STABILITY INDICATING AND COST EFFECTIVE ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF VILOXAZINE BY USING REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

The current investigation was pointed at developing and progressively validating novel, simple, responsive and stable RP-HPLC method for the measurement of active pharmaceutical ingredient of Viloxazine. A simple, selective, validated and well-defined stability that shows isocraticRP-HPLC methodology for the quantitative determination of Viloxazine. The chromatographic strategy utilized X-bridge phenyl column of dimensions 250x4.6 mm, 5 μ, using isocratic elution with a mobile phase of acetonitrile and 0.1% Tri fluoro acetic acid (60:40). A flow rate of 1 ml/min and a detector wavelength of 221 nm utilizing the PDA detector were given in the instrumental settings. Validation of the proposed method was carried out according to an international conference on harmonization (ICH) guidelines The calibration chart plotted was linear with a regression coefficient of $R^2 > 0.999$, means the linearity was within the limit. Recovery, specificity, linearity, accuracy, robustness, ruggedness were determined as a part of method validation and the results were found to be within the acceptable range. The proposed method to be fast, simple, feasible and affordable in assay condition. During stability tests, it can be used for routine analysis of the selected drug.

Key words: Viloxazine, RP-HPLC, Development, Validation, Stability.

1.   INTRODUCTION

Viloxazine, sold under the brand name Qelbree among others, is a medication [1] which is used in the treatment of attention deficit hyperactivity disorder (ADHD) in children and depression [2]. Viloxazine acts as a selective norepinephrine reuptake inhibitor (NRI) [3,4]. However, it may also act as an antagonist of the serotonin 5-HT_{2B} receptor [5,6] and as an agonist of the serotonin 5-HT_{2C} receptors [7,8], actions which may be involved in its therapeutic effects [9]. Viloxazine is indicated to treat attention deficit hyperactivity disorder (ADHD) [10,11] in people aged six through seventeen. It was previously marketed as an antidepressant [12,13] for the treatment of major depressive disorder; and is proven to be effective in mild to moderate as well as severe depression with or without co-morbid symptoms. Viloxazine is available for ADHD in the form of 100, 150, and 200 mg extended-release capsules. These capsules can be opened and sprinkled into food for easier administration. Side effects included nausea, vomiting, insomnia [14], loss of appetite [15], increased erythrocyte sedimentation [16], EKG and EEG anomalies, epigastric pain [17], diarrhea, constipation, vertigo [18], orthostatic hypotension [19,20], edema of the lower extremities, dysarthria [21], tremor [22], psychomotor agitation, mental confusion, inappropriate secretion of antidiuretic hormone [23], increased transaminases [24], seizure, (there were three cases worldwide, and most animal studies [and clinical trials that included epilepsy patients] indicated the presence of anticonvulsant [25] properties, so was not completely contraindicated in epilepsy [26]) and increased libido. Chemical structure of Viloxazine was shown in figure 1.

II.   MATERIALS AND METHOD

Chemicals

Acetonitrile (HPLC-grade), Tri fluoro acetic acid, water was purchased from Merck India Ltd, Mumbai, India. API of Viloxazine standard was procured from Glenmark, Mumbai.

Instrumentation

Waters alliance liquid chromatography (model 2695) monitored with empower 2.0 data handling system and a detector of photo diode array (model 2998) was used for this study.

Diluent

Mobile phase was used as diluent.

Preparation of the standard stock solution

For standard stock solution preparation, add 70ml of diluents to 50mg of Viloxazine taken in a 100 ml volumetric flask and sonicate for 10 minutes to fully dissolve the contents and then make up to the mark with diluent. This is called stock solution.1 ml of stock solution was drawn into a 10ml volumetric flask and diluted up to the level. The concentration of the solution was 50µg/ml.

Preparation of the standard stock solution

For sample stock solution preparation, add 70ml of diluents to 97mg of Viloxazine sample (equivalent to 50 mg of Viloxazine) taken in a 100 ml volumetric flask and sonicate for 10 minutes to fully dissolve the contents and then make up to the mark with diluent. This is called sample stock solution.1 ml of stock solution was drawn into a 10ml volumetric flask and diluted up to the level. The concentration of the solution was 50 µg/ml.

Method optimization

To optimize the chromatographic conditions, different ratios of phosphate buffer and the acetonitrile in the mobile phase with isocratic mode was tested. However the mobile phase composition was modified at each trial to enhance the resolution and also to achieve acceptable retention time. Finally 0.1% Tri fluoro acetic acid buffer and acetonitrile with isocractic elution was selected because it results in a greater response of active pharmacy ingredient. During the optimization of the method various stationary phases such as C_8, C_{18} and amino, phenyl columns were tested. From these trials the peak shape was relatively good with X-bridge phenyl column of 250 x 4.6mm, 5 µ with a PDA detector. The mobile phase flow rate has been done at 221nm in order to obtain enough sensitivity. By using above conditions we get retention time of Viloxazine was about 2.272 min with a tailing factor of 1.01. The number of theoretical plates for Viloxazine was 4533 which indicate the column's successful output the % RSD for six replicate injections was around 0.63%, the proposed approach suggests that it is extremely precise. According to ICH guidelines, the method established was validated.

Till today there were no HPLC methods were reported in the literature. Hence we developed a method for the quantification of Viloxazine. The developed HPLC method was utilized for the estimation of the drug by in vitro method.

Validation procedure

The analytical parameters such as system suitability, precision, specificity, accuracy, linearity, robustness, LOD, LOQ, forced degradation and stability were validated according to ICH Q2 (R1) guidelines.

Chromatographic conditions

The HPLC analysis was performed on reverse phase HPLC system with isocratic elution mode using a mobile phase of acetonitrile and 0.1% tri fluoro acetic acid (60:40) and X-bridge phenyl (250x4.6 mm, 5 µ) column with a flow rate of 1 ml /min.

System suitability

System suitability parameters were measured to verify the system performance. The parameters including USP plate count, USP tailing and % of RSD are calculated and found to be within the limit.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of other components, which may be expected to be present in the sample and standard solution. It was checked by examining the chromatograms of blank samples and samples spiked with Viloxazine.

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. It was assessed by the recovery studies at three different concentration levels. In each level a minimum of three injections were given and amount of the drug present, percentage recovery and related standard deviation were calculated.

Precision

Precision of the analytical method is the degree of agreement among individual test results. It was studied by analysis of multiple sampling of homogeneous sample. The precision of the present method was assessed in terms of repeatability, intraday and inter-day variations. It was checked by analyzing the samples at different time intervals of the same day as well as on different days.

Linearity

Linearity of an analytical method is its ability to obtain results directly proportional to the concentration of the analyte in the sample within a definite range. The six series of standard solutions were selected for assessing linearity range. The calibration curve was plotted using peak area versus concentration of the standard solution and the regression equations were calculated. The least squares method was used to calculate the slope, intercept and correlation coefficient.

Stress degradation

Stress degradation should be no interference between the peaks obtained for the chromatogram of forced degradation preparations. Stress degradation studies were performed as per ICH guidelines Q1 (A) R2. The degradation peaks should be well separated from each other and the resolution between the peaks should be at least 1.0 and the peak purity of the principle peaks shall pass. Forced degradation studies were performed by different types of stress conditions to obtain the degradation of about 20%.

Robustness

Robustness of an analytical procedure is a measure of its ability to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness study was performed by injecting standard solution into the HPLC system and altered chromatographic conditions such as flow rate (±0.2 ml/min), organic content in the mobile phase (±10%). The separation factor, retention time and peak asymmetry were calculated by determining the effect of the modified parameters.

III.   RESULTS AND DISCUSSION

The main analytical challenge during development of a new method was to separate active pharmacy ingredient. In order to provide a good performance the chromatographic conditions were optimized.

System suitability

In System suitability injecting standard solution and reported USP tailing and plate count values are tabulated in table 1 and the standard chromatogram was shown in figure 2.

Specificity

In this test method placebo and standard solutions were analyzed individually to examine the interference. The below figure shows that the active ingredient was well separated from blank and their excipients and there was no interference of placebo with the principal peak. Hence the method is specific. Blank chromatogram was shown in figure 3.

Linearity

The area of the linearity peak versus different concentrations has been evaluated for Viloxazine, as 10, 25, 50, 75, 100, 125, 150 percent dilutions respectively. Linearity was performed in the range of 5-75µg/ml of Viloxazine. The correlation coefficient achieved greater than 0.999 for all. Calibration plot of Viloxazine was shown in figure 4 and linearity results were represented in table 2.

Accuracy

Three kinds of concentration levels of 50, 100, and 150 percent at a specified limit were used in this process to assess the accuracy of this particular method. The developed method was found to be highly accurate and reliable. The recovery percentages, ranging from 98.2 to 100.6, were discovered. The results are given in table 3.

Precision

In method precision study prepare six different sample solutions in the concentration of Viloxazine (50 µg/ml)) are injected into HPLC system. Viloxazine % assay found to be in range of 99.6-101.5. Peak areas were calculated, which were used to calculate mean, SD and %RSD values. These results are given below table 4 and method precision chromatogram was shown in figure 5.

Intermediate precision

Separate instruments were used on different days, in different locations, for independent investigations into six different replicates of the standard solution. Mean, RSD values have been calculated and determined from the peak regions. The following table shows the results. Viloxazine (50µg/ml) was analysed on 6 different days with 6 different standards. Mean, standard deviation, and percent related standard deviation values were calculated from peak areas. Thus, it has been found that the current method yields very accurate results, with RSD values less than 2 percent and percent assay values near 100 percent. In table 5 we can see the results.

LOD and LOQ

The LOD concentration Viloxazine was 0.125 µg/ml and s/n values is 6. The LOQ concentration for Viloxazine was 0.413 µg/ml and their s/n values are 25. The method is validated as per the ICH guidelines. Results of LOD and LOQ were shown in table 6 and the chromatograms were shown in figure 6.

Robustness

The design of the experiment was intentionally altered in order to test the robustness of the system. Examples of such changes include changing the flow rate, organic to inorganic ratio, and so on. The results were robust and tabulated in Table 7.

Stability

The solution was kept at room temperature and between 2 to 8 degrees Celsius for up to 24 hours. The experiment showed that the solutions remained stable for at least two days under room temperature and 2-8°C, Viloxazine drug has no effect. The following table 8 illustrates the results of stability.

Degradation studies

Partial degradation of the drug was accomplished using various forced degradation conditions on the Viloxazine standard. Research has been carried out to see if the method works for degrading products. Additionally, the studies describe the conditions under which the drug is unstable, providing further information so that appropriate precautions are taken during the process of formulation in order to avoid possible instabilities. Degradation results were shown in table 9.

Acid degradation: Acid degradation was done by using 1N HCl and 13.4% of Viloxazine degradation was observed.

Alkali degradation: Alkali degradation was done at 1N NaOH and 13.1% of Viloxazine degradation was observed.

Peroxide degradation: Peroxide degradation was performed with 30% hydrogen peroxide and 14.9% Viloxazine degradation was observed.

Reduction degradation: Reduction degradation was performed with 30% sodium bi sulphate solution, 11.4% Viloxazine degradation was observed.

Thermal degradation: In thermal degradation the standard was degraded to 1.9% of Viloxazine.

IV. CONCLUSION

The developed method is accurate, precise and reliable for the analysis of Viloxazine in pharmaceutical formulation. This method was validated for linearity, accuracy, precision, robustness, forced degradation and stability of Viloxazine. The RSD values for all parameters were found to be less 2, which indicates the validity of method and results obtained by this method are in fair agreement. Finally this method can be used for better analysis of Viloxazine.

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Table 1: Results of system suitability

System suitability parameter	Acceptance criteria	Viloxazine
USP Plate Count	NLT 2000	4533
USP Tailing	NMT 2.0	1.05
USP Resolution	NLT 2.0	-
% RSD	NMT 2.0	0.63

Table 2: Linearity of Viloxazine

S.No	Conc µg/ml	Viloxazine area count
1	5.00	242752
2	12.50	582752
3	25.00	1225486
4	37.50	1752306
5	50.00	2457135
6	62.50	2987164
7	75.00	3575856
Correl coef		0.99971
Slope		47895.05
intercept		1440.67

Table 3: Results of accuracy

S. No	% Level	Viloxazine % Recovery	Average % Recovery
1		98.2	
	50	99.3	99.2
		100.0	
2		100.4	
	100	100.6	100.2
		99.7	
3		99.2	
	150	99.9	99.6
		99.8	

Table 4: Intraday precision results of Viloxazine

Viloxazine			
S.No	Conc.(µg/ml)	Area counts	% assay as is
1		2456187	100.9
2		2458963	99.6
3		2425784	99.7
4	50	2436215	100.8
5		2419578	99.4
6		2430160	100.7
% RSD	0.66		
mean			100.2
SD			0.6853

Table 5: Inter-day outcomes of accuracy of Viloxazine

Viloxazine			
S.No.	Conc.(µg/ml)	Area counts	% assay as is
1		2446301	99.6
2		2485794	100.5
3	50	2455631	99.1
4		2412589	98.4
5		2424247	98.5
6		2436508	98.7
%RSD	1.054		
Mean	99.1		
SD	0.8016		

Table 6: LOD and LOQ for Viloxazine

Viloxazine				
LOD		LOQ		
Concentration	S/N	Concentration	S/N	
0.063µg/ml	7	0.206µg/ml	25	

Table 7: Robustness data of Viloxazine

Parameter name	% RSD
	Viloxazine
Flow minus (0.8 ml/min)	0.44
Flow plus (1.2 ml/min)	0.31
Organic minus (54:46)	1.28
Organic plus (66:34)	0.76

Table 8: Stability results of Viloxazine

Stability	Stability at RT		Stability at 2-8°C	
	% assay	% of deviation	% assay	% of deviation
Initial	100	0.00	100	0.00
6 h	99.5	0.50	99.4	0.60
12 h	99.2	0.80	99.1	0.90
18 h	98.5	1.50	97.5	2.50
24 h	96.4	3.60	97.3	2.70

Table 9: Forced degradation results of Viloxazine

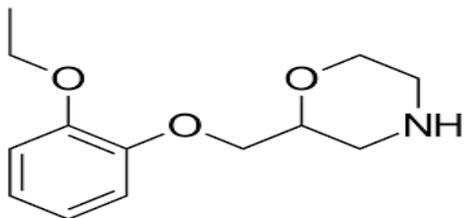| Degradation condition	Viloxazine	
	% assay	%Deg
Acid degradation	86.6	13.4
Alkali degradation	86.9	13.1
Peroxide degradation	85.1	14.9
Reduction degradation	88.6	11.4
Thermal degradation	88.1	1.9

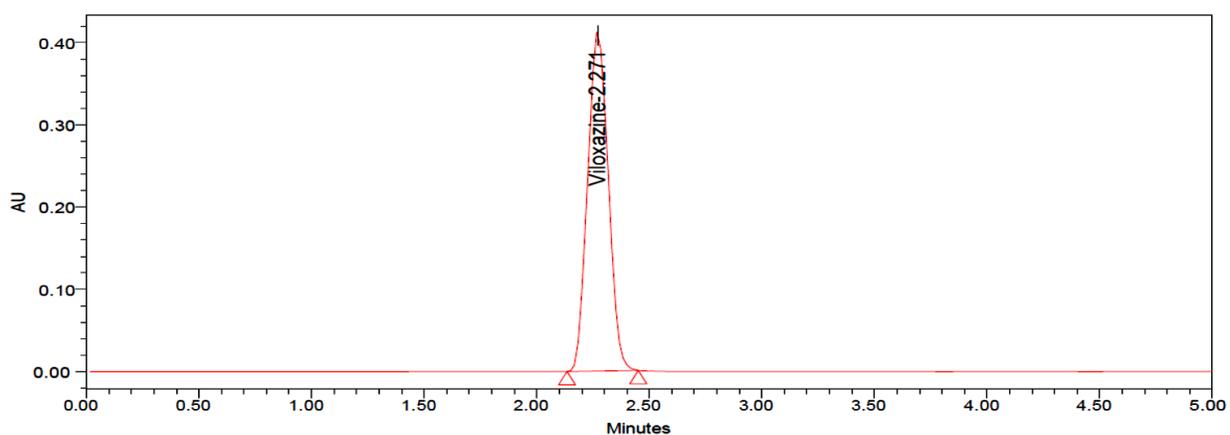**Figure 1:** Chemical structure of Viloxazine

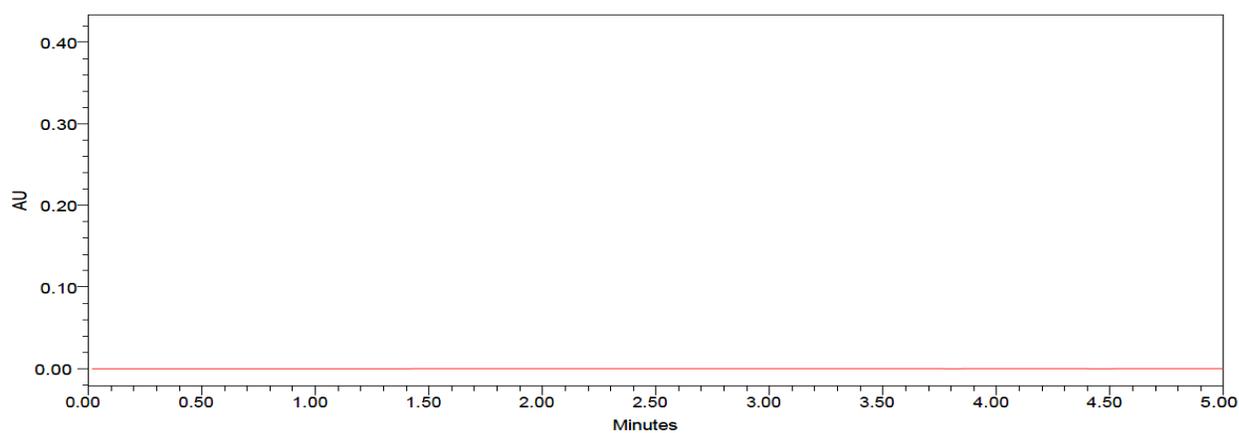
Figure 2: Chromatogram of Standard

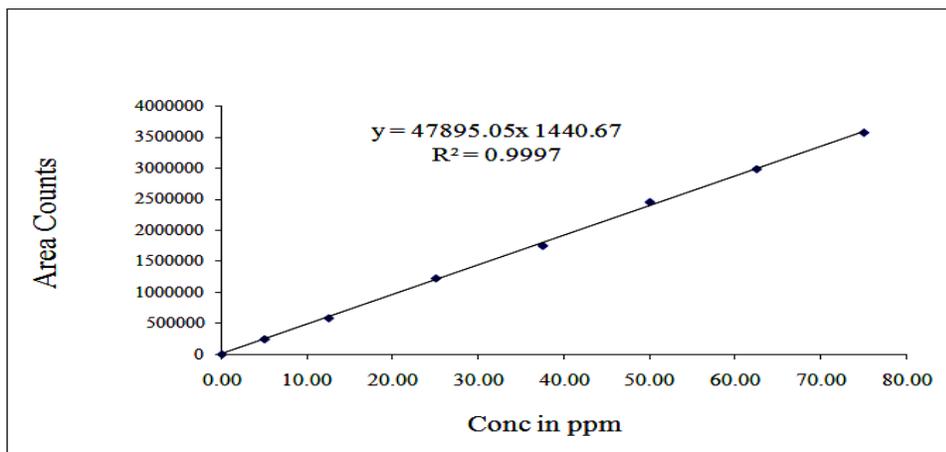**Figure 3:** Chromatogram of blank
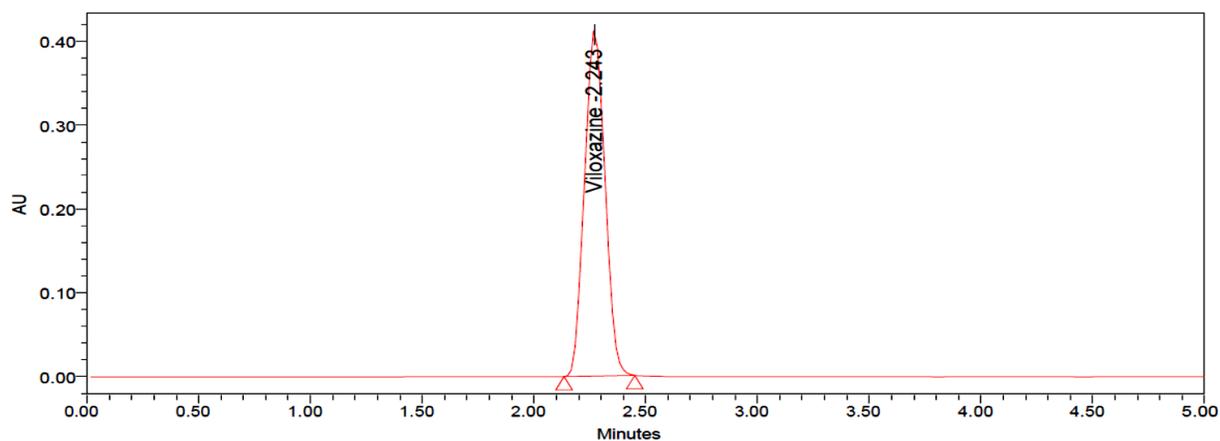
Figure 4: Calibration plot of Viloxazine

Figure 5: Chromatogram of method precision

Figure 6: Chromatogram of (A) LOD and (B) LOQ