INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences **Research Article……!!!**

Received; accepted

ANALYTICAL SPECTROPHOTOMETRIC METHOD DEVELOPMENT & VALIDATION OF VENLAFAXINE HYDROCHLORIDE IN PHARMACEUTICAL SOLID DOSAGE FORMS

Brijesh Patel*[1], Japan Patel[1], Hardeepsingh Banwait[2], Nilesh Darji[2], Dhara Patel[3], Anjali Gabhawala[2], Bhagirath Patel[2], Manish Patel[1]

1. Jodhpur National University, Jodhpur, Rajsthan, India

2. Sat Kaival College of Pharmacy, Sarsa, Anand, Gujarat, India

3. S.K.Patel College of Pharmaceutical Education and Research, Ganpat University, Kherva, Gujarat, India

Keywords:
Venlafaxine hydrochloride; Spectrophotometer; Validation

For Correspondence:

Brijesh Patel

Jodhpur National University, Jodhpur, Rajsthan, India.

E-mail:
brijesh.272@gmail.com

ABSTRACT

New, simple and cost effective UV-spectrophotometric method was developed for the estimation of Venlafaxine hydrochloride in bulk and pharmaceutical formulations. Venlafaxine hydrochloride was estimated at 225.27 nm in distilled water. Linearity range was found to be 4 - 24µg/ml. The apparent molar absorptivity was found to be3.4 × 104 $L \cdot mol^{-1} cm^{-1}$ in distilled water. 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 (tablets and capsules). The results demonstrated that the procedure is accurate, precise and reproducible (relative standard deviation < 2%), while being simple, cheap and less time consuming and can be suitably applied for the estimation of Venlafaxine hydrochloride in different dosage forms and dissolution studies.

INTRODUCTION

Venlafaxine is an antidepressant of the serotonin-nor epinephrine reuptake inhibitor. Venlafaxine hydrochloride is an important drug in neurological armentarium used for treatment of depression and general anxiety disorders like Generalized anxiety disorder, Social anxiety disorder and Panic disorder, etc [(1)].The drug in its hydrochloride salt form is administered to adults in the range of 75 to 350 mg/day. The structure of Venlafaxine hydrochloride is as given in Fig. 1.

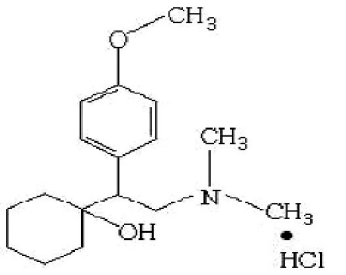


Fig. 1. Structure of Venlafaxine hydrochloride

Multiple double blind studies show venlafaxine's effectiveness in treating depression. Venlafaxine has similar efficacy to the tricyclic antidepressants amitriptiline and imipramine, and is better tolerated than amitriptyline. Its efficacy is similar to or better than sertraline and fluoxetine, depending on the criteria and rating scales used. Higher doses of venlafaxine are more effective, and more patients achieved remission or were "very much improved". The efficacy was similar if the number of patients who achieved "response" or were "improved" was considered. A meta-analysis comparing venlafaxine and combined groups of SSRI or tricyclic antidepressants showed venlafaxine's superiority [(2)]. Judged by the same criteria, venlafaxine was similar in efficacy to the atypical antidepressant bupropion; however, the remission rate was significantly lower for venlafaxine[(3)].Hence, there has been an increase in number of Venlafaxine formulations being prescribed.

Venlafaxine hydrochloride drug is official in British Pharmacopeia 2007.The assay of drug according to British Pharmacopeia is by Potentiometric titration. For routine analysis a simple, rapid and cost effective analytical method is required and preferred. A survey of literature has not

revealed any UV-spectrophotometric method for estimation of Venlafaxine in bulk drug, formulations. High performance liquid chromatography(HPLC) reported for the estimation of Venlafaxine in biological fluids such as such as plasma, serum and urine[4,5].But, chromatographic techniques are time consuming, costly and require expertise. A simple and accurate UV-spectrophotometric method can be highly useful for routine analysis of bulk, formulations and dissolution samples.

The objective of the present study was to develop simple, precise, accurate and economic analytical methods with the better detection range for estimation of Venlafaxine hydrochloride in bulk, pharmaceutical formulations and in-vitro dissolution studies. Analytical method has been developed using distilled water for estimation of Venlafaxine hydrochloride. The developed methods were validated as per ICH guidelines and USP requirements [6, 7]. Suitable statistical tests were performed on validation data [8,9].

EXPERIMENTAL PROCEDURES

Instruments

A double-beam Shimadzu UV–Vis Spectrophotometer, model UV-1700 loaded with UV-Probe software. It had an automatic wavelength accuracy of 0.1 nm and matched quartz cells of 10 mm path length.

Materials

Venlafaxine hydrochloride was obtained as gift samples from Lupin Pharma Ltd., India. Formulations were purchased from local market. The labelled content of formulations was Venlafaxine hydrochloride equivalent to 75 mg of Venlafaxine. All other chemicals and reagents were of analytical grade.

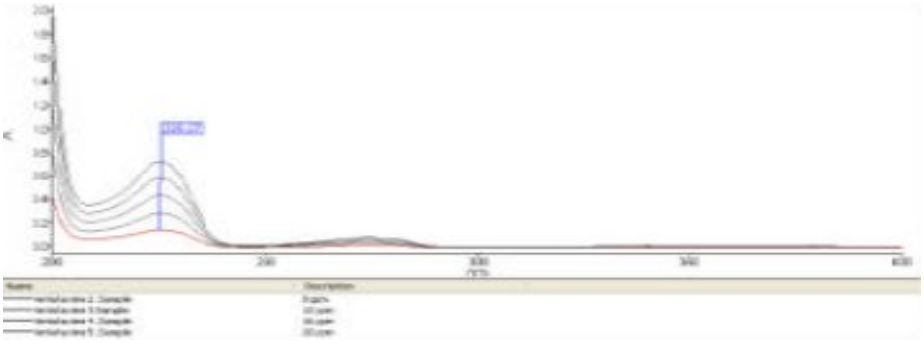
Analytical method development

Distilled water was investigated to develop a suitable UV-spectrophotometric method for the analysis of Venlafaxine hydrochloride in formulations. For selection of media the criteria employed were sensitivity of the method, ease of sample preparation, solubility of the drug, and cost of solvents and applicability of method to various purposes. An UV spectroscopic scanning run (400 - 200 nm) was carried out to select the best UV wavelength (λ max = 225.27 nm) for detection of Venlafaxine hydrochloride in an aqueous solution. The analyses were carried out using distilled water as blank. Absorbance of Venlafaxine hydrochloride was determined and apparent molar absorptivity was calculated according to standard formula.

Calibration standards

A stock solution of 100 µg/ml of Venlafaxine hydrochloride was prepared in Distilled water by dissolving 10 mg in 100 ml media. For preparation of different concentrations, aliquots of stock solutions were transferred into a series of 10 ml standard flasks and volumes were made with respective media. Five different concentrations were prepared in the range of 1–24 µg/ml of Venlafaxine hydrochloride in water for standard curve. The calibration data are presented in (Table1).

Fig. 2. Absorption spectra of Venlafaxine hydrochloride s in distilled water. The samples taken are in increasing order of 4, 8, 12, 16, 20 mcg/ml.



Analytical Validation:

Specificity and selectivity

Venlafaxine hydrochloride solutions (15µg/ml) were prepared in media along with and without common excipients (MCC, lactose, methylcellulose, talc) separately. All solutions were scanned from 400 to 200nm and checked for change in absorbance. In separate study, drug concentration of 15µg/ml was prepared independently from pure drug stock and commercial sample stock and analysed (N=5). Paired t-test at 95% confidence limit of significance was performed to compare the means of absorbance (Table 2).

Accuracy

As a part of determining accuracy of the proposed method, different levels of drug concentrations (LQC, MQC and HQC) were prepared from independent stock solution and analysed (N=6). Accuracy was assessed as the percentage relative error and mean percentage recovery(Table 3).To give additional support to accuracy of the developed assay method, standard addition method was done. In this study, different concentrations of pure drug (12, 15 and 18 mcg/ml) were added to a known pre-analysed formulation sample and the total concentration was determined using the proposed methods (N=3). A sample solution of 15µg/ml and standard solution (1 µg/ml) was prepared. The percent recovery of the added pure drug was calculated as, %Recovery = [A/(B+C)]×100, where A is total amount of drug estimated after standard addition; B is amount of drug on a pre-analysed basis and C is amount of drug added (Table 4).

Precision

Repeatability was determined by using different levels of drug concentrations (same concentration levels taken in accuracy study), prepared from independent stock solution and analysed (N=6) (Table 3).Inter-day and intra-day variation and instrument variation were taken to determine intermediate precision of the proposed methods. Different levels of drug concentrations in triplicates were prepared three different times in a day and studied for intra-day variation. Same

protocol was followed for three different days to study inter-day variation (N=18). The relative standard deviation (in %) of the predicted concentrations from the regression equation was taken as precision (Table 5).

Linearity

To establish linearity of the proposed method six separate series of solutions of the drug (1 -24 µg/ml) was prepared from stock solution and analysed. Least square regression analysis was done for the obtained data. ANOVA test (one-way) was performed based on the absorbance values observed for each pure drug concentration during replicate measurement of standard solutions (Table 2).

Estimation from formulations

Tablets

Twenty tablets were weighed and grinded. Amount of the powder equivalent to 75 mg of Venlafaxine hydrochloride was taken and sonicated for 15 mins. These solutions were suitably diluted to prepare a100 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 (Table 6).

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 (Table 6).

RESULTS AND DISCUSSIONS

Distilled water was chosen as the solvent. Initially, an UV spectroscopic scanning run allowed selecting the wavelength of 225.27 nm as the best for the detection of Venlafaxine hydrochloride in

the standard solution as well as in sample solutions. The spectra of Venlafaxine hydrochloride in distilled water is as given in Fig. 2.Apparent molar absorptivity of drug was found to be $3.9 \times 10^4 L \cdot mol^{-1} cm^{-1}$ in distilled water (Table 2).

Calibration curve

The linear regression equation obtained was: absorbance at 225.27nm = [0.0684 × Concentration in µg/ml + 0.0038]; r^2= 0.9997; with a regression coefficient of 0.9991 (Table 2).

Specificity and selectivity

The UV-spectrum of Venlafaxine hydrochloride was not changed in the presence of common excipients in media. The calculated t-values were found to be less than the critical t-value, indicating that statistically there was no significant difference between mean absorbance of solutions prepared from pure drug sample and one with excipients(Table 2). Therefore proposed methods are specific and selective for the drug.

Accuracy

Accuracy ranged from -0.38% to 0.41% (Table 3). The excellent mean% recovery values (nearly 100%) and their low standard deviation values (S.D. < 1.5) represent accuracy. The validity and reliability of the proposed methods was evaluated by recovery studies of standard addition method (Table 4). In the mean percentage recoveries (%R.S.D.) for lower, intermediate and higher concentrations were found to be 99.98±0.602, 99.32±0.455 and 99.75±0.442 respectively. The mean percentage recoveries (% R.S.D.) for lower, intermediate and higher concentrations were found to be -0.38%, 24% and 0.41% respectively. This result revealed that any small change in the drug concentration in the solution can be accurately determined by these proposed methods.

Precision

Precision determined by studying repeatability and intermediate precision. Repeatability (% R.S.D.) ranged from 0.40% to 1.39%, at all three levels of concentration (Table 3). Repeatability results indicate the precision under the same operating conditions over a short interval of time and inter-

assay precision. Intermediate precision expresses within-laboratory variations in different days. In intermediate precision study, R.S.D. values were not more than 1.5% in all the cases(Table 5). R.S.D. values were within the acceptable range indicating that these methods have excellent repeatability and intermediate precision.

Linearity

The linearity range was found to be 1- 24 µg at 225.37 nm. Lower values of parameters like standard error of slope and intercept (Table2) indicated high precision of the proposed methods. The mean slope and intercept values are within the 95% confidence interval. Goodness of fit of regression equations was supported by high regression coefficient values.

Estimation of formulations

The assay values for formulations ranged from 99.89% to 100.23%with standard deviation of not more than 0.68%. Assay values of formulations were same as mentioned in the label claim; this indicated that the interference of excipient matrix is insignificant in estimation of Venlafaxine hydrochloride by proposed method. The estimated drug content with low values of standard deviation established the precision of proposed method. The student's t-value did not exceed the tabulated values (for four degrees of freedom) indicating no significant difference between the methods, as far as accuracy and precision are concerned.

Table 1.Calibration data for the method development (each value is a result of six separate determinations.

Sr no.	Drug Concentration(µg/ml)	Absorbance at 225.37nm(±S.D.*)	% R.S.D#
1	1	0.0529±0.004	0.85
2	4	0.1994±0.003	0.71
3	8	0.3266±0.007	1.12
4	12	0.4684±0.003	0.80
5	16	0.6085±0.005	1.02
6	20	0.7502±0.004	0.94
7	24	0.9370±0.005	0.72

*Standard deviation.
Relative standard deviation

Table 2. Optical characteristics, statistical data of the regressionequations and validation parameters for Venlafaxine hydrochloride(each value is result of six separate determinations).

Parameter	Result
Optical characteristics	
Appearent molar absorptivity(1/mol.cm)	3.9×10^4
Regression analysis	
Slope	0.0375
95% confidence limits of slope	0.03722 ; 0.3772
Intercept	0.0038
95% confidence limits of Intercept	0.0041; 0.00372
Regression coefficient(r2)	0.9991
Calculated F-value	1.154
Validation parameters	
Specificity & selectivity	1.32
Linearity(µg/ml)	1-24
Robustness(Mean % recovery (±S.D.)	99.72 ± 1.082

Table 3. Accuracy and precision data for the developed methods(each value is result of six separate determinations)

Level	Predicted Conc.Range(µg/ml)*		% R.S.D.	Mean % recovery(± S.D)	Accuracy(%)#
	Range	Mean(± S.D)			
LQC	1.98 – 2.07	2.01 ± 0.028	1.39	99.98 ± 0.602	-0.38
MQC	6.98 – 7.07	7.01 ± 0.028	0.40	99.32 ± 0.455	0.24
HQC	14.81 – 15.01	14.91 ± 0.07	0.48	99.75 ± 0.442	0.41

* Predicted concentration was calculated by linear regression equation.
# Accuracy is given in % relative error (= 100 × [(predicted concentration – nominal concentration)/nominal concentration)].

Table 4. Results of standard addition method (each value is result of three separate determinations).

Sr. No.	Conc. of drug taken in sample solution	Drug amount added	% Analytical recovery (± S.D)
1	15µg	12µg	99.98 ± 0.203
2	15µg	15µg	99.42 ± 0.224
3	15µg	18µg	99.75 ± 0.382

Table 5. Results of intermediate precision study

Concentration (µg)	Intra-day repeatability %R.S.D.* (N=6)			Inter-day repeatability %R.S.D.* (N=18)
	Day-1	Day-2	Day-3	
4	0.991	0.988	0.982	1.322
12	1.023	0.987	1.032	0.988
24	1.116	0.891	1.124	1.117

* Percentage relative standard deviation

Table 6. Application of the proposed spectrophotometric methods for determination of Venlafaxine hydrochloride in dosage forms (each value is the average of five separate determinations.)

Formulation	Percent assay	t#
Brand A(Tablet)	100.23 ± 0.68	1.90(2.30)
Brand B(Capsule)	99.89 ± 0.23	2.01 (2.30)

The values in parenthesis are the tabulated values of t at P = 0.05.

CONCLUSION

In summary, the proposed methods were simple, rapid, accurate, precise and inexpensive and can be used for routine analysis of Venlafaxine hydrochloride in bulk, pharmaceutical formulations and for dissolution studies and therefore, developed analytical methods will be useful for normal dissolution studies. The sample recoveries in all formulations were in good agreement with their respective label claims and thus suggested non-interference of formulations excipients in the estimation.

REFERENCES

1."Effexor Medicines Data Sheet"; Wyeth Pharmaceuticals Inc.http://www.wyeth.com/content/showlabeling.asp?id=99. Retrieved on17 April 2009.

2. Golden RN, Nicholas L (2000). "Antidepressant efficacy of venlafaxine". Depression and anxiety 12 Suppl 1: 45–9.

3. Thase ME, Clayton AH, Haight BR, Thompson AH, Modell JG, Johnston JA . "A double-blind comparison between bupropionXL and venlafaxine XR: sexual functioning, antidepressant efficacy and tolerability". Journal of clinical psychopharmacology 26 (5):482–8.

4. Baldania SL, Bhatt KK, Mehta RS, Shah DA, Gandhi TR. " RP-HPLCestimation of venlafaxine hydrochloride in tablet dosage forms" Indian J Pharm Sci 2008;70:124-8

5. Gursharanjit S., Ghosh B., Dave D. " Screening of Venlafaxine Hydrochloride for delivery". AAPS Pharma Sci Tech, Vol.9, No. 3, Sept2008.

6. The European Agency for the Evaluation of Medical/Products. ICH Topic Q2BNote for Guideline on Validation of Analytical Procedures: Methodology GPMP/ICH/281/95, 1996.

7. United States Pharmacopoeia, in: Validation of Compendial Methods, 26th ed, Pharmacopoeial Convention Inc., Rockville, MD, 2003,pp. 2439–2442.

8. S. Bolton, in: Pharmaceutical Statistics: practical and clinical application,3rd ed, Marcel Dekker, New York, 1997, pp. 216–264.

9. J.C. Miller, J.N. Miller, in:Statistics for Analytical Chemistry, 2nd ed, Wiley, New York, 1984, pp. 83-117.