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A Novel Validated RP HPLC Method for the Estimation of Vilazodone in Bulk and Pharmaceutical Dosage Form

P. Ravisankar[1*], S. Gowthami[1], CH. Devadasu[1], P. Srinivasa Babu[1], P.Venkateswar Reddy[2]
1. Department of Pharmaceutical Analysis and Quality Assurance, Vignan Pharmacy College, Vadlamudi, Guntur- 522 213. Andhra Pradesh, India.
2. Hetero drugs Limited, Jeedimetla, Hyderabad-500 055,Andhra Pradesh, India.

ABSTRACT

A novel, convenient, accurate, precise and reproducible reverse phase high performance liquid chromatography was developed and validated for the estimation of Vilazodone in bulk and pharmaceutical tablet dosage form. Objective was achieved under optimized chromatographic conditions on Shimadzu LC-20AT Prominence Liquid Chromatograph with Welchrom C_{18} isocratic column, (250 mm × 4.6 mm i.d., particle size 5 µm, maintained at ambient temperature), is used as stationary phase. An isocratic mode with mobile phase consisting of Acetonitrile: Water (50:50 v/v), with apparent pH of 3.3, at a flow rate of 1.0 mL/minutes**.** The effluent was monitored at 240 nm using Shimadzu SPD-20A prominence UV-Vis detector. The retention time of Vilazodone was found to be 4.103 minutes. The linearity range was found to be 1-5 µg/mL with correlation coefficient (R^2) is 0.999. Validation parameters such as specificity, linearity, precision, accuracy, and robustness, limit of detection (LOD) and limit of quantitation (LOQ) were evaluated for the method according to the International Conference on Harmonization ICH Q2 (R1) guidelines. The LOD and the LOQ were found to be 0.044 µg/mL and 0.135 µg/mL respectively. Recovery of Vilazodone was found to be in the range of 99.80 % - 99.92 %. The method was validated statistically using the % RSD and the values are found to be within the limits. Therefore this method was conveniently and easily applied for the quantitative determination of Vilazodone in pharmaceutical dosage forms.

Keywords: Vilazodone, LOD, LOQ, Recovery studies, ICH guidelines.

*Corresponding Author Email: banuman35@gmail.com
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INTRODUCTION

Vilazodone is an anti-depressant drug. Chemically 5-[4-[4-(5-cyano-1H-indole-3-yl) butyl]-1-piperazinyl]-2 benzofurancarboxamide Hydrochloride, with molecular formula of $C_{26}H_{27}N_5O_2$.HCl and it has the molecular weight of 477.99. Vilazodone belongs to the benzofurans[1-2]. These are organic compounds containing benzene ring fused to a furan and chemical structure was shown in Figure 1.

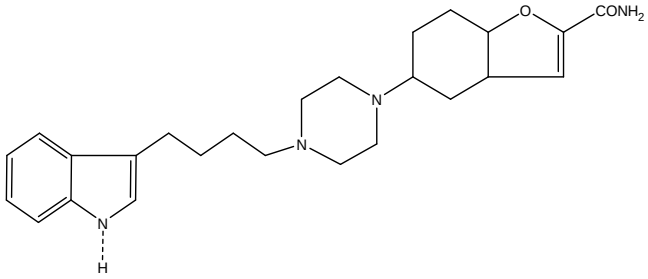

Figure 1: Chemical structure of Vilazodone

Vilazodone is a novel compound combined with high affinity and a selective serotonin reuptake inhibitor[3] (SSRI) and a 5-HT receptor partial agonist. Because of these characteristics, Vilazodone has termed a serotonin partial agonist- reuptake inhibitor (SPARI). Vilazodone was approved by the FDA for the treatment of major depressive disorder in January 2011. Vilazodone is approved for treatment of acute episodes of major depression. A thorough literature survey reveals that only one RP-HPLC technique has been reported for the estimation of Vilazodone in bulk and pharmaceutical dosage forms. However up to some extent the reported HPLC[4] method was having some limitations such as low sensitivity and specificity. Therefore it is necessary to develop a new rapid, precise, cost effective RP-HPLC method for the estimation of Vilazodone in bulk and pharmaceutical dosage forms. Therefore it is necessary to develop a convenient, sensitive and rapid RP-HPLC method for quantitative determination of Vilazodone in pharmaceutical dosage form. The optimized method was developed and validated as per ICH Q2 (R1) guidelines[5].

MATERIALS AND METHODS

Chemicals and Reagents

An analytically pure sample of Vilazodone was procured as gift sample from Hetero Labs Ltd., Hyderabad, Andhra Pradesh, India. All the chemicals were analytical grade. HPLC grade acetonitrile, ortho phosphoric acid and triethylamine were procured from Merck Pharmaceuticals Private Ltd., Mumbai, India. Methanol was utilized of HPLC grade and purchased from Merck Specialties Private Ltd., Mumbai, India. Triple distilled water procured from Vignan Pharmacy College, Vadlamudi, Guntur. Commercial tablets of Vilazodone formulation was procured from

forest laboratories. VIIBRYD tablets containing Vilazodone with labeled amount of 20 mg per tablet.

Instrumentation & Chromatographic conditions

The HPLC analysis was performed on Shimadzu LC-20AT Prominence Liquid Chromatograph comprising a LC-20AT VP pump, Shimadzu SPD-20A, variable wavelength programmable UV/VIS detector SPD-20AVP and Welchrom C_{18} column (4.6 mm X 250 mm, 5 micron particle size). A manually operating Rheodyne injector with 20 µL fixed sample loop was equipped with the HPLC system. The HPLC system was equipped with "Spinchrom" data acquisition software. The mobile phase consists of a mixture of acetonitrile and triple distilled water (pH was adjusted to 3.3 using triethylamine) in ratio of 50:50, v/v. The mobile phase was set at a flow rate of 1 mL/min. Eluate was monitored at 240 nm. An electronic balance (Shimadzu TX223L), digital pH meter (Systronic model 802), UV-Visible Spectrophotometer (Systronic model 2203), a sonicator (spectra lab, model UCB 40) were used in this present study.

Preparation of Reagents and Standards

Mobile phase

Phosphate buffer was prepared by dissolving 1.488 gm of potassium dihydrogen orthophosphate (KH_2PO_4) and 0.288 gm dipotassium hydrogen phosphate (K_2HPO_4) in 500 mL of HPLC grade water. pH was adjusted to 3.3 with ortho phosphoric acid. Triethylamine is used as column modifier. The above prepared buffer and acetonitrile were mixed in the ratio of 50:50 v/v and was filtered through 0.45 µm nylon membrane filter and degassed by sonication. (Note: water mixture with buffer should not be added to acetonitrile it may cause precipitation of the complete mobile phase).

Stock and Working Standard Solutions

Accurately 10 mg of pure Vilazodone was weighed and transferred in to a 10 ml clean and dry volumetric flask and mobile phase was added, if necessary sonicate to dissolve. The volume was brought up to the mark with mobile phase. This is primary stock solution of Vilazodone with concentration of 1000 µg/mL. Secondary stock solution is prepared by adding 1ml of primary stock solution in 10 ml volumetric flask and made up the volume with buffer having the concentration range 100 µg/mL. Five working standard solutions were prepared for calibration graph by adding defined volumes of the secondary stock solution and diluting with mobile phase. The concentrations of Vilazodone are 0.1, 0.2, 0.3, 0.4 and 0.5 µg/mL respectively.

Tablet Sample preparation

Accurately weighed not less than twenty tablets of Vilazodone and average weight was calculated. The Vilazodone (Viibryd) tablets were crushed to get homogeneous powder. Weigh accurately an amount of tablet powder equivalent to 50 mg of Vilazodone were taken and transfer into 50 ml volumetric flask. 40 ml mobile phase was added and place it in an ultrasonicator bath until dissolution was completed. Mobile phase was added to bring up the volume to 50 ml. From the above solution pipette out 1.0 ml of the sample solution into a 10 ml volumetric flask and dilute with mobile phase up to the mark, mix well. This solution was filtered using 0.45µ nylon membrane filter and degassed by sonication. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the sample solution was loaded in the 20µl fixed – sample loop of the injection port.

Selection of detection wavelength

The UV spectrum of diluted solutions for various concentrations of Vilazodone in mobile phase was recorded using UV spectrophotometer. The wavelength of maximum absorbance was scanned over a range of 200-400 nm and the UV spectrum was obtained is shown in Figure 2. The maximum absorbance was found at 240 nm.

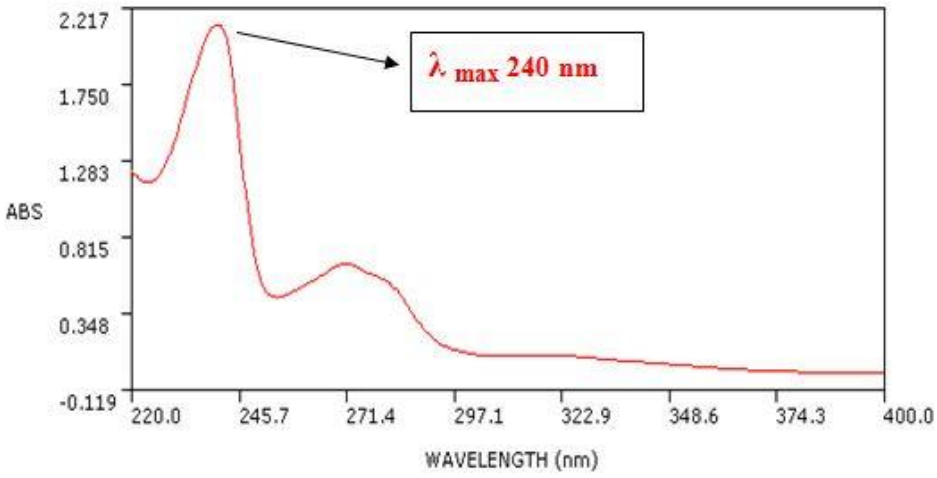

Figure 2: UV spectrum of Vilazodone in methanol

Calibration curve for Vilazodone

Aliquots of standard Vilazodone stock solution were taken in a different 10 mL volumetric flasks and diluted up to the mark with the mobile phase such that the final concentration of Vilazodone were in the range of 1µg/mL, 2µg/mL, 3µg/mL, 4µg/mL,5µg/ml. Replicates of each calibration standard solutions of 1, 2, 3, 4, 5µg/mL were injected using a 20µL fixed loop system and the chromatograms were recorded at 240 nm and a Calibration graph was obtained by plotting peak area versus concentration of Vilazodone. The calibration data is presented in Table 2.

METHOD VALIDATION

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 as per ICH guidelines.

System suitability

System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validation has been completed. The system suitability parameters like Theoretical plates, retention time, tailing factor, were studied and found satisfactory. The results are shown in Table 1.

Table 1: System suitability parameters

Parameter	Chromatographic conditions
Instrument	SHIMADZU LC-20AT prominence liquid chromatograph
Column	WELCHROM C_{18} Column (4.6 mm i.d. X 250 mm, 5 µm particle size)
Detector	SHIMADZU SPD-20A prominence UV-Vis detector
Diluents	Acetonitrile : water (50 : 50) pH : 3.3 using o-phosphoric acid)
Mobile phase	Acetonitrile : water (50 : 50) pH : 3.3 using o-phosphoric acid)
Column modifier	Triethylamine (0.5 mL)
Flow rate	1 mL/min.
Detection wave length	UV at 240 nm.
Run time	8 minutes
Column back pressure	90 kgf
Temperature	Ambient temperature (25^{o}C)
Volume of injection loop	20 µL
Retention time (t_R)	4.103 minutes
Theoretical plates [th.pl] (Efficiency)	11460
Theoretical plates per meter [t.p/m]	2229197
Tailing factor (asymmetry)	1.126

Linearity

The linearity of the method is a measure of how well a calibration plot of response verses concentration, approximates a straight line. The linearity of the method was confirmed over the concentration range of 1-5 µg /mL. A Series of dilutions were prepared by using the working standard solution. From the working standard solution 0.1, 0.2, 0.3, 0.4 and 0.5mL were pipette out into a 10 mL volumetric flasks and diluted with methanol and finally make up to the volume with

methanol. The resulting solutions were labeled as 1, 2, 3, 4 and 5µg/mL. The calibration curves were constructed by plotting absorbance versus concentration and the linearity was calculated by the least square regression method. The linearity data of Vilazodone is presented Table 2. The linearity curve is shown in Figure 3. Optical characteristics, regression data of the proposed method is tabulated in Table 3.

Precision

Precision can be defined as "The degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample". The precision of the method was determined by repeatability (intraday) and intermediate precision (inter-day) and reported as %RSD. For this 10 µg /mL concentration solution was prepared from the working standard solution by taking 1mL of the solution into a 10 mL volumetric flask and diluted with methanol. It was measured six times in the same day for intraday precision and on three different days for interday precision. The percent relative standard deviation (% RSD) was calculated which is within the acceptable criteria of not more than 2.0. The results for intra-day and inter-day precision are presented in Table 4 and Table 5 respectively.

Accuracy (Recovery studies)

The accuracy of the method was evaluated by standard addition method. In this method the volume of the test solution was taken as constant and standard Vilazodone solution was added in increasing amounts equivalent to 80%, 100% and 120% level to each test solution. Known amount of standard Vilazodone of 5 µg/mL concentrations was added in pre-analyzed sample for 8, 10 and 12 µg/mL in triplicate. The percent recovery of the triplicate solutions was determined and average of the percent recovery was calculated. The results are presented in Table 6.

Procedure for the Preparation of Solution for 80% Recovery

From the working standard solution of Vilazodone 5 mL was taken into a 10 mL volumetric flask. To this 8 mL of working test solution was added and mixed well. Volume was filled up to the mark with methanol.

Procedure for the Preparation of Solution for 100% Recovery

From the working standard solution of Vilazodone 5 mL was taken into a 10 mL volumetric flask. To this 10 mL of working test solution was added, mixed well and volume was brought up to the mark with methanol.

Procedure for the Preparation of Solution for 120% Recovery

From the working standard solution of Vilazodone 5 mL was taken into a 10 mL volumetric flask. To this 12 mL of working test solution was added, mixed well and the volume was filled up to the mark with methanol.

Specificity

Specificity of the method was evaluated by assessing whether excipients and other additives that are usually present in pharmaceutical formulations of Vilazodone do not interfered with the peaks of the analyte under optimum conditions. Specificity is shown in Table 7.

Robustness

The robustness of an analytical procedure is the measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. For the determination of a method's robustness, parameters such as variation in detector wavelength are varied within a realistic range and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method's robustness range. The absorbance was measured and assay was calculated for six times. The results of robustness are presented in Table 8.

LOD and LOQ

Limit of Detection is the lowest concentration in a sample that can be detected, but not necessarily quantified under the stated experimental conditions. The limit of quantitation is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy. The LOD & LOQ are shown in Table 9.

Assay of Vilazodone in tablets

The Viibryd tablets were analyzed using the developed method. The assay results were compiled found satisfactory and results of analysis matched with percent label claim of marketed Vilazodone tablets. The Mean percentage found and the RSD values in Table 10 showed that the proposed method can be adopted for the determination of Vilazodone in pharmaceutical tablets. The representative sample chromatogram of Vilazodone is shown in Figure 9.

RESULTS AND DISCUSSION

The present study was aimed at developing a precise, sensitive, rapid and accurate HPLC method for the analysis of Vilazodone in bulk drug and in pharmaceutical dosage forms. In order to achieve phenomenal retention time and peak asymmetry, Welchrom C_{18} isocratic column, (250 mm × 4.6 mm i.d., particle size 5 µm, maintained at ambient temperature) and mobile phase composed of Acetonitrile: Water (50:50 v/v), with pH adjusted to 3.3 using ortho-phosphoric acid

and triethylamine as column modifier at a flow rate of 1.0 mL/minutes was selected. The retention time for Vilazodone was found to be 4.103 minutes. UV spectra of Vilazodone showed that the drug absorbed maximum at 240 nm, so this wavelength was selected as the detection wavelength. The correlation coefficient (0.999) of regression was found almost equal to 1 in the range of 1-5 µg/mL which states that the method was linear to the concentration versus peak area responses. On slight variation in the mobile phase ratio of up to ± 5 %, the change in the peak asymmetry, plate count and retention time are within the limits which indicated that the method is robust and also indicating lack of influence on the test results by operational variable for the proposed method. This shows that the method is having unique system suitability parameters under given conditions. The comparison of chromatograms of placebo, standard and sample, there was no interference observed from the peaks of placebo, standard and sample. It shows that the method is specific. The precision studies were performed and the % RSD of the determinations was found to be 0.14292 for intra-day precision and 0.14031 for inter-day precision which are within the limits. Hence the developed method was found to be precise. The accuracy of the method was found to be good with the overall % RSD for recovery at 80%, 100% and 120% levels were all within the limits. This indicates that the proposed method was found to be accurate. Method validation following ICH guidelines indicates that the developed method had high sensitivity with LOD of 0.044 µg/mL and LOQ of 0.135 µg/mL. The assay results of tablets by applying the HPLC method was found to be within the pharmacopoeial limits and the assay values were found to be 99.902±0.20%.

Linearity:

The method gave a linear response to Vilazodone drug within the concentration range of 1-5 µg /ml with r^2 =0.999 as shown in Figure 3.

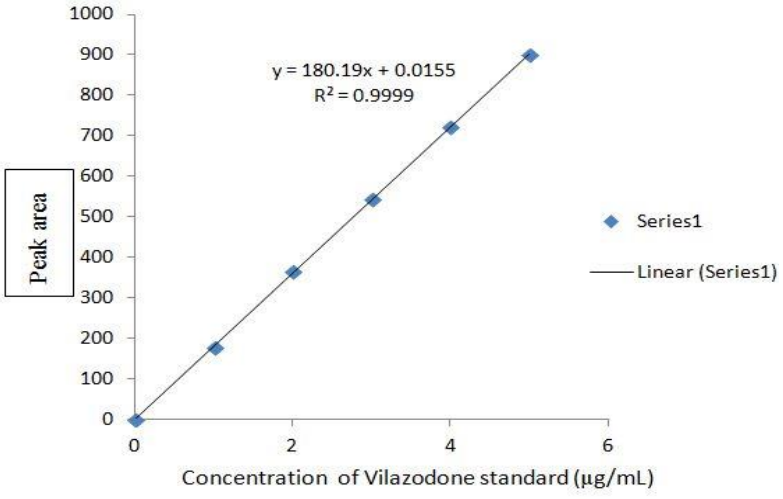

Figure 3: Linearity range graph of Vilazodone

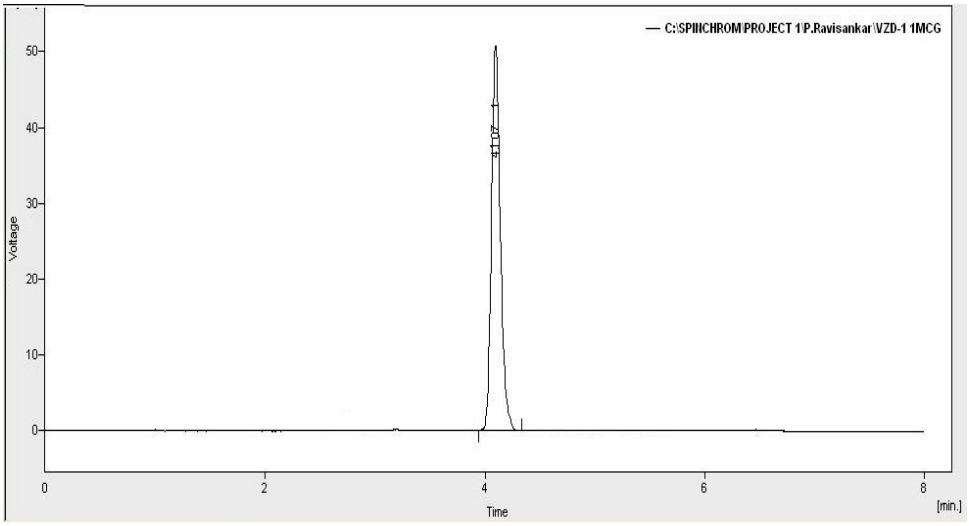

Figure 4: Standard chromatogram of Vilazodone (1 µg/ml)

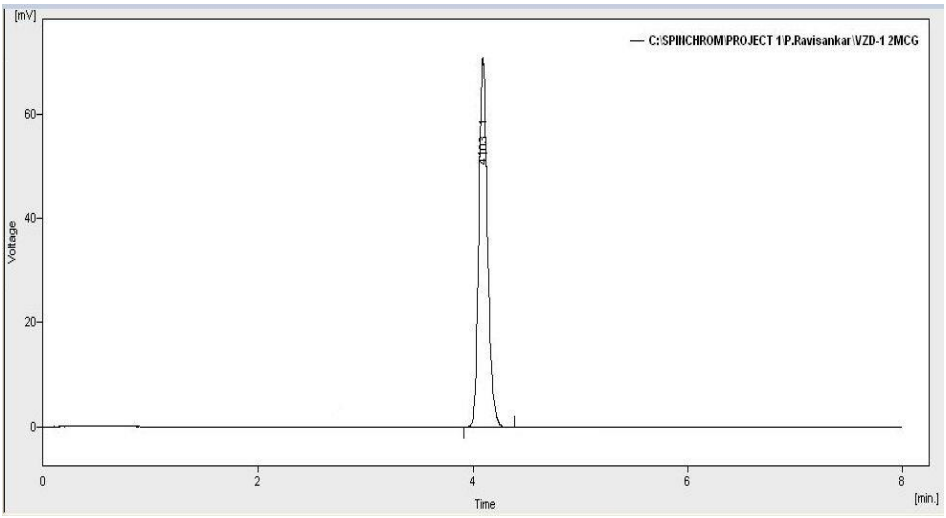

Figure 5: Standard chromatogram of Vilazodone (2 µg/ml)

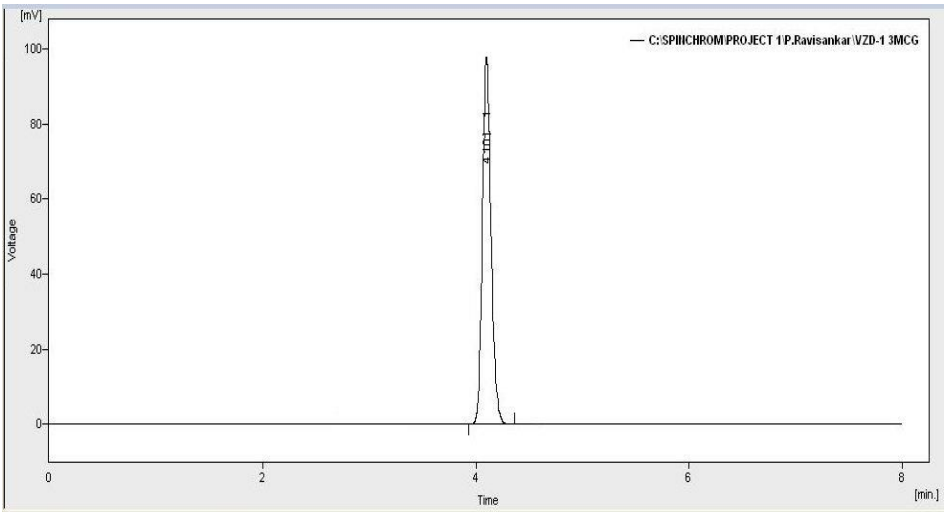

Figure 6: Standard chromatogram of Vilazodone (3 µg/ml)

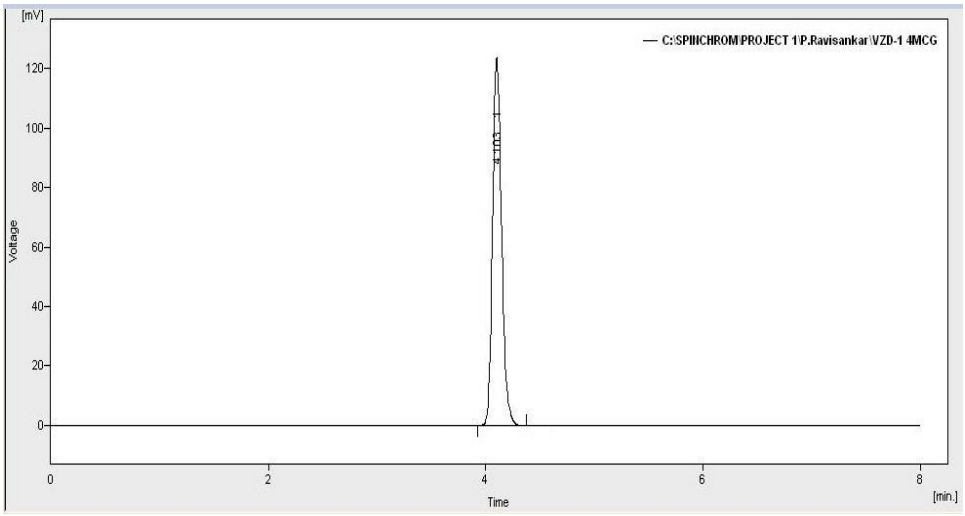

Figure 7: Standard chromatogram of Vilazodone (4 µg/ml)

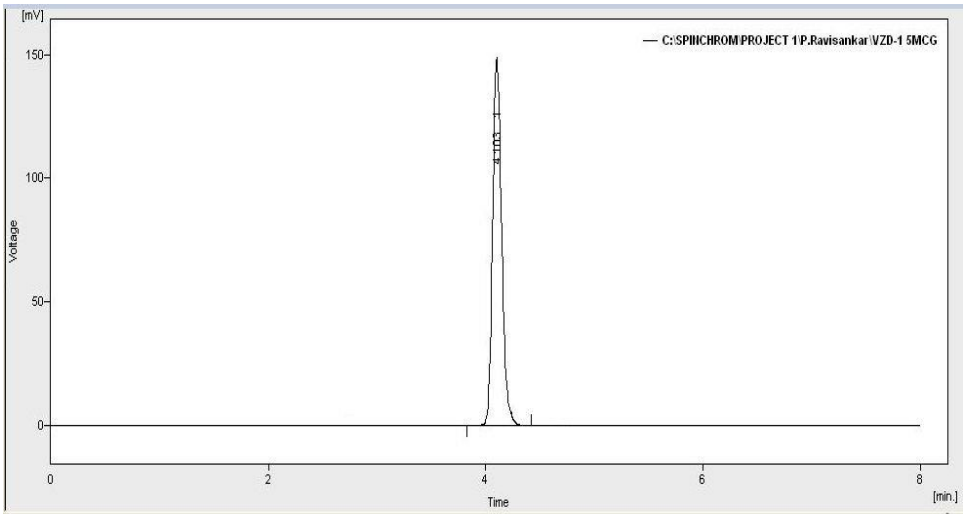

Figure 8: Standard chromatogram of Vilazodone (5 µg/ml)

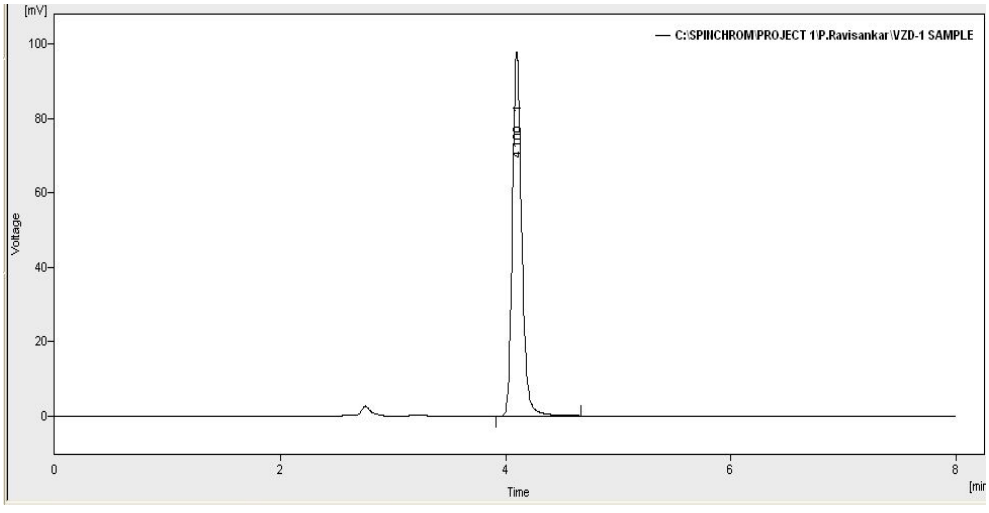

Figure 9: Vilazodone Sample chromatogram

Table 2: Linearity data of the proposed method for the estimation of Vilazodone

S. No	Concentration,µg/ml.	Retention time, (R_t) min.	Peak area, mV.s.
1	1	4.107	175.951
2	2	4.103	364.671
3	3	4.101	542.723
4	4	4.103	720.775
5	5	4.103	898.827

Table 3: Linear regression data of the proposed hplc method of Vilazodone

Parameter	Method
Detection wavelength(λ_{max})	UV at 240 nm
Linearity range (µg/mL)	1-5 µg/mL
Regression equation (Y = a + bX)	Y = 180.1x + 0.015
Slope(b)	180.190
Intercept(a)	0.015
Standard error of slope (S_b)	0.8064
Standard error of intercept (S_a)	2.4415
Standard error of estimation (S_e)	3.37353
Regression coefficient (R^2)	0.999
% Relative standard deviation* i.e., Coefficient of variation(CV)	0.142
Percentage range of errors*	
0.005significance level	0.0033
0.001 significance level	0.0044

*Average of 6 determinations; Acceptance criteria < 2.0

Table 4: Intra-day precision

Sample	Concentration(µg/mL)	Injection no.	Peak area (mV.s)	%RSD#(n=6)
Vilazodone	10	1	545.657	0.14292
		2	544.765	
		3	545.098	
		4	543.709	
		5	545.889	
		6	544.709	

Acceptance criteria < 2.0.

Table 5: Inter-day precision

Sample	Concentration(µg/mL)	Injection no.	Peak area (mV.s)	%RSD#(n=6)
Vilazodone	10	1	542.723	0.14031
		2	544.578	
		3	543.671	
		4	544.432	
		5	542.898	
		6	543.542	

Table 6: Accuracy study is the degree of agreement between a measured value and the accepted reference value.

Recovery level	Amount of sample drug taken (mg)	Amount of standard drug added (mg)	Total Amount (mg)	% recovery (mg)	Mean % Recovery	%RSD# (n=6)
80%	8	5	13	12.95	99.61	0.26
100%	10	5	15	14.98	99.86	0.17
120%	12	5	17	16.96	99.76	0.060

#Acceptance criteria < 2.0.

Table 7: Specificity study for Vilazodone

Name of the solution	Retention time, (t_R) min.
Mobile phase	No peaks
Placebo	No peaks
Vilazodone, 4µg/mL	4.103 min.

Table 8: Robustness results of Vilazodone

S.no	Parameter	Optimized	Used	Retention time (t_R), min	Plate count$	Peak asymmetry#	Remark
1.	Flow rate (±0.2 mL/min)	1.0 mL/min	0.8 mL/min	4.103	11,479	1.118	*Robust
			1.0 mL/min	4.107	11,489	1.126	*Robust
			1.2 mL/min	4.113	11,482	1.130	*Robust
2.	Detection wavelength (±5 nm)	240 nm	255nm	4.103	11,460	1.116	*Robust
			240 nm	4.103	11,479	1.118	*Robust
			220 nm	4.103	11,497	1.117	*Robust
3.	Mobile phase composition (Acetonitrile : Water)	50:50 v/v	55:45, v/v	4.118	11,477	1.122	*Robust
			50:50 v/v	4.103	11,497	1.318	*Robust
			45:55, v/v	4.111	11,516	1.319	*Robust

Acceptance criteria (Limits): #Peak Asymmetry < 1.5, $ Plate count > 3000, * Significant change in Retention time

Table 9: LOD AND LOQ results of Vilazodone

Limit of Detection(LOD)	0.044 µg/mL
Limit of Quantitation(LOQ)	0.135 µg/mL

Table 10: Assay results of Vilazodone formulation

S.no:	Formulation	Labeled amount	Amount found	Mean % recovery ± SD	% RSD(n=6)
1.	Viibryd Tablets	**20 mg**	**19.82**	99.902±0.20	0.10

CONCLUSION

A validated Reverse Phase High Performance Liquid Chromatography method has been developed for the quantitative determination of Vilazodone in bulk and pharmaceutical tablet dosage forms. The method was completely validated shows satisfactory results for all the method validation

parameters tested. The method was free from interference of the other active ingredients and additives used in the formulation. In fact, results of the study indicates that the developed method was found to be accurate, linear, sensitive, economical and reproducible and have short run time which makes the method rapid. Hence it can be concluded that this method may be employed for the routine quality control analysis of Vilazodone in active pharmaceutical ingredient (API) and pharmaceutical preparations.

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REFERENCES

1. Elizabeth choi, monika zmarlicka and megan J.Ehret. Vilazodone: A novel depressant AJHP. 2012; 69, 18, 1551-1557.
2. Laughren TP, Gobburu J. Vilazodone: clinical basis for the US Food and Drug Administration's approval of a new antidepressant. The Journal of Clinical Psychiatry 2011; 72, 9, 1166–73.
3. Hughes ZA, starr KR, langmead CJ. Neurochemical evaluation of the novel 5-ht1a receptor partial agonist/serotonin reuptake inhibitor, Vilazodone. European journal of pharmacology. 2005; 510, 1–2, 49–57.
4. B. Parameswara reddy, N. Pramod, P. Venkateswara rao, A.M.S. Sudhakar babu. Method development and validation for the assay of vilazodone in bulk and formulation by using RP-HPLC technique. International journal of biological & pharmaceutical research 2012; 3, 6, 789-795.
5. ICH Q2 (R1), Validation of analytical procedures, Text and methodology. International Conference on Harmonization Geneva 2005; 1-17.