Parameswara Reddy B. *et al.* / *International Journal of Biological & Pharmaceutical Research.* 2012; 3(6): 789-795.

e- ISSN 0976 - 3651
Print ISSN 2229 - 7480

International Journal of Biological & Pharmaceutical Research
Journal homepage: www.ijbpr.com

IJBPR

METHOD DEVELOPMENT AND VALIDATION FOR THE ASSAY OF VILAZODONE IN BULK AND FORMULATION BY USING RP-HPLC TECHNIQUE

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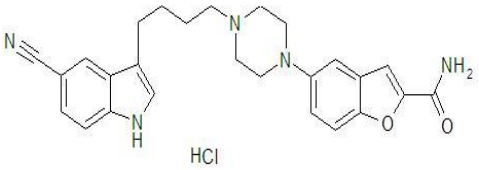
ABSTRACT

A simple, precise, rapid and accurate RP-HPLC method was developed and validated for assay of Vilazodone in tablet dosage form. Isocratic elution at a flow rate of 1mL/min was employed on a symmetry Chromosil C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of methanol: water 25: 75 v/v, (P^H 4.0 with 0.1% Ortho phosphoric Acid). The UV detection wavelength was 224nm and 20µl sample was injected. The retention time for vilazodone was 4.05 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for qualitative analysis of vilazodone in tablet dosage form and bulk drug.

Key Words: Vilazodone, RP-HPLC, UV detection, Recovery, Precise and 224nm.

INTRODUCTION

Vilazodone is chemically 5-(4-[4-(5-cyano-1*H*-indol-3-yl)butyl]piperazin-1-yl)benzofuran-2-carboxamide.



Vilazodone belongs to the category like serotonergic antidepressant (Reuters *et al.*, 2011). Vilazodone is a indole-piperazine that functions as an SSRI and 5-HT_{1A} receptor partial agonist. It has negligible affinity for other serotonin receptors. 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. The antidepressant effect is thought to be due to vilazodone's SSRI quality. It is unknown if the partial agonism of $5HT_{1a}$ receptors by vilazodone produces any beneficial effect. It has been hypothesized that vilazodone has a faster onset, compared to SSRI's alone, as the additional partial agonism of the $5HT_{1a}$ receptor results in a more rapid and specific desensitization of the somatodendritic $5HT_{1a}$ autoreceptors. Chronic administration of a SSRI results in desensitization of presynaptic $5HT_{1a}$ autoreceptors. T_{max} following oral administration is approximately 4 to 5 hrs. Mean terminal half-life is 25 hrs.

It is a strong dopamine antagonist. It has high affinity for D2 dopaminergic receptors. It has actions at several 5-HT (serotonin) receptor subtypes. The latter

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action may lead to an increased release of dopamine from mesocortical neurones in the brain. Risperidone is metabolised fairly quickly, so this potential for nausea subsides usually in two to three hours (Thomas L. Schwartz *et al*., 2011). Developed a brief pharmacological and clinical review of the novel serotonin partial agonist and reuptake inhibitor (Vilazodone). (Carol R. Reed *et al*., 2011) developed the efficacy profile of vilazodone, a novel antidepressant for the treatment of major depressive disorder. Vilazodone is a novel serotonin reuptake inhibitor and serotonin 1A receptor partial agonist approved for the treatment of major depressive disorder (MDD).

Laughren Thomas *et al* (Laughren Thomas *et al.,* 2011) developed the clinical basis for the US Food and Drug Administration's approval of a New Antidepressant-vilazodone (Laughren Thomas *et al.,* 2011). Many researchers (Timo Heinrich *et al.,* 2004; Henning Böttcher *et al*., 2002) developed the synthesis and Structure-Activity Relationship in a Class of Indole butyl piperazines as Dual 5-HT_{1A} Receptor Agonists and Serotonin Reuptake Inhibitors. Till date only pharmacological studies have been reported for the determination of vilazodone. 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.

MATERIALS AND METHODS

An isocratic high pressure liquid chromatography (PEAK HPLC instrument with peak software.Micro balance –DENVER– model SI(234), Millipore filter (0.45 µm),Millipore mill Q Water instrument and column employed Zodiac C18 column (250 mm x 4.6 mm, 5µ) with LC7000 UV detector at 224nm.

Chemicals and reagents

All the chemicals used were of HPLC grade. Double distilled water was used for making the solutions. The commercially available Vilazodone tablets were procured from the local market.

Chromatographic conditions

The content of the mobile phase was methanol: water (25:75%v/v). The mobile phase was filtered through 0.45 µm membrane filter and sonicated for 15 min. The flow rate of the mobile phase was maintained at 1.0 mL/min. The column temperature was set ambient and the detection was carried out by UV-detector wavelength at 224 nm. The run time was set at 10 min and the volume of the injection loop was 20 µL. Rheodyne injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system.

PROCEDURE

Preparation of Vilazodone Standard Solution

Vilazodone (10 mg) was accurately weighed by DENVER (SI-234) and dissolved in10 ml mobile phase in a 10 ml volumetric flask to obtain 1000 µg/ml. After filtration through 0.45µ membrane filter, required concentrations were prepared by serial dilution of this solution.

Preparation of Vilazodone tablet solution

A composite of 20 (viibryd) tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of vilazodone was accurately weighed and quantitatively transferred into a 10 ml volumetric flask. Approximately 5 ml mobile phase were added and the solution was sonicated for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration through 0.45µ membrane filter, an amount of the solution was diluted with mobile phase to a concentration of 60 µg/ml. 20 µl of this solution was injected and the chromatogram was recorded.

The content of vilazodone present in each tablet formulation was calculated by comparing the peak area of the standard and sample reports and shown in Table 1.

METHOD VALIDATION (Lloyd Synder *et al*., 1997; Hemilton *et al.,* 2006; Beckett.A.H *et al.,* 2010; Hohat Willard *et al*., 2002; Skoog *et al*., 2007)

Validation of an analytical method is a process to establish that the performance characteristics of the developed method meet the requirement of the intended analytical application.

Specificity

An investigation of specificity should be conducted during the validation of identification tests, the determination of impurities. An ICH document defines specificity as the ability to assess unequivocally the analyte in the presence compounds that may be expected to products and matrix components.

The definition has the following implications

Identification test

Suitable identification tests should be able to discriminate compounds of closely related structure which are likely to be present .Ensure identity of an analyte. The analyte should have no interference from other extraneous components and be well resolved from them.

Purity Test

To ensure that all the analytical procedures performed allow an accurate statement of the content of impurity of the content of impurity of an analyte i.e. related substances test, heavy metals, residual solvents etc.

Assay

To provide an exact result, this allows an accurate statement on the content or potency of the analyte in a sample.

Linearity

The linearity of an analytical procedure is its ability to obtain test results that are directly proportional to concentration of analyte in samples. This characteristic is determined by application of the procedure to a series of Samples having analyte concentration spanning the claimed range of procedure. When the relationship between response and concentration is not linear, standardization may be providing by means of a calibration curve. The ICH recommends that for the establishment of linearity a minimum of five concentrations normally used.

A minimum of eight concentrations were taken for linearity studies. Graph of area vs concentration is plotted and percentage curve fitting is calculated.

Limit of detection

The parameter LOD was determined by analysis of sample with known concentration of analyte and by establishing the minimum level at which the analyte can be reliably detected.

Based on visual evaluation

It may be used for non-instrumental &instrumental method of analysis. The detection limit is determined by analysis of sample with known concentration of analyte and by establishing the minimum level at which the analyte can be reliably detected.

Limit of quantification

The quantification limit of an analytical procedure is the lowest amount of analyte in a sample which can be qualitatively determined with suitable precision and accuracy.

Based on visual evaluation

For instrumental and non- instrumental methods, the quantitation limit is generally determined by the analysis of samples with known concentration of analyte and by establishing the minimum level at which the analyte can be determined with acceptable accuracy and precision.

Accuracy

The accuracy is the analytical procedure expresses the closeness of agreement between the measured values to the true value for the sample. The ICH documents recommended that accuracy should be assessed using a minimum of nine determinations over a minimum of three concentrations levels the specified range (i.e., three concentrations and three replicates of each concentration).

Accuracy is measured as the percentage of the analyte recovered by the assay spiked samples were prepared in triplicate at three intervals at a range of 80-120 % of the target concentration, and injected in to the HPLC system.

Acceptance criteria: percentage recovery should be with in 98 to 102 %.

Precision

Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample. Precision of analytical method is usually expressed as the standard deviation (or) relative standard deviation. There are two methods for determination of precision.

1) System precision
2) Method precision

Robustness

Robustness of an analytical method is a 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.

Determination

The robustness of an analytical method was determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but are still within the specified parameters of the assay. For example, change in physical parameters like flow rate, wavelength and mobile phase ratio.

Ruggedness

Ruggedness was performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different. Ruggedness also expressed in terms of percentage relative standard deviation. This parameter is determined as per ICH and USP guide lines.

System suitability studies

A Solution of 60 µg/mL of vilazodone were prepared by diluting suitably with mobile phase and same was injected.

RESULTS AND DISCUSSION

As 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 (Table 4 & 5).

Specificity of the method was found out through non-interference of the placebo identical conditions of assay. This uniforms the specificity of the proposed method. Linearity of the drug was obtained in the range of 30 to 100µg/ml for vilazodone. The linearity correlation Coefficient and percentage curve fitting was found to be 0.999 for vilazodone 99.71. The limit of detection was found to be 0.075µg/ml for vilazodone. The limit of quantification was found to be 0.25µg/ml for vilazodone.

Accuracy of the method was determined through recovery studies of the drug. Recovery of the drug was

well with in acceptance limit (97% to 102 %). Precision of the method was determined by analyzing the drug formulation by replicate injection and system precision was determined by standard solution %RSD the result was found to be with in the limits of 2%. Thus developed method was found to provide high degree of precision and reproducibility.

Ruggedness of the method was determined by performing the assay with different analyst in different days perform the assay to check the reproducibility. The test results were found with in limit 97.2% to 102%. The results were found to be reproducible. Inspite of variation in condition with could be normally expected from analyst to analyst. Robustness was determined by carrying out the assay during change in the mobile phase ration, wavelength and flow rate. The results obtained with the change in mobile phase ratio makes it possible to carryout the method for vilazodone with small variations in mobile phase ratio. System suitability was determined by performed the assay with the same sample repeatedly. The number of the theoretical plates was found to be 3439 for vilazodone.

Amount of drug in each tablet of vilazodone

$$\frac{\text{Sample Area}}{\text{Std Area}} \times \frac{\text{Std. dilution}}{\text{Sample. Dilution}} \times \frac{\text{Potency}}{100} \times \text{Average Weight of tablet}$$

$$\% \text{ Content} = \frac{\text{Amount Present}}{\text{Label Claim}} \times 100$$

Table 1. Results showing quantitative estimation of vilazodone by using the developed method

% of Recovery	Target Conc.,(µg/ml)	Spiked Conc,(µg/ml)	Final Conc,(µg/ml)	Conc., Obtained	% of Assay
50%	40	20	60	59.21	99.18
	40	20	60	59.18	98.63
	40	20	60	60.33	100.55
100%	40	40	80	78.58	98.22
	40	40	80	79.57	99.46
	40	40	80	78.81	98.51
150%	40	60	100	100.30	100.30
	40	60	100	98.88	98.88
	40	60	100	99.18	99.18

Table 2. Specificity

S.No.	Sample	Area Obtained	% Content of drug % w/v
1.	Standard	441553.9	99.77%
2.	Standard + Placebo	440538.0	99.824%
3.	Placebo	0	0

Table 3. Linearity data for Vilazodone

S.No	Concentration (µg/ml)	Area of the peak(mv.s)
1.	30	242903
2.	40	308771
3.	50	384427
4.	60	441553
5.	70	525757
6.	80	588218
7.	90	664255
8.	100	736050

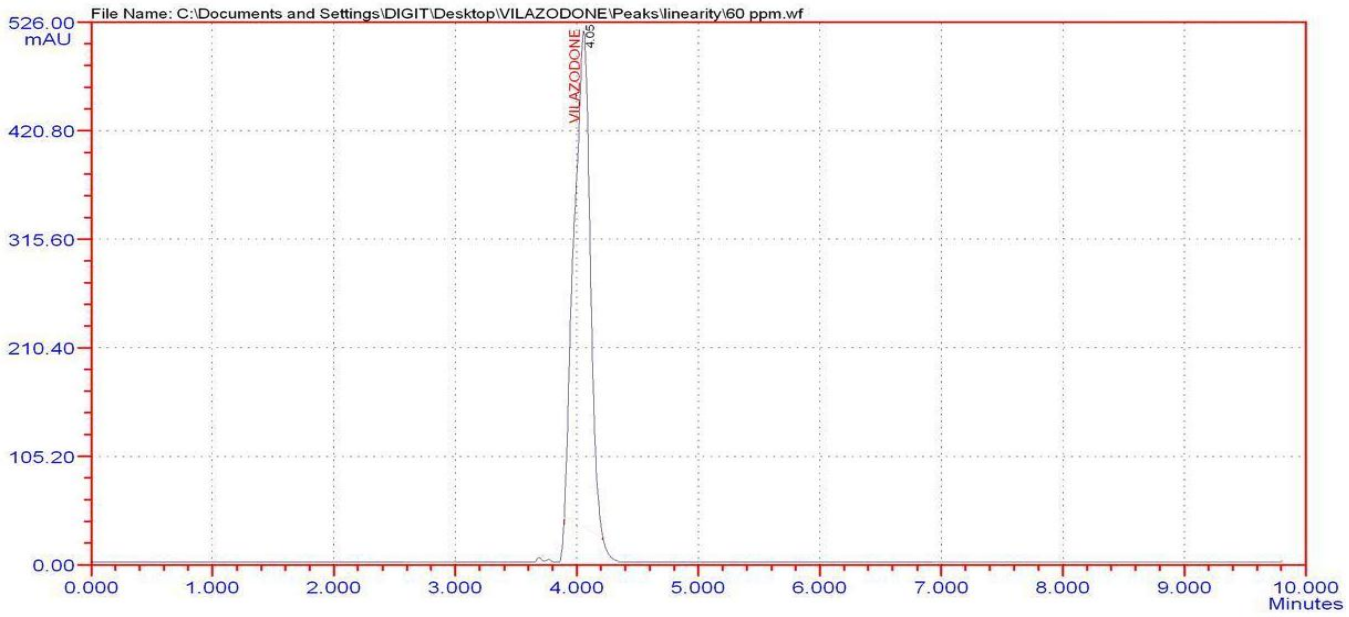
Table 4. The amount of drug present in the tablet

Formulation	Dosage	Concentration	Amount found	% Assay	%RSD
Viibryd	10 mg	60ppm	59.80	99.72	0.053
	10 mg	60ppm	59.862	99.77	
	10 mg	60ppm	59.91	99.85	

Table 5. Validation Result for Vilazodone in developed method

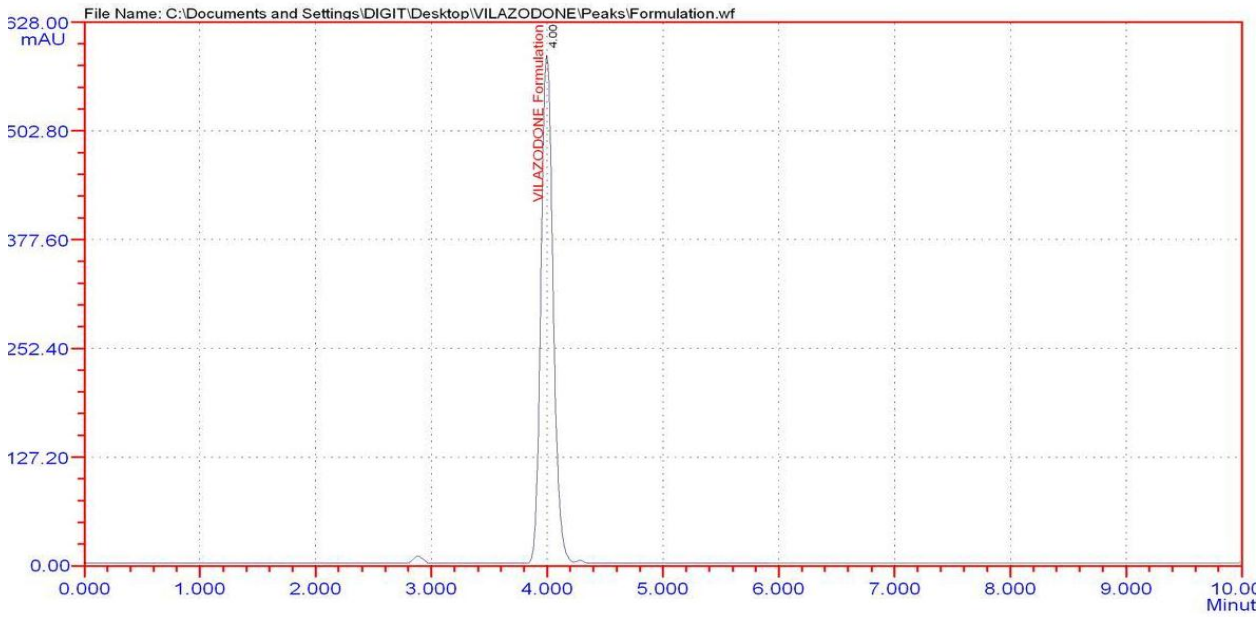
S.No.	Parameters	Results obtained		Acceptance criteria
1.	SPECIFICITY	99.797%		99 -101%
2.	LINEARITY RANGE			
	Correlation Coefficient	0.999		NLT – 0.997%
	Percentage curve fitting	99.71%		NLT – 99.7%
	Slope	7257.009		-
3.	LOD	0.075 µg/ml		-
4.	LOQ	0.25 µg/ml		-
5.	Accuracy	98.22-100.55		97-102%
6.	PRECISION			
	System Precision	0.27		2% (RSD)
	Method Precision	0.19		2% (RSD
7.	RUGGEDNESS			
	Different Analyst	99.80		99-101%
8.	ROBUSTNESS			
	MP	80:20 70:20	99.43 98.58	99-101%
	Flow	0.8ml/min 1.2ml/min	99.88 99.55	
	WL	229nm 219nm	99.04 99.53	99-101%
9.	System Suitability Parameter			
	Theoretical Plates	3934		-
	Tailing factor	0.80		Not more than 2
	Retention time	0.64		Not more than 2

Figure 1. Chromatogram of Standard vilazodone



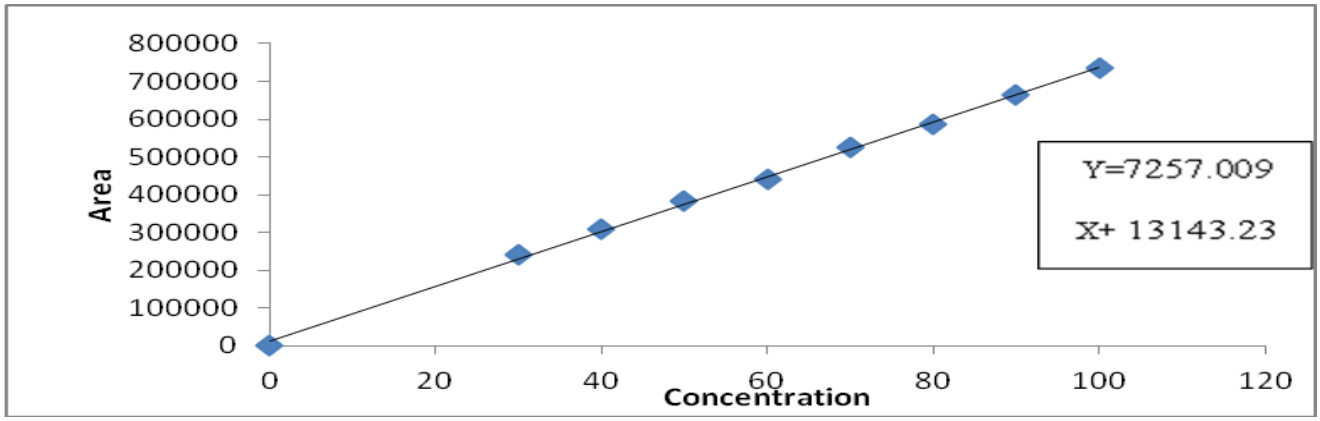
ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	VILAZODONE	4.055	48382	441553.9	100.000	0.94	3935
	Sum:		48382	441553.9	100.0000		

Figure 2. Chromatogram of Sample vilazodone



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	VILAZODONE Formulation	3.997	59104	440538.0	100.000	0.90	5730
	Sum:		59104	440538.0	100.0000		

Figure 3. Linearity data for Vilazodone



CONCLUSION

A HPLC - method is developed for the estimation of Vilazodone in tablet dosage from using RP-HPLC. LC-7000 with UV detector 224 and Zodiac C18 column (250 mm x 4.6 mm, 5µm) of standard is injected and evaluated with mobile phase of methanol: water (25:75%v/v) which is pumped at flow rate 1ml/min and detected by UV detector at 224 nm. The peak of viazodone is found well observed at 4.05 minutes respectively. The developed method is applied for the determination of vilazodone in tablet dosage form. The assay results are within the label claim of formulation. The developed method is validated with various parameters as per ICH guidelines like accuracy, precision, linearity, specificity, ruggedness and robustness, system suitability. The results are within the acceptance criteria. Hence the proposed method is found to be satisfactory and would be used for the routine analysis of vilazodone in the laboratory.

ACKNOWLEDGEMENT

The authors are thankful to A.M Reddy Memorial College of Pharmacy, Narasaraopet, Guntur, Andhra Pradesh, India, for providing necessary facilities to carry out this research work.

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