, 0.52 correspondingly. Regression equation of Dolutegravir is y = 50100x + 15520, and y= 34251x + 3054 of Rilpivirine. The approach was simple and cost-effective and can be used in industry with the standard consistency check.

INTRODUCTION

Rilpivirine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) with high potency used in the treatment of HIV infection in adults and children. Rilpivirine blocks the virus from growing and infecting more cells. Dolutegravir is an Anti-Retroviral medication used, together with other HIV medications to treat HIV/AIDS. Rilpivirine and Dolutegravir are likely to be as a nucleoside-reverse transcriptase inhibitor (NRTI)-sparing regimen primarily used for maintenance therapy in persons with stable suppressed HIV. [1-2]

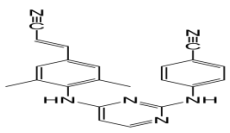

Fig- 1: Structure of Rilpivirine

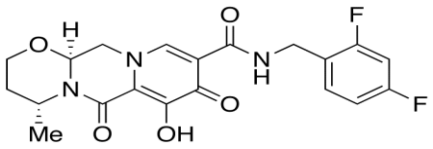

Fig-2: Structure of Dolutegravir

MATERIALS AND METHODS

Methods:

Diluent: In the ratio of 50:50v/v, diluent was selected. Methanol and Water are selected depending on the solubility of the drugs.

Stock Solution:

Preparation of Standard stock solutions: Accurately weighed 12.5mg of Dolutegravir, 6.25mg of Rilpivirine and transferred to 25ml

flasks and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (500µg/ml of Dolutegravir and 250µg/ml Rilpivirine)

Preparation of Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100ml volumetric flask, 5 ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (500µg/ml of Dolutegravir and 250µg/ml of Rilpivirine)

Working Solution:

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (50µg/ml of Dolutegravir and 25µg/ml of Rilpivirine)

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (50µg/ml of Dolutegravir and 25µg/ml of Rilpivirine). **(3)**

RESULTS AND DISCUSSION:

Method validation: Specificity, linearity, range, Accuracy, precision, Repeatability, Intermediate precision, limit of detection, limit of Quantification, Robustness.

Method development: Method development was performed by changing various chromatographic conditions like mobile phase ratios, buffers, flow rates .

SPECIFICITY: The system suitability for specificity was carried out to determine whether there is an interference of any impurities in retention time of analytical peak. The specificity study was performed by injecting blank. It was found that there was no interference of impurities in retention time of analytical peak.

LINEARITY: The regular stock solution consists of six linear concentrations (12.5 ml-75 ml Dolutegravir and 6.25 ml-37.5 ml-Rilpivirine). They were tabulated in table num-1. **(4)**

ACCURACY: Samples are prepared by sample stock solvent at three separate levels: 50%, 100%, and 150 percent each with three preparations. They were tabulated in table num-2 and 3. **(5)**

PRECISION: The precision of the analytical method was studied by injecting six replicates of standard and sample concentration on the same day and another day. The concentration of Diacerein and Glucosamine were injected at intermediate precision. The %RSD was calculated and results were reported and table no. 4 and 5. **(6)**

LOD and LOQ: The limit of detection (LOD) and limit of quantification (LOQ) were determined by injecting six replicates of mobile phase followed by three concentration of the drug. The LOD was defined as the concentration which yields a signal-to-noise ratio 3:1 while the LOQ was calculated to be the lowest concentration that could be measured with signal-to-noise ratio 10:1. The LOD & LOQ were calculated by measuring the standard deviation of the response and slope. The result of LOD &LOQ was tabulated in table no.6. **(7)**

ROBUSTNESS: There is no established improvement in the process such as the flow rate, the mobile step ratio, and the temperature, but the findings are within the range as per the ICH Guidelines. Robustness condition like flow minus (0.8ml/min), flow plus (1.2ml/min), temperature ambient was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed %RSD was found to be within the limits and results were tabulated in table no.7 and 8 . **(8-9)**

CONCLUSION:

A simple, accurate and precise method was developed for the simultaneous estimation of the Dolutegravir and Rilpivirine in tablet dosage form. Retention time of Dolutegravir and Rilpivirine were found to be 3.013 min and 2.241 min.

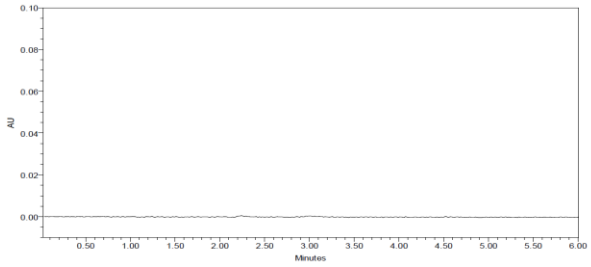

Fig-3:Chromatogram of blank

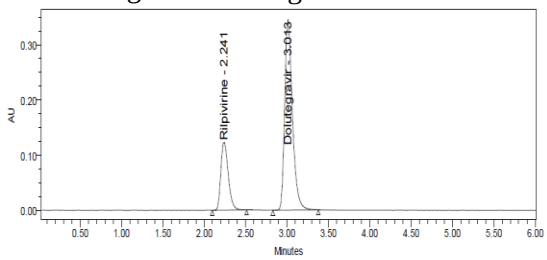

Fig-4: Optimized condition Chromatogram

Table -1:Linearity table for Dolutegravir and Rilpivirine.

Dolutegravir		Rilpivirine	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
12.5	667194	6.25	213463
25	1261917	12.5	434624
37.5	1889825	18.75	647261
50	2509543	25	862691
62.5	3172333	31.25	1083512
75	3758996	37.5	1275234

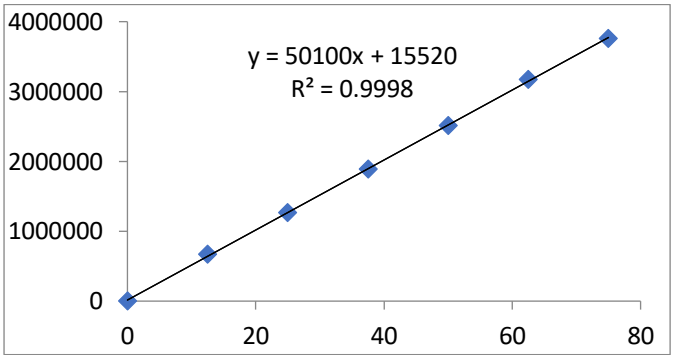

Fig-5: Calibration curve of Dolutegravir

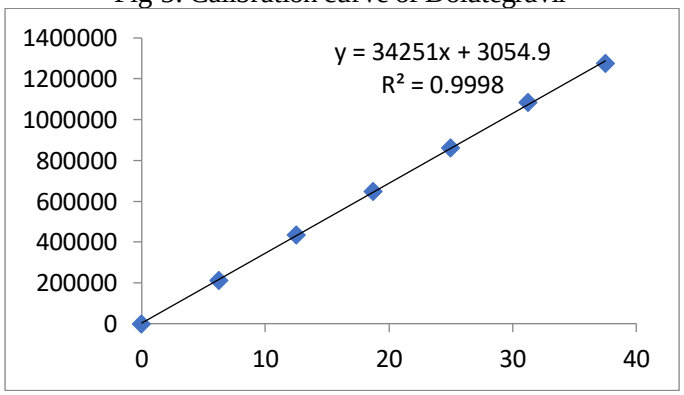

Fig-6: Calibration curve of Rilpivirine

Table- 2: Accuracy table of Dolutegravir

% Level	Amount Spiked(µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	25	24.84	99.38	99.66%
	25	24.86	99.43	
	25	24.86	99.44	
100%	50	50.00	100.00	
	50	49.80	99.60	
	50	49.71	99.42	
150%	75	74.92	99.89	
	75	75.01	100.01	
	75	74.81	99.75	

Table:3- Accuracy table of Rilpivirine

% Level	Amount Spiked(µg/mL)	Amount recovered(µg/mL)	% Recovery	Mean %Recovery
50%	12.5	12.45	99.59	99.57%
	12.5	12.44	99.52	
	12.5	12.45	99.59	
100%	25	25.03	100.13	
	25	24.84	99.38	
	25	24.84	99.37	
150%	37.5	37.34	99.58	
	37.5	37.30	99.46	
	37.5	37.32	99.52	

Table-4:System precision data for Dolutegravir and Rilpivirine

S. No	Area of Dolutegravir	Area of Rilpivirine
1.	2539562	869046
2.	2572546	870632
3.	2551357	871026
4.	2554363	873387
5.	2502627	872652
6.	2565529	869561
Mean	2547664	871051
S.D	24862.7	1699.2
%RSD	1.0	0.2

Table-5:Repeatability table

S. No	Area of Dolutegravir	Area of Rilpivirine
1.	2562596	871609
2.	2576519	870788
3.	2576519	870788
4.	2570685	869350
5.	2579416	870392
6.	2578322	869036
Mean	2574010	870327
S.D	6352.1	968.9
%RSD	0.2	0.1

Table-6: LOD and LOQ data for Dolutegravir and Rilpivirine

Molecule	LOD	LOQ
Dolutegravir	0.48	1.44
Rilpivirine	0.17	0.52

Table-7: Robustness data for Dolutegravir and Rilpivirine.

S.no	Condition	%RSD of Dolutegravir	%RSD of Rilpivirine
1	Flow rate (-) 0.7ml/min	0.8	0.9
2	Flow rate (+) 0.9ml/min	0.4	0.3
3	Mobile phase (-) 65W:35M	0.9	1.1
4	Mobile phase (+) 75W:25W	0.7	0.4
5	Temperature (-) 25°C	0.5	0.5
6	Temperature (+) 35°C	0.6	0.5

%Recovery was obtained as 99.66% and 99.57% for Dolutegravir and Rilpivirine respectively. LOD, LOQ values obtained from regression equations of Dolutegravir and Rilpivirine were 0.48, 1.44 and 0.17, 0.52 respectively. Regression equation of Dolutegravir was Y = 50100x + 15520 and Y = 34251x + 3054.9 for Rilpivirine. Retention times were decreased and that run time was also decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in industries.

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REFERENCES

1. www.drugbank.ca/**drugs**/DB08930
2. www.drugbank.ca/drugs/DB08864
3. Ll-Yord R. Snyder, Joseph J. Kirkland and Joseph L. Glajch. Practical HPLC Method development. 2nd edition, John Wiley & Sons, INC, New York, (1997).
4. SatinderAhuja and Michael W. Dong. Handbook of Pharmaceutical Analysis by HPLC, 1st edition, vol.6, Elsevier academic press, (2005).
5. M. Thompson, S. L. R. Ellison and R. Wood. Harmonized guidelines for single laboratory validation of methods of analysis. Pure Appl. Chem. (2002) 74(5): 835- 855.
6. USP 31/NF 26, United States Pharmacopoeia, 31st rev. and the National Formulary, 26 ed. United States Pharamcopoeial Convention, Rockville, (2008).
7. Girija B. Bhavar. The HPLC and HPTLC technique for the Quantitative Estimation of Rilpivirine in Bulk and Dosage Form Sci Pharm, (2016). 84(2): 305–320.
8. Valeria Cozzi. Development and Validation of a Chromatographic Ultraviolet Method for the Simultaneous Quantification of Rilpivirine and dolutegravir in Human Plasma. Therapeutic Drug Monitoring, (2016); 38(3):407-13.
9. NitinCharbe. Development of an HPLC–UV assay method for the simultaneous quantification of nine antiretroviral agents in the plasma of HIV-infected patients Journal of Pharmaceutical Analysis. (2016); 6(6):396-403.