International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.7, No.1, pp 204-211, 2014-2015

Development of Stability Indicating RP-HPLC Method and Validation for the Estimation of Vilazodone Hydrochloride

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Abstract: An accurate, precise, rapid & economical RP-HPCL method has been developed for the estimation of Vilazodone as per ICH guideline in pharmaceutical dosage from use ultra violet (UV) detector. Elution was carried out using a mobile phase consistion of Methanol and Phosphate buffer adjusted at pH 7.4 in a ratio (40:60v/v) and flow rate was set on 1ml/min at 232 nm, retention time fro Vilazodone was found to be 3.104 min. The method was found to be linear in the concentration range of 05-15 ug/ml, in the linearity study regression equation was found to be y = 16616x & correlation coefficient was found to be 0.999. This method was Rugged and Robust in different testing criteria, LOD and LOQ was found to be 0.005 µg / ml & 10 µg / ml respectively. Accuracy study was done in 3 different concentration level i.e 50, 100, 150% & % recovery of the method was found to be 99.9%, 99.8%, 99.6% respectively in 3 different levels & mean recovery was 99.77%, so method was accurate. Results of all validation parameter was within the limit as per ICH guideline.
Key Words: Vilazodone Hydrochloride, Method Development, Validation, HPLC.

Introduction:

Vilazodone IUPAC Name is 5-(4-[4-(5-cyano-1*H*-indol-3-yl)butyl]piperazin-1-yl) benzofuran-2carbox amide. It belongs to the category serotonergic antidepressant. Vilazodone was approved by the FDA for use in the United States to treat major depressive disorder in January 21, 2011. Vilazodone acts as a serotonin reuptake inhibitor and 5-HT_{1A} receptor partial agonist It has negligible affinity for other serotonin receptors such as 5-HT_{1D}, 5-HT_{2A}, and 5-HT_{2C}[(1-2)]. According to literature review [(3-11)] there are very few method reported for the determination of Vilazodone in different Instrumental techniques, out of these methods only 1 method was reported by using RP- HPLC.

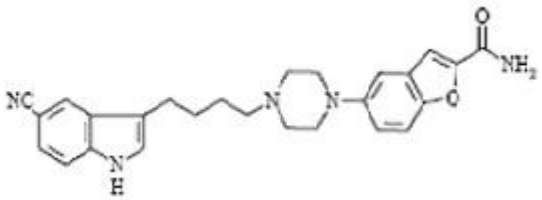

Figure no 01: Shows Chemical structure of Vilazodone

Materials and Methods

Standard Drugs: Vilazodone hydrochloride.

Chemicals and Reagents:

Vilazodone hydrochloride (Matrix), HPLC Water (Lichrosolv, Merck), KH_2PO_4 **(**Finer chemical Ltd.**),** Methanol for HPLC (Lichrosolv, Merck), Water for HPLC (Lichrosolv, Merck), Orthophosphoric acid (Merck).

Apparatus:

HPLC (WATERS software: Empower-2, 2695 separation module.2487 PDA detector), UV/VIS spectrophotometer (LABINDIA UV 3000^{+}), pH meter (Adwa – AD 1020), Weighing machine (Afcoset ER-200A), Pipettes and Burettes (Borosil), Beakers Borosil.

Preparation of Standard Solution:

About 50 mg of Vilazodone hydrochloride is weighed and transferred to 50ml volumetric flask, it was dissolved with methanol and the volume was made up to the mark with HPLC water. Further 5 ml of above solution was diluted to 100ml with water. Further 5ml is diluted to 25ml with water to get 10 µg/ml Vilazodone hydrochloride.

Preparation of Sample Solution:

A composite of 20 (Viibryd) tablets was prepared by grinding them to a fine, uniform size powder. Weight equivalent to 50 mg of Vilazodone was accurately weighed and quantitatively transferred into a 50 ml volumetric flask, it was dissolved with methanol and the volume was made up to the mark with HPLC water. Further 5 ml of above solution was diluted to 100ml with water. Further 5ml is diluted to 25ml with water to get 10 µg/ml Vilazodone hydrochloride.

Preparation of Phosphate buffer:

Accurately weighed 17.418 grams of K_2HPO_4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH with Orthophosphoric acid.

Preparation of mobile phase:

Accurately measured 600 ml (60%) of above buffer and 400 ml (40%) of HPLC methanol were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

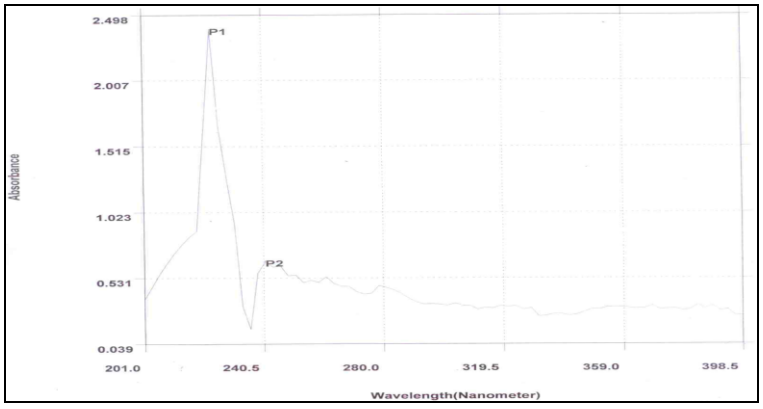

Fig. no.2: Shows UV spectrum of Vilazodone hydrochloride

From the above spectrum the wavelength selected for estimation of drug is 232 nm as λ_{max} of Vilazodone hydrocloride.

Table no 01: Optimized Chromatographic Conditions:

Instrument used	**Waters HPLC with auto sampler and UV detector.**
Temperature	Ambient
Column	Symmetry C8 (4.6 x 250mm, 5µm, Make:waters)
Buffer	c7.0 grams of potassium dihydrogen ortho phosphate in 1000 ml water pH adjusted with Orthophosphoric acid.
pH	7.4
Mobile phase	60%K_2HPO_4 and 40%methanol
Flow rate	1 ml per min
Wavelength	232 nm
Injection volume	10 µl
Run time	6min.

Validation Parameter:

The following parameters were considered for the analytical method validation of Artemether in bulk form.

System Suitability:

Chromatograph the standard preparations (6 replicate injections) and peak area responses for the analyte peak was measured and the system suitability parameters are evaluated.

Accuracy:

For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.

Precision:

The standard solution was injected for six times and the area was measured for all six injections in HPLC. The % RSD for the area of six replicate injections was found to be within the specified limits.

Robustness:

As part of the Robustness, deliberate change in the temperature and flow rate Variation was made to evaluate the impact on the method.

Linearity and range:

Linearity of the analytical method for assay by injecting the linearity solutions prepared in the range of 100µg to 600 µg (33.3% to 200%) of test concentration, into the chromatograph, covering minimum 6 different concentrations.

Ruggedness:

Establish the ruggedness of the analytical method by using the assay of 6 different sample preparations of same batch by a different analyst using a different HPLC System.

Results and Discussion:

The developed method was validated based on ICH guidelines which detect and quantitate drug in bulk form with use of HPLC system equipped with PDA detector

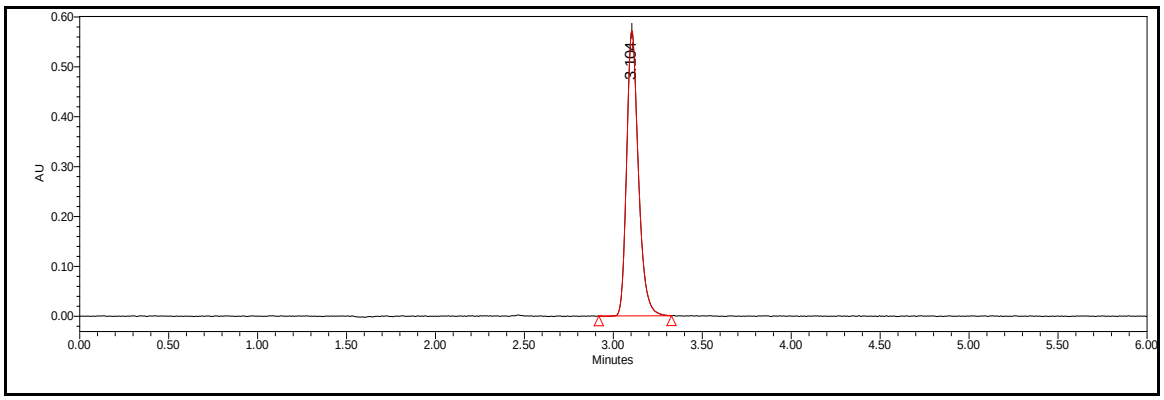

Figure no 03: Shows Chromatogram for Vilazodone hydrochloride

Table no 02: shows Method Development Parameters

Retention Time	Area	% Area	Height	s/n	USP Tailing	USP Plate Count
3.104	2628219	100.00	571605	394	1.29	10877

Validation of developed RP-HPLC method:

Validation of an analytical method is the process to establish by laboratory studies that the performance characteristic of the method meets the requirements for the intended analytical application. Performance characteristics were expressed in terms of analytical parameters.

Accuracy:

The RP-HPLC method developed in the present study has been used to quantify vilazodone hydrochloride. The average area was taken and % accuracy was calculated. The mean recoveries were found in the range of 99.6-99.9%. The results are presented in table no 03.

Table no 03: Shows Accuracy results for Vilazodone hydrochloride

Sample name	Area	Amount added in µg/ml	Amount found in µg/ml	% recovery	Mean recovery
50%	1316417	5	4.99	99.8	
50%	1312470	5	4.98	99.6	
50%	1314429	5	4.99	99.8	99.9
50%	1316351	5	4.99	99.8	
50%	1317997	5	4.97	99.4	
50%	1315867	5	5.08	101.6	
100%	2631181	10	9.98	99.8	
100%	2631961	10	9.98	99.8	99.8
100%	2635290	10	9.99	99.9	
150%	3950109	15	14.99	99.9	
150%	3952582	15	14.98	99.8	
150%	3958125	15	14.85	99	99.6
150%	3955227	15	14.89	99.2	
150%	3956218	15	14.97	99.8	
150%	3956234	15	15.01	100.06	

Precision:

The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision results were expressed as standard deviation or Relative standard Deviation

Table no 04: Shows Method Precision Results for Vilazodone hydrochloride

	RT	**Area**
Injection-1	3.103	2630448
Injection-2	3.101	2634681
Injection-3	3.102	2633307
Injection-4	3.100	2631763
Injection-5	3.099	2633495
Injection-6	3.098	2631948
Average	3.1005	2632607
Standard Deviation	0.001871	1509.167
%RSD	0.0603	0.0573

Table no 05: Shows ID-Precision Results for Vilazodone hydrochloride

Injection	**RT**	**Area**
Injection-1	3.098	2631948
Injection-2	3.101	2634681
Injection-3	3.100	2631763
Injection-4	3.099	2633495
Injection-5	3.100	2631763
Injection-6	3.098	2631843
Average	3.09933	2632582
Standard Deviation	0.001211	1227.108
%RSD	0.0390	0.0466

Linearity& Range :

The calibration curves were linear in the range 50-150 µg/ml. The correlation coefficient ('r') value was found to be >0.999 for Vilazodone hydrochloride.

Table no 06: Shows Linearity results for Vilazodone hydrochloride

Vilazodone				
S. No	**Linearity Level**	**CONC%**	**Area**	**µg/ml**
1	I	50	1318543	5
2	II	75	1978842	7.50
3	III	100	2632340	10.00
4	IV	125	3290035	12.5
5	V	150	3959003	15

Correlation coefficient: 0.99

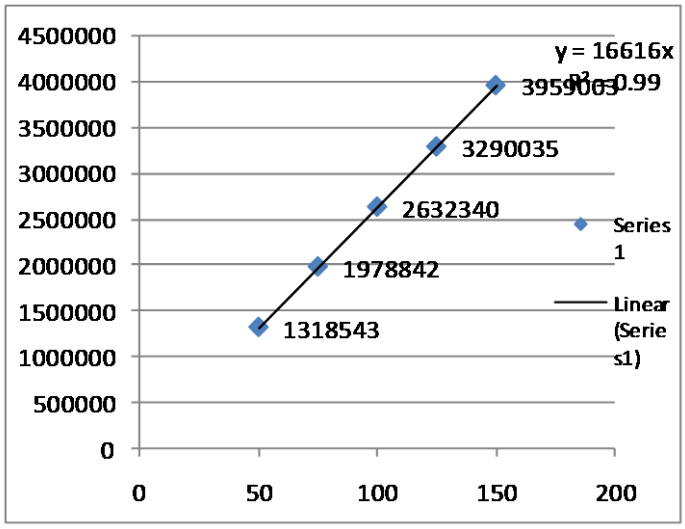

Figure no 04: Shows Calibration graph of Vilazodone Hydrochloride

Robustness:

The robustness of an analytical procedure 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.

Table no 07: Shows Study of Robustness results for Vilazodone hydrochloride (Flow Rate)

S.No	Flow Rate (ml/min)	System Suitability Results				
		RT	AREA	USP Plate Count	SP Tailing	S/N
	0.8	3.628	3296981	12085	1.345	402.707
	1.0	3.104	2628219	10877	1.29	394
	1.2	2.563	2104137	9914	1.305	31.939

Table no 08: Shows Study of Robustness (Effect of Temperature)

S. No	Temperature in °c	System Suitability Results				
		RT	AREA	USP Plate Count	SP Tailing	N
	20 °C	3.076	2560365	10887	1.315	342.249
	25 °C	3.104	2628219	10877	1.29	394.00
	30 °C	3.064	2587241	10728	1.298	347.857

LOD:

The **limit of detection** is determined by the analysis of samples with known concentration of analyte and by establishing that minimum level at which the analyte can reliably detected**,** The LOD are calculated by formula **LOD = 3.3 x SD/ b** where, **SD**- standard deviation of the peak area of the drugs, **b** -is slope of the corresponding calibration curve. LOD for Vilazodone hydocloride was 0.01µg/ml.

LOQ:

The **limit of quantification** is generally determined by the analysis of sample with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision, The LOQ are calculated by formula **LOQ = 10 x SD/ b** where, **SD**- standard deviation of the peak area of the drugs, **b** -is slope of the corresponding calibration curve. LOQ for Vilazodone hydrochloride was 0.05 µg/ml.

Table: 9. Shows LOD & LOQ results of Vilazodone hydrocloride

Parameters	Vilazodone hydrocloride
LOD	0.01µg/ml
LOQ	0.05µg/ ml

Degradation Results:

Table no 10: Shows degradation studies of Vilazodone hydrochloride

S.No	Degradation	Rt	Area
1	Acid degradation	3.078	2606188
2	Base degradation	3.021	26012343
3	Thermal degradation	3.079	2612568
4	Photolytic degradation	3.081	2615387
5	Oxidative degradation	3.081	2625387

Table no 11: Shows validation summary of Vilazodone hydrochloride

S.NO	Parameter	Acceptance criteria	HPLC
1	Linearity range (µg/ml)	-	40-120(µg/ml)
2	Correlation coefficient	NLT 0.999	0.999
3	No of Theoretical plates	NLT 2500	10877
4	Method precision	% RSD(NMT 2%)	0.0573
5	Intermediate precision	% RSD(NMT 2%)	0.0466
6	% recovery	98-102%	99.6-99.9%
7	LOD	-	0.01(µg/ml)
8	LOQ	-	0.05(µg/ml)

Conclusion:

Method development & validation of Vilazodone hydrochloride was done by RP-HPLC method. The estimation was done by using Symmetry C_{18} (4.6 x 250 mm, 5µm, Make: Waters). Mobile phase was used as Phosphate Buffer and Methanol in (60:40) ratio at a flow rate 1 ml/min, retaintion time was 3.104 min. at λ_{max} 232 nm. The linearity range of Vilazodone hydrochloride was found to be within 50-150 µg/ml. Mean recovery was 99.8 %, which is within 98-102%. Correlation coefficient value was 0.999, % RSD was 0.057 % which is within the limit. These results show the method is accurate, precise, sensitive, economic & rugged. The HPLC method is more rapid. The proposed method can be successfully applied to estimate bulk drug & Tablet dosage form. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

References:

1. Available at: http://www.drugs.com, retrieved on 28th August 2013.
2. Laughren T. P, Gobburu J, Vilazodone: clinical basis for the US Food and Drug Administration's approval of a new antidepressant, The Journal of Clinical Psychiatry, 2011, 72(9), 1166–73.
3. Lovett L. J, Nygarda G. A, Khalil S. K, A Simple HPLC Method for the Determination of Trazodone Human Serum, Journal of Liquid Chromatography, 1987, 10(5), 909-919.
4. Rao D. S, Geetha S, Srinivasu M. K, Reddy G. O, LC determination and purity evaluation of Nefazodone HCl in bulk drug and pharmaceutical formulations, Journal of Pharmaceutical and Biomedical Analysis, 2001, 26 (4), 629–636.
5. Gindy A. E, Farouk M, Aziz O. E, Abdullah E. A, Stability Indicating Assay of Trazodone Hydrochloride Using High Performance Liquid Chromatography, Journal of Applied Sciences Research, 2009, 5(11), 2028-2034.

6. Pai N. R, Pusalkar D. A, Development and validation of liquid chromatogrphic method for Trazodone hydrochloride, Journal of Chemical and Pharmaceutical Research, 2010, 2(2), 478-488.
7. Longstreth J, Alcorn H, Swan S. K, Vilazodone pharmacokinetics in subjects with mild to moderate hepatic impairment, Presented at annual meeting of the American Psychiatric Association, New Orleans, 2010.
8. Yasser A. Z, Farag R. S, Elnawawy M, Ahmed M. A, Alsalam S. R, Spectrophotometric method for the determination of Trazodone hydrochloride in pharmaceutical formulations, International Journal of Pharmaceutical Research, 2011, 2(11), 2798-2800.
9. Sharma M, Jawa P. R, Gill R. S, Bansal G, Citalopram Hydrobromide: degradation product characterization and a validated stability-indicating LC-UV method, Journal of the Brazilian Chemical Society, 2011, 22(5), 564-571.
10. Reddy B. P, Pramod N, Venkateswararao P, Sudhakar babu A. M. S, Method development and validation for the assay of Vilazodone in bulk and formulation by using RP-HPLC technique, International Journal of Biological & Pharmaceutical Research, 2012, 3(6), 789-795.
11. Ravi Prakash P. V. D. L. S, Sumadhuri B, Srikanth M, Validated HPLC-MS/MS Method for Determination of Trazodone in Human Plasma, Open Access Scientific Reports, 2013, 2(2), 1-5.

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