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METHOD DEVELOPMENT AND VALIDATION FOR VENLAFAXINE BY RP-HPLC METHOD

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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,C18, 150 mm X 4.6 mm, 5µ column an ambient temperature of 30 ^{0} C and at a flow rate of 0.8 ml. / minute with isocratic elution was used. 20 µ L of the solution was injected and at 267nm 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 - 75 µ g with correlation coefficient of 1. The %of RSD in precision and accuracy was less than 2. The present method can be successfully used for the routine assay determination of Venlafaxine in pure form and pharmaceutical dosage forms.

Keywords: *Venlafaxine, RP-HPLC, Method development, Validation*

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INTRODUCTION:

Venlafaxine is indicated for the management of major depressive disorder (MDD), generalized anxiety disorder (GAD), social anxiety disorder (SAD), and panic disorder.[1] The exact mechanism of action of venlafaxine in the treatment of various psychiatric conditions has not been fully elucidated; however, it is understood that venlafaxine and its active metabolite O-desmethylvenlafaxine (ODV) potently and selectively inhibits the reuptake of both serotonin and norepinephrine at the presynaptic terminal.[2-3] This results in increased levels of neurotransmitters available at the synapse that can stimulate postsynaptic receptors. It is suggested that venlafaxine has a 30-fold selectivity for serotonin compared to norepinephrine: venlafaxine initially inhibits serotonin reuptake at low doses, and with higher doses, it inhibits norepinephrine reuptake in addition to serotonin. Venlafaxine and ODV are also weak inhibitors of dopamine reuptake. IUPAC name is 1-[2-(dimethylamino)-1-(4-methoxyphenyl) ethyl] cyclohexan-1-ol. Molecular formula $C_{17}H_{27}NO_2$. Molecular Weight is 277.4. Venlafaxine hydrochloride is a white to off-white crystalline solid with a solubility of 572 mg/mL in water (adjusted to ionic strength of 0.2 M with sodium chloride).

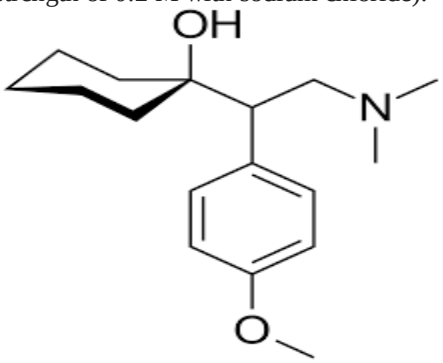

Figure 1: Structure of Venlafaxine

Various methods have been reported for estimation of venlafaxine hydrochloride in biological matrices such as plasma, which includes the use of LC with UV detection [4], LC with electrospray ionization mass spectrometry [5], LC with coulometric detection [6], LC with fluorimetric detection [7-8], LC with diode array detection [9-10], GC-MS [11], LC-MS-MS [12] and for estimation of it in serum by use of LC [13]. Stability indicating methods have also been reported for its invitro determination in gastric and intestinal fluids [14] and pharmaceutical formulations.[15]

Both the reported stability indicating methods uses acetonitrile and buffer in various proportions for quantification of venlafaxine hydrochloride. Present study involves development of RP-HPLC method using simple mobile phase for quantitative estimation of venlafaxine hydrochloride in tablet dosage forms which is sensitive and requires shorter analysis time. The developed method was validated as per ICH guidelines [16-17].

MATERIALS AND METHODS:

Chemicals and Reagents: Venlafaxine were Purchased from spansules pharmatech. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 250 nm with column INERTSIL column, C18(150x4.6 ID) 5µm, dimensions at Ambient temperature. The optimized mobile phase consists of Methanol: Acetonitrile: water (30:50:20 v/v/v). Flow rate was maintained at 1 ml/min.

Preparation of solutions:

Preparation of buffer:

The buffer solution was prepared by dissolving accurately weighed 2.95gms of potassium dihydrogen orthophosphate and 0.58gms of potassium hydrogen orthophosphate were dissolved in 1000ml. of distilled water and sonicated to dissolve.

Preparation of mobile phase:

The Mobile Phase was prepared by mixing 2.95gms of potassium dihydrogen orthophosphate and 0.58gms of potassium hydrogen orthophosphate were dissolved in 1000ml. of distilled water and sonicated to dissolve. Then the resultant solution was filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

Mobile phase is used as Diluent.

Preparation of the individual standard preparation: 50 mg of working standard was accurately weighed and transferred into a 100 ml clean dry volumetric flask and about 70 ml diluent is added. Then it is sonicated to dissolve it completely and made volume up to the mark with the diluent. (Stock solution). Further 1.5 ml from the above stock solution is pipette into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of Sample Solution :(Tablet)

The sample solution was prepared by Nearly 10 tablets of venlafaxine were taken and grind to powder in mortar and pestle 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

Procedure:

20µL of the standard, sample are injected into the chromatographic system and the areas for peaks are measured and the %Assay are calculated by using the formulae.

METHOD:

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 30 minutes to equilibrate the column at ambient temperature. The overlay spectrum of venlafaxine was obtained and the venlafaxine showed absorbance's maxima at 267 nm. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into INERTSIL column, C18(150x4.6 ID) 5µm column, the mobile phase of composition Methanol: Acetonitrile: water (30:50:20 v/v/v) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Venlafaxine in tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method:

Linearity: The linearity study was performed for the concentration of 0 ppm to 60 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 75%, 100%,125%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Venlafaxine and calculate the individual recovery and mean recovery values. The results are shown in table 4.

Precision Studies: precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 5.

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 6.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition was made to evaluate the impact on the method. The results are shown in table 7.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 8.

$LOD = 3.3\sigma/S$ and

$LOQ = 10\ \sigma/S$, where

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

RESULTS AND DISCUSSION:

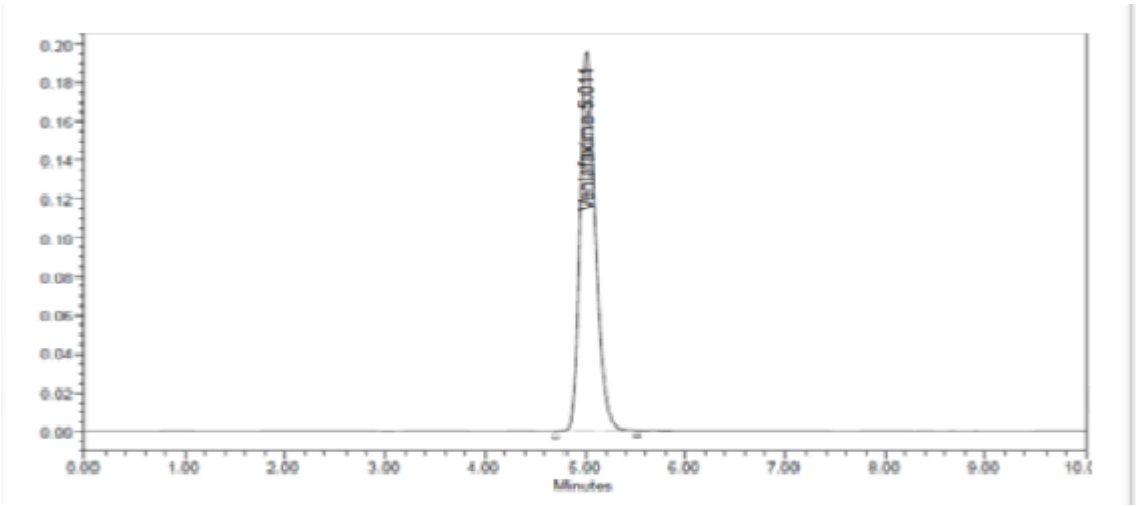

Figure 2: Standard chromatogram

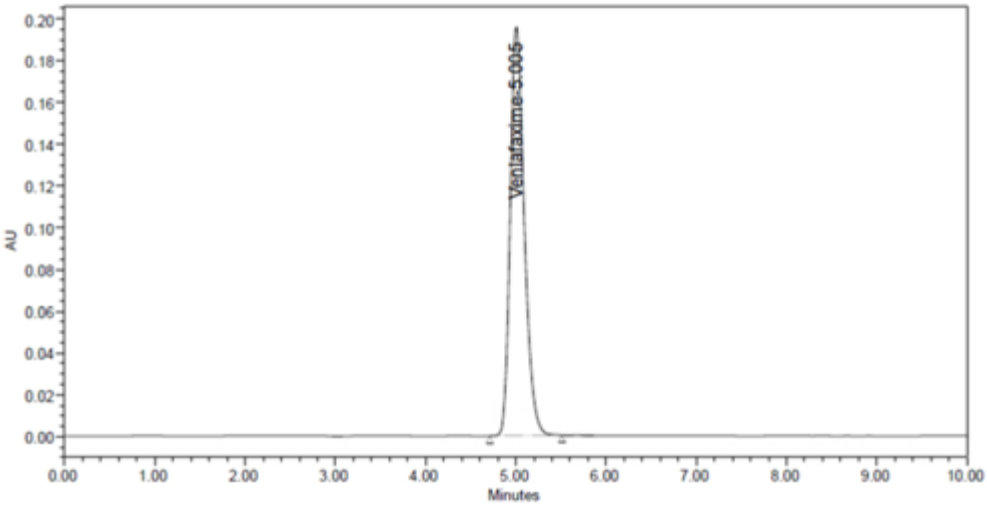

Figure 3: Sample chromatogram

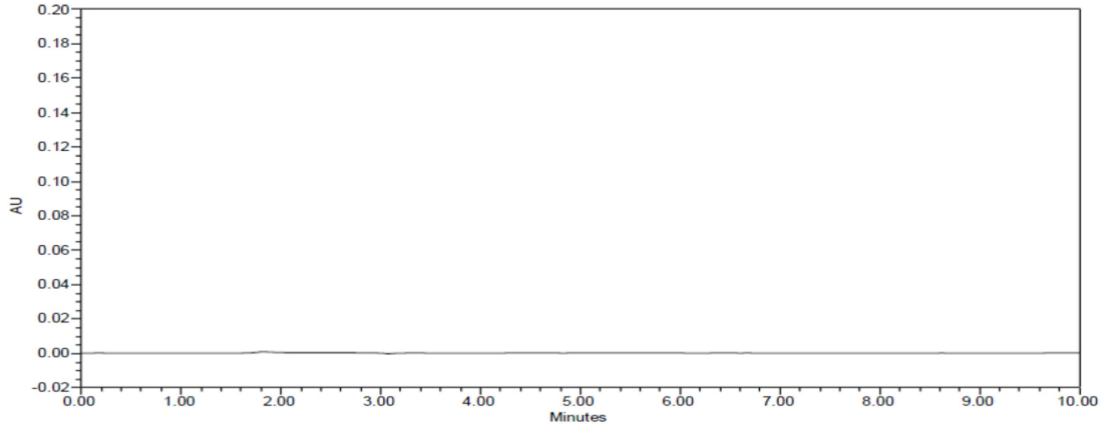

Figure 4: Blank chromatogram

Table 1: System suitability parameters

	Peak Name	RT	Area	%Area	Height	USP Plate count	USP Tailing
1	Venlafaxine	5.005	211554	100	196125	4952	1.15

Table 2: Assay results for Venlafaxine

Sl.No	Dosage in mg	Found	% Assay
1	25	24.915	99.65
2	37.5	37.421	99.74
3	50	50.06	100.11
4	75	75.54	100.73
5	100	100.31	100.31

Table 3: Linearity results of Venlafaxine

Conc (mcg)	Area
12.5	541656
25	1067131
37.5	1587197
50	2116076
62.5	2639479
75	3166192

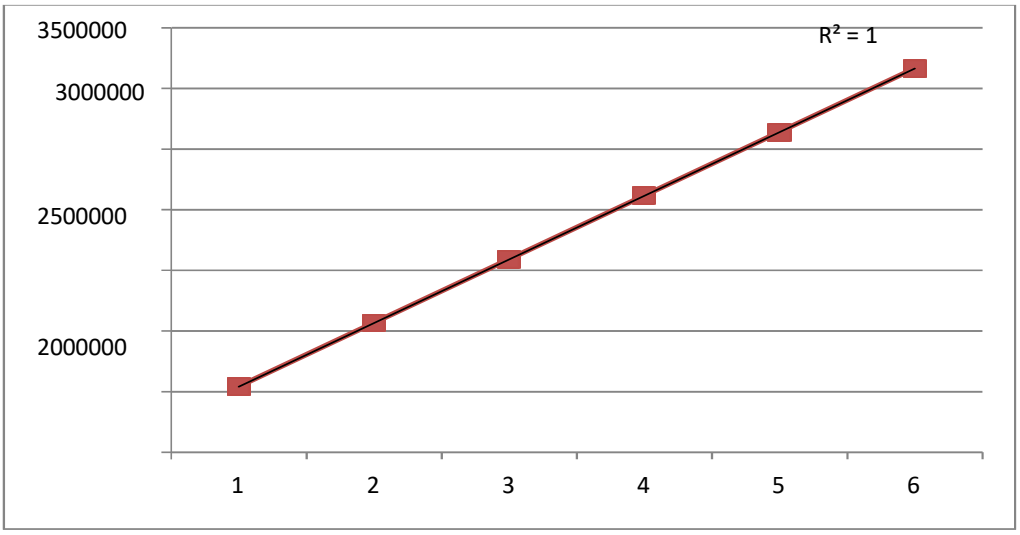

Figure 5: Linearity graph for Venlafaxine

Table 4: Showing accuracy results for Venlafaxine

S.No	Level%	Amount added(mg)	Amount Recovered(mg)	%Recovery
1	80	40	39.69	99.20
2	100	50	49.83	99.61
3	120	60	59.77	99.63

Table 5: Precision results for Venlafaxine

S No	Venlafaxine	
	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	4813.563
RSD	0.175	0.228

Table 6. Ruggedness results of Venlafaxine

S No	Venlafaxine	
	RT	Area
1	5.01	2128251
2	5.015	2130214
3	5.016	2125452
4	5.018	2124078
5	5.008	2126265
6	5.01	2118975
Avg	5.013	2125539
Std Dev	0.004	3872.101
RSD	0.08	0.181

Robustness results

Table 7: Flow variation results for Venlafaxine

		Venlafaxine	
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

Table 8: LOD, LOQ of Venlafaxine

Drug	LOD	LOQ
Venlafaxine	61.74	187.03

CONCLUSION:

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the estimation of Venlafaxine in its pure form and in its pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Venlafaxine in pure and its pharmaceutical dosage forms.

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