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Development and Validation of RP-HPLC Method For Determination of Venlafaxine Hcl In Pure and Pharmaceutical Dosage Form

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ABSTRACT

A simple, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed and validated for the estimation of Venlafaxine hydrochloride in pure and Pharmaceutical dosage form. Kromasil C_{18} column having 150 mm x 4.6 mm internal diameter, 5 µm particle sizes in isocratic mode with mobile phase containing mixture of methanol and water in the ratio of 65:35 v/v was used. The flow rate was 1.0 ml/min and effluents were monitored at 225 nm. The retention time for Venlafaxine was 2.424 min. The method was validated for linearity, accuracy, precision, specificity, limit of detection, limit of quantification and robustness. Limit of detection and limit of quantification were found 2.97µg/ml and 9.92 µg/ml respectively and recovery of Venlafaxine from tablet formulation was found 100.4 %. The proposed method was successfully applied for the quantitative determination of Venlafaxine in tablet dosage form.

Keywords: Venlafaxine, HPLC, Validation, Tablets.

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INTRODUCTION

Venlafaxine (Fig.1) is an example of synthetic novel anti-depressant[1]drug, which acts by inhibiting the reuptake of serotonin and nor adrenaline.[2] It is chemically 1-[(1 RS)-2(dimethyl amino)-1-(4-methoxy phenyl) ethyl] cyclohexanol hydrochloride.[3] It is official in European Pharmacopoeia.[4]

The literature survey revealed that some UV methods [5-11] and RP-HPLC methods. [12-19] are reported for the determination of Venlafaxine HCL individually and with other drugs till date. Present study involves the development and validation of a new RP_HPLC method for the determination of Venlafaxine HCL in pure and its pharmaceutical formulations with good accuracy and economy. The analytical method was validated according to ICH validation parameters. [20]

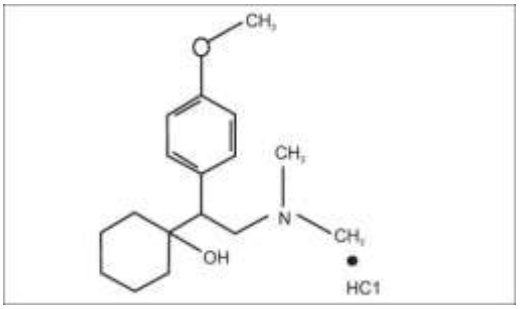

Figure 1: Chemical structure of Venlafaxine HCl

MATERIALS AND METHOD

Instrumentation:

The liquid chromatographic system consisted of Waters HPLC system equipped with a reverse phase C_{18} column having 150 mm x 4.6mm internal diameter, 5 µm particle size, a 2695 binary pump, a 20 µL injection loop and a 2487 dual absorbance detector and running on Waters Empower 2 software. Shimadzu electronic balance (AX-200) was used for weighing purpose.

Chemicals and Solvents:

The working standard of Venlafaxine HCl was provided as gift sample from Spectrum Pharma Research Solutions, Hyderabad, India. The market formulation (Venlaf-ER) tablets containing Venlafaxine hydrochloride 75 mg were procured from local market. Methanol of HPLC grade was purchased from E. Merck, Mumbai, India. HPLC grade water was obtained by double distillation and purification through milli-Q water purification system.

Preparation of Standard Solution:

10 mg of working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 7 ml of diluent and sonicate to dissolve it completely and make

volume up to the mark with the same solvent (Stock solution).Further pipette out 0.9 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of Sample Solution:

10 mg equivalent Venlafaxine hydrochloride tablet powder were accurately weighed and transferred into a 10ml clean dry volumetric flask, add about 1ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent. 20 µL of the blank, standard and sample were injected into the chromatographic system and peak areas of the Venlafaxine hydrochloride was used for calculating the % assay by using the formula.

Chromatographic conditions

HPLC was connected with Kromasil C_{18} column (150 mm x 4.6 mm, 5 µm) as Stationary phase. A mixture of methanol and water in the ratio of 65:35 v/v was used as mobile phase. Injection volume was 20 µL and flow rate was1.0 ml/min and run time was 7.0 min. The column was maintained at ambient temperature and the eluent was monitored at 225 nm.

METHOD VALIDATION

The method was validated for Specificity, linearity, accuracy, precision, limit of detection, limit of quantification and robustness by following procedures.

Specificity:

Commonly used excipients (colloidal silicon dioxide, lactose, magnesium stearate, starch and talc) were spiked into a pre-weighed quantity of drug. The chromatogram was taken by appropriate dilutions and the quantity of drug was determined.

Linearity:

The linearity of the method was determined at six concentration levels ranging from 10 -50 µg/ml for Venlafaxine. Evaluation of the drug was performed with UV detector at 225 nm, peak area was recorded for all the peaks. The correlation coefficient value of Venlafaxine was 0.997. The results show that an excellent correlation exists between peak area and concentration of drug within the concentration range indicated.

Accuracy:

The accuracy of the method was determined by calculating recovery of Venlafaxine by the method of standard addition. Known amount of Venlafaxine was added to a pre-quantified sample solution and the amount of Venlafaxine was estimated by measuring the peak area ratios and by fitting

these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range of 50%, 100% and 150% levels. The amount of Venlafaxine was estimated by measuring the peak area ratios by fitting these values to the straight line equation of calibration curve. From the above determination, percentage recovery and standard deviation of percentage recovery were calculated.

Precision:

The intra-day precision study of Venlafaxine was carried out by estimating the correspondence responses six times on the same day with 100 % concentration and inter-day precision study of Venlafaxine was carried out by estimating the correspondence responses six times next day with 100 % concentration.

Limit of detection and limit of quantification:

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solution using the developed HPLC method. The LOD for Venlafaxine was found to be 2.97µg/ml and the LOQ for Venlafaxine was found to be 9.92 µg/ml.

Robustness:

The robustness was performed for the flow rate variations from 0.8ml/min to 1.2 ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Venlafaxine hydrochloride . The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±0.2ml/min. The method is robust only in less flow condition.

RESULTS AND DISCUSSION

The procedure was optimized with a view to develop an accurate and precise HPLC method for estimation of Venlafaxine in pure and Pharmaceutical dosage form by using a Kromasil C_{18} column having 150 x 4.6 mm internal diameter, 5 µm particle sizes in isocratic mode with mobile phase containing mixture of methanol and water in the ratio of 65: 35 v/v was used. The flow rate was 1.0 ml/min and effluents were monitored at 225 nm. The results of optimized HPLC conditions were shown in Table 1. The method was linear in the range of 10-50 µg/ml for Venlafaxine with correlation coefficient of 0.997. The regression equation of Venlafaxine concentration over its peak area ratio was found to be Y=11859x+32187, where X is the concentration of Venlafaxine and Y is the respective peak area. The linearity results were shown in Table 2 and the linearity curve was shown in Figure 2.

The mean % recovery was found to be 100.04 % for Venlafaxine, which indicate the method is accurate. The results of recovery studies were shown in Table 3. The %RSD for intra-day precision and inter-day precision for Venlafaxine were found to be 0.15 and 0.24, the values were less than 2% which indicate the method is precise. The results of precision studies were shown in Table 4. The retention time of Venlafaxine was 2.42 min. The number of theoretical plates was 4042 and tailing factor was 1.2 for Venlafaxine, which indicates efficient performance of the column. The limit of detection and limit of quantification for Venlafaxine were found to be 2.97µg/ml and 9.92 µg/ml, which indicate the sensitivity of the method. The summary of system suitability parameters and validation parameters were shown in Table 5. Validated method was applied for the determination of Venlafaxine in commercial formulations. The % assay was found to be 99.92 % for Venlafaxine and the assay results were shown in Table 6. Typical chromatogram of drug Venlafaxine was shown in Figure 3. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in the formulation did not interfere with the estimation of the drug by the proposed HPLC method.

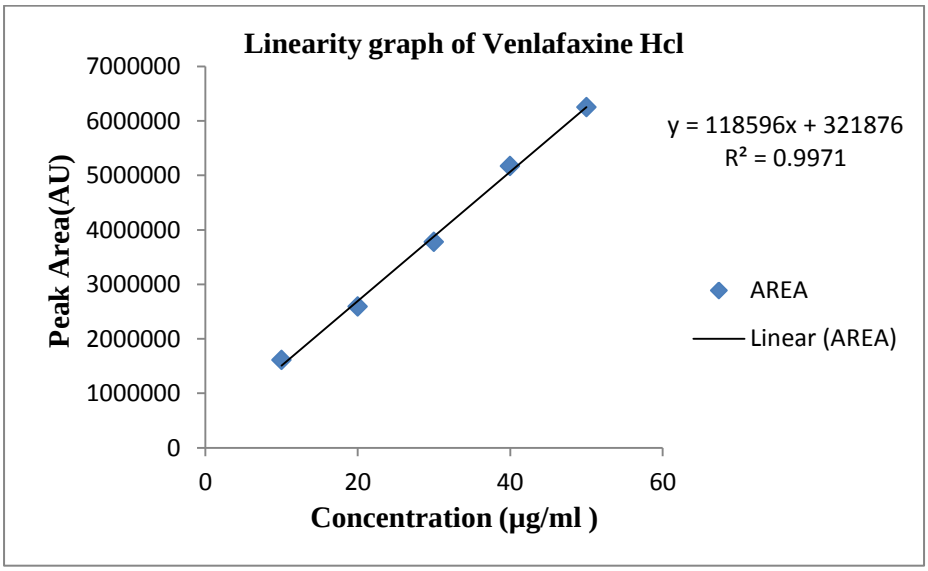

Figure 2: Linearity graph of Venlafaxine HCl

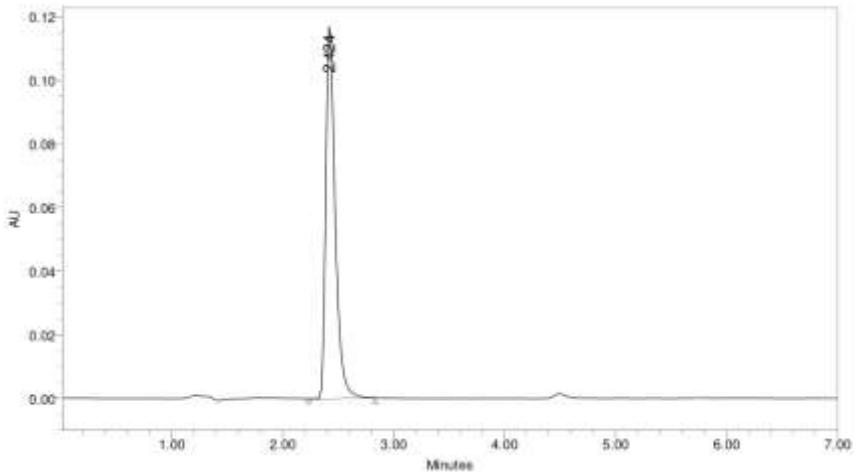

Figure 3: Chromatogram of Venlafaxine HCl

Table 1: Optimized chromatographic conditions

Parameter	Condition
Mobile phase and ratio	Methanol: Water; 65:35% v/v
Column	Kromasil C_{18} 150mm×4.5mm;5.0 µm
Wave length	225 nm
Column Temperature	Ambient
Injection volume	20 µl
Flow rate	1.0 min/ml
Run Time	7 minutes

Table 2: Linearity results of Venlafaxine HCl

S. No	Concentration (µg/ml)	Retention time(min)	Peak area (AU)
1	10	2.428	1608152
2	20	2.422	2592905
3	30	2.430	3778327
4	40	2.426	5170038
5	50	2.433	6249400

Table 3: Accuracy result of Venlafaxine HCl

Spike Level	Amount added (µg/ml)	Amount found (µg/ml)	% recovery	mean % recovery
50%	5	5.14	100.2	
50%	5	4.94	98.8	99.26
50%	5	4.94	98.8	
100 %	10	9.98	99.8	
100 %	10	10.01	98.8	99.53
100%	10	10.02	100.2	
150%	15	14.96	99.73	
150%	15	14.92	99.46	99.55
150%	15	14.96	99.46	

Table 4: Intraday and Inter day Precision Results of Venlafaxine HCl

	S. No.	Intraday Precision	Inter day Precision	Peak Area
1	2.423	693078	2.423	693877
2	2.424	693338	2.424	696531
3	2.424	695080	2.424	693977
4	2.424	694843	2.424	695278
5	2.423	695336	2.423	697676
Average				695468
694335				1642.7
Std. Dev		1047.5		0.24
% RSD		0.15		

Table 5: System Suitability and Validation Parameters

S. No.	Parameter	Results
1	Linearity range (µg/ml)	10-50
2	Slope (m)	11859
3	Intercept (c)	32187
4	Correlation coefficient (r^2)	0.997
5	Retention times (min)	2.482
6	Theoretical plates (N)	4042
7	Tailing factor	1.2
8	Repeatability (%RSD)	0.15
9	Reproducibility (%RSD)	0.24
10	LOD (µg/ml)	2.97
11	LOQ (µg/ml)	9.92

Table 6: Assay results of Venlafaxine HCL in Tablets

Formulation	Label Claim	Amount Found	% Assay
Venlaf-ER	Venlafaxine HCl 75mg/tab	Venlafaxine HCl 74.94 mg/tab	99.92

CONCLUSION

Proposed Study describes new HPLC method for the estimation of Venlafaxine in Pharmaceutical formulation. The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore the proposed method can be used for routine analysis of estimation of Venlafaxine in bulk and its tablet formulation.

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