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"Development and Validation of an RP-HPLC Method for Vortioxetine Hydrobromide Quantification: Enhancing Pharmaceutical Quality Control"

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ABSTRACT:
Analytical techniques play a crucial role in the pharmaceutical industry, aiding in the determination of drug identification, potency, purity, and physical attributes. Among these techniques, high-performance liquid chromatography (HPLC) stands out as one of the most practical methods for qualitative and quantitative analysis, particularly for drug components. This study focuses on the development and validation of an RP-HPLC method for the estimation of vortioxetine hydrobromide (HBR) in bulk drugs and dosage forms, with subsequent application to commercial dosage forms. Vortioxetine HBR, a key compound in the treatment of major depressive illness, functions by modulating serotonin levels in the central nervous system. It acts as a serotonin modulator and simulator, exerting its antidepressant effect through various receptor interactions. Despite its therapeutic importance, there is a scarcity of analytical techniques available for the HPLC assay of vortioxetine HBR. Hence, this study aims to fill this gap by establishing a reliable and accurate analytical method. The materials and methods section details the instrumentation, chromatographic conditions, and preparation of standard solutions. Methanol was selected as the solvent for dissolving vortioxetine hydrobromide, and an analytical wavelength of 226 nm was chosen based on UV scans. Chromatographic separation was achieved using a Phenomenex ODS-3 C-18 column with an isocratic mobile phase composition of acetonitrile and water. Method development involved several trial runs to optimize chromatographic conditions, ensuring satisfactory peak resolution and retention time. The final method was validated according to ICH guidelines, covering parameters such as filtration study, solution stability, specificity, linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, and precision. Results indicate that the developed RP-HPLC method is robust and reliable for the estimation of vortioxetine HBR in bulk drug and dosage forms. System suitability tests demonstrate the method's consistency, while linearity studies confirm its ability to produce proportional test results within a specified concentration range. Moreover, the method exhibits satisfactory LOD and LOQ values, ensuring sensitive detection and quantitation of vortioxetine HBR. Overall, this study contributes a validated RP-HPLC method for the analysis of vortioxetine HBR, providing a valuable tool for quality control and assurance in pharmaceutical manufacturing and formulation. The method's accuracy, precision, and robustness make it suitable for routine analysis, facilitating the development and assessment of vortioxetine-containing formulations for the treatment of depressive disorders.

Result: According to the study, vortioxetine HBr is odorless, beige, and soluble in methanol with a melting point of 232-238°C. HPLC optimization was used in the method development process to provide acceptable chromatography. The assay findings for the marketed sample fell between 99.13% and 100.34%. Filtration, stability, specificity, linearity, LOD/LOQ, accuracy, precision, and robustness studies were all included in the validation process to verify the applicability of the approach.

Conclusion: The developed RP-HPLC method offers reliable vortioxetine HBr analysis, vital in depression treatment. It passed validation, ensuring accuracy, precision, and applicability for pharmaceutical quality control.

KEYWORD: Vortioxetine hydrobromide, High-Performance Liquid Chromatography (HPLC), Analytical method development, Validation, Pharmaceutical analysis, Antidepressant, Serotonin modulator, RP-HPLC, Bulk drug analysis, Dosageforms, Chromatographicconditions, Method

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optimization, ICH guidelines, Limit of detection (LOD), Limit of quantitation (LOQ)

I. INTRODUCTION

Analytical techniques are used to determine the identification, potency, purity, and physical attributes of the pharmaceutical formulation and active ingredient. The most practical method, both qualitatively and quantitatively, is HPLC. For the quality control of drug components, it is the most effective, fastest, safest, and most adaptable chromatographic method available. The benefit of these techniques is that they will shorten the analysis time while producing results that are more dependable and accurate. In [2] The chemical formula for vortioxetine HBR is 1-{2-[2,4-dimethylphenyl) sulphynyl] phenyl} piprazinemonohydrobromide. In [3]
Major depressive illness is treated with vortioxetine HBR. In combination with serotonin, vortioxetine HBR has an antidepressant effect by raising serotonin levels in the central nervous system through a decrease in serotonin reuptake. Serotonin modulator and simulator Vortioxetine HBR. It is an antagonist of the 5HT3 receptor, a partial agonist of the 5 HT1B receptor, and an agonist of the 5 HT1A receptor.[4]

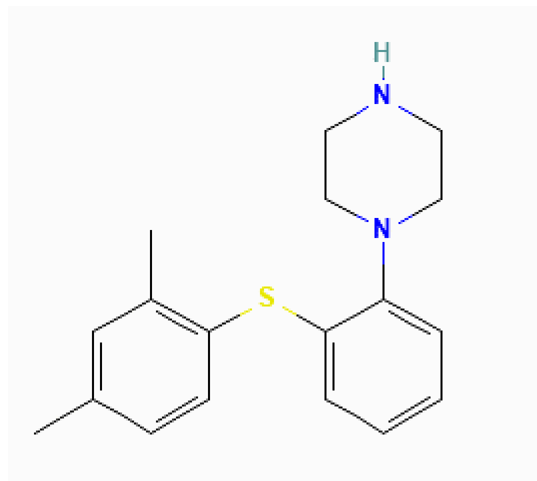

FIGURE: VORTIOXETINE HBR.

Monohydrobromide1-{2-[(2,4-dimethylphenyl) sulfanyl] phenyl} piperazine.[5] A review of the literature indicates that there aren't many techniques available for HPLC assay of vortioxetine HBR [3,4]. In order to estimate Vortioxetine HBR in bulk drug and dosage form, an attempt has been made to create and validate the rp-hplc method. The established approachhas then been applied to commercial dosage form.

II. MATERIALS AND METHODS

Materials and Reagents: An API sample of Vortioxetine HBR was received as a gift sample from INTAS PHARMACEUTICAL LTD. (Mumbai, India). The VANTAXA 20 mg tablet was purchased from a nearby store. The analysis also included the use of HPLC-quality water, analytical-grade methanol, and acetonitrile (HPLC-grade).

Instrumentation and Chromatographic Conditions: The HPLC apparatus (Agilent Model) consisted of a gradient quaternary pump and a multiple-wavelength PDA detector. Chromatographic separation was performed isocratically using a Phenomenex ODS-3 C-18 column (250mm x 4.6mm x i.d.) with a 5 µm mobile phase composition of a mixture of acetonitrile (0.1% TFAA) and water (80:20) at a flow rate of 1.0 ml/min, and a sample injection of 20 µL was injected. The eluent was monitored with a PDA detector set at 226 nm. The diluent was a mixture of acetonitrile and water (80:20).

Preparation of the mobile phase: The mobile phase consists of acetonitrile and water in a ratio of 80:20, and the pH was adjusted to 0.1% TFAA. The solution was filtered through a 0.45-µ (Nylon syringe) membrane filter and sonicated for 15 minutes with intermittent shaking. This solution was used as a mobile phase for further analysis.

Selection of an analytical wavelength
Selection of solvents
Methanol was selected as the solvent for dissolving Vortioxetine hydrobromide.

Preparation of standard solutions for UV scans In order to prepare the stock solution, we accurately weighed 31.77 mg of Vortioxetine hydrobromide (equivalent to 25 mg of Vortioxetine) and transferred it into a 50-ml volumetric flask. We added 35 ml of methanol, sonicated to dissolve the standard completely, and diluted it up to the mark with methanol (500 PPM). Further diluted 1 mL to 25 mL with methanol. (20 PPM)

Selection of an analytical wavelength Methanol was used as a blank, and Vortioxetine standard solution (20 PPM) was scanned from 800 nm to 200 nm. Absorption maxima were determined for the drug. Vortioxetine showed maximum absorbance at 226 nm, as shown in the results.

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Method Development by RP-HPLC

Preparation of a standard stock solution for chromatographic development:

Vortioxetine Standard Stock Solution was prepared by dissolving 31.77 mg of Vortioxetine hydrobromide (equivalent to 25 mg of Vortioxetine) into a 50-mL clean and dried volumetric flask, adding about 35 mL of methanol to dissolve it completely, and making the volume up to the mark with methanol (500 PPM).

Further diluted 2 ml of stock solution to 10 mL with methanol (100 PPM).

Analytical wavelength selection for the development of HPLC methods:

The analytical wavelength for the examination was selected from the wavelength of maximum absorption from the spectrophotometric analysis, which was 226 nm**.**

Chromatographic Conditions:

Standard solution:	Vortioxetine 100 PPM
Column:	Phenomenex ODS 3
Column Length:	(250 mm x 4.6 mm i.d.) 5µm
Column oven temperature:	35 °C
Detector:	U.V. Detector
Wavelength:	226 nm
Flow Rate:	1.0 ml/min
Mobile phase:	Acetonitrile: 0.1% TFAA in water (80:20)
Injection Volume:	20 µl

Validation of the RP-HPLC Method

As per the ICH recommendations for the following parameters, the suggested method for the Vortioxetine estimate was verified.

FILTRATION STUDY

This analytical procedure's filter study examines the interference of extraneous components from the filter, deposition on the filter bed, and compatibility of the filter with the sample. The Vortioxetine Test sample (tablet solution) was used in this investigation. Filtration analysis is performed using both unfiltered and filtered test solutions. 5 milliliters of aliquot material were discarded in favor of 0.45 µm PVDF and 0.45 µm Nylon syringe filters during the filtration process.

Stability of Analytical Solution

A stability analysis was performed on both the test sample and the standard solution. The stability research was carried out in a typical lab setting. After 12 and 24 hours, the solution was examined under standard laboratory lighting conditions. By computing the difference between the test solution findings at each stability time point and the initial results, a standard and test solution stability study were carried out.

Specificity

The capacity to clearly identify the analyte in the presence of components that may be anticipated to be present is called specificity. To demonstrate the method's specificity, the following solution will be made and injected: Peak purity for the standard and test sample solutions was verified

1. **Blank (methanol as a diluent)**
2. **Placebo**
3. **Vortioxetine Standard Solution**
4. **Tablet test sample solution**

Excipients, or additives, used in the analysis of marketing test samples are completely unknown. Thus, a placebo was created in a lab setting using the following formula:

Sr. No.	**Ingredients**	**Role**	**Qty (mg)**
1	Lactose	Filler	80
2	Starch	Binder	5
3	Magnesium stearate	Lubricant	5

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4	Talc	Glidant	5
5	crospovidone	Disintegrants	5
Total			100 mg

Overall, 10 grams of placebo were made:

Placebo Sample solution preparation:

measured and transferred 252.48 mg of placebo material—equivalent to 25 mg of vortioxetine—to a 50 mL volumetric flask that had been cleaned and dried. added 35 milliliters of methanol and sonicated with sporadic shaking for ten minutes. Allow the solution to cool to ambient temperature after ten minutes, then add methanol to bring the volume up to the desired level. 3-5 mL of the initial filtrate were discarded after filtering the mixture using an appropriate 0.45 µ nylon syringe filter. Chromatograms were recorded after injecting the resulting solution after further diluting 1.0 ml of the filtered stock solution to 25 ml with methanol.

LINEARITY AND RANGE

Preparation of a linear solution

The capacity of an analytical method to produce test findings that are exactly proportionate to the concentration (amount) of analyte in the sample, within a specified range, is known as linearity. Five different linearity levels, ranging from 10% to 150% of the working concentration, were tested.

Linearity of the **Vortioxetine stock solution:** measured and dissolved in 25 mL of methanol 31.77 mg of vortioxetine hydrobromide, which is equal to 25 mg of vortioxetine. I added more methanol to the stock solution, diluting 5.0 mL to 50 mL (100 parts per million).

Linearity levels are prepared as follows:

Sr. No.	Level (%)	mL of stock solution	Diluted with Methanol (mL)	Vortioxetine Concentration (µg/mL)
1	10%	0.20	10	2.00
2	50%	1.00	10	10.00
3	100%	2.00	10	20.00
4	125%	2.50	10	25.00
5	150%	3.00	10	30.00

Determination

Each level is injected in triplicate, and the mean area is calculated. The calibration curve was plotted graphically as a function of analyte concentration in µg/mL on the X-axis and mean area on the y-axis, as given in the results.

Acceptance criteria

Correlation Coefficient: NLT 0.98
Intercept: To be reported
Slope: To be reported

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

Detection limit:

The lowest concentration of analyte in a sample that can be identified but may not always be quantified as an exact number is known as the detection limit of a particular analytical technique.

Quantitation limit:The lowest concentration of analyte in a sample that can be quantitatively identified with appropriate precision and accuracy is known as the quantitation limit of a particular analytical process.

As per ICH Q2R1 guidelines, LOD and LOQ were determined by using the approach Based on the Calibration Curve, in which the residual standard deviation of a regression line was calculated and the LOD and LOQ were determined by using the following formula:

$LOD = 3.3\ \sigma / S$

$LOQ = 10\ \sigma / S$

Where,

σ = residual regression line standard deviation

S = Slope of regression line

Accuracy (% Recovery)

The degree of agreement between the value found and the value that is recognized as either a conventional true value or an acceptable reference value is expressed by the analytical procedure's accuracy. Between fifty percent and

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one hundred fifty percent of working concentration will be used for accuracy. Three copies of each accuracy level's solution were made. % recovery for each sample, mean recovery for every level, and total recovery were computed. Additionally, % RSD for every level and % RSD for the total recovery were computed.

Accuracy level details:
Refer The following table is for each sample:

Level (%)	Vortioxetine HBr Std (mg)	Placebo (mg)	Diluted to (mL)	Volume taken (mL)	Diluted to (mL)	Vortioxetine Concentration (µg/mL)
50	15.9	252.8	50	1	25	10.01
	16.2	252.3	50	1	25	10.20
	16.1	252.6	50	1	25	10.14
100	31.9	252.4	50	1	25	20.08
	31.8	252.9	50	1	25	20.02
	32.0	252.5	50	1	25	20.15
150	47.9	252.3	50	1	25	30.16
	47.7	252.8	50	1	25	30.03
	47.8	252.6	50	1	25	30.09

Procedure for preparation of the Accuracy Sample Solution:

Take a clean and dried 9-volumetric flask of 50 ml. Weighed approximately 252.48 mg of placebo and transferred it into each 50-mL volumetric flask. Weighed Vortioxetine hydrobromide API as per accuracy level and transferred in the same 50-ml volumetric flask. Add 35 ml of methanol and sonicate it for 10 minutes with intermittent shaking. Allowed to cool the solution at room temperature and made the volume up to the mark with methanol. Filter the solution through suitable 0.45 µ Nylon syringe filter, discarding 5 mL of filtrate. Further dilute 1.0 ml of filtrate to 25 ml with methanol.

Acceptance criteria

1. The percent recovery for each sample and the mean recovery should be in the range of 98–102%.
2. A relative standard deviation of no more than 2.0% is acceptable.

PRECISION

When several measurements are taken from numerous samplings of the same homogenous test under specified conditions, the precision of an analytical technique is expressed as how closely the measurements match. Two forms of precision exist: intermediate precision and repeatability. A test sample of tablets is used for the procedure.

1. **Repeatability**

Sample solution preparation (6 samples are made):

I estimated the average weight of 20 tablets, moved them to a mortar and pestle, and ground them into a fine powder. Equally combine the items with the butter paper. The powder material equivalent to 25 mg of vortioxetine was weighed and then placed in a 50-mL volumetric flask that had been cleaned and dried. added 35 milliliters of methanol and sonicated with sporadic shaking for ten minutes. Allow the solution to cool to ambient temperature after ten minutes, then add methanol to bring the volume up to the desired level. 3-5 mL of the initial filtrate were discarded after the solution was filtered using an appropriate 0.45 µ Nylon syringe filter. Use methanol to further dilute 1.0 ml of the filtered stock solution to 25 ml. After injecting the resulting solution with 20 milligrams of vortioxetine, the chromatograms were

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Precision (repeatability) sample details are as follows:

Sample No.	Test powder material (mg)	Diluted to (mL)	Volume taken (mL)	Diluted to (mL)
1	284.2	50	1	25
2	284.6	50	1	25
3	284.4	50	1	25
4	283.9	50	1	25
5	284.3	50	1	25
6	283.8	50	1	25

Acceptance criteria
% Assay: 90–100% for each sample and mean assay value
% RSD for the assay of 6 samples: NMT 2%

1. **Intermediate precision**

It is performed by doing analysis on another day to check the reproducibility of results. Samples are prepared in the same manner as the repeatability parameter (6 samples prepared).

Intermediate precision sample details are as follows:

Sample No.	Test powder material (mg)	Diluted to (mL)	Volume taken (mL)	Diluted to (mL)
1	284.4	50	1	25
2	284.6	50	1	25
3	283.8	50	1	25
4	284.3	50	1	25
5	284.1	50	1	25
6	284.5	50	1	25

Acceptance criteria:
% Assay: 90–100% for each sample and mean assay value
% RSD for the assay of 6 samples of intermediate precision**:** NMT 2
% RSD for total 12 samples: NMT 2% for test results (6 of repeatability and 6 of intermediate precision)

ROBUSTNESS
An analytical procedure's robustness indicates how reliable it is under typical operating conditions and can withstand intentional, modest changes in method parameters.

Determination: Blank andstandard solutions were injected under different chromatographic conditions, as shown below.
a). Changes in flow rate by ±10%. (± 0.1 ml/min)
b). Change in column oven temperature. (± 2ºC)
c) Change in wavelength (± 3 nm)

III. RESULTS AND DISCUSSION

Primary Characterization and Identification of Drugs
Color, odor, and appearance of the drug

Sr. No	Name	Color, odor, and appearance of the drug
1	Vortioxetine HBr	Beige, odorless, and crystalline powder

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Melting point determination

Melting point of a drug

Sr. No.	Name	Melting point standard. value (°C)	Melting point observed (°C)
1	Vortioxetine HBr	230-236 °C	232-238 °C

Solubility study

Solubility study of Vortioxetine HBr

Sr. No.	Name of Solvent	Observation	Conclusion	Summary
1	Water	Drug particles seen after sonication	The drug was not found to be soluble in water.	Methanol is used as a diluent for preparing stock solutions.
2	Methanol	No drug particles were seen after sonication.	The drug was found to be soluble in methanol.	

Selection of solvent

Methanol was selected as the solvent for dissolving Vortioxetine HBr.

Selection of an analytical wavelength

1) Blank Methanol:

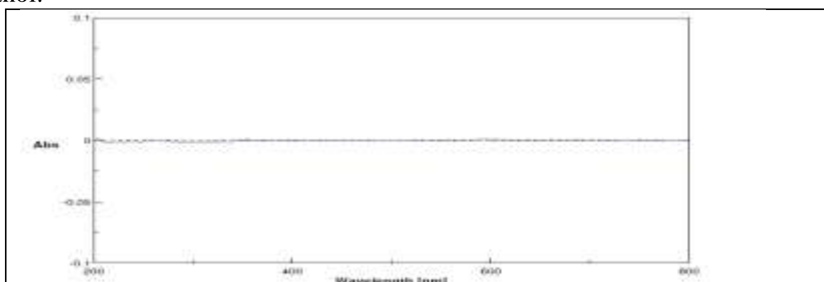

Fig. No. 1 UV spectrum of Methanol as a blank

2) Vortioxetine STD solution (20 PPM)

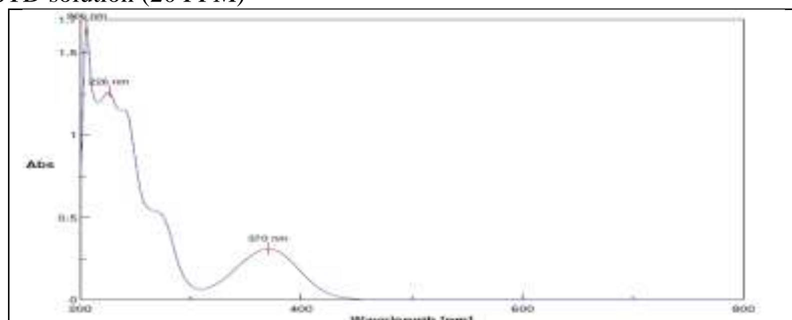

Fig. No. 2 UV spectrum of Vortioxetine

Observation: The standard solution was scanned between 200 nm and 800 nm. The wavelength of maximum absorption was determined for the drug. Vortioxetine showed maximum absorbance at 226 nm. It is shown in **Figure No. 2.** Therefore, 226 nm is considered an analytical wavelength for further determination.

Method Development by RP-HPLC

Optimization of the HPLC method

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Trial 1:
Chromatogram:

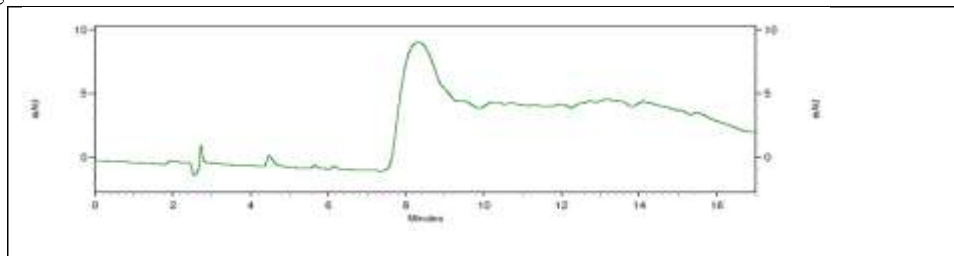

Fig. No. 3 Typical chromatogram of Trial 1

Observation: Vortioxetine is not eluted.
Conclusion: Method rejected.

Trial 2:
Chromatogram:

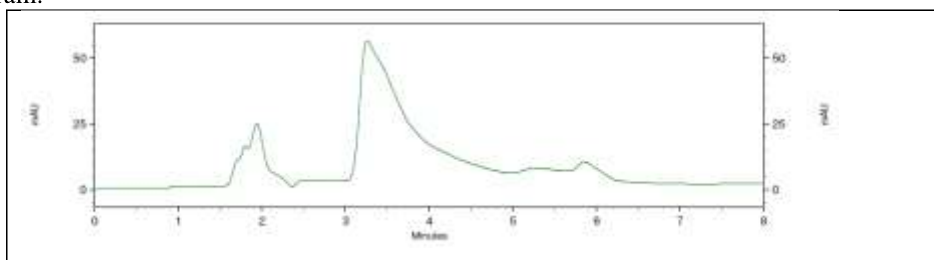

Fig. No. 4 Typical chromatogram of Trial 2

Observation: Vortioxetine eluted with unacceptable chromatography
Conclusion: Method rejected.

Trial 3:
Chromatogram:

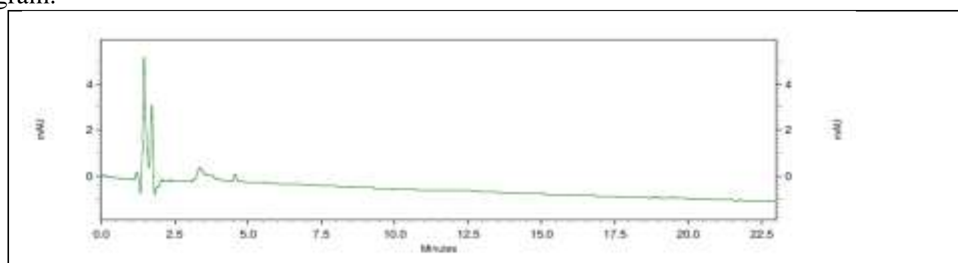

Fig. No. 5 Typical chromatogram of Trial 3

Observation: Vortioxetine is not eluted.
Conclusion: Method rejected.

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Trial 4:
Chromatogram:

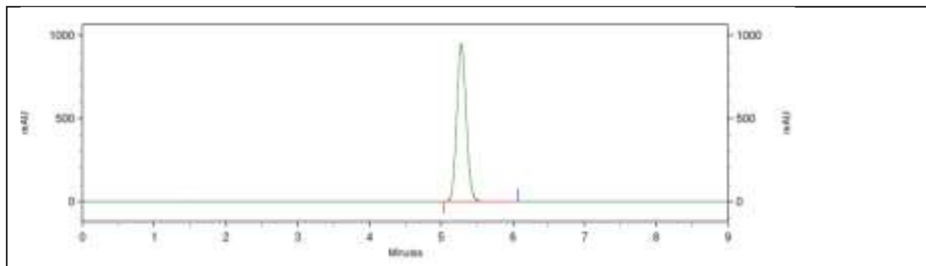

Fig. No. 6 Typical chromatogram of Trial 4

Observation: Vortioxetine was eluted at a RT of 5.32, and good chromatography was observed.
Conclusion: Try to reduce the R.T. of vortioxetine by changing the mobile phase composition in the next trial.

Trial 5:
Chromatogram:

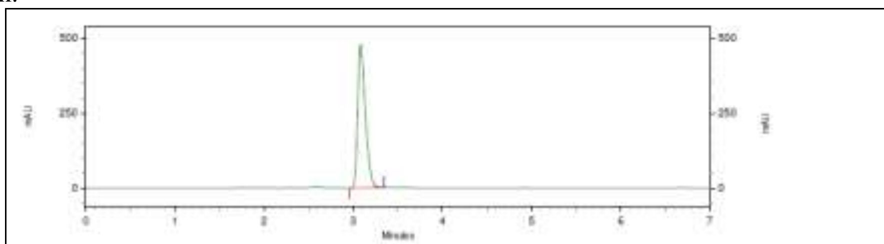

Fig. No. 7 Typical chromatogram of Trial 5

Observation: Vortioxetine eluted at RT of 3.07 and good chromatography observed.
Conclusion: Method Accepted.
Conclusion: From the observations of trials first to five, it was concluded that chromatographic conditions in trial five gives better peak, good retention time and tailing factor therefore chromatographic conditions in trial five was used for method validation.

Optimized Chromatographic Conditions

Parameter	Description
Mode	Isocratic
Column Name	Phenomenex ODS 3, 250 mm*4.6mm, 5µ
Detector	UV Detector
Injection Volume	20 µl
Wavelength	226 nm
Column Oven temperature	35ºC
Mobile Phase	Acetonitrile: 0.1% TFAA in Water (80:20% V/V)
Flow Rate	1.0 ml/min
Run time	7 Minutes
Retention Time	3.07

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System suitability test

Results for the System Suitability Test of Vortioxetine

Sr No.	Standard solution	Area	Asymmetry	Theoretical plates
1	Standard_1	8935740	1.31	7160
2	Standard_2	8971024	1.32	7153
3	Standard_3	8922562	1.31	7174
4	Standard_4	8969764	1.31	7148
5	Standard_5	8952479	1.31	7164
Mean		8950314	1.31	7160
STD Dev		21180.58548		
% RSD		0.24		

System Suitability Acceptance Criteria:

1. The relative standard deviation of the area of analyte peaks in standard chromatograms should not be more than 2.0%.
2. The theoretical plates of the analyte peak in standard chromatograms should not be less than 2000.
3. Tailing factor (asymmetry) of analyte peaks in standard chromatograms should be less than 2.0.

Data interpretation:

As can be seen from the data given above, the procedure conforms with the requirements for system appropriateness. Therefore, it may be said that the intended analysis can be conducted using the chromatographic method.

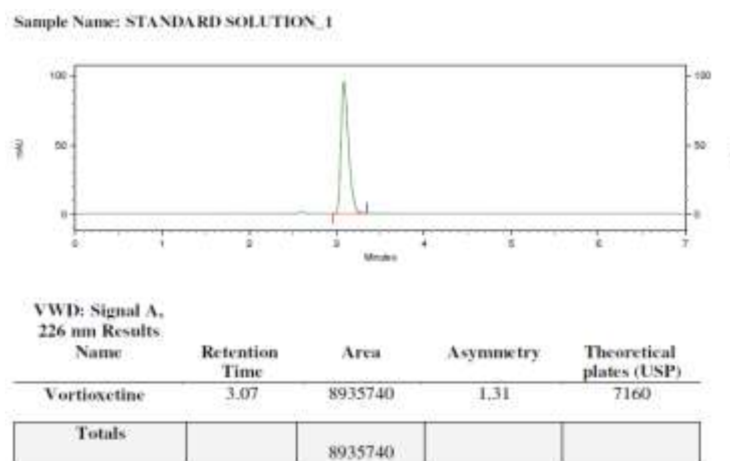

Fig. No. 8 Typical chromatogram Standard solution 1 of system suitability solution.

Analysis of Marketed Test Samples (Assay)

1. **a) Vantaxa 20 mg Tablet:**

Weight of 20 tablets = 4.5480 g

Average weight of tablet = 4.5480/20 = 0.2274 gm = 227.4 mg

Assay results for Vantaxa 20 mg Tablet

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Sample	Area	% Assay	Mean Assay
Sample 1	8993491	100.34	99.73
Sample 2	8873217	99.13	

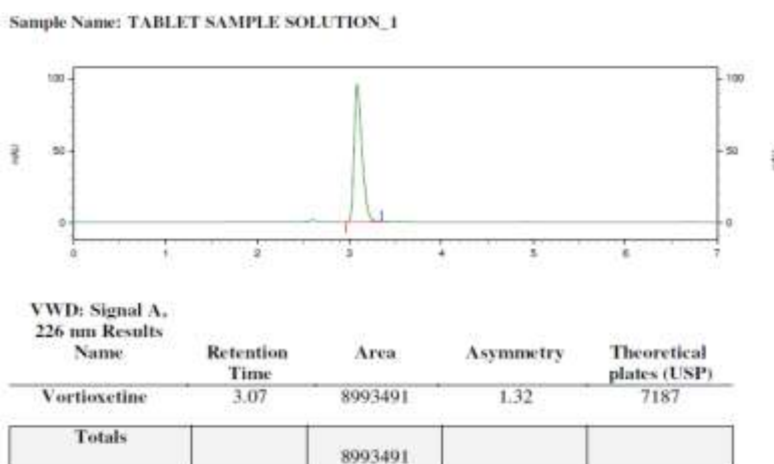

Fig. No. 9 Typical chromatogram Vantaxa 20 mg Tablet sample.

Acceptance criteria:
1) % Assay found should be in the range of 90-110%.

Data interpretation:
From the above results, it can be concluded that the assay result is within the limit for the selected marketed test sample, and the sample can be used for validation.

Validation of the RP-HPLC Method

- **FILTRATION STUDY:**

Filtration studies of an analytical procedure check the interference of extraneous components from the filter, deposition on the filter bed, and compatibility of the filter with the sample. Performed on a tablet test sample.

Results of Filter study

Sample description	Area	% Absolute difference
Unfiltered	8943569	NA
0.45 µ PVDF filter	8882497	0.68
0.45 µ Nylon filter	8983258	0.44

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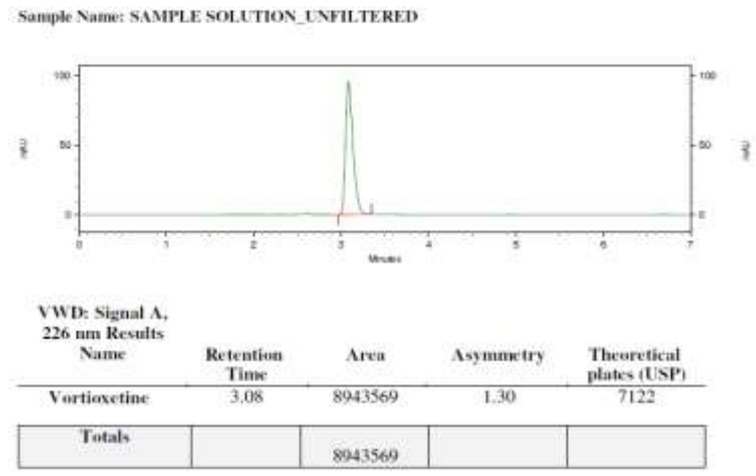
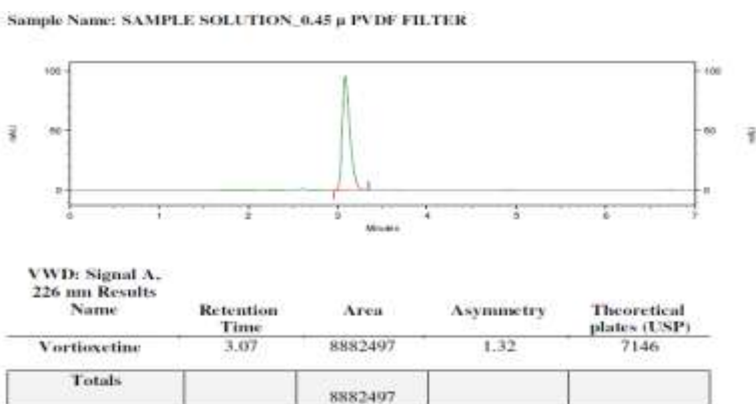
Chromatograms:



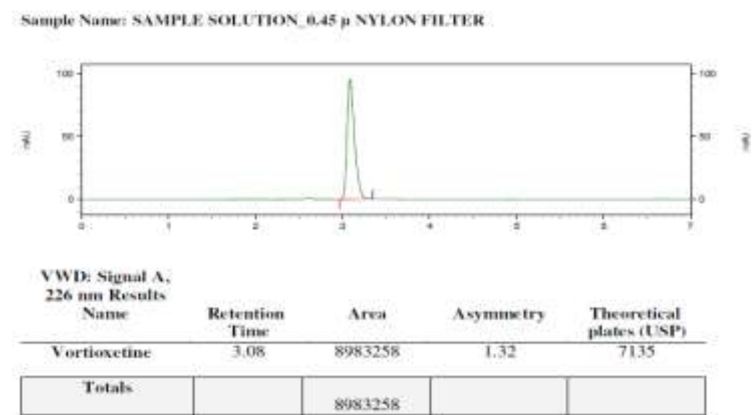
VWD: Signal A, 226 nm Results Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Vortioxetine	3.08	8943569	1.30	7122
Totals		8943569		

Fig. No. 10 Typical chromatogram of unfiltered sample.



VWD: Signal A, 226 nm Results Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Vortioxetine	3.07	8882497	1.32	7146
Totals		8882497		

Fig. No. 11 Typical chromatogram of sample filtered through 0.45µ PVDF filter.



VWD: Signal A, 226 nm Results Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Vortioxetine	3.08	8983258	1.32	7135
Totals		8983258		

Fig. No. 12 Typical chromatogram of sample filtered through 0.45µ Nylon filter.

Acceptance criteria: % absolute difference of filtered samples NMT 2.0 w.r.t. Unfiltered sample.
Data interpretation: Both filters, PVDF and Nylon, pass the criteria for filter study; hence, both filters can be used. We used the nylon filter because it showed a lower absolute difference than the PVDF filter.

SOLUTION STABILITY: Both the Standard Sample and the Test Sample underwent stability

testing. Under standard laboratory settings, a stability investigation was carried out. The solution was initially, 12 hours later, and 24 hours later examined under standard laboratory lighting.

Results of Solution stability.

Sample solution			Standard solution		
Time point	**Area**	**% Absolute difference**	**Time point**	**Area**	**% Absolute difference**
Initial	8950913	NA	**Initial**	8968458	NA
12 Hours	8912689	0.43	**12 Hours**	8935860	0.36
24 Hours	8876907	0.83	**24 Hours**	8909863	0.65

Chromatograms:

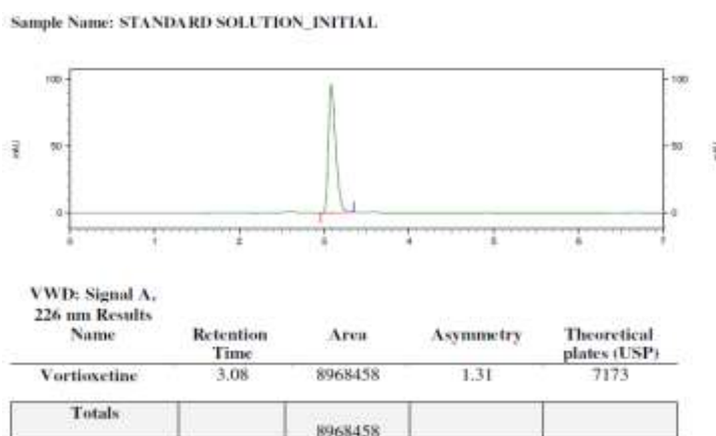


Fig. No. 13 Typical chromatogram of Standard solution Initial.

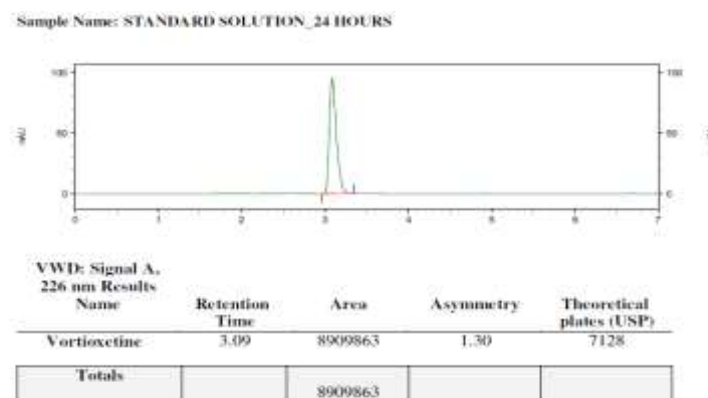


Fig. No. 14 Typical chromatogram of Standard solution After 24 Hrs.

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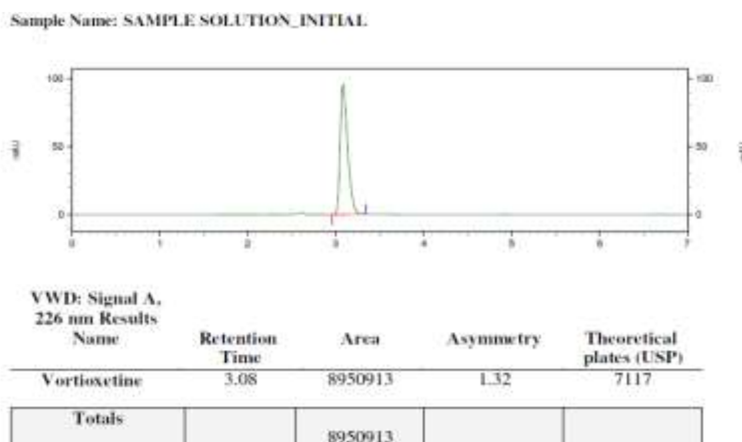
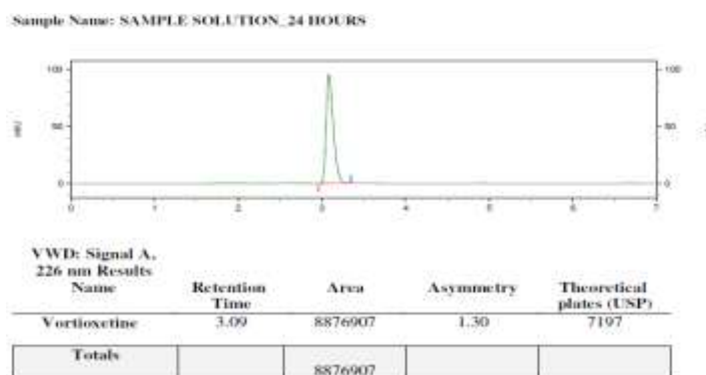


Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Vortioxetine	3.08	8950913	1.32	7117
Totals		8950913		

Fig. No. 15 Typical chromatogram of Test solution Initial.



Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Vortioxetine	3.09	8876907	1.30	7197
Totals		8876907		

Fig. No. 16 Typical chromatogram of Test solution After 24 Hrs.

Acceptance criteria: % Absolute difference of stability solution: NMT 2.0 w.r.t. Initial solution.

Data interpretation: The standard and test solutions were found stable up to 24 hours. Hence, both solutions can be used for up to 24 hours.

- **SPECIFICITY:**

To assess peak purity, a blank, standard solution is prepared and injected. Specificity is the capacity to access the analyte unambiguously in the presence of components that may be expected to be present.

Results of Specificity.

Description	Observation
Blank	No interference at R.T. of Vortioxetine due to blank
Placebo	No interference at R.T. of Vortioxetine due to placebo
Standard solution	Peak purity was 0.996.
Test Solution	Peak purity was 0.991.

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Chromatograms:

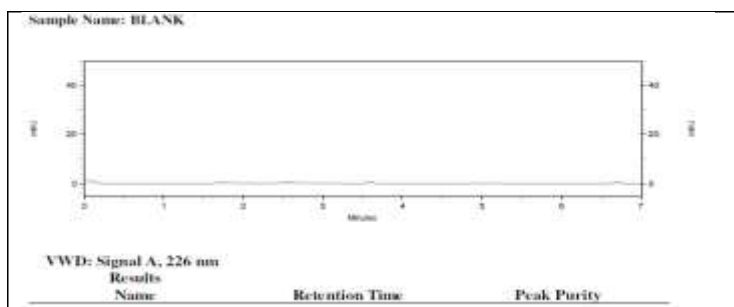


Fig. No. 17 Typical chromatogram of Blank solution.

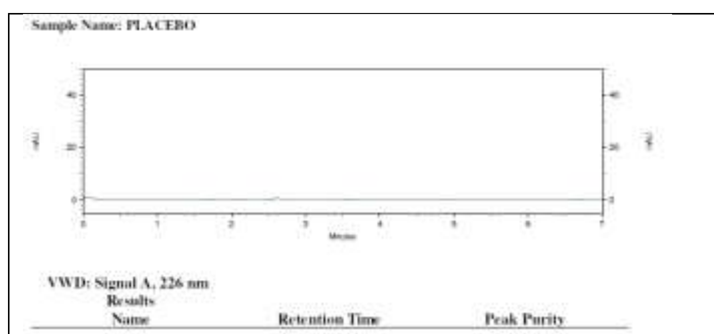
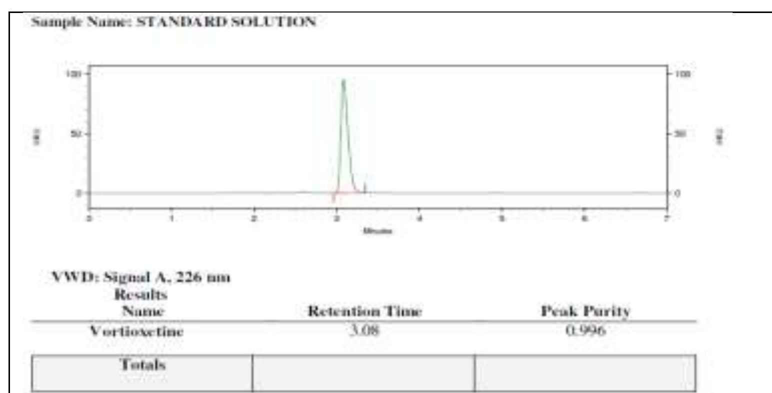


Fig. No. 18 Typical chromatogram of Placebo solution.



Name	Retention Time	Peak Purity
Vortioxetine	3.08	0.996
Totals		

Fig. No. 19 Typical chromatogram of Peak purity of Standard solution.

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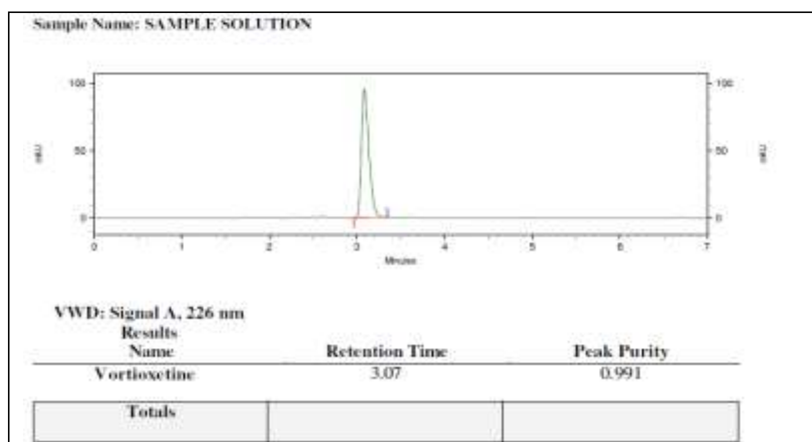

Fig. No. 20 Typical chromatogram of Peak purity of Test sample solution.

Acceptance criteria:
Blank: There should be no interference at R.T. of Vortioxetine.
Placebo: There should be no interference at the R.T. of Vortioxetine.
Standard and test sample solutions: Peak purity: NLT 0.95
Data interpretation: Blank and placebo were not having interference at R.T. of Vortioxetine. Peak purity for the standard as well as the test solution was well within limits. Hence, the developed chromatographic method passed the criteria for specificity.

- **Linearity and Range**

The capacity of an analytical method to yield test findings that are proportionate to the analyte concentration in samples within a specified range is known as linearity.

Linearity Data for Vortioxetine

Level	Conc (µg/mL)	Area	Mean	% RSD
10%	2.00	889641	888527	0.110
		888136		
		887803		
50%	10.00	4456943	4445293	0.232
		4437256		
		4441680		
100%	20.00	8963014	8973248	0.121
		8984690		
		8972040		
125%	25.00	11260679	11295334	0.302
		11328976		
		11296348		
150%	30.00	13527944	13497614	0.218
		13469070		
		13495829		

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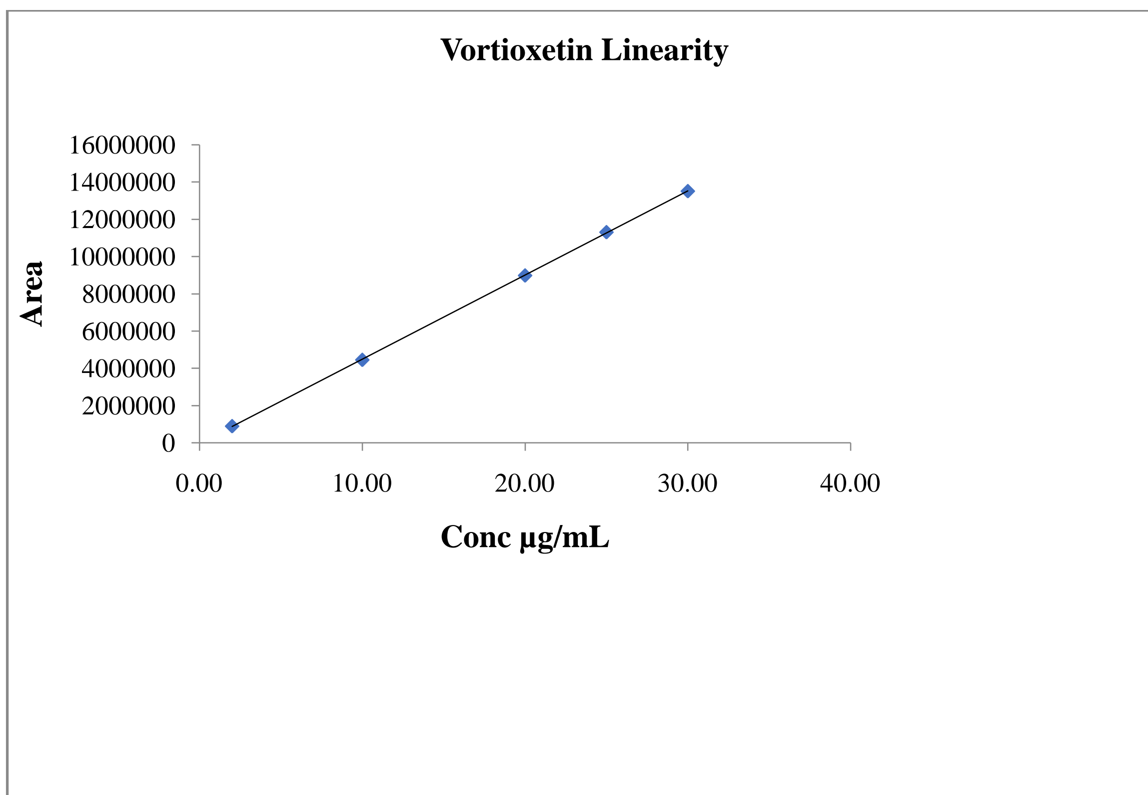

Fig. No. 21 Calibration curve of Vortioxetine

Data of linearity of Vortioxetine:

Sr no.	Parameter	Result value	Acceptance criteria
1	Beer's linearity range	2.0–30.0 µg/mL	NA
2	Correlation coefficient (R^2)	0.99998	NLT 0.98
3	Intercept	-37908.251	To be reported
4	Slope	451550.101	To be reported
5	% RSD for area at each level	NA	NMT 2.0

The respective linear equation for Vortioxetine was

Y = M X + C

Y = 451550.101 x + -37908.251

where x = concentration of analyte in µg/mL

y = is area of peak.

M = Slope

C= Intercept

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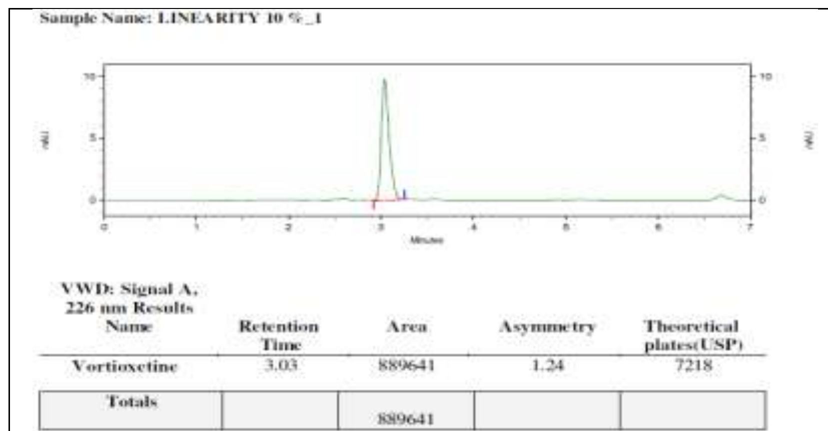
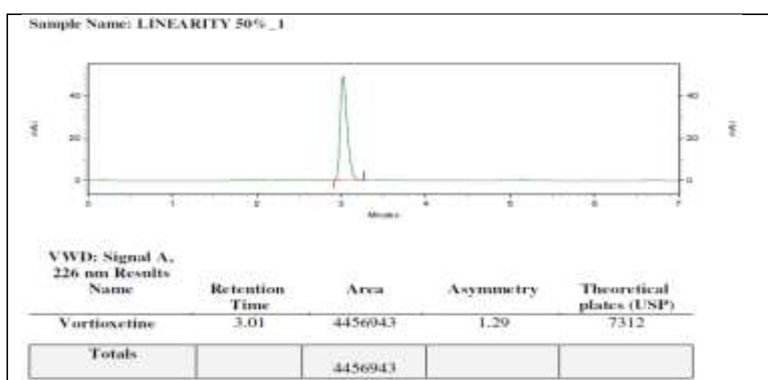
Chromatograms:



VWD: Signal A, 226 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates(USP)
Vortioxetine	3.03	889641	1.24	7218
Totals		889641		

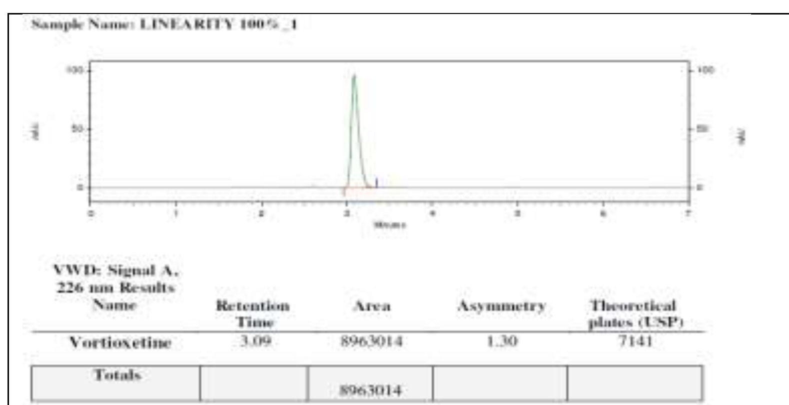
Fig. No. 22 Typical chromatogram of Linearity 10%.



VWD: Signal A, 226 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Vortioxetine	3.01	4456943	1.29	7312
Totals		4456943		

Fig. No. 23 Typical chromatogram of Linearity 50%.



VWD: Signal A, 226 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Vortioxetine	3.09	8963014	1.30	7141
Totals		8963014		

Fig. No. 24 Typical chromatogram of Linearity 100%.

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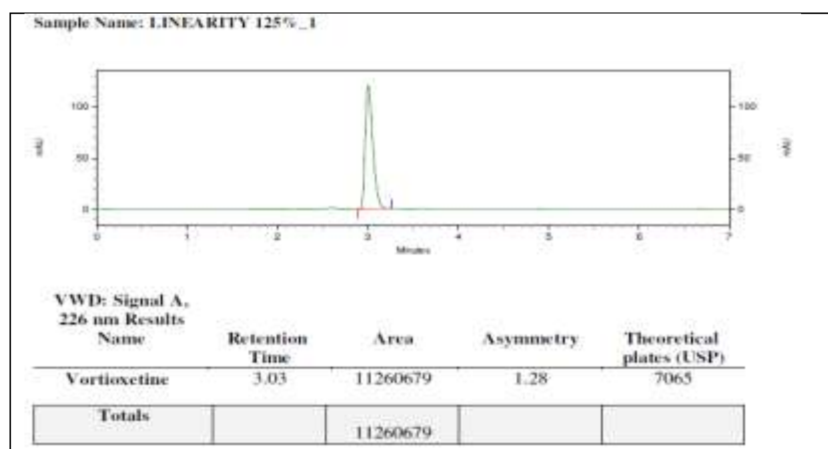
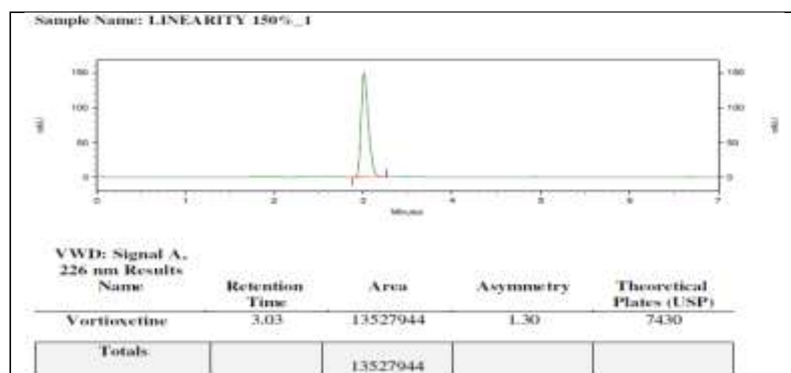

Sample Name: LINEARITY 125%_1

VWD: Signal A, 226 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Vortioxetine	3.03	11260679	1.28	7065
Totals		11260679		

Fig. No. 25 Typical chromatogram of Linearity 125%.


Sample Name: LINEARITY 150%_1

VWD: Signal A, 226 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical Plates (USP)
Vortioxetine	3.03	13527944	1.30	7430
Totals		13527944		

Fig. No. 26 Typical chromatogram of Linearity 150%.

IV. CONCLUSION:

From the calibration curve, it was concluded that Vortioxetine shows a linear response in the range of 2.0–30.0 µg/mL.. The regression value was found to be well within the limit.

.

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

σ = 31920.40482 (the residual standard deviation of a regression line).

s = 451550.101

Detection limit (LOD):

LOD = 3.3 σ / S

LOD = 3.3 x 31920.40482 / 451550.101

LOD = 0.233 µg/mL

Quantitation limit (LOQ):

LOQ = 10 σ / S

LOQ = 10 x 31920.40482 / 451550.101

LOQ = 0.707 µg/mL

- **ACCURACY (RECOVERY):**

Applying the method to examined samples that have known amounts of analyte added will yield an accurate method based on how closely the test results generated by the method match the true value.

Result and statistical data of Accuracy of Vortioxetine

Level (%)	Area	Recovered conc (µg/mL)	Added conc (µg/mL)	% Recovery	Mean Recovery	% RSD
50	4425035	9.89	10.01	98.80	99.47	0.830
	4583256	10.24	10.20	100.39		
	4502519	10.06	10.14	99.21		
100	8900365	19.89	20.08	99.05	99.02	0.405
	8833586	19.74	20.02	98.60		
	8965217	20.03	20.15	99.40		
150	13379675	29.90	30.16	99.14	99.95	1.204
	13356048	29.84	30.03	99.37		
	13645823	30.49	30.09	101.33		

Overall Recovery: 99.48 %
% RSD for Overall Recovery: 0.862

Chromatograms:

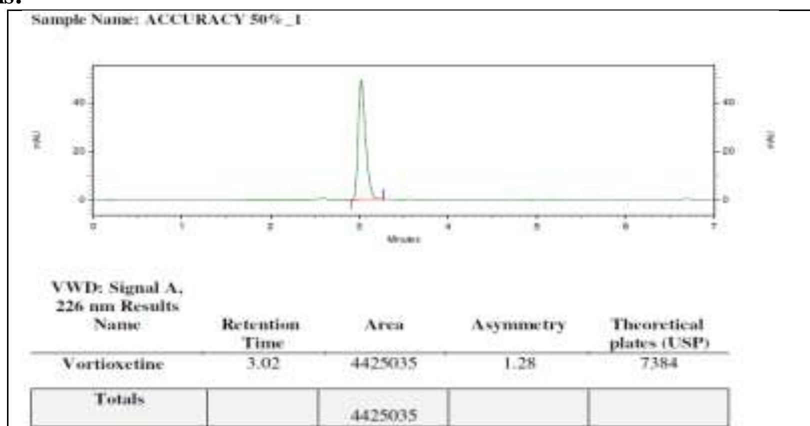


Fig. No. 27 Typical chromatogram of Accuracy 50%.

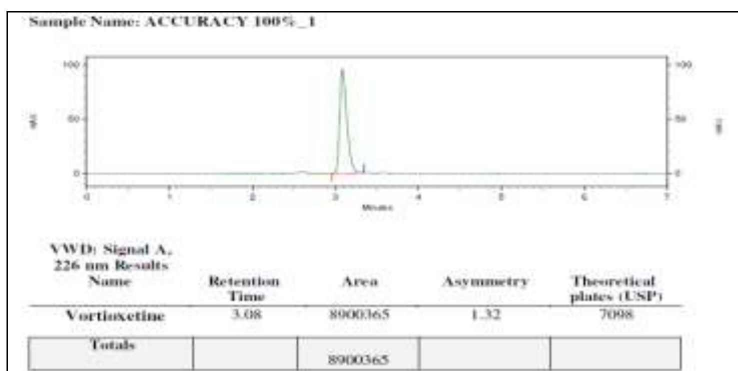
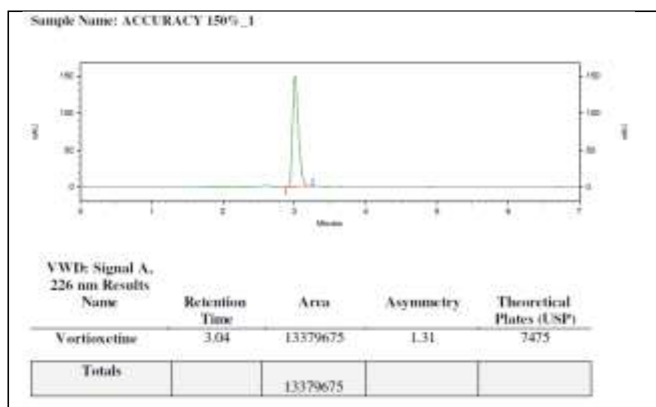


Fig. No. 28 Typical chromatogram of Accuracy 100%.



VWD: Signal A, 226 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical Plates (USP)
Vortioxetine	3.04	13379675	1.31	7475
Totals		13379675		

Fig. No. 29 Typical chromatogram of Accuracy 150%.

Acceptance criteria:
98.0 to 102.0 percent recovery overall and for each stage

Overall recovery and % RSD for each level: NMT 2.0

Data interpretation: It was determined that the analytical procedure's recovery fell comfortably within the acceptable range on all three levels. Adjustments in analyte concentration do not interfere with recovery.

PRECISION The degree of consistency between individual test findings obtained from multiple samplings of a homogenous sample is known as the precision of an analytical method. Standard deviation or relative standard deviation are commonly used to express an analytical method's precision. The test sample underwent precision work.

Result of Intra-day and Inter-Day Precision for Vortioxetine Test Sample Assay:

	Sample	Test Sample (mg)	Area	% Assay
Repeatability	Sample 1	284.2	8840251	98.80
	Sample 2	284.6	8885210	99.16
	Sample 3	284.4	8765823	97.90
	Sample 4	283.9	8825022	98.73
	Sample 5	284.3	9019581	100.77
	Sample 6	283.8	8803024	98.52
	Mean			**98.98**
	STD DEV			**0.970505**
	% RSD			**0.981**
Intermediate precision (Inter-Day)	Sample 1	284.4	9063561	101.22
	Sample 2	284.6	8758318	97.75
	Sample 3	283.8	8923045	99.87
	Sample 4	284.3	8830256	98.65

	Sample 5	284.1	8978103	100.38
	Sample 6	284.5	8896020	99.32
	Mean			**99.53**
	STD DEV			**1.239442**
	% RSD			**1.245**
Repeatability Plus Inter-day	**Mean**			**99.256**
	STD DEV			**1.09973**
	% RSD			**1.108**

Chromatograms:

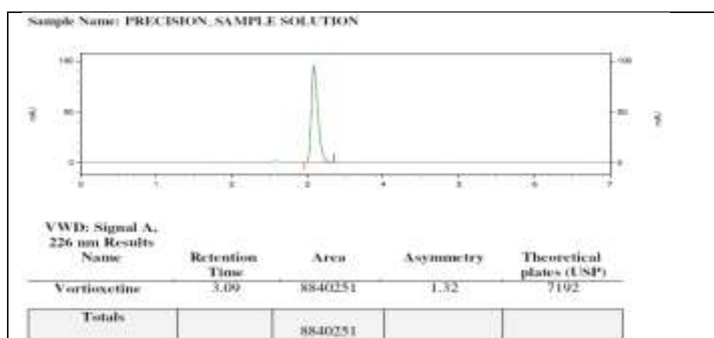


Fig. No. 30 Typical chromatogram of Repeatability precision (Sample 1).

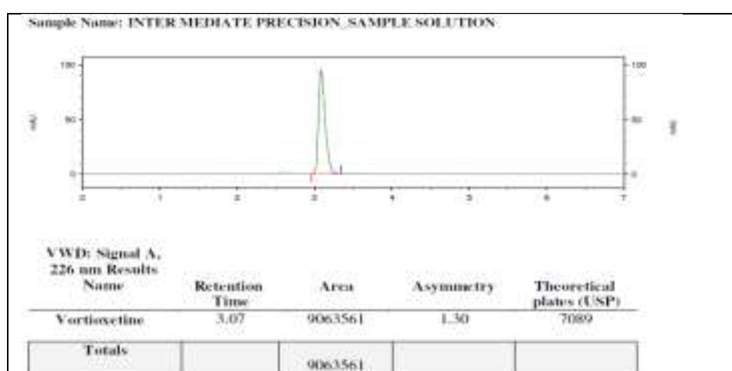


Fig. No. 31 Typical chromatogram of Inter-day precision (Sample 1).

Acceptance criteria:
% Assay: % Assay value for each sample (individual sample) and mean assay value for precision (6 sample), mean assay value for intermediate precision (6 sample), and mean assay value for precision plus intermediate precision (12 sample): 90-110%
% RSD: % RSD for precision study samples (6 sample), intermediate precision study samples (6 sample), and precision plus intermediate precision sample (12 sample): NMT 2.0
Data interpretation: % Assay and % RSD were found to be well within the acceptance limit, and hence the method is precise (reproducible).

- **ROBUSTNESS:**

An analytical technique's robustness gives an indication of its dependability under typical operating conditions by measuring its ability to

withstand slight but intentional changes in method parameters.

Following changes made under robustness:

- Change in Wavelength
- Change in flow rate
- Change in column oven temperature

Result of the robustness study of Vortioxetine

Change in Parameter	R.T.	Standard area	Asymmetry	Theoretical plates
Wavelength by +3 NM (229 NM)	2.98	8709563	1.25	7687
Wavelength by -3 NM (223 NM)	2.97	8693046	1.23	7710
Flow rate by +10% (1.1 mL/min)	2.67	8469594	1.27	7523
Flow rate by -10% (0.9 mL/min)	3.28	9860253	1.25	7958
Column oven temp by +2ºC (37 ºC)	3.07	8929673	1.29	7069
Column oven temperature by -2ºC (33ºC)	3.09	8906975	1.31	7116

Chromatograms:

A. Change in Wavelength by +3 NM:

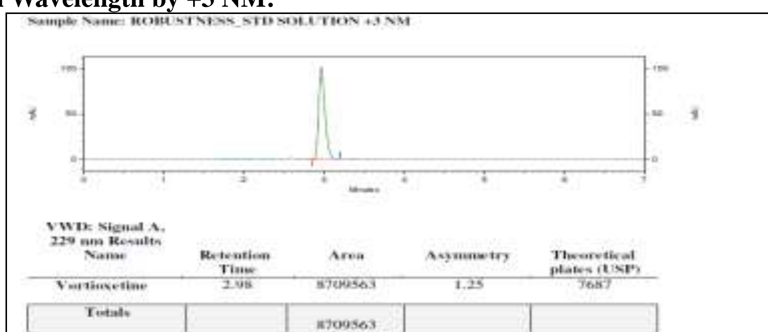


Fig. No. 32 Typical chromatogram of Standard +3 NM.

B. Change in Wavelength by -3 NM:

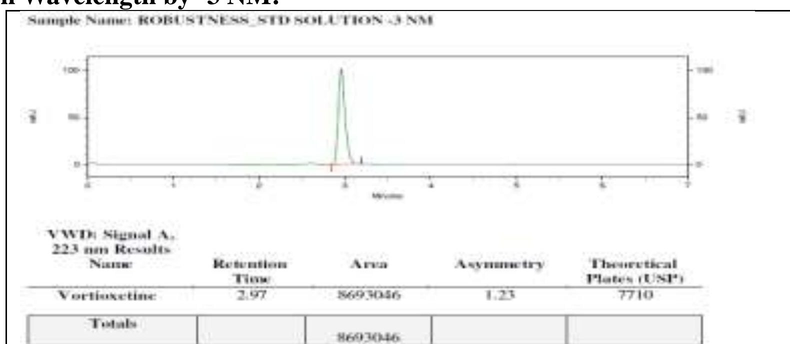


Fig. No. 33 Typical chromatogram of Standard -3 NM.

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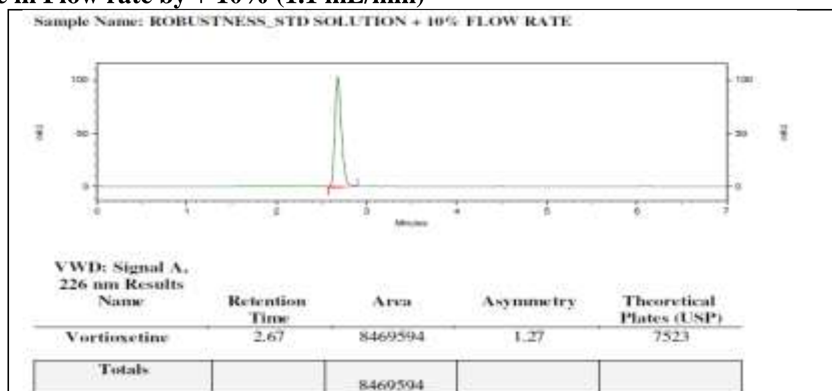
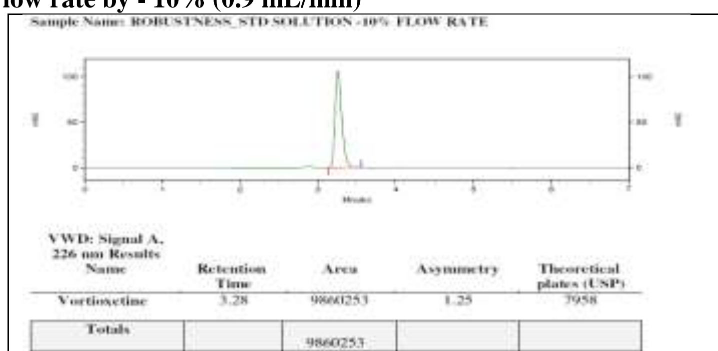
C. Change in Flow rate by + 10% (1.1 mL/min)


Sample Name: ROBUSTNESS_STD SOLUTION + 10% FLOW RATE

VWD: Signal A, 226 nm Results Name	Retention Time	Area	Asymmetry	Theoretical Plates (USP)
Vortioxetine	2.67	8469594	1.27	7523
Totals		8469594		

Fig. No. 34 Typical chromatogram of Standard +10 F.R.%.

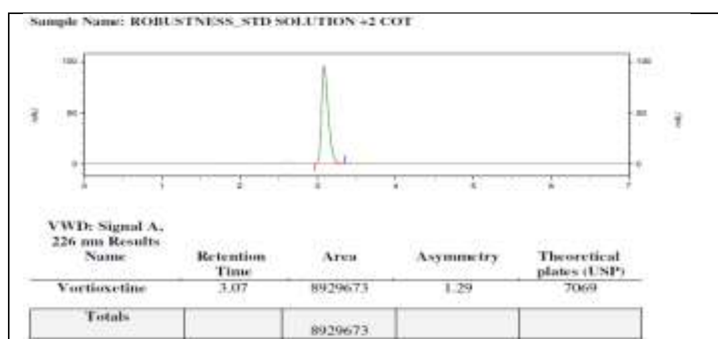
D. Change in Flow rate by - 10% (0.9 mL/min)


Sample Name: ROBUSTNESS_STD SOLUTION -10% FLOW RATE

VWD: Signal A, 226 nm Results Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Vortioxetine	3.28	9860253	1.25	7958
Totals		9860253		

Fig. No. 35 Typical chromatogram of Standard -10 F.R.%.

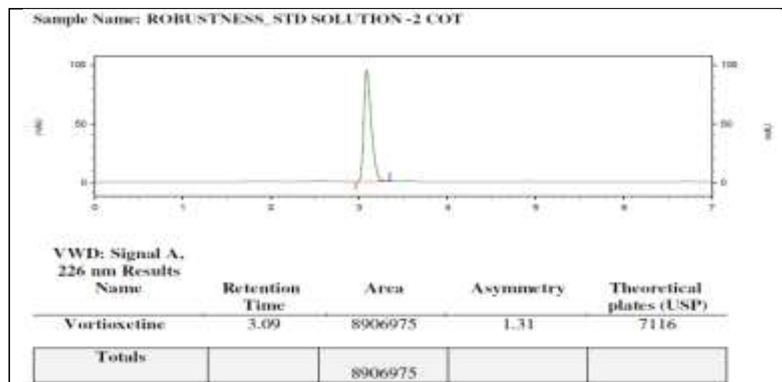
E. Change in Column Oven temperature by +2°C:


Sample Name: ROBUSTNESS_STD SOLUTION +2 COT

VWD: Signal A, 226 nm Results Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Vortioxetine	3.07	8929673	1.29	7069
Totals		8929673		

Fig. No. 36 Typical chromatogram of Standard +2°C C.O.T.

F. Change in Column Oven temperature by -2°C:



Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Vortioxetine	3.09	8906975	1.31	7116
Totals		8906975		

Fig. No. 37 Typical chromatogram of Standard -2ºC C.O.T.

Acceptance criteria:
Chromatography (system suitability) acceptance criteria should not fail.

Data interpretation: From the above results, it was concluded that the system suitability test result was well within the limits and the analytical method was robust.

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