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RESEARCH ARTICLE

Development and Validation of Stability Indicating HPTLC Method for Estimation of Vilazodone Hydrochloride

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ABSTRACT

A simple and sensitive stability indicating HPTLC method has been developed and validated for estimation of Vilazodone hydrochloride. Separation of the drug was carried on aluminium plates precoated with silica gel 60 F_{254} using Toluene: Methanol (7:3 v/v) as mobile phase. The retention factor (R_f) for Vilazodone hydrochloride was found to be 0.50 ± 0.04. The detection was carried at 240 nm. Stress testing of Vilazodone hydrochloride was carried out according to the International conference of harmonization (ICH) guideline Q1A (R2). The drug was subjected to acid, base, neutral hydrolysis, oxidation, thermal degradation and photolysis. The method was successfully validated according to ICH guidelines Q2 (R1). The data of linear regression analysis indicated a good linear relationship over the range of 100–500 ng/band concentrations with correlation coefficient 0.995. The accuracy of the method was established based on the recovery studies. The LOD and LOQ were 2.21 and 6.75 ng/band respectively. Vilazodone hydrochloride showed considerable degradation under alkaline, acidic, oxidative and neutral hydrolytic condition.

KEYWORDS

Vilazodone Hydrochloride, High Performance Thin Layer Chromatography (HPTLC), Validation, Stability-Indicating Method

INTRODUCTION

Vilazodone HCl is an anti-depressant drug. Chemically 5-[4-[4-(5-cyano-1H-indole-3-yl) butyl]-1- piperazinyl]-2 benzofuran carboxamide Hydrochloride (Fig.1), 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 class of benzofurans. These are organic compounds containing benzene ring fused to a furan ring. Vilazodone is a selective serotonin (5-HT) reuptake inhibitor (SSRI) and 5-HT1A receptor partial agonist with a novel chemical structure unrelated to conventional SSRIs.

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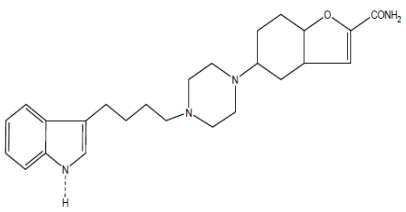
Because of these characteristics, Vilazodone has been termed as a serotonin partial agonist-reuptake inhibitor (SPARI). Vilazodone was approved by the FDA for the treatment of major depressive disorder in January 2011[1].

Literature search reveals following methods reported viz., Simple spectrophotometric method for the estimation in pharmaceutical dosage form[2], Simple RP-HPLC method for estimation in bulk and pharmaceutical dosage form[3], Stability indicating RP-HPLC method for estimation in pharmaceutical dosage form[4], Isolation and structure elucidation of major alkaline degradation products by HPLC in bulk form[5], Spectrofluorimetric and RP-HPLC with Fluorimetric Detection Methods for the

determination in bulk and pharmaceutical prepration[6] and LC-MS/MS method for estimation in rat plasma[7]. To the best of our knowledge, no stability indicating HPTLC method has been reported. The present work describes a simple stability indicating HPTLC method for the determination of Vilazodone hydrochloride, according to the international conference on harmonization (ICH) guidelines[8-10].



.HCl

Figure 1: Chemical structure of Vilazodone Hydrochloride

MATERIAL AND METHODS

Chemicals and Reagents

Vilazodone hydrochloride was provided as a gift sample by Manus Aktteva biopharm ltd, Surat and used as such, without any further purification. Aluminum sheets precoated with silica gel (60 F_{254}, 20 cm × 20 cm with 250 µm layer thickness) were purchased from E-Merck, Darmstadt, Merck (Germany). Methanol (AR grade), Toluene (AR grade) were purchased from S. D. fine chemical Laboratories, Mumbai. Hydrochloric acid (HCl), hydrogen peroxide (H_2O_2) and sodium hydroxide (NaOH) were purchased from Loba Chemie Pvt. Ltd. Mumbai.

Chromatographic Conditions and Instrumentation

Chromatographic separation of drug was performed on Aluminum plates precoated with silica gel 60 F_{254}, (10 cm × 10 cm with 250 µm layer thickness). Samples were applied on the plate as a band with 6 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). The mobile phase was composed of Toluene: Methanol (7:3v/v). 10 cm × 10 cm CAMAG twin trough glass chamber was used for linear ascending development of TLC plate under 20 mins of chamber saturation time and 10 mL of mobile phase was used per run, migration distance was 90 mm. Densitometric scanning was performed using Camag TLC scanner 3 in the range of 400-200 nm, operated by win CATS software (Version 1.4.3, Camag), slit dimensions were 5.00 x 0.45 mm and Deuterium lamp was used as a radiation source.

Selection of Detection Wavelength

From the standard stock solution further dilutions were done using methanol and scanned over the range of 200 – 400 nm and the spectrum was obtained. It was observed that the drug showed considerable absorbance at 240 and 270 nm (Fig.2).

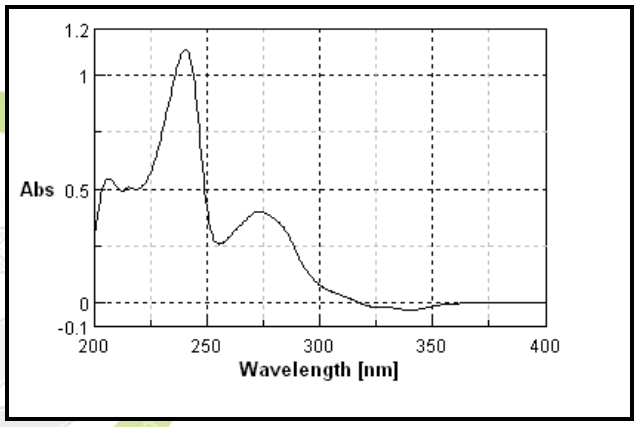


Figure 2: UV Spectrum of Vilazodone Hydrochloride (6µg/ml)

Preparation of Standard Stock Solution

Standard stock solution of Vilazodone hydrochloride was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000µg/ml. From the standard stock solution, working standard solution was prepared to contain 50µg/ml of Vilazodone hydrochloride.

Preparation of Sample Solution Containing Blend

Strength of marketed tablets: 40mg of Vilazodone hydrochloride/tablet.

Preparation of Blend

Blend containing 40mg Vilazodone hydrochloride was prepared by spiking drug into blank blend (80mg starch, 5mg Magnesium

Stearate, 80mg lactose). Mixing was done by geometric addition method.

Preparation of Test Solution

Bend equivalent to 10 mg of Vilazodone hydrochloride was weighed and dispersed in 8ml methanol. This solution was centrifuged, filtered and the filtrate volume was made up to 10 ml with methanol which contains 1mg/ml Vilazodone hydrochloride (A). From solution A, Further dilution was made with methanol to get a concentration of 50µg/ml of Vilazodone hydrochloride (B), 6µL of the resultant solution was then applied at TLC plate and densitogram was developed.

Densitogram

Solution of Vilazodone hydrochloride (50µg/ml) was prepared. 6µl (300ng/band) of solution was applied on pre-activated TLC plate with the help of Hamilton syringe (100µl), using Linomat 5 sample applicator. The development chamber was saturated with mobile phase for 20 mins. The spotted plate was placed in the saturated chamber and developed up to 90 mm distance. The plate was dried and was scanned over 90 mm distance at 240nm. The retention factor was found to be: 0.50± 0.04 respectively (Fig.3).

Stress Degradation Study of Bulk Drug

Stress degradation studies were carried under condition of acid/ base/ neutral hydrolysis, oxidation, dry heat and photolysis. For each study, samples were prepared as follows

1. Vilazodone hydrochloride working standard solution subjected to stress condition
2. The blank subjected to stress in the same manner as the drug solution.

Dry heat and photolytic degradation were carried out in solid state. Stress conditions were optimized in terms of strength of reagent and time of exposure to achieve 10-30% degradation.

Degradation under Alkali Catalyzed Hydrolytic Condition

To 5mL of 100 µg.mL^{-1}solution of Vilazodone hydrochloride, 1mL of 0.1 N NaOH was added. The volume was made up to 10 mL with methanol. The above solution was kept for 2 hours at room temperature in dark.

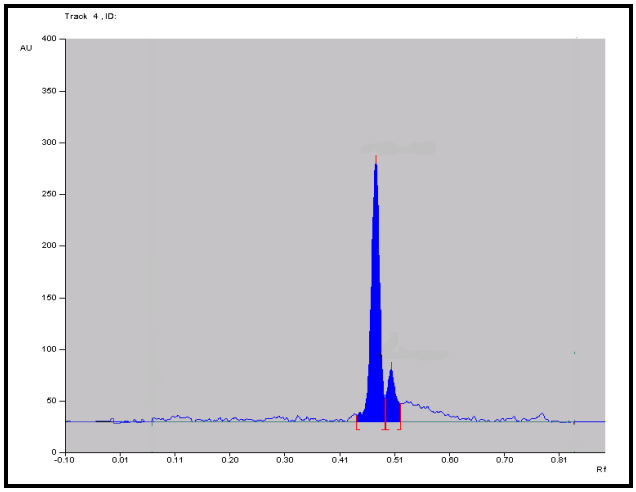


Figure 3: Densitogram of Standard Solution of Vilazodone Hydrochloride (300ng/band)

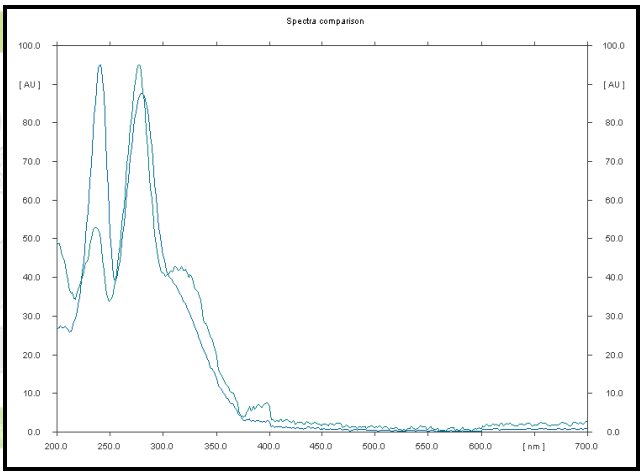


Figure 4: Overlay of the above Two Adjacent Peaks

Degradation under Acid Catalyzed Hydrolytic Condition

To 5 mL of 100 µg.mL^{-1} solution of Vilazodone hydrochloride, 1mL of 0.1N HCl was added. The volume was made upto 10 mL with methanol. The above solution was kept for 2.5 hours at room temperature in dark.

Degradation under Neutral Hydrolytic Condition

5mL of 100 µg.mL^{-1} solution of Vilazodone hydrochloride was taken in a round bottom flask, 5 mL of distilled water was added. The volume was again made upto 50 mL with distilled water.

The above solution was refluxed for 5 hours. After cooling to room temperature the contents were transferred to a 50 mL volumetric flask and the volume was again made upto 50 mL.

Degradation under Oxidative Condition

To 5 mL of 100 µg.mL^{-1} solution of Vilazodone hydrochloride, 1 mL of 3% H_2O_2 was added. The volume was made upto 10 mL with methanol. The above solution was kept for 2 hours at room temperature

Degradation under Dry Heat

Dry heat studies were performed by keeping drug sample in oven (70^0 C) for a period of 12 hours.

Photo-Degradation Studies

The photo degradation study of the drug was carried out by exposing the drug to UV light providing illumination of NLT 200 watt hr/m^2, and was subsequently exposed to cool white fluorescence light to achieve 1.2 million Lux-Hr.

RESULTS AND DISCUSSION

Summary of stress degradation studies is shown in the table below

Table 1: Summary of Stress Degradation Studies

Stress Degradation Condition	VZD			
	Peak Area	% Recovery	Peak Purity	
			r(s,m)	r(m,e)
Initial	3260.78	100%	0.999898	0.999897
Base (0.1 N NaOH, kept for 2 hrs)	2411.02	73.94 %	0.999383	0.997882
Acid (0.1 N HCl, kept for 2 1/2 hrs)	2722.75	83.5 %	0.999384	0.979726
H_2O_2 3% (kept for 2 hrs)	2816.24	77.78 %	0.998884	0.991154
Neutral (5 hrs reflux)	2905.02	89.09 %	0.998895	0.991155
Dry heat (70^0C, 12 hrs)	2572.75	78.09 %	0.999646	0.997883
Photo stability(UV, 200 watt hrs/square meter and Florescence 1.2 million Lux. Hrs)	1470.28	45.09 %	0.999484	0.99787

Though the peak area is reduced under stress; no peak of degraded product was observed. This was confirmed by spotting 10 times high (3000 ng/band) concentration.

Validation of Analytical Method

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 0.991, indicating the non interference of any other peak of degradation product or impurity, at the Rf of Vilazodone HCl.

Linearity

From the standard stock solution (1000µg/ml) of Vilazodone hydrochloride, solution was prepared containing 50µg/ml of Vilazodone hydrochloride. This solution was further used for spotting. Five replicates per concentration were spotted. The linearity (relationship between peak area and concentration) was determined by analyzing five solutions over the concentration range of 100-500 ng/band for Vilazodone hydrochloride. The equation of the calibration curve was found to be -

y = 9.937x + 176.7 the coefficient of determination was found to be 0.995 respectively. (Fig 5)

Range

Vilazodone hydrochloride - 100-500 ng/band

Precision

The precision of the method was demonstrated by Intra-day and Inter-day variation studies. In

the Intra-day studies 3 replicates of 3 concentrations were analyzed on the same day, and percentage RSD was calculated. For the inter day variation studies, 3 replicates of 3 concentrations were analyzed on 3 consecutive days and percentage RSD were calculated. For intraday precision % RSD found to be 0.94% and for inter-day precision % RSD found to be 0.86%. (Table 2)

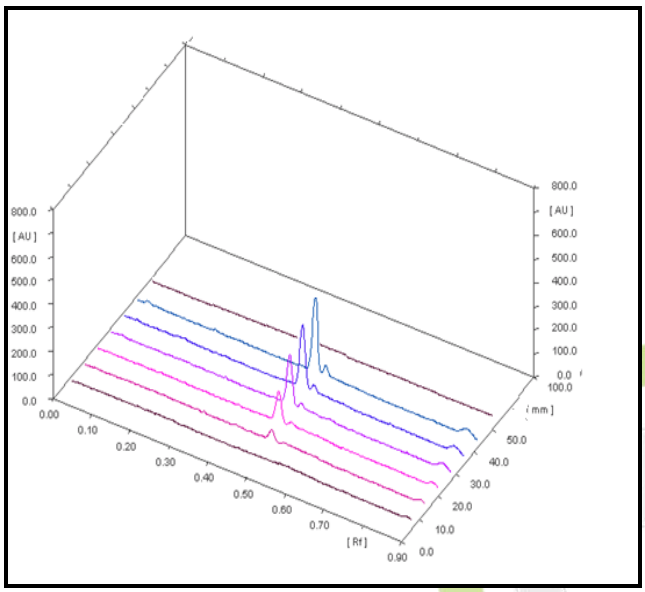


Figure 5: Densitogram of linearity of Vilazodone Hydrochloride (100-500 ng/band)

Table 2: Intra-day precision of Vilazodone Hydrochloride

Concentration (ng/spot)	Area	SD	% RSD	Mean % RSD
200	2272.3	27.79	1.22%	0.94%
	2229.3			
	2281.3			
300	3265.7	27.85	0.85%	
	3294.0			
	3259.4			
400	4124.0	0.755	0.755%	
	4163.2			
	4186.1			

Table 3: Inter-day precision of Vilazodone Hydrochloride

Concentration (ng/spot)	Area	SD	% RSD	Mean % RSD
200	2227.9	11.00	0.49%	0.86%
	2249.9			
	2239.9			
300	3219.9	34.54	1.06%	
	3238.8			
	3286.1			
400	4174.0	42.77	1.03%	
	4104.1			
	4186.1			

Assay

Spiked blend analysis was carried out as mentioned under section test solution procedure. Analysis was repeated six times. Sample solution was spotted and area was recorded. % assay was determined from linearity equation (Table 4).

Table 4: Assay of Spiked Blend

Sr. No.	Peak area of Vilazodone hydrochloride	Amount Recovered (ng/band)	% Recovery
1	3150.8	299.2	99.7
2	3165.9	301.9	100.6
3	3154.7	299.8	99.9
4	3191.4	303.3	101.1
5	3145.9	298.8	99.6
6	3167.8	301.0	100.3
Mean	3162.75	300.6	100.2
%RSD	0.51	0.57	0.57

Table 5: Recovery studies Vilazodone Hydrochloride

Level	Conc. (ng/band)		Area	Mean	Amount Recovered	% Recovery ± SD
	Sample	Std.				
80 %	200	160	3750.89	3755.39	360.13	100.03 ± 0.66
			3724.80			
			3790.48			
100 %	200	200	4174.50	4111.66	397.47	99.36 ± 0.28
			4004.10			
			4156.40			
120 %	200	240	4609.60	4619.12	448.74	101.98 ± 0.58

*Mean area of 3 determination

Accuracy

To check accuracy of the method, recovery studies were carried by spiking the standard drug to the blend, at three different levels 80, 100 and 120 %, Basic concentration of blend sample chosen was 200ng/band. % recovery was determined from linearity equation. The results obtained are shown in (Table 5).

Limit of Detection and Quantification (LOD and LOQ)

From the linearity data the limit of detection and Quantitation was calculated, using the following formula.

LOD= 3.3 σ/ S and LOQ = 10 σ/ S

σ = standard deviation of the lowest response of linearity equation.

S = slope of the calibration curve of the analyte.

LOD= 2.23ng/ band

LOQ= 6.75ng/band

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase ratio, detection wavelength, chamber saturation time were altered, time was also changed from spotting to development and development to scanning and the effects on the area were noted. It was found that method is robust (Table 6)

Table 6: Robustness Study

Sr. No.	Parameters	Variation	% RSD
1.	Wavelength	± 2 nm	1.88
2.	Chamber saturation period	±2 min	0.60
3	Time from application to development	0,30,60 min	0.32
4	Time from development to scanning	0,30,60 min	0.77

CONCLUSION

The developed method was found to be simple, sensitive, specific, accurate, and repeatable for analysis of Vilazodone hydrochloride in the blend without any interference from the excipients. The result of validation parameters are summarized in Table 7, The results indicated the suitability of the method to study stability of Vilazodone hydrochloride under various forced degradation conditions like hydrolysis, oxidation, dry heat and photolytic degradation.

Table 7: Summary of Validation Parameters

Sr. No.	Validation Parameter	Results
1.	Linearity	Y= 9.937x + 176.7
2.	Range	100 – 500 ng/band
3.	Precision	% RSD
	A) Intraday precision	0.94%
	B) Interday precision	0.86%
4.	Accuracy	% Recovery
	80%	100.03
	100%	99.36
	120%	101.98
5.	LOD	2.21 ng/band
6.	LOQ	6.75 ng/band
7.	Specificity	Specific
8.	Robustness	Robust

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