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A NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF DOLUTEGRAVIR AND RILPIVIRINE IN A BULK AND TABLET BY RP-HPLC

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Abstract:

A new, simple, precise, accurate and reproducible RP-HPLC method for Simultaneous estimation of Dolutegravir and Rilpivirine in bulk and pharmaceutical formulations. Separation of Dolutegravir and Rilpivirine was successfully achieved on a Phenomenex Luna C18 (4.6×250mm, 5µm) particle size or equivalent in an isocratic mode utilizing Acetonitrile: Phosphate Buffer (pH-4.6) (45:55 v/v) at a flow rate of 1.0mL/min and elutes was monitored at 245nm, with a retention time of 2.102 and 3.537 minutes for Dolutegravir and Rilpivirine respectively. The method was validated and the response was found to be linear in the drug concentration range of 6µg/mL to 14µg/mL for Dolutegravir and 18µg/mL to 42µg/mL for Rilpivirine. The values of the slope and the correlation coefficient were found to be 77824 and 0.999 for Dolutegravir and 10515 and 0.999 for Rilpivirine respectively. The LOD and LOQ for Dolutegravir were found to be 0.6µg/mL and 1.8µg/mL respectively. The LOD and LOQ for Rilpivirine were found to be 0.8 µg/mL and 2.4µg/mL respectively. This method was found to be good percentage recovery for Dolutegravir and Rilpivirine were found to be 100.351 and 100.93 respectively indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analytes in the sample without interference from excipients of tablet dosage forms. The method was extensively validated according to ICH guidelines for Linearity, Range, Accuracy, Precision, Specificity and Robustness.

Keywords: *Dolutegravir and Rilpivirine, RP-HPLC, Accuracy, Precision, ICH Guidelines.*

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INTRODUCTION:

Pharmaceutical analysis comprises those procedures necessary to determine "identity, strength, quality and purity of the drug substances and drug products. Pharmaceutical analyst plays a major role in all quality controlling divisions of industry. Analytical chemistry involves separating, identifying, and determining the relative amounts of components in a sample matrix. The number of new drugs is constantly growing. This requires new methods for controlling the quality. Modern pharmaceutical analysis must need the following requirements [1].

1. The analysis should take a minimal time.
2. The accuracy of the analysis should meet the demands of the Pharmacopoeia.
3. The analysis should be performed with a minimal cost.
4. Precision and selectivity of the selected method should be good.

Typical Instrumental Techniques [2,3]:

The methods of estimation of drugs are divided into physical, chemical, physicochemical and biological ones of them, physical and physicochemical methods are used mostly. Physical methods of analysis involve the studying of the physical properties of a substance. They include determination of the solubility, transparency or degree of turbidity, colour density or specific gravity (for liquids), moisture content, melting, freezing and boiling points. Physicochemical methods are used to study the physical phenomenon that occurs as a result of chemical reactions. Among the physicochemical methods are optical refractometry, polarimetry, emission and fluorescent methods of analysis, photometry including photocolorimetry, spectrophotometry, nephelometry and turbidometry, electrochemical (potentiometry, amperometry, coulometer, polarography) and chromatography (column, paper, thin layer, gas, high performance liquid) methods are generally preferable.

Methods involving nuclear reactions such as nuclear magnetic resonance (NMR) and paramagnetic resonance (PMR) are becoming more popular. The combination of mass spectroscopy with gas chromatography is one of the most powerful tools available. The chemical methods include the gravimetric and volumetric procedures, which are based on complex formation, acid-base and precipitation and redox reactions. Titrations in non-aqueous media and complexometry have been widely used in pharmaceutical analysis whenever the existing amounts are in milligram level and the interference is negligible. The methods (LC-MS,[4] HPLC, GLC, NMR and Mass Spectroscopy) of choice for assay involve sophisticated equipment that are very costly and pose problems of maintenance. Hence, they are not in the reach of most laboratories and small-scale industries, which produce bulk drugs and pharmaceutical formulations.

The visible Spectrophotometric methods which fall in the wavelength region 400-800 nm and fluorimetric methods (may fall in UV & Visible regions) are very simple, cheap and easy to carry out estimations of drugs in bulk form and their formulations. The limitations of many colorimetric or fluorimetric methods of analysis lie in the chemical reactions upon which the procedures are based rather than the instruments available. Many of the reactions involve colour or fluorescence of a drug are quite selective or can be rendered selective through the introduction of masking agents, control of PH, use of solvent extraction technique, adjustment of oxidation states or by prior removal of interfering ingredients with the aid of chromatographic separation.

1. This is preferably followed by general methodology for UV-Visible and HPLC method developments.
2. Followed by literature of drugs used in Analysis

INTRODUCTION TO HPLC:

Russian botanist Tswett invented chromatography as a separation technique. He describes in detail the separation of pigments, the colour substances by filtration through column, followed by developments with pure solvents.

High-performance liquid chromatography (HPLC) [5] is the fastest growing analytical technique for analysis of drugs. Its simplicity, high specificity and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage forms and biological fluids.

According to IUPAC, chromatography [6] is a physical method of separation in which components will be separated or distributed between stationary and mobile phases. The importance of chromatography is increasing rapidly in pharmaceutical analysis for the exact differentiation, selective identification and quantitative determination of structurally closely related compounds. Another important field of application of chromatographic methods is the purity testing of final products and the intermediates. The reasons for the popularity of the method is its sensitivity, its ready adaptability to accurate quantitative determinations, its suitability for separating non-volatile species or thermally fragile ones and its wide spread applicability to substances that are of prime interest to the industry. Sensitive detectors have transformed liquid column

chromatography into high speed, efficient, accurate and highly resolved method of separation.

The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

- Speed (many analysis can be accomplished in 20 min or less)
- Greater sensitivity (various detectors can be employed)
- Improved resolution (wide variety of stationary phases)
- Reusable columns (expensive columns but can be used for many analysis)
- Ideal for the substances of low viscosity
- Easy sample recovery, handling and maintenance.
- Instrumentation leads itself to automation and quantification (less time and less labour)
- Precise and reproducible
- Integrator itself does calculations.

INSTRUMENTATION [7]

The essential parts of the High Performance Liquid Chromatography are:

1) Solvent reservoir and Treatment system
2) Mobile phase
3) Pump system[17]
4) Sample Injection System
5) Column
6) Detector

MATERIALS AND METHODS:

Dolutegravir-Sura labs, Rilpivirine-Sura labs,Water and Methanol for HPLC-LICHROSOLV (MERCK),Acetonitrile for HPLC Merck

Hplc method development:

Trails

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Dolutegravir and Rilpivirine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.1ml of the above Dolutegravirand 0.3ml of the Rilpivirine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Optimized chromatographic conditions:

Instrument used	:	Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature	:	35ºC
Column	:	Phenomenex Luna C18 (4.6×250mm, 5µm) particle size
Buffer	:	Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.
pH	:	4.6
Mobile phase	:	Acetonitrile: Phosphate Buffer (45:55 v/v)
Flow rate	:	1ml/min
Wavelength	:	245 nm
Injection volume	:	10 µl
Run time	:	7 min

VALIDATION:

Preparation of mobile phase:

Preparation of mobile phase:

Accurately measured 450 ml (45%) of Methanol, 550 ml of Phosphate buffer (55%) were mixed and degassed in digital ultrasonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION:

Optimized Chromatogram (Standard)

Mobile phase : Acetonitrile: Phosphate Buffer (pH-4.6) (45:55 v/v)
Column : Phenomenex Luna C18 (4.6×250mm, 5µm) particle size
Flow rate : 1 ml/min
Wavelength : 245 nm
Column temp : 35ºC
Injection Volume : 10 µl
Run time : 7 minutes

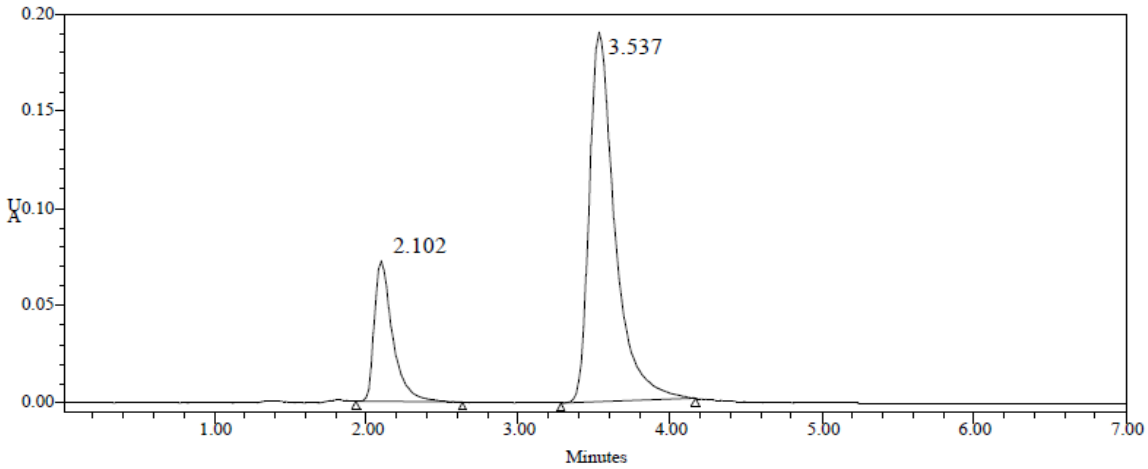


Figure-: Optimized Chromatogram (Standard)

Table No.18: Optimized Chromatogram (Standard)

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Dolutegravir	2.102	765788	69583		0.98	5588.0
2	Rilpivirine	3.537	2532157	190048	2.98	1.27	5399.0

Optimized Chromatogram (Sample)

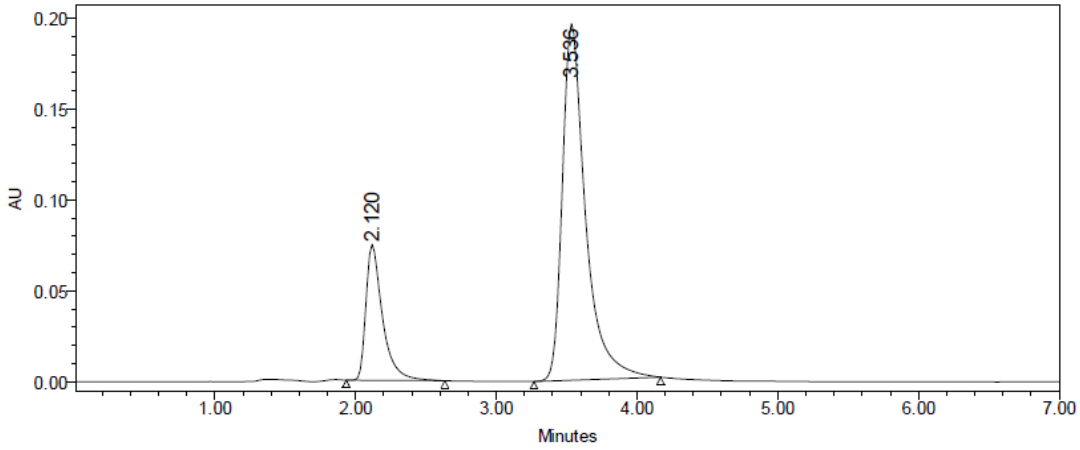


Figure-: Optimized Chromatogram (Sample)

Table No. 19: Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Dolutegravir	2.120	775683	13123		0.98	6364.0
2	Rilpivirine	3.536	2658479	937406	5.07	1.24	7459.0

Acceptance Criteria:

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

Assay (Standard):

Table-: Peak results for assay standard

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Dolutegravir	2.102	759869	71256		1.7	5688	1
2	Rilpivirine	3.537	2458753	215653	2.03	1.6	5363	1
3	Dolutegravir	2.105	759459	72542		1.7	5747	2
4	Rilpivirine	3.552	2465886	226566	2.01	1.6	5451	2
5	Dolutegravir	2.112	759244	72583		1.7	5585	3
6	Rilpivirine	3.560	2489577	221541	2.05	1.6	5457	3

Assay (Sample):

Table-: Peak results for Assay sample

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Dolutegravir	2.120	756986	68959		0.97	7254	1
2	Rilpivirine	3.536	2569857	198563	2.06	1.24	8837	1
3	Dolutegravir	2.120	758744	69858		1.06	6531	2
4	Rilpivirine	3.537	2598653	195681	2.05	0.98	7272	2
5	Dolutegravir	2.102	756849	69587		1.8	7587	3
6	Rilpivirine	3.537	2587457	192542	2.04	1.5	8372	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

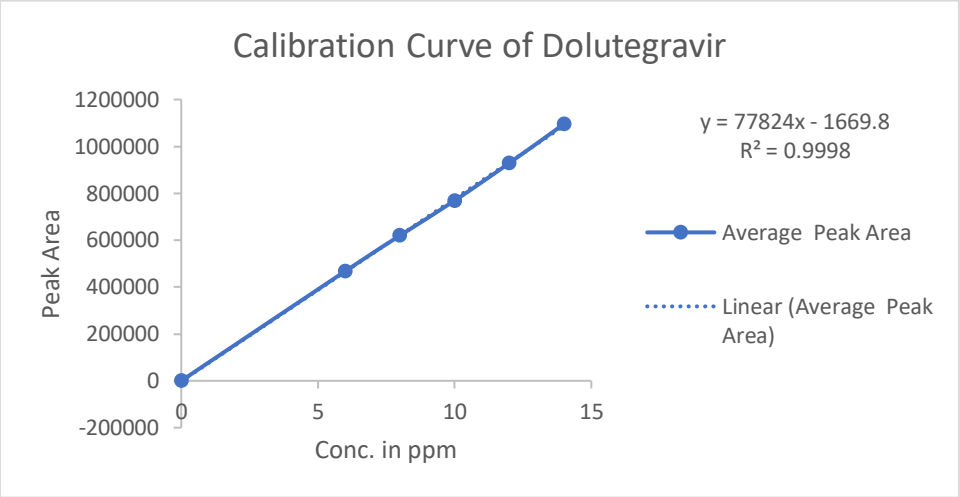
The % purity of Dolutegravir and Rilpivirine in pharmaceutical dosage form was found to be 99.8%.

LINEARITY:

Chromatographic data for linearity study:

Dolutegravir:

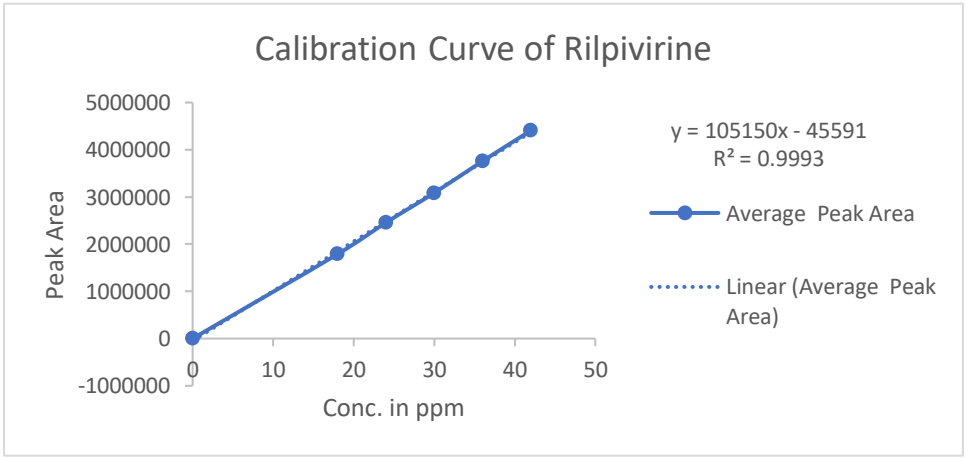
Concentration µg/ml	Average Peak Area
6	467848
8	619853
10	768785
12	928978
14	1095699



Rilpivirine:

Concentration µg/ml	Average Peak Area
18	1789547
24	2456988
30	3085986
36	3759863
42	4406588

Fig: Chromatogram showing linearity level



REPEATABILITY:

Table-: Results of Repeatability for Dolutegravir:

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Dolutegravir	2.108	766853	702563	5686	1.6
2	Dolutegravir	2.105	765885	698788	5583	1.4
3	Dolutegravir	2.113	765843	701236	5522	1.6
4	Dolutegravir	2.109	768986	700125	5526	1.9
5	Dolutegravir	2.109	765844	698987	5579	1.7
Mean			766682.2			
Std. Dev			1358.219			
% RSD			0.177155			

Acceptance Criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table : Results of Repeatability for Rilpivirine:

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Rilpivirine	3.552	2569866	2231112	5366	1.6
2	Rilpivirine	3.550	2578473	2674211	5424	1.6
3	Rilpivirine	3.564	2568986	2231262	5369	1.5
4	Rilpivirine	3.564	2586844	2421303	5358	1.5
5	Rilpivirine	3.565	2545899	2324714	5497	1.6
Mean			2570014			
Std. Dev			15308.62			
% RSD			0.595663			

Intermediate precision:

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Dolutegravir	2.108	758956	68987	5786	1.6
2	Dolutegravir	2.105	759868	68958	5699	1.4
3	Dolutegravir	2.113	758984	68546	5688	1.6
4	Dolutegravir	2.109	756893	68953	5782	1.9
5	Dolutegravir	2.109	759855	68596	5786	1.7
6	Dolutegravir	2.102	756986	68953	5694	1.6
Mean			758590.3			
Std. Dev			1339.793			
% RSD			0.176616			

Acceptance Criteria:

- %RSD of six different sample solutions should not more than 2.

Table : Results of Intermediate precision day1 for Rilpivirine

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Rilpivirine	3.552	2659853	190026	5486	1.5	2.04
2	Rilpivirine	3.550	2648572	190049	5422	1.6	2.03
3	Rilpivirine	3.564	2659866	190053	5469	1.6	2.01
4	Rilpivirine	3.564	2658548	190079	5488	1.6	2.05
5	Rilpivirine	3.565	2648982	190017	5493	1.6	2.02
6	Rilpivirine	3.537	2654653	190058	5464	1.6	2.03
Mean			2655079				
Std. Dev			5242.086				
% RSD			0.197436				

Acceptance Criteria:

- %RSD of six different sample solutions should not more than 2.

Table-: Results of Intermediate precision Day 2 for Dolutegravir

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Dolutegravir	2.102	766896	69859	5587	1.5
2	Dolutegravir	2.105	765989	69853	5635	1.6
3	Dolutegravir	2.112	766533	69825	5433	1.6
4	Dolutegravir	2.113	766215	69876	5469	1.6
5	Dolutegravir	2.109	765898	69855	5547	1.9
6	Dolutegravir	2.109	765246	69849	5508	1.7
Mean			766128.5			
Std. Dev			567.7234			
% RSD			0.074103			

Acceptance Criteria:

- %RSD of six different sample solutions should not more than 2.

Table: Results of Intermediate precision Day 2 for Rilpivirine

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Rilpivirine	3.537	2653253	190111	5429	1.6	7.99
2	Rilpivirine	3.552	2648986	190059	5453	1.6	6.5
3	Rilpivirine	3.560	2658212	190143	5497	1.6	8.8
4	Rilpivirine	3.564	2653651	190033	5443	1.5	8.2
5	Rilpivirine	3.564	2648979	190059	5488	1.5	7.6
6	Rilpivirine	3.565	2658986	190048	5462	1.6	5.4
Mean			2653678				
Std. Dev			4313.355				
% RSD			0.162543				

Acceptance Criteria:

- %RSD of six different sample solutions should not more than 2.

ACCURACY:

Table-: The accuracy results for Dolutegravir

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	392892.7	5	5.028	100.541%	100.352%
100%	781997	10	10.027	100.262%	
150%	1171989	15	15.039	100.254%	

Table : The accuracy results for Rilpivirine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	204963	15	15.157	101.041%	100.94%
100%	365019	30	30.379	101.261%	
150%	521063.3	45	45.217	100.485%	

Robustness

Table-: Results for Robustness
Results for Robustness - Dolutegravir

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	765788	2.102	5588	1.7
Less Flow rate of 0.9 mL/min	758699	2.330	5459	1.7
More Flow rate of 1.1 mL/min	7689585	1.950	5697	1.7
Less organic phase	758413	2.290	5585	1.4
More organic phase	769851	1.998	5354	1.5

Table : Results for Robustness- Rilpivirine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	2532159	3.537	5399	1.6
Less Flow rate of 0.9 mL/min	2458693	3.885	5328	1.7
More Flow rate of 1.1 mL/min	2658641	3.263	5257	1.7
Less organic phase	2452149	4.435	5213	1.2
More organic phase	2653895	3.009	5525	1.0

Acceptance Criteria:
The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION:

A new method was established for simultaneous estimation of Dolutegravir and Rilpivirine by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Dolutegravir and Rilpivirine by using Phenomenex Luna C18 (4.6×250mm, 5µm) particle size, flow rate was 1ml/min, mobile phase ratio was (45:55 v/v) Acetonitrile: Phosphate Buffer (pH-4.6 was adjusted with orthophosphoric acid), detection wave length was 245nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.102mins and 3.537mins. The % purity of Dolutegravir and Rilpivirine was found to be 99.8%. The system suitability parameters for Dolutegravir and Rilpivirine such as theoretical plates and tailing factor were found to be within limits. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Dolutegravir and Rilpivirine was found in concentration range of 6µg-14µg and 18µg-42µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 100.351% and 100.93%, %RSD for repeatability was 0.177 and 0.595. The precision study was precise, robust, and repeatable. LOD value was 0.6 and 0.8, and LOQ value was 1.8 and 2.4 respectively.

Hence the suggested RP-HPLC method can be used for routine analysis of Dolutegravir and Rilpivirine in API and Pharmaceutical dosage form.

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