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Novel RP-HPLC Method Development and Validation for Estimation of Venlafaxinein Bulk Dosage Form

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ABSTRACT:

A simple and selective LC method is described for the determination of Venlafaxine dosage forms. Chromatographic separation was achieved on a c18 column using mobile phase consisting of a mixture of 0.02M phosphate buffer: ACN (60:40v/v), with detection of 226 nm. Linearity was observed in the range 50-150 µg /ml for Hydrochlorothiazide (r2 =0.999) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form

Key words:

1. Introduction:

Venlafaxine hydrochloride (VEN) is a phenethylamine bicyclic derivative, chemically known as 1-[2 -(dimethylamino)-1-(4- methoxy-phenyl) ethyl] cyclohexanol hydrochloride.

It is a novel, non-tricyclic antidepressant, and the mechanism of action in humans is believed to be associated with potential of neurotransmitter activity in the central nervous system. Preclinical studies have shown that VEN is a potent inhibitor of neuronal serotonin and norepinephrine reuptake and a weak inhibitor of dopamine reuptake, but in contrast to other tricyclic amines (TCAs), it does not interact with cholinergic, adrenergic or histaminergic receptors. It is white to off-white crystalline solid, soluble in water, with Molecular formula $C_{17}H_{28}C_lNO_2$, and is of 313.866 g/mol weight. The chemical structure of Venlafaxine hydrochloride is shown in Fig. 1.

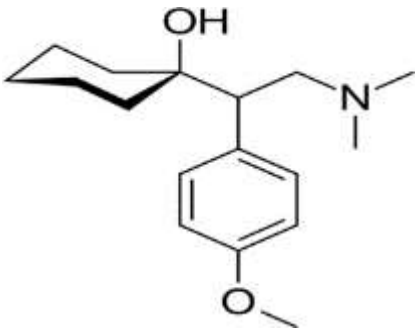


Figure.1. Structure of Venlafaxine

Literature Review reveals that few methods were reported for the determination of impurities in Venlafaxine API. The Aim of present work is to develop a simple, specific, rapid and sensitive analytical method for the quantification of venlafaxine hydrochloride. The present method is fast, accurate as compared to already existing methods. This work followed the validation as per the ICH guidelines to develop an analytical method with acceptable characteristics of suitability, reliability and feasibility.

2. MATERIALS AND METHODS

2.1. Standard:

Venlafaxine

2.2. Reagents and Chemicals:

Water, Methanol, Acetonitrile (HPLC grade) and Potassium Dihydrogen ortho Phosphate, Ammonium acetate buffer, Sodium dihydrogen phosphate (AR Grade).

2.3 .Instrumentations:

UV-Visible Spectrophotometer(Nicolet evolution 100), HPLC(Shimadzu(LC 20 AT VP)), HPLC(Agilent 1200 series), Ultra sonicator(Citizen, Digital Ultrasonic Cleaner), pH meter(Global digital), Electronic balance(Shimadzu), Syringe(Hamilton), HPL Column(INERTSILcolumn, C18(150x4.6 ID) 5µm)

3.Methodology:

3.1. Mobile Phase preparation:

A mixture of 60 volumes of 0.02M phosphate buffer (pH.5): and 40 volumes of Acetonitrile were prepared. The mobile phase was sonicated for 10min to remove gases.

3.2. Preparation of Venlafaxine standard solution:

Weigh accurately 25mg of Venlafaxine in 25ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 100µg/ml of Venlafaxine is prepared by diluting 1ml of Venlafaxine to 10ml with mobile phase. This solution is used for recording chromatogram.

3.3. Preparation of sample solution:

10 tablets (each tablet contains 25mg of Venlafaxine) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of 100µg/ml were prepared by dissolving weight equivalent to 50mg of Venlafaxine dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 50ml with mobile phase. Further dilutions are prepared in 5 replicates of 100µg/ml of Venlafaxine was made by adding 5ml of stock solution to 50 ml of mobile phase.

4. Results and Discussion:

4.1. Solubility Studies

These studies are carried out at 25^0C

Venlafaxine: It is freely soluble in water, soluble in acetonitrile, soluble in methanol, and sparingly soluble in ethanol.

Preparation of standard stock solution of Venlafaxine:25mg of Venlafaxine was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 0.4ml to 10ml with methanol.

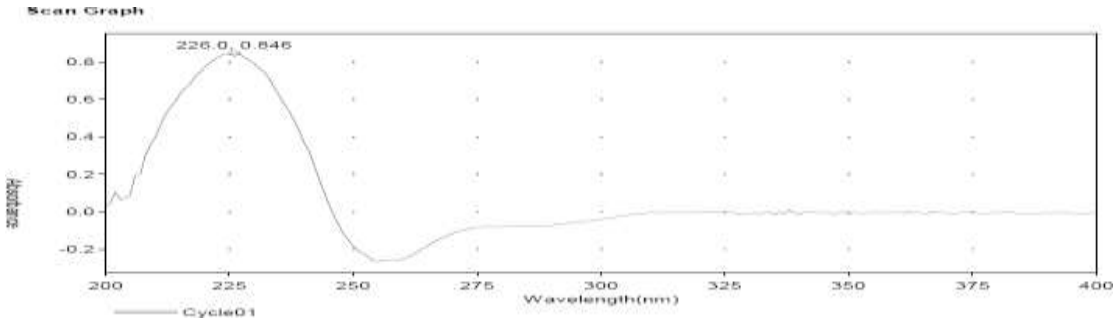


Figure.1. UV-VIS spectrum of Venlafaxine

Discussion: The wavelength of maximum absorption (λmax) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in the fig. no.1 and the absorption curve shows characteristic absorption maxima at 226nm for Venlafaxine.

4.2. Optimization of Chromatographic method:

According to the information collected from literature there is few methods reported for determination of Venlafaxine HCl using HPLC .By following pharmacopoeia the column, temperature, wavelength, flow rate and mobile phase composition with acetonitrile: buffer is selected for initial trail was done. Based on the trails observation of peak shape and area obtained the method was optimized. The optimized chromatographic condition was INERTSILcolumn,C18(150x4.6 ID) 5µm with temperature at 30oCusing mobile phase consisting of a mixture of 0.02M phosphate buffer: ACN (60:40v/v), injection volume is 10ul with detection of 226 nm.

4.3. Method validation:

Validation of proposed analytical method involves linearity and range, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness study. It was validated according to ICH Q2 (R1) guideline.

A. System Suitability:

System suitability is an integral part of chromatographic system. The calculation and comparison of verified resolution, capacity factor, tailing factor, and theoretical plate count with standard specification of system. Equilibrate the column with the mobile phase for 45 min before analysis. The chromatographic system should satisfy the system suitability limits before analysing sample. Tailing factor (T), theoretical plate number (N) and resolution (Rs) for standard Venlafaxine and standard impurity A were tested.

Column Description	**Name of Component**	**Evaluation parameters**				
		Avg. Area	**% RSD**	**Retention time (tr)**	**Tailing factor (Tf)**	**Plate count (N)**
INERTSIL column, C 18(150x4.6 ID) 5µm	Venlafaxine hcl	2833.862	0.4	3.317min	0.8	8738

Acceptance Criteria: When calculating the peak area of Venlafaxine using six injections of the standard solution, the % RSD should not exceed 0.85. For Venlafaxine spikes, the USP tail factor should not be greater than 2.0.Column efficiency: More than 2000 theoretical plates are required on top of the Venlafaxine column.

Conclusion: System suitability parameters were acceptable and met the criteria.

B. Specificity / Placebo Interference:

It is necessary to demonstrate that dissolution results are not affected by placebo constituents, other active ingredients in the drug product. Placebo interference was evaluated by weighing samples of placebo blend and dissolving or dispersing it into the dissolution medium at concentrations that would normally be encountered during testing. The chromatograms of blank, placebo, test sample and standard are used to justify the specificity of target analyte.

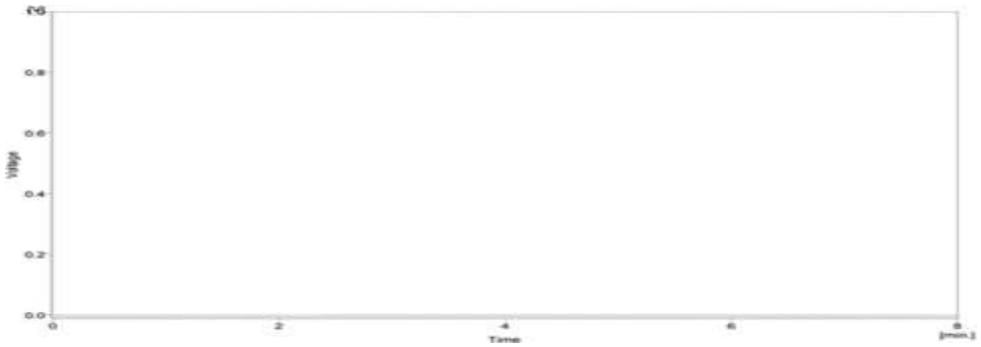


Figure.2. Chromatogram of blank

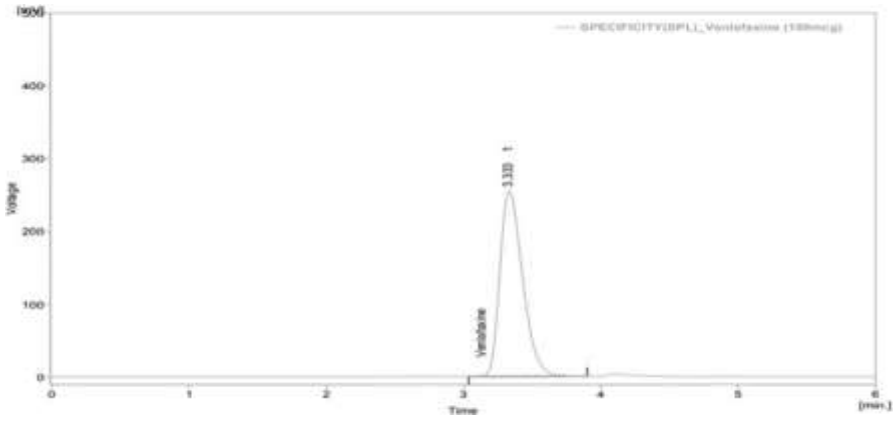
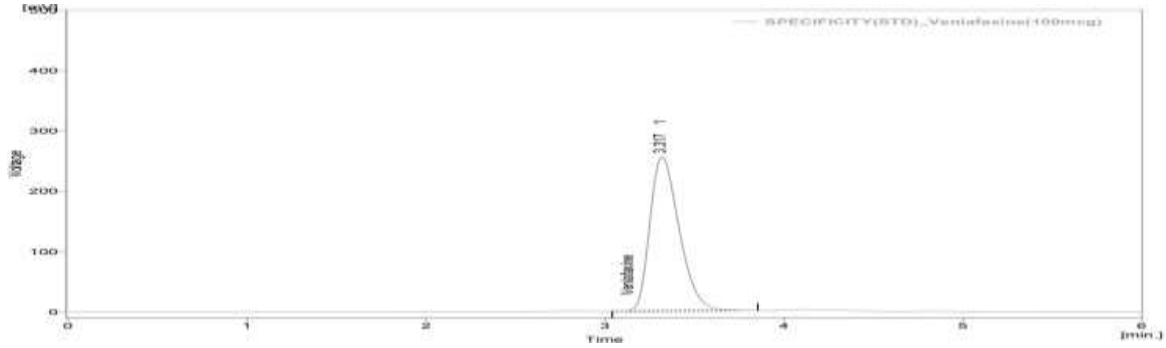


Figure.3. Chromatogram of standard

Figure.4.



Chromatogram of sample

Conclusion: No interference was found in the blank at the retention time of Venlafaxine. No interference was found in the Placebo at the retention time of Venlafaxine

C. Linearity:

Weigh accurately 50mg of Venlafaxine in 100 ml of volumetric flask and from this, 5ml dissolve in 10ml of mobile phase and make up the volume with mobile phase.

Measurements using clean standard preparations were performed to demonstrate method linearity. The solutions for different levels of drug (50% to 150% of target concentration of Venlafaxine standard) comprising of 5 levels were prepared and analyzed and the average peak areas were plotted against the concentration to determine the concentration range with linearity. The data is given below:

Table.2. Linearity of Venlafaxine

S. No.	Conc. (µg/ml)	Area
1	50	1638.768
2	75	2197.919
3	100	2882.593
4	125	3550.790
5	150	4155.542

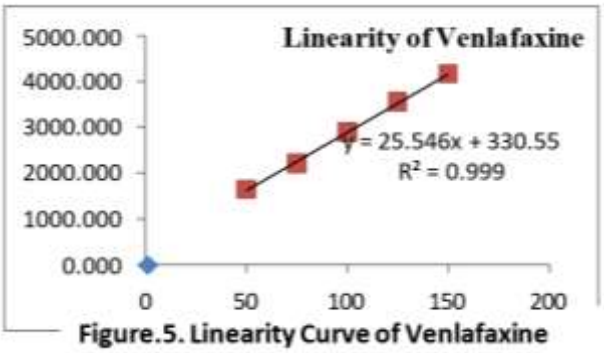


Figure.5. Linearity Curve of Venlafaxine

Acceptance criteria: Correlation coefficient should be not less than 0.999

Observation: The correlation coefficient for linear curve obtained between concentrations vs. Area for standard preparations of Venlafaxine is 0.999. The relationship between the concentration of Venlafaxine and area of Venlafaxine is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

Conclusion: Correlation coefficient between Venlafaxine concentration and peak area was calculated by linear regression and was found to be within the acceptance criteria.

D. Accuracy:

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100% & 150%.

Table.3. Recovery results for Venlafaxine

Recovery level	**Accuracy Venlafaxine**					**Average % Recovery**
	Amount taken(mcg/ml)	**Area**	**Average area**	**Amount recovered(mcg/ml)**	**% Recovery**	
50%	50	2069.772	2060.856	74.69	99.58	99.89
	50	2054.232				
	50	2058.565				
100%	100	2882.593	2871.680	99.10	99.10	
	100	2875.659				
	100	2856.789				
150%	150	3535.008	3502.665	126.26	101.01	
	150	3421.987				
	150	3551.001				

Acceptance criteria: The % recovery of Venlafaxine should lie between 98% and 102%.

Observation: The percentage mean recovery of Venlafaxine is 99.89%.

Conclusion: Recovery for analyte was within acceptance criteria of NLT 95% and NMT 105 %.

E. Precision:

Precision of the method refers to the reproducibility of value on repeated measurements. Six samples were injected and RSD of the values is estimated to understand the precision of the method.

Procedure for Sample preparation: Pipetted 2 mL of the above solution to 20 mL volumetric flask and made up the volume with diluent. Filtered the samples through 0.45µm nylon filter (Make: Chromsource), discarded about first 2 mL of filtrate. Prepared sample preparations of Venlafaxine as per test method and injected 5 times in to the column.

Table 4. Results for Method precision of Venlafaxine

Venlafaxine		
S.No.	**Rt**	**Area**
1	3.367	2912.901
2	3.367	2932.566
3	3.353	2946.873
4	3.333	2920.975
5	3.717	2911.577
avg	3.3474	2924.978
stdev	0.0220	14.819
%RSD	**0.66**	**0.51**

Acceptance criteria: The % Relative standard deviation of Assay preparations of Venlafaxine should be not more than 2.0%.

Observation: Test results for Venlafaxine are showing that the %RSD of Assay results are within limits. The results were shown in table

Conclusion: Precision data at Q point time was within acceptance criteria of NLT 80% and RSD below 10%.

F. Robustness: To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like Temperature and wavelength. System suitability parameters were compared with that of method precision.

Table 5. Result of Robustness study

Parameter	Changes	Retention time(min)	Tailing factor
Flow Rate	**0.8ml/min**	4.187	1.717
	1.0 ml/min	3.367	1.632
	1.2ml/min	2.830	1.656
Wavelength	**224nm**	3.353	1.605
	226nm 228nm	3.367	1.632
		3.353	1.605

Acceptance criteria: The system suitability should pass as per the test method at variable conditions.

Observation: From the observation it was found that the system suitability parameters were within limit at all variable conditions.

G. Ruggedness: The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts

Table.6. Results for Ruggedness

Venlafaxine	%Assay
Analyst 01	99.67
Analyst 02	98.34
%RSD	0.94%

Acceptance criteria: The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%.

Observation: From the observation the %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged.

5. Conclusion:

The gradient RP-HPLC method developed for determination of Venlafaxine HCl in pharamceutical dosage form. The developed method is validated with results of precise, accurate and specific. Chromatographic separation was achieved on a c18 column using mobile phase consisting of a mixture of 0.02M phosphate buffer: ACN (60:40v/v), with detection of 226 nm. Linearity was observed in the range 50-150 µg /ml for Hydrochlorothiazide (r2 =0.999) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2.The method can be used for routine analysis.

6. Acknowledgement:

1. Dr. M. Ajitha, Head of Department, Centre for Pharmaceutical Sciences, UCEST, JNTUH Hyderabad

7. References:

1. Swartz ME and Krull IS: Handbook of analytical validation, HPLC method development and optimization with validation in mind, edition 1st, 2012; 38-59.

2. Maryam hosseini application of uv-spectrophotometry and HPLC for determination of venlafaxine and its four related substances in pharmaceutical dosage forms. turk J Pharm Sci 2011; 8(2): 91- 04.

3. Kaur J, Srinivasan KK, Joseph A, Gupta A, Singh Y, Srinivas KS and Jain G: Development and validation of stability indicating method for the quantitative determination of venlafaxine hydrochloride in extended release formulation using high performance liquid chromatography. J Pharm Bioall Sci 2010; 2: 22-6.

4. Larissa SB, Paulo O, Fabio M, Borgmann HM, Silvia, Marcela ZA and Simone GC: Development and validation of a stability-indicating LC method for the determination of venlafaxine in extended-release capsules and dissolution kinetic studies, Article in Journal of Chromatographic Science 2009; 47(9): 770-6.

5. Vanita S, Dannana G and Shivakumar N: Development and validation of a rapid RP-HPLC method for the determination of venlafaxine hydrochloride in pharmaceutical dosage forms using experimental design, EJournal of Chemistry 2009; 6(4): 1091-02.

6. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for M.A. Gutierrez, G.L. Stimmel, J.Y. Aiso, Venlafaxine: a 2003 update, Clin. Ther. 25 (2003) 2138–2154.

7. A.T. Harvey, R.L. Rudolph, S.H. Preskorn, Evidence of the dual mechanisms of action of venlafaxine, Arch. Gen. Psychiatry 57 (2000) 503–509.

8. ICH European Medicines Agency, Q2(R1) Validation Of Analytical Procedure, cpmp/ich/381/95, http://www.emea. eu.int., June 1995; 2-15.

9. ICH Guidance for Industry Q3C Impurities: residual solvents, Center For Drug Evaluation And Research (CDER), published in Federal Register on 1997; 1-3.

10. J.M. Andrews, P.T. Ninan, and C.B. Nemeroff. Venlafaxine: a novel antidepressant that has a dual mechanism of action. Depression. 4: 48–56 (1996).

11. M.A. Gutierrez, G.L. Stimmel, and J.Y. Aiso. Venlafaxine: A 2003 Update. Clin. Ther. 25: 2138– 2154 (2003).

12. Effexor XR (venlafaxine hydrochloride) extended release capsules. (2008) Available at: http://www.wyeth.com/content/ showlabeling.asp?id=100.