RP-HPLC Method development, validation and stability indicating studies for simultaneous estimation of Cabotegravir and Rilpivirine in bulk and its pharmaceutical formulations

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ABSTRACT:

A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography method has been developed for the quantitative analysis of Cabotegravir and Rilpivirine in pharmaceutical dosage form. Chromatographic separation of these are achieved on Waters Alliance-e2695, by using Waters X-Bridge Phenyl (150x4.6mm, 3.5µm) column and the mobile phase containing 0.1% formic acid & ACN in the ratio of 60:40% v/v. The flow rate was 1.0 ml/min; detection was carried out by absorption at 231nm using a photodiode array detector at ambient temperature. The retention times were found to be 7.732 and 4.363 min respectively. Linearity was established for cabotegravir and rilpivirine in the range of 10-60 µg/mL and 15-90 µg/mL respectively, with correlation coefficient (r^2) for both the drugs are 0.9993 and 0.9995. Both drugs were exposed to a variety of stressors, such as oxidative, photolytic, basic, acidic, and thermal stress. With the exception of heat, UV, and neutral environments, with the presence of the degradation products exhibiting a good separation of drug peaks. The proposed method was validated according to ICH guidelines.

Key words: RP- HPLC Cabotegravir and Rilpivirine

I.INTRODUCTION:

HIV-1 integrase strand transfer inhibitor (INSTI). Cabotegravir (CAB), an analogue of dolutegravir, prevents viral DNA integration into the host genome and inhibits HIV replication. CAB is FDA-approved for HIV treatment and is currently in Phase 3 development for HIV prevention.

Cabotegravir is a drug that has been approved under the brand name Vocabria by the U.S. Food and Drug Administration (FDA) for use with oral rilpivirine (brand name: Edurant) for the short-term treatment of HIV infection. Cabotegravir is also being studied as an investigational drug to prevent HIV infection.

Rilpivirine is a non- competitive NNRTI that binds it to reverse transcriptase. It's binding results in the blocking of DNA-dependent DNA polymerase activities such as replication of HIV-1. It does not present action against human DNA polymerase 5-007, β and Δ.12 rilpivirine binds to HIV-1reverse transcriptase (RT) and its versatile structure around the aromatic rings makes it possible to adapt to changes in the non-nucleoside RT binding pocket.

Rilpivirine is used along with other therapeutics to treat human immunodeficiency virus infection (HIV) in some adults and children 12 years of age and older who weight at least 77Ib (35kg) and have not received antiretroviral therapy in the past.

The present study is to develop a stability indicating RP-HPLC method for CAB and RIL. The objective of the study is to subject the drugs for acid, base, peroxide, light, thermal degradation and estimate its extent of degradation. A Literature survey reveals that several analytical methods for the estimation of CAB and RIL were reported.

Although different analytical methods are available, a more economical stability indicating analytical method was developed for estimation of Cabotegravir and Rilpivirine. We have forcefully degraded the drugs (standards) under different stress conditions and developed an HPLC method that can differentiate the pure drug from its degradants. The International Conference on Harmonization (ICH) guideline entitled "Stability Testing of New Drug Substances and Products" requires that stress testing be carried out to elucidate the inherent.

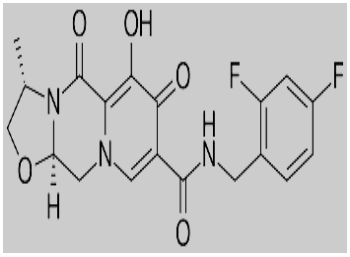

Figure 1: Chemical structure of Cabotegravir

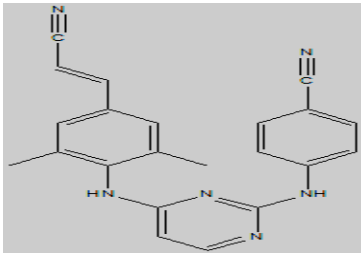

Fig.2: Chemical structure of Rilpivirine

II.MATERIALS AND METHOD:

A. Chemicals and Reagents

1. Acetonitrile and Methanol were of HPLC grade and obtained from SD fine chem. and J. T. Bakers
2. Tri-ethylamine and Ortho phosphoric acid was procured from Fischer scientific, Hyderabad
3. Sodium acetate was purchased from Qualigens, Hyderabad
4. HPLC grade water (Merck)

B. HPLC instruments

- Agilent Zorbax XDB-C18 [150 x 2.1 mm, 5 µ]
- Shiseido C18 [250 x 4.6 mm, 5 µ]

- Phenomenex Kinetex [150 x 2.1 mm, 5 µ]
- Waters symmetry -C18[150 x 4.6 mm, 3. 5 µ]
- X-Bridge phenyl [150 x 4.6 mm, 3. 5 µ]

C. Preparation of standard stock solution

- Accurately weigh and transfer 40 mg of Cabotegravir, 60 mg of Rilpivirine working standard into a 100 ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
- Further pipette 5 ml of the above stock solutions into a 50 ml volumetric flask and dilute up to the mark with diluent. (40ppm of Cabotegravir, 60ppm of Rilpivirine)
- **Sample Solution Preparation:**
  The 200 µl of injection formulation, equivalent to 40 mg of CBV and 60 mg of RLV was precisely pipetted out, followed by the formulation being transferred into a clean dry 1000 ml volumetric flask. Then it was dissolved in acetonitrile and sonicated for 30 minutes for the complete dissolution of drugs. The solution was centrifuged up to 15 minutes at 4000 RPM and the above supernatant was pipetted out. An aliquot of 5 ml of supernatant was diluted 10 times to get 40 ppm of CBV and 60 ppm of RLV.
- **Preparation of Mobile Phase:**
  Mobile phase was prepared by mixing 0.1% formic acid and ACN taken in the ratio 60:40. It was filtered through 0.45µ membrane filter to remove the impurities which may interfere in the final chromatogram

Method Validation

The established method in this study was validated following the quality guidelines of the international conference on harmonization, ICH Q2 (R1). The parameters are system suitability, specificity, sensitivity, robustness, accuracy, and precision were studied by using the developed method [10 and 11]. The system suitability of the method was assessed by injecting a stock solution containing 10 ppm CBV and 15 ppm RLV six times into the HPLC system. The tailing factor, theoretical plate count, and resolution for the peaks were observed in chromatogram tables

DEGRADATION STUDIES:

Preparation of stock:

Accurately weigh and transfer 40mg of Cabotegravir, 60mg of Rilpivirine working standard into a 100 ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Acid degradation

Pipette 5 ml of above solution into a 50ml volumetric flask and 3 ml of 1N HCl was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

Alkali degradation

Pipette 5 ml of above solution into a 50ml volumetric flask and add 3ml of 1N NaOH was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 1N HCl and make up to 10ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

Peroxide degradation

Pipette 5 ml above stock solution into a 50ml volumetric flask, 1 ml of 3% w/v of hydrogen peroxide added in 50 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

Thermal degradation

Cabotegravir, Rilpivirine sample was taken in Petri dish and kept in Hot air oven at 110^0 C for 24 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analyzed.

Photolytic degradation

Cabotegravir, Rilpivirine sample was placed in sun light for 24 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analysed.

III.RESULTS AND DISCUSSION:

A. Method development and optimization:

The choice of the detection wavelength was based on the scanned absorption spectrum of cabotegravir and Rilpivirine 10mg of drugs were dissolved in 10ml of Acetonitrile and Formic acid 0.1% (40:60) separately. The UV spectrum of cabotegravir and Rilpivirine was separately scanned in the wave length range 200-400nm. After correlation of the spectrum 231nm wavelength was selected for analysis. (Fig.3) Trails were performed using different columns (Agilent Zorbax XDB-C18, Phenomenex Kinetex, X-Bridge phenyl, Waters Symmetry C_{18} and Shiseido C18), organic phases (Acetonitrile, Formic acid 0.1%). X-Bridge Phenyl (150X4.6mm 3.5µ) produced good separation with efficient resolution and more theoretical plates. The drugs were eluted at a flow rate of 1.0 ml/min using a mobile phase consisting of (Acetonitrile and Formic acid 0.1%) in the ratio of 40: 60 v/v respectively. The retention times for Rilpivirine and Cabotegravir were found to be 4.363 and 7.732 min respectively.

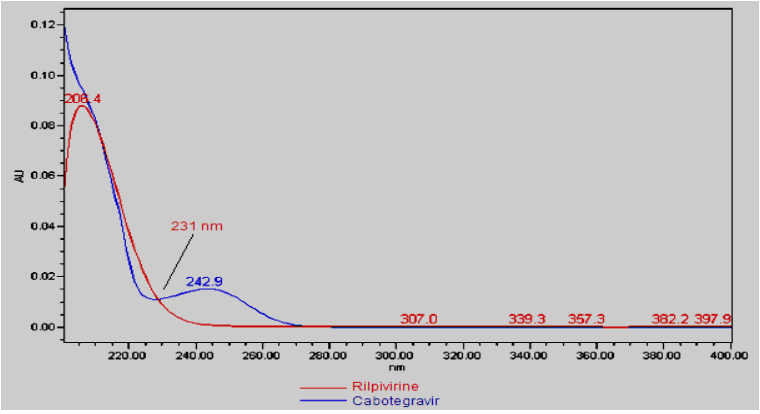

Fig.3: UV Overlay spectrum of Cabotegravir and Rilpivirine

A. System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines

Table 1. System suitability results for Cabotegravir and Rilpivirine

S.no	Parameter	Rilpivirine	Cabotegravir
1	Retention time	4.363	7.732
2	Plate count	5362	8956
3	Tailing factor	1.01	1.10
4	Resolution	--	12.41
5	%RSD	0.61	0.38

Acceptance Criteria: According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

C. Specificity: The HPLC chromatograms were recorded for blank (Fig.4a) and standard (Fig.4b) under optimized analytical conditions and compared for additional peaks, however no additional peaks were found. The three peaks were completely separated in HPLC chromatogram and the resolution was found to be more than 2.

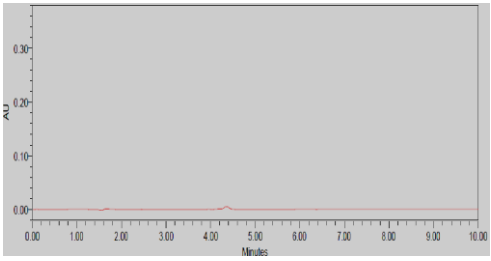

Fig.4a: Blank chromatogram

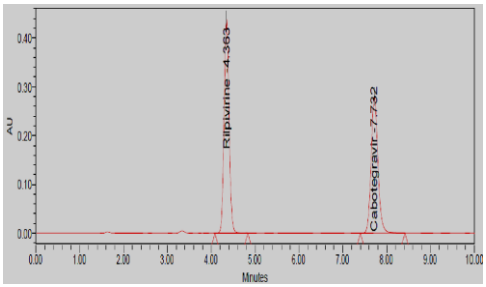

Fig 4b: optimized chromatogram

D. Linearity: Linearity was established over the range of 10ppm -60ppm for Cabotegravir and 15ppm-90ppm for Rilpivirine using the weighted least square regression analysis and the results were shown in table 2, linearity graphs were down as fig 5a and 5b.

Table 2. Linearity of Cabotegravir and Rilpivirine

S.NO	Cabotegravir		Rilpivirine	
	Conc.(µg/ml)	**Peak area**	**Conc.(µg/ml)**	**Peak area**
1	10.00	635013	15.00	942105
2	20.00	1252468	30.00	1802635
3	30.00	1761545	45.00	2764981
4	40.00	2354785	60.00	3600412
5	50.00	2925436	75.00	4365329
6	60.00	3405684	90.00	5254521
Regression equation	y = 56786.48x +58538.54		y = 58113.7x +60591.7	
Slope	56786.48		58113.7	
Intercept	58538.54		60591.7	
R^2	0.9993		0.9995	

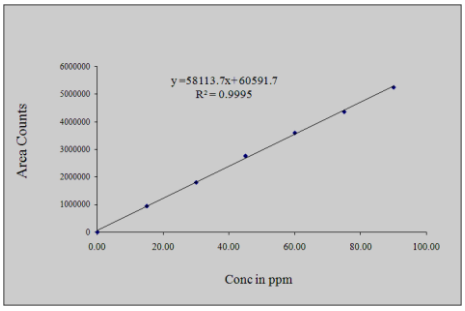

Figure 5a: Linearity plot of Rilpivirine

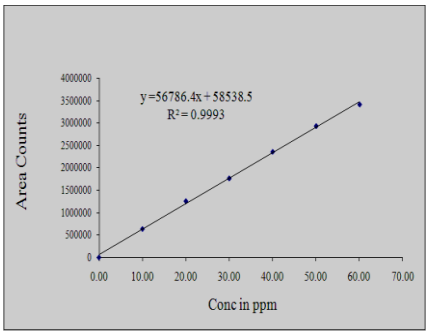

Figure 5b: Linearity plot of Cabotegravir

E.ACCURACY:

The accuracy for proposed method was determined, recovery studies were performed in mentioned levels and recorded (Table 3a and 3b), Obtained results were found to be within the limits of 100.8% and 100.0% for Cabotegravir and Rilpivirine respectively.

Table3a: Accuracy results of Cabotegravir

%Concentration(at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1174872	20	20.23	101.2	100.8
100%	2345194	40	40.38	101.0	
150%	3491753	60	60.13	100.2	

Table 3b. Accuracy results for Rilpivirine

%Concentration(at specification Level)	Area	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	1834687	30	30.24	100.8	100.0
100%	3622554	60	59.71	99.5	
150%	5450641	90	89.85	99.8	

F.PRECISION:

System Precision:

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.38% and 0.61% respectively for Cabotegravir and Rilpivirine. As the limit of Precision was less than “2” the system precision was passed in this method. System precision, Method precision and Intermediate precision for Cabotegravir and Rilpivirine were found to be in Acceptance limits .Results were showed in Tables of 4a,4b and 4c.

Table 4a: System precision table of Cabotegravir and Rilpivirine

S. No	Concentration Cabotegravir (µg/ml)	Area of Cabotegravir	Concentration of Rilpivirine (µg/ml)	Area of Rilpivirine
1.	40	2311078	60	3629082
2.	40	2323358	60	3627503
3.	40	2332502	60	3680656

4.	40	2313639	60	3621863
5.	40	2329357	60	3650588
6.	40	2327574	60	3629914
Mean		2322918		3639934
S.D		8734.66		22230.82
%RSD		0.376		0.611

Repeatability:

Table 4b: Method Precision for Cabotegravir and Rilpivirine

S. No.	**Area for Cabotegravir**	**Area for Rilpivirine**
1	2348714	3631871
2	2337242	3654952
3	2328741	3629874
4	2301478	3672478
5	2316719	3664736
6	2319417	3651897
Average	2325385	3650968
Standard Deviation	16603.34	17196.65936
%RSD	0.71	0.471016436

Acceptance Criteria: The % RSD for the area of six standard injections results should not be more than 2%**.**

Intermediate precision (Day_ Day Precision):

Table4c: Intermediate Precision (Day variation) for Cabotegravir and Rilpivirine

S. No.	**Area for Cabotegravir**		**Area for Rilpivirine**	
	Day-1	**Day-2**	**Day-1**	**Day-2**
1	2348714	2354865	3658812	3646217
2	2337242	2330142	3641841	3663940
3	2328741	2317994	3624984	3628945
4	2301478	2325690	3628483	3632483
5	2316719	2341589	3603657	3614726
6	2319417	2322647	3621788	3653012
Average	2325385	2332154	3629927	3639887
Standard Deviation	16603.34	13722.45	18757.19	17880.18
%RSD	0.71	0.59	0.52	0.49

Acceptance Criteria: The % RSD for the area of six standard injections results should not be more than 2%.

G. Sensitivity:

It is expressed as Limit of detection and Limit of quantitation. LOD is the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) with a stated confidence level (generally 99%). LOQ is the lowest concentration at which the analyte can not only be reliably detected but at which some predefined goals for bias and imprecision are met.

Table 5: Sensitivity parameters (LOD & LOQ)

Name of drug	LOD(µg/ml)	LOQ(µg/ml)
Cabotegravir	0.05	0.165
Rilpivirine	0.075	0.247

H. Robustness:

Table 6a: Robustness results of Cabotegravir

Parameter	**Condition**	**Retention time(min)**	**Peak area**	**Tailing**	**Plate count**
Flow rate Change(mL/min)	Less flow(0.8ml)	9.544	2681464	1.12	13908
	Actual(1ml)	7.732	2311078	1.13	8956
	More flow(1.2ml)	6.410	2027894	1.11	10042
Organic Phase change	Less Org (36:64)	9.414	2567921	1.13	13390
	Actual(40:60)	7.730	2323358	1.11	8941
	More Org(44:56)	6.372	2134567	1.12	10376

Table 6b: Robustness results of Rilpivirine

Parameter	Condition	Retention time(min)	Peak area	Tailing	Plate count
Flow rate Change(mL/min)	Less flow(0.8ml)	5.389	3782894	1.09	8583
	Actual(1ml)	4.363	3629082	1.00	5362
	More flow(1.2ml)	3.623	3328948	1.06	5257
Organic Phase change	Less Org (36:64)	4.939	3852691	1.08	7860
	Actual(40:60)	4.369	3627503	1.04	5354
	More Org(44:56)	3.859	3142978	1.06	5875

I. Stress testing studies:

Stress studies were performed to the analyte by exposing the drug sample to acidic, alkali, peroxide, Photolytic and thermal environment. The degradation peaks were confirmed by witnessing different peaks at different Rt and also there was a decrement in the peak area of the analyte. The forced degradation data of Cabotegravir and Rilpivirine was given in Table 7

Table 7: Forced Degradation results for Cabotegravir and Rilpivirine

Results: % Degradation results	Cabotegravir		Rilpivirine	
	Area	% Degradation	Area	% Degradation
Control	2321781	0	3641524	0
Acid	2025241	12.8	3236257	11.1
Alkali	2082365	10.3	3192148	12.4
Peroxide	1941024	16.4	3083625	15.3
Thermal	2312541	0.4	3623528	0.5
Photolytic	2010026	13.4	3109658	14.6

Degradation chromatograms of Cabotegravir and Rilpivirine in different conditions

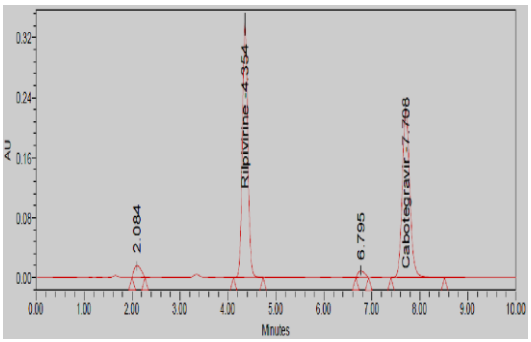

Fig 6a: Chromatogram of Acid degradation

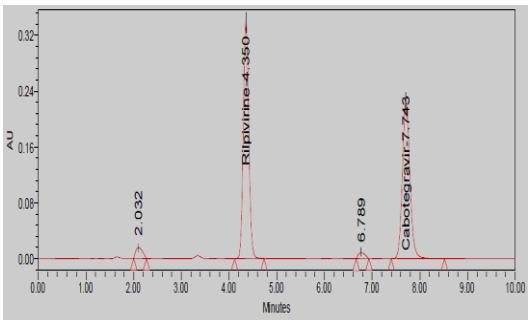

Fig 6b: chromatogram of alkali degradation

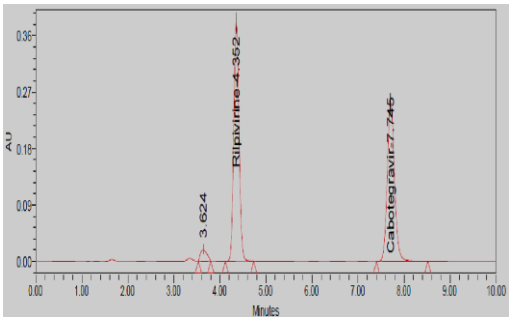

Fig6c: Chromatogram of Peroxide degradation

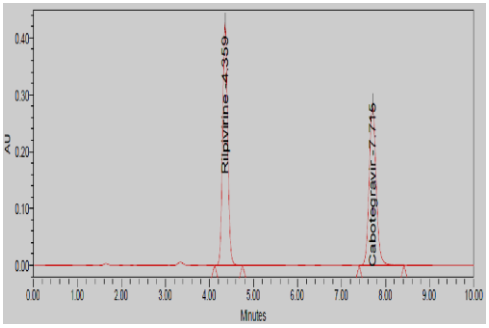

Fig 6d: Chromatogram of photo degradation

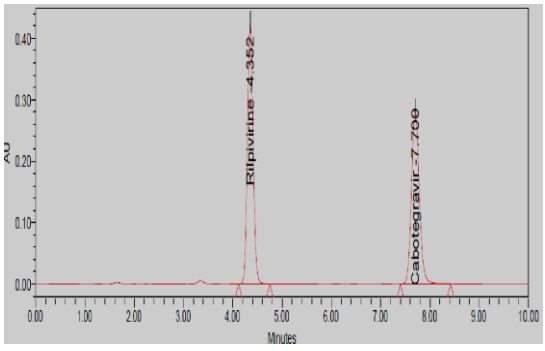

Fig 6e: Chromatogram of thermal degradation

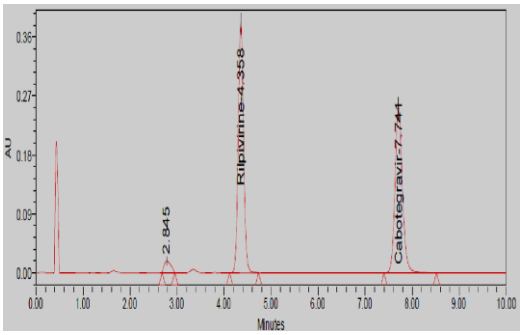

Fig 6f: Chromatogram of Control degradation

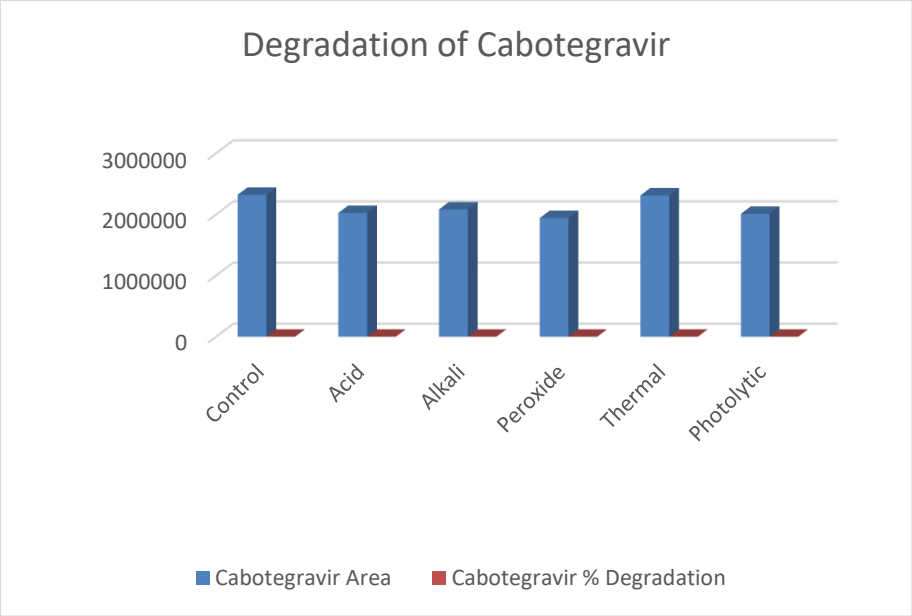

Fig7a: Degradation pattern of Cabotegravir

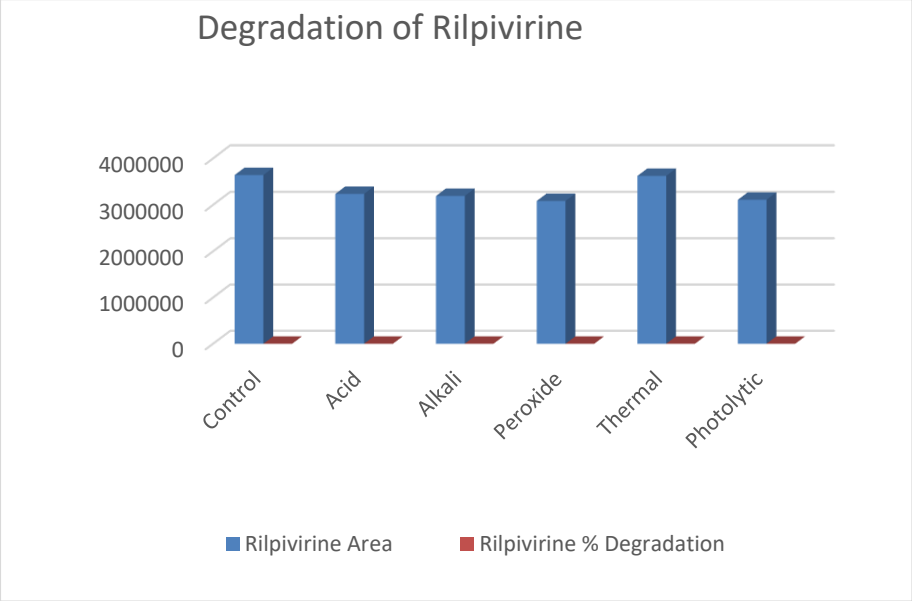

Fig 7b: Degradation pattern of Rilpivirine

IV. CONCLUSION:

The developed HPLC method for the estimation of selected drugs is simple, rapid, accurate, precise, robust and economical. The mobile phase and solvents are simple to prepare and economical, reliable, sensitive and less time consuming.

The sample recoveries were in good agreement with their respective label claims and they suggested noninterference of formulation excipients in the estimation and can be used in laboratories for the routine analysis of selected drugs.

Since the system validation parameters of HPLC method used for estimation of selected drugs in pure and have shown satisfactory, accurate and reproducible results (without any interference of excipients) as well, it is deduced that the simple and short proposed methods be most useful for analysis purpose.

The present work concluded that stability indicating assay method by RP-HPLC was simple, accurate, precise, and specific and has no interference with the placebo and degradation products. Hence these can be used for routine analysis of Cabotegravir and Rilpivirine.

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