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Analytical Method Development and Estimation of Venlafaxine Hydrochloride in Bulk and Pharmaceutical Dosage Form by UV- Visible Spectrophotometer

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Abstract:
A simple, sensitive and reproducible spectrophotometric method for the analysis of venlafaxine in pure form and in pharmaceutical formulations has been developed. Venlafaxine is 1-[2-(di methylamino)-1-(4-methoxyphenyl) ethyl] cyclohexan-1-ol, used as an antidepressant of the neither serotonin- nor epinephrine reuptake inhibitor (SNRI) class. It is prescribed for the treatment of clinical depression and anxiety disorders. In present work, an absorption maximum was found to be at 225.20 nm with the distilled water as solvent system. The drug shows linearity range of 4 – 20 µg/ml with correlation coefficient of 0.9991. These methods were tested and validated for various parameters according to ICH guidelines and USP. The proposed method was successfully applied for the determination of Venlafaxine hydrochloride in pharmaceutical formulations (capsules).The developed method was validated in terms of accuracy, precision, linearity, limit of detection and limit of quantitation which proves suitability of proposed method for routine estimation of venlafaxine hydrochloride in bulk and pharmaceutical formulations.

***Key words**: Venlafaxine, Spectrophotometric method, Beer's law, ICH, antidepressant.*

1. Introduction

Venlafaxine Hydrochloride is an established anti-depressant drug. Chemically it is known as [2-(Dimethyl amino)-1-(4-methoxyphenyl) ethyl] cyclohexanol hydrochloride with molecular weight 313.9.Freely soluble in water and elimination half-life of 4-5 hrs.[1- 4] Venlafaxine hydrochloride is a third generation antidepressant& it is BCS class 1 drug. The drug in its hydrochloride salt form is administered to adults in the rang 75 mg to 350 mg/day. The structure of Venlafaxine hydrochloride is as given in Fig. 1.

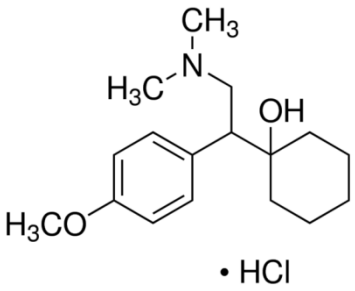

Fig.1: Structure of Venlafaxine HCL

Literature survey tells about the estimation of Venlafaxine by using UPLC. In addition it also tells about the estimation of Venlafaxine with other drug by HPLC and UV Spectrophotometric method.[5-12] Apart from these some other methods are also available for determination of Venlafaxine in bulk and dosage form. The objective of the present work was to develop a simple spectrophotometric method.[13-16]

2. Experimental

2.1. Materials and methods:

Venlafaxine hydrochloride was received as a gift sample from Alembic Pharma LTd, Vadodara, India. All analytical grade chemicals and solvents were supplied by Pallav chemical, Mumbai, India. Distilled water was used to prepare all solution. Freshly prepared solutions were always employed.The instrument used for the study was an UV-VIS double beam spectrophotometer (Shimadzu, UV-1800)) with 1cm matched pair quartz cells.

2.2 Selection of Media

Main criteria for media selection are solubility and stability, i.e. drug should be soluble as well as stable for sufficient time in selected media. The literature survey revealed venlafaxine hydrochloride is highly water soluble and stable for sufficient time in water. The venlafaxine hydrochloride also exhibited high solubility in DMSO.

2.3 Preparation of Standard Stock Solution

Standard drug solution of venlafaxine hydrochloride was prepared by dissolving 10 mg in 100 ml distilled water. The concentration of prepared stock 1 solution was 100µg/ml.

2.4 Selection of λ max: -

The standard stock solution was further diluted with distilled water to get a 10 µg/mL of concentration. The solution was scanned between 400 and 200 nm using distilled water as blank. From the spectrum obtained, 225.20 nm was selected as λmax for the analysis of venlafaxine hydrochloride.

2.5 Preparation of working Standard Dilutions

The serial dilution from the stock 2 in the range of 1, 4, 8, 12, 16 and 20 µg/ml was prepared. The absorbance was measured at λmax 225.20 nm.

2.6 Calibration curve of Venlafaxine Hydrochloride:

Prepared dilution of 2, 4, 6, 8and 10 µg/ml. The absorbance was measured at λmax 225.20 nm. Then, the calibration curve was plotted by taking concentration on x-axis and absorbance on y-axis (in Tab no.1 & fig.2).

2.7 U.V Method Validation

2.7.1 Linearity Range

The linearity of the response of the drug was verified at 4 to 20 µg/ml concentrations. The calibration graphs were obtained by plotting the absorbance versus the concentration data and were treated by linear regression analysis. (Table.03)

2.7.2 Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies. In intra-day studies, three repeated measurements of standard and sample solutions were made in a day and percentage RSD were calculated. In inter-day studies, three repeated measurements of standard and sample solutions were made on three consecutive days and percentage RSD were calculated (Table.04&05).

2.7.3 Accuracy (Recovery Test)

The accuracy of the method was determined by preparing solutions of different concentrations that is 80%, 100% and 120% in which the amount of marketed formulation (10 mg) was kept constant and the amount of pure drug was varied that is 8mg, 10mg and 12mg for 80%, 100% and 120% respectively. The solutions were prepared in triplicates and the accuracy was indicated by % recovery. (Table.06)

2.7.4 Limit of Detection (LOD) and Limit of Quantification (LOQ):

LOD is 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. The **LOQ** is the concentration that can be quantitated reliably with a specified level of accuracy and precision.LOD and LOQ were calculated by the equations;

$$LOD = 3.3\sigma / S$$
$$LOQ = 10\sigma / S$$

Where S is slope of the calibration curve and σ is the residual standard deviation. The LOD and LOQ were calculated. (Table.02)

2.7.5 Ruggedness:

Ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective absorbance was noted. The result was indicated by % RSD. (Table.07)

2.7.6 Robustness:

Robustness of the method was determined by carrying out the analysis at two different temperatures i.e. at room temperature (25^0c) and at 17^0c. The respective absorbance was noted and the result was indicated by % RSD. (Table.08)

2.8 Estimation From Formulations

2.8.1 Capsules

Contents of twenty capsules were weighed and grinded. Amount mg of Venlafaxine hydrochloride was taken and sonicated for 15 mins. These solutions were suitably diluted to prepare a 100 µg/ml concentration. Finally solutions were filtered through Whatman filter paper number 40 and the filtrate was suitably diluted to prepare 15 µg/ml concentration and the samples were analysed using proposed method. The amount of venlafaxine was computed from the calibration curve.

3 Results And Discussion:

3.1 Analytical method Development

3.1.1 Estimation of λmax:

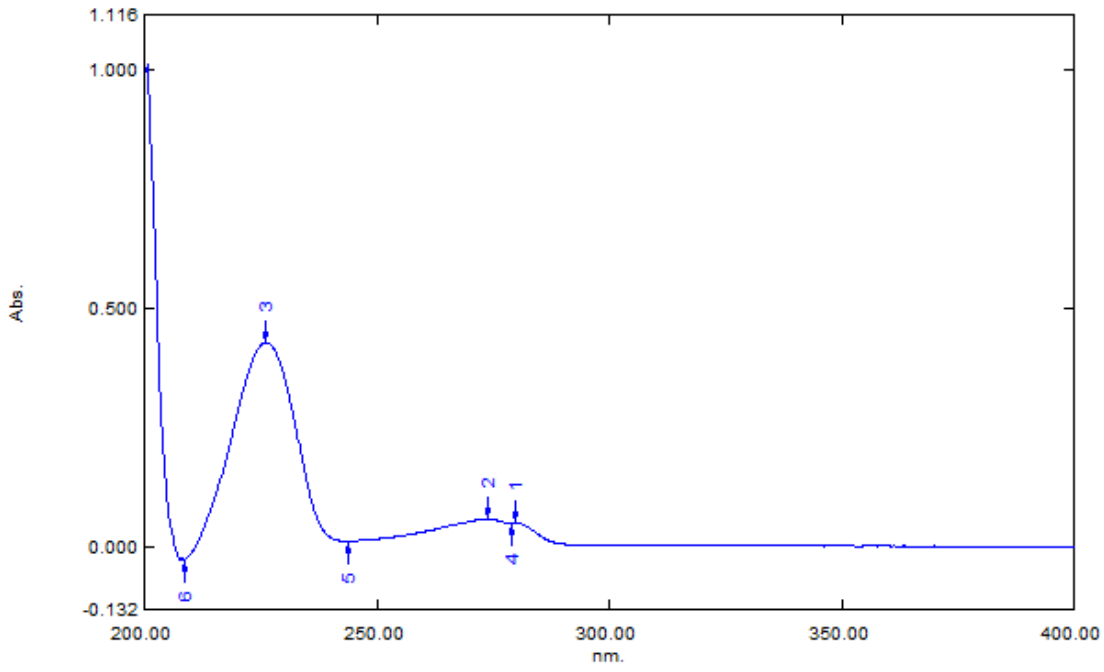

Fig. 2: λmax of venlafaxine Hydrochloride

3.1.2 Calibration curve of Venlafaxine Hydrochloride:

The absorbance of the prepared stock solutions was measured at 225.20 nm in an UV spectrophotometer. Plot a graph between concentration (in µg/ml) vs absorbance (in nm) on X-axis and Y-axis respectively.

Table. 1: Calibration curve of Venlafaxine HCL

Sr.no.	Concentration (in µg/ml)	Absorbance (in nm)
1	00	000
2	2	0.102
3	4	0.208
4	6	0.312
5	8	0.398
6	10	0.4978

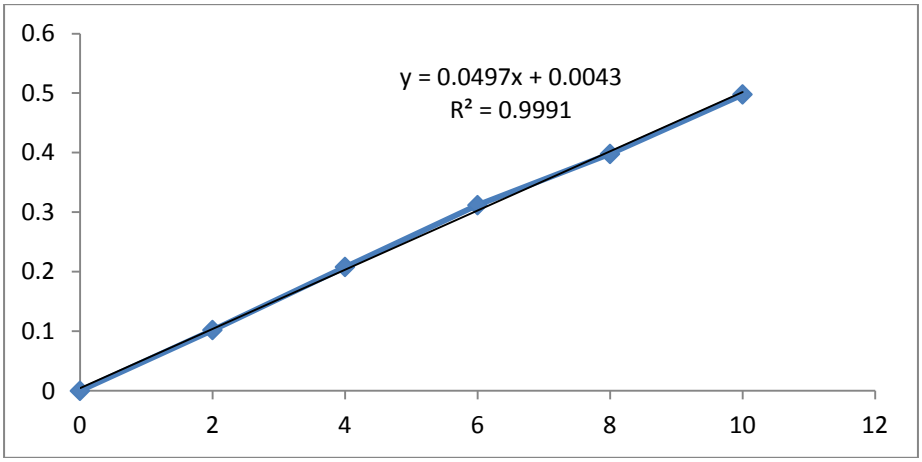

Fig. 3: Calibration curve of Venlafaxine HCL

3.1.3 UV Method Validation:

The developed method was found to be precise as the %RSD values for intra-day and inter-day were found to be less than 2%. Good recoveries (98% to 101%) of the drug were obtained at each added concentration, indicating that the method was accurate. The LOD and LOQ were found to be in sub microgram level indicating the sensitivity of the method. The method was also found to be robust and rugged as indicated by the %RSD values which are less than 2%.

Table. 2: Validation Parameters

Sr.No.	Parameter	Result
1	Absorption maxima(nm)	225.20
2	Linearity range (µg/ml)	4- 20 µg/ml
3	Standard regression equation	y = 0.0562x - 0.0081
4	Correlation coefficient (r2)	0.999
5	Standard Deviation	0.01395
6	LOD (µg/ml)	0.8191
7	LOQ (µg/ml)	2.4822
8	Assay indicated by % recover	100.05%

3.1.4 Linearity Range

The linearity of the response of the drug was verified at 4 to 20 µg/ml concentrations. The calibration graphs were obtained by plotting the absorbance versus the concentration data and were treated by linear regression analysis. The equation of the calibration curve for venlafaxine hydrochloride obtained y = 0.0562x - 0.0081The linearity range of calibration curve was found to be 4-20 µg/ml concentrations. The correlation coefficient (r2) of determination was 0.9991.

Table. 3: Linearity study

Sr. no	Concentration (µg/ml)	A 1 (225.2nm)	A 2 (225.2nm)	A 3 (225.2nm)	Statistical result		
					mean	SD	% RSD
1	1	0.0601	0.0611	0.0599	0.060366	0.000643	1.065008367
2	4	0.211	0.214	0.216	0.2136	0.002517	1.177821285
3	8	0.413	0.4033	0.462	0.4261	0.031466	7.384729222
4	12	0.656	0.686	0.635	0.659	0.025632	3.889531295
5	16	0.901	0.895	0.925	0.907	0.015875	1.750221374
6	20	1.123	1.111	1.109	1.1143	0.007572	0.679498456

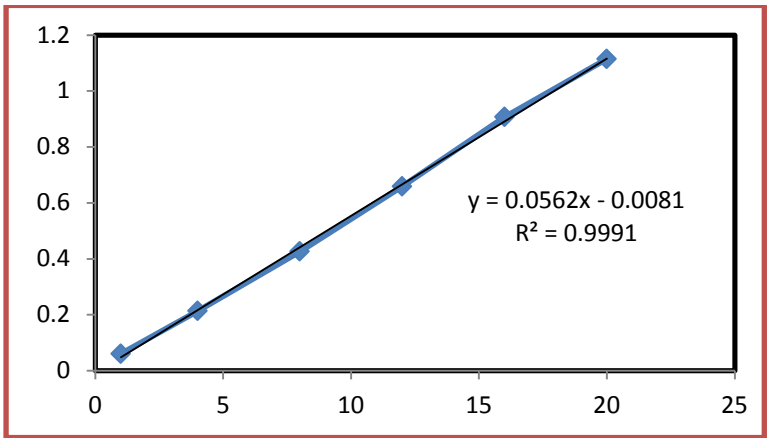

Fig. 4: Linearity graph of Venlafaxine HCL

3.1.5 Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies.

Table. 4: Intraday absorbance of precision study

Sr. no	Concentration (µg/ml)	Morning	afternoon	Evening	Statistical result		
					mean	SD	% RSD
1	4	0.214	0.213	0.218	0.215	0.00264575	1.230582 %
2	4	0.213	0.217	0.211	0.2136667	0.00305505	1.429820 %
3	4	0.216	0.217	0.211	0.2146667	0.00321455	1.4974613%
4	12	0.745	0.755	0.757	0.7523333	0.0064291	0.8545548%
5	12	0.744	0.751	0.758	0.751	0.007	0.9320905%
6	12	0.748	0.734	0.754	0.7453333	0.0102632	1.376995%
7	24	1.117	1.111	1.121	1.1163333	0.00503322	0.450871%
8	24	1.172	1.188	1.178	1.1793333	0.0080829	0.6853791
9	24	1.171	1.181	1.178	1.1766667	0.0051316	0.4361134%

Table no. 05: Interday precision absorbance of precision study

Sr. no	Concentration (µg/ml)	Day 1	Day 2	Day 3	Statistical result		
					mean	SD	%RSD
1	4	0.214	0.211	0.209	0.2113333	0.00251661	1.1908256 %
2	4	0.213	0.217	0.211	0.2136667	0.00305505	1.4298208 %
3	4	0.216	0.211	0.209	0.212	0.00360555	1.7007317%
4	12	0.745	0.732	0.7296	0.7355333	0.00828573	1.1264929%
5	12	0.758	0.744	0.739	0.747	0.00984886	1.3184549%
6	12	0.785	0.765	0.758	0.7693333	0.0140119	1.8213041%
7	24	1.125	1.117	1.109	1.117	0.008	0.7162041%
8	24	1.121	1.119	1.109	1.1163333	0.0064291	0.5759123%
9	24	1.131	1.125	1.129	1.1283333	0.00305505	0.2707578%

3.1.6 Accuracy (Recovery Test)

Accuracy of the method was studied by recovery experiments. The recovery was performed at three levels, 80, 100 and 120% of venlafaxine hydrochloride standard concentration. The recovery values for venlafaxine hydrochloride were summarized in Table.06

Table no. 06: Accuracy study of venlafaxine HCl

Sr no.	Fix conc. Taken ppm	Conc. Added ppm	% added	% recovery	Average recovery	% RSD
1	10	8	80%	81.40%	80.48%	1.33
2	10	8	80%	79.3%		
3	10	8	80%	80.74%		
4	10	10	100%	100.43%	100.14 %	0.3175
5	10	10	100%	100.19%		
6	10	10	100%	99.80%		
7	10	12	120%	121.84%	120.71%	0.8421
8	10	12	120%	119.86%		
9	10	12	120%	120.45%		

3.1.7 Ruggedness study:

Table. 7: Ruggedness study of venlafaxine HCl

ANALYST 1				ANALYST 2			
Sr no.	Conc. (µg/ml)	Absorbance	Statistical Analysis	Sr no.	Conc. µg/ml)	Absorbance	Statistical Analysis
1	10	0.620	Mean =0.622 SD= 0.001414 RSD= 0.2273%	1	10	0.623	Mean = 0.6254 SD= 0.002059 RSD= 0.3401%
2	10	0.622		2	10	0.625	
3	10	0.621		3	10	0.624	
4	10	0.623		4	10	0.626	
5	10	0.624		5	10	0.629	

3.1.8 Robustness study:

Table. 8: Robustness study of Venlafaxine HCl

At Room temperature (at 25^{0}C)				At Temperature 17^{0}C			
Sr no.	Conc. (µg/ml)	Absorbance	Statistical Analysis	Sr no.	Conc. µg/ml)	Absorbance	Statistical Analysis
1	12	0.745	Mean = 0.7528 SD= 0.00598 RSD= 0.7943%	1	12	0.732	Mean = 0.7356 SD= 0.005886 RSD= 0.8001%
2	12	0.748		2	12	0.743	
3	12	0.761		3	12	0.728	
4	12	0.752		4	12	0.733	
5	12	0.758		5	12	0.742	

4 Conclusion

Although several studies have provided different methods for determination of Venlafaxine hydrochloride, this study provide another alternative method, which is rapid, specific, and sensitive for venlafaxine hydrochloride. The method linearity range of 4 – 20 µg/ml with correlation coefficient of 0.9991.

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