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Efficient RP-HPLC Method Development for the Analysis of Vilazodone HCl in Pharmaceutical Dosage Form

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Abstract Vilazodone is an anti-depressant drug. A simple, precise and rapid RP-HPLC method was developed for the estimation of Vilazodone in pharmaceutical dosage forms. The mobile phase used for the development of method was phosphate buffer, methanol and ACN in ratio of 55:10:35, v/v. (pH adjusted 6.5). Detection was carried out at 220 nm. The linearity was observed in the concentration range of 30-150 µg/mL. Run time for the method was 10 min and the mean retention time for Vilazodone HCl was found to be at 2.342 min at a flow rate of 1.0 ml/min. The result of the analysis by the proposed method is sensitive, simple, reliable, precise, accurate and robust which indicated good agreement with the label claim of the drug. The method can be used for the routine analysis of the Vilazodone HCl.

Keywords Vilazodone HCl, RP-HPLC, Pharmaceutical Dosage Form

Introduction

Vilazodone is a novel antidepressant agent, approved by the US Food and Drug Administration (US FDA) for the treatment of major depressive disorder. Chemically it is 5-[4-[4-(5-cyano-1H-indole-3-yl) butyl]-1-piperazinyl]-2 benzofurancarboxamide Hydrochloride. [1,2] Vilazodone belongs to the benzofurans. Vilazodone's antidepressant effects are thought to be due to enhancement of serotonergic activity in the central nervous system (CNS) through selective inhibition of serotonin reuptake. [3,4] Literature review revealed that only pharmacological and clinical studies have been reported for the determination of Vilazodone and a pharmacokinetic study which used LC-MS method for the determination of vilazodone. HPLC has become a widely used tool for the routine determination and separation of drugs either alone in pure form or in admixture with other drugs or degradation products and in pharmaceutical formulations. Existing literature reveals that there are only few methods for the assay of vilazodone in bulk and dosage forms. [5-8]

There is no official method for the estimation of vilazodone in the pharmacopoeia`s, it was necessary to develop a new sensitive method for the estimation of the parameters used for the developed method. 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. The proposed method was validated as per ICH guidelines. [9-10]

Material and Methods

Reagents and Materials

The pure drug of VLD HCl was procured as gift sample from Jubilant Life Sciences, Ghaziabad. All the chemicals were used of analytical grade. HPLC grade methanol, acetonitrile, disodium hydrogen phosphate, ortho-phosphoric acid and distilled water were procured from Merck Pharmaceutical Private Ltd., Mumbai, India used. Vilano tablet-Sun Pharma (each tablet contains 20 mg of vilazodone HCl) were purchased from local market. Membrane filters 0.45 µm and 0.2 µm were procured from Millipore Pvt. Ltd. Bangalore, India.

Chromatographic System and Conditions

The chromatographic separation was performed using a C8 Chromasil column (150 × 4.6 mm, 5µm particle size) in isocratic mode. The mobile phase consists of a mixture of phosphate buffer, methanol and ACN in ratio of 55:10:35, v/v. The mobile phase was set at a flow rate of 1 mL/min and the analyte was monitored at 220 nm. The column was maintained at 25°C temperature and injected volume was 20 µl. The total runtime was 10 min. The mobile phase was filtered through 0.2 µm membrane filter prior to use. [11-13]

Mobile Phase Preparation

The mobile phase was prepared by mixing of phosphate buffer (2.72 gm pot. dihydrogen phosphate transferred in 1000 ml volumetric and adjusted pH 6.5 with 0.1N sodium hydroxide), methanol and ACN in ratio of 55:10:35, v/v. It was filtered through 0.2 µm membrane filter and then sonicated for degassing.[14-15]

Preparation of Stock and Working Standard Solutions

Accurately weighed powder equivalent to 30 mg of VLD HCl was transferred in a 100 mL clean, dry volumetric flask and mobile phase was added and sonicated to dissolve. The volume was made up to the mark with mobile phase to prepare 300 µg/mL stock solution. 3 mL of this solution was transferred into 10 mL volumetric flask and volume was made up to the mark with mobile phase to prepare 90µg/mL working standard solution. [16-18]

Preparation of Calibration Curve

Aliquots of standard stock solution of VLD HCl (1.0, 2.0, 3.0, 4.0 and 5.0 mL) were taken in a series of 10 mL volumetric flasks. The volume was made up to the mark with mobile phase to give the concentration range 30-150 µg/mL. Each solution was injected and a chromatogram was recorded. The calibration curves were plotted using the peak areas against the respective concentrations of the drug. [19-20]

Test Sample Preparation

For the estimation of VLD HCl in the tablet formulation, 20 tablets (label claim 20 mg) were accurately weighed and the average weight per tablet was calculated. The tablets were crushed and finely powdered in glass mortar. Powder equivalent to 10 mg of VLD HCl was accurately weighed andtransferred into a 10 mL volumetric flask and sonicated to dissolve. The volume was made up to the mark with mobile phase, mixed well to prepare 1000 µg/mL stock solution. The solution was filtered using 0.2 µm membrane filter and degassed by sonication. 0.9 mL of this solution was transferred into 10mL volumetric flask and volume was made up to the mark with mobile phase to prepare 90 µg/mL test solution. The resulting solution was used as the sample solution for chromatographic analysis. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the sample solution was injected three times and the peak areas were recorded.

Method Validation

The method was validated according to ICH guidelines to study system suitability, linearity, precision, accuracy and robustness, limit of detection (LOD) and limit of quantitation (LOQ).

System Suitability

The chromatographic system used for analysis must pass the system suitability limits before sample analysis. Set up the chromatographic system, allow the HPLC system to stabilize for 30 min. system suitability of VLD HClwas evaluated by injecting blank preparation (single injection) and standard preparation (five replicates) then recorded the chromatograms to evaluate the system suitability parameters like tailing factor (NMT 2.0), theoretical plate count (NLT 2000), peak area and retention time of VLD HCl standard (% RSD NMT 2.0).

Linearity

Linearity was established by least square linear regression analysis of the calibration curve. The linearity response was determined by analyzing calibration curve in the range of 30-150 µg/ml for VLD HCl. Peak area of different concentrations of VLD HCl was plotted versus their respective concentrations. The data was analyzed by linear regression analysis.

Accuracy/Recovery

The accuracy of the method was tested by triplicate samples at 3 different concentrations equivalent to 80%, 100% and 120% of the active ingredient, by adding a known amount of VLD HCl standard to a fixed amount of the pre-analyzed sample of VLD HCl. The recovered amount of VLD HCl, % recovery and % RSD of each level was calculated to determine the accuracy.

Precision

Intra-day precision was investigated by replicate applications and measurements of peak area for VLD HCl for three times on the same day under similar conditions. Inter-day precision was obtained from % RSD values obtained by repeating the assay three times on different days. The percent relative standard deviation (% RSD) was calculated.

Robustness

Robustness can be determined by analysis of solution by changing physical parameters like composition of mobile phase and flow rate. In order to measure the extent of method robustness, the parameters were interchanged while keeping the other parameters unchanged and in parallel, the chromatographic profile was observed and recorded.

Limit of Detection and Limit of Quantitation

LOD and the LOQ of the drugs were calculated using the following equations as per International conference on harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma /S$

Where σ = the standard deviation of the response

S = Slope of calibration curve.

Results and Discussion

Method Development and Optimization

The present study was aimed at developing a precise, sensitive, rapid and accurate HPLC method for the analysis of VLD HCl. In order to achieve phenomenal retention time and peak asymmetry, a C8 Kromasil column (150 mm X 4.6mm i.d, 5 µm particle size) and to optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for VLD HCl was obtained with a mobile phase containing phosphate buffer (pH 6.5), methanol and ACN in a ratio of 55:10:35, v/v at a flow rate of 1 mL/min to get better reproducibility and repeatability. The retention time for VLD HCl was found to be 2.342 min. UV spectra of VLD HCl showed absorbance maxima at 220 nm, so this wavelength was selected as the detection wavelength. Results from method development and optimization studies are given in Table 1.

Table 1: Optimized Chromatographic Conditions for Estimation of VLD HCl by HPLC

Parameter	Chromatographic conditions
Stationary Phase	C8 Kromasil column (150 mm × 4.6 mm i.d, 5 µm particle size)
Mobile phase	mixture of phosphate buffer (pH 6.5), methanol and ACN in ratio of 55:10:35, v/v
Flow rate	1 mL/min
Column temperature	Ambient temperature
Detection wavelength	220 nm
Injection volume	20 µl
Run time	10 min
Retention time	2.342 min

Method Validation

System Suitability

Suitability test parameters of VLD HCl for the developed method are reported in Table 2. % RSD for tailing factor, theoretical plate count, peak area and retention time for VLD HCl were found to be within the limit of 2 %, which indicates suitability of the system. The number of theoretical plates and tailing factor were found within the acceptance criteria of >2000 and ≤ 2.0, respectively, indicating good column efficiency and optimum mobile phase composition.

Table 2: Results from System Suitability Study of VLD HCl by HPLC

Parameter	VLD HCl (90 µg/mL)	
	Mean (n = 5)	%RSD
Retention time (t_R) min	2.335	0.241
Peak area (A)	907322	1.432
Tailing factor (T)	1.403	0.119
No. of theoretical plates (N)	2023.12	0.332

Linearity: The calibration curve was found to be linear over the range of 30-150 µg/ml (Table 3).

Table 3: Linearity Data of VLD HCl by HPLC

S. No.	Conc. (µg/ml)	*Peak Area
1.	30	323712
2.	60	612610
3.	90	901203
4.	120	1193663
5.	150	1499404

*Peak area mean of three replicates

The standard chromatogram of VLD HCl is given in Figure 1.

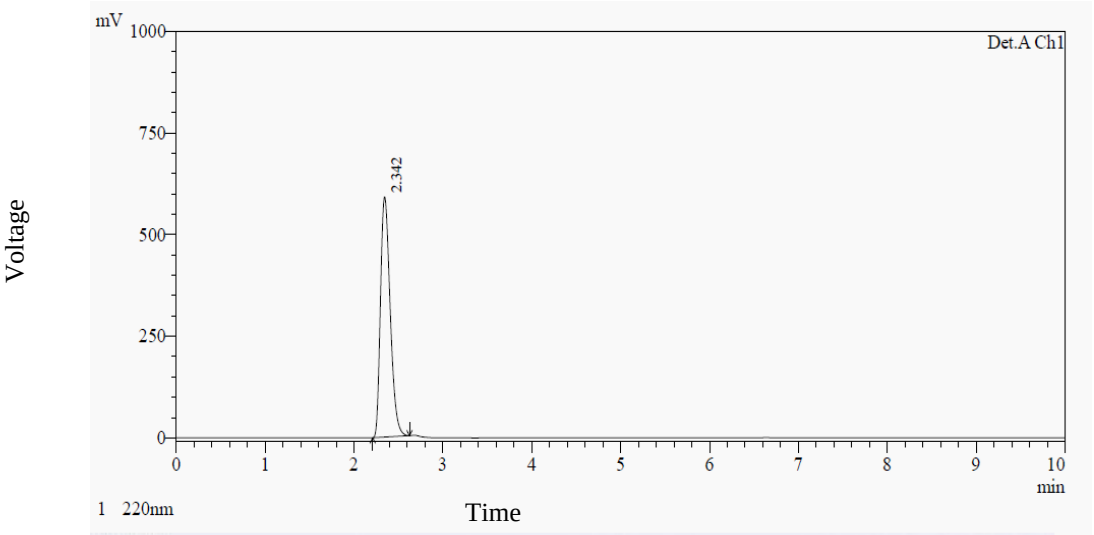

Figure 1: Chromatogram of Standard Solution of VLD HCl

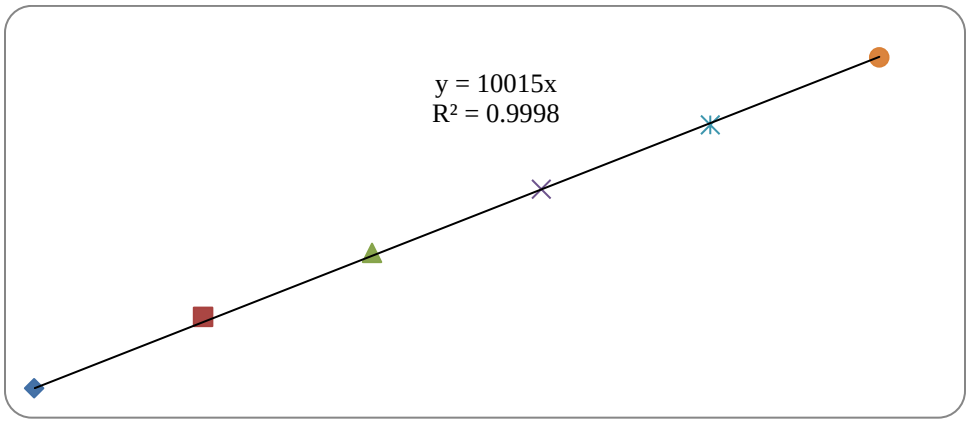

Figure 2: Calibration Curve of Standard Solution of VLD HCl

After linear regression analysis, the correlation coefficient was found to be 0.999, which shows good correlation between peak area and concentration of drug. The regression graph for VLD HCl is presented in Figure 2. The data of regression analysis is presented in Table 4.

Table 4: Results of Regression Analysis of VLD HCl by HPLC

Parameter	**VLD HCl**
Linearity range (µg/mL)	30-150
Regression equation (y = mx + c)	y = 10015x
Slope (m)	10015
Intercept (c)	0
Correlation coefficient (R^2)	0.999
Limit of detection (µg/mL)	2.335
Limit of quantitation (µg/mL)	7.077

Accuracy/Recovery

The mean recovery range of VLD HCl was between 100.06-100.43 % and percentage RSD of recoveries was between 0.01-0.22 percent, which indicates accuracy of the method **(Table 5).**

Table 5: Accuracy Study for VLD HCl by HPLC

Accuracy Level (%)	Amount Added (µg/mL)	Amount Recovered (µg/mL)	% Recovery	Mean	SD	% RSD
	24	23.86	99.40			
80	24	24.05	100.22	100.09	0.10	0.10
	24	24.16	100.65			
	30	30.02	100.05			
100	30	30.02	100.06	100.06	0.01	0.01
	30	30.02	100.08			
	36	36.24	100.66			
120	36	36.15	100.43	100.43	0.22	0.22
	36	36.08	100.22			

Precision: The RSD value of repeatability for VLD HCl was found to be 1.432 (**Table 6**). The RSD value was found to be < 2 %, which indicates that the developed method is repeatable.

Table 6: Repeatability Study for VLD HCl by HPLC

S. No.	VLD HCl	
	Conc. (µg/mL)	Peak Area
1.		890302
2.		906421
3.	90.0	906553
4.		906431
5.		926904
	Avg	907322
	SD	12993.58
	% RSD	1.432

The precision studies were performed and the % RSD of the determinations was found to be 1.06-1.09 for intra-day precision and 1.28-1.37 for inter-day precision (**Table 7**) which are within the limits. Hence the developed method was found to be precise.

Table 7: Precision Study for VLD HCl by HPLC

Conc.	Intra-day (n=3)		Inter-day (n=3)	
(µg/mL)	Mean ± SD	% RSD	Mean ± SD	% RSD
30	313160 ± 3384	1.08	322910±4435	1.37
90	901253 ± 9553	1.06	920059 ±11802	1.28
150	1440591±15688	1.09	1474815 ±19776	1.34

Limit of Detection and Limit of Quantitation: Limit of detection was calculated from standard deviation and slope of calibration curve and it was found to be 2.335 µg/ml for VLD HCl. Limit of quantitation was calculated from standard deviation and slope of calibration curve and it was found to be 7.077 µg/ml for VLD HCl.

Robustness

Two parameters were changed for the study of robustness i.e. composition of mobile phase and flow rate. Small change in these parameters indicated that it did not significantly affect the determination of VLD HCl. Results for robustness are given in table 8.

Table 8: Robustness Study for VLD HCl by HPLC

S. No	Parameter	Optimized Values	Robust Conditions	Retention Time (t_R), min	Plate Count (N)	Tailing Factor (T)
1.	Flow rate	1.0 mL/min	1.1 mL/min	2.197	1973	1.391
			0.9 mL/min	2.483	2044	1.388
2.	Mobile phase composition (Buffer: Methanol: ACN)	55:10:35	55.5:10: 34.5	2.554	2024	1.391
			54.5:10: 35.5	2.136	2045	1.366

Acceptance Criteria: Tailing Factor (T) < 2.0, Plate count (N) > 2000, No significant change in Retention time (t_R).

Assay of Tablet Formulation by HPLC

The developed method was applied to the assay of VLD HCl tablets. From the peak areas the amount of drug present in tablet was estimated. The drug content was calculated as an average of three determinations and assay results were shown in Table 9. The results were very close to the labeled value of commercial tablets. The representative sample chromatogram of VLD HCl is shown in Figure 3.

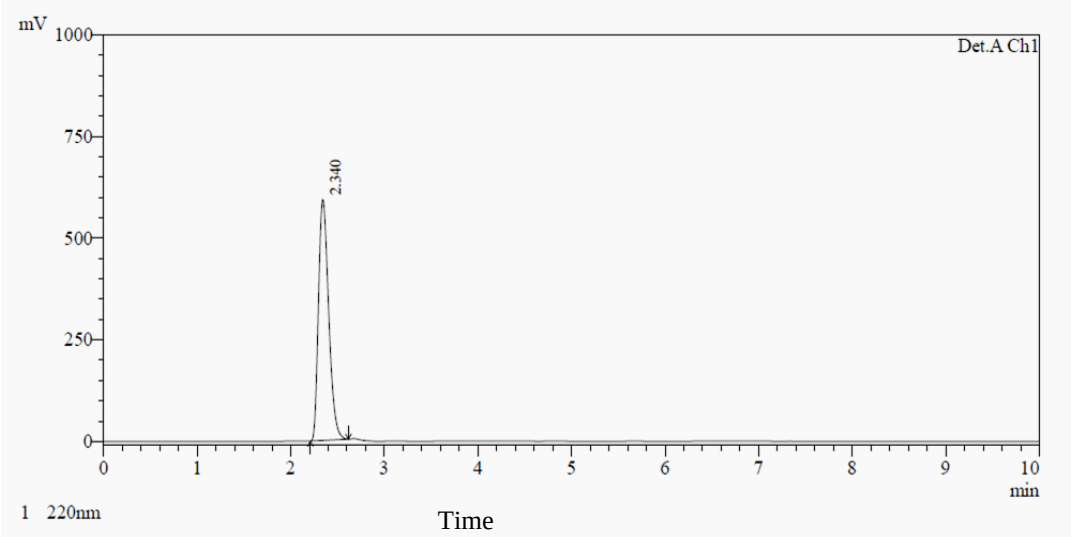

Figure 3: Chromatogram of VLD HCl in Tablet Formulation

Table 9: Results of Analysis of Tablet Formulation of VLD HCl by HPLC

Formulation Name	Label Claim (mg)	Amount Found (mg)	% Label Claim
Vilano Tablet (Sun Pharma)	20	20.093	100.46
	20	20.021	100.20
	20	19.976	99.88
Mean		**20.030**	**100.15**
SD		**0.058**	**0.293**
% RSD		**0.293**	**0.293**

Conclusion

In the present research work, HPLC method have been developed for estimation of Vilazodone HCl from its tablet formulation. RP-HPLC method was used for the estimation of Vilazodone HCl by using C8 Kromasil column. The mobile phase used for the development of method was phosphate buffer, methanol and ACN in ratio of 55:10:35,

v/v. (pH adjusted 6.5). Detection was carried out at 220 nm. The linearity was observed in the concentration range of 30-150 µg/mL. Run time for the method was 10 min and the mean retention time for Vilazodone HCl was found to be at 2.342 min at a flow rate of 1.0 ml/min. The result of the analysis by the proposed method is sensitive, simple, reliable, precise, accurate and robust which indicated good agreement with the label claim of the drug. The method can be used for the routine analysis of the Vilazodone HCl.

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