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Stability Indicating RP-HPLC Method Development And Validation For Estimation Of Daridorexant In Tablets

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ABSTRACT: Rapid, precise, and accurate RP-HPLC method developed for estimation of Daridorexant in tablets. Daridorexant was studied under clinical trail phase III. It was proved that Daridorexant is effective in Insomnia patient. The separation was achieved by C18(250mm×4.6mm× 5µm) column and Buffer (potassium Phosphate) at a f low rate of 1ml/min. Detection was carried out 304.8 nm. Retention time 7.977 min for Daridorexant. The method has been validated for linearity, accuracy and precision. Linearity observed for range of 10-30 µg/ml. The percentage recovery obtained for Daridorexant was found to be in range 100.85 ± 0.034. Development method was found to be accurate, precise and rapid for estimation of Daridorexant in tablets.

KEYWORDS: Daridorexant, Stability Indicating RP-HPLC Method, Validation.

I. INTRODUCTION [1-7]: Daridorexant is chemically names as [(2S)-2-(5-chloro-4-methyl-1H-benzimidazol-2-yl)-2-methylpyrrolidine-1-yl]-[5-methoxy-2-(triazol-2-yl) phenyl] methanone. Daridorexant is a dual orexin receptor antagonist. The orexin neuropeptide signalling system plays a role in wakefulness. Blocking the binding of wake-promoting neuropeptides orexin A and orexin B to receptors OX1R and OX2R is thought to suppress wake drive. Daridorexant is used to treatment of insomnia. Side effects of Daridorexant are sleepiness, dizziness, headache, nausea, depression, etc.Various methods are reported for the analysis of drug but no stability indicating HPLC method reported for Daridorexant. Therefore, it was thought worthwhile to develop stability indicating RP-HPLC method for estimation of Daridorexant in tablets.

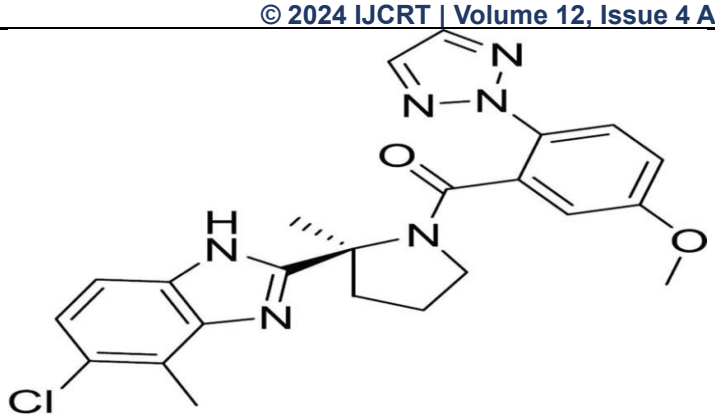

Fig. 1 Structure of daridorexant

II. MATERIALS AND METHODS

A RP-HPLC(LC-10-AD) model and spinchrom software was used. Acetonitrile, Methanol and Water of HPLC grade from final chemicals Ltd. Marketed formulation was purchased from local market.

III. IR IDENTIFICATION AND WAVELENGTH SELECTION

FT-IR spectra of Daridorexant standard was obtained by FT-IR spectrophotometer. Small quantity of standard was kept directly in the sample compartment of FT-IR and they were scanned in the range of 400-4000 cm^{-1}. And then FT-IR spectra was interpreted, and result was co-related with M.P, UV spectra and solubility to confirm identity of drug. Wavelength was selected from spectra of above solutions.

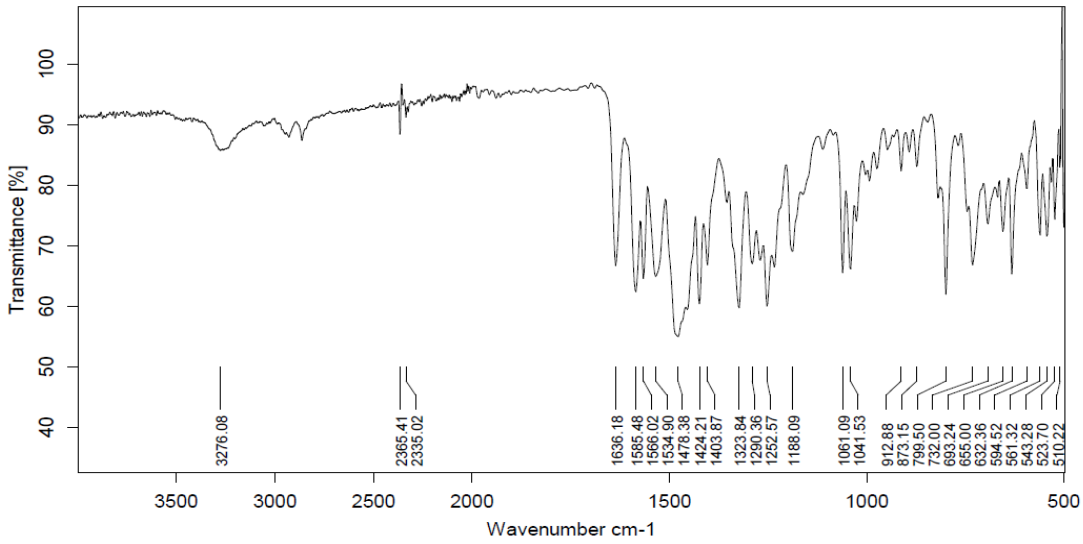

Fig. 2 IR Spectra of Daridorexant

Table. 1 IR Interpretation of Daridorexant

Sr.no	Functional group	Observed frequency
1	C-H Stretching	2335.02
2	O-H Stretching	3276.08
3	N-H Stretching	2365.41
4	C=O Stretching	1252.57
5	C-Cl Stretching	873.12

Standard solution of [20mg daridorexant made in methanol: water (50:50)] was scanned between 200-400 nm using UV-visible spectrophotometer. Wavelength what gives maximum absorbance was selected from the spectra.

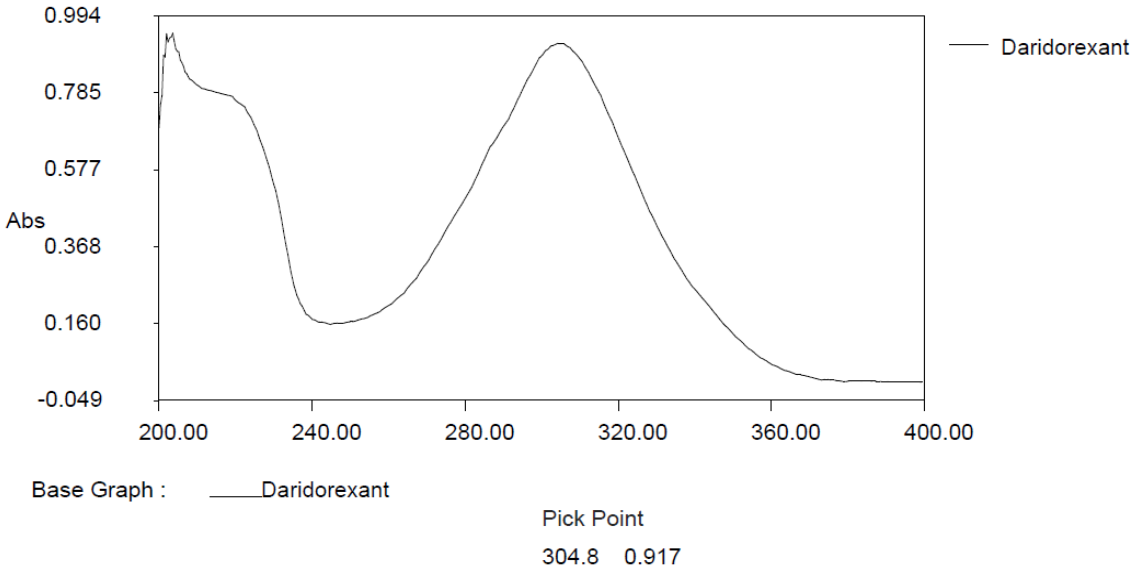

Fig. 3 Wavelength maximum (304.8 nm)

Selection of Mobile Phase:

The composition and flow rate of mobile phase were changed to optimize the separation using combined solution. After number of trail experiments, it was established that the mobile phase buffer: ACN (30:70 %v/v) shows good peak shape and resolution.

METHOD DEVELOPMENT:

Trail-1:

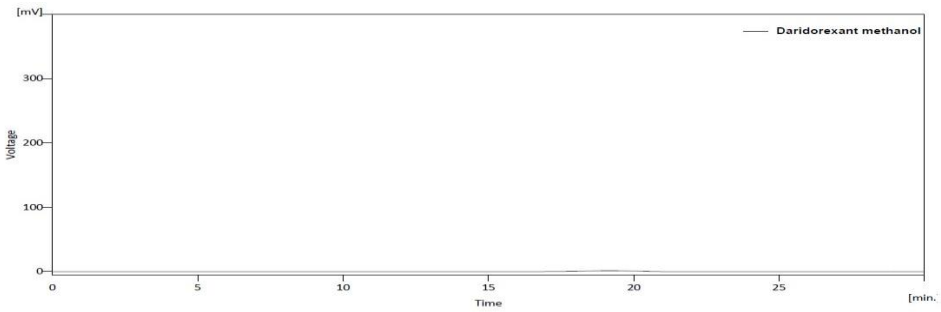

Fig. 4 RP-HPLC Chromatography of daridorexant in Methanol at 304.8 nm (flow rate: 1ml/min)

Trail-2:

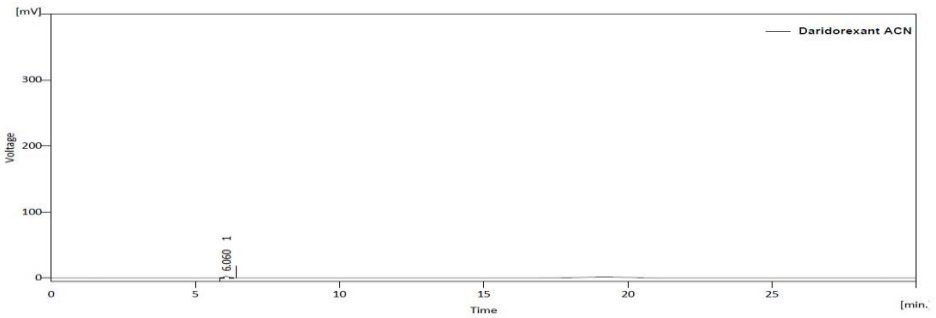

Fig. 5 RP-HPLC Chromatogram of daridorexant in ACN at 304.8 nm (flow rate: 1ml/min)

Trail-3:

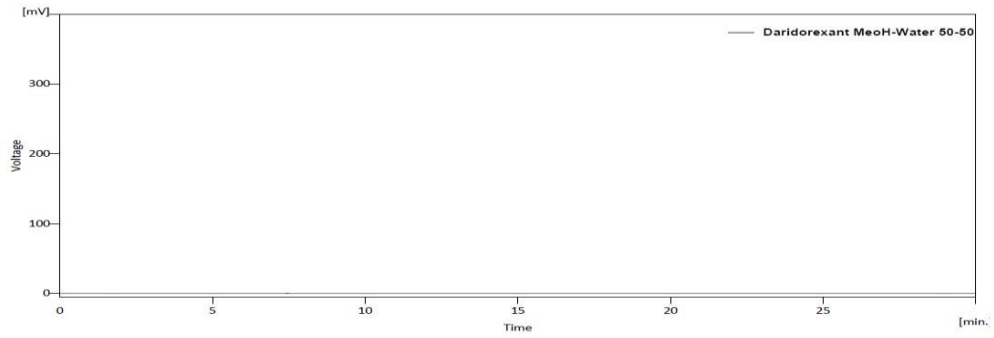

Fig. 6 RP-HPLC Chromatogram of daridorexant in Methanol: Water (50:50 %v/v) at 304.8 nm (flow rate: 1ml/min)

Trail-4:

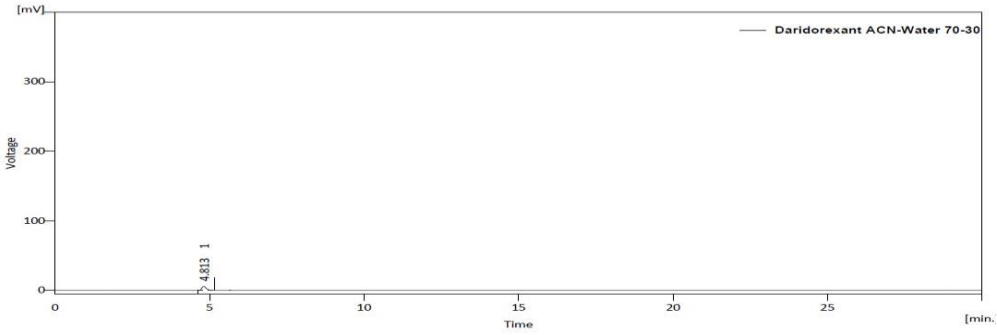

Fig. 6 RP-HPLC Chromatogram of daridorexant in ACN: Water (70:30 %v/v) at 304.8 nm (flow rate: 1ml/min)

Trail-5:

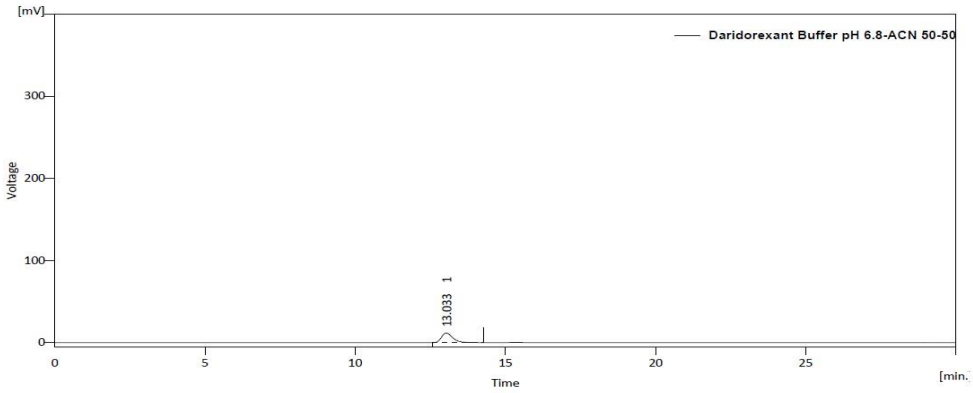

Fig. 7 RP-HPLC Chromatogram of daridorexant in Buffer: ACN (50:50 %v/v) at 304.8 nm (flow rate: 1ml/min)

Trail-6:

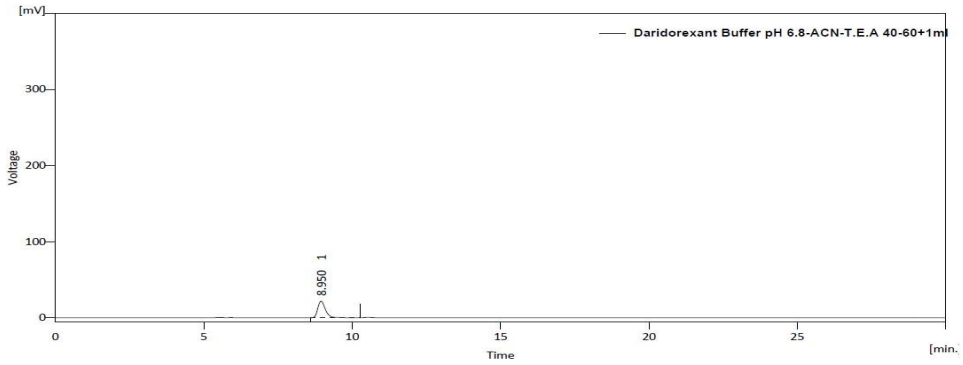

Fig. 8 RP-HPLC Chromatogram of daridorexant in ACN: T.E.A (40:60 %v/v) at 304.8 nm (flow rate: 1ml/min)

Trail-7:

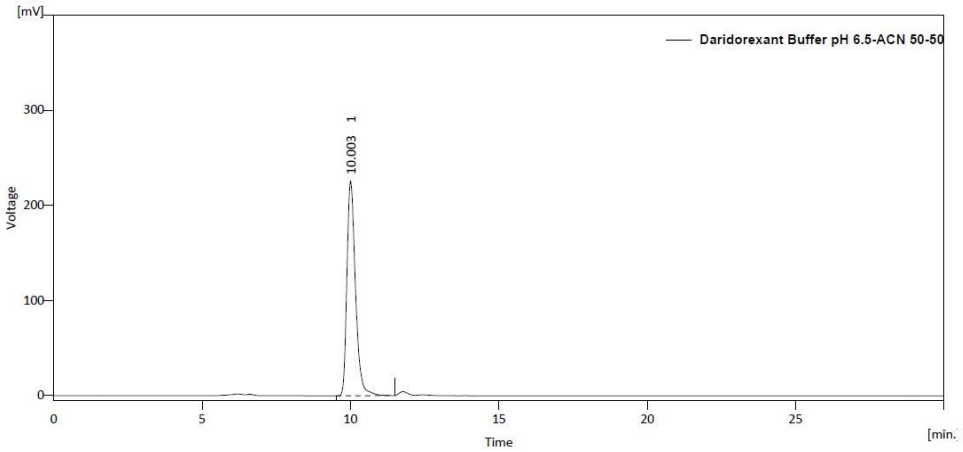

Fig. 9 RP-HPLC Chromatogram of daridorexant in Buffer: ACN (50:50 %v/v) at 304.8 nm (flow rate: 1ml/min)

Trail-8:

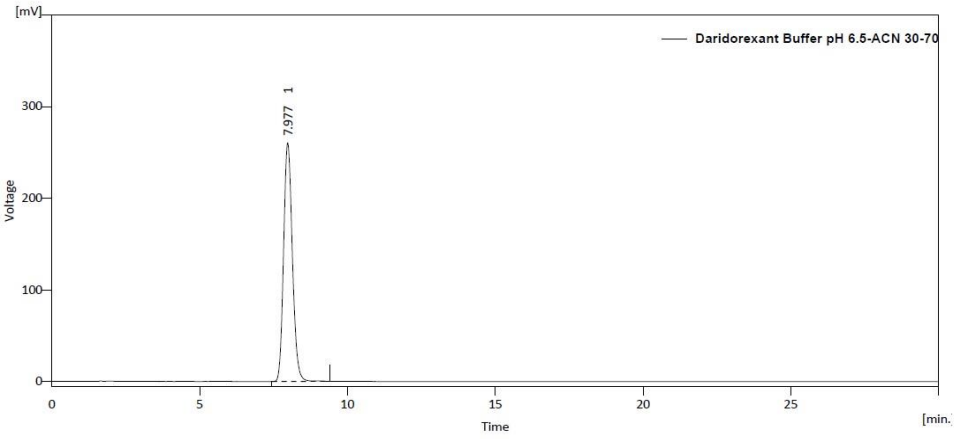

Fig. 10 RP-HPLC Chromatogram of daridorexant in Buffer: ACN (30:70 %v/v) at 304.8 nm (flow rate: 1ml/min)

Table 2 List of Mobile Phase trails for Daridorexant

Trail	Mobile Phase	Ratio	Remark
1	Methanol	-	No peak
2	ACN	-	Peak but not proper. Retention time-6.060
3	Methanol- Water	50:50%v/v	No peak
4	ACN- Water	70:30%v/v	Peak but not proper. Retention time-4.813
5	Buffer: ACN	50:50%v/v	Peak but not proper. Retention time- 13.033
6	ACN-T.E. A	40:60%v/v	Peak but not proper. Retention time- 8.950
7	Buffer: ACN	50:50%v/v	Peak but not proper. Retention time- 10.003
8	Buffer: ACN	30:70%v/v	Proper peak Retention time- 7.977

IV. METHOD VALIDATION

1. Linearity:

The linearity of Daridorexant was found to be 10-30 µg/ml.

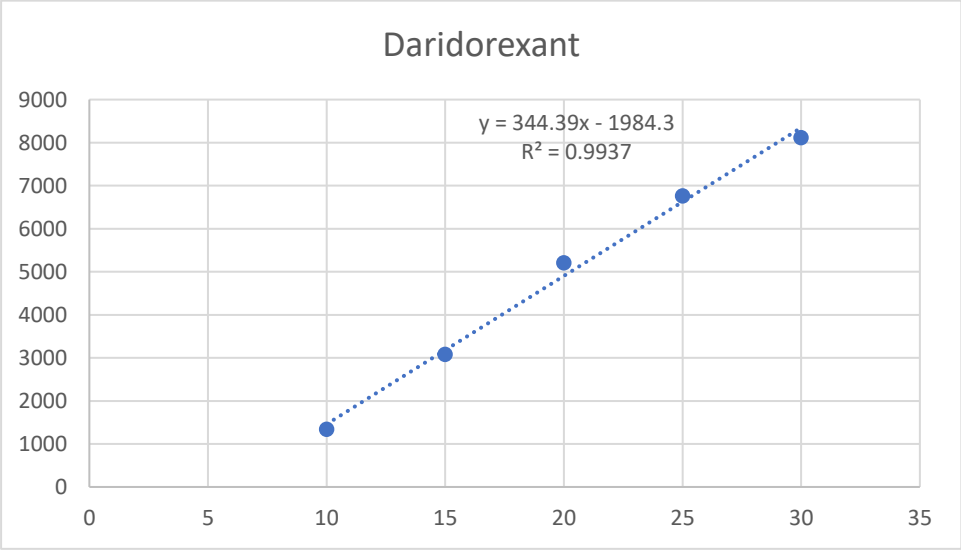

Fig. 11 Calibration curve of Daridorexant (10-30µg/ml)

Table 3: Calibration data for daridorexant

Sr. No	Concentration (µg/ml)	Area
1	10	1344.952
2	15	3084.426
3	20	5209.863
4	25	6762.55
5	30	8115.675

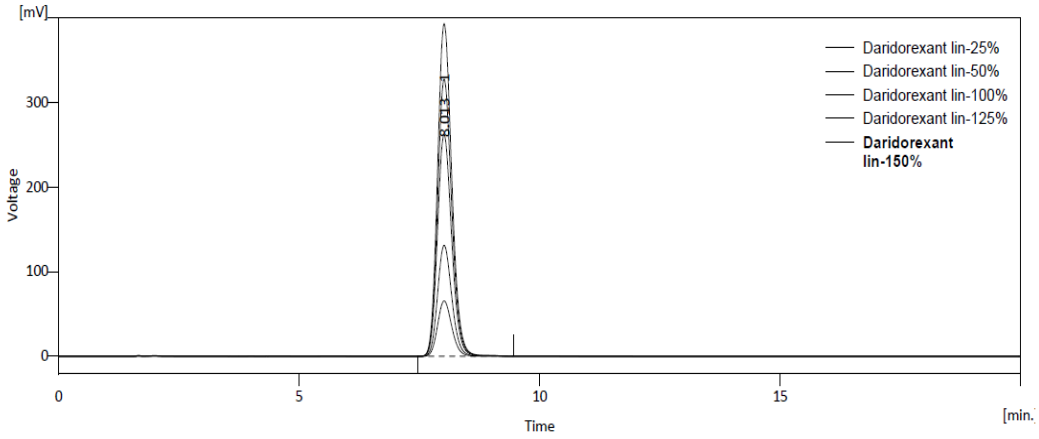

Fig. 12 Overlay chromatogram of different concentration of mixtures of Daridorexant

2. Precision:

Table 4: Repeatability data for daridorexant

Daridorexant				
Sr. No	**Conc. (µg/ml)**	**Area**	**Mean ± S.D (n=6)**	**% R. S. D**
1.	20	5209.106	5208.351±1.247	0.023
		5209.140		
		5208.630		
		5206.125		
		5209.412		
		5207.695		

Table 5: Intraday precision data for daridorexant

Daridorexant			
Sr. No.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D.**
1	10	1343.509 ± 1.124	0.083
2	20	5208.801 ± 0.902	0.017
3	30	8113.882 ± 3.183	0.039

Table 6: Interday precision data for daridorexant

Daridorexant			
Sr. No.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D.**
1	10	1343.875 ± 0.534	0.039
2	20	5208.644 ± 0.873	0.016
3	30	8113.761 ± 2.848	0.035

3. Accuracy

Table 7: Recovery study for daridorexant

Sr. No.	Conc. (%)	Sample amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean recovery ± S. D
1	80%	10	8	8.104	101.30	101.34 ± 0.04
2		10	8	8.107	101.34	
3		10	8	8.111	101.39	
4	100%	10	10	10.130	101.30	101.14 ± 0.14
5		10	10	10.108	101.08	
6		10	10	10.102	101.02	
7	120%	10	12	12.100	100.83	100.85 ± 0.03
8		10	12	12.106	100.88	
9		10	12	12.098	100.82	

4. Robustness

Table 8: Robustness data for daridorexant

Parameter	Deviation	Area	% Mean ± S. D	% R.S.D.
Flow rate	+0.2	5209.612	5209.057 ± 0.57	0.011
		5208.456		
		5209.103		
	-0.2	5216.598	5215.699 ± 0.81	0.015
		5215.496		
		5215.003		
Mobile phase	+2	5208.456	5208.560 ± 1.17	0.022
		5209.789		
		5207.436		
	-2	5209.124	5210.469 ± 1.90	0.036
		5212.650		
		5209.635		
		5208.132		

PH	+0.2	5207.659	5207.971 ± 0.27	0.005
		5208.123		
	-0.2	5216.025	5216.835 ± 0.74	0.014
		5216.986		
		5217.496		

5. Specificity

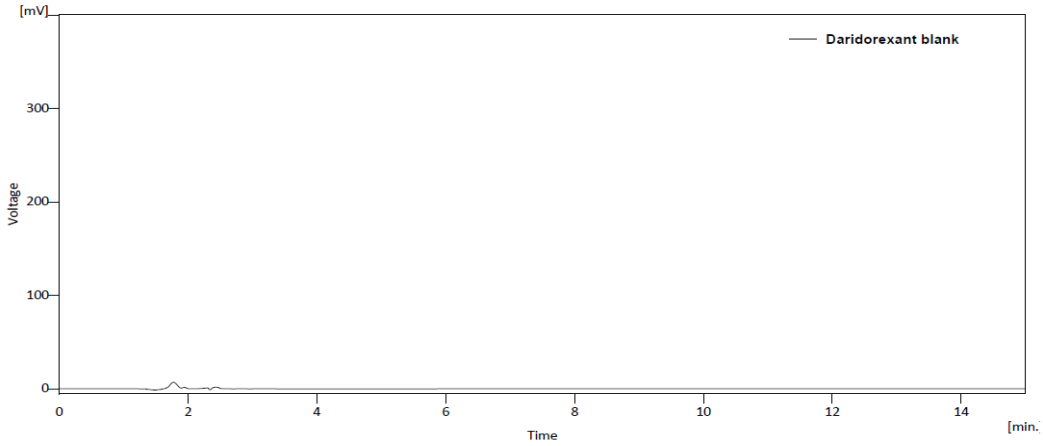

Fig. 13 Chromatogram of Daridorexant Blank

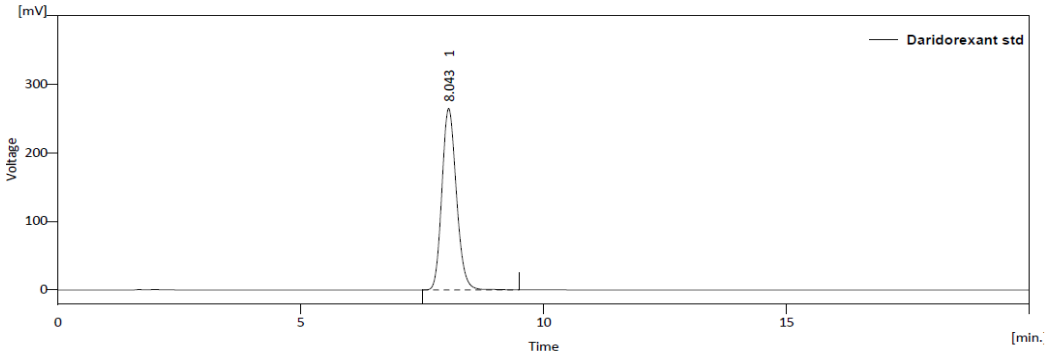

Fig. 14 Chromatogram of Daridorexant Standard

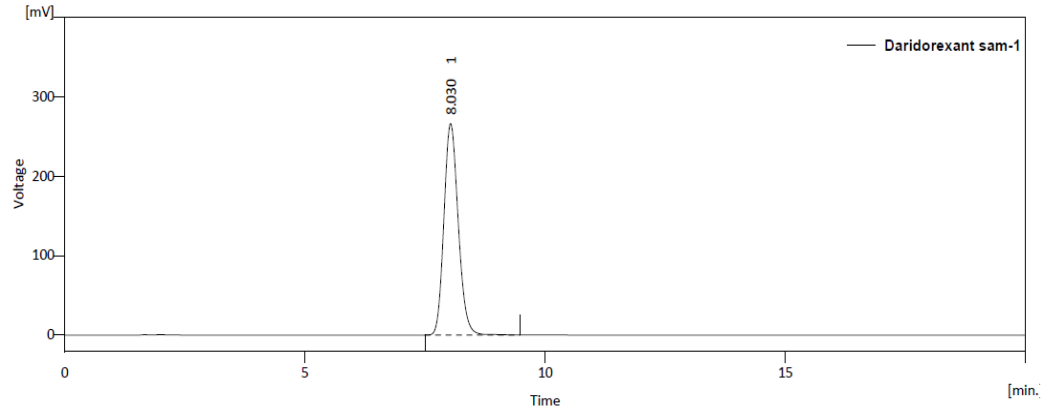

Fig. 15 Chromatogram of Daridorexant Sample-1

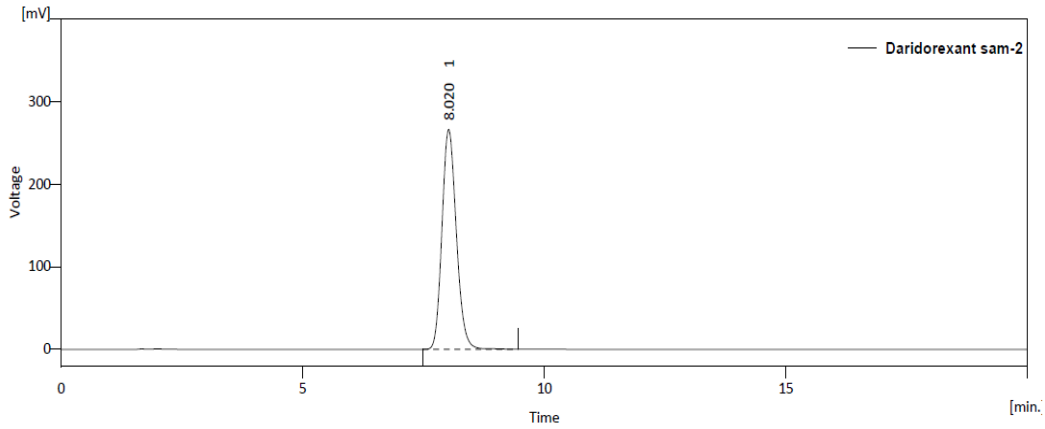

Fig. 16 Chromatogram of Daridorexant Sample-2

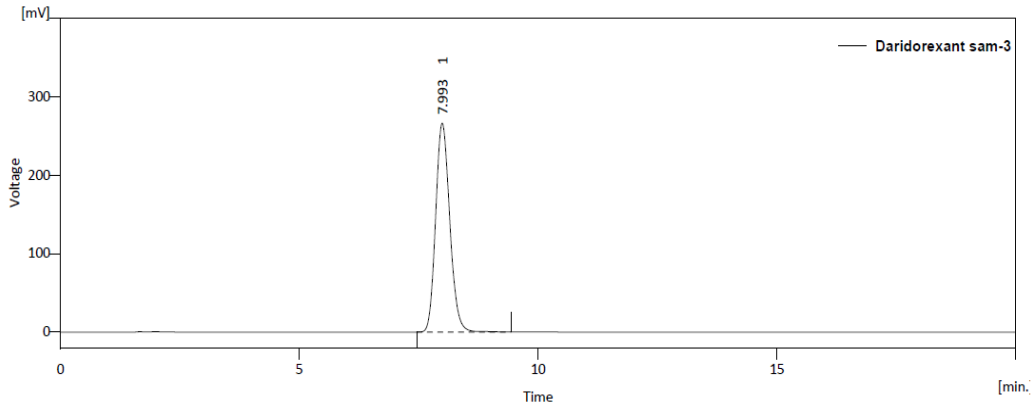

Fig. 17 Chromatogram of Daridorexant Sample-3

V. Force Degradation

1. **Acid degradation** 1 ml of Daridorexant standard stock solution were taken into 10ml volumetric flask. 1ml 0.1 N HCL was added into the flask. The was kept on table top at room temperature for 1.5 hours. After time period 1ml of NaOH added to neutralize the solution and make up the volume with diluent.

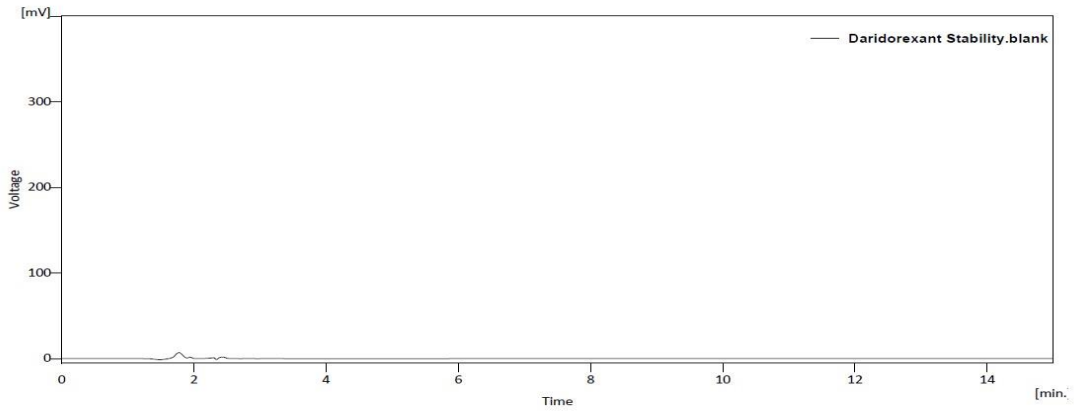

Fig. 18 Acid degradation Blank

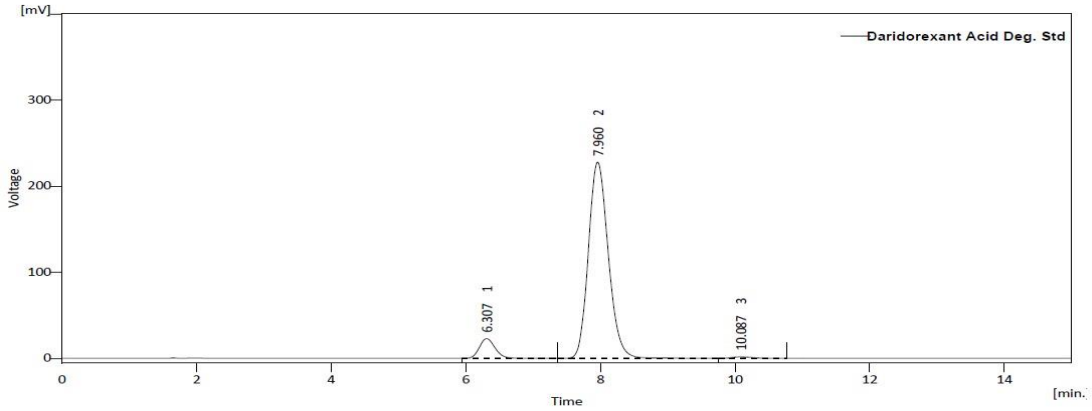

Fig. 19 Acid degradation Standard

Table. 9 Calculation of acid degradation standard

	Ret. time	Area (mV.s)	Area (%)	Height (mV)	Asymmetry	Efficiency [th.pl]	Resolution
1	6.307	372.968	7.3	22.977	1.274	3433	-
2	7.960	4682.021	91.9	227.814	1.253	3501	3.414
3	10.087	37.563	0.7	1.891	1.465	5993	4.015
	Totle	5092.552	100.0	252.682			

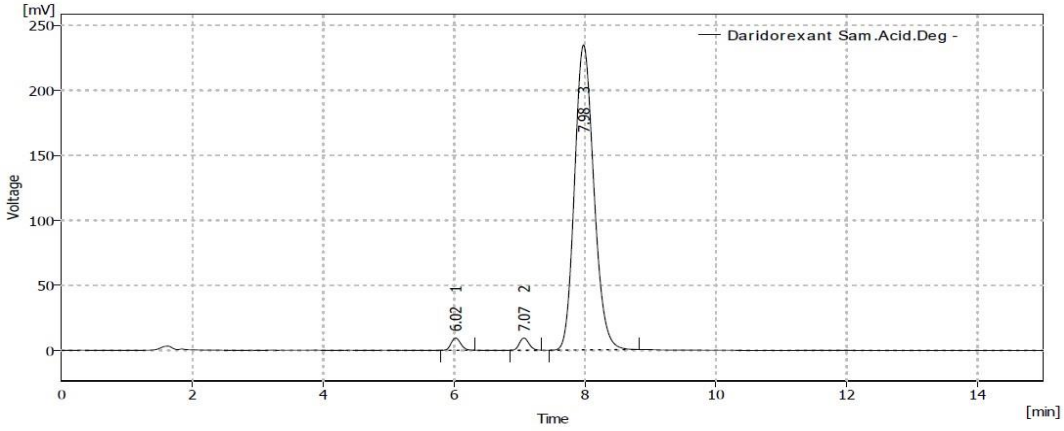

Fig. 20 Acid degradation Sample

Table. 10 Calculation of acid degradation sample

	Ret. Time	Area (mV.s)	Area (%)	Height (mV)	Asymmetry	Efficiency (th.pl)	Resolution
1	6.023	95.094	1.9	9.359	1.227	7722	-
2	7.068	94.380	1.9	9.335	1.213	10864	3.840
3	7.979	4801.509	96.2	234.730	1.258	3499	2.253
	Total	4990.984	100.0	253.424			

2. Base degradation:

1ml of standard stock solution were taken 10ml volumetric flask. 1ml 0.1 N NaOH was added into 10ml volumetric flask. The flask was kept on table top at room temperature for 1.5 hours. After time period 1 ml of 0.1 N HCL added to neutralize the solution and make up the volume with diluent.

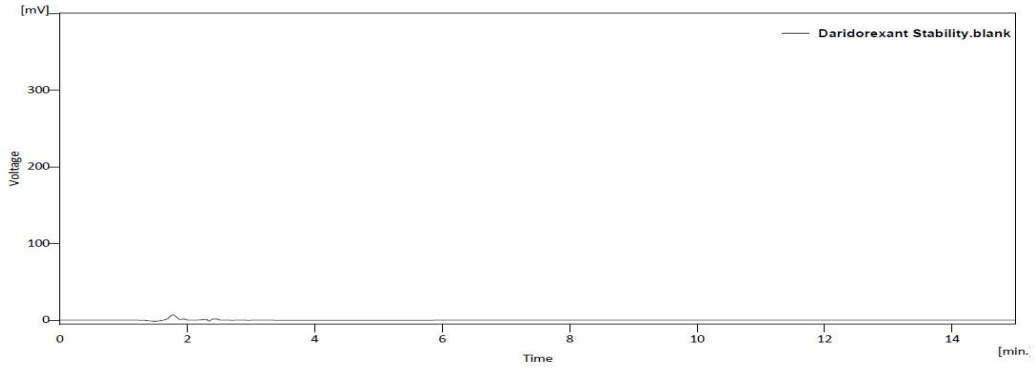

Fig.21 Base degradation Blank

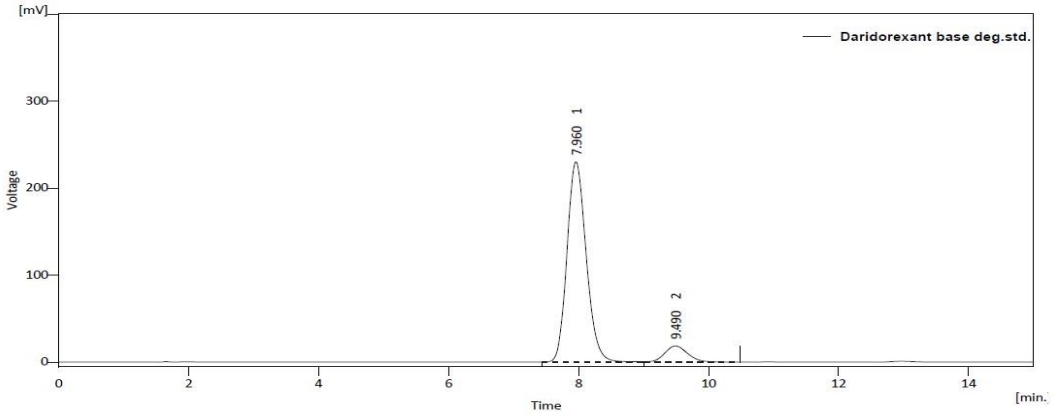

Fig. 22 Base degradation Standard

Table. 11 Calculation of base degradation standard

	Ret. Time (min)	Area (mV.s)	Area (%)	Height (mV)	Asymmetry	Efficiency (th.pl)	Resolution
1	7.960	4723.481	91.2	230.154	1.253	3501	-
2	9.490	455.136	8.8	18.527	1.229	3455	2.585
	Total	5178.616	100.0	248.681			

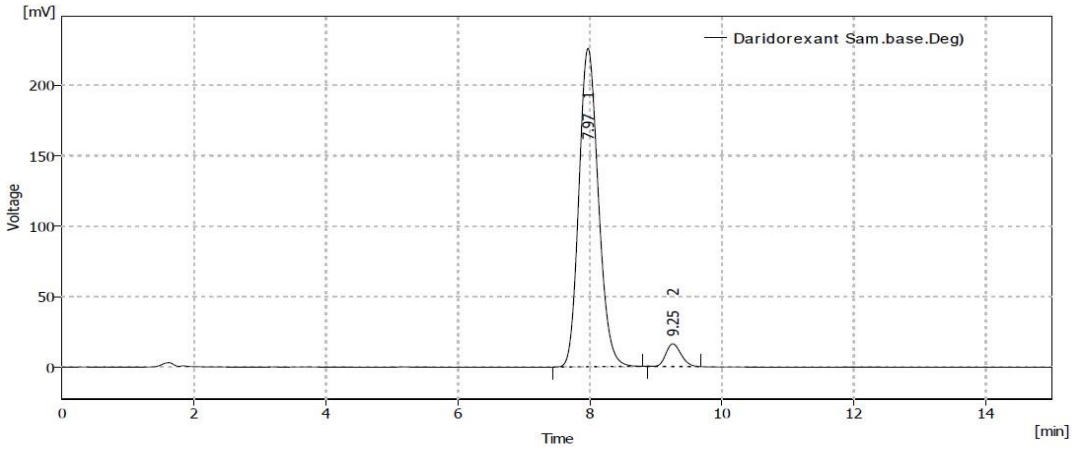

Fig. 23 Base degradation Sample

Table. 12 Calculation of base degradation sample

	Ret. Time	Area (mV.s)	Area (%)	Height (mV)	Asymmetry	Efficiency (th.pl)	Resolution
1	7.971	4616.097	94.8	225.877	1.251	3521	-
2	9.255	254.385	5.2	16.083	1.355	7654	2.680
	Total	4870.483	100.0	241.915			

3. Oxidation degradation:

1ml of standard stock solution was taken into 10 ml volumetric flask. 1ml 3% H_2O_2 was added into the flask. The flask was kept at room temperature for 12 hours. Volume was made up to the mark with diluent and chromatographed.

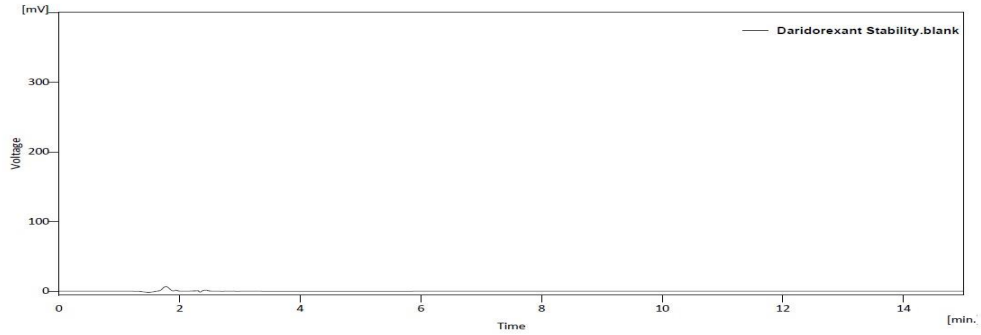

Fig. 24 Oxidation degradation blank

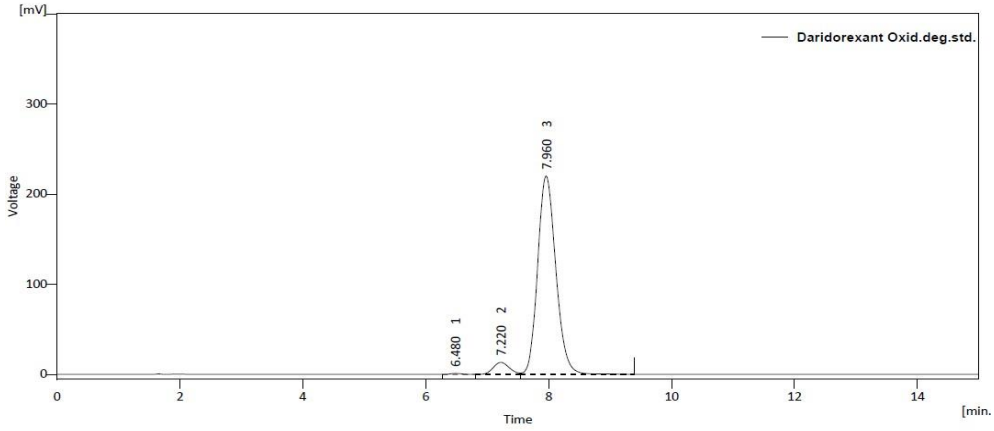

Fig. 25 Oxidation degradation Standard

Table. 13 Calculation of oxidation degradation standard

	Ret. Time	Area (mV.s)	Area (%)	Height (mV)	Asymmetry	Efficiency (th.pl)	Resolution
1	6.480	13.578	0.3	1.098	1.477	6224	-
2	7.220	242.500	5.1	13.349	1.352	3434	1.802
3	7.960	4519.197	94.6	219.993	1.253	3501	1.436
	Total	4775.275	100.0	234.440			

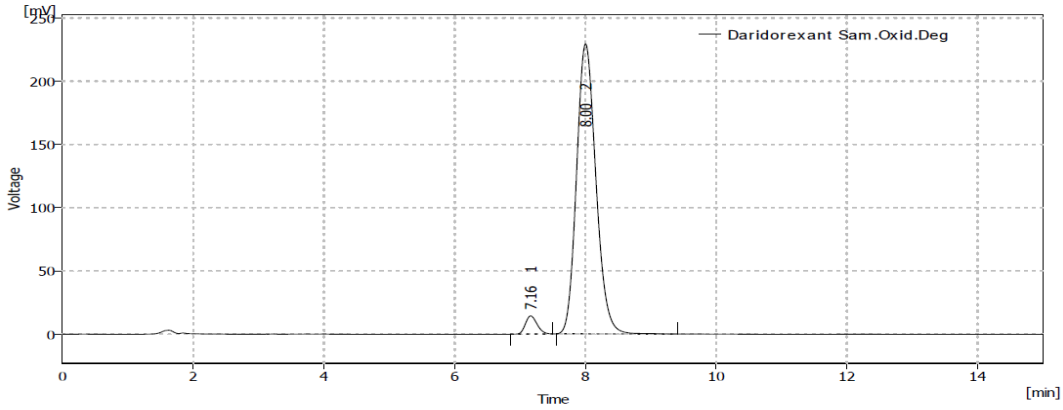

Fig. 26 Oxidation degradation Sample

Table. 14 Calculation of oxidation degradation sample

	Ret. Time	Area (mV.s)	Area (%)	Height (mV)	Asymmetry	Efficiency (th.pl)	Resolution
1	7.163	176.514	3.6	14.307	1.347	7477	-
2	8.003	4719.486	96.4	229.275	1.256	3521	1.934
	Total	4896.000	100.0	243.581			

4. Photo degradation:

1 ml of standard stock solution was transferred in to 10 ml of volumetric flask. The volumetric flask was kept in presence of sunlight for 1 hour. Volume was made up to the mark with diluent and chromatographed.

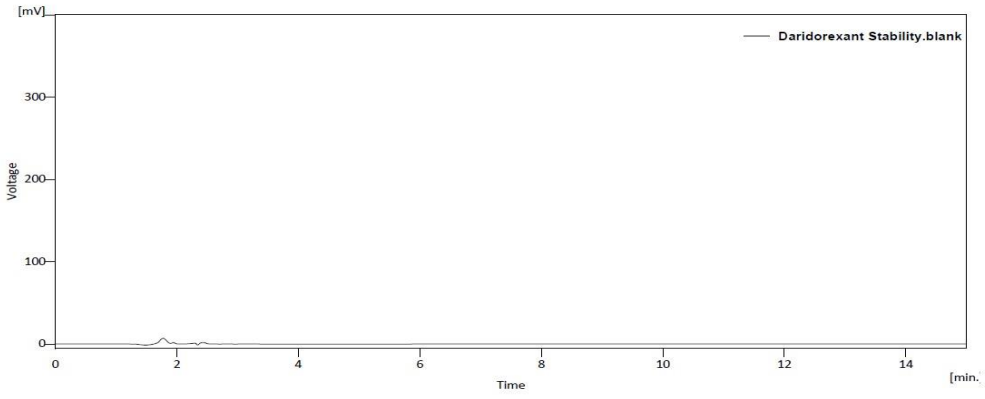

Fig. 27 Photo degradation blank

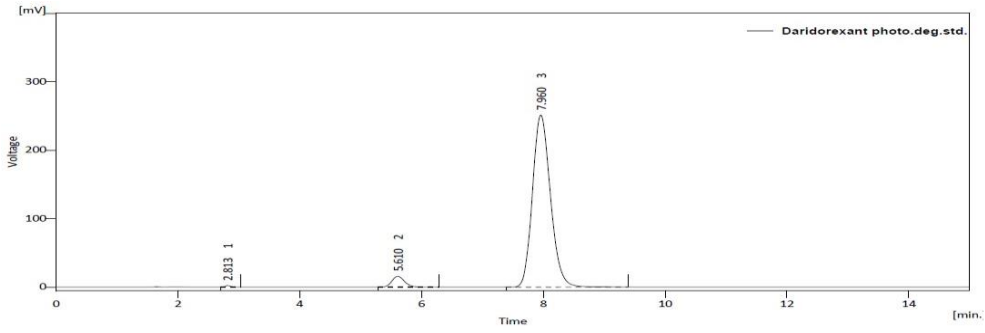

Fig. 28 Photo degradation Standard

Table. 15 Calculation of photo degradation standard

	Ret. Time	Area (mV.s)	Area (%)	Height (mV)	Asym metry	Efficiency (th.pl)	Resolut ion
1	2.813	12.794	0.2	2.320	1.579	5838	-
2	5.610	226.071	4.2	15.716	1.250	3496	10.617
3	7.960	5156.692	95.6	251.225	1.253	3501	5.122
	Total	5395.557	100.0	269.261			

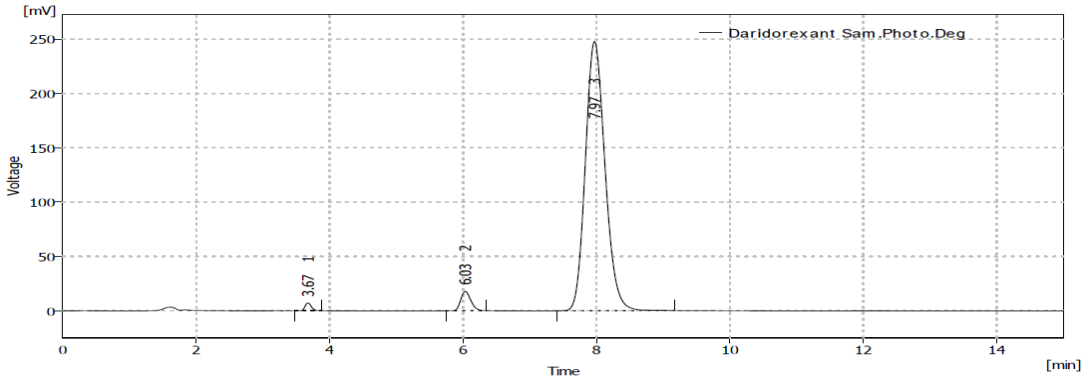

Fig. 29 Photo degradation Sample

Table. 16 Calculation of photo degradation sample

	Ret. Time	Area (mV.s)	Area (%)	Height (%)	Asym metry	Efficiency (th.pl)	Resoluti on
1	3.674	46.814	0.9	2.7	1.381	7353	-
2	6.033	186.186	3.5	6.5	6.033	7467	10.498
3	7.971	5080.968	95.6	90.8	7.971	3521	4.760
	Total	5313.968	100.0	100.0			

5. Thermal degradation:

1 ml of standard stock solution was transferred in to 10 ml of volumetric flask. The volumetric flask was stored in oven at 80°C for 4 hours. Then the volume was adjusted with diluent and chromatographed.

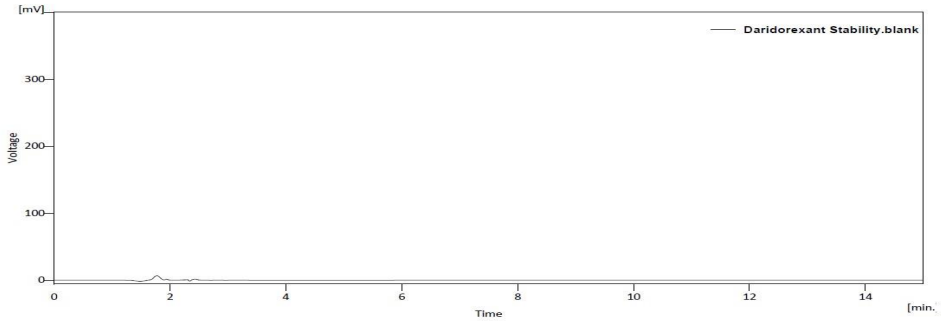

Fig.30 Thermal degradation blank

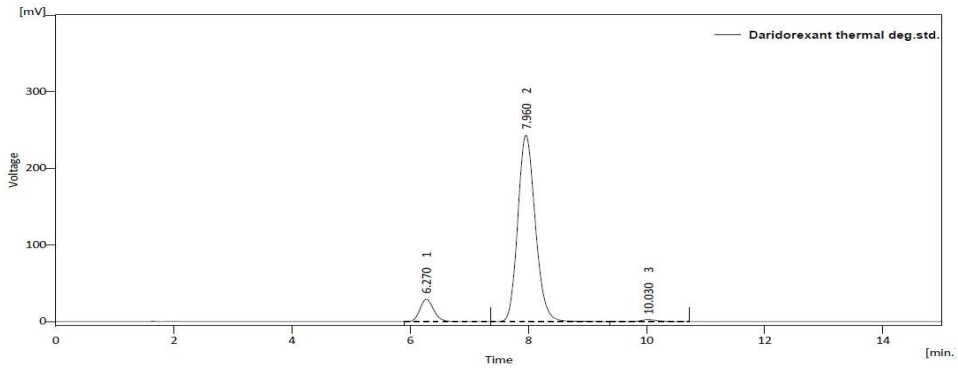

Fig. 31 Thermal degradation Standard

Table. 17 Calculation of thermal degradation standard

	Ret. Time	Area (mV.s)	Area (%)	Height (mV)	Asymmetry	Efficiency (th.pl)	Resolution
1	6.270	471.203	8.5	29.192	1.274	3485	-
2	7.960	4995.625	90.6	243.174	1.253	3501	3.510
3	10.030	49.154	0.9	2.395	1.444	6057	3.929
	Total	5515.983	100.0	274.761			

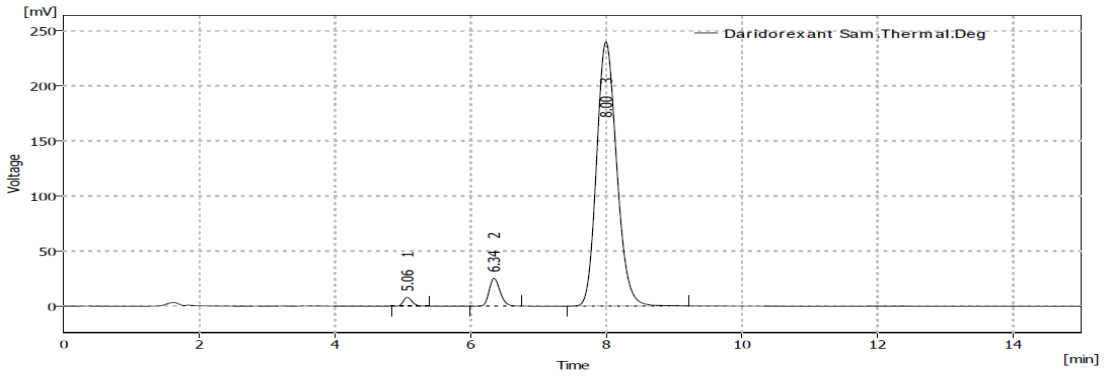

Fig. 32 Thermal degradation Sample

Table. 18 Calculation of thermal degradation sample

	Ret. Time	Area (mV.s)	Area (%)	Height (%)	Asymmetry	Efficiency (th.pl)	Resolution
1	5.065	70.011	1.3	2.9	1.372	7397	-
2	6.343	278.609	5.3	9.2	1.393	7400	4.833
3	7.995	4937.344	93.4	87.9	1.256	3521	3.972
	Total	5285.964	100.0	100.0			

CONCLUSION:

A rapid, sensitive, accurate and precise Stability indicating RP-HPLC method has been developed and validated for routine analysis of daridorexant.The RP-HPLC method is suitable for estimation of daridorexant. The developed method was successfully applied in daridorexant powder.The proposed method can be utilized for the routine analysis of daridorexant. Linearity Range of 10-30 µg/ml for daridorexant with Correlation Coefficient for daridorexant 0.996. And the precision data was obtained with less than 2% of RSD. Accuracy was carried out by the Recovery study and obtained in range of between 98-102 % for daridorexant. Precision was carried out by repeatability, intraday precision and interday precision. In Repeatability % RSD was found to be 0.023. In intraday precision % RSD was found to be 0.083- 0.039. In interday precision % RSD was found to be 0.039- 0.035. Robustness data was obtained with less than 2% of RSD.
After acid degradation, 10.14 % degradation was obtained in daridorexant. After base degradation, 13.61 % degradation was obtained in daridorexant. After oxidation degradation 11.68 % degradation was obtained in daridorexant. After photo degradation 4.49 % degradation was obtained in daridorexant. After thermal degradation 7.60 % degradation was obtained in daridorexant. There was no co-elusion of any degradation with main peak and the results obtained were found within the acceptance criteria. Hence, the proposed stability indicating RP-HPLC method can be applied for the estimation of daridorexant in tables.

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