Research Article

Analytical Method Validation for the Determination and Quantification of Venlafaxine Hydrochloride and Its Stress Degradation Studies

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ABSTRACT

The present study describes a simple, accurate, precise and cost effective UV-Visible Spectrophotometric method for the estimation of Venlafaxine Hydrochloride. The absorbance values were observed for different dilutions of drug at 225nm and which were used for the estimation and quantification of the drug. The solvents used for the dilution was Distilled water, 0.1 N Hydrochloric acid 1.2 pH and Phosphate buffer 6.8 pH. The method obeys Beer's Lambert's law in the selected concentration range respectively in different media. The parameters like linearity, precision, accuracy, specificity, robustness, limit of detection and limit of quantitation were studied according to International Conference on Harmonization guidelines. The degradation studies of Venlafaxine Hydrochloride was studied for the purpose of stability indicating method and demonstrate specificity of method for Venlafaxine Hydrochloride in presence of potentially interfering materials that could occur and cause interference. Thus it helps to establish stability characteristics and to support the suitability of the proposed analytical method. The results of analysis have been validated statistically and the recovery studies confirmed the accuracy of this proposed method.

Keywords: UV-Vis Spectrophotometer, Method development, Venlafaxine hydrochloride.

INTRODUCTION

Venlafaxine Hydrochloride, chemically (R/S)-1-[2-(dimethylamino)-1- (4-methoxyphenyl) ethyl] cyclohexanol hydrochloride (chemical structure as in figure 1) [1], a suicidality and antidepressant drug [2]. Venlafaxine Hydrochloride acts by a selectively inhibiting reuptake of serotonin- and norepinephrine-reuptake [3].Generally Venlafaxine Hydrochloride is prescribed in Major Depressive Disorder (MDD) and other psychiatric disorders[4]. It is official in European Pharmacopoeia[5]. Few analytical methods have been reported for its quantitative estimation in pharmaceutical formulations, which includes few HPLC methods [6, 7, 8] and spectrophotometric methods [9, 10]. The drug has following chemical structure as shown in figure 1.

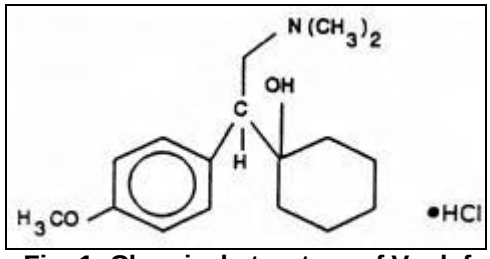

Fig. 1: Chemical structure of Venlafaxine Hydrochloride

The present work reports the development and validation of a UV-Visible Spectrophotometric method for the estimation of Venlafaxine Hydrochloride accurately and its degradation studies. The method can be used for the routine Q. C. analysis and quantification of the drug in the formulations.

MATERIAL AND METHOD

Venlafaxine Hydrochloride was kindly supplied as a gift sample by Sun Pharmaceuticals Pvt. Ltd. (Mumbai),

Analytical grade Sodium Hydroxide, Hydrochloric Acid, Potassium Dihydrogen Phosphate were procured from RFCL Ltd. (New Delhi), Analytical grade Potassium Chloride was procured from Loba Chemie Laboratory and Fine Chemicals (Mumbai).

Determination of solubility

A double-beam Shimadzu UV–Vis Spectrophotometer, model UV-1601-220V automatic wavelength accuracy of 0.1 nm and matched quartz cells of 10 mm path length were used, REPTECH E.M.F.C. Technology based digital balance (RA/RS-Series) was used for weighing the samples, Class-A volumetric glass wares were used for the preparation of the samples.

Method development

Reported solubility of Venlafaxine Hydrochloride is 572 mg/ml in water [11]. Solubility of the drug Venlafaxine Hydrochloride was determined in various solvents like distilled water, methanol, ethanol, acetonitrile, 0.1N Hydrochloric acid 1.2 pH, and Phosphate buffer 6.8 pH. The drug is freely soluble in distilled water, 0.1N Hydrochloric acid 1.2 pH, Phosphate buffer 6.8 pH hence were chosen as a solvent for developing the method.

Determination of λ max

Preparation of Stock Solution

About 100 mg of Venlafaxine Hydrochloride was weighed accurately and dissolved separately and diluted to 100 ml with distilled water, 0.1N Hydrochloric acid 1.2 pH, and Phosphate buffer 6.8 pH respectively and were used as working standards. From the above three working standards 10 ml solutions were diluted to 100 ml with distilled water to produce a concentration of 100µg/ml, and used as standard stock solution.

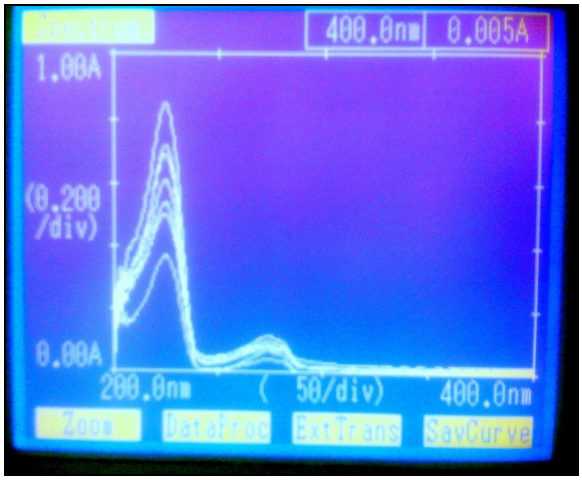

Fig. 2: UV Spectrum of Venlafaxine hydrochloride (λmax determination)

Preparation of Working Standard Solution

From the above stock solutions, 1.0 ml of the solution from each stock solution was diluted to 100 ml with distilled water to produce a concentration of 10µg/ml. The solution was scanned in UV-Visible Spectrophotometer in the range 400-200 nm using distilled water as a blank. The wavelength corresponding to maximum absorbance (λmax) was found at 225 nm (**Figure2**).

Method Validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics[12]. The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. Typical validation characteristics which should be considered are listed below:

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present.

Accuracy
The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

Precision
The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Detection Limit
The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Quantitation Limit
The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

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

Range
The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.
The method was validated for different parameters like linearity, accuracy, precision, range, specificity, limit of detection (LOD) and limit of quantification (LOQ).

Linearity and Range
Preparation of calibration curve in distilled water
The calibration curve was prepared from standard stock solution in distilled water and diluted to produce working standards of concentrations 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml, 12µg/ml solutions. The calibration curve was constructed by taking the solution concentrations ranged from 2-12µg/ml. The absorbance values were plotted against concentration. (**Figure3, Table1**).

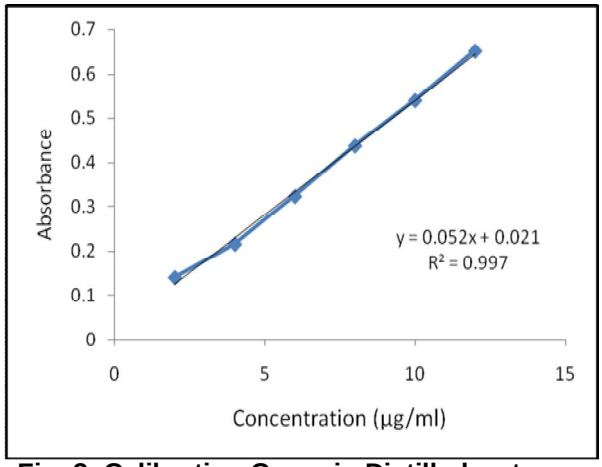

Fig. 3: Calibration Curve in Distilled water

Preparation of calibration curve in 0.1n hydrochloric acid 1.2pH
The calibration curve was prepared from standard stock solution in 0.1N Hydrochloride acid 1.2 pH and diluted to produce working standards of concentrations 8µg/ml 10µg/ml, 12µg/ml, 14µg/ml, 16µg/ml, 18µg/ml solutions. The calibration curve was constructed by taking the solution concentrations ranged from 8-18µg/ml. The absorbance values were plotted against concentration. (**Figure4, Table1**).

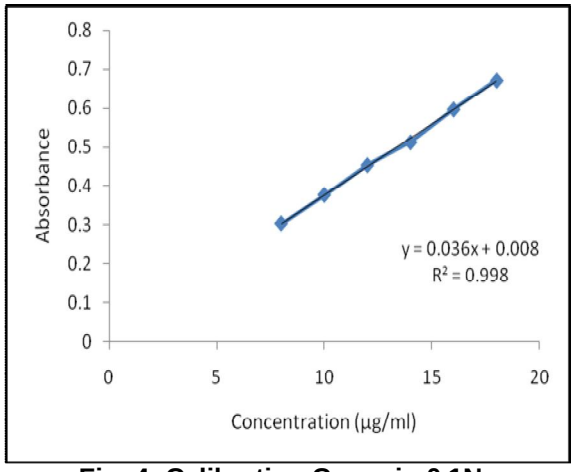

Fig. 4: Calibration Curve in 0.1N Hydrochloric acid 1.2 pH

Preparation of calibration curve in phosphate buffer 6.8 pH

The calibration curve was prepared from standard stock solution in Phosphate buffer 6.8pH and diluted to produce 10µg/ml, 12µg/ml, 14µg/ml, 16µg/ml, 18µg/ml, 20µg/ml, 22µg/ml solutions respectively. The calibration curve was constructed by taking the solution concentrations ranged from 10-22µg/ml. The absorbance values were plotted against concentration. (**Figure5, Table1**).

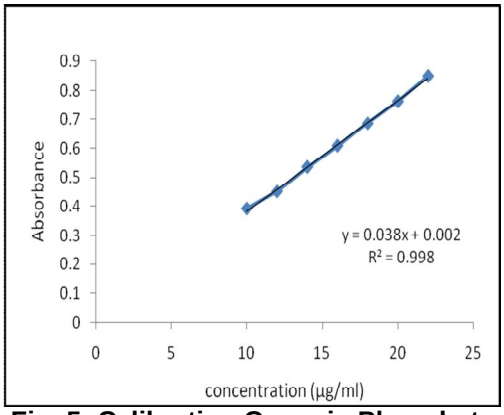

Fig. 5: Calibration Curve in Phosphate buffer 6.8 pH

Table 1: Linearity

Distilled Water		0.1N Hydrochloric acid 1.2 pH		Phosphate buffer 6.8 pH	
Concentartion (µg/ml)	Absorbance	Concentartion (µg/ml)	Absorbance	Concentartion (µg/ml)	Absorbance
2	0.141	8	0.303	10	0.393
4	0.216	10	0.376	12	0.453
6	0.323	12	0.453	14	0.537
8	0.438	14	0.512	16	0.609
10	0.541	16	0.597	18	0.686
12	0.651	18	0.672	20	0.763
				22	0.849

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ for Venlafaxine Hydrochloride by the proposed method were determined using calibration standards. To determine the LOD and LOQ, a specific calibration curve were studied using samples containing the analyte in the range of detection limit (DL) and quantitation limit (QL).

LOD = 3.3 σ/S and

LOQ = 10 σ/S

Where, σ = the standard deviation of the response

S = slope of the calibration curve

Accuracy (Recovery Test)

The accuracy was determined by recovery of known amounts of Venlafaxine Hydrochloride reference standard added to the samples at the beginning of the process. An accurately weighed 20 mg of Venlafaxine Hydrochloride was transferred to 200 ml volumetric flask and dissolved in 0.1N Hydrochloric acid 1.2 pH (100µg/ml). Aliquots of 2.0 ml of this solution were transferred into 20 ml volumetric flasks containing 1.0, 2.0 and 3.0 ml of Venlafaxine Hydrochloride standard solution (100 µg/ml) and 0.1N Hydrochloric acid 1.2 pH was added to make up to volume to give a final

concentrations of 15, 20 and 25 µg/ml. All solutions were prepared in triplicate and assayed. The percentage recovery of added Venlafaxine Hydrochloride standard was calculated using the equation proposed by AOAC[13].
Similar procedure was repeated using Phosphate buffer 6.8 pH and distilled water as solvents to prepare solutions and dilutions.

Method Reproducibility (Precision)
The system precision is the measure of method variability, by measuring the absorbance of five replicates of the same working solution. The percent relative standard deviation should be less than 2. Precision of the method was demonstrated by intra-day and inter-day variation studies. In intra-day variation study three different solutions of three different concentration, 12µg/ml, 14µg/ml, 16µg/ml, were analyzed for three times in a day i.e. zero hours, three hours and six hours and the absorbance was measured. %RSD was calculated from the absorbance values. In the inter-day precision, solution of three different concentration, 12µg/ml, 14µg/ml, 16µg/ml, was analyzed on three different days.

Degradation Studies
The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and drug products requires stress testing to elucidate the inherent stability characteristics of the active pharmaceutical ingredients. The degradation studies of Venlafaxine Hydrochloride was carried out for the purpose of stability indicating method and demonstrate specificity of method for Venlafaxine Hydrochloride in presence of potentially interfering materials that could occur and cause interference. Thus it helps to establish stability characteristics and to support the suitability of the proposed analytical method. The purpose of the work was to perform the stress degradation studies of Venlafaxine Hydrochloride
(**Table 6**) [12]

Acid Degradation (Hydrolytic Degradation under Acidic Condition)
To 1 ml of stock solution (1000 µg/ml) of Venlafaxine Hydrochloride, 1 ml of 0.1N HCl was added in 100 ml of volumetric flask, the volume was made up with distilled water, the solution was kept at room temperature for the period of 90 minutes. 10 ml of solution was neutralized and diluted with distilled water to 100 ml after 90 minute. UV spectra of the solution between 200 to 400 nm was scanned and calculated for the percent of Venlafaxine Hydrochloride.

Alkaline Degradation (Hydrolytic Degradation under Alkaline Condition)
To 1 ml of stock solution (1000 µg/ml) of Venlafaxine Hydrochloride, 1 ml of 0.1N NaOH was added in 100 ml of volumetric flask, the volume was made up with distilled water, the solution was kept at room temperature for the period of 90 minutes. 10 ml of solution was neutralized and diluted with distilled water to 100 ml after 90 minute. UV spectra of the solution between 200 to 400 nm was scanned and calculated for the percent of Venlafaxine Hydrochloride.

Oxidative Degradation
To 1 ml of stock solution (1000 µg/ml) of Venlafaxine Hydrochloride, 1 ml of 30 % v/v H_2O_2 was added in 100 ml of volumetric flask, the volume was made up with distilled water, the solution was kept at room temperature for the period of 30 minutes. 10 ml of solution was neutralized and diluted with distilled water to 100 ml after 30 minute. UV spectra of the solution between 200 to 400 nm was scanned and calculated for the percent of Venlafaxine Hydrochloride.

Thermal Degradation
Sample of Venlafaxine Hydrochloride was kept in a petridish and exposed to the temperature of 70°C for 24 hours in an oven. After 24 hour 100 mg of Venlafaxine Hydrochloride was weighed and transferred accurately and dissolved and diluted in 100ml distilled water. A solution was prepared for concentration of 10 µg/ml of Venlafaxine Hydrochloride from

stock solution. UV spectra of the solution between 200 to 400 nm was scanned and calculated for the percent of Venlafaxine Hydrochloride.

Photolytic Degradation

Sample of Venlafaxine Hydrochloride was exposed to ultra violet radiations for photostability in UV chamber providing illumination of not less than 1.2 million flux per hour 180 minutes. After 180 minutes 100 mg of Venlafaxine Hydrochloride was weighed and transferred accurately and dissolved and diluted in 100ml distilled water. A solution was prepared for concentration of 10 µg/ml of Venlafaxine Hydrochloride from stock solution. UV spectra of the solution between 200 to 400 nm was scanned and calculated for the percent of Venlafaxine Hydrochloride.

Solution Stability

The stability of the sample solutions was performed at intervals of zero hour, 6 hours and 12 hours. The stability of solution was determined in terms of the assay of the drug in sample solutions against the freshly prepared standard solutions. The relative standard deviation for the assay values determined up to 12 hours.

RESULT AND DISCUSSION

Linearity and Range

The calibration curve in water showed linearity in the concentration range of 2-12µg/ml. The correlation coefficient for Venlafaxine hydrochloride was 0.997.

The calibration curve in 0.1N HCl 1.2 pH showed linearity in the concentration range of 8-18µg/ml. The correlation coefficient for Venlafaxine hydrochloride was 0.998.

The calibration curve in Phosphate buffer 6.8 pH showed linearity in the concentration range of 10-22µg/ml. The correlation coefficient for Venlafaxine hydrochloride was 0.998.

The method is validated and shown to be linear in the mentioned concentrations

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The limit of detection for Venlafaxine hydrochloride was found to be 1.3326 µg/ml, 0.7893 µg/ml, 0.1976 µg/ml in distilled water, 0.1N Hydrochloric acid 1.2 pH, Phosphate buffer 6.8 pH respectively. **(Table2)**

The limit of quantitation for Venlafaxine hydrochloride was found to be 4.0384 µg/ml, 2.3918 µg/ml, 0.5989 µg/ml in distilled water, 0.1N Hydrochloric acid 1.2 pH, Phosphate buffer 6.8 pH respectively. (**Table2)**

Table 2: Summary

Parameter	Distilled Water	0.1N Hydrochloric acid 1.2 pH	Phosphate buffer 6.8 pH
Linearity (Correlation coefficient)	0.997	0.998	0.998
Precision (% RSD)	0.975	1.342	1.613
Limit of Detection (LOD)	1.3326 µg/ml	0.7893 µg/ml	0.1976 µg/ml
Limit of Quantitation (LOQ)	4.0384 µg/ml	2.3918 µg/ml	0.5989 µg/ml
Range	2-12 µg/ml	8-18 µg/ml	10-22 µg/ml
Linear regression equation	y = 0.052x + 0.021	y = 0.036x + 0.008	y = 0.038x + 0.002
Slope	0.0521	0.0366	0.0381
Intercept	0.0211	0.0087	0.0022

Accuracy (Recovery Test)

The recovery results showed that the proposed method had acceptable level of accuracy for Venlafaxine Hydrochloride. **(Table3**).

Table 3: Recovery test for Venlafaxine hydrochloride by the proposed methods

Media	Fixed sample concentration µg/ml	Sample of added concentration µg/ml	% Recovery	±SD	% R.S.D
Distilled water	1	0.5	102.4%	1.1718	1.1595
	1	1	100.6%		
	1	1.5	100.2%		
0.1N Hydrochloric acid 1.2 pH	2	1	99.2%	2.0033	1.9743
	2	2	103%		
	2	3	102.2%		
Phosphate buffer 6.8 pH	2	1	101%	1.5662	1.5738
	2	2	98.1%		
	2	3	99.26%		

Method Reproducibility (Precision)

Percent relative standard deviation for inter-day assay was found to be 1.55-1.99%, 1.04-1.77% in 0.1N Hydrochloric acid 1.2 pH, Phosphate buffer 6.8 pH respectively. (**Table 4 and Table 5**).

Percent relative standard deviation for inter-day assay was found to be 1.66-1.86%, 1.24-1.58% in 0.1N Hydrochloric acid 1.2 pH, Phosphate buffer 6.8 pH respectively. **(Table 4 and Table 5).**

Table 4: Intraday

Media	Concentration	Hours			±SD	% RSD
		0	3	6		
Distilled Water	8 µg/ml	0.431	0.445	0.435	0.0072	1.6501
	10 µg/ml	0.537	0.539	0.544	0.0036	0.6676
	12 µg/ml	0.647	0.667	0.654	0.0101	1.5470
0.1N Hydrochloric acid 1.2 pH	12 µg/ml	0.461	0.449	0.447	0.0075	1.6739
	14 µg/ml	0.525	0.51	0.507	0.0096	1.8761
	16 µg/ml	0.601	0.587	0.609	0.0111	1.8590
Phosphate buffer 6.8 pH	12 µg/ml	0.468	0.466	0.476	0.0052	1.1258
	14 µg/ml	0.532	0.541	0.549	0.0085	1.5730
	16 µg/ml	0.611	0.629	0.617	0.0091	1.4806

Table 5: Interday

Media	Concentration	Day			±SD	% RSD
		1st day	2nd day	3rd day		
Distilled Water	8 µg/ml	0.438	0.431	0.44	0.0047	1.0830
	10 µg/ml	0.541	0.537	0.555	0.0094	1.7363
	12 µg/ml	0.651	0.647	0.641	0.0050	0.7787
0.1N Hydrochloric acid 1.2 pH	12 µg/ml	0.453	0.461	0.443	0.0090	1.9937
	14 µg/ml	0.512	0.525	0.507	0.0092	1.8053
	16 µg/ml	0.597	0.601	0.615	0.0094	1.5639
Phosphate buffer 6.8 pH	12 µg/ml	0.453	0.468	0.466	0.0081	1.7616
	14 µg/ml	0.537	0.532	0.551	0.0098	1.8238
	16 µg/ml	0.609	0.611	0.621	0.0064	1.0476

Degradation Studies

Results of degradation studies were obtained in distilled water as media and compared with the calibration data obtained. (**Table6**).

**Table 6: Degradation Stud
y by UV-Visible Spectrophotometer**

Sr. No.	Degradation type	Duration	% Degradation
1	Acid Degradation	90 min	0.699%
2	Alkaline Degradation	90 min	1.50%
3	Oxidative Degradation	30 min	6.80%
4	Thermal Degradation	24 hours	9.50%
5	Photolytic Degradation	180 min	5.87%

Solution Stability

The relative standard deviation is less than 2 % up to 12 hour. The results indicate that the solutions were stable for 12 hours at an ambient temperature.

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Table 7: Solution Stability

Hours	Absorbance of 10 µg/ml drug solution		
	Distilled Water	0.1N Hydrochloric acid 1.2 pH	Phosphate buffer 6.8 pH
0	0.541	0.376	0.393
6	0.551	0.375	0.391
12	0.551	0.375	0.382
±SD	0.0057	0.0005	0.0058
%RSD	1.0542	0.1538	1.5075

CONCLUSION

The proposed method development and validation of UV-Vis Spectrophotometric method was to determine Venlafaxine Hydrochloride. The developed method was validated in all the three media's distilled water, in 0.1N Hydrochloric acid 1.2 pH and Phosphate buffer 6.8 pH according to ICH guideline and shown to be accurate, precise and cost effective. It do not require expensive or sophisticated and chemicals in contrast with chromatographic method. It can be used for the routine Q. C. analysis and quantification of the drug in the formulations.

Form the results obtained by stress degradation studies the stability of the drug in different stress conditions were found which should be taken care of in routine analysis of drug.

LIST OF ABBREVIATION AND SYMBOLS

MDD - Major depressive disorder
LOD - Limit of detection
LOQ - Limit of quantification
σ - Standard deviation
S - Slope of the calibration curve
AOAC - Association of Official Analytical Chemists
%RSD - % Relative standard deviation
ICH - The International Conference on Harmonization

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