International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 2 Mar-Apr 2022, pp: 1248-1255 www.ijprajournal.com ISSN: 2456-4494

Development and Validation of RP-HPLC method for Estimation of Vortioxetine in bulk drug and In Pharmaceutical products

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Submitted: 05-04-2022 Accepted: 17-04-2022

ABSTRACT
The depression is a mood disorder that causes a persistent feeling of sadness and loss of interest. Also called as major depressive disorder (MDD) or Clinical depression.Vortioxetine is a novel anti-depressant drug, it may effectively treat for depression and cognitive dysfunction in adults with MDD. A rapid and specific reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed for the validated of Vortioxetine in its pure form as well as in tablet dosage form. The reagents was used Vortioxetine, Merck provided HPLC grade methanol, acetonitrile, orthophosphoric acid (OPA), and analytical grade ethanol, DMF, DMSO, and HCL and the HPLC grade water. The developed method was validated for Linearity and Range, Precision, Specificity, Accuracy, Limit of detection (LOD), Limit of quantitation (LOQ), and System Suitability. The linearity was accessed by plotting calibration curve of Vortioxetine. For these, five different concentrations of ranging from 10-50 µg/ml were taken. Additionally, the degradation products were identified by the high-resolution liquid chromatography coupled with electrospray ionization-quadrupole-time of flight-mass spectrometry method. The RP-HPLC method was successfully applied for the quantification of Vortioxetine in tablets. Different parameters affecting chromatographic separation were studied, including column efficiency (number of theoretical plates), tailing factors, percentage relative standard deviation (%RSD) of the peak area, and retention time of six injections. The methods validated for specificity, LOQ, LOD, linearity, precision, accuracy, and robustnesswas performed.

KEYWORDS: Vortioxetine, RP-HPLC; PDA Detection, Validation; Tablet dosage forms

I. INTRODUCTION

Major depressive disorder (MDD) is a medical illness that affects how people think, feel, and behave, causing persistent feelings of sadness and loss of interest in previously enjoyed activity.[1] Major depression frequently goes untreated and unrecognized and may foster tragic consequences, such as suicide and impaired interpersonal relationships at work and at home.[1-3] some people may experience only a single episode in their lifetimes, but more often a person may have multiple episodes.[1-3] MDD is one of the most common mentally disorders in the United States (US), affecting 6.7% of the population annually. Women are 70% more likely to experience MDD than men. [2]

Vortioxetine is a novel anti-depressant drug, it may effectively treat for depression and cognitive dysfunction in adults with MDD.[4,5] it is chemically 4-{2-[(2,4-dimethylphenyl)sulfanyl] piperazine-1-ium vortioxetine and its Molecular Formula and Molecular Weight C18H22N2S and 298.45 gm/mole.[6,7]Vortioxetine is a medication used to treat major depressive disorder (MDD).[8] Effectiveness is viewed as similar to that of other antidepressants.[8]Common side effects include nausea, vomiting, constipation, and sexual dysfunction.[8,9] Serious side effects may include suicide in those under the age of 25, serotonin syndrome, bleeding, mania, andSyndrome of inappropriate antidiuretic hormone secretion (SIADH).[8] A withdrawal syndrome may occur if the dose is rapidly decreased[8] Use during pregnancy and breastfeeding is not generally recommended.[10] It is classified as a serotonin modulator and stimulator.[8,11] How it works is not entirely clear but is believed to be related to increasing serotonin levels and possibly interacting with certain receptors for serotonin.[8,12,13]

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Volume 7, Issue 2 Mar-Apr 2022, pp: 1248-1255 www.ijprajournal.com ISSN: 2456-4494

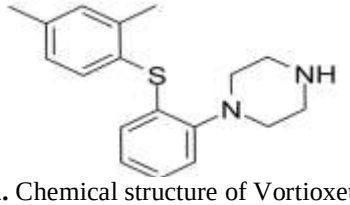

Fig 1. Chemical structure of Vortioxetine

Pharmaceutical Analysis plays a very vital role in the quality assurance and quality control of bulk drugs and their formulations. Pharmaceutical analysis is a specialized branch of analytical chemistry which involves separating, identifying and determining the relative amounts of components in a sample of matter. It is concerned with the chemical characterization of matter both quantitative and qualitative.[14]

A new, sensitive, suitable, clear, accurate, and robust reversed-phase high-performance liquid chromatography (RP-HPLC) method for the determination of vortioxetine in bulk drug and pharmaceutical product was developed and validated in this research. Surface methodology was used to optimize the data, with a three-level Box-Behnken design. Method concentration in the mobile phase, flow rate, and PH were chosen as the three variables.[15] The separation was performed using an HPLC method with a UV detector and Open lab EZchrom program, as well as a Water spherisorb C_{18} column (100 mm × 4.6; 5m). Acetonitrile was pumped at a flow rate of 1.0 mL/min with a 10mm phosphate buffer balanced to a pH of 2.50.05 by diluted OPA (65:35% v/v) and detected at 216 nm.

The aim of the present review is to develop and validate a sample, fast and reliable isocratic RP-HPLC method detection for the determination of Vortioxetine in bulk and in tablet dosage forms. The important features and novelty of the proposed method included simple sample treatment with sonication of small amount of powder sample at ambient temperature, short elution time (less than 5 min) Vortioxetine , good precision (R.S.D.less than 2%) and high recovery (greater than 98%). Confirmation of the applicability of the developed method validated according to the International Conference on Harmonization (ICH) [15-17] for the determination of Vortioxetine in bulk and in tablet dosage form.[14,18]

II. EXPERIMENTAL

Chemical reagents:

Vortioxetine, Merck provided HPLC grade methanol, acetonitrile, orthophosphoric acid (OPA), and analytical grade ethanol, DMF, DMSO, and HCL and the HPLC grade water.[15,16]

Instrumentation and analytical conditions:

The HPLC system (Waters alliance 2695 HPLC) consisted of a pump (LC-10 ATVP series pump) equipped l loop (Rheodyne Inc.,Cotati, CA, USA), an PDAμwith a Rheodyne model -7161 injection valve with a 20 4.6mm×(type SPD 10 AVP) set at 254 nm. The Analytical column, a Phenomenax Luna C18 (150mm c). Isocratic elution with Acetonitrile 1± particle size) was operated at ambient temperature (20 μi.d.,5 was used at a flow rate of 1ml/ min. The mobile phase was prepared freshly and degassed by sonicating for 10 min before use (Soltec, Soluzioni tecnologiche, Luglio, Italy). The UV spectrum of Vortioxetine for selecting the working wavelength of detection was taken using a Lab India UV - Visible spectrophotometer. [17,18]

Method Development:

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Vortioxetine working standard into a 10ml of clean dryvolumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of aircompletely and make volume up to the mark with the same Methanol.

Further pipette 0.3ml of the above Vortioxetine stock solution into a 10ml volumetric flask anddilute up to the mark with Methanol. [15,19]

VALIDATION

Preparation of mobile phase:

Accurately measured 1000 ml (100%) of HPLC Acetonitrile in a 1000ml volumetric flask.

Diluent Preparation:

The Mobile phase was used as the diluent.

Table no 1: Optimized chromatographic conditions

Parameters	Methods
Stationary phase (column)	Inertsil -ODS C18 (250 x 4.6 mm, packed with 5 micron)
Mobile Phase	Acetonitrile and Methanol (70:30)
Flow rate (ml/min)	1.0 ml

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Run time (minutes)	6
Volume of injection loop (µl)	20
Detection wavelength (nm)	274nm
Drug RT (min)	2.922

VALIDATION PARAMETERS

System suitability:

Accurately weigh and transfer 10 mg of Vortioxetine working standard into a 10ml of clean dryvolumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and makevolume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3ml of the above Vortioxetine stock solution into a 10ml volumetric flask anddilute up to the mark with diluents.[15,18]

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Vortioxetine working standard into a 10ml of clean dryvolumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and makevolume up to the mark with the same solvent.

Further pipette 0.3ml of the above Vortioxetine stock solutions into a 10ml volumetric flask anddilute up to the mark with diluents.[18,20]

Preparation of Sample Solution:

Take average weight of the Tablet and crush in a mortar by using pestle and weight 10 mgequivalent weight of Vortioxetine sample into a 10mL clean dry volumetric flask and add about7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with thesame solvent.

Further pipette 0.3ml of Vortioxetine above stock solution into a 10ml volumetric flask anddilute up to the mark with diluent.[18,19]

Preparation of Drug solution for Linearity:

Accurately weigh and transfer 10 mg of Vortioxetine working standard into a 10ml of clean dryvolumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and makevolume up to the mark with the same solvent. (Stock solution)[18-20]

Specificity:

The blank solution was performed and recorded the chromatogram.

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:

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.[21]

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 (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. [21,22]

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Precision:

Precision was estimated at three different concentrations 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.[22]

APPLICATION OF DEVELOPED METHOD FOR MARKETED FORMULATION

Vortioxetine 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 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.[15] Volume was adjusted for 10 ml with methanol. further dilutions was made to make final concentration of 5 ug/ml of Vortioxetine and volume was adjusted with diluent.[23]

FORCED DEGRADATION STUDIES OF VORTIOXETINE USING VALIDATED METHOD

Forced degradation studies were carried out by exposing the stock solution of the drug to the following conditions: [21,24]

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. [22-24]

Base Hydrolysis:

2 ml of stock solution was pipette 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. [22-24]

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.[22-24]

Oxidative degradation:

2ml of stock solution was pipette 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.[22-24]

Photolytic degradation:

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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.[22-24]

III. RESULTS AND DISCUSSION

Linearity:

The linearity was accessed by plotting calibration curve of Vortioxetine HBr. For these, five different concentrations of ranging from 10-50 µg/ml were taken. (Figure 2 and Table 2)

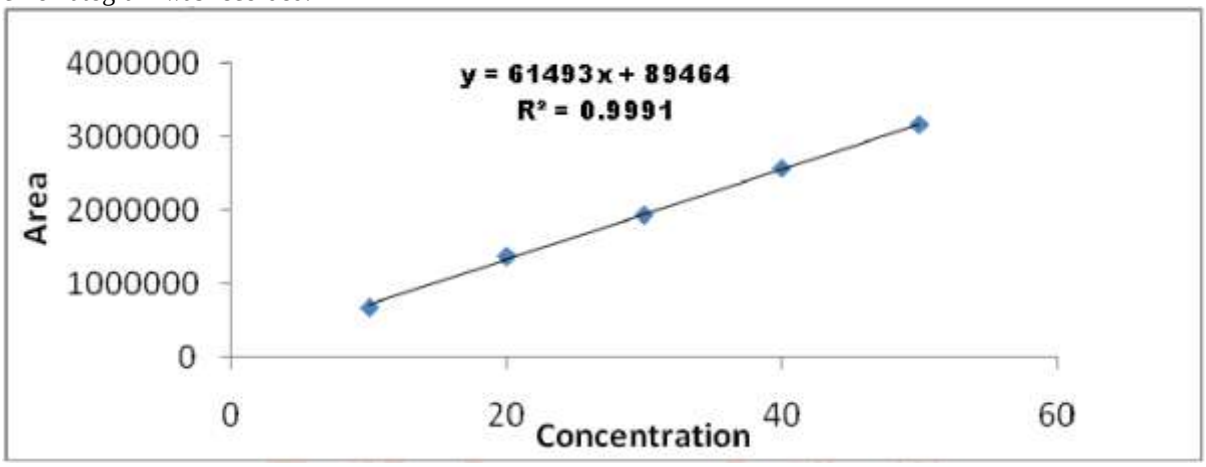

Fig no. 2: Calibration curve for Vortioxetine

Table no. 2: Data of Calibration curve of Vortioxetine

Sr. No.	Conc. (µg/ml)	Area
1	10	672090
2	20	1365679
3	30	1924578
4	40	2558598
5	50	3150259

Analysis of tablet formulation:[25-30]

Line equation obtained from calibration plot was used to calculate label claim of marketed formulation of Vortioxetine. (Table 3)

Table No 3: Data of Tablet Analysis of Vortioxetine

Drug Name	Mean	SD	%RSD
Brintellix (Vortioxetine)	100.26	0.2900	0.2861

*Average of six determinations
SD=Standard Deviation, RSD=Relative Standard Deviation

Accuracy:[25-30]

Accuracy was determined by standard addition method. The study was determined by spiking known amount of standard stock to the test solution prepared from tablet formulation at three different spiking level 50%, 100%, 150% of the target concentration. (Table No. 4)

Table no 4: Data for recovery study of Vortioxetine

Level of addition	Tablet drug conc.(µg/ml)	Standard added (µg/ml)	Total conc. (µg/ml)	% Mean* recovery	SD	%RSD
50%	10	20	30	100.4	0.8079	0.804828

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100%	20	20	40	99.16	1.2364	1.246803
150%	30	20	50	99.81	0.356	0.356701

*Average of three determination

Precision:[25-30]

Precision of an analytical method was ascertained by replicate analysis of homogeneous sample. It involves intraday and interday precision. For intraday precision three concentrations of 10, 20, 30 µg/mL were analyzed three times on the same day and for intraday precision solutions were analyzed for the days at the same concentration level. (Table No. 5 and 6)

Table no 5: Data for intraday precision of Vortioxetine

Sr. no.	Conc. (µg/ml)	Mean*	SD	%RSD
1	10	675403	8240.745	1.220123
2	30	1922588.33	4458.668	0.23191
3	50	3156396.33	5375.561	0.170307

*Average of three determination

Table no 6: Data for interday precision of Vortioxetine

Sr. no.	Conc. (µg/ml)	Mean*	SD	%RSD
1	10	679485	8637.90293	1.27124262
2	20	1930531.33	6758.75805	0.35009833
3	30	3151484.67	4927.78473	0.15636391

*Average of three determination

Robustness:[25-30]

Robustness is the measure of a method unaffected by small, deliberate changes in method parameters like flow rate. The small but deliberate variations in the optimized method parameters were done to evaluate the robustness of the proposed method. The study was conducted to determine the effect of variation in flow rate. Standard solutions of Vortioxetine was prepared and injected into the HPLC system by keeping flow rates 0.7 ml/min and 0.9 ml/min (Table 7 and 8). The effect of variations in flow rate was evaluated.

Table no 7: Data for Robustness study of Vortioxetine

Sr. No.	Parameters	Flow rate mL/min	Area	Mean*Area	SD	%RSD
1	Change in Flow Rate (ml/min) (20ppm)	0.7	1362540	1355854	6813.93	0.50256
2		0.8	1356104			
3		0.9	1348919			

*Average of three determination

Table no 8: Data for robustness study of Vortioxetine

Sr.no.	Parameters	Wavelength (nm)	Area	Mean*Area	SD	%RSD
1	Change in Wavelength (nm) (20ppm)	368	1342515	1345004	10229.7	0.76057
2		370	1356249			
3		372	1336249			

*Average of three determination

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

The LOD and LOQ of Vortioxetine HBr were found to be 0.23927µg/ml and 0.72507µg/ml respectively. The low LOD and LOQ values for

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Vortioxetine HBr indicate the sensitivity of the method.

Specificity: [25-30]

Specificity study is the ability to asses unequivocally the analyte in the presence of component which may be expected to be present. For the specificity study of proposed method the sample may be spiked with excipients or possible interfering components. Results of specificity study were found in analytical limits shown in table. (Table 9)

Table no 9: Data for specificity study of Vortioxetine

Level of addition	Drug conc.	Excipients	Total conc. (µg/ml)	mean	SD	%RSD
50%	10	20	30	672550	438.32	0.0652
100%	2	20	40	1362348	12324.8	0.9047
150%	30	20	50	1929299	3113.5	0.1614

*Average of three determination

IV. CONCLUSION

The symmetry, resolution, and repeatability of the chromatographic peaks were assessed using system suitability tests. Repeatability and symmetry factors show the accuracy of quantitative analysis, and resolution can be used to confirm that closely eluting compound are resolved from each other. c. Based on validation results, the RP-HPLC method developed was considered suitable for the quantitation vortioxetine residues in VT.

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