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Validation of stability indicating RP-HPLC method for the assay of venlafaxine in pharmaceutical dosage form

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ABSTRACT

A simple, rapid and accurate stability indicating RP- HPLC method was developed for the quantitative estimation of Venlafaxine in pure and pharmaceutical formulations and the method was validated as per ICH guidelines. In this method Inertsil ODS,C_{18}, 150 mm X 4.6 mm, 5μ column at an ambient temperature of 30 ^{0} C and at a flow rate of 0.8 ml. / minute with isocratic elution was used. 20 μL of the solution was injected and at 268nm wave length the UV detection was made. Phosphate buffer and Acetonitrile in the ratio 30:70 (v/v) was used as mobile Phase and diluent. The retention time for Venlafaxine standard (sample) was found to be 5.011 min and linearity was observed in the concentration range of 12.5 - 75μg with correlation coefficient of 1. The %of RSD in precision and accuracy was less than 2. Degradation under different stress conditions was studied and the drug was found to be stable. The degradation was estimated to be 3.95 – 5.42%. It was found that Venlafaxine degraded more in oxidation, least in UV light and acidic conditions. The present method can be successfully used for the routine assay determination of Venlafaxine in pure form and Pharmaceutical dosage forms.

Key words: Venlafaxine, Pharmaceutical dosage forms, RP-HPLC assay, Forced degradation.

INTRODUCTION

Venlafaxine is used as an antidepressant which is administered into the human body orally. The molecular formula of Venlafaxine is $C_{17}H_{27}NO_2$, and molecular weight is 277.402.

Chemically, it is (RS) – 1[2- (dimethylamino) -1- (4-methoxyphenyl) ethyl] – cyclo hexanol as shown in Figure1.

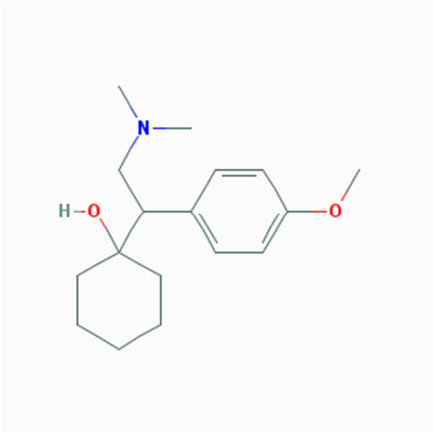


Fig- 1 Chemical Structure of Venlafaxine

It was referred to as a Serotonin – norepinephrine – dopamine reuptake inhibitor. Venlafaxine was available in the market in the name of "Effexor" tablets. (Venlafaxine Hydrochloride). Compressed tablets contain Venlafaxine hydrochloride equivalent to 25mg, 37.5mg, 50mg, 75mg and 100mg. In the literature, various methods were reported for the determination of venlafaxine. Several spectrophotometric [1-2], RP-HPLC[3-6], in plasma [7,8], stability indicating LC [9] and simultaneous determinations[10,11] have been reported. Even though, there is one stability indicating HPLC method [12] reported in the literature, it confined to fewer stress effects such as acid,base and oxidation.The present stability indicating RP- HPLC method was rapid, accurate and precise for the quantitative estimation of Venlafaxine and a varied stress conditions for its degradation was studied with a different mobile phase and experimental conditions.

MATERIALS AND METHODS

Chemicals & Method:
Chemicals: Venlafaxine pure sample was donated by Hetero Drugs, Hyderabad. It was available in the market as Effexor tablets and procured from the market. All the chemicals used in the validation were Merk brand and they were supplied by Bharat Scientifics, Hyderabad. In the present experiment, ultrapure water was used.

Buffer Solution:
2.95gms of potassium dihydrogen orthophosphate and 0.58gms of potassium hydrogen orthophosphate were dissolved in 1000ml. of distilled water and sonicated to dissolve.

Mobile Phase(Diluent):- Buffer and Acetonitrile were mixed in the ratio 30:70 (v/v), sonicated to dissolve, filter the solution using 0.4 micron membrane filter and degassed by vacuum filtration. The mobile phase was also used as diluent.

Standard Stock Solution:
Exactly 50mg of venlafaxine working standard was taken in 100ml volumetric flask, some diluent was added, sonicated to dissolve and made up to the mark by the diluent. By diluting the above solution, different required concentrated solutions were prepared.

Assay Sample Solutions:
Nearly 10 tablets of venlafaxine were taken and grind to powder in mortar and pestile and an amount equivalent to 100mg of venlafaxine (API) was weighed and transferred to 100ml volumetric flask and 50ml of diluent was added, sonicated to dissolve and made up to the mark with the diluent.

Instrumentation:
In this method, Waters HPLC 2 2695 series consisting 4pump, autosampler with 5 racks was used. Each rack has 24 vials holding capacity with temperature control. Auto injector has capacity to inject 5µL to 500µL and UV-Visible detector with PDA. Waters (alliance) HPLC system was equipped with Empower Software – 2 Software.

The Chromatographic studies were carried out with Inertsil ODS, C_{18}, 150x4.6, 5µ column at a temperature of 30^0C with mobile phase flow rate of 0.8ml/minute. A 30:70(v/v) buffer and acetonitrile mixture acts as mobile phase with isocratic elusion. Measurements were made by injecting 20µL of solution at a wave length of 268 nm, by UV – visible detector with PDA.

Validation of the method:

1. **Specificity**: As no difference was observed between the chromatograms of the standard and the assay sample of venlafaxine, it is concluded that the developed method removes the interference of the excipients in the tablets. The chromatograms of blank, standard and sample were shown in Figs 2,3, 4.

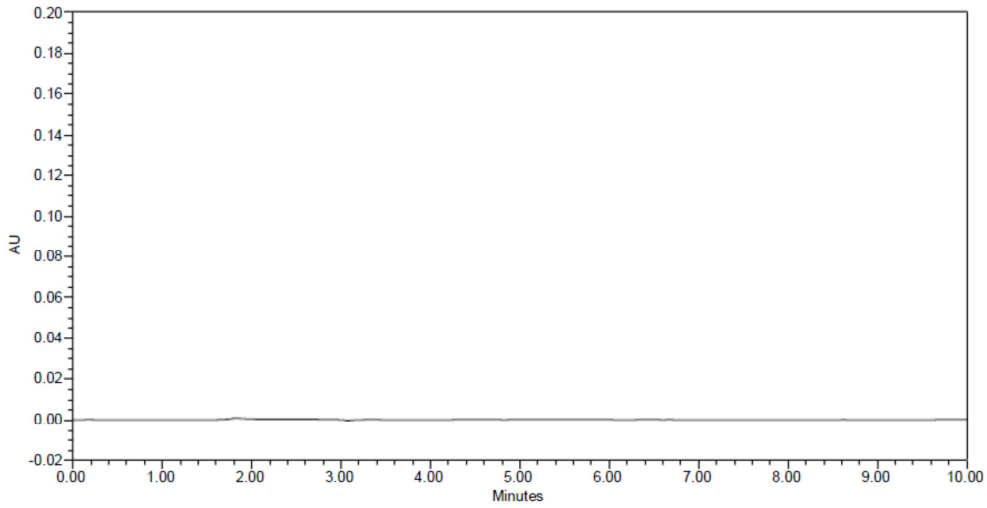
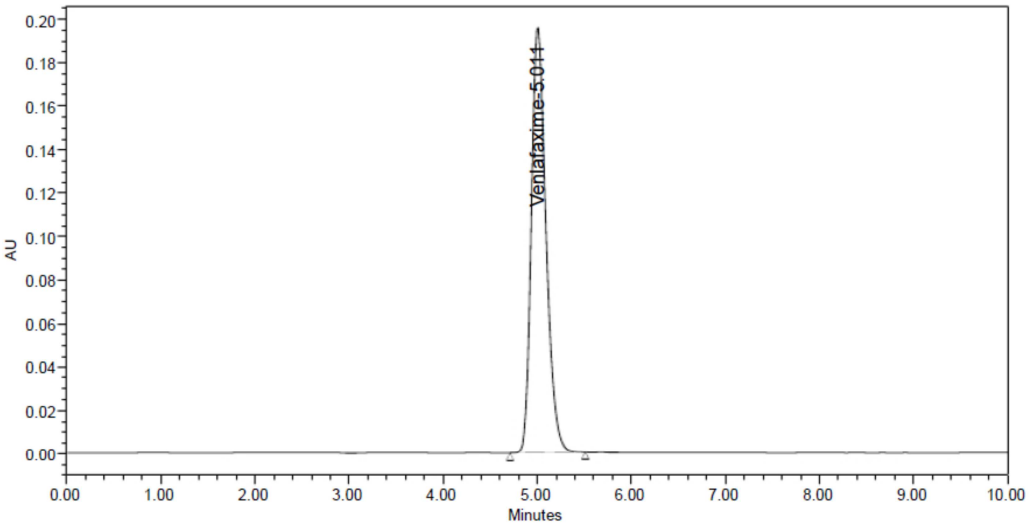
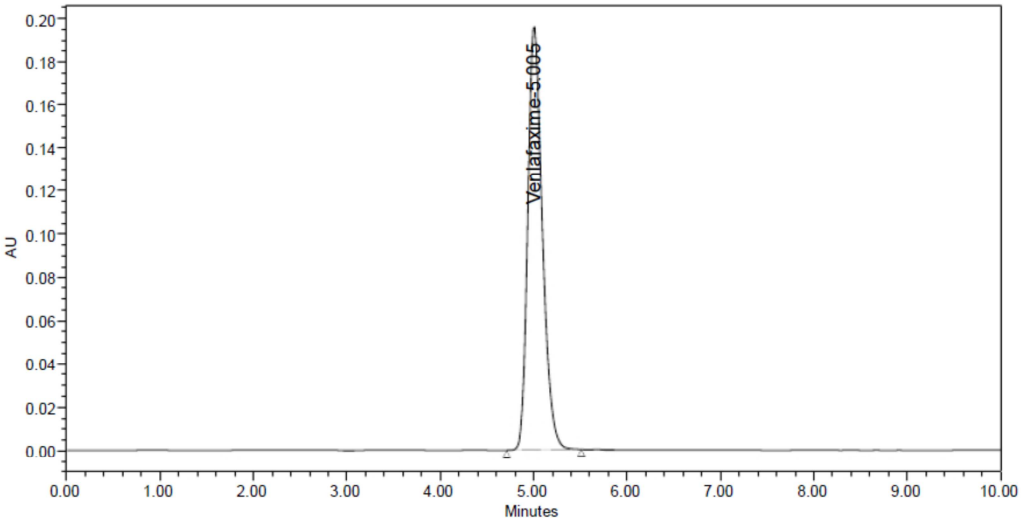


Fig 2.Chromatogram Of The Blank



	Peak Name	RT	Area	% Area	Height	USP Plate Count	USP Tailing
1	Venlafaxime	5.011	2118148	100.00	196104	4957	1.18

Fig.3. Chromatogram of the Venlafaxine standard



	Peak Name	RT	Area	% Area	Height	USP Plate Count	USP Tailing
1	Venlafaxime	5.005	2118554	100.00	196125	4952	1.15

Fig 4. Chromatogram of the Venlafaxine sample

2. **Linearity:** Verified the linearity of the method by preparing six different solutions from the standard stock solution i.e.,(25%,50%,75%,100%,125% & 150%) in the concentration range of 12.5 – 75 µg/ml and 20 ml each of the solutions was injected and chromatograms were taken. A concentration Vs peak area graph was drawn which is shown in Fig 5. A straight line was obtained. The slope, intercept, correlation coefficient of the calibration curve of the present method were determined and they are 52481, 16095 & 1 respectively.

Conc(mcg)	Area
12.50	541656
25.00	1067131
37.50	1587197
50.00	2116076
62.50	2639479
75.00	3166192

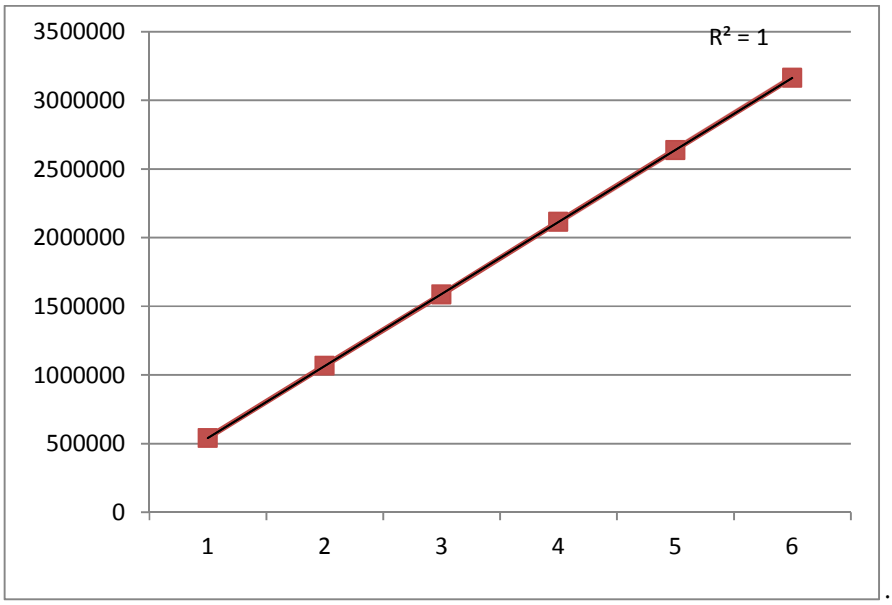


Fig 5. Linearity curve of venlafaxine

Table 1 Results showing linearity

Sl.No	Typeof Solution	Volume of Stock Solution Taken in ml.	Final Dilution in ml.	Concentration of the Solution µg/ml	Peak Area
1	25%	0.25	100	12.5	541656
2	50%	0.5	100	25	1067131
3	75%	0.75	100	37.5	1587197
4	100%	1.0	100	50.0	2116076
5	125	1.25	100	62.5	2639479
6	150%	1.5	100	75	3166192

The LOD and LOQ of the proposed method were calculated by using slope (s) and standard deviation of the intercept of the calibration curve (σ). LOD is 61.74 and LOQ is 187.03.

3. **Accuracy:** By the recovery studies of venlafaxine, the accuracy of the present method was determined. By adding known amounts of venlafaxine standard to the preanalysed sample and the resulting mixture was subjected to the present RP-HPLC method. The analysis was carriedout at three different concentrations. The results of recovery studies were shown in Table 2.

Table 2: Results of recovery studies - Accuracy

Sl.No	Level%	Amount added(µg)	Amount Recovered(µg)	% Recovery
1	80	40	39.69	99.23
2	100	50	49.83	99.65
3	120	60	59.77	99.62

The % recovery was in between 100±2, hence the method was accurate.

4. **Precision:**

a) System Precision: It was determined from the repeatability of the analytical method by assaying six solutions of venlafaxine during the same day, under same experimental conditions, the results of system precision were shown in Table 3.

Table 3:Results showing system precision

S No	Venlafaxime	
	RT	Area
1	5.011	2118148
2	5.009	2117956
3	4.988	2124629
4	4.995	2130645
5	5.002	2125204
6	5.005	2122015
Avg	5.002	2123100
Std Dev	0.0088	4813.5630
RSD	0.175	0.227

b) Method Precision: Six replicate solutions of venlafaxine of concentration 20 µg/ml were prepared from the standard solution and the chromatograms were taken under identical conditions separately. The average, Standard Deviation and % of RSD are determined by the statistical methods which were shown in Table 4.

Table 4: Results showing method precision of the present method

S No	Venlafaxime	
	RT	Area
1	4.985	2124562
2	4.992	2130545
3	5.012	2135245
4	5.010	2128252
5	5.021	2133005
6	5.006	2125215
Avg	5.004	2129471
Std Dev	0.0134	4258.6262
RSD	0.268	0.200

The % of RSD for system precision and method precision were less than 2. Hence the method was precise.

5. **Ruggedness:** Under the same experimental conditions, experiments were carried out with different instruments, different columns and on different days. The mean, SD, % of RSD of ruggedness were found and shown in table 5.

Table 5: Results of Ruggedness

S No	Venlafaxime	
	RT	Area
1	5.011	2118148
2	5.009	2117956
3	4.988	2124629
4	4.995	2130645
5	5.002	2125204
6	5.005	2122015
Avg	5.002	2123100
Std Dev	0.0088	4813.5630
RSD	0.175	0.227

S No	Venlafaxime	
	RT	Area
1	5.010	2128251
2	5.015	2130214
3	5.016	2125452
4	5.018	2124078
5	5.008	2126265
6	5.010	2118975
Avg	5.013	2125539
Std Dev	0.0040	3872.1010
RSD	0.080	0.182

The % of RSD of ruggedness was less than 2 and hence the method was rugged.

6. **Robustness:** By making intentional method variations like mobile phase flow rate changes, column oven temperature changes etc were made and the robustness of the method was determined. The % of RSD of areas and RT's from repeated injections were not more than 2%. The results of robustness were shown in Table 6.

Table 6: Results of Robustness

		Venlafaxime	
S No	Parameter	RT	Area
1	Standard	3.612	1450316
2	Robustness-Flow-1	3.173	1249667
3	Robustness-Flow-2	3.763	1595609
4	Robustness-Oven Temp-1	5.093	2128634
5	Robustness-Oven Temp-2	4.879	2106600

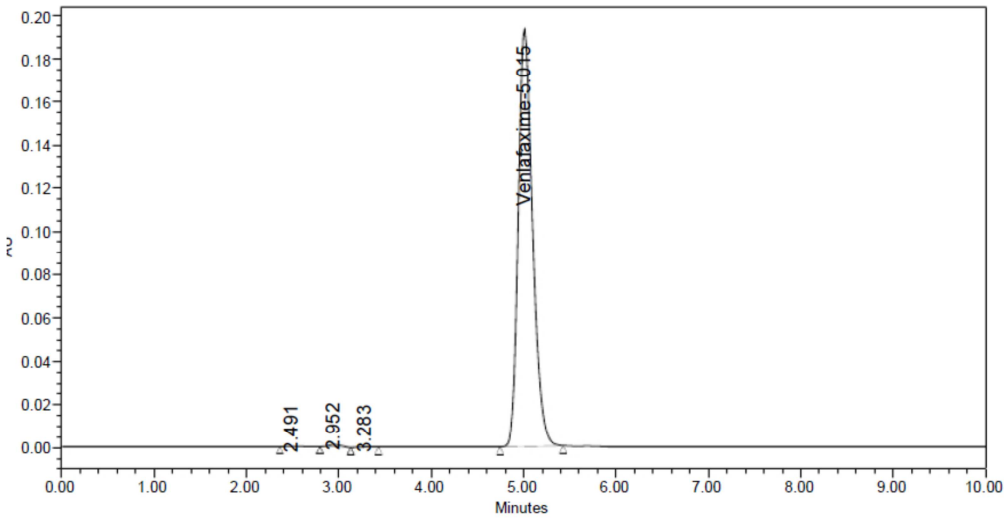


Fig 6. Chromatogram of venlafaxine in Acid Hydrolysis

7. Study of Degradation:

a) Acid Hydrolysis: Accurately 50mg of venlafaxine was transferred into a 200 ml RB flask and 100ml of freshly prepared 0.1N HCl was added and left the solution for 10hours. After 10 hours the solution was filtered and neutralized with a suitable base. 10ml of the filtrate was diluted to 100ml with the diluent and chromatogram was taken, which was shown in Figure 6.

b)Base Hydrolysis: Accurately 50mg of venlafaxine was transferred into a 200 ml RB flask, 100ml of freshly prepared 0.1N NaOH and left the solution for 10hours. After 10 hours the solution was filtered and neutralized with a suitable acid. 10ml of the filtrate was diluted to 100ml with the diluent and chromatogram was taken, which was shown in Figure 7.

c)

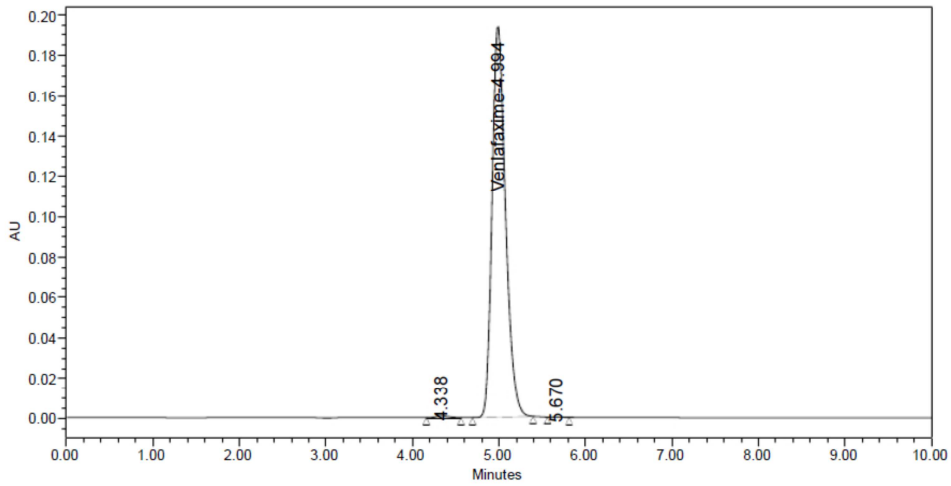


Fig 7. Chromatogram of venlafaxine in Base Hydrolysis

d) Oxidation with Hydrogen Peroxide: Transferred quantitatively 50mg of Venlafaxine into a 200ml. RB flask, 100 ml. of freshly prepared 1.0% Hydrogen Peroxide was added and left it for 10hours. Filtered through the filter paper and diluted 10ml. of the filtrate to 100ml. with diluent and the chromatogram was taken which was shown in Figure 8.

e)

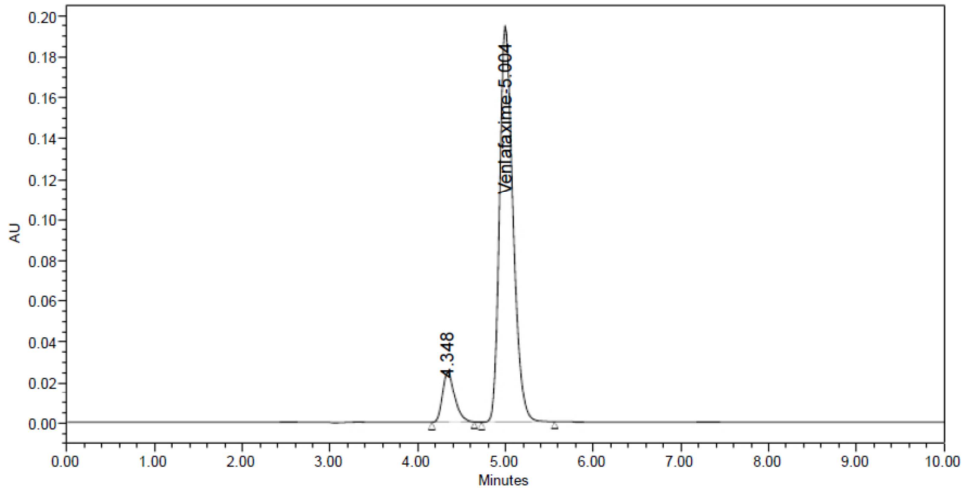


Figure 8.Chromatogram of Venlafaxine on Oxidation with H_2O_2.

f) Thermal Degradation: Transferred quantitatively 50mg. of Venlafaxine on to a clean and dry petri dish. Spread it through out the plate. Placed the petri dish in a oven which was maintained at 100^0C over night. After heating the contents were transferred into 100ml volumetric flask, 50ml. of diluent was added and sonicated it for 10minutes and made up to the volume and filtered. Dilute 10ml. of the filtrate with diluent and Chromatogram was taken. The chromatogram was shown in Figure 9.

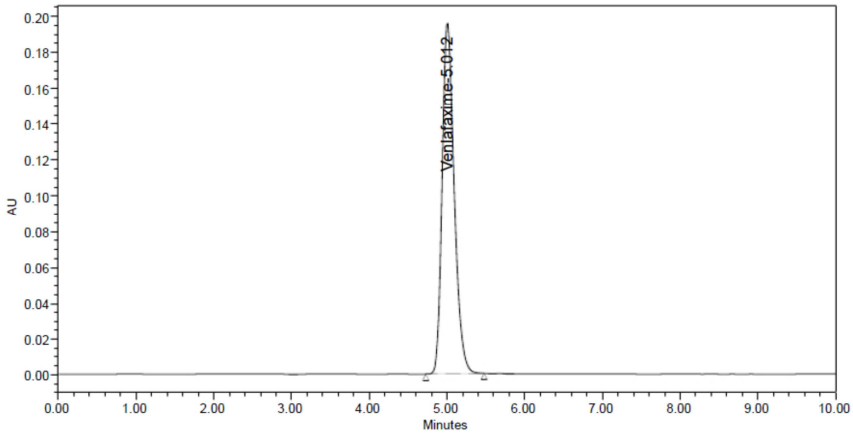


Figure 9.Chromatogram of Venlafaxine on Thermal Degradation

g) UV Exposure: 50mg of Venlafaxine was taken on a clean and dry petri dish and the plate was placed in the UV chamber for 10hours. After exposure the contents were transferred to 100ml volumetric flask 50ml of diluent was added, sonicated and made up to the volume and filtered. Dilute 10ml of the filtrate to 100ml and chromatogram was taken. The chromatogram was shown in Figure 10.

Table 6 showing results of Degradation

Acid Hydrolysis				
	Peak Area	Found Assay	% of Assay	% Degradation
Degraded API	2046462			
Standard	2135265	95.75	99.71	3.96
Base Hydrolysis				
	Peak Area	Found Assay	% of Assay	%Degradation
Degraded API	2025467			
Standard	2135265	95.33	99.71	4.38
Oxdation with H_2O_2				
	Peak Area	Found Assay	% of Assay	% Degradation
Degraded API	2034511			
Standard	2135265	94.29	99.71	5.4
Thermal Degradation				
	Peak Area	Found Assay	% of Assay	% Degradation
Degraded API	2007413			
Standard	2135265	95.38	99.71	4.33
U.V Exposure				
	Peak Area	Found Assay	% of Assay	% Degradation
Degraded API	2034611			
Standard	2135265	95.76	99.71	3.95

As per the above results, the degradation varies from 3.9 to 5.4% under different stress conditions.

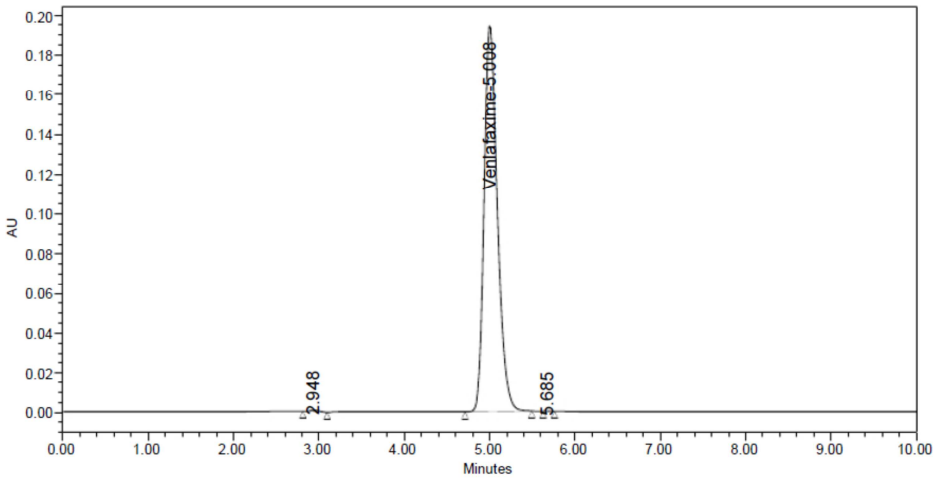


Figure 10. Chromatogram of Venlafaxine on UV Exposure

Assay of Venlafaxine:

20µl of blank, standard and sample solutions were injected into the HPLC system and the chromatograms were recorded. The average % of assay of Venlafaxine was 99.71%.

Assay of Pharmaceutical Formulation:

20µL solution of Effexor tablets of different dosages were injected into the HPLC system separately and chromatograms were recorded. The results were shown in table 7.

Table 7 Showing results of Assay of Pharmaceutical Formulations(Effexor)

Sl.No	Dosage in mg	Found Assay mg	% Assay
1	25	24.915	99.66
2	37.5	37.421	99.79
3	50	50.06	100.12
4	75	75.54	100.72
5	100	100.31	100.31

The % of Assay was found to be within the limits of 99-101.

CONCLUSION

The presently developed stability indicating RP-HPLC method for the determination of Venlafaxine in Pharmaceutical Formulations in presence of its degradation products is precise, accurate, rapid, linear and specific. This method may be used in routine analysis of Venlafaxine in quality control laboratories.

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