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Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Venlafaxine Hydrochloride in Bulk and Capsule Dosage Form

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ABSTRACT

A simple, rapid, precise, stability-indicating RP-HPLC method has developed the estimation of Venlafaxine HCl from bulk and capsule dosage form on Waters HPLC-1595 having UV Detector 2489 binary system by using Chemsil ODS C18 (4.6 X 150mm 5µm) column. Mobile phase composed of Methanol: Water in the proportion of 85:15 with 0.5% triethylamine and pH adjusted to 7.4 by using O-phosphoric acid. The flow rate was set 1 ml/min and the injection volume was 10 µl. Detection was carried out at 230 nm. The retention time of Venlafaxine HCl was found to be 6.8± 0.3 min. the method was then validated as per the ICH Q2 R1 guideline. The method was found to be linear for venlafaxine HCl with a correlation coefficient of 0.9945, the method was found to be precise, accurate & robust with RSD of <2. Stability was performed by using various stress conditions as per ICH guidelines for the stability of new drug products. And the degradation products which are generated during the stress testing of the drug were successfully resolved from drug peak with resolution >1.5. Complying all the guidelines, hence concluding that it can be used for routine analysis of drugs.

INTRODUCTION:

Chromatography is a technique by which a mixture sample is separated into components. Although originally intended to separate and recover (isolate and purify) the components of a sample, today complete chromatography systems are often used to both separation and quantitation sample components. The Stability Indicating Method can be defined as "Validated Quantitative analytical method that can detect the changes with time in the chemical, physical or microbiological properties of the drug substance and drug product, and that are specific so that the content of active ingredient, degradation can be accurately measured without interference." Venlafaxine Hydrochloride;(±)-1-[α-[(dimethyl-amino) methyl]-p-methoxybenzyl] cyclohexanol hydrochloride (Fig.1), and its active metabolite O-desmethylvenlafaxine (ODV) was considered to be Serotonin and norepinephrine reuptake inhibitor (SSRI) which is used in the treatment of depression and related conditions sometimes it is also used in the treatment of neuropathic pain. Thereby increasing the reduced levels of those neurotransmitters. It shows no significant activity towards Muscarinic, Histaminic, and alpha-adrenergic; Thereby reducing possible side effects. Literature survey reveals that drug has been estimated by using UV spectrophotometric, RP-HPLC either alone in its dosage forms or in the presence of its metabolites.

Comprehensive literature shows that there are various methods developed for the estimation of Venlafaxine HCl. Simple method development, Stability indicating RP-HPLC method[6-8], Stability indicating for in-vitro determination[9] as well as LC-MS-MS method[10] is developed. To predict drugs instability and to gain knowledge of degradation pathways stability study through is useful. The objective of this study was to develop simple, accurate, precise and rapid stability indicating RP-HPLC method for routine analysis. This chromatographic method is distinct from literature, here use of methanol and water for mobile phase makes it very economical method.

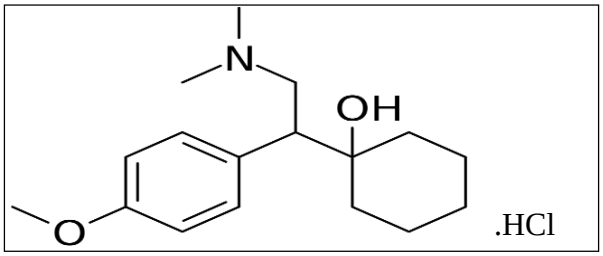


Figure No. 01: Chemical structure of Venlafaxine Hydrochloride.

MATERIALS AND METHODS:

Chemicals and reagents:

Water (HPLC Grade), Methanol (HPLC Grade), Triethylamine (AR Grade), O-Phosphoric Acid (AR Grade)

Selection of Mobile phase: Mobile Phase was selected based on the solubility of Drugs and trials.

Preliminary optimization of mobile phase and other chromatographic conditions:

Table No. 01: List of trials taken for method development

Sr. No.	Mobile phase	Column	Lambda max	Flow rate
1	Methanol : Water (95:5)	Orosil C18	273 nm	1ml/min
2	Methanol : Water (90:10)	Orosil C18	225 nm	1ml/min
3	Methanol : Water (95: 5)	Chemsil C18	225 nm	1ml/min
4	Methanol : Water (90:10)	Chemsil C18	225 nm	1ml/min
5	Methanol : Water pH-6.8 (80:20)*	Chemsil C18	225 nm	1ml/min
6	Methanol : Water (90:10) pH 7.2	Chemsil C18	225 nm	1ml/min
7	Methanol : Water (85:15) pH 7.2	Chemsil C18	273 nm	1ml/min
8	Methanol : Water (85:15) pH7.4	Chemsil C18	225 nm	1ml/min
9	Methanol : water (85:15) pH 7.4	Chemsil C18	273 nm	1ml/min
10	Methanol : water (85:15) pH 7.4	Chemsil C18	235 nm	1ml/min
11	**Methanol : water (85:15) pH 7.4**	**Chemsil C18**	**230 nm**	**1ml/min**

Optimized chromatographic conditions:

Table No. 02: List of trials taken for method development

Parameter	Condition
Column name	Chemsil ODS C18 column
Detection	230nm.
Flow rate	1 ml/min
Injection volume	10 µl
Mobile phase	Methanol: Water (85:15) 0.5% Triethylamine, pH adjusted to 7.4 by OPA

Preparation of standard solution:

a. Standard stock solution: 25mg Drug was weighed transferred into 25 ml volumetric flask and dissolved sufficient quantity of Methanol up to the mark on 25 ml volumetric flask which gives 1000ppm (S.S.)

b. Working Standard Solution: the required amount of standard stock solution was pipetted out and transferred into 10 ml volumetric flask & ultra-sonicated 5 min. for which gives following concentration of 200, 400, 600, 800, 1000 ppm.

c. **Aqueous Phase: T**he aqueous phase composed of 0.5% Triethylamine pH adjusted to 7.4 by OPA (Orthophosphoric acid).

Analysis of marketed capsule formulation:

Preparation of sample solution (test solution):

For analysis of marketed formulation, 10 capsules of venlafaxine hydrochloride were purchased from a local pharmacy. These capsules are then weighed and content is crushed to a fine powder. Accurately weigh and transferred several samples equivalent to 25 mg venlafaxine hydrochloride in 25 ml volumetric flask, a sufficient quantity of diluent was added up to the mark to produce a 25 ml solution. This solution then subjected to ultra-sonication, filtered through a 0.45µm membrane filter. Pipette out 2.5ml of the stock solution into 10ml volumetric flask and diluted up to the mark by diluent which gives 250 ppm test solution. This test solution is then used for accuracy study to determine % recovery.

Method Validation:

Method validation was performed as per ICH Q2 R1 guideline and the following parameters were performed: System suitability, Linearity, accuracy, precision, limit of detection, the limit of quantitation, robustness.

System suitability:

System suitability is performed to determine that the selected instrument and method is suitable for its intended purpose, it is performed by taking 5 replicates of the standard sample.

Linearity:

Linearity was performed by diluting the stock solution to give a final concentration of 200 to 1000 ppm of venlafaxine Hydrochloride. 2 ml of standard stock solution was pipette out and transferred into 10 ml volumetric flask and remaining volume was adjusted with diluent to give 200 ppm venlafaxine HCl. Similarly, other dilutions are also prepared for analysis. 10 µl of each concentration was injected and the calibration curve was plotted as peak area vs. concentration.

Accuracy:

Accuracy was performed by using a mixture of 1. Standard stock solution & 2. Test solution of marketed capsules. Mixing is done by taking a known quantity of drug as either of the solution concentration remains constant and % recovery was performed at 50,100 & 150% levels of analyte. From the results, the obtained % recovery was calculated.

Table No. 03. Accuracy Solution

% solution	Amount of test	Amount of std.	Total
50	250	0	250
100	250	250	500
150	250	500	750

Preparation

Precision:

Precision can be performed on either 3 concentration & 3 replication or 6 replication of the same concentration usually 100%.

Robustness:

The robustness of the method was performed by using a homogeneous sample of analyte and by changing the physical parameters of the method. To carry out robustness following chromatographic conditions are varied: Change in flow rate, change in buffer pH, and change in mobile phase composition.

Limit of Detection & Limit of Quantitation:

Limit of detection & Limit of quantitation were calculated by the standard deviation of response and slope method.

$$\mathbf{LOD = 3.3X\frac{(SD)}{S}}$$

Where **SD =** Standard deviation

S = slope

$$\mathbf{LOQ = 10\ X\ \frac{(SD)}{S}}$$

Where **SD =** Standard deviation

S = slope

FORCED DEGRADATION STUDIES:

Acidic hydrolysis:

Accurately weigh 100 mg of Venlafaxine Hydrochloride and transferred into a 10 ml volumetric flask. A sufficient amount of hydrochloric acid (HCl) was added to produce 10 ml of solution (0.1, 0.5 & 1N). These samples were set aside for up to 24Hrs. then for analysis 0.5ml of the solution was pipette out in 10 ml volumetric flask remaining volume was made up by diluent.

Basic degradation:

Accurately weighed 100 mg of venlafaxine HCl. Transferred into a 10 ml volumetric flask, volume was made up by 0.1 N Sodium Hydroxide (NaOH) sample was set aside for up to 24Hrs. then from this 0.5 ml solution was pipette out into 10 ml volumetric flask and the remaining volume was made up by diluent.

Oxidative degradation:

Accurately weighed 100 mg of venlafaxine HCl transferred into 10 ml volumetric flask and 6% H_2O_2 (Hydrogen peroxide) was added up to the mark. The resultant solution was set aside

for up to 24 hrs. Then from this solution, 0.5 ml was pipette out and transferred into 10 ml volumetric flask remaining volume was made up by diluent.

Thermal degradation:

For thermal degradation, 100 mg of drug was weighed and transferred into the Petri plate and spread evenly then the plate was covered with aluminum foil. And stored in the oven for 24 hrs. At various temperature conditions (40, 50, 60, 70ºC). 12.5 mg of drug was weighed and transferred into 25 ml volumetric flask and diluent was added up to the mark to produce 25 ml of the resultant solution.

Preparation of solutions used for stress testing:

1. Hydrochloric acid: 0.1, 0.5, 1N solutions of HCl were prepared according to IP 2007 by diluting 8.5×0.1, 0.5, 1ml of hydrochloric acid to 100 ml with water.

2. Sodium Hydroxide: 0.1 N solution of NaOH was prepared according to IP 2007, by dissolving 0.4 g of sodium hydroxide insufficient water to produce 100 ml.

RESULT AND DISCUSSION:

The wavelength of 230nm seems to be the most suitable for the detection of venlafaxine.

By considering different system suitability parameters like RT, tailing, Number of theoretical plates, the mobile phase was found to be most suitable consisting of Methanol: Water (85:15) with 0.5% triethylamine & pH adjusted to 7.4 by OPA.

The correlation coefficient for linearity was found to be 0.9945 with the working range which shows good linearity. For Precision, Accuracy and robustness RSD was found to be less than 2. Hence, all the validation parameters are complying with Guidelines. Force degradation which is applied for the generation of degradation products successfully generated various degradants and methods successfully separated Drug as well as Degradants with resolution more than 1.5.

SothemethodcanbeusedroutineanalysisofVenlafaxine HClitsdegradantsandalso its marketed formulations.

SYSTEM SUITABILITY PARAMETERS:

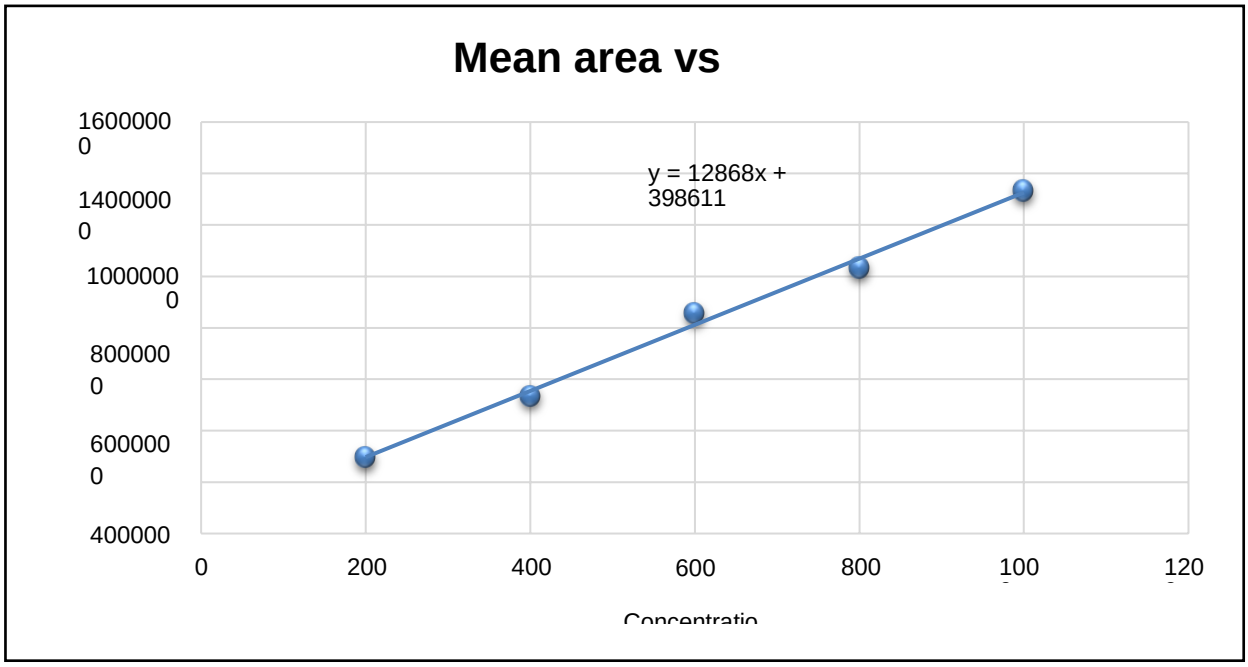
Table. No.04: System suitability table

Sample name	Retention time	Area	USP Plate count	Tailing factor
SDT.1	6.793	13808812	4467	1.35
SDT.2	6.749	14827324	5278	1.37
SDT.3	6.737	12836589	4436	1.33
SDT.4	6.444	14790903	3879	1.23
SDT.5	6.459	11504386	4382	1.30
	Average	13553602	4488.4	1.316
	S.D.	127605	10.5	0.00028
	RSD	0.94	0.23	0.021

Linearity:

Table No. 05: Result of linearity data

Sr. No.	Conc.	Area1	Area2	Area3	Mean area	SD
1	200	3032976	3098766	2785292	2972344.667	165298.5363
2	400	5325792	5401651	5355085	5360842.667	38255.84941
3	600	8673791	8513678	8528327	8571932	88516.04494
4	800	10433369	10211458	10381401	10342076	116064.4851
5	1000	13306094	13371893	13370997	13349661.33	37733.07706
		Regression	0.9945			
		Slope	12868			



Graph 01: Linearity plot of Venlafaxine Hydrochloride

Accuracy:

Table No. 06: Result of % recovery study

Sr. No.	% Conc	Area	Mean	SD	RSD	% Accuracy
1	50	3327811	3489735	54773.4	1.56	95.59%
2	50	3560999				
3	50	3480395				
4	100	6759399	6669692	67323.21	1.00	97.61%
5	100	6652463				
6	100	6597214				
7	150	9492540	9425498	56740.04	0.60	93.78%
8	150	9430164				
9	150	9353791				

Precision:

Table No. 07: Result of Intraday precision study

Intraday precision				
Sr. No.	Concentration	Area	S.D.	% RSD
1	500	6667609	93316.09	1.413%
2	500	6715758		
3	500	6599788		
4	500	6476714		
5	500	6558429		

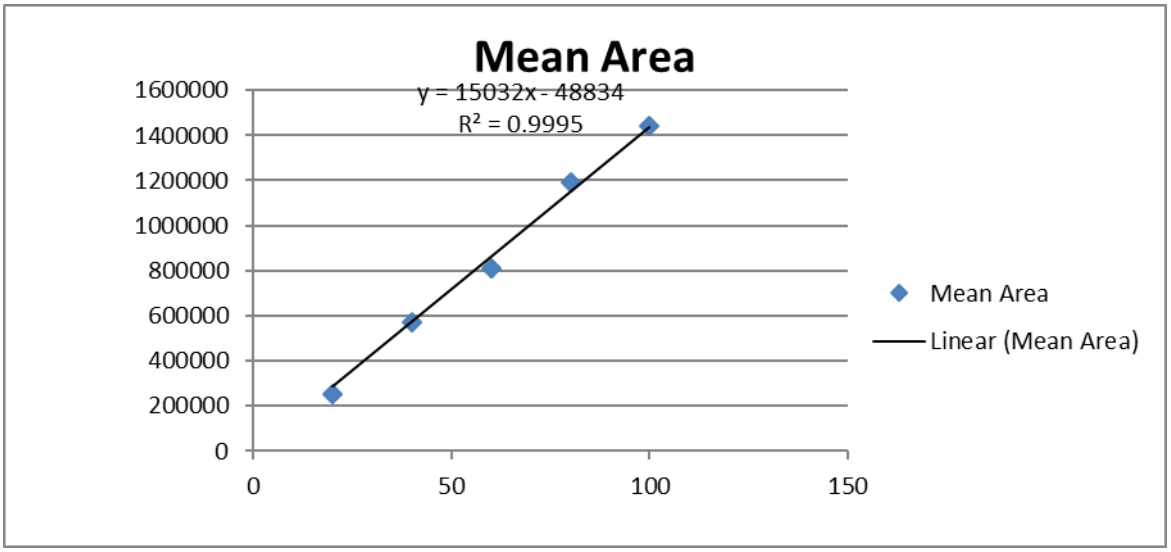
Table No. 08: Result of Interday precision study

Inter day precision				
Sr. No.	Concentration	Area	S.D.	%RSD
1	500	7218542	1005910.8	1.467
2	500	7129554		
3	500	7132094		
4	500	7216986		
5	500	7391700		

LOD & LOQ:

Table No. 09: LOD & LOQ Table

Sr. No.	Conc.	Area	Regression	STD DEV	Slope	LOD	LOQ
1	20	250747	0.9959	476318.7	15032	104.567	316.8698
2	40	571104					
3	60	809146					
4	80	1189847					
5	100	1444570					



Graph. 02: Calibration Curve for LOD & LOQ of Venlafaxine HCl

Robustness:

Table No. 10: **Robustness**

Parameter		Area	Mean	S.D.	R.S.D.
Change in flow rate	0.8 ml/min	6697030	6590544	106486	1.61
	1.2 ml/min	6484059			
Change in pH	pH-7.2	7086531	6968408	118123	1.69
	pH-7.6	6850285			
Change in Analyst	Analyst-1	7673310	7680072	6762	0.08
	Analyst-2	7686835			

Forced Degradation Studies:

Table No. 11: **Results of Forced Degradation Studies**

Sr. No.	Parameter	% of Drug	% Degradation
1	Acidic hydrolysis (0.5 N HCl for 12 Hrs.)	99.31	0.69
2	Basic degradation (0.1 N NaOH for 12 Hrs.)	94.33	5.67
3	Oxidative degradation (6% H_2O_2 for 12 Hrs.)	99.11	i. 0.80% ii.0.08%
4	Thermal degradation	100	0

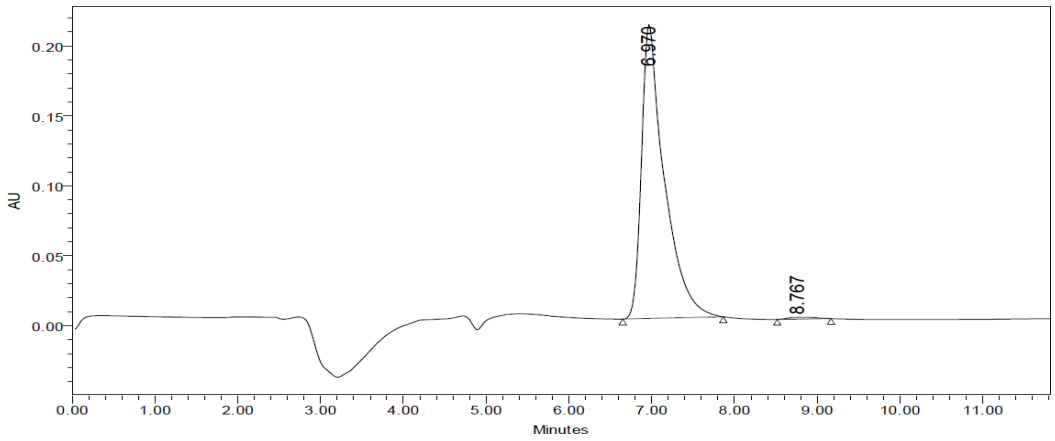


Figure No. 02: Chromatogram of Acidic degradation

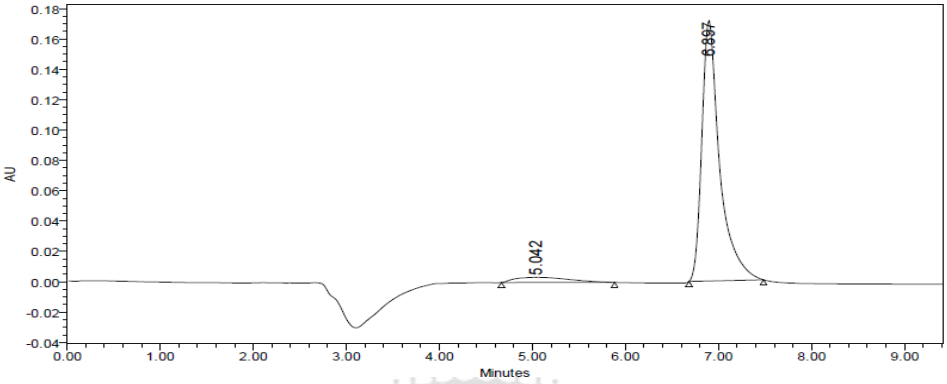


Figure No. 03: Chromatogram of Basic degradation

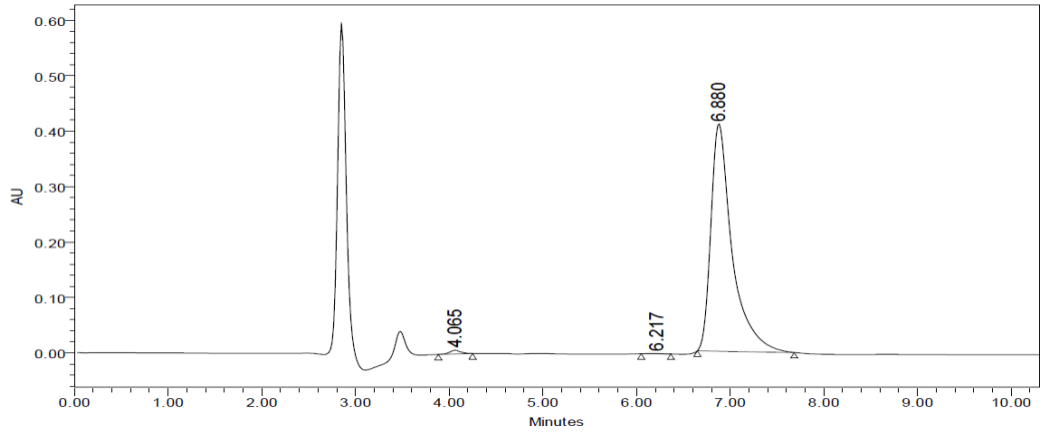


Figure No. 04: Chromatogram of Oxidative degradation

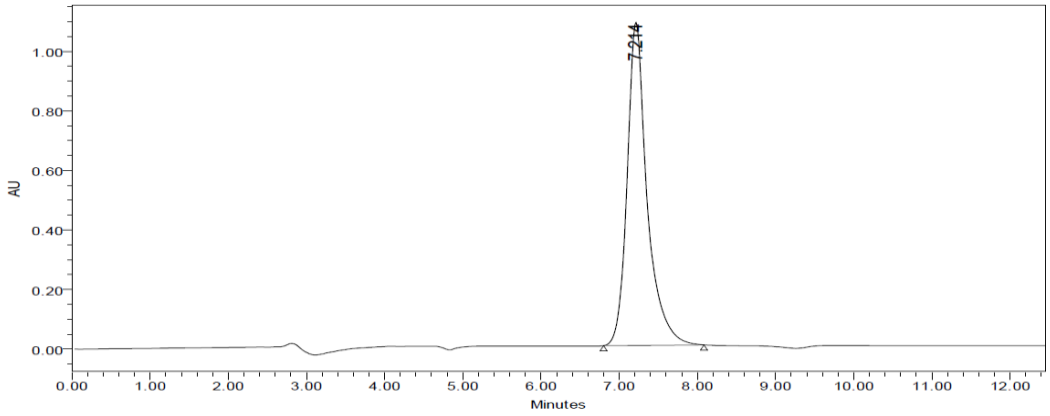


Figure No. 05: Chromatogram of Thermal degradation

Result: No degradation peak was obtained after thermal degradation which shows that the drug is stable over a wide range of temperatures.

CONCLUSION:

The present work involves development and validation of stability indicating RP-HPLC method for estimation of venlafaxine HCl in bulk and capsule dosage form. In reversed-phase HPLC method sample was resolved by using isocratic elution and mobile phase used was (85:15) Methanol: Water, 0.5% triethylamine pH adjusted to 7.4 by using OPA. Chemsil ODS C18 Column. (150mm x 4.6 mm) was used as a stationary phase. Flowrate used 1.0 ml/ min. linearity was observed in the range of 200 to 1000 ppm for venlafaxine HCl. The retention time was found to be 6.8 ± 0.5 min. the method was validated as per the ICH Q2 R1 guideline. Standard deviation and % RSD <2% are within the range indicating accuracy and precision of the method. Recovery studies showed good accuracy of the method. Hence, it can be concluded that the method developed is simple, accurate, precise, reproducible, and economic and can be employed successfully for estimation of venlafaxine HCl in bulk and capsule dosage form. Forced degradation study of venlafaxine HCl was performed under various stress conditions like acidic hydrolysis, basic hydrolysis, oxidative and thermal degradation. Venlafaxine shows 0.69% degradation in acidic condition (0.5N), 5.67% degradation in Basic condition. 0.88% in Oxidative condition and found to be stable at high temperature (70°C) condition.

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