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STABILITY INDICATING HPLC METHOD FOR RILPIVIRINE AND DOLUTEGRAVIR SODIUM

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ABSTRACT

Objective: To develop and validate stability indicating HPLC method for determination of Rilpivirine and Dolutegravir Sodium. **Methods:** The HPLC method was developed using mobile phase p^H adjusted to 3 (Distilled water: MeOH) (40:60) v/v stationary phase was HiQSiL C_{18} Column (250* 4.6 mm) at the flow rate 1 ml/min. The retention time for the drug was found to be: Rilpivirine = 4.54 ± 0.2 min, Dolutegravir Sodium = 10.04 ± 0.2 min.The eluted compounds were detected using PDA detector. **Result:** The drugs were subjected to stress testing as per ICH Q1A (R2). There was no interference of any degradant at RT of Rilpivirine and Dolutegravir Sodium. The developed method was successfully validated according to ICH Q2R1 guidelines. The calibration curve was found to be linear over a range of 10 - 50 µg/ml. The accuracy of the method is indicated by good recovery in the range of for Rilpivirine 99.52% - 101.48% and of Dolutegravir Sodium 97.02% - 111.97. The limit of detection and limit of quantification of Rilpivirine was found to be LOD-1.743 µg/ml and LOQ-5.284 µg/ml and for Dolutegravir Sodium was found to be LOD-1.267 µg/ml and LOQ-3.840µg/ml. **Conclusion:** A new simple, accurate, precise and specific stability- indicating high performance liquid chromatographic (HPLC) method has been developed and validated for determination of Rilpivirine and Dolutegravir Sodium in combination.

KEYWORDS: Stability Indicating, HPLC, Rilpivirine and Dolutegravir Sodium.

INTRODUCTION

Rilpivirine[1] is chemically 4-{[4-({4-[(1E)-2- cyanoeth-1-en-1-yl]-2, 6 dimethylphenyl} amino) pyrimidin-2 yl] amino}benzonitrile. It is the second generation of non-nucleoside reverse transcriptase inhibitors (NNRTIS) recently marketed for the treatment of HIV infection. It is superior to the first generation NNRTI in that it is active against NNRTI resistant HIV-I. It is a diarylpyrimidine, a class of molecules that resemble pyrimidine nucleotides found in DNA. Because of its flexible chemical structure, resistance to Rilpivirine is less likely to develop than other NNRTI's. Dolutegravir[2-3], (4R,12aS)-N-(2,4-difluorobenzyl)-7-hydroxy-4-methyl-6,8-dioxo-3 4,6, 8,12,12a-hexahydro-2H-pyrido[1',2':4,5]pyrazino[2,1-b][1,3]oxazine-9-carboxamide.It is integrase inhibitor used in the treatment of HIV and was approved by FDA. Dolutegravir works by blocking integrase, an HIV enzyme. This prevents HIV from replicating and lowers the amount of HIV in the blood. It can be used in the treatment of HIV in both adults and children of 12 years age and older and weighing at least 40 kilograms. Literature review revealed that UV,[4] HPTLC[5,6] and[7-13] HPLC methods have been reported for analysis of Rilpivirine and Dolutegravir sodium as a single form and in combination with other drugs. There is no reported method is available for simultaneous estimation of Rilpivirine and Dolutegravir sodium by HPTLC in combination. The work describes new adoptable method for simultaneous quantitation of Rilpivirine and Dolutegravir Sodium by HPLC in combination. Janssen[14] announces Two-drug Combination of Dolutegravir and Rilpivirine Demonstrates Efficacy in Maintaining Viral Suppression in Phase III. Study results presented by Janssen indicates that combination of Rilpivirine and Dolutegravir Sodium can be very promising, Two Drug regimen; for HIV patients. Hence stability indicating method has been developed for this combination as per ICH Q1A R2[15-16] guidelines & the method was validated.

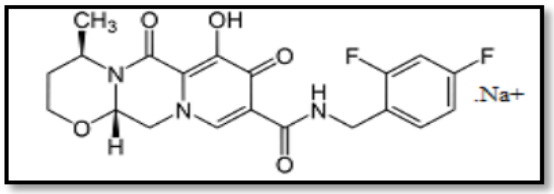

Fig 1: Structure of Dolutegravir Sodium.

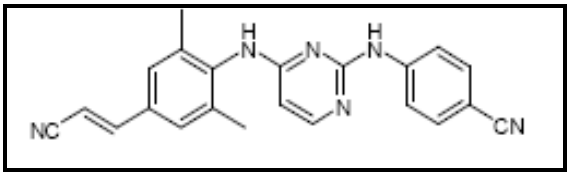

Fig 2: Structure of Rilpivirine.

MATERIALS AND METHODS

Reagents and chemicals

Working standard of Rilpivirine and Dolutegravir Sodium were obtained from Mylan Labs, Hyderabad and Lupin Laboratories Ltd, Aurangabad, respectively. Methanol (AR grade), Hydrochloric acid (HCl), Hydrogen peroxide (H_2O_2, 30% & 6% v/v), Sodium hydroxide (NaOH) were purchased from LOBA CHEMIE Pvt. Ltd Mumbai.

Method development

Method development for resolution of Rilpivirine and Dolutegravir Sodium was started with the development of chromatogram with solvents in different ratios and combinations of methanol, Acetonitrile, p^H adjusted to 3 of Distilled water. Finally, (Distilled water: MeOH (40:60) v/v was selected as a mobile phase with a good resolution at RT Rilpivirine = 4.54 ± 0.2 min, Dolutegravir Sodium = 10.04 ± 0.2 min for Rilpivirine and Dolutegravir Sodium respectively.

Instruments

Quantitative HPLC was performed using isocratic high performance liquid chromatography liquid chromatography (JASCO HPLC system) with a LC-PU 2080 plus pump, manual injector with loop volume of 20 µL (Rheodyne),programmable MD 2010 PDA detector and HiQSiL C_{18} Column (250 * 4.6 mm).The HPLC system was equipped with BORWIN-PDA software (version 1.5). An electronic balance (Shimadzu AY-120), UV-Visible (make JASCO, Model V -730) spectrophotometer.

Selection of Analytical Wavelength

From the standard stock solution (1000 µg/ml) further dilutions were done using methanol and scanned over the range of 200 - 400 nm and the spectra was obtained. It was observed that showed considerable absorbance at 272nm (Rilpivirine) & 258 nm (Dolutegravir Sodium).

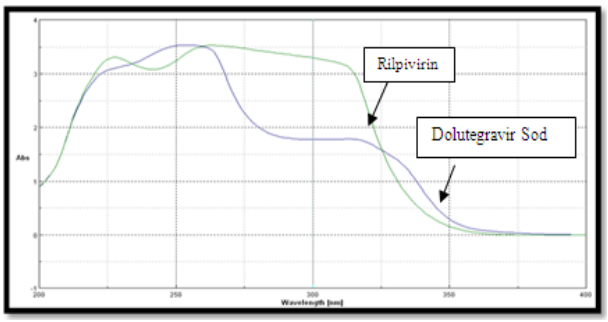

Fig 3: Absorbance spectra of 272nm (Rilpivirine) & 258 nm (Dolutegravir Sodium).

Optimized chromatographic conditions

The mobile phase consisted of (Distilled water p^H adjusted to 3: MeOH) (40:60) v/v. It was then filtered through 0.45 µ membrane filter paper using vacuum filtration assembly and then sonicated using ultrasonic water bath for 15 min. The flow rate of mobile phase was maintained at 1ml/min. The column was kept at ambient temperature.

Preparation of solutions

Preparation of Standard Stock Solution

Standard stock solutions of Rilpivirine and Dolutegravir Sodium were prepared by separately dissolving 10 mg of drug in 10 ml of methanol to get concentrations of 1000 µg/ml. Respective stock solutions of strength 100 µg/ml were diluted appropriately to get standard stock solution.

Stress Degradation Studies of Bulk Drugs

Stress testing studies were carried out separately on each drug to provide evidence on how the quality of drug varies under the influence of variety of stress conditions like hydrolysis under different pH conditions, oxidation, photolysis and thermal etc. Optimization of stress conditions was done by changing strength of reagent and duration of exposure to obtain degradation preferably in the 10-30% range. The analysis was carried out by HPLC with a PDA detector.

Alkali catalyzed hydrolysis

First the degradation was tried with 0.1 N NaOH for 1 hr, degradation observed was too low. So later harsh condition was used 0.5N NaOH for Rilpivirine. Base hydrolysis was done using 1 ml of solution of Rilpivirine mixed with 1 ml of 0.5N NaOH. The solution was diluted to 10 ml with methanol and injected after 1 hr. same procedure was repeated for working standard solution of Dolutegravir Sodium using 0.1N NaOH, and injected after 1 hr.

Acid Catalyzed Hydrolysis

The solution was exposed to Acid hydrolysis 1 ml of solution of Rilpivirine was mixed with 1 ml of 0.5N HCl. The solution was diluted to 10 ml with methanol and injected after 1 hr. same procedure was repeated for working standard solution of Dolutegravir Sodium with 0.1N HCl, and spotted after 1 hr. First the degradation was tried with 0.1 N HCl for 1 hour degradation observed was too low. So later harsh condition was used 0.5N HCl for Rilpivirine was used.

Oxidation Degradation

For Oxidative studies, 30% v/v of H_2O_2 for Rilpivirine and 6% v/v of H_2O_2 for Dolutegravir Sodium was used.1 ml of solution of Rilpivirine was mixed with 1 ml of 30% H_2O_2. The solution was diluted to 10 ml with methanol and injected after 1 hr. Same procedure was repeated for working standard solution of Dolutegravir Sodium with 6% H_2O_2, and injected after 1 hr. First the degradation was tried with 6% H202 for 1 hour

degradation observed was too low. So later harsh conditions were used 30% H_2O_2 for Rilpivirine was used.

Degradation under Dry Heat

Dry heat studies were performed by keeping drug sample of Rilpivirine in oven (60°C) for a period of 2 hrs. Sample were withdrawn and dissolved in methanol and diluted to get 1000µg/ml as stock solution. Stock solution was diluted to100 µg/ml concentration of 4 µl was injected. Dolutegravir Sodium was exposed to 60°c for 2 hrs and solution was prepared as sample were withdrawn and dissolved in methanol and diluted to get 1000µg/ml as stock solution. From Stock 1 ml was pipette out i.e. 100 µg/ml concentrations.

Photo-Degradation

Photolytic studies were also carried out by exposure of drug to UV light up to 200 watt hours/square meter and subsequently to cool fluorescent light to achieve an illumination of 1.2 million Lux hr. Samples were withdrawn and dissolved in methanol and diluted to get 1000µg/ml as stock solution. Dolutegravir Sodium was exposed to UV and Fluorescence solution was prepared as sample were withdrawn and dissolved in methanol and diluted to get 1000µg/ml as stock solution. From Stock 1 ml was pipette out i.e. 100 µg/ml concentrations further diluted to 50µg/ml and injected.

RESULTS AND DISCUSSION

Chromatogram

Solution of Rilpivirine and Dolutegravir Sodium (1000 µg/mL) was prepared and from this 100 µg/mL was used and (10-50 µg/ml) of solution was injected with the help of Hamilton syringe (100 µl). The column was equilibrated with the mobile phase (indicated by constant back pressure at desired flow rate). Working standard solution of drug (10µg/ml) was injected into the system. The retention time for the drug was found to be: Rilpivirine = 4.54 ± 0.2 min, Dolutegravir Sodium = 10.04 ± 0.2 min.

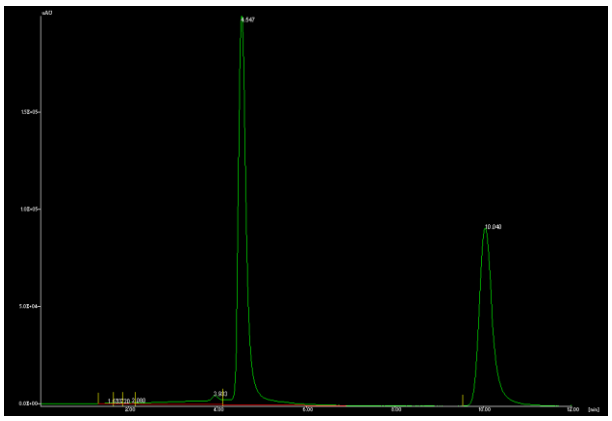

Fig 4: Representative Chromatogram of standard solution of Rilpivirine (50µg/ml)R.T-4.54 min and Dolutegravir Sodium (50µg/ml) R.T-10.04min.

Summary of chromatographic parameters selected

Table 1: Chromatographic parameters.

Sr. No.	Parameter	Conditions used for Analysis
1.	Stationary phase	Column: HiQSiL C_{18} Column HS 511
2.	Mobile phase	Distilled Water (P^h 3): MeOH(40:60)
3.	Detection Wavelength	258nm
4.	Flow rate	1ml/min
5.	Sample Volume	20µl
6.	Column temperature	Ambient

Forced degradation studies

Forced degradation studies were conducted to evaluate the stability and specificity of the method. No degradation products was observed when the drug was subjected to acidic, basic and neutral treatment, exposure to UV light, heat and to oxidation conditions. The drug peaks obtained from all the stressed samples were found to be homogenous and pure as confirmed by peak purity study. This method is found to be specific.

Table 2: Summary of stress degradation of Rilpivirine Stress degradation conditions.

Sr. No.	Stress degradation conditions of Rilpivirine	Percent degraded (%)
1.	Base (0.5 N NaOH, kept for 1 hr)	14.02
2.	Acid (0.5 N HCl, Kept for 1 hr)	11.20
3.	H_2O_2 30% v/v (kept for 1 hr)	15.17
4.	Dry Heat (60ºC, 2hrs)	18.98
5.	Photo stability (UV, 200 watt hrs/square meter)	22.52
6.	Florescence (1.2 million Lux. Hrs)	20.70

Table 3: Summary of stress degradation of Dolutegravir Sodium Stress degradation conditions (Dolutegravir Sodium was more sensitive to oxidation and hydrolysis stress conditions).

Sr. No.	Stress degradation conditions of Dolutegravir Sodium	Percent degraded (%)
1.	Base (0.1 N NaOH, kept for 1 hr)	19.55
2.	Acid (0.1 N HCl, Kept for 1 hr)	12.88
3.	H_2O_2 6% v/v (kept for 1 hr)	13.79
4.	Dry Heat (60ºC, 2hrs)	16.36
5.	Photo stability (UV, 200 watt hrs/square meter)	14.80
6.	Florescence (1.2 million Lux. Hrs)	19.55

Validation of Analytical Method

The method was validated for various parameters in accordance with ICH guidelines.

Specificity
The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than, indicating the non interference of any other peak of degradation product or impurity.

Table 4: Specificity.

Sr. No.	Conditions	Rilpivirine Percent degraded (%)	Dolutegravir Sodium Percent degraded (%)	Rilpivirine		Dolutegravir Sodium	
				Peak Tail	Peak Front	Peak Tail	Peak Front
1.	Base Hydrolysis	14.02	19.55	997.7	998.8	999.6	999.6
2.	Acid Hydrolysis	11.20	12.88	999.7	999.8	990.9	993.6
3.	Oxidation	15.17	13.79	990.2	998.7	999.2	999.7
4.	Heat(60ºC 2 hrs)	18.98	16.36	993.9	993.2	998.5	980.3
5.	UV(200 watt hrs/square meter)	22.52	14.80	998.2	998.2	999.9	987.7
6.	Fluorescence(1.2 million Lux.hrs)	20.70	19.55	999.5	999.7	998.2	999.0

Linearity
From the standard stock solution (1000 µg/mL) five replicates per concentration were injected. The linearity was determined by analyzing five concentrations over the concentration range of 10-50 µg/ml for Rilpivirine and Dolutegravir Sodium.

Range
Rilpivirine = 10-50 µg/ml
Dolutegravir Sodium= 10-50 µg/ml

Assay
Assay was performed on blend of bulk drugs plus excipients. It was determined by extrapolation of peak area was found to be Rilpivirine - 102.97% and for Dolutegravir Sod -99.32%.

Accuracy
The accuracy of the developed method was established by standard addition method by adding known amount standard to the pre-analyzed samples in different levels, i.e., 80, 100 and 120 %. The samples were analyzed for five times at each level. The mean recovery and RSD values were calculated. This is in accordance with ICH guidelines. Therefore method was found to be accurate. Recovery of Rilpivirine was found to be 99.52% - 101.48% with less than 0.96%of RSD value and of Dolutegravir Sodium was found to be 97.02% -102.97 % with less than 0.70% indicating that the proposed method.

Table 5: Recovery Studies of Rilpivirine and Dolutegravir Sodium.

Drug	Amount Taken (µg/ml)	Total amount found (µg/ml)	% Recovery	% RSD
1. Rilpivirine	80%	9.952	99.52%	0.5648
	100%	19.69	98.48%	0.6531
	120%	30.44	101.48%	1.7548
2. Dolutegravir Sodium	80%	9.702	97.02%	0.7885
	100%	10.102	101.02%	1.2799
	120%	11.197	102.97%	1.0669

Precision
Interday precision of the system was evaluated by analyzing three independent standard preparations on three different days. % RSD value obtained was calculated to determine system precision (Table 3.8). Intraday precision of the method was evaluated by analyzing six independent sample preparations in a day. % RSD value obtained was calculated to determine method precision.

Table 6: Precision (INTRA-DAY).

Conc. (µg/ml)	Area		Mean Area		Mean SD		% RSD	
	RILP	DOLU	RILP	DOLU	RILP	DOLU	RILP	DOLU
	170267.11	158022.12						
10	164629.72	159603.22	16653	15960	2.20	1.41	1.08	1.23
	164709.75	158706.23						
	223339.3	257399.2						
20	223224.6	256940.3	22322	25694	1.29	1.35	1.80	1.98
	216720.8	265158.2						
	443543.87	505851.6						
30	447280.84	504282.6	44728	50428	1.18	0.91	1.20	0.80
	436992.05	513065.6						

Table 7: Precision (INTER- DAY).

Conc. (µg/ml)	Area		Mean Area		SD		%RSD	
	RILPI	DOLU	RILPI	DOLU	RILPI	DOLU	RILPI	DOLU
	152168.9	165459.3						
10	155122.1	163456.2	15512	16345	1.49	1.13	1.53	1.50
	156450.2	161565.2						
	224233.8	278633.2						
20	222249.4	278634.1	22224	27863	0.38	1.50	0.53	1.62
	224226.4	269634.4						
	425414.7	501682.1						
30	414626.6	511528.2	41462	51152	1.25	0.95	1.35	1.04
	422257.6	506142.1						

Limit of Detection (LOD)

The LOD (Limit of Detection) and LOQ (Limit of Quantitation) were estimated from the standard deviation of the lowest response and the slope of the calibration curve.

LOD and LOQ were calculated from the formula:

$$LOD = \frac{3.3\ \sigma}{S} \qquad LOQ = \frac{10\ \sigma}{S}$$

Where,

σ = standard deviation of the response at lowest concentration in range.

S = slope of the calibration curve.

Table 8: LOD and LOQ.

Drugs	LOD(µg/ml)	LOQ(µg/ml)
Rilpivirine	1.74	5.28
Dolutegravir Sodium	1.26	3.84

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which Mobile phase composition, Meniscus change, Flow rate and the effects on the peak area was noted. The %RSD values of all robustness parameters were examined and found to be within the limit of 2%, showed that the proposed method was robust.

Table 9: Robustness.

Sr. No.	Parameter	Robust condition	% RSD Rilpivirine	% RSD Dolutegravir Sodium
1.	**Flow rate** 1ml/min	0.8	0.0715	0.6942
		1.3	0.4347	0.0399
2.	**Mobile phase ratio** Distilled Water: MeOH(40:60)	38:62	1.6445	0.2468
		42:58	1.6266	0.5023
3.	**P^H**	2.9	0.4292	0.6942
		3.1	1.1715	0.0399

Summary of validation study

Table 10: Summary of Rilpivirine and Dolutegravir Sodium validation studies.

Sr. No.	Validation parameters		Results			
			(Rilpivirine)		(Dolutegravir Sodium)	
1.	Specificity		Specific		Specific	
2.	Linearity Ra		y = 14671x +71921 r^2 = 0.992		y = 17223x – 32367 r^2 = 0.987	
3.	% Recovery		80%	99.52%	80%	97.02%
			100%	98.48%	100%	101.02%
			120%	101.48%	120%	102.97%
4.	Precision					
	A) Intraday precision		%RSD		%RSD	
		10	1.08		1.23	
		20	1.80		1.98	
		30	1.20		0.80	
	A) Interday precision	10	1.53		1.50	
		20	0.53		1.62	
		30	1.35		1.04	
5.	Limit of Detection (µg/ml)		1.74		1.26	
6.	Limit of Quantitation (µg/ml)		5.28		3.84	
7.	Robustness		Robust		Robust	

DISCUSSION

S.S.Chitlange.et al.[6] have reported Stability Indicating HPTLC method for the Simultaneous estimation of Rilpivirin Emtricitabine and Tenofovir in Bulk and Combined Pharmaceutical Dosage Form. In this work, mild conditions were used like 0.1N for Acid and Base hydrolysis, 6% for oxidation but no degradation was observed for Rilpivirine.

T. Sreenivasulu Reddy et al.[7] have reported a validated stability indicating HPLC assay method for Rilpivirine HCl in bulk drug. In this work, HPLC method was developed by using gradient elution and harsh stress conditions were used like 1N& 5Nacid/base for hydrolysis 30% H2O2 for Oxidation and very long duration of 7 days for Thermal stress at 60ºC, but no degradation was observed.

We have developed HPLC method wherein the stress conditions were optimized to achieve 10-30% degradation.

CONCLUSION

The developed method is simple, rapid and stability indicating. It may be used to monitor stability of Rilpivirine and Dolutegravir Sodium

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