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A VALIDATED RP-HPLC METHOD FOR ESTIMATION OF VENLAFAXINE FROM TABLETS

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ABSTRACT

An accurate, precise and simple rapid reversed phase high performance liquid chromatographic method has been developed and validated for estimation of venlafaxine in tablet dosage forms. The mobile phase consisted of buffer (pH 3.9; adjusted with ortho phosphoric acid) and acetonitrile at a ratio of 35:65 v/v and mobile phase pumped at a flow rate of 0.6 mL/min with PDA detection at 227nm. The linearity range was found to be 20-60 µg/mL. The method was successfully validated and it was concluded that the developed method was accurate, sensitive, precise, robust and useful for the routine quality control of venlafaxine in pharmaceutical dosage forms.

Key words: Venlafaxine, Tablets, HPLC and Validation

INTRODUCTION

Venlafaxine, (*RS*)-1-[2-dimethylamino-1-(4-methoxyphenyl)-ethyl] cyclohexanol is an antidepressant of the serotonin-norepinephrine reuptake inhibitor (SNRI). It lacks monoamine oxidase activity and more importantly, the adverse effect profile of tricyclic antidepressants. Venlafaxine has no affinity for brain muscarinic, cholinergic, histaminergic or adrenergic receptors [1-4]. A detailed literature survey revealed that several analytical methods were reported for the determination of venlafaxine in pharmaceutical dosage forms by HPLC[5-7], and determination of venlafaxine and its active metabolite O-desmethyl venlafaxine in human plasma by HPLC[8] and LC-MS-MS[9]. The aim of the present work is to develop and validate a simple liquid chromatography method to be applied for the quantification of venlafaxine in tablets, which therefore serves as a tool for the quality control and safety of this type of pharmaceutical preparations.

EXPERIMENTAL

Materials

Venlafaxine (VAF) was received as a gift sample from Cipla Ltd., Mumbai, India. Acetonitrile (HPLC-grade) was purchased from Merck, India. Millipore purification system was used for high purity water. All other chemicals and reagents employed were of analytical grade and were purchased from S.D. Fine Chemicals, India.

Instrumentation and conditions

The chromatograph system comprised of a Water 2695 binary gradient pump, an inbuilt auto sampler, a column oven and Water 2487 dual wavelength absorbance detector (DAD) was employed throughout the analysis. The data was acquired using Empower-2 software. The column used was XTerra symmetry C_{18} (150×4.6 mm, 3.5µm). The eluent was monitored at 227 nm and these conditions the retention time observed for VAF was 2.801min.

Preparation of mobile phase
The mobile phase contained 35% buffer and 65% acetonitrile and delivered at a rate of 0.6 mL/min. An accurately weighed 7.0 g of potassium dihydrogen phosphate transferred to 1000 mL of water and adjusted the pH to 3.0 with orthophosphoric acid. Before use, the mobile phase was degassed by an ultrasonic bath and filtered through a 0.45 Nylon filter.

Preparation of the standard solution
A stock solution of VAF (1000µg/mL) was prepared in water. A standard solution was prepared by dilution of the stock solution with mobile phase to give solution of 100µg/mL. Further dilutions were made with mobile phase to give a solution in concentration range of 20-60 µg/mL.

Preparation of the sample solutions
Twenty tablets (Brands: FLAVIX and VENFAX-PR) were accurately weighed and crushed into a fine powder. The powder equivalent to one tablet (75mg) was taken in 100mL volumetric flask containing 70 mL water, shakes for 15 min on rotary shaker and then sonicated for 20 mins with intermediate shaking. Then the solution was finally made up to mark with water. The resulting solution was centrifuged at 3000 rpm/min for 10 mins to get a clear solution. Then supernatant solution was used to get final concentration of 30µg/mL with mobile phase.

Method development
Various solvent systems were tried for the development of suitable HPLC method for the analysis of VAF in the tablet dosage forms. The suitability of the solvent system was decided on the basis of the sensitivity of the assay, accuracy of the method and availability of cost effective solvents.

Method Validation[10]
Analytical method validation is a process of performing several tests designed to verify that an analytical test system is suitable for its intended purpose and is capable of providing useful and valid analytical data. A validation study involves testing linearity, limit of detection, limit of quantification, precision, accuracy and robustness of a method to determine that it can provide useful and valid data when used routinely. The developed method was validated in terms of specificity, linearity, accuracy, limit of detection, limit of quantification, intra-day and inter-day precision and robustness as per ICH guidelines.

RESULT AND DISCUSSIONS

The method was chosen after several trails with various proportions of buffer and acetonitrile and at different pH values. A mobile phase consisting of buffer (pH 3.0) and acetonitrile at a ratio of 35:65% v/v was selected to achieve best chromatographic peak and sensitivity. The flow rate was 0.6 mL/min, a detection wavelength of 227 nm and an injection volume of 20 µL. System suitability tests were carried out as per USP XXIV requirements. The results were obtained by five replicate injections and shown in Table 1. The linearity of the VAF was verified at five concentration level ranging from 20-60µg/mL. The calibration curve was constructed by plotting peak area against concentration (µg/mL). The regression equation obtained for the VAF was $Y=68655X+57914$ ($r^2= 0.9992$, n=3). The results show that an excellent correlation existed between peak area and concentration of VAF within the concentration range tested (Table 1). The Limit of Detection (LOD) and Limit of Quantitation (LOQ) values were calculated based on the standard deviation (SD) of the response and the slope (S) of the calibration curve at levels approximating the LOD and LOQ, LOD= 3.3 (SD/S) and LOQ= 10 (SD/S) is shown in Table 1.

The accuracy of the method was assessed by determination of recovery for three concentrations (corresponding to 50, 100 and 150% of test solution concentration) covering the range of the method. For each concentration three sets were prepared and injected in duplicate. The mean recovery of VAF was between 99.82-100.45% and %RSD of recoveries between 0.764-0.912. The obtained results were indicating that the method was accurate for the determination of the VAF in tablet dosage forms. Precision was studied to find out intra and inter day variations in the test methods of VAF in the concentration range of 20-60 µg/mL for three times on the same day and different day. Precision was determined by analysing corresponding standard daily for a period of three days. The inter-day and intra-day precision obtained were % RSD (<2) indicates that the proposed method is quite precise and reproducible as shown in Table 2.

The robustness of the method was assessed by assaying test solutions under different analytical conditions deliberately changed from the original conditions. For each different analytical condition the standard solution and test solution were prepared separately. The result obtained from assay of the test solution was not affected by varying the conditions and was in accordance with the true value. System

suitability data were also found to be satisfactory during variation of the analytical conditions. The analytical method therefore remained unaffected by slight but deliberate changes in the analytical conditions (Table 3).

The proposed validated method was successfully applied to determine VAF in tablet dosage forms. The estimated results of VAF formulation were given in Table 4. The results indicating that there was no interference from the excipients in the formulation.

CONCLUSION

The developed method was found to be simple and have short run time which makes the method rapid. The results of the study indicate that the proposed HPLC method is simple, precise, accurate and less time consuming. It can be conveniently adopted for routine quality control testing of VAF in pharmaceutical dosage forms.

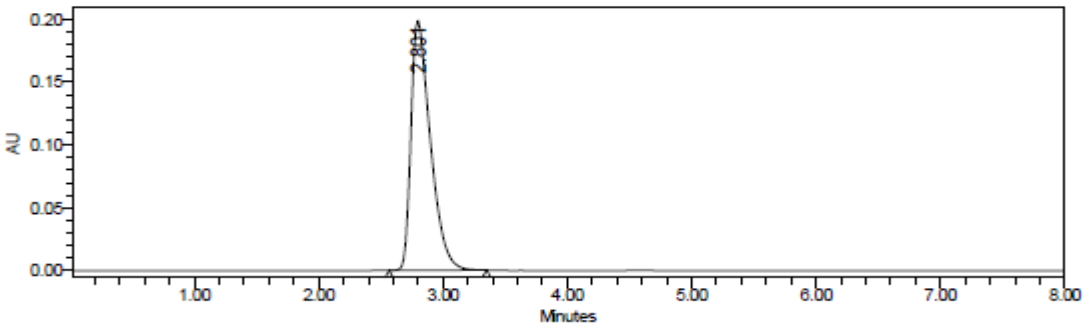

Figure 1: A typical chromatogram of venlafaxine

Table1. Results from regression analysis and system suitability of venlafaxine

Description	Value
Retention time (min)	2.801
Linear range (µg/mL)	20-60
Limit of detection (µg/mL)	0.05
Limit of quantification (µg/mL)	0.18
Regression line	Y=68655X+57914
Correlation coefficient (r)	0.9992
Theoretical plates*	4682
Tailing factor*	1.16

*Five replicate injections

Table2. Results of recovery studies and precision

Actual Conc. (µg/mL)	% Recovery		Precision			
			Intra-day		Inter-day	
	Mean±SD	%RSD	Mean±SD	%RSD	Mean±SD	%RSD
20	99.93±0.887	0.887	99.61±0.742	0.7448	99.46±1.051	1.0571
40	100.45±0.917	0.912	100.44±0.908	0.9083	100.54±1.018	1.0125
60	99.82±0.763	0.764	99.82±0.856	0.8574	99.66±0.665	0.6674

Table 3: Results for robustness of the proposed method

Parameters	Original	Used	Retention time		Assay		Theoretical Plates	Tailing Factor
			Mean±SD	%RSD	Mean±SD	%RSD		
pH of Buffer (±0.1 units)	3.0	2.9	2.845±0.053	1.855	99.66±0.734	0.737	4432	1.26
		3.0	2.801±0.015	0.549	99.77±0.639	0.641	4723	1.18
		3.1	2.857±0.026	0.925	99.53±1.059	1.064	4126	1.21
Organic Strength (±2%)	65	63.7	2.824±0.016	0.559	99.69±0.64	0.642	3952	1.26
		65	2.803±0.007	0.248	100.27±0.673	0.671	4765	1.19
		66.3	2.766±0.027	0.987	100.17±0.88	0.878	4169	1.23

Table 4: Analysis of venlafaxine in tablets

Brand Name	Label Claim per Tablet (mg)	Assay Results		
		% Drug found ± SD (n=6)	%RSD	SEM
FLAVIX	75	100.24±0.682	0.68	0.2784
VENFAX-PR	75	99.81±0.581	0.582	0.2371

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