Open Access Contents
Int. J. Drug Dev. & Res.
| October - December 2013 | Vol. 5 | Issue 4 | ISSN 0975-9344 |
www.ijddr.in

Method development and estimation of Venlafaxine Hydrochloride in bulk and Pharmaceutical dosage forms using UV-VIS

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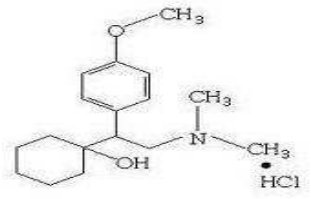
Abstract:
The present study describes a simple, accurate, precise and cost effective UV-VIS Spectrophotometric method for the estimation of Venlafaxine hydrochloride, an antidepressant drug in bulk and pharmaceutical dosage form. The solvent used was Water and Methanol in the ratio of 50:50 and the λmax or the absorption maxima of the drug was found to be 227nm. A linear response was observed in the range of 4-70µg/ml with a regression coefficient of 0.999. The method was then validated for different parameters as per the ICH (International Conference for Harmonization) guidelines. This method can be used for the determination of Venlfaxine in quality control of formulation without interference of the excipients.

Keywords: Venlafaxine, Antidepressant, λmax, ICH, UV-VIS spectroscopy.

Full Length Original Research Paper

Introduction

[R/S]-1-[2-dimethylamine)-1-[4-Methoxy phenyl) ethyl] cyclohexanol hydrochloride or (+)-1-[2-[(dimethylamino)methyl]p-Methoxybenzyl] cyclohexanol hydrochloride.



Venlafaxine hydrochloride is a bicyclic antidepressant, and is usually categorized as a serotonin-norepinephrine reuptake inhibitor (SNRI), but it has been referred to as a serotonin-norepinephrine-dopamine reuptake inhibitor (SNDRI). It works by blocking the transporter "reuptake" proteins for key neurotransmitters affecting mood, thereby leaving more active neurotransmitters in the synapse. The neurotransmitters affected are serotonin and norepinephrine. Additionally, in high doses it weakly inhibits the reuptake of dopamine. [1,2,3]

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. Apart from these some other methods are also available for determination of Venlafaxine in bulk and dosage form.[4,5,6,7,8,9.10,11,12,13].

Materials and Methods:

The instrument used for the study was an UV-VIS double beam spectrophotometer (Model T60, Analytical Technologies Limited) with 1cm

matched pair quartz cells. The solvent used was Distilled Water and Methanol AR grade, purchased from SD Fine Chemicals Limited, India and Venlafaxine was supplied from Alkem Laboratories Limited as a gift sample.

METHOD DEVELOPMENT:

Solubility Test: Solubility test for the drug Venlafaxine was performed by using various solvents. The solvents include Ethanol, Distilled water, Acetic Acid mixture, 0.1 N Hydrochloric Acid (HCl), 0.1 N Sodium Hydroxide (NaOH) and Chloroform. However, Water and Methanol in the ratio of 50:50 was chosen as a solvent for developing the method.

Determination of λ_{max}:

Preparation of Stock Solution: Standard stock solution of Venlafaxine hydrochloride was prepared by dissolving 100mg of Venlafaxine hydrochloride in 100 ml mixture of Water and Methanol in the ratio of 50:50 in a order to produce a concentration of 1000µg/ml. 10ml of this stock solution was taken and then diluted up to 100ml with Water and Methanol in the ratio of 50:50, to produce a concentration of 100µg/ml which is the standard stock solution.

Preparation of Working Standard Solution: From the above stock solution, 1ml was pipetted into a 10ml volumetric flask and the volume was made up to the mark with Water and Methanol mixture (50:50) to prepare a concentration of 10µg/ml. Then from this 100µg/ml stock solution 2ml, 3 ml, 4ml, 5ml, 6ml, 7ml, 8ml, 9ml, and 10ml was pipetted into a 10ml volumetric flask and the volume was made up to the mark with Water and Methanol mixture(50:50), to prepare a concentration of 20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml, 60µg/ml, 70µg/ml, 80µg/ml, 90µg/ml and 100µg/ml. Then 40µg/ml sample was scanned in UV-VIS Spectrophotometer in the range 400-200nm using Water and Methanol mixture (50:50) as a blank and the wavelength corresponding to maximum absorbance (λmax) was found to be 227 nm (fig.1).

Preparation of Calibration Curve: 1ml of the 100µg/ml solution was diluted to 10ml by using mixture of solvent to produce 10µg/ml solution. From this 100µg/ml 1ml, 2ml, 3 ml, 4ml, 5ml, 6ml, 7ml, 8ml, 9ml, and 10ml solution were pipetted and diluted to 10ml Water and Methanol mixture (50:50) to produce 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml, 60µg/ml, 70µg/ml, 80µg/ml, 90µg/ml and 100µg/ml solutions respectively. Then the construction of calibration curve was done by taking the above prepared solutions of different concentration ranging from 10-100µg/ml. Then, the calibration curve was plotted by taking concentration on x-axis and absorbance on y-axis (in fig.2). The curve showed linearity in the concentration range of 10-100µg/ml. The correlation coefficient (r^2) was found to be 0.999.

Assay of Venlafaxine tablet:

A quantity of powder equivalent to 100mg of Venlafaxine hydrochloride was taken in a 100ml volumetric flask and it was dissolved in 10 ml of Water and Methanol mixture (50:50) and diluted up to the mark with Water and Methanol mixture (50:50). The resultant solution was ultrasonicated for 5 minutes. The solution was then filtered using Whatmann filter paper No.40. From the filtrate, appropriate dilutions were made in Water and Methanol mixture (50:50). To obtain the desired concentration (10µg/ml). This solution was then analysed in UV and the result was indicated by % recovery given in table 1.

METHOD VALIDATION:

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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.

The method was validated for different parameters like Linearity, Accuracy, Precision, Specificity, Robustness, Ruggedness, Limit of Detection (LOD) and Limit of Quantitation (LOQ).[14]

Linearity: Various aliquots were prepared form the stock solution (100µg/ml) ranging from 10-100µg/ml. The samples were scanned in UV-VIS Spectrophotometer using Water and Methanol mixture (50:50) as a blank. It was found that the selected drug shows linearity between the 0-70µg/ml (table 1&3).

Accuracy: 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 5).

Precision: Precision of the method was demonstrated by intraday and interday variation studies. In intraday variation study, 6 different solutions of same concentration that is 10µg/ml were prepared and analysed two times in a day i.e. morning, and evening and the absorbance were noted. The result was indicated by % RSD (table no.6, & table no.7).

In the interday variation study, solutions of same concentration 10µg/ml were prepared and analysed two times for two consecutive days and the absorbance were noted. The result was indicated by % RSD (table no.8).

Specificity: 10mg of Venlafaxine hydrochloride was spiked with 50% (3.75mg), 100% (7.5 mg), and 150% (11.25mg) of excipient mix (Magnesium Stearate) and the sample was analysed for % recovery of Venlafaxine hydrochloride (table no.9).

Robustness: Robustness of the method was determined by carrying out the analysis at two different temperatures i.e. at room temperature (25ºc) and at 18ºc. The respective absorbance were noted and the result was indicated by % RSD (tableno.10 &).

Ruggedness: Ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective absorbance were noted. The result was indicated by % RSD (table no.10).

Limit of Detection (LOD): The limit of detection (LOD) was determined by preparing solutions of different concentrations ranging from 0.1-1µg/ml. 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 (table no.1).

Limit of Quantitation: The LOQ is the concentration that can be quantitated reliably with a specified level of accuracy and precision. The LOQ was calculated using the formula involving standard deviation of response and slope of calibration curve (table no.1).

Results and Discussion:

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 method was also found to be specific indicated by the % recoveries ranging from 99.8% to 101.2%. 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%. The results of Assay show that the amount of drug was in good agreement with the label claim of the formulation as indicated by % recovery (100.03%). Summary of validation parameters of proposed spectrophotometric method is shown in table 1.

Table No.1: SUMMARY OF VALIDATION:

PARAMETER	RESULT
Linearity indicated by correlation coefficient	0.9991
Precision indicated by %RSD	0.34%
Accuracy indicated by % recovery	99.3%
Specificity indicated by % recovery	100.03%
Limit of Detection	0.8 µg/ml
Limit of Quantification	2.64µg/ml
Range	0-100µg/ml
Linear regression equation	y = 0.0038x
Robustness indicated by %RSD	0.68%
Assay indicated by % recovery	100.03

Determination of λ_{max}:

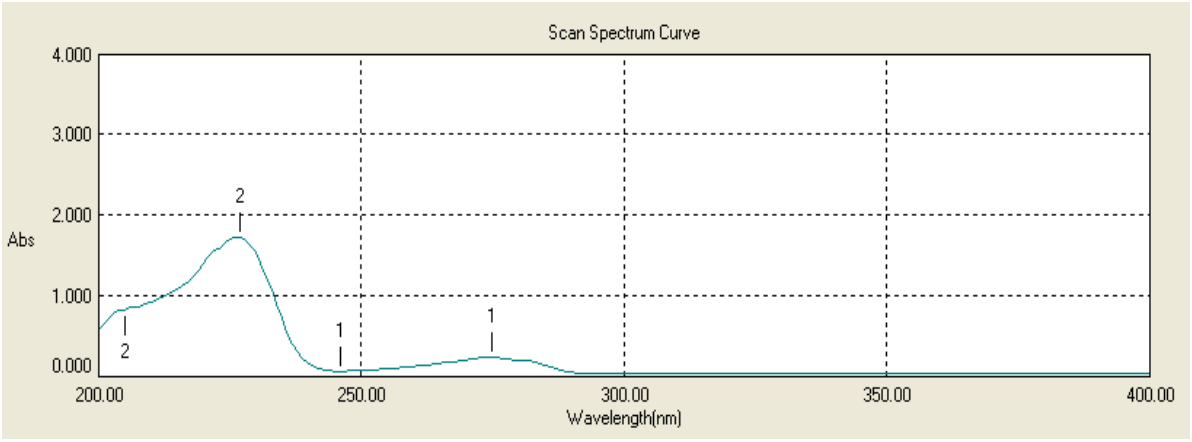

Fig. 1: λmax of Venlafaxine hydrochloride showing at 227nm

Table 3: Preparation of Calibration Curve

Conc.	Abs
0	0
10	0.305
20	0.599
30	0.875
40	1.2
50	1.447
60	1.75
70	2.0

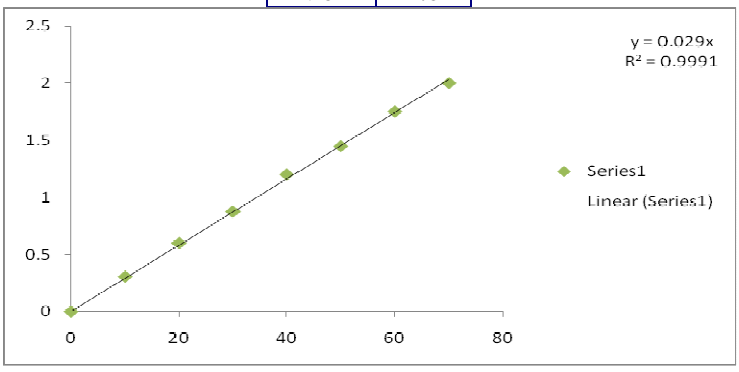

Fig 2: Calibration Curve of Venlafaxine hydrochloride

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VALIDATION:

Table for Linearity:

Table 3: Linearity Table of Venlaflaxine hydrochloride in Working Standard

Concentration (µg/ml)	Absorbance
0	0
10	0.305
20	0.599
30	0.875
40	1.2
50	1.447
60	1.75
70	2.0

Table 4: Optical characteristics

Beer's Law limit (µg/mL)	0-70µg/ml
Molar extinction coefficient (1 mole-1 c.m-1)	1.22
Correlation coefficient	0. 9991
Regression equation (Y*)	0.029x
Slope (a)	0.029
Intercept (b)	0

Accuracy:

Table 5: Accuracy Readings of Venlafaxine hydrochloride

OBSERVATION / RESULTS						
No. of preparations	Concentration (µg/ml)		% Recovery	Statistical Results		
	Formulation	Pure Drug		Mean	SD	%RSD
S_1 : 80 %	10	8	98	98	0	0
S_2 : 80 %	10	8	98			
S_3 : 80 %	10	8	98			
S_4 : 100 %	10	10	101	100.3	0.59	0.58
S_5 : 100 %	10	10	100			
S_6 : 100 %	10	10	101			
S_7 : 120 %	10	12	99	100	0.81	0.81
S_8 : 120 %	10	12	100			
S_9 : 120 %	10	12	101			

PRECISION:

Table 6: Precision

Concentrations (µg/ml)	Absorbance Morning	Absorbance Evening	Statistical Analysis
10	0.305	0.301	Mean = 0.192 SD = 0.0005 %RSD = 0.66%
10	0.300	0.305	
10	0.301	0.303	
10	0.306	0.305	
10	0.302	0.303	
10	0.304	0.305	
Average	0.303	0.304	

Table 7: Intra-assay Precision

Concentrations (µg/ml)	Absorbance 1	Absorbance 2	Average %RSD
10	0.302	0.305	
10	0.301	0.300	
10	0.303	0.301	
10	0.305	0.306	
10	0.307	0.302	
10	0.305	0.304	
Average	0.303	0.303	
%RSD	0.73%	0.78%	0.76%

Table 8: Inter-assay Precision

Concentrations (µg/ml)	%RSD		Average %RSD
	Day 1	Day2	
10	0.66%	0.76%	0.71%

Test for Specificity:

Table 9: Test for Specificity showing no effect of excipient

Sample No.	Excipient Conc.(%)	Venlafaxine Input(mg)	Venlafaxine Recovered(mg)	Venlafaxine Recovered(%)	Mean Recovered(%)	S.D.	%R.S.D.
1	100%	10	9.95	99.8	100.03	1.06	1.06%
2	50%	10	9.12	101.2			
3	150%	10	9.91	99.1			

Ruggedness & Robustness:

Table 10: Results Showing Ruggedness of Method for Venlaflaxine hydrochloride

Analyst-1			Analyst-2		
Conc. (µg/ml)	Abs.	Statistical Analysis	Conc. (µg/ml)	Abs.	Statistical Analysis
20	0.0595	Mean = 0.059417 SD = 0.000512 %RSD = 0.86%	20	0.0584	Mean = 0.0589 SD = 0.000497 %RSD = 0.84%
20	0.0587		20	0.0585	
20	0.0598		20	0.0598	
20	0.0589		20	0.0590	
20	0.0596		20	0.059	
20	0.06		20	0.059	
Room Temperature			Temp. 18°C		
Conc. (µg/ml)	Abs.	Statistical Analysis	Conc. (µg/ml)	Abs.	Statistical Analysis
20	0.0598	Mean = 0.59533 SD = 0.000446 %RSD = 0.74%	20	0.0593	Mean= 0.593 SD = 0.000345 %RSD = 0.58%
20	0.0599		20	0.0589	
20	0.0598		20	0.0598	
20	0.0587		20	0.0591	
20	0.0594		20	0.0593	
20	0.0596		20	0.0597	

Limit of Detection (LOD)

The LOD for Venlafaxine was found to be 0.8 µg/ml µg/ml.

Limit of Quantitation (LOQ)

The LOQ for Venlafaxine was found to be 2.45µg/ml

Acknowledgements:

I Mr. Dhiraj Kumar is very much thankful to Prof. S.A.Srinivas, Principal, Guru Nanak Institute of Pharmacy, Hyderabad for providing the necessary chemicals for our work. I am also thankful to the management of Guru Nanak Institute of Pharmacy for giving the facilities for this kind of research work.

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Article History:----------

Date of Submission: 28-06-2013

Date of Acceptance: 11-07-2013

Conflict of Interest: NIL

Source of Support: NONE