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NEW HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR DETERMINATION OF VILAZODONE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, precise, rapid and accurate reverse phase HPLC method developed for the estimation of Vilazodone in Tablets dosage form. A Inertsil ODS 3V C18, 250x4.6 mm i.d, 5 µm partical size, with mobile phase containing solvent-A as 0.1% Ammonium dihydrogen phosphate in water with pH adusted to 3.2 with *ortho*-phosphoric acid in 1000ml water and solvent-B as acetonitrile in gradient mode of separation was used. The flow rate was 1 ml/min and the effluents were monitored at 265 nm. The retention time was 4.1 min. The detector response was linear in the concentration of 20-240 mcg/ml. The respective linear regression equation being Y= 43515.779x+22939.55. The limit of detection and limit of quantification was 0.05 and 0.15 mcg/ml respectively. The percentage assay of Vilazodone was 99.16 %. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate; this is useful for the routine determination of Vilazodone in bulk drug and in its pharmaceutical dosage form.

KEYWORDS: Vilazodone, RP-HPLC, Estimation, and Tablets.

INTRODUCTION

Vilazodone (marketed as Viibryd, Forest Pharmaceuticals, St. Louis, MO) is the first in a new class of antidepressants that combine the effect of a serotonin selective reuptake inhibitors (SSRI) with 5-HT1A receptor partial agonist activity.[1] Vilazodone HCl is 2-benzofurancarboxamide, 5-[4-[4-(5cyano-1H-indol-3-yl) butyl]-1-piperazinyl]-, hydrochloride (1:1). This compound belongs to the class of organic compounds known as n-arylpiperazines. These are organic compounds containing a piperazine ring where the nitrogen ring atom carries an aryl group. Its molecular weight is 477.99. Viibryd® Tablets for oral administration contain polymorph Form IV vilazodone hydrochloride (HCl), a selective serotonin reuptake inhibitor and a $5HT_{1A}$ receptor partial agonist. It was approved on January 21, 2011, by the Food and Drug Administration (FDA) for the treatment of major depressive disorder (MDD) in adult patients in the US. Vilazodone acts similarly to SSRIs in that it inhibits the serotonin transporter, desensitizes serotonin receptors, and consequently enhances serotonergic neurotransmission.[2] The drug is classified as a Selective Serotonin Reuptake Inhibitor (SSRI) and is manufactured by Forest Laboratories, Inc. in 10, 20, and 40 mg tablets. Vilazodone also has an inherent selectivity for serotonin-1A receptors, acting as a partial agonist which stimulates the production of serotonin. Vilazodone HCl drug substance is a white to cream-colored solid. It is achiral and slightly hygroscopic. Solid state form analysis demonstrates that it exists in multiple polymorphs. Polymorph-IV is biologically effective and promising agent. The solubility in water is 0.32mg/mL. The partition coefficient between n-octanol and water is 3.71. The pKa is 7.1. Its partial agonist actions at the 5-HT1A receptor may potentially increase the antidepressant effect of the drug by reducing the negative feedback of endogenous serotonin.[3,4] Vilazodone activity primarily comes out of the parent compound. Active metabolites have not been found.[5] Vilazodone is well absorbed after oral administration and its absolute biavailability is 72% in the fed state.[6–8] Vilazodone showed dose proportional pharmacokinetics in the dosage range of 5–80 mg after single and multiple administrations.[6, 9] Peak plasma concentrations were usually reached at 4 hours post-dose. Vilazodone is extensively metabolized by cytochrome P450 (CYP), principally by CYP3A4.[10] Only 3% of the dose excreted from urine and feces as unchanged drug.[9] In the present study, we developed and validated a rapid and sensitive HPLC–UV method to determine vilazodone in pharmaceutical formulations in a run-time of 4.1 min. The short run time enables efficient analysis of large number of formulation samples obtained for regular

qualitative control analysis. To the best of our knowledge, this is the first report of a fully validated HPLC-UV method for the quantification of vilazodone in formulations. This method was successfully applied to the estimation studies of vilazodone in marketed products. Existing literature reveals that there are only few methods for the assay of vilazodone in bulk and dosage forms. Hence an attempt has been made to develop new simple, reliable, and reproducible, isocratic RP-HPLC methods to estimate the vilazodone in bulk and pharmaceutical formulation with good precision, accuracy, linearity and reproducibility respectively. Hence in this present study, we developed a simple, rapid and inexpensive liquid chromatographic method for the analysis of vilazodone in pure and pharmaceutical dosage form. The proposed HPLC method is validated using standard ICH guidelines.

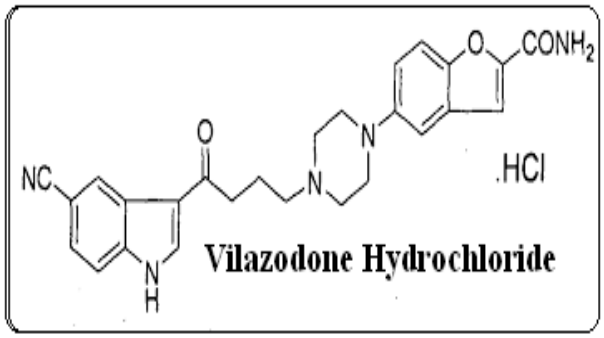

Figure-1: Chemical structure of Vilazodone.

EXPERIMENTAL

MATERIALS AND METHODS

Vilazodone was obtained as a gift sample from Mylan Laboratories, Hyderabad. Ammonium dihydrogen phosphate and *Ortho*-Phosphric acid was of analytical grade, and supplied by M/s S.D.Fine Chem Limited, Mumbai. Acetonitrile and water used were of HPLC grade (Qualigens). Commercially available Vilazodone Tablet (Viibryd® 20 mg, Film coated tablets, Zuventus),) was procured from local market.

Instrument: Quantitative HPLC was performed on the Waters Alliance 2695 Separations Module is a high performance liquid chromatographic system with a quaternary, low-pressure mixing pump and inline vacuum degassing. Flow rates from50 uL/min to 5 mL/min can be generated for use with 2.1 mm ID columns and larger. The auto-sampler has a maximum capacity of 120 vials (12x32, 2-mL) with programmable temperature control from 4 to 40°C. A heated column compartment provides temperatures from 5 degrees above ambient to 65°C. The detector is a photodiode array (model 2996) with a wavelength range of 190-800 nm and sensitivity settings from 0.0001-2.0000 absorbance units. Inertsil ODS 3V-C18 Column (250x4.6 mm i.d; particle size 5 µm) was used. The HPLC system was equipped with LC solution software.

Preparation of the mobile phase: The contents of the mobile phase were 0.05M Ammonium dihydrogen buffer orthophosphate in 1000 ml of water (mobile phase solvent-A) and acetonitrile (mobile phase solvent-B) in a gradient mode of separation was used to resolute the Vilazodone.. They were filtered before use through a 0.45 µm membrane filter, and pumped from the respective solvent reservoirs to the column at a flow rate of 1.0 ml/min in gradient mode. The gradient program has been shown in Table-. The run time was set at 15.0 min and the column temperature was ambient. Prior to the injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluents were monitored at 252 nm.

Table-1: Gradient program for the chromatography of Vilazodone

Time in minutes	Mobile phase solvent-A	Mobile phase Solvent-B
0	70	30
2	70	30
5	20	80
10	20	80
11	70	30
15	70	30

Preparation of Standard drug solution: A standard stock solution of the drug was prepared by dissolving 200 mg of Vilazodone in 100 ml volumetric flask containing 30 ml of acetonitrile and water in the ratio of 40:60 as diluent, sonicated for about 15 min and then made up to 100 ml with mobile phase to get approximately 2000µg/mL.

Working Standard Solution: 5ml of the primary standard stock solution of 2000µg/mL was taken in 10 ml volumetric flask and thereafter made up to 50 ml with mobile phase to get a concentration of 200µg/ml.

Preparation of Sample solution: 20 film coated tablets of Vilazodone (Viiryd® 20 mg, Film coated tablets, Zuventus) were and then powdered. A sample of the powdered tablets, equivalent to 20 mg of the active ingredient, was mixed with 7 ml of mobile phase in 10 ml volumetric flask. The mixture was allowed to stand for 1 hr with intermittent sonication for complete solubility of the drug, and then filtered through a 0.45 µm membrane filter, followed by addition of mobile phase up 100 ml to obtain a stock solution of 2000µg/mL. The resultant solution was further diluted by taking 5 ml of the stock solution with 50 ml of mobile phase to get the concentration of 200µg/mL.

Chromatographic Conditions

Column: Inertsil ODS 3V-C18 Column (250x4.6 mm i.d; particle size 5 µm) Mobile phase—Solvent-A is 0.1% Ammonium dihydrogen phosphate in water with pH adusted to 3.2 with *ortho*-phosphoric acid in 1000ml water and Solvent-B is acetonitrile in gradient mode.

Diluent- Water: Acetonitrile 30:70 (v/v)
Flow rate – 1.0 ml/min. Run time—15 min
Temperature- ambient. Injection volume--20 µl
Detection wavelength--265 nm Retention time—8.07 min

Linearity: Aliquots of standard Vilazodone stock solution were taken in different 10 ml volumetric flasks and diluted up to the mark with the diluent such that the final concentrations of Vilazodone are in the range of 15-180 mcg/ml. Each of these drug solutions (20 µL) was injected three time into the column, and the peak area and retention time were recorded. Evaluation was performed with PDA detector at 265 nm and a Calibration graph was obtained by plotting peak area versus concentration of Vilazodone (Fig 2). The plot of peak area of each sample against respective concentration of Vilazodone was found to be linear in the range of 50-180 µg/ml. µg/ml with correlation coefficient of 0.9999. Linear regression least square fit data obtained from the measurements are given in table I. The respective linear regression equation being Y= 43515.779x+22939.55. The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in **Table II.**

To find out the suitability of the proposed method for the assay of Vilazodone in pharmaceutical dosage forms (Viibryd® 20 mg,Film coaed tablets) the sample solutions from tablets containing Vilazodone were analyzed by the proposed method. A homogenized powder of Viibryd® 20 mg tablets of Vilazodone equivalent to 20 mg of the active ingredient was mixed with 5 ml of diluent in 10 ml volumetric flask. The mixture was allowed to stand for 30 minutes with intermittent sonication for complete solubility of the bulk drug, and then filtered through a 0.45 µm membrane filter, followed by addition of mobile phase up 10 ml to obtain a stock solution of 2000µg/mL as the working sample solution. The mixture was allowed to stand for 1 hr with intermittent sonication for complete solubility of the drug, and then filtered through a 0.45 µm membrane filter, followed by addition of mobile phase up 10 ml to obtain a stock solution of 2000µg/mL. The resultant solution was further diluted by taking 5 ml of the stock solution with 50 ml of mobile phase to get the concentration of 200µg/mL. The results are recorded in Table-4. The retention time was found to be 4.07 mins. The amount of drug present per tablet was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in **Table II**.

Precision studies: The precision of the method was ascertained separately from the peak area obtained by actual determination of 6 replicas of a fixed amount of drug and formulation. The HPLC systems was set up the described Chromatographic conditions, mentioned as above and follow the system to equilibrate, and then injected the 200 µg/ml concentration of Vilazodone standard 6 times and recorded the response (peak area). The proposed method was extended to the pharmaceutical dosage forms by injecting the 200 µg/ml of Vilazodone sample (Bulk drug synthesized in-houses) 6times recorded the response (peak area). The precision was repeated with the formulated sample from (Viibryd® 20 mg, Film coaed tablets) contains Vilazodone of same concentration 6 times and recorded the response (peak area). The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated and presented in Table-III.

Recovery Studies: Accuracy was determined by recovery studies of Vilazodone, known amount of standard was added to the pre-analyzed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in Table II. The study was done at three different concentration levels. Recovery studies were conducted by analyzing pharmaceutical formulation in the first instance for the active ingredient in the concentration of 80% of the working standard (contains 160 µg/mL of Vilazodone); 100% of the working standard solution (contains 200 µg/mL of Vilazodone) and 120% of the working standard solution (contains 240 µg/mL of Vilazodone) by the proposed method. Each concentration was injected 3 times and the peak area was recorded. Known amounts of pure drug [10% of the working standard solution contains 20 µg/mL of Vilazodone for 80% of the working standard, for 100% of the working standard, for 120% of the working standard] was then added to each 3 previously analyzed formulation and the total amount of the drug was once again determined by the proposed method (each concentration was again injected 3 times) after keeping the active ingredient concentration within the linearity limits. The results for recovery studies were shown in **Table-4.**

RESULTS AND DISCUSSION

System suitability studies: The system suitability tests were carried out on freshly prepared standard stock solution of Vilazodone. The system was suitable for use, the tailing factors for Vilazodone were 1.01 and USP theoretical plates were found to be significantly high around 22280.84. Parameters that were studied to evaluate the suitability of the system are given in **Table III.**

Specificity: The effect of wide range of intermediates and other precursors, generally used in pharmaceutical formulations of Vilazodone were investigated under optimized chromatographic conditions. Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. The common excipients present in the pharmaceutical dosage form did not interfere with the elution or quantification of the method. Each Viibryd® 20 mg, Film coated tablets contains

Vilazodone, contains equivalent to Vilazodone 20 mg and the tablets include the following inactive ingredients: lactose monohydrate, microcrystalline cellulose, silicon dioxide, croscarmellose sodium, hydroxyl propyl cellulose, sodium lauryl sulfate, and magnesium stearate. The tablets are film-coated with a coating material containing indigo carmine (FD&C Blue #2) aluminum lake, polyethylene glycol, polyvinyl alcohol, talc, titanium dioxide, and yellow iron oxide. As per the acceptance criteria, the specificity, RSD were found to be less than 2%.

Robustness: A method is robust if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at three different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of the mobile phase flow rate was varied by ±10%), and different column had no significant effect on the retention time and chromatographic response of the method, indicating that the method was robust. When the chromatographic conditions were deliberately altered, system suitability results remained within acceptance limits and selectivity for individual substance was not affected. The results of the study were shown in **Table-5** which proved the robust nature of the method.

Limit of Detection (LOD) and Limit of Quantification (LOQ): The detection limit of the method was investigated by injecting standard solutions Vilazodone into the HPLC column. By using the signal-to-noise method the peak-to-peak noise around the analyte retention time is measured, and subsequently, the concentration of the analyte that would yield a signal equal to certain value of noise to signal ratio is estimated. A signal-to-noise ratio (S/N) of 3 is generally accepted for estimating LOD and signal-to-noise ratio of 10 is used for estimating LOQ. This method is commonly applied to analytical methods that exhibit baseline noise. Chromatograms illustrating the LOD are shown in figure 2.10. The limit of detection (LOD) and limit of quantification (LOQ) for Vilazodone were found to be 0.1 µg/ml and 0.3 µg/ml respectively. The results were shown in Table-II.

Table 2: Linear Regression Data for Calibration curves.

Concentration of drug (µg/mL)	Retention time	Peak Area	Regression Data
20	4.066	848079	Regression Equation:
40	4.063	1768360	Y= 43515.779x+22939.55
80	4.1	3494463	Slope (m): 43515.779
120	4.09	5367711	Intercept (c):22939.55
160	4.1	6992239	Correlation coefficient: 0.99999
200	4.1	8607390	Relative Standard deviation*: 0.1
240	4.11	10505905	% error in bulk samples: 0.2

Table 3: Results of HPLC method

Parameter	Results of the proposed HPLC method
Retention time (min)	4.14
Theoretical plates (n)	5998.04
Plates per meter (N)	23992.16
HETP	4.168028×10^{-5}
Peak asymmetry (T)	1.28
Linearity range (µg/mL)	20-240
Limit of Detection (µg/mL)	0.05
Limit of Quantification (µg/mL)	0.15

*Average of three different concentration levels.

Table-4: Results of Precision Studies

Injection No.	Name of the drug & conc. (200 µg/ml)	Retention time in min.	Peak Area
1	Vibryd® injection-1	4.04	8724653
2	Vibryd® injection-2	4.04	8736646
3	Vibryd® injection-3	4.04	8738289
4	Vibryd® injection-4	4.07	8723406
5	Vibryd® injection-5	4.07	8683831
6	Vibryd® injection-6	4.06	8733638
Mean		4.1	**8723410.5**
% RSD.		0.0	20343.2
Std. Deviation		0.4	**0.2**

Table-5: Results of Accuracy Studies

S.No	Recovery at 80% dilution Level Peak areas		Recovery at 100% dilution Level Peak areas		Recovery at 120% dilution Level Peak areas	
	Standard	Spiked	Standard	Spiked	Standard	Spiked
1	7029652	7893957	8717049	9683958	10447002	11327423
2	7045251	7875675	8746621	9582060	10463601	11338408
3	7055538	7863796	8748010	9608765	10415940	11343795
Avg	7043480.3	7877809.3	8737226.7	9624927.7	10442181.0	11336542.0
Std.Dev	13033.5	15193.4	17488.2	52836.8	24193.5	8344.0
%RSD	0.2	0.2	0.2	0.5	0.2	0.1
Recovery	98.60 %		101.60 %		102.40 %	

Table-6: Results of Robustness Studies:

Parameter	Peak areas of Viibryd® in Flow increase study	Peak areas of Viibryd® in Flow decrease study	Peak areas of Viibryd® in Variable column study
Injection-1	7919868	9840885	8814971
Injection-2	7918609	9814553	8802626
Injection-3	7935173	9792595	8816740
Mean	7924550.0	9816011.0	8811445.7
Std. Dev	9221.3	24178.0	7689.1
% RSD	0.1	0.2	0.1

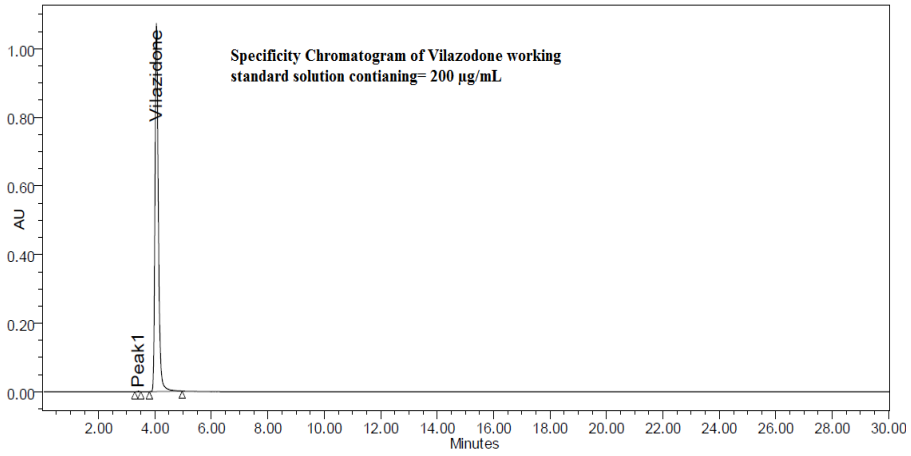

Fig 2: Typical Chromatogram of Vilazodone by RP-HPLC.

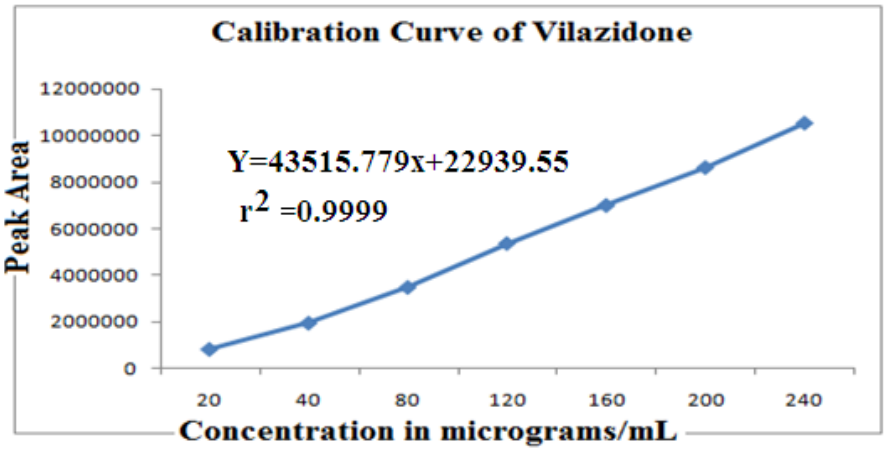

Fig 3: Calibration curve of Vilazodone by RP-HPLC.

CONCLUSION

The author has developed a sensitive, accurate and precise HPLC for the estimation of Vilazodone in bulk drug. From the typical chromatogram of Vilazodone as shown in fig 3.1.2, it was it found that the retention time was 4.41 min. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear

relationship (r^2=0.9998) was observed between the concentration range of 20-240 µg/mL. The assay of Vilazodone in tablet dosage forms was found to be 99.19%. From the recovery studies it was found that about --- % on average of Vilazodone was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the sterile powder for injection. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and sterile powder for injection dosage form of Vilazodone within a short analysis time.

It can be seen from the results presented that the proposed procedure has good precision and accuracy. Results of the analysis of pharmaceutical formulations revealed that proposed methods are suitable for their analysis with virtually no interference of the usual additives present in the pharmaceutical formulations.

The above proposed method obviates the need for any preliminary treatment and is the method could be of use for process development as well as quality assurance of Vilazodone.

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