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DEVELOPMENT AND VALIDATION OF RP- HPLC METHOD AND FORCE DEGRADATION STUDIES FOR ESTIMATION OF VORTIOXETINE HBR IN BULK DRUG AND DOSAGE FORM

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ABSTRACT

A simple, rapid high-performance liquid chromatographic method with PDA detection has been developed and validated according to the ICH guidelines for the estimation of Vortioxetine HBR in bulk drugs and d dosage form. Chromatographic separation was carried out in a INERTSIL ODS-3V C-18 column (250mm x 4.6mm x 5µ) with mobile phase composition of a mixture of Ammonium acetate(pH 4.5) and Acetonitrile (40:60) at a flow rate of 1ml/min and sample injection of 10 µL was injected. The eluent was monitored with a chrom nav software with PDA detector set at 228 nm with a total run time of 15 minutes. The method was linear over the concentration range of 2.5 to 7.5 µg/ml for Vortioxetine HBR with a correlation coefficient of 0.9960 Accuracy was found to be 99.58 to 101.17%. The developed and validated method was successfully applied to assay of Vortioxetine HBR In dosage form.

KEYWORDS: Vortioxetine HBR, method development, RP-HPLC, PDA detector, stability indicating method, validation.

INTRODUCTION

Analytical methods are applied to find out identity, purity, physical characteristics and potency of the active pharmaceutical ingredient and formulation.[1] HPLC is most useful qualitative and quantitative technique. It is the foremost versatile, safest, dependable and fastest chromatographic technique for the quality control of drug components. advantage of these technique is that it will more accurate and reliable result and also reduce the time of analysis.[2]

Vortioxetine HBR is chemically 1-{2-[2,4-dimethylphenyl)sulphynyl]phenyl}piprazine monohydrobromide.[3] Vortioxetine HBR is used for the treatment of major depressive disorder. Vortioxetine HBR combines with serotonin transporter and its antidepressant action is due to enhancing serotonin level in central nervous system by reduction in reuptake of serotonin. Vortioxetine HBR is a serotonin modulator and simulator. It is partial agonist of 5 HT1B receptor and agonist of 5 HT1A and antagonist of 5HT3 receptor.[4]

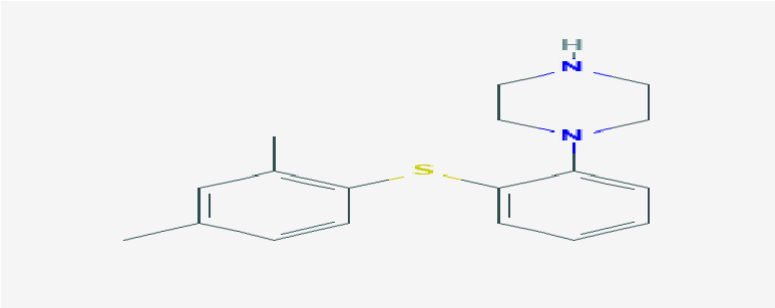


Fig. 1: Structure of Vortioxetine.

1-{2-[(2,4-dimethylphenyl)sulfanyl]phenyl}piperazine monohydrobromide.[5]

Literature survey reveals that there are very few methods available for assay of Vortioxetine HBR by using HPLC[3,4] hence attempt has been made to develope and validate stability indicating rp- hplc method for estimation of Vortioxetine HBR in bulk drug and dosage form And developed method was applied to marketed dosage form.

MATERIALS AND METHODS

Chemicals and Reagents: API sample of Vortioxetine HBR was obtained from Piramal pharma Mumbai as a gift sample. Brintellix 10 mg tablet was obtained from local market. Acetonitrile (HPLC Grade from MERCK)), Ammonium acetate (analytical grade from RANKEM), glacial acetic acid), and HPLC-grade water (from J. K. LAB) were among the other chemicals and reagents used in the analysis.

Instrumentation and Chromatographic Conditions: HPLC apparatus (JASCO; Model) consisted of gradient quaternary pump, multiple wavelength PDA detector, and chromnav software. Chromatographic separation was performed isocratically using a INERTSIL ODS-3V C-18 column (250mm x 4.6mm x 5µ) mobile phase composition of a mixture of Ammonium acetate(pH 4.5) and Acetonitrile (40:60) at a flow rate of 1ml/min and sample injection of 10 µL was injected. The eluent was monitored with a PDA detector set at 228 nm. Diluent was mixture of Acetonitrile and water (60:40).

Preparation of mobile phase: Mobile phase was consist of Ammonium acetate buffer and Acetonitrile in ratio of 40:60 and pH of buffer was adjusted to 4.5 using Glacial acetic acid. The mixture was sonicated for 15 mins with intermittent shaking. This solution used as mobile phase for further analysis.

Preparation of Stock and Working Solutions

For hplc method development and validation: 12.5 mg of vortioxetin HBR was accurately weighed and transferred to 100 ml volumetric flask and adjust volume with methanol (125 ug/ml solution A). 10ml of solution A was pipette out and transferred to 100 ml of volumetric flask and adjust the volume with diluent (12.5 ug/ml. solution B).

For force degradation studies: 50 mg of API was accurately weighed and transferred to 50 ml of volumetric flask and 25 ml of methanol was added and sonicated it for 15 min and volume was adjusted for 50 ml with methanol (1000 ug / ml Solution C). this solution was used as stock solution for force degradation studies.

Initial method development

Initially method development was started with 50:50 ACN water but peak eluted after long time. Then 70:30 ACN water combination used but peak elute to early and retention time is also fluctuating hence the final combination was ACN:Amm. acetate buffer with Ph 4.5 was used and diluent was mixture of ACN and buffer then all peak parameter within specfication but interfering peaks of diluent was observed hence buffer was remove from diluent and only ACN water mixture is used as diluent. And finally peaks was observed with proper system suitability parameter.

Method Validation[6]: The developed method was validated for Linearity and Range, Precision, Specificity, Accuracy, Limit of detection (LOD), Limit of quantitation (LOQ), and System Suitability according to ICH guidelines.[6]

System suitability: System suitability testing was carried out by injecting 6 replicates of 5 µg/ml Standard Vortioxetine HBR solution. In this test, system suitability parameters like %RSD of Peak area, retention time and number of theoretical plates (NTP) were evaluated.

Specificity: Specificity of the method was determined by recording the chromatogram of the Standard stock solution of Vortioxetine HBR (5 µg/ml) and Blank chromatogram (only diluent).

Linearity and Range: The linearity of the method was evaluated in the range of 2.5 ug / ml to 7.5 ug / ml for Vortioxetine HBR. The calibration standard for linearity was prepared at a concentration of 2.5, 3.75, 5, 6.25, 7.5 ug/ml by further dilution of solution B and volume was adjust with diluent. Then each level was injected six times in HPLC, Chromatogram was recorded and peak area was calculated for all the peak. The calibration curve was plotted as mean peak area of the analyte against concentration of Vortioxetine HBR in ug / ml.

Precision: Precision was estimated at three different concentration of Vortioxetine HBR at 2.5 ug / ml, 5 ug / ml and 7.5ug/ml (50%, 100% and 150% of working level) on intraday precision and interday precision. Then mean area, standard deviation and % RSD were calculated.

Accuracy: The accuracy of the method was calculated as % recovery from blank solution spiked with 50%, 100% and 150%. The experiment was conducted in triplicate. The % recovery was calculated for each solution.

LOD and LOQ: The LOD and LOQ of method was estimated by applying injections of low concentrations of Vortioxetine HBR standard solution. The concentration of solution which give signal to noise ratio of NLT 3:1 is LOD and concentration of solution which give signal to noise ratio of NLT 10:1 is LOQ of method.

APPLICATION OF DEVELOPED METHOD FOR MARKETED FORMULATION

Vortioxetine HBR available with dose strength 10 mg. 10 tablets were weighed and average weight of tablet was calculated. 10 tablets were crushed in morter with help of pestle. Powder equivalent to 10 mg of Vortioxetine HBR was weighed on weighing balance and transferred to 10 ml of volumetric flask and 6 ml of methanol was added and continuous shake for 30 min and then sonicated for 30 min with occasional swirling. Volume was adjusted for 10 ml with methanol. further dilutions was made to make final concentration of 5 ug/ml of Vortioxetine HBR and volume was adjusted with diluent.

FORCED DEGRADATION STUDIES OF VORTIOXETINE HBR USING VALIDATED METHOD

Forced degradation studies were carried out by exposing the stock solution of the drug to the following conditions:

1. Acid hydrolysis

2. Base hydrolysis
3. Oxidative Degradation
4. Thermal degradation
5. Photolytic degradation

Acid Hydrolysis: 2 ml of stock solution (Solution C) was pipette out and transferred to a round bottom flask and 2 ml of 1N HCl was added in round bottom flask and this mixture was refluxed on a water bath for 15 min at 60°C. After the reflux, the round bottom flask containing the stressed solution was cooled to room temperature, transferred to a 10 ml volumetric flask, neutralized with the corresponding base and volume was made up with diluent. Finally, this solution was filtered through 0.45µ filter paper and was loaded into HPLC and the corresponding chromatogram was recorded.

Base Hydrolysis: 2 ml of stock solution was pippette out and transferred to a round bottom flask and 2 ml of 0.1N NaOH was added in round bottom flask and this mixture was refluxed on a water bath for 15 min at 60°C. After the reflux, the round bottom flask containing the stressed solution was cooled to room temperature, transferred to a 10 ml volumetric flask, neutralized with the corresponding acid and volume was made up with diluent. Finally, this solution was filtered through 0.45µ filter paper and was loaded into HPLC and the corresponding chromatogram was recorded.

Thermal Degradation: 2 ml stock solution was pipeette out and transferred to a round bottom flask and this was refluxed on a water bath for 1 hrs at 80°C. After the reflux, the round bottom flask containing the stressed solution was cooled to room temperature, transferred to a 10 ml volumetric flask and volume was made up with diluent. Finally, this solution was filtered through 0.45µ filter paper and was loaded into HPLC and the corresponding chromatogram was recorded.

Oxidative degradation: 2ml of stock solution was pipeette out and transferred to a round bottom flask. and 2 ml of 3% Hydrogen Peroxide was added in round bottom flask and this mixture kept as such at room temperature for 30 minutes. After 30 min, the round bottom flask containing the stressed solution was transferred to a 10 ml volumetric flask and volume was made up with diluent. Finally, this solution was filtered through 0.45µ filter paper and was loaded into HPLC and the corresponding chromatogram was recorded.

Photolytic degradation: The photolytic degradation was carried out by exposing drug substance i.e. Vortioxetine HBR (10 mg) in the UV chamber for one week. After 1 week, the drug substance was dissolved in 10 ml volumetric flask and then the volume was made up with diluent. 2 ml of this solution was further diluted to 10 ml with diluent. Finally, this solution was loaded into HPLC and the corresponding chromatogram was recorded.

RESULTS AND DISCUSSION

Table no 1: Optimized chromatographic conditions.

Parameter	Specifications
Pump	Jasco PU-2089 Plus Quaternary Gradient HPLC Pump
Detector	Jasco MD-2018 Plus intelligent PDA Detector
Software	ChromNAV Software
Column	INERTSIL ODS-3V C18 column (250mm x 4.6mm x 5µ)
Mobile Phase	Ammonium acetate buffer (Ph 4.5): ACN (40 : 60)
Wavelength	228 nm
Injection volume	10 ul
Flow Rate	1 ml / min

Representative Chromatogram of Vortioxetine HBR Standard Solution (5 ug/ml).

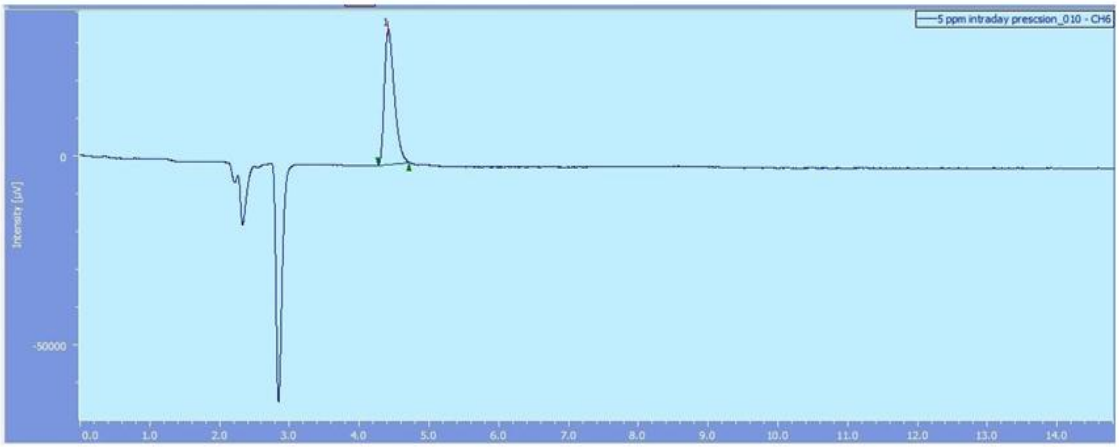


Fig. 2: Chromatogram of Vortioxetine HBR Standard (5µg/ml).

VALIDATION OF OPTIMIZED CHROMATOGRAPHIC METHOD OF VORTIOXETINE HBR BY USING HPLC

System Suitability

The Vortioxetine HBR Standard Solution of 5 µg/ml was injected in six replicates. The System Suitability parameters were obtained and are summarized in table given below.

Table no 2: System suitability data for Vortioxetine HBR.

Sr. no	System suitability parameter	Observations	Acceptance criteria
1	Vortioxetine HBR standard	5 ug/ml	
2	Area % RSD	0.4713	NMT 2%
3	Retention time (Rt)	4.4 min	
4	NTP	4775	NLT 2000
5	Symmetry factor	1.471	0.8 TO 2

The System Suitability parameters were within specification.

Specificity: The Specificity is done mainly for Identification, Interference and Peak Purity of drug. Blank (Diluent) and Vortioxetine HBR Standard Solution (5µg/ml) were injected and the representative Chromatograms for specificity are shown below.

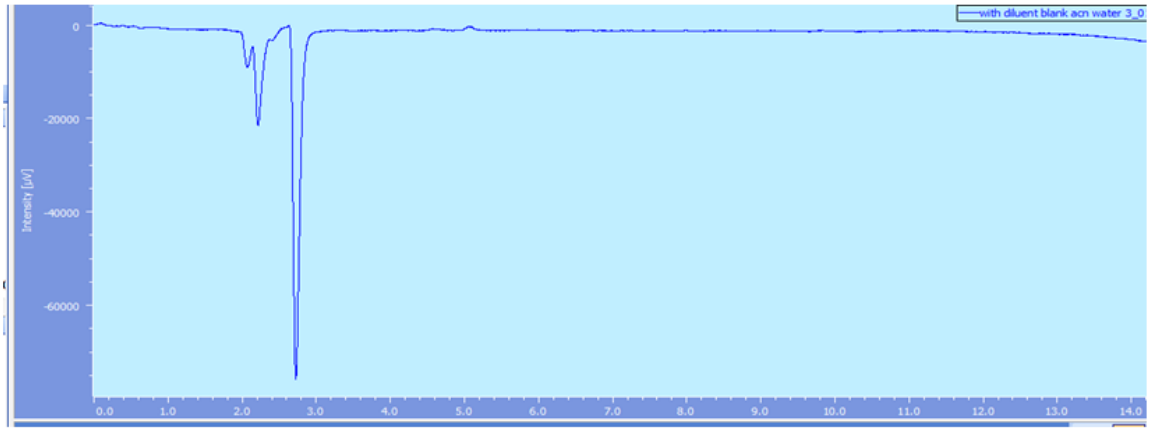


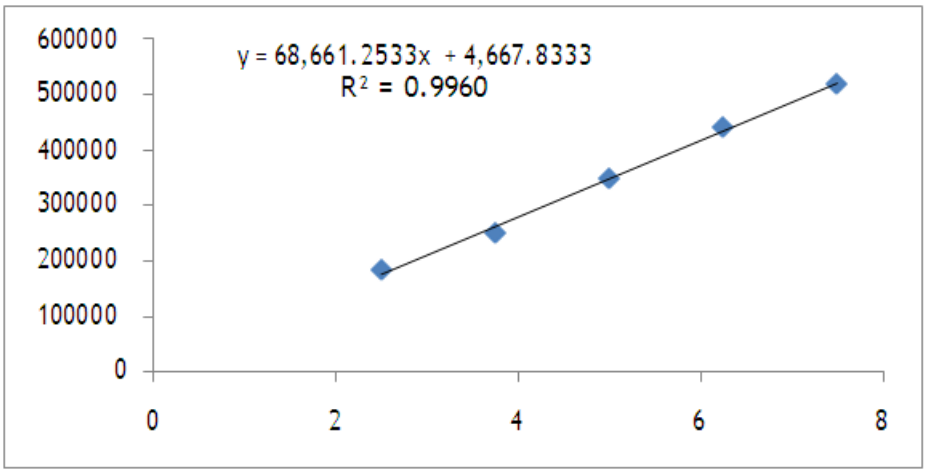
Fig no 3: Chromatogram for Blank.

There was no any interfering peak around retention time of API (4.4) hence the method was proved to specific.

Linearity and range: Linearity was done over the range of 50 to 150% of working level i.e 5ug/ml in six replicates for Vortioxetine HBR was determined and observation is as follows.

Table no 3: Linearity for Vortioxetine HBR.

Concentration (ug/ml)	Peak area						
	Lin 1	**Lin 2**	**Lin 3**	**Lin 4**	**Lin 5**	**Lin 6**	**Mean**
2.5	184682	186362	182756	185340	180847	186881	184478
3.75	24886	249056	248851	249466	248240	248543	248840.3
5	350197	348420	349719	346637	345021	348762	348142.7
6.25	441393	442766	440240	440422	439167	440554	440757
7.5	518069	516428	517937	515565	519979	517937	517652.5



Graph no 1: Calibration graph for Vortioxetine HBR.

The Linearity was done in the range of **2.5 to 7.5 ug/ml**. The Correlation Coefficient (r^2) was found to be **0.9960** Thus, the data shows that the response was found to be linear.

Precision

Intraday Precision: Intraday precision of Vortioxetine HBR was determined by taking six replicates of 50%, 100% and 150% of working level i.e. 2.5µg/ml, 5µg/ml and 7.5µg/ml concentration at different time intervals on same day.

Interday Precision: Interday precision of Vortioxetine HBR was determined by taking six replicates of same 3 concentration on two consecutive days.

The % RSD for Intraday and Interday Precision of three concentration levels was found to be 0.45 and 1.69 respectively which is below 2% which shows that method was found to be precise.

E) Accuracy

% Recovery study was performed using a minimum of 3 concentration level i.e 50%, 100% and 150% of working level concentration. Results for % Recovery summarized below.

Table no 4: Accuracy Data of Vortioxetine HBR.

Sr. no	Conc. (ug/ml)	% Recovery
1	2.5	101.17
2	5	100.42
3	7.5	99.58

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

For LOQ signal to noise ratio is 10:1 and for LOD signal to noise ratio is 3:1.

Limit of Quantification (LOQ)

The limit of quantitation was found to be 12.5 ng. Hence method was found to be sensitive.

Limit of Detection (LOD)

The LOD was found to be 6.5 ng. This proves that method was sensitive.

APPLICATION OF DEVELOPED AND VALIDATED METHOD FOR ASSAY OF MARKETED FORMULATION

The developed and validated method is applied sucessfully for assay determination of marketed formulation. The representative chromatogram of is as shown below.

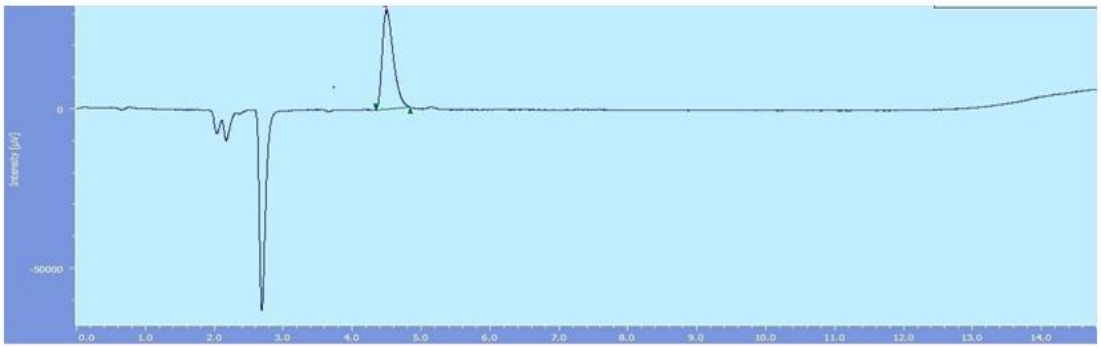


Fig No. 3: Chromatogram of Marketed Formulation.

Table no 5: Assay of Marketed formulation.

Sample	Assay
Marketed Formulation	99.61%

STABILITY INDICATING ASSAY METHOD OF VORTIOXETINE HBR BY USING HPLC

To prove that the developed method method is stability-indicating Forced degradation is performed. The result obtained for various stress studies are shown below.

Table no 6: Force degradation data for stability studies.

Sr no	degradation	Concentration	% degradation
1	undegraded	200 ug/ml	0.00
2	Acid hydrolysis	200 ug / ml	9.9%
3	Base hydrolysis	200 ug/ml	1 %
4	Oxidation	200 ug/ml	8%

5	Thermal	200ug/ml	2%
6	photolysis	200ug/ml	0.00
7	Total degradations		19.9%

CONCLUSION

Here, we have developed and validated an HPLC-PDA method that has provides simple mobile phase composition for chromatographic separation, shorter run time for analysis, simple sample preparation as well as improved sensitivity. Therefore, this method leads to a simple, feasible, cost-effective, rapid method with a high degree of accuracy and specificity to quantify Vortioxetine HBR in bulk and tablet dosage form. The developed method was proved to be stability indicating assay method.

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