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METHOD DEVELOPMENT AND VALIDATION FOR THE ASSAY OF VENLAFAXINE HYDROCHLORIDE CAPSULES AND ITS IMPURITIY BY RP-HPLC

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ABSTRACT

This paper describes the reverse phase high performance liquid chromatographic method and it was validated as per ICH guidelines for the determination of impurity-F in Venlafaxine hydrochloride. Analysis was carried on shimadzu HPLC system with inertsil ODS-3V C_{18} column, (150mm x 4.6mm, 5µm) using filtered and degassed solutions containing a mixture of buffer and methanol (55:45) in isocratic mode as mobile phase with flow rate of 1.0ml/minute. The detection was carried out using UV detector set at 225nm. This method obeyed Beer's law in the concentration range from 80% - 120% of venlafaxine hydrochloride. The validation was performed according to the ICH Guidelines Q2 (R1). The % RSD for system suitability was 0.23

& 0.64 for Venlafaxine hydrochloride and impurity-F respectively. Linearity was observed in the concentration range of 80%-120% were linear (R_1=0.9999 & R_2=0.9994). For precision the %RSD was 0.8 & 0.2 for Venlafaxine hydrochloride and impurity-F respectively. The percentage recovery was 100.76% from the capsule formulation. Signal to noise ratio for limit of detection and limit of quantification was found to be within 3-5 and less than 10 for Venlafaxine hydrochloride and impurity-F respectively. The percentage recover was good and the results obtained in this study demonstrate that the venlafaxine hydrochloride HPLC method described in the protocol is selective, linear, precise, rugged and robust for the determination of impurity in venlafaxine hydrochloride.

Key words: Venlafaxine hydrochloride, impurity F, Reverse phase liquid chromatography, assay, validation, percentage recovery

INTRODUCTION

Venlafaxine hydrochloride is chemically (R/S)-1-[2-(dimethylamino)-1-(4-methoxy-phenyl) ethyl] cyclohexanol hydrochloride or (±)-1-[α-[(dimethyl-amino) methyl]-p-methoxybenzyl] cyclohexanol hydrochloride. Its molecular formula is $C_{17}H_{27}NO_2$ and molecular weight is about 313.87g/mol **[1]**. Venlafaxine is a new generation antidepressant, and is usually categorized as a serotonin-norepinephrine reuptake inhibitor (SNRI) and drug showing effective anti-depressant properties, but it has been referred to as a serotonin-norepinephrine-dopamine reuptake inhibitor, Depression is the most common of the affective disorders **[2]**. It may range from very mild condition bordering on normality to severe psychotic, depression accompanied by hallucination and delusions. Emotional symptoms include misery, indecisiveness, lack of motivation and the biological symptoms include retardation of thought and action **[3-5]**.

Venlafaxine selectively inhibits the neuronal re-uptake of serotonin, norepinephrine and to a lesser extent dopamine. It has minimal affinity for muscarinic, histamine or alpha1-adrenergic receptors **[6-8]**. It appears to be as effective as standard antidepressants with a lower incidence of the anti-cholinergic side effects. Venlafaxine hydrochloride is freely soluble in methanol, acetonitrile and soluble in water. Its partition coefficient is 0.43 with pH 5.2 and pKa 8.2. It has 90% absolute bioavailability **[9]**.

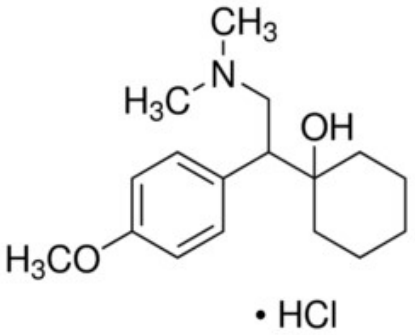

Figure 1: Venlafaxine hydrochloride

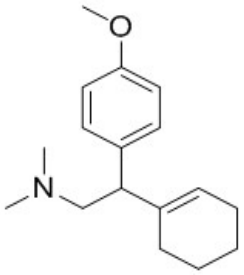

Figure 2: impurity-F

MATERIALS AND METHODS

Estimation of absorption maxima (λ_{max}) by UV-visible spectroscopy

Preparation of UV sample solution: Weigh about 48.0mg of Venlafaxine hydrochloride in to a 25mL volumetric flask. Add 10mL of mobile phase and sonicate to dissolve and dilute to volume with mobile phase. Transfer the above solution into a 50mLvolumetric flask and make up to mark with mobile phase. Filter through 0.45µ nylon membrane filter.

Adjust the baseline to zero sing water as a blank. Take the UV spectrum for Venlafaxine hydrochloride **(Figure 3)**.

Method for the estimation of Venlafaxine hydrochloride Capsules

Chromatographic conditions

Chromatographic conditions were achieved using ODS-3V, 150 x 4.6mm, 5µm or its equivalent column for separation. Flow rate of 1.0mL/min. wavelength at 225nm with an injection volume of 20µL and run was about 12 minutes.

Preparation of Mixed phosphate buffer

Weigh about 1.6gm of anhydrous ammonium acetate in to a volumetric flask containing 1000mL water and mix well. pH is adjusted to 3.0 with glacial acetic acid and the solution was filtered using 0.45µm nylon filter.

Preparation of Mobile phase

A mixture of mixed buffer and Acetonitrile in the ratio of 45:55v/v% was prepared and filtered.

Assay of venlafaxine Hcl 150mg capsules

Preparation of standard solution

Weigh about 25mg of Venlafaxine hydrochloride and transfer into a 50mL volumetric flask and dissolve in the mobile phase and make up to 50mL to obtain 500µg/mL of concentration. Mix and filter the solution.

Transfer 5.0mL of the above solution to 50mL volumetric flask and make up to

50mLwith the mobile phase to obtain 50µg/mL of concentration. Mix and filter the solution **(Figure 4)**.

Preparation of sample solution

Accurately weigh the quantity of powdered pellets equivalent to 30mg of Venlafaxine hydrochloride to 250mL volumetric flask. Add 60mL of mobile phase and sonicate to dissolve for 15min with intermediate shaking and make up to volume with mobile phase. Mix the solution and filter through 0.45µm nylon membrane filter. Transfer 10mL of this solution to a 25mL volumetric flask, dilute with mobile phase up to the volume to obtain a concentration of 50µg/mL, and mix **(Figure 5)**.

Preparation of placebo solution

Accurately weigh the quantity of placebo powdered pellets equivalent to 31mg of Venlafaxine hydrochloride to 250mL volumetric flask. Add 60mL of mobile phase and sonicate to dissolve for 15min with intermediate shaking and make up to volume with mobile phase. Mix the solution and filter through 0.45µm nylon membrane filter. Transfer 10mL of this solution to a 25mL volumetric flask, dilute with mobile phase up to the volume, and mix **(Figure 6)**.

Procedure

Separately inject diluent as a blank, single injection of placebo solution, five replicate injections of standard solution and two replicate injections of sample solution into the chromatograph, record the chromatograms and measure the peak responses.

METHOD VALIDATION

System suitability

Preparation of System suitability solution

Mix 1mL of impurity-F preparation, 1mL of unknown impurity preparation and 1mL of Venlafaxine hydrochloride diluted standard into a 10mL volumetric flask and dilute to volume with diluent.

System suitability of Venlafaxine HCl and impurity-F (Table 1)

Acceptance criteria

The resolution between impurity-F and Venlafaxine hydrochloride should not be less than 1.5 from system suitability solution.

Specificity

According to ICH, the term specific generally refers to a method that produces a response for a single analyte only while the term selective refers to a method which produces responses for a number of chemical entities that may or may not be distinguished from all other responses, the method is said to be specific **(Figure 7)**.

Chromatographic conditions

Chromatographic conditions were achieved using Altima C8, 150 x 4.6mm, 5µm or its

equivalent column for separation. Flow rate of 1.0mL/min. wavelength at 225nm with an injection volume of 20µL and run was about 90 minutes

Preparation of Buffer solution

Mix 1.6mL of ortho phosphoric acid with 1000mL of distilled water and adjust the pH to 3.0 with Trimethyl amine.

Preparation of Mobile phase

A mixture of mixed buffer and Acetonitrile in the ratio of 45:55v/v% was prepared and filtered.

Preparation of standard stock solution

Preparation of Impurity-F

Transfer 20mg of impurity-F in 20mL volumetric flask, add 10mL of mobile phase, sonicate to dissolve and make p to the volume with mobile phase. Transfer 1mL of this solution into 100mL volumetric flask, make up to the volume with mobile phase to obtain 10µg/mL of concentration (10ppm) **(Figure 8)**.

Standard preparation of diluted Venlafaxine hydrochloride

Transfer 25mg of Venlafaxine hydrochloride WS in 25mL volumetric flask, add 20mL of mobile phase sonicate to dissolve and make up to the volume with mobile phase. Transfer 1mL of this solution into 100mL volumetric flask, make p to the volume with the mobile phase to obtain 10µg/mL of concentration (10ppm) **(Figure 9)**.

Preparation of test solution

Transfer an accurately weighed quantity of powdered pellets equivalent to about 100mg of Venlafaxine hydrochloride to a 100mL volumetric flask. Add 50mL of diluent sonicate for 30 minutes and make up the volume with diluents. Filter the solution through 0.45 PVDF filter. Final concentration of the resulting solution is 1mg/mL of Venlafaxine hydrochloride **(Figure 10)**.

Linearity

Linearity of an analytical procedure is its ability within a given range to obtain test results which are directly proportional to the concentration of analyte in the sample.

Linearity of Venlafaxine HCl and impurity-F (Table 2).

Preparation of linearity stock solution of Venlafaxine hydrochloride and impurity-F solution

Accurately weigh and quantitatively transfer about 25mg of Venlafaxine hydrochloride WRS and 25mg of impurity-F separately into a 50mL volumetric flasks. Add 10mL of methanol to each and sonicate to dissolve and dilute to volume with methanol to obtain 50µg/mL of concentration **(Figure 11, 12)**.

From the above solution prepare the linearity from 20% to 160% using methanol as a diluent. Construct a plot of concentration verses peak area of Venlafaxine hydrochloride and impurity-F.

Acceptance criteria

The plot of concentration verses peak area of Venlafaxine hydrochloride and impurity-F at 20% to 160% level should be linear with a correlation coefficient (r) value is not less than 0.999.

Accuracy

Accuracy is the measure of the closeness of the experimental value to the true value. Accuracy should be established across the specified range of the analytical procedure.

Preparation of recovery stock solution

Accurately weigh and quantitatively transfer about 25mg of Venlafaxine hydrochloride WRS and 25mg of impurity-F separately into a 50mL volumetric flasks. Add 10mL of methanol to each and sonicate to dissolve and dilute to volume with methanol.

Recovery at 50% level: 250mg of Venlafaxine hydrochloride was weighed into a 50mL volumetric flask, to this 0.5mL of recovery stock solution was added and make up to the mark with diluent.

Recovery at 100% level: 250mg of Venlafaxine hydrochloride was weighed into a 50mL volumetric flask, to this 1.0mL of recovery stock solution was added and make up to the mark with diluent.

Recovery at 150% level: 250mg of Venlafaxine hydrochloride was weighed into a 50mL volumetric flask, to this 1.5mL of recovery stock solution was added and make up to the mark with diluent.

The above solutions were injected into the HPLC system thrice along with standards to check the recovery.

Accuracy of Venlafaxine HCl and impurity-F (Table 3)

Acceptance criteria

The recovery of impurities at three levels (50%, 100%, and 150%) should be in the range of 80.0% and 120.0% and relative standard deviation should be not more than 10.0%.

Precision

Precision is a measure of how close the data values are to each other for a series of measurement under the same analytical conditions obtained from the multiple sampling of the same homogenous sample .Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

The precision of this method was determined b analyzing a sample at 100% of the specification limit i.e. six replicate sample preparations.

Precision of Venlafaxine HCl and impurity-F (Table 4)

Preparation of test solution

Accurately weigh and quantitatively transfer about 25mg of Venlafaxine hydrochloride WRS and 25mg of impurity-F separately into a 50mL volumetric flasks. Add 10mL of methanol to each and sonicate to dissolve and dilute to volume with methanol to obtain a Concentration of 0.2 mg/mL for Venlafaxine hydrochloride and impurity-F (Solution –A). 1.0mL of solution-A was pipetted out into 100mL volumetric flask and diluted up to the mark with methanol to obtain Concentration of 0.002mg/mL for Venlafaxine hydrochloride and impurity-F. The same procedure was repeated for six replications.

Acceptance criteria

The %RSD of six replicate injections of Venlafaxine hydrochloride and impurity-F should not be more than 2.0

The theoretical plates of Venlafaxine hydrochloride and impurity-F should be not less than 2000.

The tailing factor of Venlafaxine hydrochloride and impurity-F should be not more than 2.0

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Conditions

- Flow rate to be changed by 1.0 mL/min and 1.4 mL/min
- Buffer pH to be changed by 4.2 to 4.6
- Buffer composition to be changed by 65% to 67%
- Oven temperature changed from 25ºC to 30ºC

Acceptance criteria

The resolution between Venlafaxine hydrochloride and impurity-F peaks should not be less than 2.0 from resolution solution.

Robustness of Venlafaxine HCl and impurity-F (Table 5)

Limit of Quantification (LOQ)

The limit of quantification is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable accuracy and precision.

Limit of quantification (LOQ) of Venlafaxine HCl and impurity-F (Table 6)

Acceptance criteria

The signal to noise ratio is 10:1 and % RSD of replicate injections of Venlafaxine hydrochloride and impurity-F is not more than 10.0%

Limit of detection (LOD)

The limit of detection is determined by calculating the signal to noise ratio and by comparing test results from sample with unknown concentration of analyte with those blank samples and establishing the minimum level at which the analyte can be reliably detected **Table 8, 9**.

Limit of detection of Venlafaxine HCl and impurity-F (Table 8)

Acceptance criteria

The signal to noise ratio is 3:1 and % RSD of replicate injections of Venlafaxine hydrochloride and impurity-F is not more than 10.0%

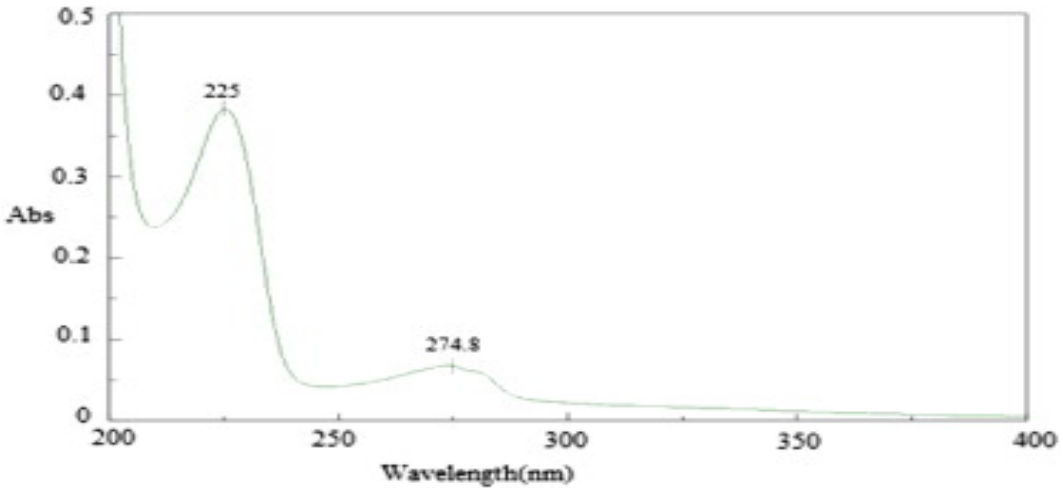

Figure: 3 UV spectrum of Venlafaxine hydrochloride

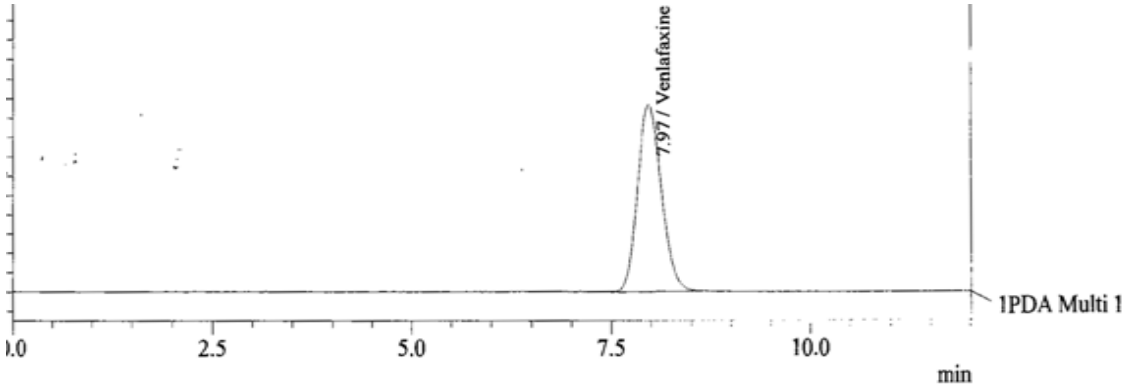

Figure 4: Chromatogram of Standard solution (API)

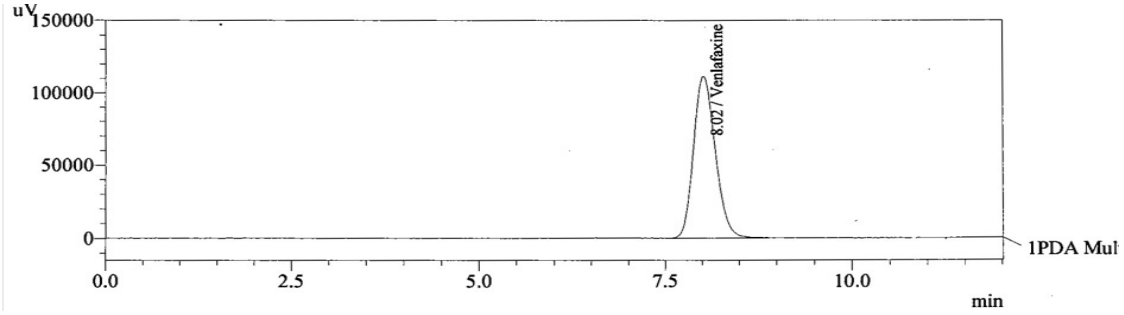

Figure: 5 Chromatogram of Sample solution

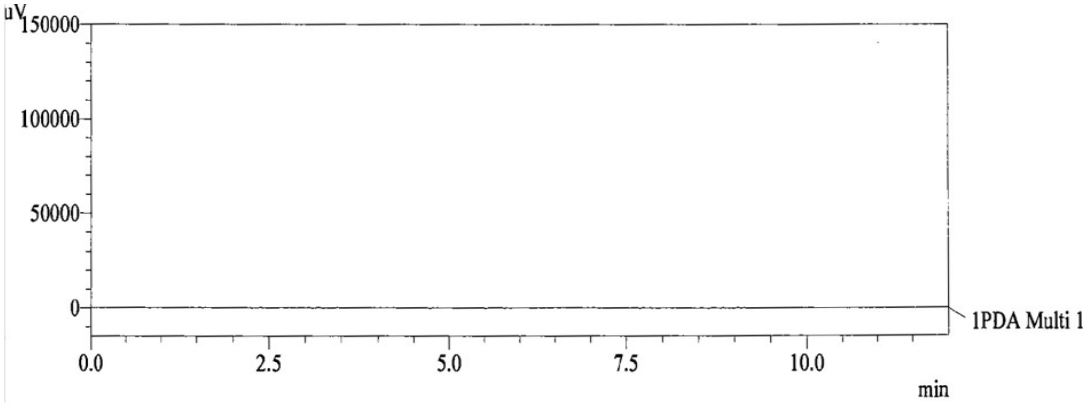

Figure: 6 Chromatogram of Placebo solution

Table 1: System suitability

No. of injections	Retention time (min)		Peak area		Tailing factor		Theoretical plates	
	Venlafaxine Hcl	impurity-F	Venlafaxine Hcl	impurity-F	Venlafaxine Hcl	impurity-F	Venlafaxine Hcl	impurity-F
1	7.97	32.69	4258669	27222	1.3	1.18	4300	12579
2	7.97	32.69	4256701	27121	1.3	1.17	4288	12614
3	7.97	32.69	4255988	27037	1.3	1.18	4282	12678
4	7.97	32.69	4256255	27069	1.3	1.18	4276	12689
5	7.97	32.69	4255740	27175	1.3	1.18	4275	12632
6	7.97	32.69	4256471	27268	1.3	1.17	4280	12589
Mean	7.97	32.69	4256650	27149	1.3	1.17	4284	12630
%RSD	------	-------	0.03	89.323	0.18	0.64	0.23	0.82

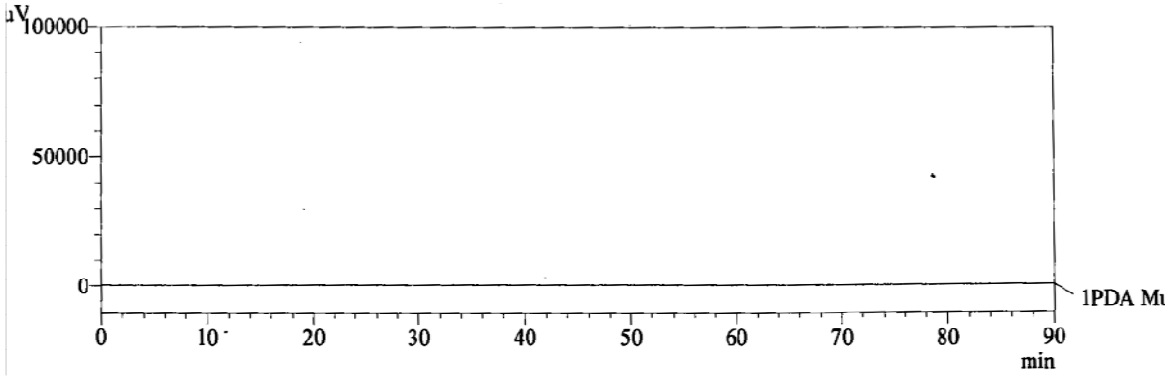

Figure: 7 Specificity chromatogram of blank solution

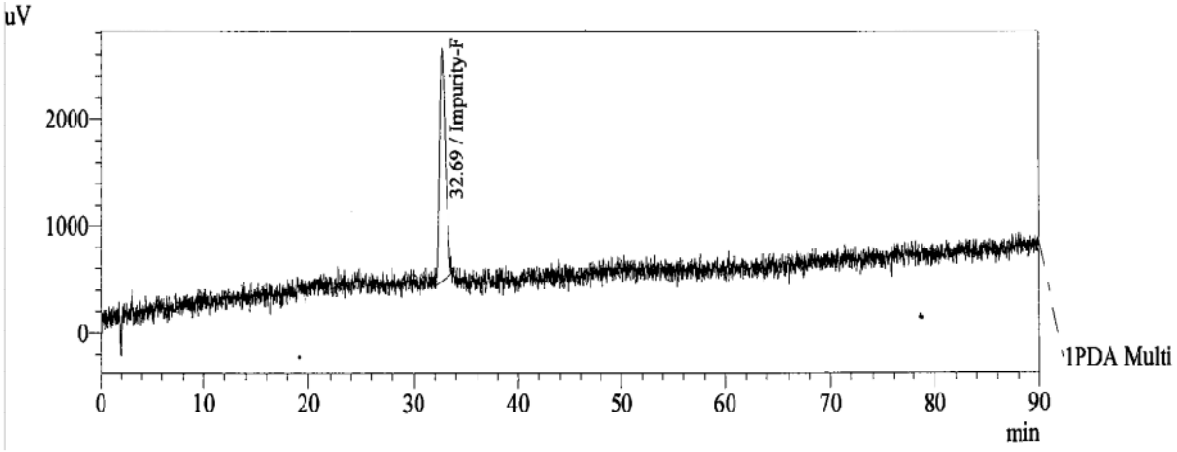

Figure: 8 Chromatogram for impurity-F solution

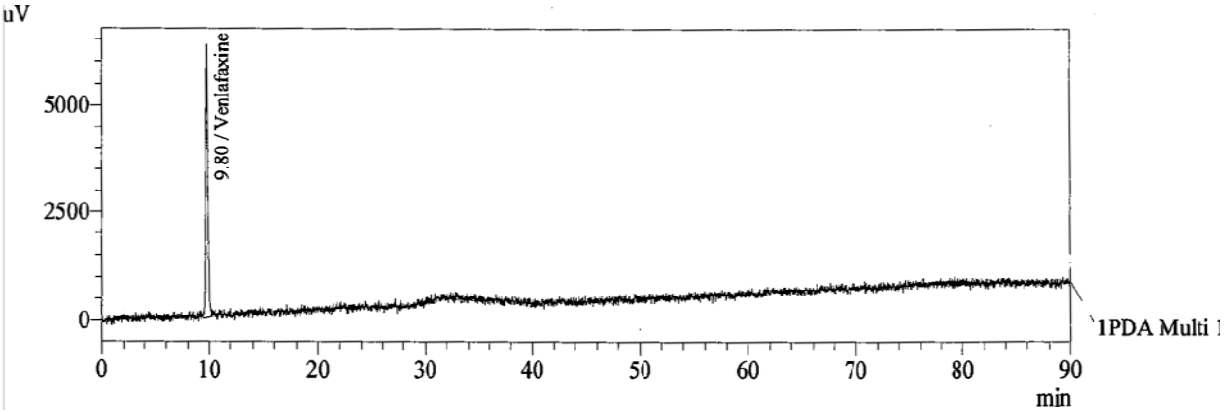

Figure: 9 Chromatogram for working standard solution of drug

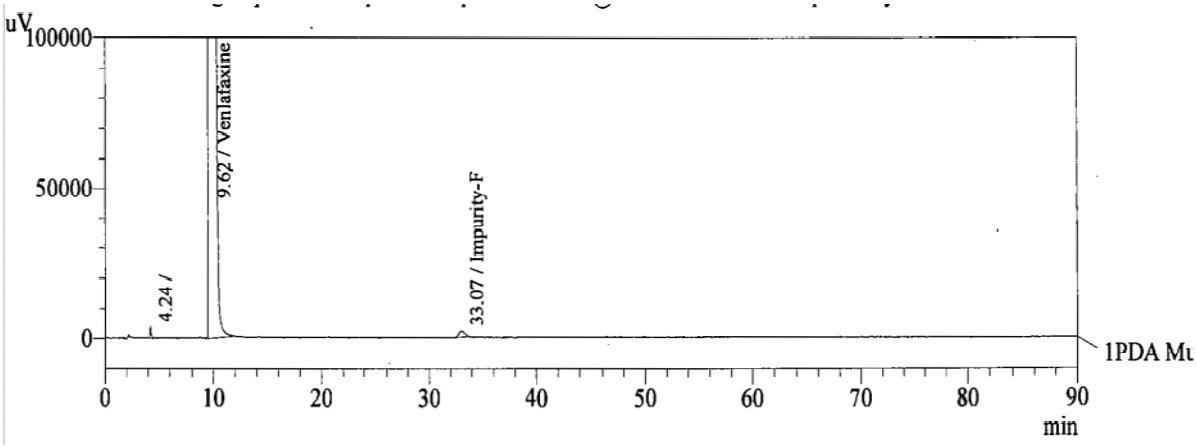

Figure: 10 Specificity chromatogram of Venlafaxine hydrochloride and impurity-F solution

Table 2: Linearity

Venlafaxine hydrochloride		Impurity-F	
Level	**Peak area**	**Level**	**Peak area**
80%	**1570991**	**20%**	**893**
90%	**1767996**	**40%**	**8625**
100%	**1959098**	**80%**	**13842**
110%	**2162998**	**100%**	**17194**
120%	**2396471**	**120%**	**25107**
Slope	**38704994.7**	**Slope**	**33314453.38**
Intercept	**12993.0**	**Intercept**	**189.91**
Correlation coefficient	**0.9999**	**Correlation coefficient**	**0.9997**
R^2	**0.9999**	**R^2**	**0.9994**

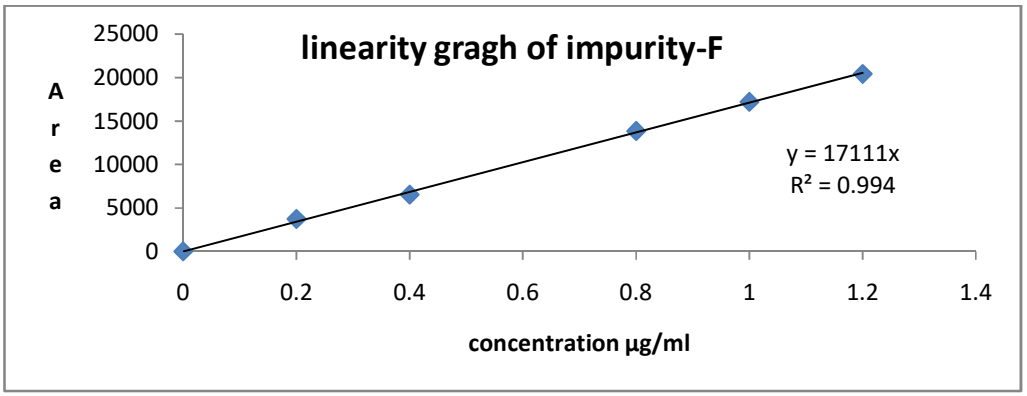

Figure: 11 Linearity of impurity-F

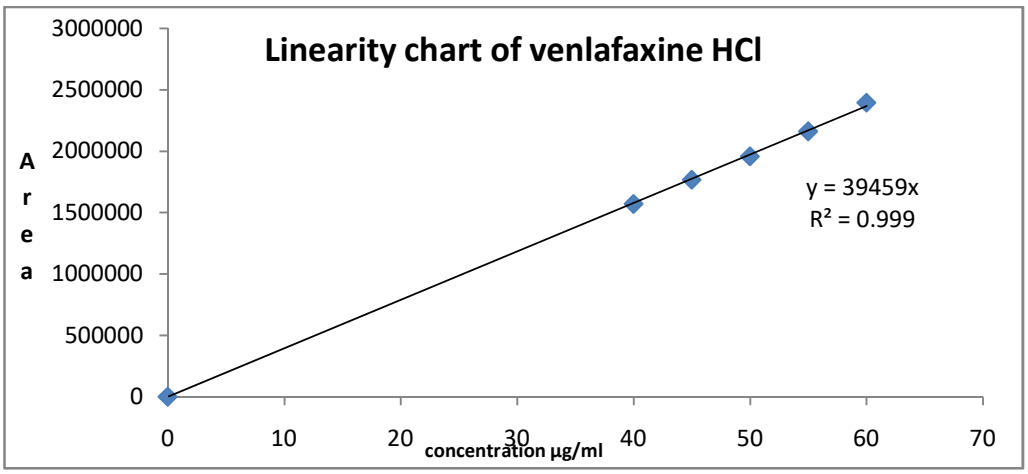

Figure: 12 Linearity of Venlafaxine HCl

Table 3: Accuracy

Level	Venlafaxine hydrochloride		Impurity-F	
	% Recovery	**%RSD**	**% Recovery**	**%RSD**
At 50%	**99.6**	**0.06**	**103.34**	**0.97**
	99.6		**105.26**	
	99.5		**104.86**	
At 100%	**100.2**	**0.06**	**103.10**	**0.82**
	100.1		**101.80**	
	100.2		**101.48**	
At 150%	**99.6**	**0.00**	**103.39**	**1.57**
	99.6		**100.21**	
	99.6		**101.27**	

Table: 4 Precision

No. of injections	Retention time (min)		Peak area		Tailing factor		Theoretical plates	
	Venlafaxine Hcl	impurity-F	Venlafaxine Hcl	impurity-F	Venlafaxine Hcl	impurity-F	Venlafaxine Hcl	impurity-F
1	7.97	32.69	2027834	82228	1.2	1.2	21469	86177
2	7.97	32.80	2052705	82492	1.2	1.2	21645	83928
3	7.97	32.69	2060510	82469	1.2	1.3	21672	84845
4	7.97	33.07	2065025	82108	1.2	1.2	21721	85234
5	7.97	32.69	2031696	82564	1.2	1.2	22736	86057
6	7.97	33.07	2041741	82383	1.2	1.1	21641	86730
Avg	7.97	32.83	2047554	82374	1.2	1.2	21849	85495
SD	------	-------	16881.80	173.90	-----	-----	--------	-------
%RSD	------	-------	0.8	0.2	-----	-----	--------	-------

Table 5: Robustness

Parameter condition	The resolution between Venlafaxine hydrochloride and impurity-F
Actual	4.5
High flow rate: 1.1 mL/min	4.9
Low flow rate: 0.9 mL/min	5.0
High oven temperature: 30ºC	5.0
Low oven temperature: 20ºC	4.9
High buffer variation: 67%	6.2
Low buffer variation: 63%	4.3
Buffer pH: 3.4	5.0
Buffer pH: 3.0	4.9

Table: 6 LOQ for analyte peak and its impurity

Name of the peak	%LOQ
Venlafaxine hydrochloride	0.006
Impurity-F	0.003

Table: 7 LOQ for area of Venlafaxine hydrochloride and impurity

No. of injections	Venlafaxine hydrochloride	Impurity-F
1	2838	1301
2	2882	1352
3	2601	1347
4	2660	1340
5	2856	1355
6	2733	1391
Mean	2762	1348
SD	115.05	28.94
%RSD	4.17	2.15

Table: 8 LOD for analyte peak and its impurity

Name of the peak	%LOD
Venlafaxine hydrochloride	0.0058
Impurity-F	0.0069

Table: 9 LOD for area of Venlafaxine hydrochloride and impurity-F

No. of injections	Venlafaxine hydrochloride	Impurity-F
1	1676	902
2	1674	899
3	1613	883
4	1612	864
5	1612	869
6	1600	893
Mean	1626	885
SD	28.07	15.81
%RSD	1.73	1.79

RESULTS AND DISCUSSION

The method was validated for specificity, linearity, accuracy, precision, limit of detection, limit of quantification, robustness and solution stability. Optimization of mobile phase was performed based on resolution, asymmetric factor and peak area obtained. Different mobile phases were tired but satisfactory separation, well resolved and good symmetry peaks were obtained with the mobile phase buffer and methanol (55:45). Calibration curve was prepared using concentrations in the range of 0.08µg/mL-0.12µg/mL the standard deviation of y–intercepts of regression line was determined and kept in the equation for the detection limit and quantification limit. Detection limit=3.3σ/s; Quantification limit=10σ/s, where σ is the standard deviation of y–intercepts of regression lines and s is the slope of the calibration curve. A graph was plotted against peak area verses concentration of Venlafaxine hydrochloride over the range of 0.08µg/mL-0.12µg/mL, slope and intercept value for calibration curve was y= 12993.0 and it was found to be linear over entire calibration range studied with r^2 value of 0.999.

The recovery studies were carried out three times over a specified concentration range and the amount of Venlafaxine hydrochloride and its impurity was estimated b measuring the peak ratios by fitting the values to the straight line equation of calibration curve. From the above results percentage recovery and standard deviation of percentage recovery was calculated. The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation.

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