International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 9, Issue 4, 2017

Original Article

STABILITY INDICATING HPLC METHOD FOR DETERMINATION OF VILAZODONE HYDROCHLORIDE

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Received: 28 Jan 2017, Revised and Accepted: 20 Apr 2017

ABSTRACT

Objective: The aim and objective of this study was to develop and validate Stability Indicating HPLC method for determination of Vilazodone Hydrochloride.

Methods: The method was carried out on a Phenomenex, C_{18} (250x4.6 mm, 5 µm) Column using a mixture of Acetonitrile: Water (50:50v/v), pH adjusted to 3.3 with Glacial Acetic Acid for separation. The flow rate was adjusted at 1 ml/min and Detection was carried out at 240 nm.

Results: The retention time of vilazodone hydrochloride was found to be 2.3 min. The calibration curve was found to be linear in the range 25-75µg/ml with a correlation coefficient (R^2=0.996). The limit of detection and limit of quantitation were found to be 4.78µg/ml and 14.48µg/ml respectively. The % recovery of vilazodone hydrochloride was found to be in the range of 98.21±0.08 % to 99.07±0.64%. The proposed method was successfully applied for the estimation of vilazodone hydrochloride in marketed tablet formulation.

Vilazodone Hydrochloride was subjected to forced degradation under Acidic, Alkaline, Oxidation, Dry Heat and Photolytic degradation conditions. Vilazodone hydrochloride showed 3.12% degradation under acidic condition, 4.78% under alkaline condition, 7.8% under oxidation condition, 3.53% under dry heat condition and 4.9% under photolytic condition.

Acid degradation impurity was identified and characterised by LC-MS/MS was found to be 1-(4-Penten-1-yl) piperazine having molecular weight 154.253 (m/z 155.08) and Molecular Formula $C_9H_{18}N_2$.

Conclusion: A simple, precise, rapid and accurate Stability Indicating HPLC method has been developed and validated for the determination of Vilazodone Hydrochloride in presence of its degradation products as per the ICH Guidelines.

Keywords: Vilazodone Hydrochloride, HPLC, Stability Indicating Method, Acid Degradation Impurity

DOI: http://dx.doi.org/10.22159/ijcpr.2017v9i4.20975

INTRODUCTION

Vilazodone contains polymorph Form IV vilazodone hydrochloride for oral administration. Vilazodone belongs serotonergic antidepressant category. Vilazodone is an indole-piperazine derivative. It is a selective serotonin reuptake inhibitor (SSRI) and a 5-HT_{1A} receptor partial agonist. It has negligible affinity for other serotonin receptors. Vilazodone's antidepressant effects are thought to be due to the enhancement of serotonergic activity in the central nervous system (CNS) through selective inhibition of serotonin reuptake. Vilazodone Hydrochloride is indicated for the treatment of Major Depressive Disorder (MDD). It works by helping to restore the balance of serotonin in the brain. Vilazodone Hydrochloride is chemically 5-{4-[4-(5-Cyano-1H-indol-3-yl) butyl] piperazin-1-yl} benzofuran-2-carboxamide monohydrochloride [2] (fig. 1). Vilazodone Hydrochloride was approved by the FDA for the treatment of Major Depressive Disorder in January 2011. It is not official in any pharmacopoeia.

Stability is an essential factor for quality, safety and efficacy of a drug product. Literature search reveals that there are very few methods reported for the determination of Vilazodone Hydrochloride in different instrumental techniques like HPLC [3-6], UV [7], HPTLC [8], LC-ESI-MS/MS [9], UPLC-MS/MS [10], LC-MS/MS [11], LC-PDA [12], etc. Literature Survey also revealed that few Stability Indicating HPLC methods have been reported for drugs like Aripiprazole [13], Quetiapine Fumarate [14], Risperidone [15] and other antipsychotic drugs.

The aim of present study was to develop and validate stability indicating HPLC method for determination of Vilazodone Hydrochloride. As compared to other analytical techniques, HPLC method is highly powerful, quick, automated, accurate, efficient, reproducible, sensitive and extremely precise analytical technique. Also, there is no official method for the estimation of Vilazodone Hydrochloride in the pharmacopoeia so it was thought to develop and validate a new simple, sensitive, reliable and reproducible stability indicating HPLC method for Vilazodone Hydrochloride and to identify and characterize acid degradant impurity of Vilazodone Hydrochloride.

MATERIALS AND METHODS

Materials

Pure sample of Vilazodone Hydrochloride was received from SYMED LABS LIMITED, Hyderabad as a gift sample. All Chemicals and Reagents used were of Analytical Grade and HPLC Grade. Marketed Formulation used for assay was Valz-20 by Torrent Pharmaceuticals Ltd, Ahmedabad; having Label Claim 20 mg of Vilazodone Hydrochloride, Batch No. VAC51003, Mfg. OCT.2015, Exp. SEP.2017.

Instruments

The HPLC System used was Liquid Chromatograph: LC-2010 CHT (Shimadzu) with photodiode array detector and Class VP software. The column used was phenomenex, C_{18} (250 x 4.6; 5 µm). The LC instrument used for LC-MS analysis was surveyor plus HPLC system with autosampler and PDA detector.

Optimization of HPLC method

Optimization of Chromatographic condition

Chromatographic separation was achieved on reversed phase column Phenomenex, C_{18} (250x4.6 mm, 5 µm) using mobile phase

Acetonitrile: Water (50:50, v/v), pH 3.3 adjusted with Glacial Acetic Acid at ambient temperature. The flow rate was 1 ml/minute and injection volume was 10 µl. The detection was carried out at 240 nm.

Validation of proposed HPLC method

Preparation of standard

The standard stock solution of 1000µg/ml was prepared by dissolving 50 mg of Vilazodone Hydrochloride in 50 ml mobile phase. The standard stock solution of 100µg/ml was prepared by diluting 5 ml of standard stock solution (1000µg/ml) to 50 ml with mobile phase.

Linearity

The linearity response was determined by preparing and injecting solutions with concentration about 25, 37.5, 50, 62.5 and 75µg/ml prepared from standard stock solution (100µg/ml) with mobile phase.

Precision

The injection system precision was determined by performing 6 replicate injection for Repeatability at 50 µg/ml and 3 replicate injections for Intra-day and Inter-day Precision at 37.5, 50 and 62.5µg/ml.

Accuracy

The accuracy of the method was confirmed by Recovery study from Marketed Formulation at three level of standard addition. The quantity of tablet powder equivalent to 10 mg Vilazodone Hydrochloride was transferred to four individual 100 ml volumetric flasks about 25 ml of mobile phase were added and the flask was sonicated for 15 min. The volume was made up to the mark with mobile phase and mixed well. The sample solutions were prepared by spiking known amount of Vilazodone Hydrochloride at 80%, 100 % and 120% level to a pre-analyzed sample of Vilazodone Hydrochloride. The 5 ml of above solution further diluted to 10 ml with mobile phase. The 10 µl was injected for HPLC analysis.

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ for Vilazodone Hydrochloride were determined at a signal-to-noise ratio of 3:1 and 10:1 respectively by injecting a series of dilute solutions of known concentrations.

Robustness

Robustness of the method was determined by subjecting the method to a slight change in method condition using 50µg/ml solution of Vilazodone Hydrochloride; by changing Pump Flow Rate and by changing pH of Mobile Phase and % RSD was calculated.

Assay

20 tablets of Vilazodone Hydrochloride were weighed and powdered. The tablet powder equivalent to 10 mg of Vilazodone Hydrochloride was accurately weighed and transferred to a 100 ml volumetric flask, about 25 ml of mobile phase was added and the flask was sonicated for 15 min. The volume was made up to the mark with mobile phase and mixed well. The 5 ml of above solution was diluted to 10 ml with mobile phase. The 10 µl of 50µg/ml was injected for HPLC analysis.

Forced degradation study

Preparation of standard solution

The standard stock solution 100µg/ml of Vilazodone Hydrochloride was prepared by dissolving accurately weighed 10 mg of the drug, transferred to 100 ml volumetric flask, dissolved and made up to the volume using mobile phase. The 5 ml of above solution further diluted to 10 ml with mobile phase to give 50µg/ml solution.

Acidic degradation

Accurately weighed 10 mg of Vilazodone Hydrochloride was transferred to 100 ml volumetric flask. To the above 1 ml 1M HCl was added and 25 ml mobile phase was added. The solution was refluxed for 1hour at 80-100 °C in water bath. After that, the solution was cooled and neutralised by 1 ml 1M NaOH and the volume was made up to mark with mobile phase. Dilute 5 ml of above solution with mobile phase up to 10 ml.

Alkaline degradation

Accurately weighed 10 mg of Vilazodone Hydrochloride was transferred to 100 ml volumetric flask. To the above 1 ml 1M NaOH was added and 25 ml mobile phase was added. The solution was refluxed for 1hour at 80-100 °C in water bath. After that, the solution was cooled and neutralised by 1 ml 1M HCl and the volume was made up to mark with the mobile phase. Dilute 5 ml of above solution with mobile phase up to 10 ml.

Oxidative degradation

Accurately weighed 10 mg of Vilazodone Hydrochloride was transferred to 100 ml volumetric flask. To the above 1 ml 3%H_2O_2 was added and 25 ml mobile phase was added. The solution was refluxed for 1hour at 80-100 °C in water bath. After that, the solution was cooled and the volume was made up to mark with the mobile phase. Dilute 5 ml of above solution with mobile phase up to 10 ml.

Dry Heat degradation

Accurately weighed 10 mg of Vilazodone Hydrochloride was taken in a porcelain dish and kept in hot air oven at 100 °C for 6 h. After that Vilazodone Hydrochloride was transferred into 100 ml volumetric flask and volume was made up to the mark by mobile phase. Dilute 5 ml of above solution with mobile phase up to 10 ml.

Photolytic degradation

Accurately weighed 10 mg of Vilazodone Hydrochloride was taken in a porcelain dish with a closed lid and was exposed to sunlight in an open atmosphere for 24 h. After that Vilazodone Hydrochloride was transferred into 100 ml volumetric flask and volume were made up to the mark by mobile phase. Dilute 5 ml of above solution with mobile phase up to 10 ml.

Characterization of acid degradation impurity by LC-MS/MS

LC-MS experiment was carried out on LCQ Fleet Ion Trap LC/MSn equipped with Entrap Triple Quadrupole Analyzer. The LC instrument used for LC-MS experiment was Surveyor plus HPLC System with Auto Sampler and PDA Detector. The analysis was carried out using Hypersil C_{18} (250 x 4.6 mm; 5 µm) column, using mobile phase Acetonitrile: Water (50: 50; v/v) pH 3.3 adjusted with Glacial Acetic Acid at 240 nm detection wavelength, flow rate 1 ml/min, injection volume 10 µl. The Mass Spectra of Vilazodone Hydrochloride and its acid degradation product were recorded on LCQ Fleet Ion Trap LC/MSn mass spectrometer. The detection of ions was performed in Electron Spray Ionization positive ion mode. The data was collected and processed using Xcalibur software.

RESULTS AND DISCUSSION

Optimisation of Chromatographic condition

The chromatographic conditions were optimised with a view to developing a stability-indicating assay method. The method was optimized using Phenomenex, C18 (250x4.6 mm, 5 µm) using mobile phase Acetonitrile: Water (50:50, v/v), pH 3.3 adjusted with Glacial Acetic Acid at ambient temperature. The flow rate was 1 ml/minute and the injection volume was 10 µl. The detection was carried out at 240 nm.

Validation of proposed HPLC method

Linearity

The linearity of Vilazodone Hydrochloride was determined in the concentration range of 25, 37.5, 50, 62.5 and 75µg/ml by diluting stock solution. The calibration curve was found to be linear in this range. The correlation coefficient obtained was greater than 0.996.

Precision

The %RSD value of Vilazodone Hydrochloride for Repeatability was found to be 0.53%, for Intra-Day Precision was found to be in the range 0.72-1.41% and for Inter-Day Precision was found to be in the

range 0.47-0.80%. The %RSD value of Vilazodone Hydrochloride was found to be less than 2%, which indicates that the developed method is precise.

Accuracy

The percentage recovery of Vilazodone Hydrochloride was found to be in range 98.21±0.08%-99.07±0.64%.

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ of Vilazodone Hydrochloride were found to be 4.78µg/ml and 14.48µg/ml respectively.

Robustness

Robustness of the method was determined by slightly changing the pH and the flow rate of mobile phase using 50µg/ml solution of Vilazodone Hydrochloride. % RSD was found to be in the range 0.22-0.98 %.

Assay of marketed formulation

The proposed method was successfully applied to the marketed tablet dosage form Valz-20 having a label claim 20 mg of Vilazodone Hydrochloride. The % Assay was found to be 98.09±1.22 %.

Force degradation studies

Degradation under acidic condition

After refluxing, the drug solution with 1N HCl at 80-100 °C for 1 hour, the percentage degradation of Vilazodone Hydrochloride in acidic condition was found to be 3.1%.

Degradation in alkaline condition

After refluxing, the drug solution with 1N NaOH at 80-100 °C for 1 hour, the percentage degradation of Vilazodone Hydrochloride in alkaline condition was found to be 4.78%.

Oxidative degradation

After refluxing the drug solution with 3% hydrogen peroxide at 80-100 °C for 1 hour, the percentage degradation of Vilazodone Hydrochloride in Oxidative Condition was found to be 7.8%.

Dry heat degradation

After exposing the drug powder to dry heat at 100 °C for 6 h, the percentage degradation of Vilazodone Hydrochloride in Dry Heat condition was found to be 3.53%.

Photolytic degradation

After exposing the drug powder to direct sunlight in petri dish covered with lid, the percentage degradation of Vilazodone Hydrochloride in Photolytic condition was found to be 4.9%.

Characterization of impurity by LC-MS/MS

The base ion peak at retention time 3.44 min was m/z 442.33. The probable acid degradant with retention time 3.03 min showed base ion peak at m/z 155.08. Acid degradation impurity was identified and characterised by LC-MS/MS was found to be 1-(4-Penten-1-yl) piperazine having Molecular Weight 154.253 (m/z 155.08) and molecular formula $C_9H_{18}N_2$.

Table 1: Linearity of vilazodone hydrochloride

S. No.	Concentration (µg/ml)	Peak area (mean±SD); (n=3)
1	25	2351891.33±11650.43
2	37.5	3756985.00±23711.85
3	50	4763628.00±40607.60
4	62.5	6124760.33±16316.36
5	75	7113593.33±115875.76

Table 2: Intra-day and Inter-day precision

S. No.	Precision period	Concentration (µg/ml)	Mean (n=3)	SD	%RSD
1	Intra-Day Precision	37.5	3728892.67	52723.83	1.41
		50	4694937.67	62944.68	1.34
		62.5	6187850.33	44314.56	0.72
2	Inter-Day Precision	37.5	3749863.33	30067.72	0.80
		50	4699207.00	22055.11	0.47
		62.5	6136864.67	34131.21	0.56

Table 3: Results for recovery of vilazodone hydrochloride

Level	Amount from the sample(mg)	Amount of standard vilazodone spiked (mg)	Total Amount (mg)	Peak area	Total amount found (mg)	Amount recovered (mg)±SD (n= 3)	% amount recovered±SD (n= 3)
Blank	10	-	10	4731337.66±58029.53	9.81±1.24	-	-
80%	10	8	18	7739185±65986.01	16.13±0.14	7.90±0.05	98.81±0.68
100%	10	10	20	9443750.33±41570.78	19.72±0.09	9.91±0.06	99.07±0.64
120%	10	12	22	11457940.67±56480.16	23.95±0.12	11.78±0.01	98.21±0.08

Table 4: Summary of validation parameters

Parameters	Results
Linearity and Range	25-75µg/ml
Correlation Coefficient R^2	0.996
Regression Equation	y = 95129x+65700
Repeatability (%RSD)	0.53%
Intra-Day Precision (%RSD)	0.72-1.41%
Inter-Day Precision (%RSD)	0.47-0.80%
Accuracy (%)	98.21±0.08%-99.07±0.64%
LOD (µg/ml)	4.78 µg/ml
LOQ (µg/ml)	14.48 µg/ml
Robustness (%RSD)	0.22–0.98%
Assay (%)	98.09±1.22%

Table 5: Summary of degradation study under various stress condition

S. No.	Exposure condition		Number of degradation products (t_R) min	% Drug recovered	% degradation
1.	Acid Degradation	1N HCl (1h Reflux at 80-100 °C)	3(1.984, 2.560, 4.128)	96.88%	3.12%
2.	Alkaline Degradation	1N NaOH (1h Reflux at 80-100 °C)	4(2.517, 3.115, 4.053, 5.056)	95.22%	4.78%
3.	Oxidative Degradation	3%H_2O_2 (1h Reflux at 80-100 °C)	3(1.920, 2.763, 4.032)	92.2%	7.8%
4.	Dry Heat Degradation	Dry Heat (100 °C, 6 h.)	4(1.931, 2.229, 4.064, 5.099)	96.47%	3.53%
5	Photolytic Degradation	Photolytic (Sun Light, 24 h)	4(1.920, 2.123, 4.053, 5.120)	95.1%	4.9%

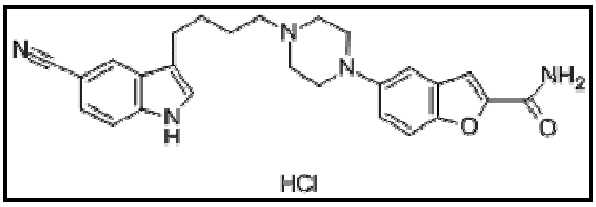

Fig. 1: Structure of vilazodone hydrochloride

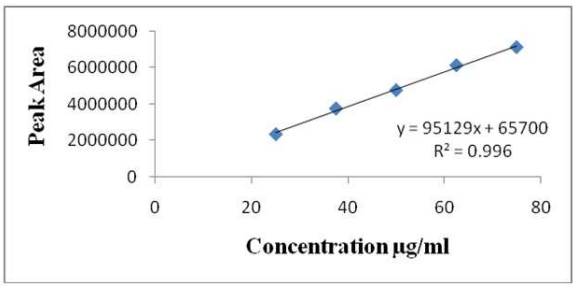

Fig. 2: Calibration curve for vilazodone hydrochloride (25-75µg/ml)

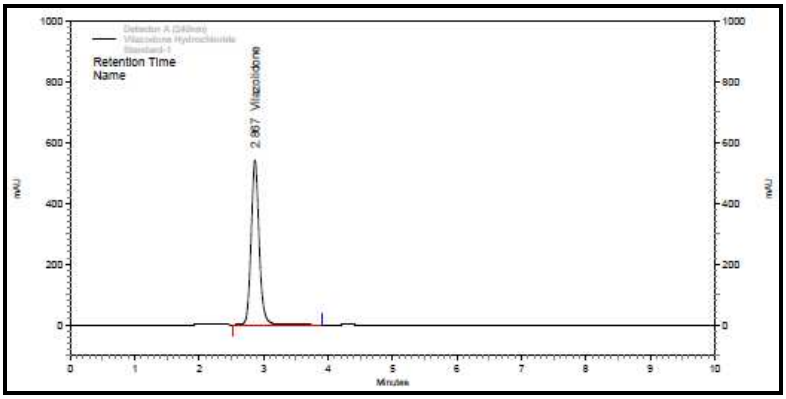

Fig. 3: Chromatogram of standard vilazodone hydrochloride (50µg/ml)

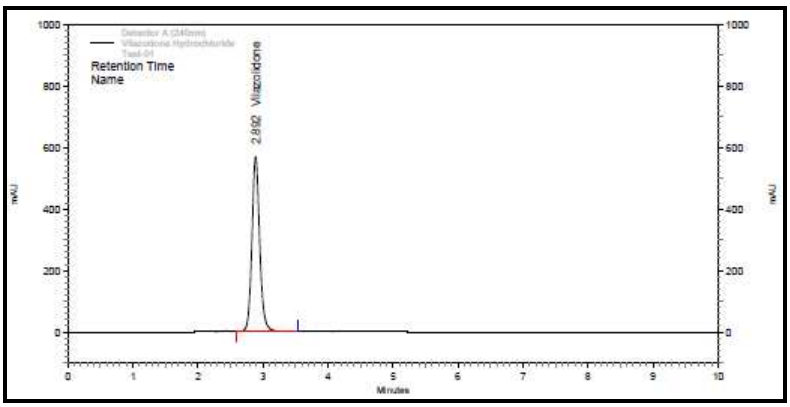

Fig. 4: Chromatogram of marketed formulation of vilazodone hydrochloride (50µg/ml)

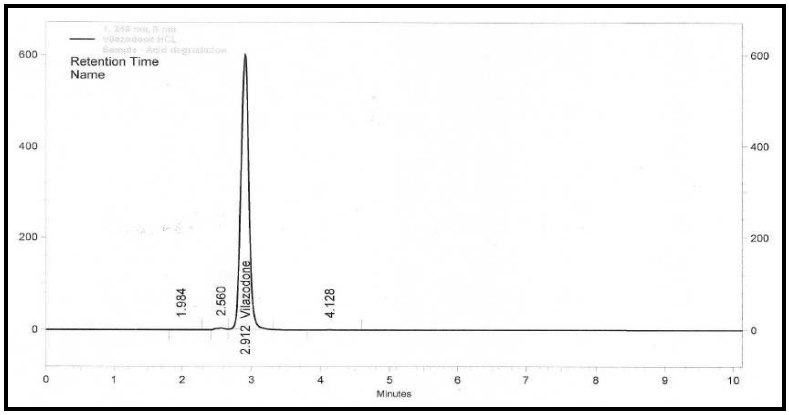

Fig. 5: Chromatogram of vilazodone hydrochloride in acidic degradation condition (50µg/ml)

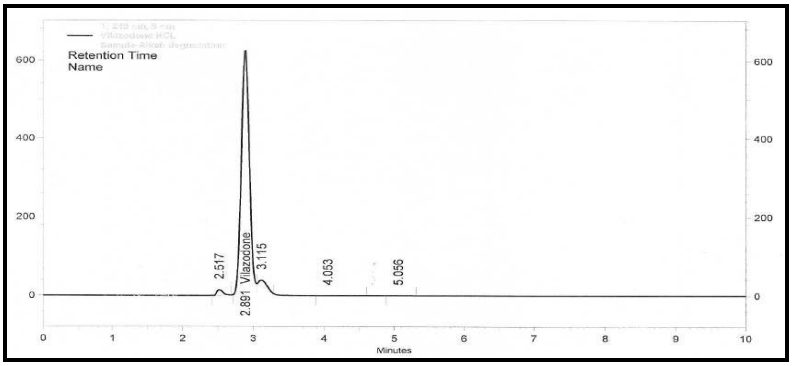

Fig. 6: Chromatogram of vilazodone hydrochloride in alkaline degradation condition (50µg/ml)

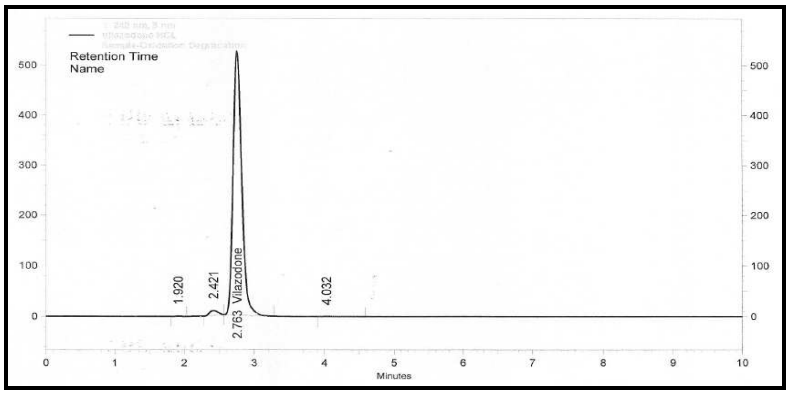

Fig. 7: Chromatogram of vilazodone hydrochloride in oxidative degradation condition (50µg/ml)

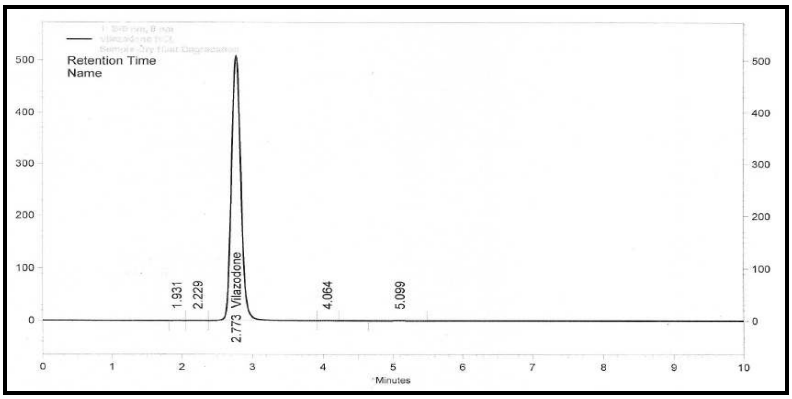

Fig. 8: Chromatogram of vilazodone hydrochloride in thermal degradation condition (50µg/ml)

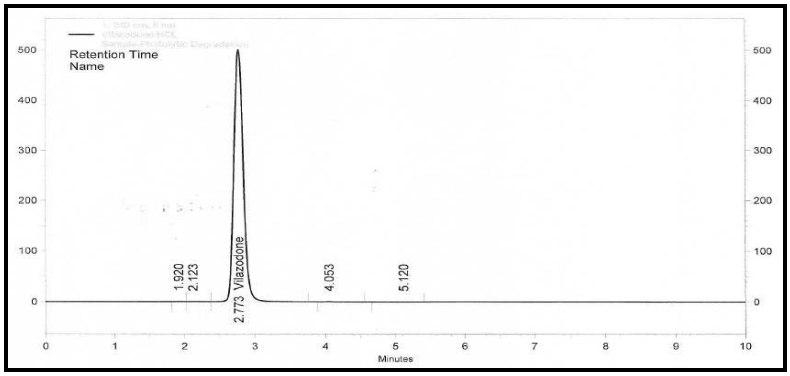

Fig. 9: Chromatogram of vilazodone hydrochloride in photolytic degradation condition (50µg/ml)

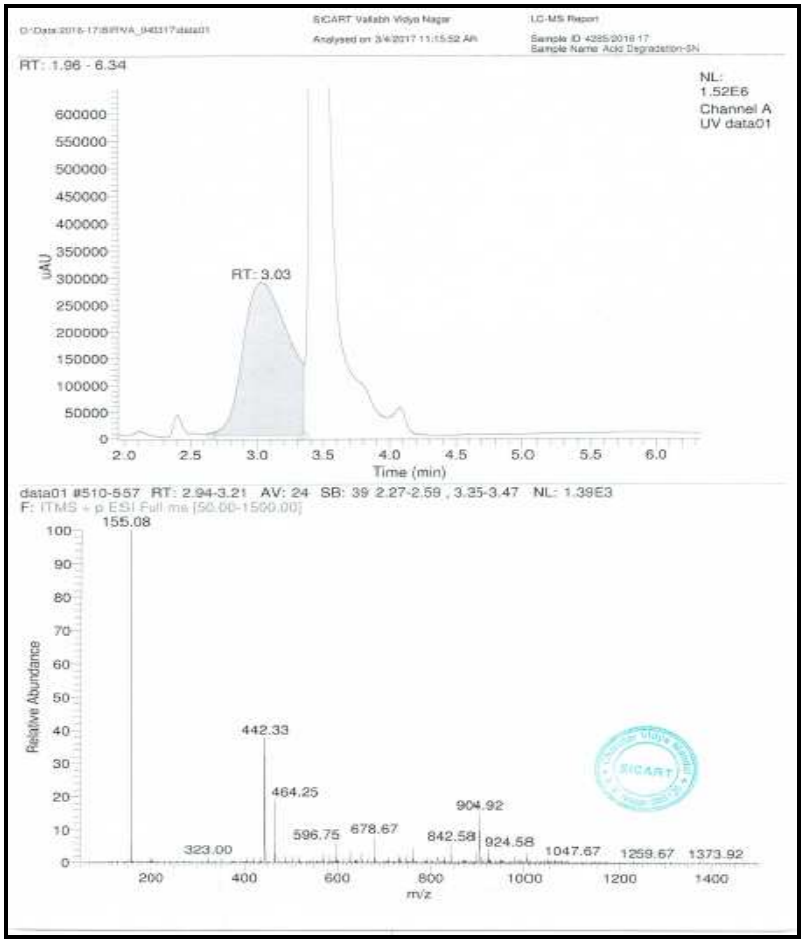

Fig. 10: LC-MS/MS spectrum of vilazodone hydrochloride in acid degradation condition at retention time 3.03 min

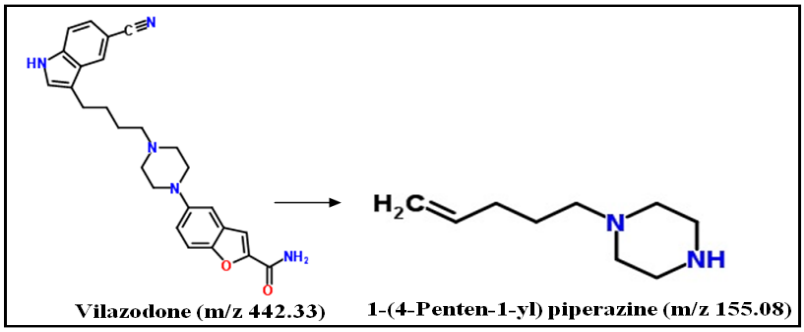

Fig. 11: Acid degradant of vilazodone hydrochloride

CONCLUSION

The proposed HPLC method was developed and validated according to ICH Guidelines and was found to be precise, accurate and stability indicating. The proposed method was successfully applied for the estimation of Vilazodone Hydrochloride in Marketed Tablet Formulation. The method can also be used for determining the drug purity by being able to detect related impurities. The method can be used in small laboratories with very high accuracy and also can save much time and money.

ACKNOWLEDGEMENT

The author would like to thank SYMED LABS LTD for providing the gift sample of Vilazodone Hydrochloride. The author would also like to thank the Bhagwan Mahavir College of Pharmacy, Surat and SICART, Vallabh Vidyanagar for providing facilities to carry out research work.

CONFLICT OF INTERESTS

Declare none

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How to cite this article

- Birva A Athavia, Zarna R Dedania, Ronak R Dedania, SM Vijayendra Swamy, Chetana B Prajapati. Stability indicating HPLC method for determination of vilazodone hydrochloride. Int J Curr Pharm Res 2017;9(4):123-129.