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Development and Validation of Vilazodone Hcl by Rp-Hplc In Bulk and in Pharmaceutical Formulation Along with its Application in Dissolution

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Abstract

The aim of present work is to develop and validate accurate stability indicating RP-HPLC method for Vilazodone HCl in bulk drug and in tablets and the same is to be applied to dissolution. In RP-HPLC method, the drug was eluted from a Qualisil BDS C18 reversed phase column with methanol as mobile phase at a flow rate of 1 mL/min, UV detection at 242 nm and column temperature 30°C. The retention time for Vilazodone HCl was found to be 3.5 min. The linear response (r2 = 0.9987) was observed in the range of 0.4-1.2 µg/mL with Limits of Detection (LOD) and Limits of Quantitation (LOQ) being 0.04 and 0.12 µg, respectively. The proposed method was validated across the several parameters in accordance with ICH guidelines. The stability indicating studies showed that drug undergoes slight degradation in acidic and basic conditions while it is stable against oxidative and photo degradation. As the method could affectively separate the drugs from their degradation products it can be employed as stability indicating method. In dissolution studies, the medium used is acetic acid PH 3.1 using a paddle apparatus at a stirring rate of 50 rpm. The drug release was evaluated by UV spectrophotometric method at 241 nm for Vilazodone HCl. The method was validated to meet requirements for a global regulatory filing which includes linearity, precision, accuracy, robustness, sensitivity and ruggedness.

Keywords: Vilazodone HCl; RP-HPLC; validation; stability studies; dissolution; ICH guidelines

Introduction

Vilazodone HCl (VLN) is chemically 5-(4-[4-(5-cyano-1H-indol-3-yl) butyl] piperazin-1-yl) benzofuran-2-carboxamide hydrochloride [1] (Figure 1). It contains an indole piperazine that utilizes its function as an SSRI and 5-HT1a receptor partial agonist [2]. It belongs to the category of serotonergic antidepressant approved by FDA (Food and Drug Administration) for treatment of depressive disorder [3]. It is a serotonin reuptake inhibitor and serotonin 1A receptor partial agonist having strong affinity for D2 dopaminergic receptors [4]. It has actions at several 5-HT (serotonin) receptor subtypes. The extensive literature review highlighted the therapeutic and pharmacological profile of drug but no published methods validated for its estimation in pharmaceutical formulations.

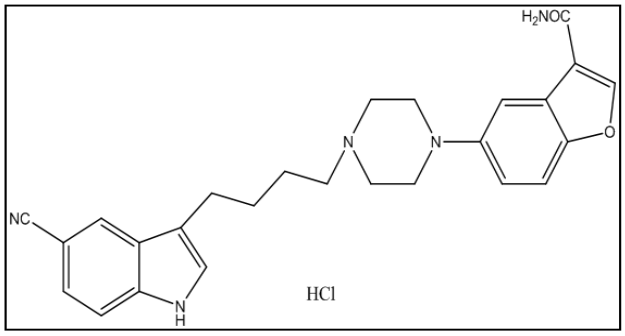

Figure 1: Chemical structure of Vilazodone HCl

Dissolution test has emerged in the pharmaceutical field as a very important tool to characterize drug product performance. It provides measurements of the bioavailability of a drug as well as demonstrates bioequivalence from batch-to-batch. Besides, it is a requirement for regulatory approval for product marketing and is vital component of overall quality control programme.

In an attempt to assess the drug, recently we have published its estimation by spectroscopic method. In continuation to that, in the present study the same was applied for validation of dissolution test for quality control of Vilazodone HCl tablets. This encourages undertaking this work, so that quantitative estimation of VLN can be done and hence can be used for routine analysis of bulk and tablet formulation as well. The present study describes development and validation of rapid, simple, specific, sensitive, accurate and precise RP-HPLC Chromatographic methods for the determination of VLN in bulk and in tablet dosage form.

Dissolution methods, as well as other analytical methods, are validated to ensure that they are suitable for their intended

use and give accurate and reliable data. Guidance on validation characteristics and considerations has been published. Validation of a dissolution method typically involves validation of the end analysis method for linearity, accuracy, precision, range, robustness and solution stability studies [5,6].

Materials and Methods

Materials

Standard gift sample of Vilazodone HCl was provided by Glenmark Pharmaceuticals Ltd., Mumbai (India). Marketed tablets Viibryd (Forest laboratory Ltd.) were used for analysis as formulation. Methanol used for mobile phase is of HPLC grade and from Merck chem. Ltd., Mumbai (India). All reagents and solvents used were of analytical grade. Methanol and 0.01 M HCl of pH 2.0, pH 3.1 acetic acid, pH 10.4 phosphate buffer solutions were prepared according to USP Pharmacopoeia.

Instrument

The chromatographic analysis was performed on Shimadzu HPLC system equipped with PDA detector. The output signals were monitored and processed using LC Solution software. The analytical column was Qualisil BDS C18 (4.6 mm x 250 mm, 5 µ) and the samples were introduced through a Rheodyne injection valve with 20 µL sample loop.

USP Standards tablet dissolution test multi-bath (n = 6) apparatus form Electro lab, Model: TDT-06L was used for dissolution. The medium were vacuum degassed under in house vacuum and maintained at 37.0 ± 0.5°C by using a thermostatic bath. A double-beam UV-Visible double beam spectrophotometer, make: SHIMADZU (model UV-2450) with a pair of 1cm matched quartz cells with spectral band width of 1nm, was used for all absorbance measurements. Systronics pH system (Model: 362) was used to determine the pH of all solutions.

Methods

Determination of Vilazodone HCl in Bulk and in Tablet Formulation by RP-HPLC Detection of wavelength

The drug was accurately weighed 10mg and transferred in 100mL volumetric flask, methanol was added up to the mark. From that solution 0.3 mL was pipette out and diluted with methanol in 10mL volumetric flask. The scanning was done between 200-400nm by UV spectroscopy.

Chromatographic conditions

After performing number of trials by changing the mobile phase composition in various proportions and pH of buffer, finally methanol was selected as mobile phase that gives symmetrical peak. Injection volume was 20 µL, flow rate was 1.0 mL/min and the eluent was detected at 242 nm at column temperature 30°C. The retention time of VLN was obtained at 3.58min, showing a sharp peak. The total run time of analysis was less than 10 min. The chromatogram of Vilazodone HCl is shown in Figure 2.

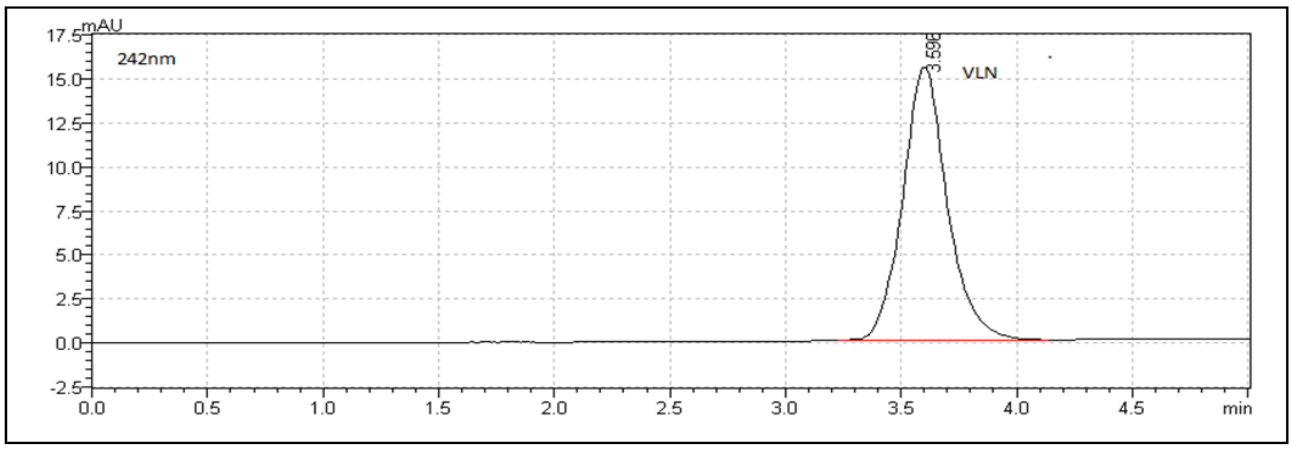

Figure 2: Chromatogram of Vilazodone HCl showing retention time 3.58min

Preparation of stock and standard solution

Standard stock solution was prepared by dissolving 10 mg of VLN in 100 mL methanol that gives concentration of 100 µg/mL. This solution was diluted with mobile phase as needed to prepare different standard solutions.

Validation of proposed method

The proposed method was validated as per ICH guidelines across several parameters like linearity, precision, accuracy, robustness, ruggedness and system suitability test [7,8].

Linearity

From the stock solution of VLN aliquots of 0.4, 0.6, 0.8, 1.0 1.2, and 1.4 mL were taken in 10mL volumetric flasks and diluted up to the mark with mobile phase to get the final concentration in range of 0.4-1.4 µg/mL. Calibration curve was constructed by plotting the peak area vs. the drug concentration.

Precision

Precision can be performed at two different levels-repeatability and intermediate precision. Repeatability refers to

the use of the analytical procedure within the laboratory over the shorter period of the time that was evaluated by assaying the samples during the same day. Repeatability was carried out using six replicates of the sample injection.

Intra-day precision was determined by analyzing, the three different concentrations 0.4, 0.6 and 0.8 µg/mL. of VLN, for three times in the same day. Day to day variability was assessed using above mentioned three concentrations analyzed on three consecutive days for inter day precision.

Accuracy

Accuracy was done by recovery study using standard addition method at 80 %, 100 % and 120 % level; known amount of standard VLN was added to the sample and subjected to the proposed HPLC method. The accuracy studies were carried out three times and the % recovery and % RSD was calculated.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Sensitivity of the proposed method was estimated in terms of LOD and LOQ. LOD is the lowest concentration in a sample that can be detected, but not necessarily quantified; under the stated experimental conditions. LOQ is the lowest concentration of analytes in a sample that can be determined with acceptable precision. In order to determine LOD and LOQ, VLN concentrations in the lower part of the linear range of the calibration curve was used. Dilutions of 0.4, 0.6, 0.8, 1.0, and1.2 µg/mL were prepared for analysis.

Application of proposed method marketed tablets

Twenty tablets, each containing 10 mg VLN, were accurately weighed and finely powdered. A quantity equivalent to 10mg of VLN was transferred to 100 mL volumetric flask and diluted using methanol. The resulting solution was filtered using 0.45 µm filter. It was further diluted for analysis to get a concentration of 1.2 µg/mL. The proposed method was validated in accordance with ICH guidelines.

Robustness

Robustness of the method was studied by making small deliberate changes in few parameters. The flow rate and mobile phase composition were varied by ± 0.2 mL/min and ± 5 %, respectively. The effects on the results were studied by injecting 0.8 µg/mL of VLN.

Ruggedness

From stock solution, sample solution of VLN (0.8 µg/mL) was prepared and analyzed by two different analysts using similar operational and environmental conditions. Peak area was measured for same concentration solutions, six times.

System Suitability Test

System suitability testing is essential for the assurance of the quality performance of chromatographic system. Earlier prepared solutions for chromatographic conditions were tested for system suitability testing.

Forced degradation studies

Initially 10 mg VLN was kept in 0.1N HCl, 0.1N NaOH and 3 % H_2O_2 (10mL each), at room temperature. Simultaneously, 10mg of VLN was exposed to direct sunlight for 12hr. The drug was exposed to higher conditions in gradual increasing manner. Finally an intentional degradation was carried out by refluxing 10mg of VLN in 10 mL 1N HCl and 1N NaOH; while 10 mg drug was dissolved in 30 % H_2O_2 and kept for 24hr. Exposure time to direct sunlight was increased up to 24hr. Acidic and basic samples were neutralized and 20 µL of sample solutions were injected and analyzed [9].

Application of proposed method to dissolution study

Solubility determination and dissolution test optimization

The solubility of Vilazodone HCl was determined in 1000 mL of 0.01 M HCl, pH 3.1 acetic acid, pH 10.4 phosphate buffer using an amount of the drug equivalent to three times of dose in the pharmaceutical formulation [10]. Drug release tests were carried out according to conventional dissolution procedures recommended for single entity products, using paddle (USP Apparatus II) at 50 rpm.

Dissolution study of Vilazodone HCl tablets using absorbance method

The release kinetics of VLN from tablets was studied by conducting dissolution tests. Dissolution tests were performed using (USP Apparatus II) dissolution apparatus and 1000 mL of pH 3.1 acetic acid as dissolution medium at 37 ± 0.5°C at 50 rpm [11]. Sampling aliquots of 10 mL were withdrawn at 0, 5, 10, 15, 20, 30, 45, 60 and 75 min and replaced with an equal volume of the fresh medium. The absorbance of solution was recorded at 241 nm using pH 3.1 acetic acid dissolution medium as blank and drug release was calculated.

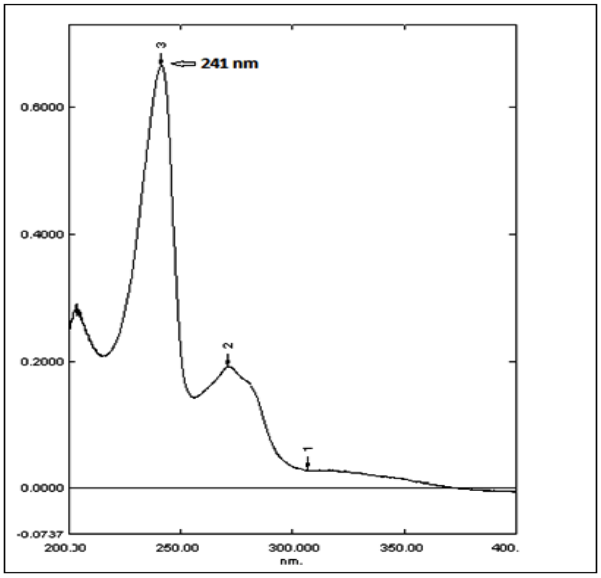

Figure 3: UV absorbance spectra of Vilazodone HCl showing λ max at 241 nm

Linearity

The linearity of drug response is evaluated in the range of 1-5 µg/mL and showed a good correlation coefficient. To assess linearity, the standard curves of VLN was constructed by plotting concentration (µg/ml) vs absorbance as shown in Figure 4.

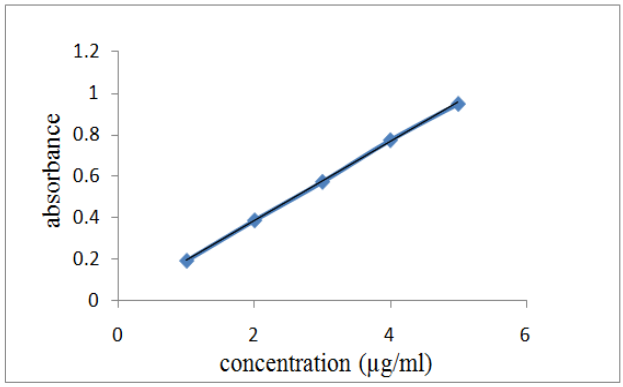

Figure 4: Calibration curve of Vilazodone HCl

Precision

Precision of the method was studied as intra-day and inter-day variations. Intra-day precision was determined by analyzing 2, 3 and 4 µg/mL of solution for three times in the same day. Inter-day precision was determined by analyzing same concentration daily for three consecutive days over a period of week.

Accuracy (Recovery studies)

Accuracy was evaluated by applying proposed method to the analysis of mixture of the tablet and with known amount of working standard, corresponding to the concentrations of 80, 100 and 120 %.

Repeatability

Repeatability was determined by analyzing 3 µg/mL concentration of solution for six times. The absorbance of solution was recorded and spectra were recorded.

Sensitivity

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ were calculated by the use of equation,

$$\mathbf{LOD = SD/S \times 3.3 \text{ and } LOQ = SD/S \times 10,}$$

Where

SD is the residual standard deviation of the peak areas of the drug (n = 6).

'S' is the slope of the line. Sensitivity was performed between 2-3 µg/mL, for method.

Ruggedness

Ruggedness of the proposed method was determined by analysis of from homogenous slot by two different analysts using same operational and environmental conditions.

Results and Discussion

Method A: Determination of Vilazodone HCl in Bulk and in Tablet Formulation by RP-HPLC

A simple, accurate, precise and specific stability indicating RP-HPLC method for estimation of Vilazodone HCl using stressed samples, various mobile phases with different composition and flow rate were tried. After several permutation and combinations, chromatographic condition has been optimized and established. Satisfactory estimation of VLN with good peak symmetry and steady baseline was obtained with the mobile phase methanol at a flow rate of 1.0 mL/min. Drug showed single sharp peak at retention time (RT) of 3.58 min with clear baseline at 242 nm. The detail validation parameters are summarized in Table 1. The standard curve for VLN was linear over the investigated concentration range 0.2-1.0 µg/mL.

Table 1: Summary of Validation Parameters for method A

Parameters	Conc. (µg/mL)	Amt. found (µg) Mean ± S.D.	% R.S.D.	% Recovered
Precision				
Intra-day (n=3)	0.4	0.40 ± 0.021	1.24	-
	0.6	0.60 ± 0.021	0.03	-
	0.8	0.79 ± 0.022	0.73	-
Inter-day (n=3)	0.4	0.40 ± 0.002	1.23	-
	0.6	0.60 ± 0.008	0.60	-
	0.8	0.79 ± 0.015	0.57	-
Recovery studies				
80 %	0.4	0.71 ± 0.029	1.86	99.34
100 %	0.4	0.79 ± 0.040	0.11	98.87
120 %	0.4	0.87 ± 0.012	0.63	99.59
Tablet assay	0.8	0.81 ± 0.020	1.29	101.61
Ruggedness				
Analyst I	0.8	0.81 ± 0.012	1.46	99.51
Analyst II	0.8	0.80 ± 0.016	1.32	100.04

Precision

The precision of an analytical method is the degree of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample. Precision studies of proposed method were determined by repeatability and intermediate precision (intra-day and inter-day precision). Repeatability was measured by multiple injections of 0.8 µg/mL of VLN that indicates the performance of the HPLC instrument under chromatographic conditions. The % RSD was found to be within the limit indicating the proposed method is more precise.

Recovery

The mean recovery data of VLN in sample was 100.03 %, while % RSD was 0.71, that satisfying the acceptance criteria for the study. It proved that there is no interference of excipients used in tablet.

Limit of detection (LOD) and Limit of Quantitation (LOQ)

The LOD with signal-to-noise (S/N) ratio of 3:1 and the LOQ with S/N ratio of 10:1 were calculated for VLN using the equations

$$LOD = 3.3 \times N/B \text{ and } LOQ = 10 \times N/B$$

Where

'N' is the standard deviation of the peak areas of the drug (n=3), taken as measure of the noise

'B' is the slope of corresponding calibration plot.

The signal to noise ratio was determined. LOD and LOQ were found to be 0.15 and 0.47 µg respectively.

Tablet assay

By taking the average of six determinations, the amount found for VLN was 101.61 %. From the data obtained, % RSD of drug was found to be within the limits, thus it can be concluded that excepients do not interfere.

Robustness

The robustness of the proposed method was studied by introducing small deliberate changes in flow rate (±0.2 mL/min) and temperature (± 0.5°C). With respect to these, changes in retention time and tailing factor were observed and the detail results are tabulated in Table 2.

Table 2: Robustness of the proposed method

Runs	Temperature (± 0.5°C).	Flow rate (± 0.2 mL/ min)	Area	Retention time (min)	Tailing factor
1	29.5	0.8	1.182640	3.28	1.14
2	29.5	1.2	0.948374	3.47	1.17
3	30	1.0	0.505412	3.57	1.05
3	30.5	1.2	0.797948	3.78	1.19
4	30.5	0.8	1.178875	3.20	1.20

Recovery

Ruggedness of the method was studied by two different analysts. Method proved to be rugged as it showed low values of % RSD.

System suitability test

The number of theoretical plates and other system suitability parameters were calculated, and were found to be within the limits.

Degradation studies

After exposing the drug to different degradation conditions like acidic, alkaline and oxidation, a single peak of degradation sample was found in acidic and alkaline conditions with less amount of degradent. Finally, VLN was refluxed with 1N NaOH and 1N HCl at 60°C for 3 hr; drug was kept in 30 % H2O2 at room temperature for 24 hr and 10 mg of VLN was exposed to sunlight for 24 hr (6 hr per day). This stress study gives a single peak of degrade in acidic and alkaline conditions with increased amount of degrade. This indicates that the drug is unstable at acidic and alkaline condition. This fact also gives an additional advantage in designing the formulation across the shelf life of VLN (Figure 5 and 6) (Table 3).

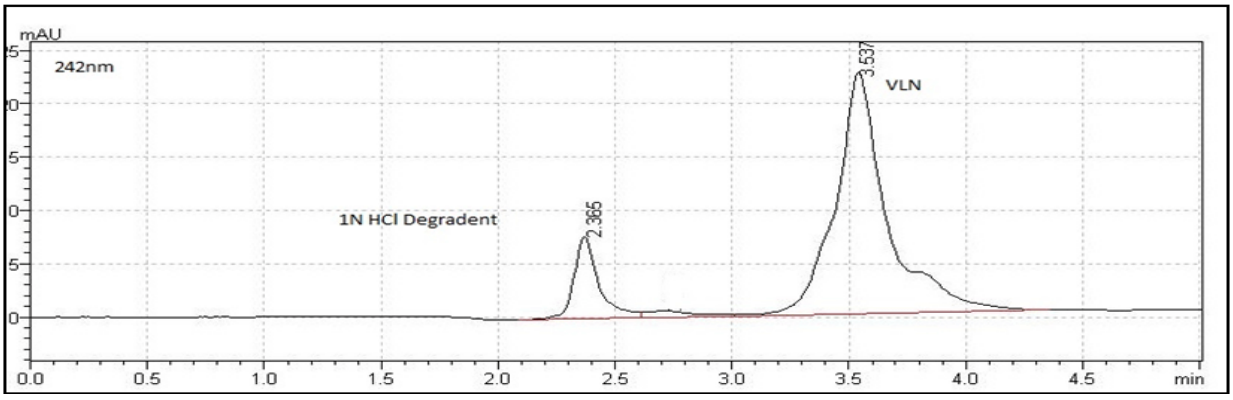

Figure 5: Chromatogram of Vilazodone HCl Degradation in 1N HCl

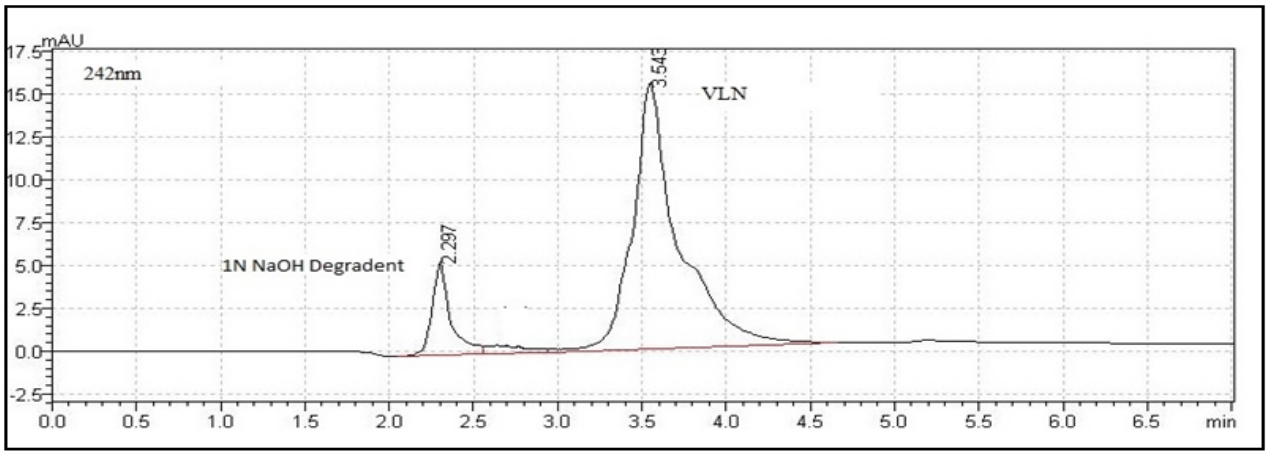

Figure 6: Chromatogram of Vilazodone HCl Degradation in 1N NaOH

Table 3: Forced degradation study

Agent	Exposure time	Conditions	No. of Degradant peak	RT (min)	% Degradation
1N HCl	3 hr	60° C (Reflux)	1	2.35	18.60
1N NaOH	3 hr	60° C (Reflux)	1	2.37	17.55
30% H_2O_2	24 hr	-	-	-	-
Light	24 hr	Sunlight	-	-	-

Method B: Application of proposed method to dissolution study

For dissolution test method the conditions that allowed the dissolution are 1000 mL pH 3.1 acetic acid 37.0 ± 0.5°C paddle apparatus, 50 rpm stirring speed. The in vitro dissolution profiles in different physiological pH mediums at higher speed i.e. 50 rpm, the drug release is more compared to that at 25. This is because of the less solubility of the Vilazodone across all medium. The pH 3.1 shows the faster and completes dissolution, indicates that the drug is to be given with food, to have maximum effect, as the pH of the stomach is around 3 to 4.5 under FED condition. The in vitro dissolution data showed the release was found to be almost 95 % at the end of 75min. The results are tabulated in Table 4 and the release pattern is shown in Figure 7

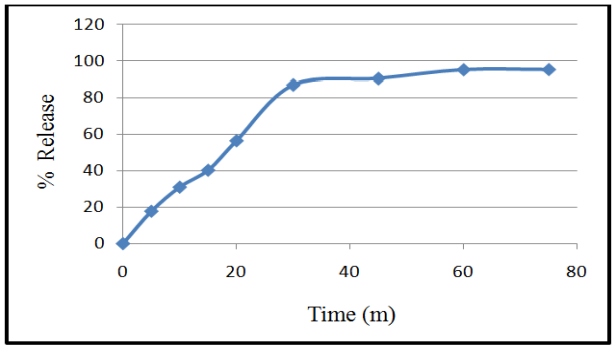

Figure 7: *In vitro* drug release pattern of Vilazodone HCl

Table 4: *In vitro* drug release data of Vilazodone HCl

Sr. No	Sampling Time (min)	Absorbance of Vilazodone HCl	Percentage release of Vilazodone HCl
1	0	0	0
2	5	0.112	17.81
3	10	0.171	13.06
4	15	0.212	40.36
5	20	0.284	56.58
6	30	0.419	97.11
7	45	0.435	90.57
8	60	0.455	95.23
9	75	0.456	95.30

In this method Vilazodone HCl followed linearity in concentration range 1-5 µg/ml. The developed methods were applied for pharmaceutical tablet formulations. The % amount of Vilazodone HCl from tablet formulation was found to be 98.32 %. Precision study at different time and day interval in method showed low standard deviation and % RSD less than 2 indicate that the proposed methods are precise for determination of Vilazodone HCl. High recovery and low standard deviation confirmed that proposed method is accurate for its determination in pharmaceutical tablet formulation. The method was found to be rugged as indicated by low value of % RSD. Results obtained for

LOD and LOQ is a sign of adequate sensitivity of the method. The detailed results showing summary of all validation parameters of proposed methods are tabulated in Table 5. Thus, the method was found to be simple, economical and can suitably apply for the routine analysis of Vilazodone HCl in pharmaceutical tablet formulation.

Table 5: Summary of validation parameter for method B

Parameters	Results
Linearity(µg/ml)	1-5
Y= mx + C	Y = 0.1895x + 0.0063
Correlation coefficient	0.9996
LOD (µg/ml)	0.112
LOQ (µg/ml)	0.341
% Recovery*	99.82
% RSD (Recovery)	0.14
Precision (% RSD)	
Intra- Day*	0.45-0.71
Inter- Day*	0.42-0.74
Repeatability#	1.05
Ruggedness (% RSD) #	
Analyst I	1.02
Analyst II	1.50

**n = 3, #n = 6*

Conclusion

A validated stability indicating RP-HPLC method has been developed for the determination of VLN in bulk and in tablet dosage form. The proposed method is found to be simple, rapid, accurate and precise. The statistical evaluation of the proposed method was revealed its good linearity and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of VLN in tablet formulation. The modalities adopted in experiment were successfully validated as per ICH guidelines, analytical procedures laid down in routine analysis. The proposed method was validated by preliminary analysis of standard sample and by recovery studies. From the results obtained, it concluded that the method is suitable for estimation of Vilazodone HCl. The dissolution study of Vilazodone HCl by UV-Spectroscopy has been done and the dissolution study shows that the drug release at 75 min up to 95 %, which is according to ICH guidelines, hence it can be used for tablet formulation. Therefore the proposed method was successfully applied and suggested for the quality control studies of Vilazodone HCl in tablet dosage forms contributing to assure the therapeutic efficacy of the drug.

Acknowledgement

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