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Analytical Method Development and Validation of Vortioxetine Hydrobromide by RP-HPLC on Bulk and Tablet Dosage Form

Avinash Gajanan Tapkire[1], Rishikesh S. Bachhav[2]

[1]Department of Quality Assurance Techniques, [2]Department of Pharmacology,
[1,2]R. G. Sapkal College of Pharmacy, Anjaneri, Nashik, Maharashtra, India

ABSTRACT

Purpose: A simple, rapid, highly sensitive, selective, specific, robust RP-HPLC method has been developed and validated for the estimation of Vortioxetine HBr in bulk and Tablet Dosage form. **Methods:** The Chromatographic separation was obtained using a mobile phase ACN: Methanol: Water (40:50:10V/V) on column Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron) at 30℃ with UV detection 370 nm at flow rate 0.8 ml/min. The linearity was found to be 10-50µg/ml. The retention time was found to be 4.498 min. **Results:** The method was validated according to ICH. The correlation coefficient (r^2) of HPLC method was found to be 0.9991. The LOD and LOQ were found to be 0.23927µg/ml and 0.72507 µg/ml respectively. The intra-day and inter-day precision and accuracy values for method were found to be ≤ 2% RSD. **Conclusion:** The Proposed RP-HPLC method has been successfully applied to the commercial tablets without any interference of excipients. The method can be used for the routine analysis in industry for Vortioxetine HBr in bulk and tablet dosage forms.

KEYWORDS: *Vortioxetine HBr, RP-HPLC, Tablet Dosage form, Mobile Phase, ICH, Validation, Depression*

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INTRODUCTION

Major depressive disorder (MDD), also known basically as depression, is a mental disorder characterized by at least two weeks of general low mood. Low self-confidence, loss of interest in normally enjoyable activities, low energy, and pain without a strong reason are common symptoms. Those affected might also occasionally have delusions or hallucinations. Some people have phases of depression separated by years, while others nearly always have symptoms present. Major depression is more severe and persists longer than sadness, which remains a normal part of life.[1, 2]The Food and Drug Administration (USFDA) approved Vortioxetine (Brintillex, Takeda Pharmaceuticals) for the treatment of adults with Major depressive disorder (MDD)on year 2013.The chemically Vortioxetine hydrobromide is 1-[2-(2,4-dimethylphenyl) sulfanyl phenyl] piperazine; hydro bromide (fig no.1) corresponding to the molecular formula $C_{18}H_{23}BrN_2S$.

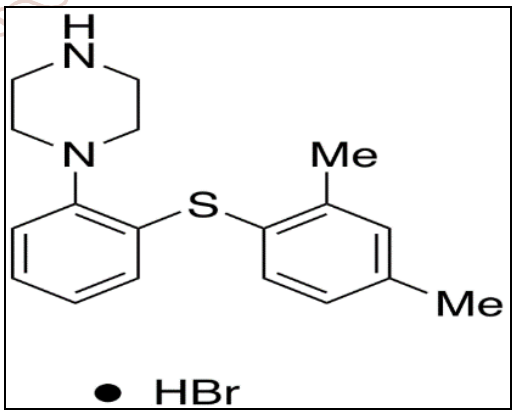

Fig No. 1: Structure of Vortioxetine Hydrobromide

Vortioxetine hydrobromide has a relative molecular mass of 379.4 g/mole.[3, 4] The Vortioxetine HBr is a drug with novel mechanism which is developed as one of a series of compounds developed from halogenated benzenes and was intentional to have combined effects on multiple 5-HT receptors and on

the serotonin transporter. It has been revealed in recombinant cell lines to combine 5-HT3 and 5-HT7 receptor antagonism, 5-HT1B receptor partial agonism, 5-HT1A receptor agonism, and serotonin transporter inhibition.[5]

The literature survey reveals that numerous methods for determinations of Vortioxetine HBr in single in pharmaceutical dosage forms, spectrophotometric methods in combination with other drugs, HPTLC, stability indicating spectrophotometric and HPLC methods in combination with other drug including HPLC, HPLC–MS/MSUPLC–MS/MS, hydrophilic interaction liquid chromatography coupled with mass spectrometry detection (HILIC–MS) are reported.[6-28]

In this paper we described very simple, sensitive, novel RP-HPLC methods. The simple RP-HPLC methods are not available for estimation of Vortioxetine HBr in single component and for simple analysis on bulk and tablet dosage form. These method show very simple and accurate approach for the analysis of Vortioxetine HBr without need of sophisticated instruments, expensive solvents or a large number of samples.

MATERIALS AND METHODS:

1. **Materials:**Pure sample of Vortioxetine HBr was kindly supplied as a gift sample by Megafine Pharma (P) Ltd. (Nashik, Maharashtra) India. All solvents and chemicals were of analytical grade. Marketed Tablet dosage form used in this research work was Brintellix 10 mg (H. Lundbeck, Denmark) acquired from local market.
2. **Instruments:** Chromatographic measurements were carried out on ANALYTICAL TECHNOLOGIES LTD HPLC. Infrared spectroscopic study was done on FTIR (Bruker,Japan).

3. Chromatographic method:

Chromatographic method and its validation

Selection of Mobile Phase:

The standard stock solution of 10µg/mL was injected into the HPLC system and run in different solvent systems. Different composition of mobile phase like Acetonitrile: Methanol (50:50 v/v), Acetonitrile: Methanol: Water (40:40:20v/v) Acetonitrile: Methanol: Water (40:50:10v/v) were tried in order to find the best composition for determination of Vortioxetine HBr. After several trials Acetonitrile: Methanol: Water (40:50:10 v/v) was chosen as mobile phase for analysis.

Preparation of mobile phase:

The optimized mobile phases consist of Acetonitrile: Methanol: Water (40:50:10 v/v). The solvents of HPLC grade are used. Mobile phase are filtered through membrane filter 0.45 µm and fill into solvent reservoir.

Preparation of standard stock solution:

Standard stock solution of Vortioxetine HBr was prepared by accurately weighing 20 mg of Vortioxetine HBr to 20 ml volumetric flask containing mobile phase. The drug was sonicated and volume was made up to mark with mobile phase to get the concentration of 1000 µg/ml.

Selection of concentration range and preparation of calibration curve:

Aliquots portion 1, 2, 3, 4, 5 ml were pipetted out from the standard stock solution of 100µg/mL and transferred to series of 10 ml volumetric flask and volume was made with mobile phase to get the concentration range from 10-50 µg/ml. The observed chromatogram was measured three times for each concentration at wavelength 370 nm. (Figure 2)

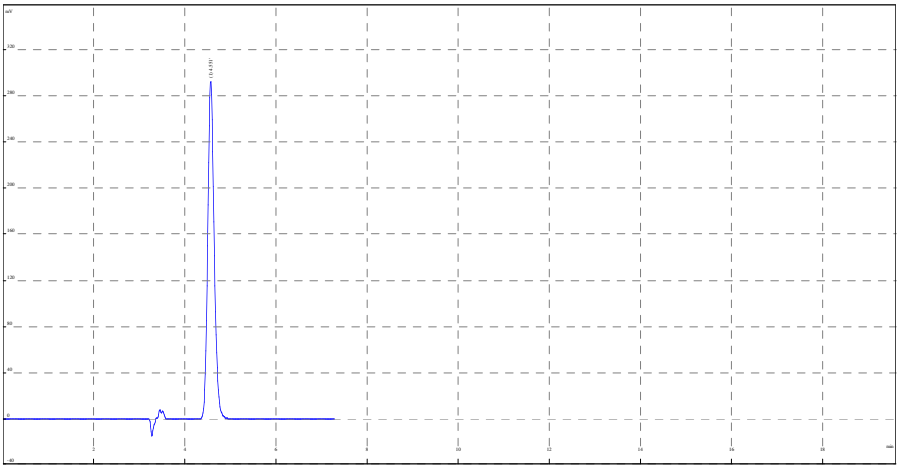

Fig. No.2: Typical chromatogram of Vortioxetine HBr

Analysis of tablet formulation (Recovery study):

Twenty tablets were weighed and finely powdered. Equivalent to 10 mg of Vortioxetine HBr was weighed and transferred to a 100 mL volumetric flask containing 40:50 ml Acetonitrile: methanol, and sonicated for 20

minutes with intermittent shaking. The solution was filtered through 0.45 µm membrane filter and volume was made up to mark with 10ml water and mixed to get 100µg/ml. An aliquot of tablet stock solution 3 mL was transferred to 10 mL volumetric flask and volume was made up to mark with methanol to get concentration of 30µg/mL Brintellix. Solution was injected and chromatogram and area was observed.

Method validation:

According to ICH Q2 (R1) guidelines the developed method was validated to assure the reliability of results of the analysis for different parameters like linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ),specificity, and robustness.

RESULT 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 3 and Table 1)**

Fig No.3: Calibration curve for Vortioxetine HBr

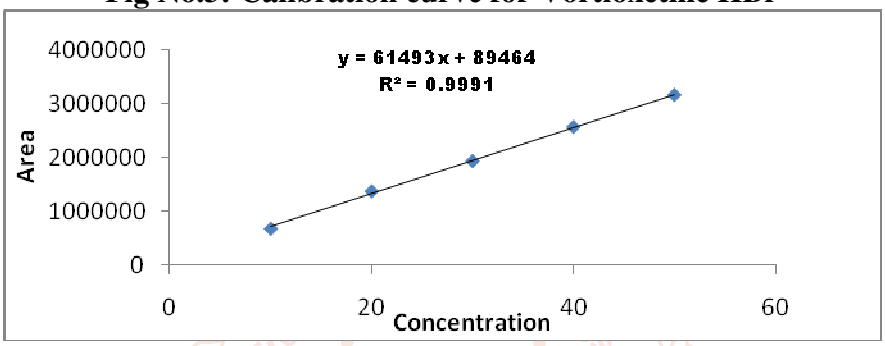

Table No. 1: Data of calibration curve of Vortioxetine HBr

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:

Line equation obtained from calibration plot was used to calculate label claim of marketed formulation of Vortioxetine HBr. (Table 2)

Table No. 2: Data of Tablet analysis of Vortioxetine HBr

Drug Name	Mean*	SD	%RSD
Brintellix	100.26	0.2900	0.2861

*Average of six determinations

SD = Standard Deviation, RSD = Relative Standard Deviation.

Accuracy:

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. 3)

Table No. 3: Data for recovery study of Vortioxetine HBr

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
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

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 interday precision solutions were analyzed for the days at the same concentration level. (Table No. 4 and 5)

Table No. 4: Data for intraday precision of Vortioxetine HBr

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. 5: Data for interday precision of Vortioxetine HBr

Sr. No.	Conc. (µg/mL)	Mean*	SD	%RSD
1	10	679485	8637.90293	1.27124262
2	30	1930531.33	6758.75805	0.35009833
3	50	3151484.67	4927.78473	0.15636391

*Average of three determination

Robustness:

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 HBr was prepared and injected into the HPLC system by keeping flow rates 0.7 ml/min and 0.9 ml/min (Table 6 and 7). The effect of variations in flow rate was evaluated.

Table No. 6: Data for Robustness study of Vortioxetine HBr

Sr. No	Parameter	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. 7: Data for Robustness study of Vortioxetine HBr

Sr. No	Parameter	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

Ruggedness:

Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions. Ruggedness study was performed to examine effect of non-procedure related factors such as instruments and analyst. Ruggedness study of Vortioxetine HBr was carried out by using two different analysts under the similar operational and environmental conditions. %RSD of ruggedness study was found to be less than 2 (Table 8)

Table No. 8: Data for ruggedness study of Vortioxetine HBr

Sr. No	Analyst	Conc. (µg/ml)	Area	Mean area*	SD	% RSD
1	Analyst-I	30	1926469	1919972.67	6607.15675	0.3441276
			1920189			
			1913260			
2	Analyst-II	30	1925645	1931669.67	12199.9689	0.6315763
			1945710			
			1923654			

*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 Vortioxetine HBr indicate the sensitivity of the method.

Specificity:

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 HBr

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

*Average of three determination

CONCLUSION:

From the above experimental parameter and results, it was concluded that the proposed RP-HPLC method is novel, sensitive, accurate, precise and cost effective and time effective with short column retention time of 4.498min indicates to less solvent consumption. The results of analysis reveal that virtually no interference of the attitudes present in marketed formulations. This proposed method can be intended for the routine analysis in industry for Vortioxetine HBr in bulk and tablet dosage form.

CONFLICT OF INTEREST: None

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