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Development and Validation of RP-HPLC Method for Estimation of Vortioxetine in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Vortioxetine in its pure form as well as in tablet dosage form. Chromatography was carried out on ODS C18 (4.6 x 250 mm, 5µm) column using Acetonitrile And Methonal (70:30) as the mobile phase at a flow rate of 1.0 mL/min, the detection was carried out at 274nm. The retention time of the Vortioxetine was 2.922 ±0.02min. The method produce linear responses in the concentration range of 20 µg/ml of Vortioxetine. The method precision for the determination of assay was below 2.0 %RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

KEYWORDS: *Vortioxetine; RP-HPLC; PDA Detection; validation; Tablet dosage forms*

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1. INTRODUCTION

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.

1.1. SPECTROPHOTOMETRIC METHODS

Spectrophotometry is generally preferred especially by small-scale industries as the cost of the equipment is less and the maintenance problems are minimal. The method of analysis is based on measuring the absorption of a monochromatic light by colorless compounds in the near ultraviolet path of spectrum (200-380nm). The photometric methods of analysis are based on the Bouger-Lambert-Beer's law, which establishes the absorbance of a solution is directly proportional to the concentration of the analyte. The fundamental principle of operation of spectrophotometer covering UV region consists in that light of definite interval of wavelength passes through a cell with solvent and falls on to the photoelectric cell that transforms the radiant energy into electrical energy measured by a galvanometer.

The important applications are

- Identification of many types of organic, inorganic molecules and ions.
- Quantitative determination of many biological, organic and inorganic species.
- Monitoring and identification of chromatographic of effluents.

1.2. HPLC METHOD DEVELOPMENT

The term 'Chromatography' covers those processes aimed at the separation of the various species of a mixture on the basis of their distribution characteristics between a stationary and a mobile phase.

1.2.1. MODES OF CHROMATOGRAPHY

Modes of chromatography are defined essentially according to the nature of the interactions between the solute and the stationary phase, which may arise from hydrogen bonding, Vander walls forces, electrostatic forces or hydrophobic forces or basing on the size of the particles (e.g. Size exclusion chromatography).

Different modes of chromatography are as follows:

- Normal Phase Chromatography
- Reversed Phase Chromatography
- Reversed Phase – ion pair Chromatography
- Ion-Exchange Chromatography
- Size Exclusion Chromatography

1.3. METHOD VALIDATION

Method validation can be defined as (ICH) "estabilishing documented evidence which provides a high degree of assurance that specific activity will consistenty produce a desired result or product meeting its predetermined specifications and quality characteristic, Method validation is an integral part of the method development; it is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, quality, purity, an and drug products. Simply, method validation is the process of proving that and potency of the drug substances analytical method is acceptable for its intended purpose.

For chromatographic methods used in analytical applications there is more consistency in validation practice with key analytical parameters

(a)Recovery (b) Response function (c) Sensitivity (d) Presicion (e) Accuracy (f) limits of detection (g) Limit of quantitation (h) Ruggedness (i) Robustness (j) stability (k) system suitability

A. Recovery:

The absolute recovery of analytical method is measured as the response of a processed spiked matrix standard expressed as a percentage of the response of pure standard which has not been subjected to sample pre treatment and indicates whether the method provides a response for the entire amount of analyte that is present in the sample.

$$\text{Absolute recovery} = \frac{\text{Response of an analyte spike into matrix (processed)}}{\text{Response of analyte of pure standard (unprocessed)}} \times 100$$

B. Sensitivity:

The method is said to be sensitive if small changes in concentration cause large changes in response function. The sensitivity of an analytical method is determined from the slope of the calibration line. The limits of quantification (LOQ) or working dynamic range of bio analytical method are defined as the highest and lowest concentrations, which can determined with acceptable accuracy. It is suggested that, this be set at ± 15% for both the upper and lower limit of quantitation respectively.

C. Precision:

The purpose of carrying out a determination is to obtain a valid estimate of a 'true' value. When one considers the criteria according to which an analytical procedure is selected, precision and accuracy are usually the first time to come to mind. Precision and accuracy together determine the error of an individual determination.

Precision refers to the reproducibility of measurement within a set, that is, to the scatter of dispersion of a set about its central value. The standard deviation S, is given by

$$S = \sqrt{\frac{1}{n-1}\sum_{i-1}^{n}\left(x_i - \bar{x}\right)^2}$$

The square of standard deviation is called variance (S^2). Relative standard deviation is the standard deviation expressed as a fraction of the mean, i.e., S/x. It is sometimes multiplied by 100 and expressed as a percent relative standard deviation. It becomes a more reliable expression of precision.

% Relative standard deviation = S x 100 / x

D. Accuracy:

Accuracy normally refers to the difference between the mean x****, of the set of results and the true or correct value for the quantity measured. According to IUPAC accuracy relates to the difference between results (or mean) and the true value. For analytical methods, there are two possible ways of determining the accuracy, absolute method and comparative method.

$$\%\text{Bias} = \frac{(\text{measured value} - \text{true value})}{\text{true value}} \text{ X } 100$$

E. Limit of detection (LOD):

The limit of detection (LOD) of an analytical method may be defined as the concentration, which gives rise to an instrument signal that is significantly different from the blank. For spectroscopic techniques or other methods that rely upon a calibration curve for quantitative measurements, the IUPAC approach employs the standard deviation of the intercept (Sa), which may be related to LOD and the slope of the calibration curve, b, by

$$LOD = 3\ Sa/\ b$$

F. Limit of quantitation (LOQ)

The LOQ is the concentration that can be quantitate reliably with a specified level of accuracy and precision. The LOQ represent the concentration of analyte that would yield a signal-to-noise ratio of 10.

$$LOQ = 10\ Sa/\ b$$

Where, Sa- the estimate is the standard deviation of the peak area ratio of analyte to IS (5 injections) of the drugs. b -is slope of the corresponding calibration curve.

G. Ruggedness

Method Ruggedness is defined as the reproducibility of results when the method is performed under actual use conditions. This includes different analysts, laboratories, columns, instruments, source of reagents, chemicals, solvents etc..

H. Robustness

The concept of robustness of an analytical procedure has been defined by the ICH as "a measure of its capacity to remain unaffected by small but deliberate variations in method parameters". The robustness of a method is the ability to remain unaffected by small changes in parameters such as pH of the mobile phase, temperature, %organic solvent strength and buffer concentration etc

I. System suitability

System suitability experiments can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validation have been completed. (or) The USP (2000) defines parameters that can be used to determine system suitability prior to analysis.

MATERIALS And Method:-Chemicals:

- Acetonitrile HPLC Grade And Methanol

Raw Material:

- Vortioxetine Working Standard.

Instruments:

- HPLC – WATERS Model NO.2695 series Compact System Consisting of
- Inertsil-C18 ODS column.
- UV-Spectroscopy 1800 (Shimadzu)
- Electronic balance (SARTORIOUS)
- Digital pH meter(POLOMAN)
- Sonicator(FAST CLEAN)

METHOD DEVELOPMENT FOR HPLC:

The objective of this experiment was to optimize the assay method for estimation of Vortioxetine based on the literature survey made. So here the trials mentioned describes how the optimization was done.

Observation: Got more assymetry. The trial 2 chromatogram result was shown in Fig:

Trail: 1.

Mobile Phase: Methanol and Acetonitrile were mixed in the ratio of 80:20 V/V and sonicated to degas.

Preparation of Standard Solution:

10mg of Vortioxetine drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml. was taken from this and diluted to 10ml.with mobile phase

hromatographic Conditions:

Flow rate	: 1.0ml/min
Column	: Inertsil - C18 ODS column
Detector wavelength	: 274nm
Column temp	: Ambient
Injection volume	: 20µl
Run time	: 5min
Retention time	: 2.910

Observation:

Got Bad Peak. The trial 3chromatogram result was shown in Fig:1

OPTIMIZED METHOD

Mobile Phase: Acetonitrile and Methanol were taken and sonicated to degas in the ratio of 70:30.

Preparation of stock solution:

10mg of Vortioxetine drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1ml. was taken from this and diluted to 10ml.with mobile phase.

Preparation of working standard solution:

The stock solution equivalent to 20ppm to 70ppm were prepared, sonicated and filtered through 0.45µ membrane.

Optimized chromatographic conditions: Table no-1

Parameters	Method
Stationary phase (column)	Inertsil -ODS C_{18} (250 x 4.6 mm, packed with 5 micron)
Mobile Phase	Acetonitrile and Methanol (70:30)
Flow rate (ml/min)	1.0 ml
Run time (minutes)	6
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	274nm
Drug RT (min)	2.922

METHOD VALIDATION

SYSTEM SUITABILITY:

A Standard solution was prepared by using 10mg of Vortioxetine drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml. was taken from this and diluted to 10ml.with mobile phase and was injected Five times into the HPLC system.

The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Vortioxetine, retention times and peak areas.

ACCEPTANCE CRITERIA:

1. The % RSD for the retention times of principal peak from 5 replicate injections of each Standard solution should be not more than 2.0 %
2. The % RSD for the peak area responses of principal peak from 5 replicate injections of each standard Solution should be not more than 2.0%.
3. The number of theoretical plates (N) for the Vortioxetine peaks is NLT 3000.
4. The Tailing factor (T) for the Vortioxetie peaks is NMT 2.0

OBSERVATION:

The %RSD for retention times and peak areas were found to be within the limit.refer table: 5 As sown in Fig6 – 10.

SPECIFICITY:-

Vortioxetie identification:

Solutions of standard and sample were prepared 10mg of Vortioxetine drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml. was taken from this and diluted to 10ml.with mobile phase and injected into chromatographic system.

ACCEPTENCE CRITERIA: Chromatogram of standard and sample should be identical with near Retention time.

OBSERVATION:

The chromatograms of Standard and Sample were same identical with same retention time. As shown in fig: 13

PRECISION:

Repeatability:

A. **System precision:** Standard solution prepared 10mg of Vortioxetine drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml. was taken from this and diluted to 10ml.with mobile phaseand injected five times.

B. **Method precision:** Prepared six sample preparations individually using single as per above method and injected each solution.

ACCEPTANCE CRITERIA: The % relative standard deviation of individual Vortioxetine, from the six units should be not more than 2.0%.

The assay of Vortioxetine should be not less than 98% and not more than 102.0%.

OBSERVATION:

Test results are showing that the test method is precise. Refer tables:- 6 for system precision and for method precision.

Intermediate precision

10mg of Vortioxetine drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml. was taken from this and diluted to 10ml.with mobile phase.4 ml was taken from this diluted to 10 ml.with mobile phase.

ACCEPTENCE CRITERIA:

The individual assays of Vortioxetine should be not less than 98% and not more than 102% and %RSD of assay should be NMT2.0% by both analysts.

OBSERVATION:

Individual %assays and %RSD of Assay are within limit and passes the intermediate precision, Refer table: 8

ACCURACY (RECOVERY):

A study of Accuracy was conducted. Drug Assay was performed in triplicate as 10mg of Vortioxetine drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml. was taken from this and diluted to 10ml.with mobile phase with equivalent amount of F Vortioxetine into each volumetric flask for each spike level to get the concentration of f Vortioxetine equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Vortioxetine was calculated.

ACCEPTANCE CRITERIA:

The mean % recovery of the Vortioxetine at each spike level should be not less than 98.0% and not more than 102.0%.

OBSERVATION:

$$\%\text{Recovery} = \frac{\text{Amount found}}{\text{Amount added}} \times 100$$

The recovery results indicating that the test method has an acceptable level of accuracy. Refer table: 9

LINEARITY OF TEST METHOD:

A Series of solutions are prepared using Vortioxetine working standard at concentration levels from 20ppm to 80 ppm of target concentration .Measure the peak area response of solution at Level 1 and Level 6 six times and Level 2 to Level 5 two times.

ACCEPTANCE CRITERIA:

Correlation Coefficient should be not less than 0.9990.
% of y- Intercept should be ±2.0.
% of RSD for level 1 and Level 6 should be not more than 2.0%.

OBSERVATION:

The linear fit of the system was illustrated graphically. The results are presented in table:4

ROBUSTNESS:

Effect of variation of flow rate:

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 1.0ml/min and 1.2ml/min. The system suitability parameters were evaluated and found to be within the limits for 1.0ml/min and 1.2ml/min flow.

Vortioxetine was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0ml/min.

ACCEPTANCE CRITERIA:

The Tailing Factor of Vortioxetine standards should be NMT 2.0 for Variation in Flow.

OBSERVATION:

The tailing factor for MF was found to be within the limits. As shown in table 10.

LIMIT OF DETECTION AND QUANTITATION (LOD and LOQ):

From the linearity data calculate the limit of detection and quantitation, using the following formula.

$$LOD = \frac{3.3\,\sigma}{S}$$

σ = standard deviation of the response
S = slope of the calibration curve of the analyte.

$$LOQ = \frac{10}{S}$$

σ = standard deviation of the response
S = slope of the calibration curve of the analyte.

Method Development:-

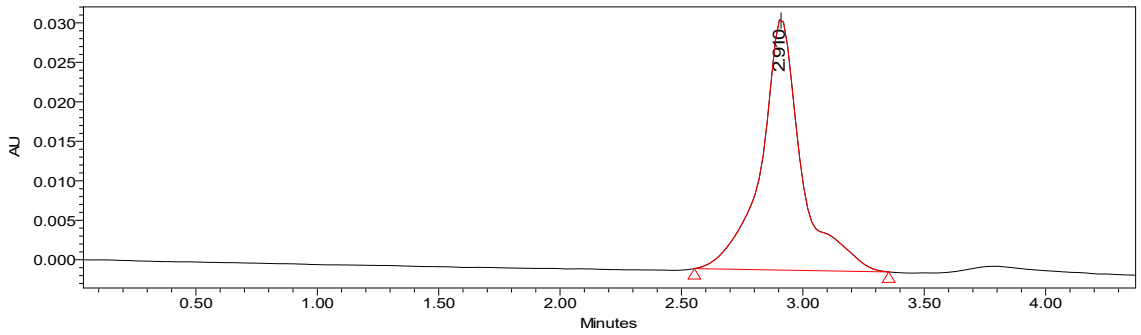


Fig1: Chromatogram of Trial Inference: Got Bad Peak.

Table No-2

Sr. NO.	Name of the peak	Retention time(min)
1.	VORTIOXETINE	2.910

OPTIMIZED METHOD

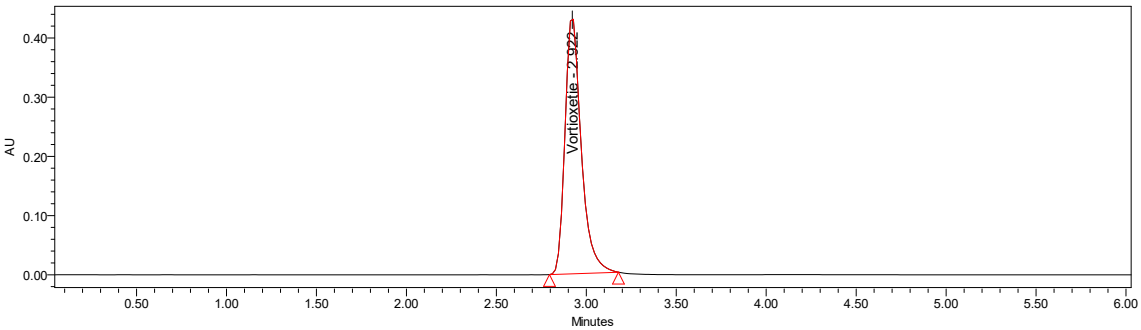


Fig2: Chromatogram of standard 1 Inference: Got chromatogram at an Rt of 2.922for standard

Table No-3

Sr. NO.	Name of the peak	Retention time(min)	Peak area	Tailing factor
1	VORTIOXETINE	2.922	1115674.56	1.014

VALIDATION DATA

LINEARITY:

TABLE No4: Data of Linearity

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	27848
20	557827.45	y-Intercept	-214.8
30	836741.48	Correlation Coefficient	0.999
40	1115655.45		
50	1381456.65		
60	1673482.32		
70	1952396.25		

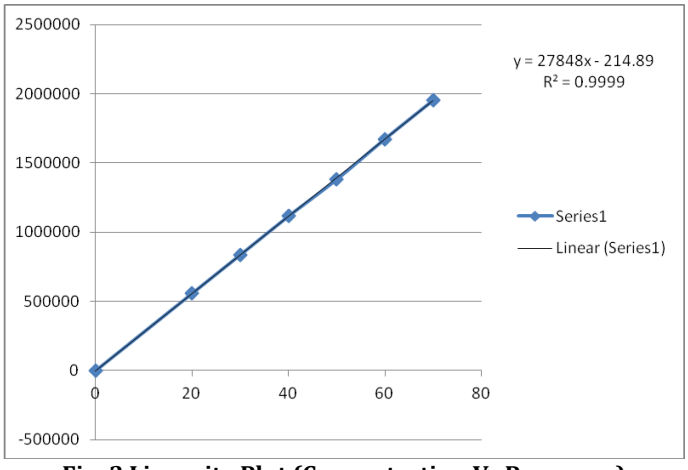


Fig: 3 Linearity Plot (Concentration Vs Response)

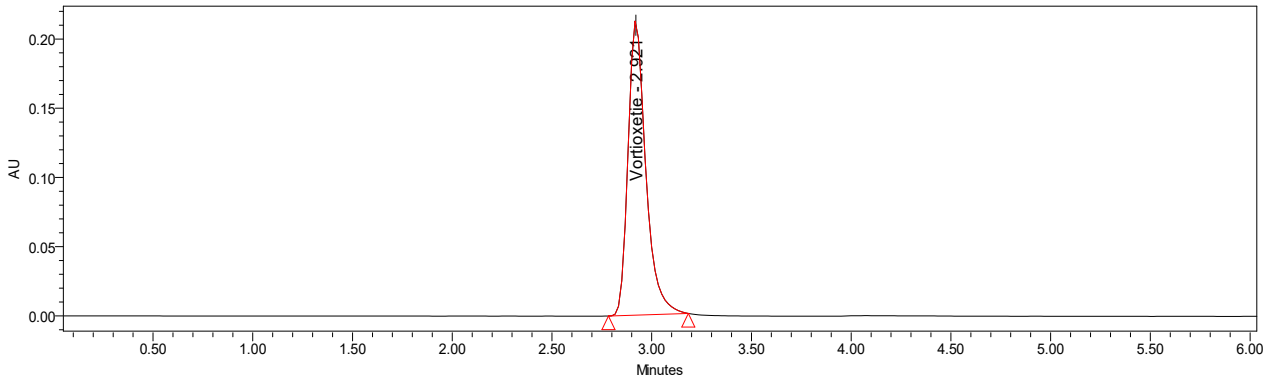


Fig: 4 Chromatograms for 20 ppm Inference: Chromatogram for 20 ppm

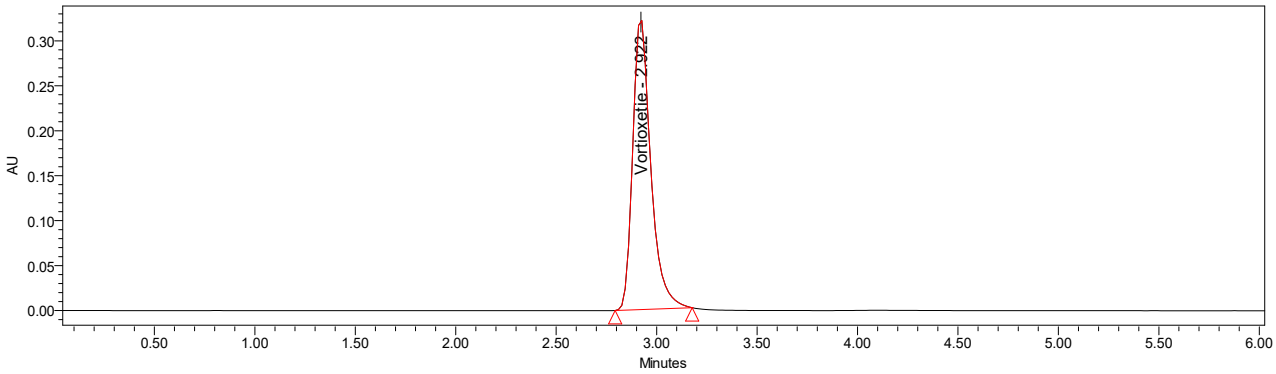
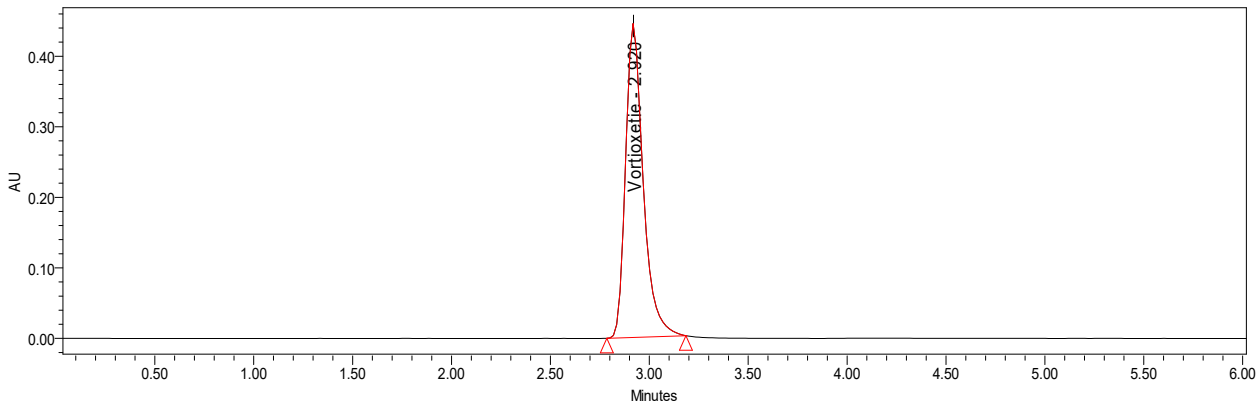


Fig: 5-6 chromatograms for 30ppm, 40 ppm Inference: Chromatogram for 30 ppm standard 1



Inference: Chromatogram for 40 ppm standard 1

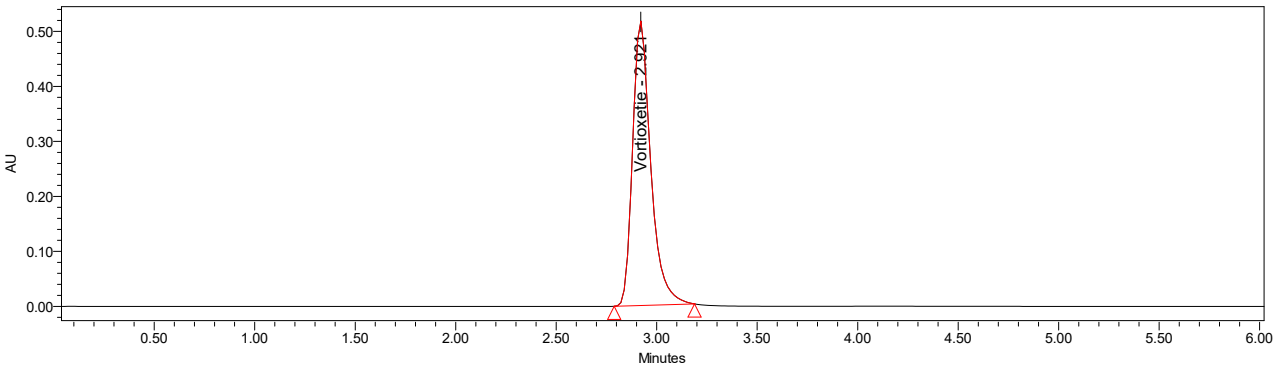
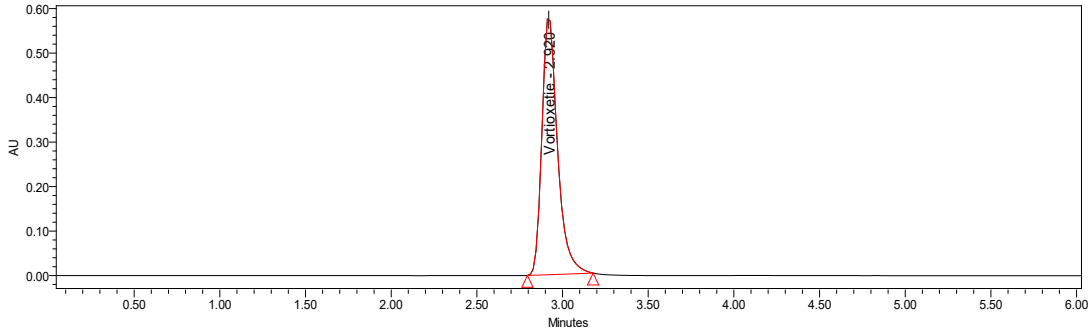
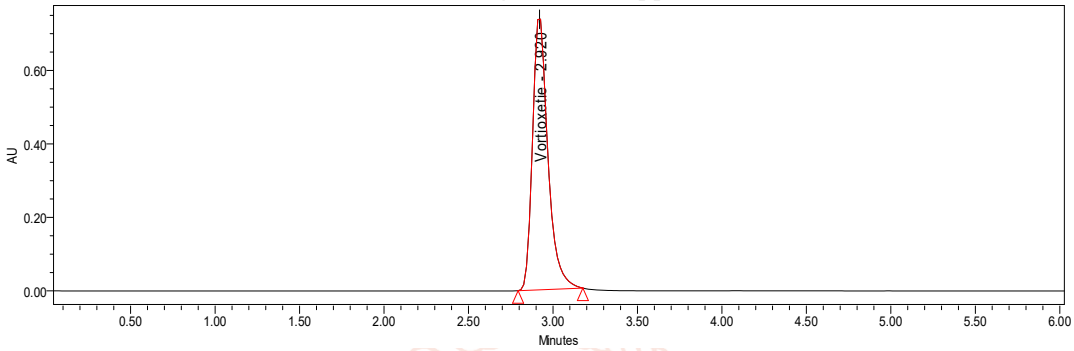


Fig: 7-8 Chromatograms for 50 ppm, 60 ppm Inference: Chromatogram for 50 ppm standard 1



Inference: Chromatogram for 60 ppm standard 1



Inference: Chromatogram for 70 ppm standard 1

SYSTEM SUITABILITY:

TABLE No-5: Data of System Suitability

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	2.921	1115623.12	11035	1.012
2	2.920	1115684.35	11042	1.016
3	2.920	1115601.99	11054	1.023
4	2.922	1115674.56	11038	1.014
5	2.923	1115688.56	11045	1.019
Mean	2.921231	1115654.51	11042	1.016
SD	0.001303	39.3557	-------	-------
% RSD	0.04460	0.00352	-------	-------

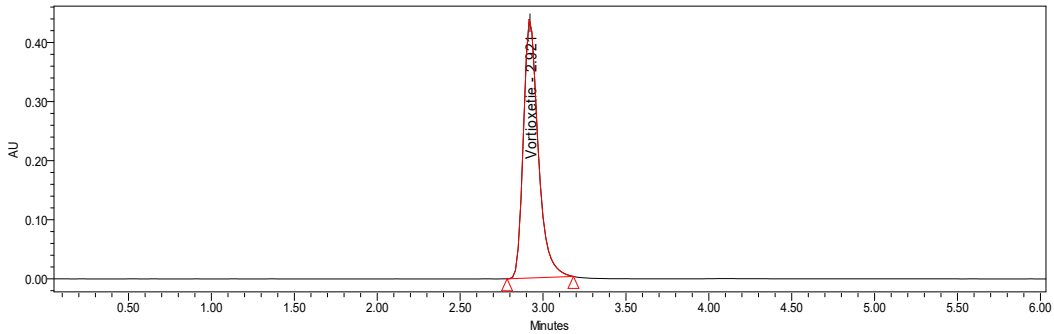
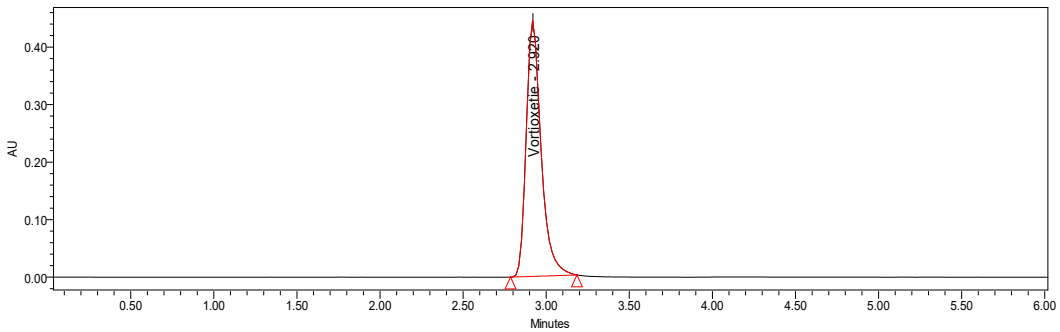
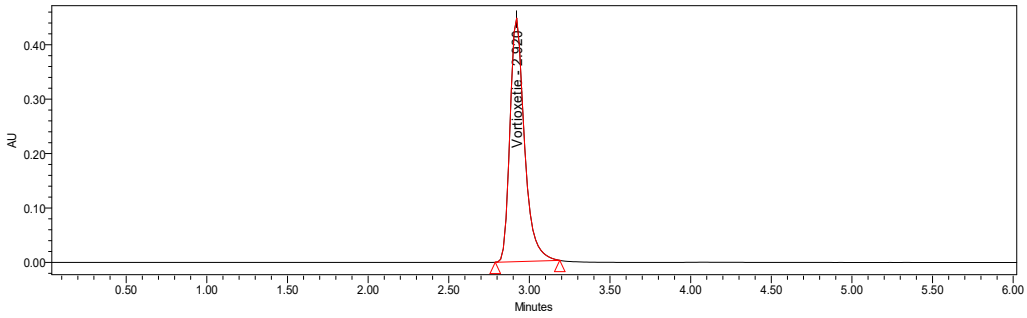


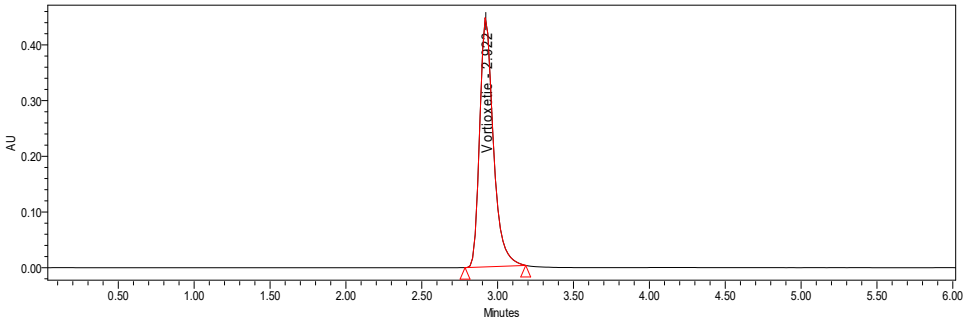
Fig: 9-13 Chromatograms of system suitability(standards 1-5) Inference: System suitability Chromatogram for standard – 1



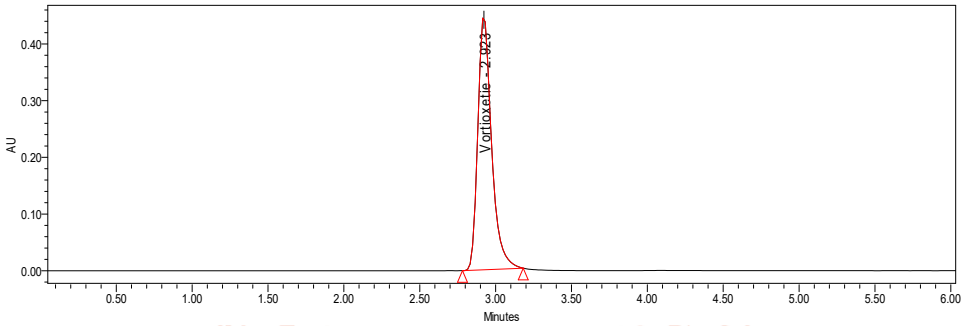
Inference: System suitability Chromatogram for standard – 2



Inference: System suitability Chromatogram for standard – 3



Inference: System suitability Chromatogram for standard - 4



Inference: System suitability Chromatogram for standard - 5

SPECIFICITY:

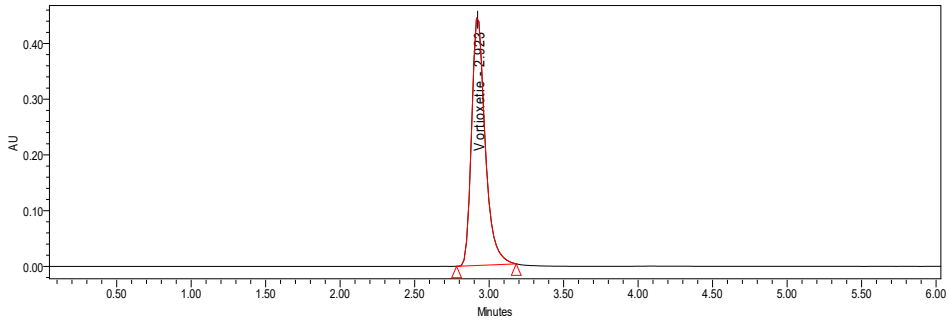


Fig14: Chromatogram of standard Inference: Got a peak for standard at an Rt of 2.923

PRECISION:

Repeatability:

A. system precision:

TABLE No -6 Data of Repeatability (System precision)

	Injection	Peak Areas of Vortioxetine	%Assay
Concentration 40ppm	1	1115589.45	100.16
	2	1115601.05	100.17
	3	1115596.58	100.16
	4	1115608.89	100.17
	5	1115582.65	100.16
Statistical Analysis	Mean	1115595.72	100.16
	SD	10.1579	0.00091
	% RSD	0.00091	0.00091

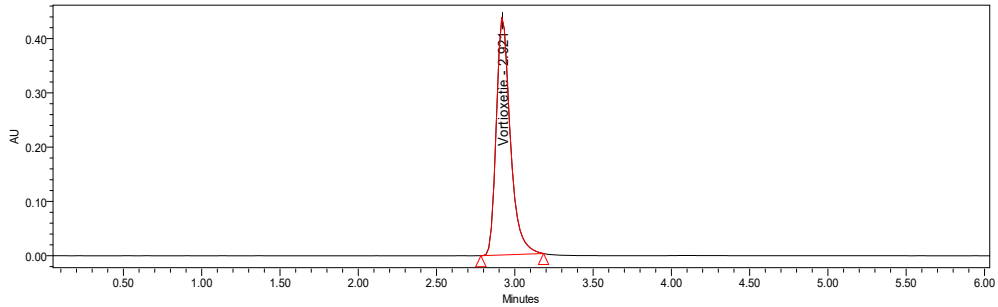
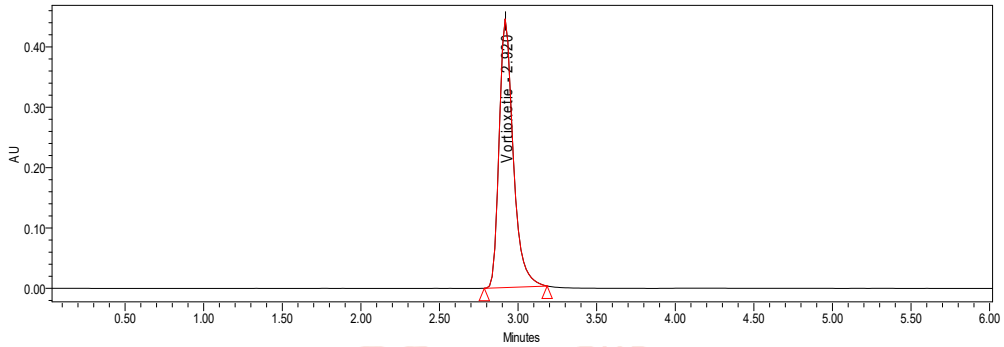
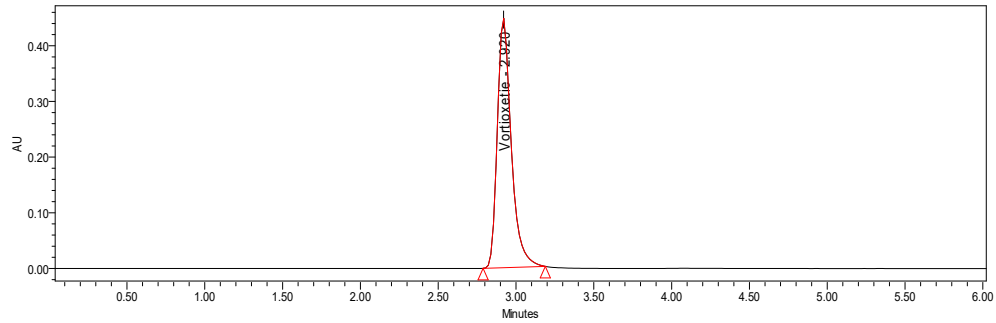


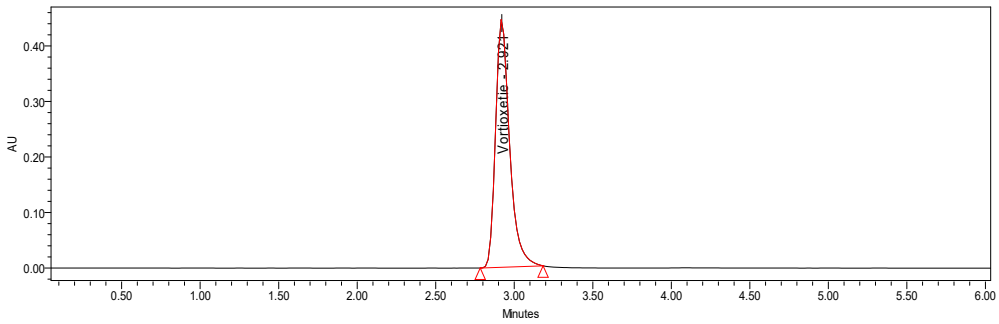
Fig15-19 Chromatograms of system precision Inference: Chromatogram for system precision (standard - 1)



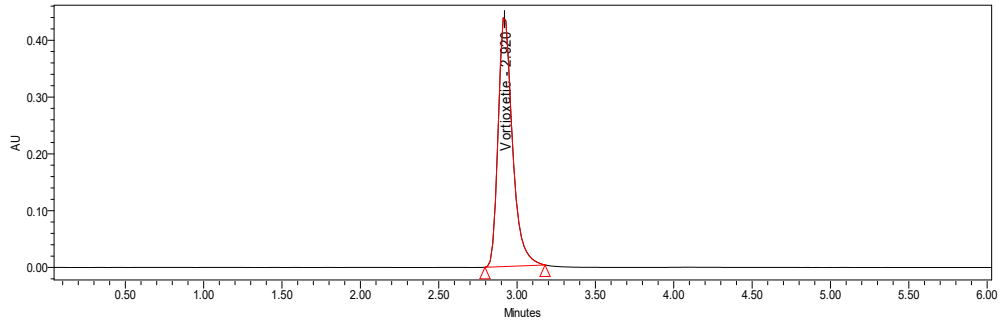
Inference: Chromatogram for system precision (standard - 2)



Inference: Chromatogram for system precision (standard - 3)



Inference: Chromatogram for system precision (standard - 4)



Inference: Chromatogram for system precision (standard - 5)

B. Method precision: TABLE No -7 Data of Repeatability (Method precision)

	Injection	Peak Areas of Vortioxetine	%Assay
Concentration 40ppm	1	1115568.87	100.16
	2	1115590.63	100.16
	3	1115579.42	100.16
	4	1115601.55	100.17
	5	1115595.45	100.16
Statistical Analysis	6	1115610.62	100.17
	Mean	1115591.09	100.16
	SD	15.09947	0.00135
	% RSD	0.001353	0.00135

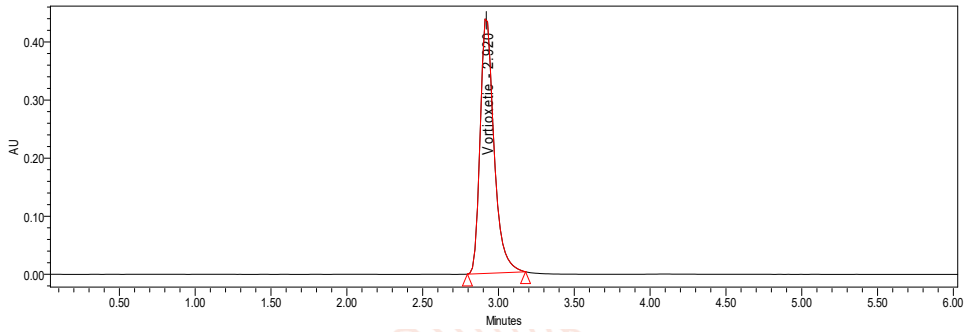
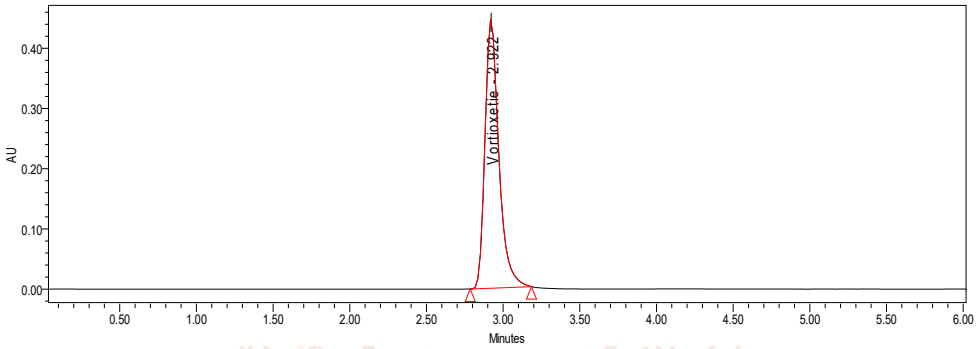
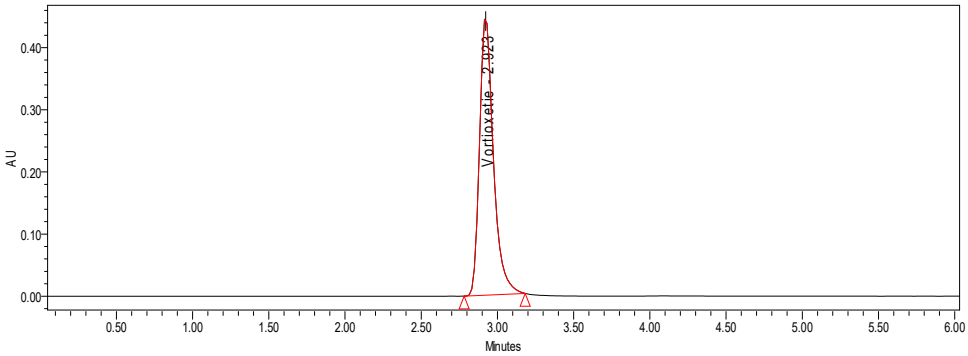


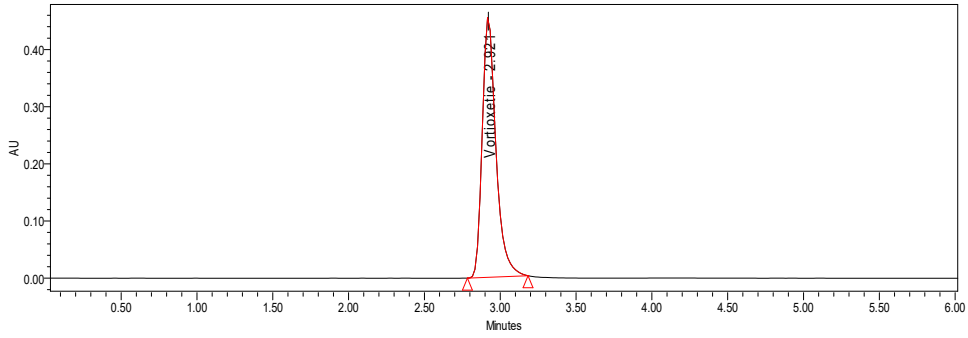
Fig: 20-24 Chromatograms of Repeatability Inference: Chromatogram for Repeatability (standard - 1)



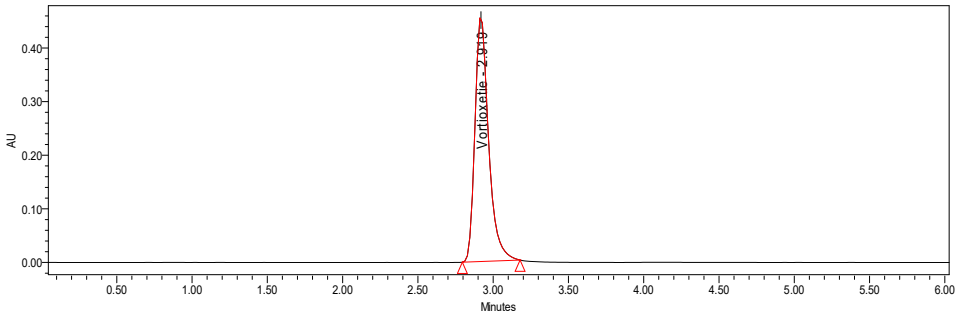
Inference: Chromatogram for Repeatability (standard -2)



Inference: Chromatogram for Repeatability (standard - 3)



Inference: Chromatogram for Repeatability (standard - 4)



Inference: Chromatogram for Repeatability (standard - 5)

Intermediate precision: Table No-8 Data of Intermediate precision

	Injection	Peak Areas of Vortioxetine	%Assay
Concentration 40ppm	1	1115593.56	100.16
	2	1115568.95	100.16
	3	1115599.15	100.17
	4	1115602.08	100.17
	5	1115618.45	100.17
	6	1115622.12	100.17
Statistical Analysis	Mean	1115600.71	100.17
	SD	19.16885	0.001720
	% RSD	0.001718	0.001717

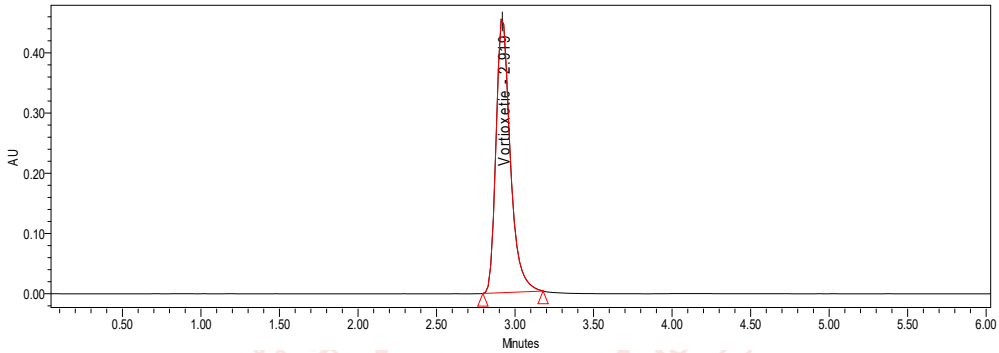
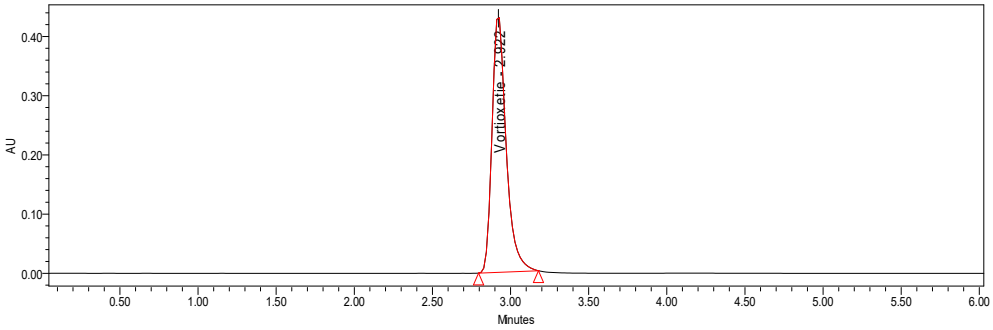


Fig: 25-26Chromatograms of Intermediate precision Inference: Chromatogram for IntermediatePrecision



Inference: Chromatogram for Intermediate Precision ACCURACY (RECOVERY)

TABLE No -9 Data of Accuracy

Concentration (40ppm) % of spiked level	Peak Area	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50%Sample 1	557853.51	20	20.03	100.19	MEAN	100.19
50% Sample 2	557823.48	20	20.03	100.19		
50% Sample 3	557860.56	20	20.04	100.19	%RSD	0.00352
100 %Sample 1	1115601.96	40	40.06	100.16	MEAN	100.17
100 % Sample 2	1115580.45	40	40.06	100.17		
100% Sample 3	1115620.56	40	40.06	100.17	%RSD	0.00179
150% Sample 1	1673484.25	60	60.10	100.16	MEAN	100.16
150% Sample 2	1673482.12	60	60.10	100.16	%RSD	0.00022
150% Sample 3	1673476.85	60	60.10	100.16		

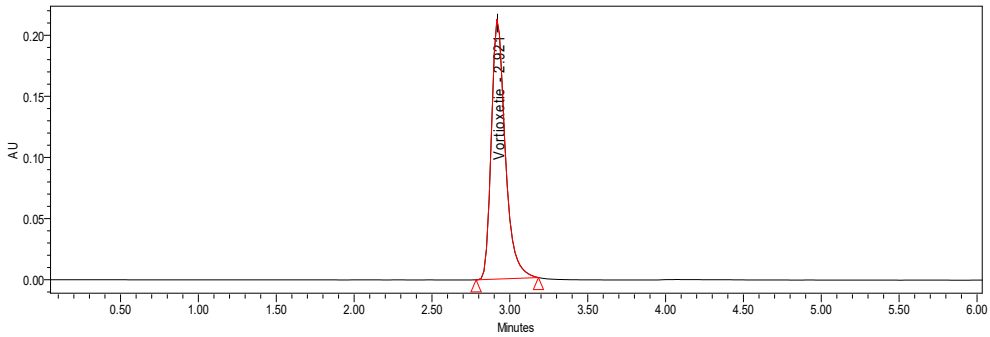
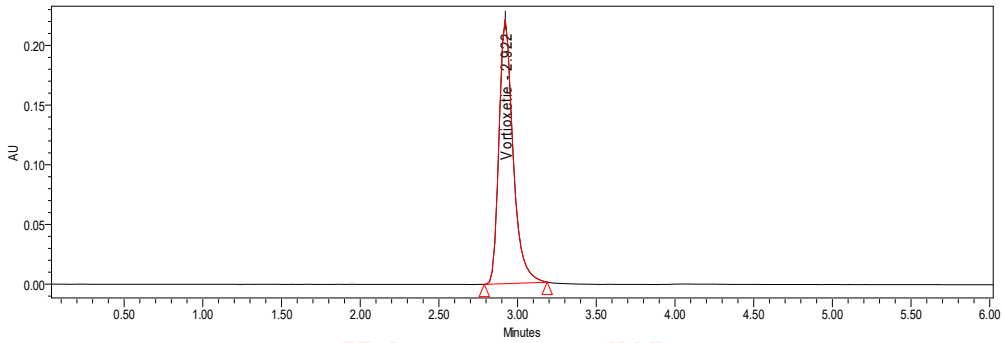


Fig: 27-28Chromatograms for accuracy (50%) Inference: Chromatogram for standard 1



Inference: Chromatogram for standard 2

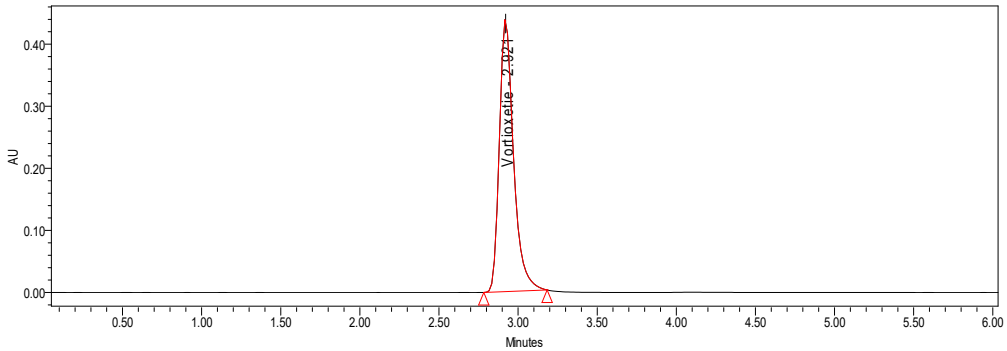
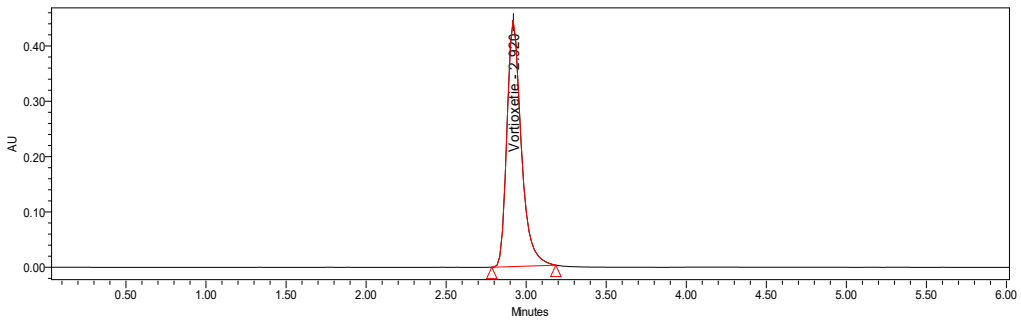


Fig: 29-30- Chromatograms for accuracy (100%) Inference: Chromatogram for standard 1



Inference: Chromatogram for standard 2

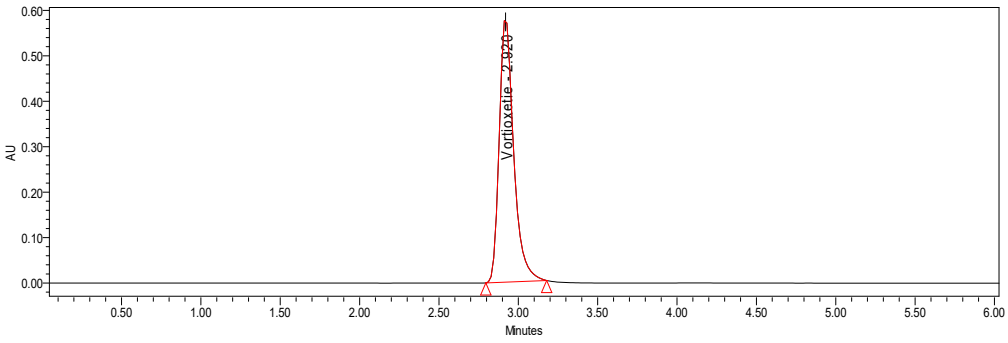
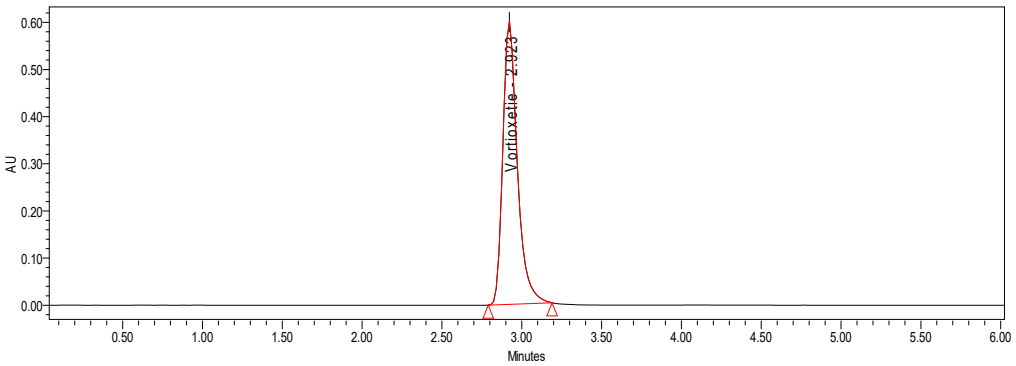


Fig: 31-32 chromatograms For Accuracy (150%) Inference: Chromatogram for standard 1



Inference: Chromatogram for standard

Robustness: TABLE No-10 Data for Effect of variation in flow rate:

	Std Area	Tailing factor		Std Area	Tailing factor		Std Area	Tailing factor
Flow 0.8 ml	1108456.25	1.111	**Flow 1.0 ml**	1115647.14	1.115	**Flow 1.2 ml**	1123864.24	1.128
	1108444.66	1.115		1115632.32	1.117		1123888.46	1.130
	1108471.52	1.117		1115639.64	1.115		1123878.23	1.129
	1108462.59	1.121		1115621.35	1.116		1123845.16	1.129
	1108473.19	1.123		1115611.54	1.117		1123854.54	1.128
Avg	1108461.64	1.117	Avg	1115630.39	1.116	Avg	1123866.12	1.129
SD	11.71848	0.0047	SD	14.2029	0.001	SD	17.4834	0.0008
%RSD	0.00105	0.4273	%RSD	0.00127	0.0896	%RSD	0.00155	0.0741

A. Effect of variation of flow rate (for 0.8 ml/min flow)

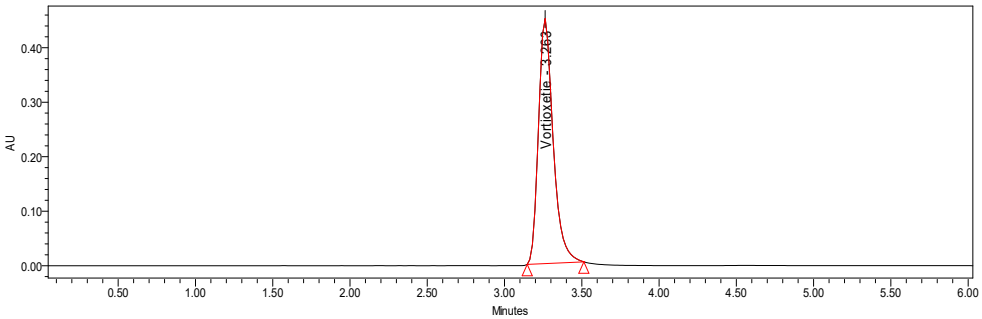
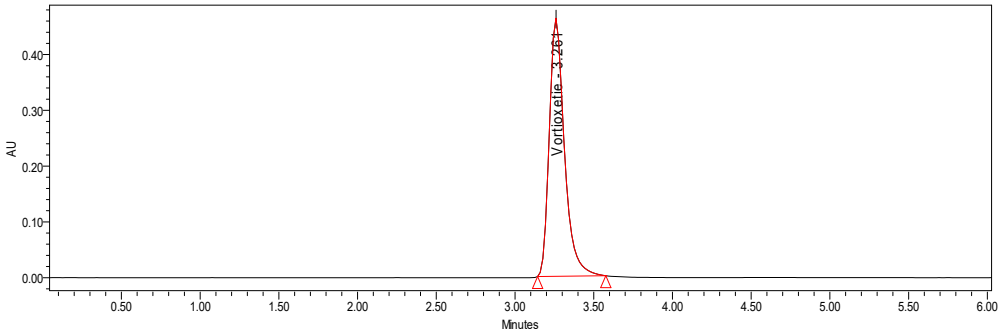
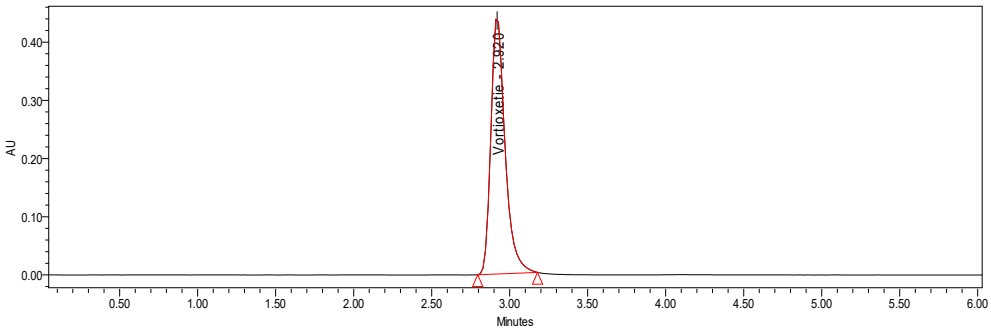


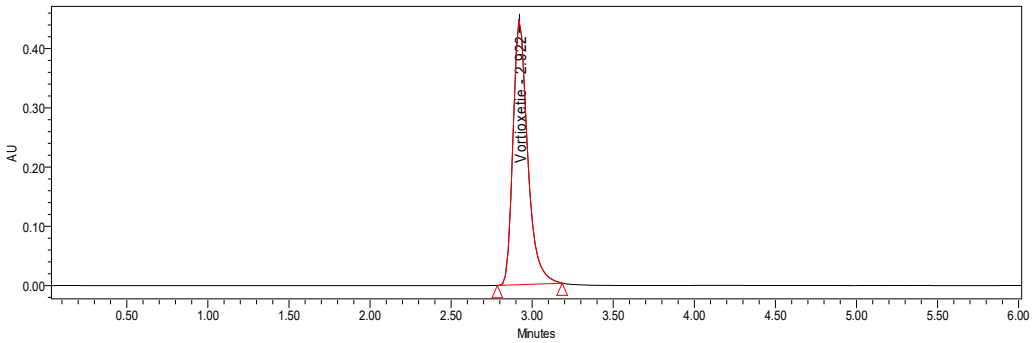
Fig: 33-34 Chromatograms of robustness Inference: Chromatogram for robustness standard – 1



Inference: Chromatogram for robustness standard – 2 Fig: 35-36 chromatograms for 1ml/min



Inference: Chromatogram for robustness standard – 1



Inference: Chromatogram for robustness standard – 2

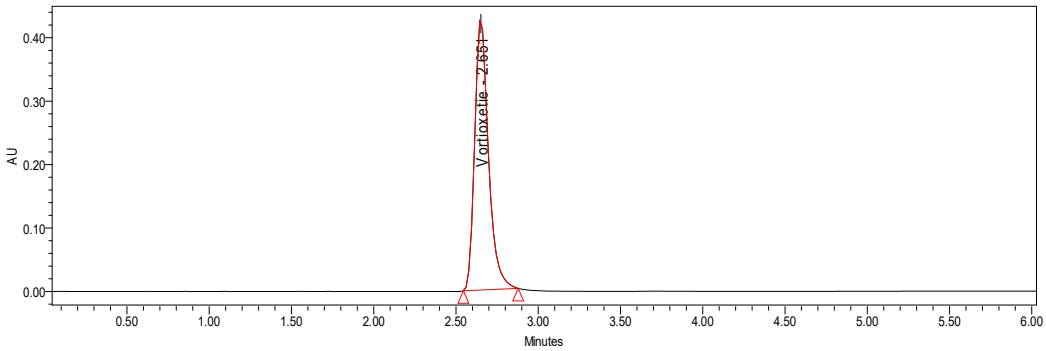
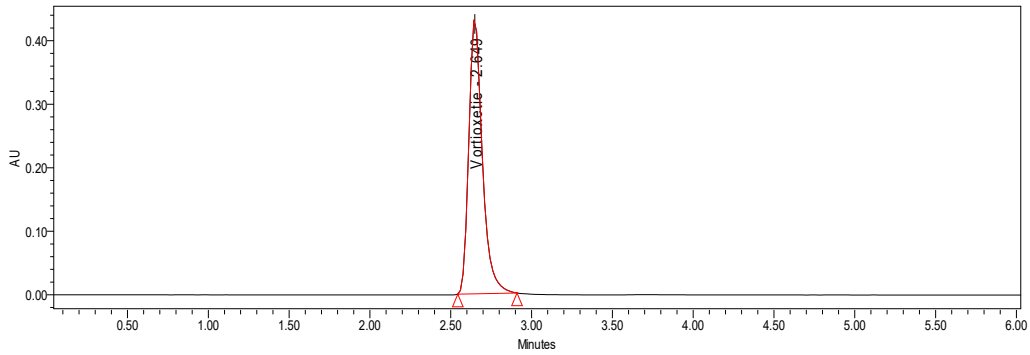


Fig: 37-38 Chromatograms for 1.2ml/min Inference: Chromatogram for robustness standard – 1



Inference: Chromatogram for robustness standard – 2

LIMIT OF DETECTION AND LIMIT OF QUANTITATION (LOD and LOQ)

From the linearity plot the LOD and LOQ are calculated: -

$$LOD = \frac{3.3\,\sigma}{S}$$

$$= \frac{3.3 \times 39.5337}{27848} = 0.00468$$

$$LOQ = \frac{10\,\sigma}{S}$$

$$= \frac{10 \times 39.5337}{27848} = 0.0141$$

Summary and conclusion

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 274nm and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Inertsil C_{18} chosen good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Different pH and ratios of mobile phase were studied, mobile phase with ratio of Acetonitrile and Methanol (70: 30) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Methanol was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. Retention time is 2.922 .

The present recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. Detection limit was found to be 0.00468. Linearity study was, correlation coefficient and curve fitting was found to be. The analytical method was found linearity over the range of 10-70ppm of the target concentration.

The method was found to be simple, accuracy,precise,economical and reproducible. So the propose method can be used for the routine quality control analysis of Vortioxetine in bulk and dosage form.

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