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Research article

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A new analytical method development and validation for estimation of rilpivirine and dolutegravir in bulk and tablet by RP-HPLC

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ABSTRACT

A New RP-HPLC Method for the Simultaneous Estimation of Dolutegravir and Rilpivirine in bulk and its Pure and Pharmaceutical Dosage Form as per ICH Guidelines. The Present work was to develop a simple, fast, accurate, precise, reproducible, Reverse Phase High Performance Liquid Chromatographic Method for simultaneous estimation of Dolutegravir and Rilpivirine in pure and combined dosage form. Chromatographic separation was done using Symmetry ODS C18 column having dimension of 4.6×250mm having particle size of 5.0µm, with mobile phase consisting of Acetonitrile: Methanol in the ratio 65:45v/v, flow rate was adjusted to 1ml/min and detection wavelength at 256nm. The retention times of Dolutegravir and Rilpivirine was found to be 2.256 and 5.427 mins. The proposed method has been validated for accuracy, precision, linearity; robustness and range were within the acceptance limit according to ICH guidelines. Linearity for Dolutegravir and Rilpivirine was found in range of 6µg-14µg and 18µg-42µg and correlation coefficient was found to be 0.999 and 0.999% RSD for intermediate precision was found to be 0.5 and 0.3, for repeatability was 0.4 and 0.1, % mean recovery for Dolutegravir and Rilpivirine was found to be 101.326% and 100.501% respectively. The method was found to be robust even by change in the mobile phase ±2% and in more and less flow conditions. The developed method can be successfully employed for the routine analysis of Dolutegravir and Rilpivirine in bulk and Pharmaceutical dosage forms.

Keywords: Dolutegravir and Rilpivirine, RP-HPLC, Validation, Accuracy.

INTRODUCTION

Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the *mobile phase*, which carries it through a structure holding another material called the *stationary phase*. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation.

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for later use, and is thus a form

of purification. Analytical chromatography is done normally with smaller amounts of material and is for establishing the presence or measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive.

Chromatography is based on the principle where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase. The factors effective on this separation process include molecular characteristics related to adsorption (liquid-solid), partition (liquid-solid), and affinity or differences among their molecular weights. Because of these differences, some components of the mixture stay longer in the stationary phase, and they move slowly in the chromatography system, while others pass rapidly into mobile phase, and leave the system

faster. Based on this approach three components form the basis of the chromatography technique.

- Stationary phase: This phase is always composed of a “solid” phase or “a layer of a liquid adsorbed on the surface a solid support”.
- Mobile phase: This phase is always composed of “liquid” or a “gaseous component.”
- Separated molecules

The type of interaction between stationary phase, mobile phase, and substances contained in the mixture is the basic component effective on separation of molecules from each other. Chromatography methods based on partition are very effective on separation, and identification of small molecules as amino acids, carbohydrates, and fatty acids. However, affinity chromatographies (ie. ion-exchange chromatography) are more effective in the separation of macromolecules as nucleic acids, and proteins. Paper chromatography is used in the separation of proteins, and in studies related to protein synthesis; gas-liquid chromatography is utilized in the separation of alcohol, ester, lipid, and amino groups, and observation of enzymatic interactions, while molecular-sieve chromatography is employed especially for the determination of molecular weights of proteins. Agarose-gel chromatography is used for the purification of RNA, DNA particles, and viruses. Stationary phase in chromatography, is a solid phase or a liquid phase coated on the surface of a solid phase. Mobile phase flowing over the stationary phase is a gaseous or liquid phase. If mobile phase is liquid it is termed as liquid chromatography (LC), and if it is gas then it is called gas chromatography (GC). Gas chromatography is applied for gases, and mixtures of volatile liquids, and solid material. Liquid chromatography is used especially for thermal unstable, and non-volatile samples.

The purpose of applying chromatography which is used as a method of quantitative analysis apart from its separation, is to achieve a satisfactory separation within a suitable time interval. Various chromatography methods have been developed to that end. Some of them include column chromatography, thin-layer chromatography (TLC), paper chromatography, gas chromatography, ion exchange chromatography, gel permeation chromatography, high-pressure liquid chromatography, and affinity chromatography.

- ✚ Column chromatography
- ✚ Ion-exchange chromatography
- ✚ Gel-permeation (molecular sieve) chromatography
- ✚ Affinity chromatography
- ✚ Paper chromatography
- ✚ Thin-layer chromatography
- ✚ Gas chromatography
- ✚ Dye-ligand chromatography
- ✚ Hydrophobic interaction chromatography
- ✚ Pseudoaffinity chromatography
- ✚ High-pressure liquid chromatography (HPLC)

High-pressure liquid chromatography (HPLC)

Using this chromatography technique it is possible to perform structural, and functional analysis, and purification of many molecules within a short time, This technique yields perfect results in the separation, and identification of amino acids, carbohydrates, lipids, nucleic acids, proteins, steroids, and other biologically active molecules, In HPLC, mobile phase passes through columns under 10–400 atmospheric pressure, and with a high (0.1–5 cm/sec) flow rate. In this technique,

use of small particles, and application of high pressure on the rate of solvent flow increases separation power, of HPLC and the analysis is completed within a short time. Essential components of a HPLC device are solvent depot, high-pressure pump, commercially prepared column, detector, and recorder. Duration of separation is controlled. Essential components of a HPLC device are solvent depot, high-pressure pump, commercially prepared column, detector, and recorder. Duration of separation is controlled with the aid of a computerized system, and material is accrued [23].

MATERIALS AND METHODS

Dolutegravir from Sura labs, Rilpivirine from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK). Acetonitrile for HPLC from 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 Dolutegravir and 0.3ml of the Rilpivirine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol. 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 : 35°C
 Column : Symmetry ODS C18 (4.6×250mm, 5µm) particle size
 Mobile phase : Acetonitrile: Methanol (65:45v/v)
 Flow rate : 1ml/min
 Wavelength : 256 nm
 Injection volume : 20 µl
 Run time : 10 min

VALIDATION

PREPARATION OF MOBILE PHASE

Preparation of mobile phase

Accurately measured 650 ml (65%) of Acetonitrile, 450 ml of Methanol (450%) were mixed and degassed in digital ultrasonicator 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: Methanol (65:45v/v)
 Column : Symmetry ODS C18 (4.6×250mm, 5µm) particle size
 Flow rate : 1 ml/min
 Wavelength : 256 nm

Column temp : 35°C
Injection Volume : 20 µl

Run time : 10 minutes

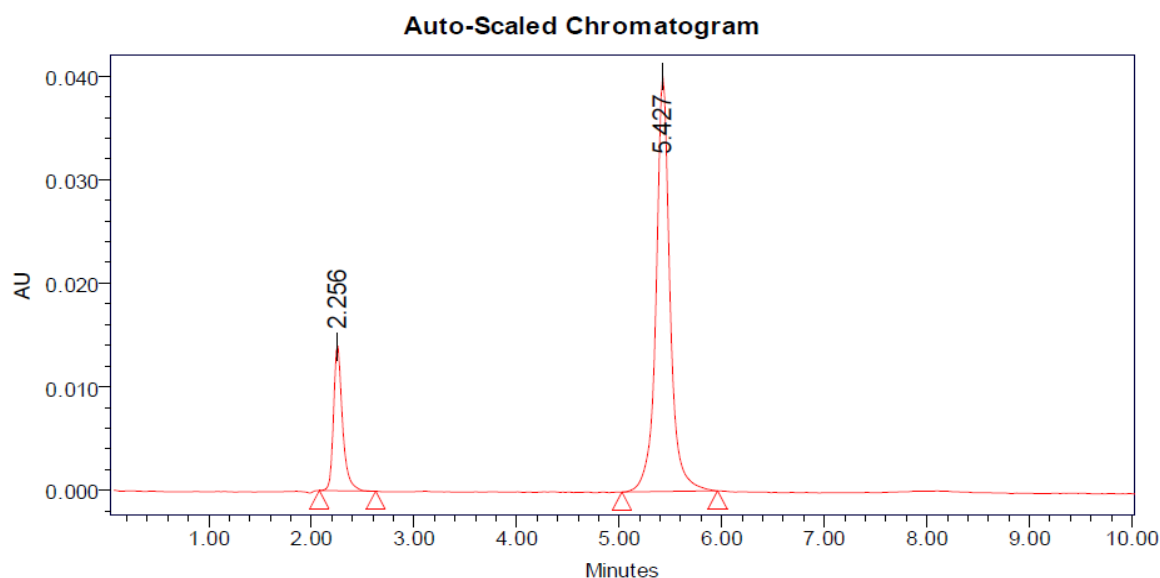


Fig 1: Optimized Chromatogram

Table 1: Peak results for Trail

S. No	Peak name	R _t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Dolutegravir	2.220	39638	2718		0.69	278
2	Rilpivirine	5.220	124267	13913	9.63	1.00	9218

The chromatogram shows void peaks, and broad peak is obtained. The trial shows tailing effect so go for further trials to obtain good separation of the peak.

Optimized Chromatogram (Sample)

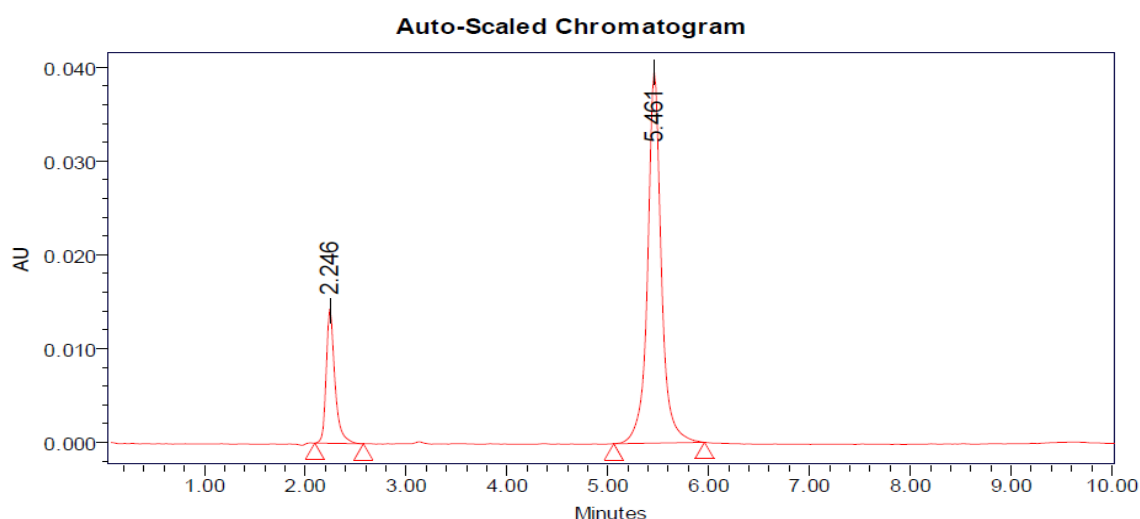


Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S. No	Peak name	R _t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Dolutegravir	2.246	87584	14254		1.32	5648

2	Rilpivirine	5.461	398565	41365	16.42	1.02	9416
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- 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 3: Results of system suitability for Dolutegravir

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Dolutegravir	2.247	86585	14451	5506	1.36
2	Dolutegravir	2.246	86598	14225	5674	1.2
3	Dolutegravir	2.248	86754	14332	5298	1.2
4	Dolutegravir	2.252	86598	14306	5132	1.0
5	Dolutegravir	2.248	86547	14252	5412	1.33
Mean			86616.4			
Std. Dev			79.70759			
% RSD			0.092024			

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Table 4: Results of system suitability for Rilpivirine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Rilpivirine	5.452	385428	41256	9546	1.04	15.0
2	Rilpivirine	5.484	385976	41365	9524	1.5	15.5
3	Rilpivirine	5.491	385951	41258	9567	1.2	15.3
4	Rilpivirine	5.482	385968	41289	9476	1.1	15.1
5	Rilpivirine	5.491	385694	41274	9327	1.2	15.2
Mean			385803.4				
Std. Dev			240.6051				
% RSD			0.062365				

- %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.

Assay (Sample)

Table 5: Peak results for Assay sample

S.No	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Dolutegravir	2.247	86985	14352		1.36	5688	1
2	Rilpivirine	5.452	386587	41365	16.42	1.38	9547	1
3	Dolutegravir	2.246	86852	14269		1.32	5628	2
4	Rilpivirine	5.461	385784	41298	16.42	1.04	9587	2
5	Dolutegravir	2.243	86542	14325		1.03	5642	3
6	Rilpivirine	5.466	385983	41354	16.48	1.02	9658	3

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

The % purity of Dolutegravir and Rilpivirine in pharmaceutical dosage form was found to be 99.9 %.

linearity

Chromatographic data for linearity study

Dolutegravir

Concentration µg/ml	Average Peak Area
6	51476
8	67598
10	84897
12	101114
14	119554

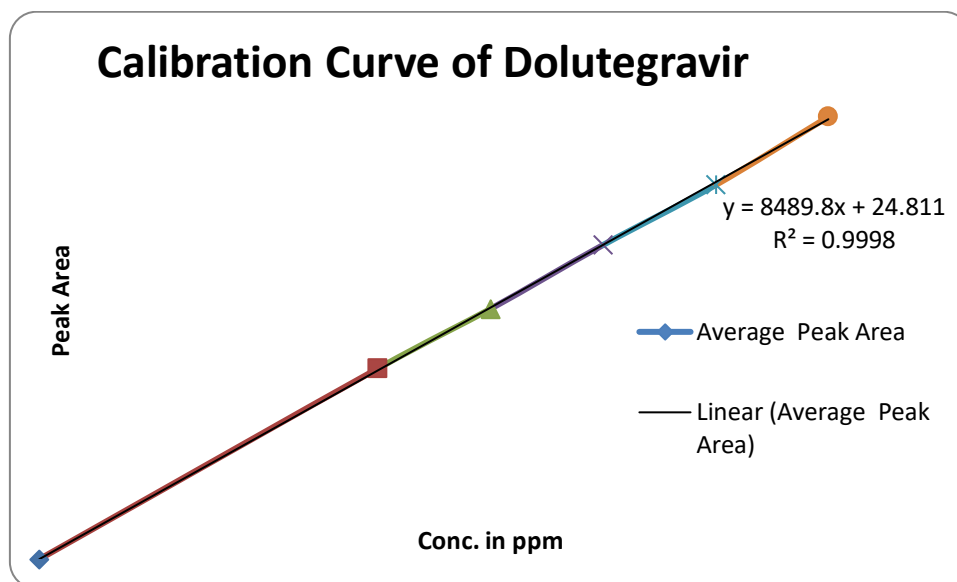


Fig 3: Calibration graph for Dolutegravir

Table 6: Chromatographic Data for Linearity Study Rilpivirine

Concentration µg/ml	Average Peak Area
18	2286598
24	3086587
30	3867579
36	4758517
42	5604874

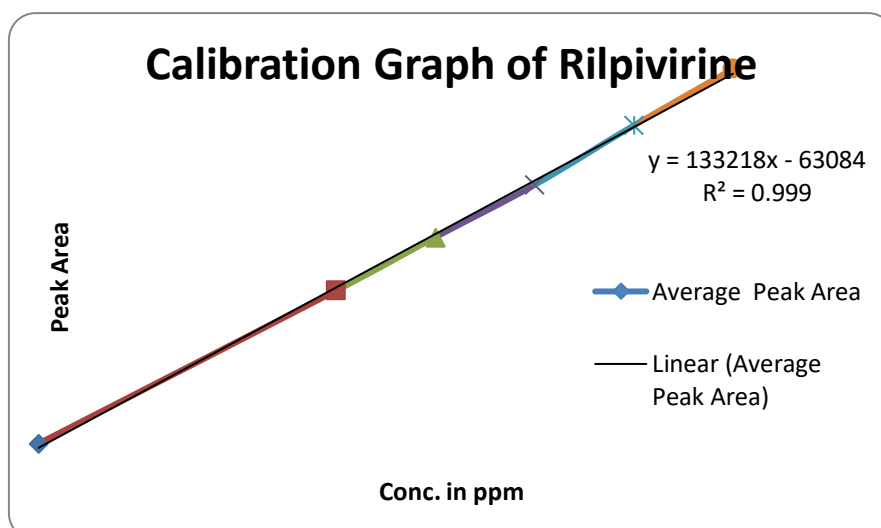


Fig 4: Calibration Graph for Rilpivirine

Epeatability

Table 7: Results of Repeatability for Dolutegravir

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Dolutegravir	2.269	86985	14565	5648	1.4
2	Dolutegravir	2.255	86879	14658	5654	1.4
3	Dolutegravir	2.252	86578	14652	5623	1.4
4	Dolutegravir	2.267	86598	14525	5713	1.4
5	Dolutegravir	2.260	86578	14632	5698	1.3
Mean			86723.6			1.4
Std. Dev			194.0703			
% RSD			0.22378			
			86723.6			

- %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 8: Results of method precision for Rilpivirine

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Rilpivirine	5.274	386598	41236	9475.5	1.1	15.4
2	Rilpivirine	5.266	385474	41365	9420.4	1.1	15.6
3	Rilpivirine	5.265	386895	41256	9489.4	1.1	15.3
4	Rilpivirine	5.278	384574	41329	9383.0	1.1	15.3
5	Rilpivirine	5.305	386548	41652	9441.5	1.1	15.3
Mean		5.319	385874	41236	9474.1	1.1	15.3
Std. Dev			385993.8				
% RSD			870.0268				
			0.225399				

- %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 9: Results of Intermediate precision day 1 forDolutegravir

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Dolutegravir	2.248	87521	14356	5632.5	1.4
2	Dolutegravir	2.245	86598	14269	5589.4	1.4
3	Dolutegravir	2.242	86987	41352	5658.2	1.4
4	Dolutegravir	2.239	87213	41269	5652.1	1.3
5	Dolutegravir	2.243	86548	41254	5703.3	1.4
6	Dolutegravir	2.246	87548	41365	5648.4	1.3
Mean			87069.17			
Std. Dev			436.918			
% RSD			0.501806			

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

Table 10: Results of Intermediate precision day 1 for Rilpivirine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Rilpivirine	5.284	386213	41565	9458	1.1	15.8
2	Rilpivirine	5.293	385698	41659	9521	1.1	15.5
3	Rilpivirine	5.306	385789	41378	9487	1.0	15.5
4	Rilpivirine	5.319	385897	41659	9456	1.1	15.8
5	Rilpivirine	5.346	385489	41665	9562	1.1	15.6
6	Rilpivirine	5.352	382764	41584	9547	1.1	15.9
Mean			385308.3				
Std. Dev			1269.181				
% RSD			0.329394				

- %RSD of Six different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

Table 11: Results of Intermediate precision Day 2 for Glipizide

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Glipizide	2.067	249499	11594	5240	1.2
2	Glipizide	2.069	240991	11357	5130	1.2
3	Glipizide	2.068	240431	11878	5136	1.2
4	Glipizide	2.069	241330	11748	5267	1.2
5	Glipizide	2.067	240519	11830	5222	1.2
6	Glipizide	2.067	240470	11475	5982	1.2
Mean			242206.7			
Std. Dev			3590.034			
% RSD			1.48222			

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

Table 12: Results of Intermediate precision Day 2 for Dolutegravir

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Dolutegravir	2.255	87584	14365	5698	1.4
2	Dolutegravir	2.260	86598	14452	5682	1.4
3	Dolutegravir	2.242	86584	14369	5647	1.4
4	Dolutegravir	2.245	86758	14524	5682	1.3
5	Dolutegravir	2.260	86462	14365	5624	1.4
6	Dolutegravir	2.255	86523	14396	5687	1.3
Mean			86751.5			
Std. Dev			419.6998			
% RSD			0.483795			

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

Table 13: Results of Intermediate precision for Rilpivirine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Rilpivirine	5.266	396585	41365	9568	1.0	15.5
2	Rilpivirine	5.265	398658	41452	9487	1.1	15.8
3	Rilpivirine	5.306	399897	41268	9587	1.1	15.6
4	Rilpivirine	5.293	395785	41365	9528	1.1	15.9
5	Rilpivirine	5.265	396879	41658	9487	1.2	15.1
6	Rilpivirine	5.266	396887	41874	9562	1.0	15.3
Mean			397448.5				
Std. Dev			1523.845				
% RSD			0.383407				

- %RSD of Six different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is rugged.

Accuracy

Table 14: The accuracy results for Dolutegravir

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	42603.33	5	5.015	100.30	101.326%
100%	86533	10	10.190	101.90	
150%	129631.667	15	15.267	101.78	

Table 15: The accuracy results for Rilpivirine

% Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	264159	15	15.094	100.626	100.501%
100%	465304.3	30	30.194	100.646	
150%	663923	45	45.104	100.231	

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Dolutegravir

Table 16: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	86895	2.256	5635	1.32
Less Flow rate of 0.9 mL/min	89897	2.505	5852	1.27
More Flow rate of 1.1 mL/min	83526	2.046	5265	1.20
Less organic phase	89865	2.505	5125	1.20
More organic phase	80898	2.046	5253	1.27

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

Rilpivirine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	385689	5.427	9452	1.01
Less Flow rate of 0.9 mL/min	398985	5.599	9456	1.03
More Flow rate of 1.1 mL/min	326538	4.576	9658	0.98
Less organic phase	396869	5.599	9454	1.02
More organic phase	341254	4.576	9584	0.99

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

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Dolutegravir and Rilpivirine in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Dolutegravir and Rilpivirine was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: TEA Buffer pH 4.5: Acetonitrile (65:15:20) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-

HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Dolutegravir and Rilpivirine in bulk drug and in pharmaceutical dosage forms.

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