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A Novel Stability-Indicating Method for Determination of Related Substances of Viloxazine Hydrochloride in a Active Pharmaceutical Ingredient (API) form Using RP-HPLC

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KEYWORDS

Validation, Stability,Related Impurities,API, RP-HPLC, Degradation, Viloxazine Hydrochloride

ABSTRACT:

Introduction: Viloxazine Hydrochloride is a pharmacologically significant compound known for its dual role as a selective norepinephrine reuptake inhibitor and an anti-asthmatic agent. Despite its clinical importance, there is a conspicuous gap in research concerning the detection of related substances that emerge during the manufacturing process of Viloxazine Hydrochloride. This oversight presents a critical concern, as the presence of impurities could significantly affect the drug's safety profile and therapeutic efficacy. The development of a robust High-Performance Liquid Chromatography (HPLC) method for the determination of related substances in Viloxazine Hydrochloride is, therefore, an urgent need.

Objectives: The primary objective is to establish a straightforward, robust, and precise approach for detecting Viloxazine hydrochloride-associated impurities within an Active Pharmaceutical Ingredient(API)through the utilization of reverse-phase high-performance liquid chromatography (RP-HPLC).

Methods: Chromatographic separation was performed on an Agilent Eclipse XDB C8 column (250 mm × 4.6 mm, 5 µm) using a gradient elution mode. The mobile phase consisted of potassium phosphate and acetonitrile in varying ratios, adjusted to pH 2.35 with phosphoric acid. The flow rate was set at 0.8 mL/min, and detection was carried out with a UV detector at 210 nm.

Results: The method was validated according to ICH guidelines, demonstrating specificity, linearity, precision, accuracy, limit of detection, and limit of quantification. Linear regression analysis of the calibration plot showed a strong linear relationship between response and concentration, with a correlation coefficient (r^2) of 0.9998, indicating excellent correlation. The accuracy of identified impurities ranged from 90% to 110%. The limits of detection (LOD) and quantification (LOQ) for impurities were established at 0.01 µg/g and 0.03 µg/g, respectively.

Conclusion: The proposed method proved effective in accurately quantifying Viloxazine Hydrochloride related impurities in Active Pharmaceutical Ingredient (API) form.

1. Introduction

Viloxazine Hydrochloride is a selective norepinephrine reuptake inhibitor and an anti-asthmatic agent. Its chemical name is(±)-2-[(2ethoxyphenoxy)methyl]morpholine hydrochloride and is recommended for the treatment of Attention Deficit Hyperactivity Disorder (ADHD) in pediatric patients 6 to 17 years of age. It is was approved by the U.S. Food and Drug Administration (FDA) in 2021. It is known to have several desirable pharmacologic uses, such as treatment of depression, nocturnal enuresis, narcolepsy, sleep disorders and alcoholism. Viloxazine Hydrochloride's molecular formula is $C_{13}H_20NO_3Cl$ and it has a molecular weight of 273.8 g mol−1. It is readily

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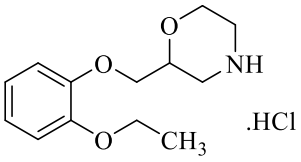
soluble in water and practically insoluble in ethyl acetate. It is a non-hygroscopic, optically active, white powder. The value of its Log P is 1.10. The primary side effects include increased heart rate and blood pressure, insomnia, somnolence, nausea, anorexia, abdominal pain, vomiting, constipation, gastroesophageal reflux disease (GERD), and weight loss with long-term use. Viloxazine Hydrochloride is stable, when exposed to light, Organic impurities might be produced during the storage and manufacturing of Active Pharmaceutical Ingredient.

For active pharmaceutical ingredients (APIs) or pharmaceutical products, adherence to permissible limits is crucial, which is established based on drug research or known safety data. If the concentration of impurities in the drugs exceeds the specified threshold, it could potentially harm patients and, in severe cases, endanger their lives. These impurities play a critical role in ensuring the safety and efficacy of drugs. Regulatory guidelines, such as those provided by The International Council for Harmonization (ICH), mandate the control of these impurities within defined limits.

Literature review indicates numerous methods for estimating Viloxazine hydrochloride assay using high-performance liquid chromatography reverse phase (RP-HPLC) coupled with UV spectrophotometry. However, there was no methods exist for determining Viloxazine hydrochloride-related impurities via RP-HPLC. To our knowledge, four potential impurities of Viloxazine hydrochloride have been identified in the synthesis and no methods available for the quantification of these impurities with their relative response factors

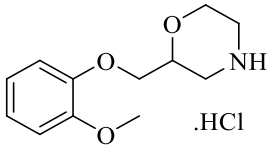
Furthermore, there is no available USP method reference for Viloxazine hydrochloride, rendering the approach described herein novel. The objective of this research is to develop a stability-indicating method capable of accurately quantifying process and degradation impurities of Viloxazine hydrochloride

The developed method underwent validation encompassing essential parameters such as precision, specificity, linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness, and ruggedness, adhering to ICH guidelines to ascertain its compliance with regulatory standards.



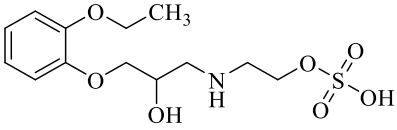
2-((2-Ethoxyphenoxy) methyl) morpholinehydrochloride

Fig 1: Viloxazine Hydrochloride



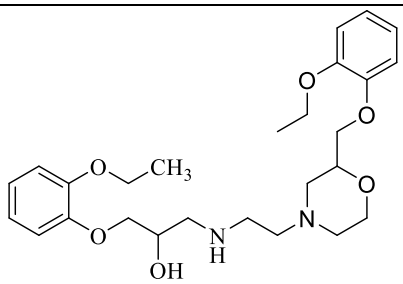
2-((2-methoxyphenoxy) methyl) morpholine

Fig 2: Methyl analog impurity



2-((3-(2-ethoxyphenoxy)-2-hydroxypropyl)amino)ethyl hydrogen sulfate

Fig 3: VXZ-3D Impurity



1-(2-ethoxyphenoxy)-3-((2-(2-((2-ethoxyphenoxy) methyl) morpholine) ethyl) amino) propan-2-ol

Fig 4: Dimer-A Impurity

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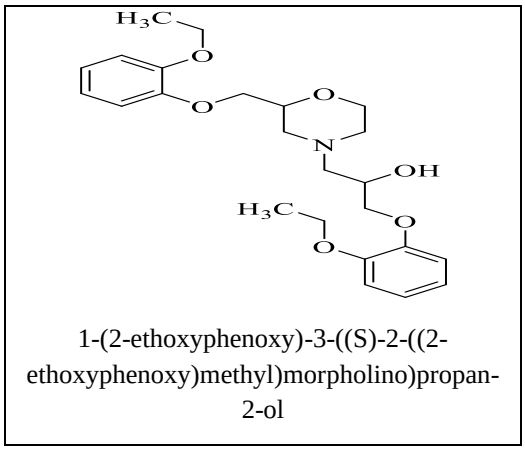

1-(2-ethoxyphenoxy)-3-((S)-2-((2-ethoxyphenoxy)methyl)morpholino)propan-2-ol

Fig 5: VXZ-4D-Dimer Impurity

2. Materials and methods

The Viloxazine Hydrochloride standard along with its impurities including the Methyl analog impurity and Dimer-A Impurity standards were sourced from Granules India Ltd. VXZ-4D-Dimer Impurity and VXZ-3D impurity were obtained from Chemxzen Labs Private Ltd. Potassium dihydrogen phosphate, phosphoric acid, hydrochloric acid, sodium hydroxide, and Hydrogen peroxide were procured from Merck-Sigma-Aldrich Chemicals Private Limited, Bangalore. Additionally, HPLC Grade solvents such as water, methanol, and acetonitrile were acquired from the same source.

2.1 Instrumentation

The development and validation procedures were carried out utilizing the Agilent 1260 Infinity HPLC system from Agilent Technologies, California, USA, featuring a low-pressure quaternary gradient pump, PDA detector, UV detector, column oven, auto-sampler, and Thermo Scientific Chromeleon software version 7.2. The weighing of mobile phase reagents, standards, and impurities was conducted using the Mettler Toledo analytical balance. The pH of the mobile phase was adjusted employing a Metrohm pH meter. Subsequently, the mobile phase underwent filtration through a 0.45 mm filter paper utilizing a glass vacuum-filtration apparatus. Any dissolved gas and entrapped air bubbles within the mobile phase were eliminated via an ultrasonic bath. Stress studies were conducted utilizing the Remi RWB 6 water bath.

2.1.1 Operating conditions

HPLC method development, validation, and analysis of solutions from forced degradation studies were conducted utilizing an Agilent 1260 series HPLC system equipped with photo diode-array detection. Chromatographic separations were accomplished on a 250 mm, 4.6 mm i.d., 5-µm particle size, Agilent Eclipse XDB C8 column. The mobile phase gradient consisted of 20 mM monobasic potassium phosphate adjusted to pH 2.40 with orthophosphoric acid (mobile phase A) and a mixture of acetonitrile and water in the ratio 90:10 %v/v (mobile phase B). The gradient programme (time (min)/percentage B) was as follows: 0/10, 3/10, 25/25, 45/90, 57/90, 58/10, and 65/10, at a constant flow rate of 0.8 mL min−1. The column temperature was held at 50 °C, and detection was performed at 210 nm. The test concentration was approximately 0.8 mg/mL with an injection volume of 10 µL. Data acquisition and Viloxazine peak purity assessment were carried out using Chromeleon software version 7.2.

The percentage of each impurity in the portion of Viloxazine Hydrochloride taken was calculated accordingly.

Calculate the percentage of each impurity in the portion of Viloxazine Hydrochloride taken

% of specified impurity = (rU/rS)×(CS /CU)×P/ RRF

rU = peak area from the sample solution,

rS = peak area from the standard solution,

CS = concentration of Viloxazine hydrochloride in the standard solution (mg mL−1),

CU =concentration of Viloxazine hydrochloride in the sample solution, RRF=relative response factor.

P= Potency of Viloxazine Hydrochloride standard.

2.1.2 Preparation of system suitability

The system suitability solution was carefully prepared, an accurately weighed quantity of Viloxazine Hydrochloride standard was dissolved in 80:20 (%v/v) water: methanol (diluent) to obtain a solution having a known concentration of about 0.8mg/mL.

2.1.3 Preparation of standard solution

The standard solution was prepared in accordance with the Viloxazine Hydrochloride standard. A precise amount of Viloxazine Hydrochloride standard was dissolved in a diluent composed of 80:20 (%v/v) water:

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methanol to achieve a solution with a predetermined concentration of approximately 0.0008 mg/mL. The sample solution was prepared by dissolving an accurately measured quantity of Viloxazine Hydrochloride sample (API) in the same diluent, resulting in a solution with a known concentration of about 0.8 mg/mL.

2.1.4 Preparation of Sample solution

The sample solution was derived from Viloxazine Hydrochloride API. It was prepared by dissolving a precisely measured amount of Viloxazine Hydrochloride sample (API) in a diluent composed of 80:20(%v/v) water: methanol. resulting in a solution with a known concentration of about 0.8 mg/mL.

3. Results and Discussion

3.1 Method development

Viloxazine hydrochloride, a drug with a logP value of approximately 8.47, poses challenges for chromatographic analysis due to its high partition coefficient into the stationary phase. This study addresses the need for a robust HPLC method to separate impurities, including methyl analog, VXZ-3D, VXZ-Dimer-A, and VXZ-4D-Dimer impurities, crucial for process and degradation monitoring. Method development involved pH optimization, flow rate variation, and column oven temperature adjustment to enhance resolution and sensitivity. Experiments were conducted using an HPLC system equipped with an octyl silane (C8) stationary phase and a variable wavelength detector (VWD). The mobile phase comprised potassium dihydrogen phosphate buffer with varying pH levels, mixed with acetonitrile in gradient mode using an Agilent column. Parameters such as flow rate (0.6-1.0 mL/min) and column oven temperature (45-55°C) were varied to optimize resolution and sensitivity.

Initial experiments at pH 7.0 revealed merging of VXZ-3D impurity with the Viloxazine peak, prompting pH adjustment to 2.6 for successful separation. Subsequent investigations identified pH 2.35 as optimal, ensuring well-resolved critical pairs: VXZ-3D impurity/Viloxazine peak and Dimer-A impurity/4D-Dimer impurity. The impact of flow rate and column oven temperature on resolution was also assessed, with marginal improvements observed with increased flow rates and temperatures.

The developed HPLC method demonstrated effective separation of impurities in Viloxazine hydrochloride, offering specificity and reliability for pharmaceutical analysis. Optimization of pH, flow rate, and column oven temperature enhanced resolution and sensitivity, ensuring accurate quantification of impurities. The method's robustness was confirmed through specificity testing and degradation studies, validating its suitability for routine pharmaceutical analysis.

These four impurities are the potential (Specifed) impurities which are controlled by acceptance criteria not more than 0.15%, controlled under any other individual impurities not more than 0.1%. Hence, the potential impurities were taken for the present validation study.

Finally, the spike solution containing all the impurities spiked at known concentrations was injected to check the specificity of the method as shown in Fig 6. From this, the retention time, relative retention time for Viloxazine hydrochloride peak and the known impurities were obtained. The minimum linearity studies provided the relative response factor values for the impurities obtained which are tabulated as shown in Table-1. The Viloxazine hydrochloride -related impurities labeled in the chromatograms are as follows: Methyl analog impurity, VXZ-3D Impurity, VXZ-Dimer-A Impurity and VXZ-4D-Dimer impurity.

Table-1: relative response factor

Impurity name	Relative retention time	Relative response factor
Methyl analog	~0.75	0.91
VXZ-3D	~0.96	0.74
VXZ-Dimer-A	~1.63	1.12
VXZ-4D-Dimer	~1.66	1.16

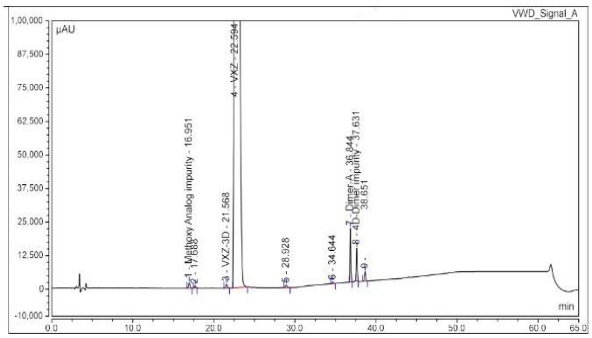

Fig 6: Spiked solution

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3.2 Validation of analytical method

3.2.1 System suitability

Standard solution was injected in six replicates and the average of the injections was used for calculation. The first injection is considered for the system suitability of the standard solution with parameters signal-to-noise ratio and plate count from system suitability solution with 0.8 mg/mL of Viloxazine hydrochloride peak, Such as USP plate count, % RSD for six injections of standard solution and signal-to-noise ratio for standard solution. The values are tabulated in table-2.

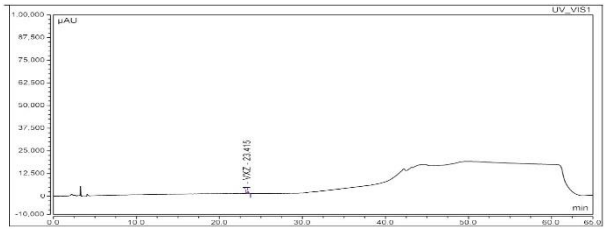

Fig 7: Chromatogram of Viloxazine hydrochloride 0.0008 mg/mL

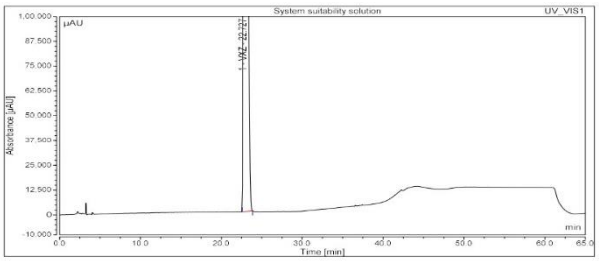

Fig 8: Chromatogram of Viloxazine hydrochloride 0.8 mg/mL

Table-2: Results of System suitability

System suitability	Acceptance criteria	Viloxazine
USP Plate count	NLT 5000	13365
S/N Ratio	NLT 30	39.74
%RSD	NMT 10	1.35

3.2.2 Precision

The precision of an analytical method is the degree of agreement amongst individual test results when the method is applied repeatedly to multiple sampling of a homogeneous sample. The precision of the analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of series of measurements. The system precision, method precision (Repeatability) and intermediate precision (Reproducibility or Ruggedness) of the proposed methods were determined by several measurements of known Impurities Methyl analog, VXZ-3D, VXZ-Dimer-A and VXZ-4D-Dimer impurities, respectively. System precision was established by performing six different measurements of the known standard Viloxazine hydrochloride (0.0008mg/mL). Method precision was determined by six individual preparations of Viloxazine hydrochloride spiked with impurities Methyl analog, VXZ-3D, VXZ-Dimer-A and VXZ-4D-Dimer impurities, respectively. Intermediate precision was performed with different instruments and different columns by different analysts on different days. The percentage RSD of each impurity and total impurities were calculated and recorded in the Table-3 and Table-4.

Table-3: Results of method precision (MP)

Name	Methyl analog	VXZ-3D	VXZ-Dimer-A	VXZ-4D-Dimer	Total Imp's
MP-1	0.156	0.151	0.743	0.426	1.80
MP-2	0.157	0.151	0.746	0.431	1.82
MP-3	0.155	0.151	0.746	0.428	1.81
MP-4	0.156	0.151	0.745	0.426	1.81
MP-5	0.156	0.154	0.746	0.429	1.82
MP-6	0.156	0.152	0.746	0.430	1.81
Average	0.16	0.15	0.75	0.43	1.81
%RSD	0.4	0.9	0.2	0.5	0.3

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Table4: Results of Intermediate precision (IP)

Name	Methyl analog	VXZ-3D	VXZ-Dimer-A	VXZ-4D-Dimer	Total Imp's
IP-1	0.157	0.148	0.736	0.449	1.79
IP-2	0.158	0.147	0.736	0.449	1.79
IP-3	0.157	0.151	0.732	0.448	1.79
IP-4	0.155	0.150	0.728	0.448	1.78
IP-5	0.156	0.151	0.714	0.450	1.76
IP-6	0.157	0.153	0.733	0.451	1.79
Average	0.16	0.15	0.73	0.45	1.78
%RSD	0.5	1.4	1.2	0.2	0.6

3.2.3 Linearity

Linearity is the ability to obtain the test results that are directly proportional to the analyte concentration. Linearity was determined by six different concentrations with respect to test (0.03, 0.15, 0.3, 0.5, 0.625 and 0.75% with respect to test concentration 0.8mg/mL of Viloxazine hydrochloride) of Viloxazine hydrochloride and its known impurities (LOQ level to 500% of specification limit of 0.15%). Linearity performed LOQ to 500% for it covers before stage impurities of Viloxazine hydrochloride, which are present in the sample that is lesser than the reporting threshold. As per the European pharmacopeia general chapter, specified impurities were taken for the linearity study and the values of their peak area versus concentration (concentration with respect to test in %) were plotted. To calculate the coefficient of correlation, slope, and intercept using linearity curve extrapolation. The values were tabulated in Table-5 and Table-6.

Table-5: Linearity of Viloxazine hydrochloride specified impurities

Name	Methyl analog		VXZ-3D		VXZ-Dimer-A		VXZ-4D-Dimer	
	Conc. (%)	Area	Conc. (%)	Area	Conc. (%)	Area	Conc. (%)	Area
L-1	0.023	3824	0.032	4226	0.012	2348	0.011	2962
L-2	0.144	21749	0.148	17644	0.151	27315	0.149	27477
L-3	0.288	47349	0.295	38111	0.302	59163	0.298	59810
L-4	0.481	76795	0.492	61837	0.504	96145	0.496	97079
L-5	0.601	96202	0.615	77611	0.630	120185	0.620	121815
L-6	0.721	114279	0.738	92421	0.755	142430	0.744	144680
Correlation coefficient		0.9998		0.9998		0.9998		0.9998
y-Intercept		35.29		42.29		108.77		268.29
Slope		159453.5		125680.3		189854.5		195123.9

Table-6: Linearity of Viloxazine hydrochloride

Name	Viloxazine Hydrochloride	
	Conc.(%)	Area
L-1	0.024	4322
L-2	0.151	25298
L-3	0.301	55253

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L-4	0.502	89785
L-5	0.628	112036
L-6	0.754	133296
	Correlation coefficient	0.9998
	y-Intercept	-30.47
	Slope	177998.9

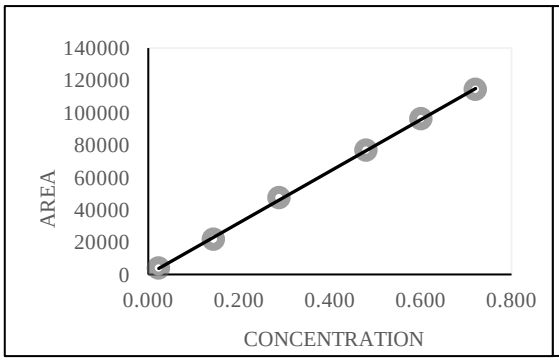

Fig 9: Methyl analog impurity

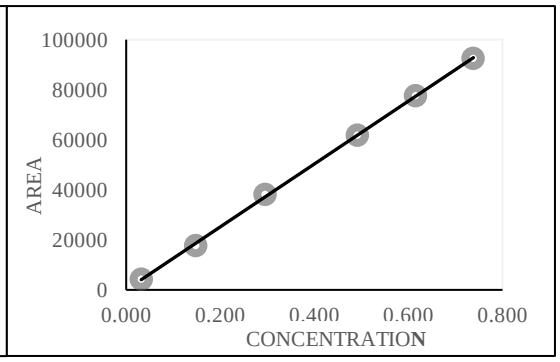

Fig 10: VXZ-3D Impurity

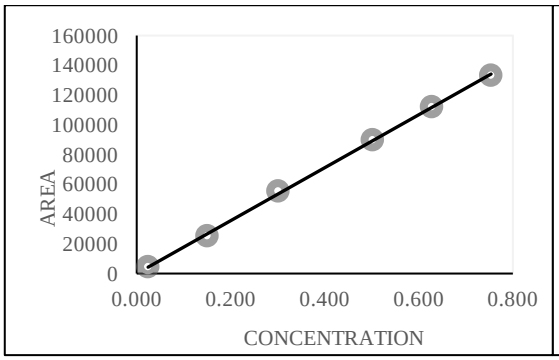

Fig 11: Viloxazine hydrochloride

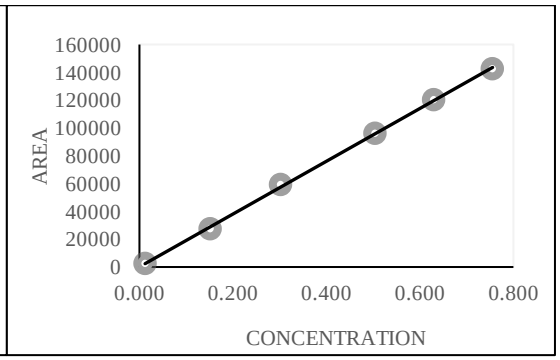

Fig 12: VXZ-Dimer-A Impurity

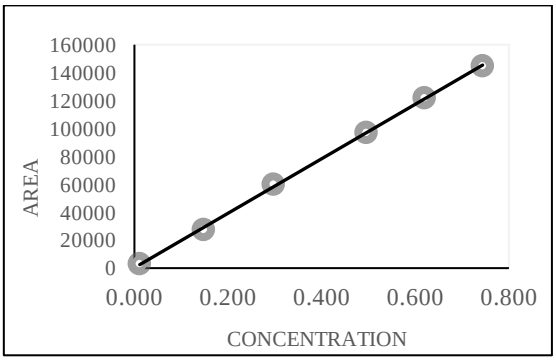

Fig 13: VXZ-4D-Dimer Impurity

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3.2.4 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) of an analytical method is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. The limit of quantification (LOQ) of an analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. The limit of detection (LOD) and the limit of quantification (LOQ) of the known Impurities Methyl analog, VXZ-3D, VXZ-Dimer-A and VXZ-4D-Dimer impurities and Viloxazine hydrochloride were calculated using the signal-to-noise ratio method as per ICH. A typical signal-to-noise ratio is 10:1 for Limit of Quantification (LOQ) and a signal-to-noise ratio between 3 and 2:1 is generally considered acceptable for estimating the detection limit (LOD). Further, the % RSD of six preparations at LOQ was calculated. The values were tabulated in Table-7.

Table-7: Results of LOQ and LOD for known impurities and Viloxazine Hydrochloride

Name of Impurity/Analyte	%Impurity/Analyte		Signal-to-noise ratio		Precision at LOQ %RSD
	LOD	LOQ	LOD	LOQ	
Methyl analog	0.008	0.023	3.057	9.176	3.1
VXZ-3D	0.011	0.032	3.105	9.658	2.1
VXZ-Dimer-A	0.004	0.012	3.344	10.041	1.5
VXZ-4D-Dimer	0.004	0.011	2.858	10.065	2.5
Viloxazine Hydrochloride	0.008	0.024	3.124	10.177	2.2

3.2.5 Accuracy

The accuracy of an analytical method is the nearness between the expected value and the value found. It is obtained by calculating the percent recovery (% R) of the analyte recovered. A study of recovery was performed on LOQ (0.03%), 50% (0.075%), 100% (0.15%) and 150% (0.225%) of the target concentration of each impurity by spiking with 0.8 mg mL−1 of Viloxazine hydrochloride. Spiked samples were extracted and analyzed. Then the % recovery and its relative standard deviation were calculated. The values were tabulated in Table-8.

Table-8: Accuracy of known impurities

Level	Parameter	Methyl analog	VXZ-3D	VXZ-Dimer-A	VXZ-4D-Dimer
LOQ	%Recovery	99.8	99.0	108.6	99.3
50%	%Recovery	110.4	104.6	104.2	102.3
100%	%Recovery	109.3	103.8	103.5	103.3
150%	%Recovery	107.1	104.2	102.4	102.6

Table-9: %RSD of Precision

Level	Parameter	Methyl analog	VXZ-3D	VXZ-Dimer-A	VXZ-4D-Dimer
LOQ	%RSD	3.1	2.1	1.5	2.5
100%	%RSD	0.4	0.9	0.2	0.5
150%	%RSD	0.6	1.1	0.3	0.3

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3.2.6 Robustness

The robustness of the developed method was investigated. The experimental conditions were deliberately changing the pH value ±0.2 units, flow rate±0.1 min mL−1 and column temperature±5 °C. The values are tabulated in Table-10.

3.2.7 Stability of solution

The stability of Viloxazine hydrochloride and its known Impurities solution is determined.

The aforementioned findings indicate that the test sample remains stable for up to 43 hours, and the stability of spiked sample solution persists for 42 hours when stored at room temperature and 2-8°C.

3.2.8 Specificity

The specificity studies were performed on Viloxazine hydrochloride to provide a stability-indicating property and also the specificity of the developed method. The degradation was performed on the Viloxazine hydrochloride intentionally by various conditions which are mentioned in the experiment part to evaluate that the proposed chromatographic method is suitable to separate the main moiety from its impurities. Also, the peak purity checked through the photodiode array detector gets passed, and the determined purity Limit more than the 950 which is refers the purity angle was lower than the calculated purity threshold which is a part of the development activity.

Table-10: Robustness data of known impurities

Name of Impurity	Observed RRT With flow rate		
	0.6mL/min	0.8mL/min	1.0mL/min
Methyl analog imp	0.80	0.77	0.75
VXZ-3D imp	0.97	0.96	0.95
VXZ-Dimer-A imp	1.37	1.51	1.63
VXZ-4D-Dimer imp	1.39	1.53	1.65
Name of Impurity	Observed RRT With column oven temperature		
	45°C	50°C	55°C
Methyl analog imp	0.78	0.77	0.76
VXZ-3D imp	0.97	0.96	0.95
VXZ-Dimer-A imp	1.46	1.51	1.55
VXZ-4D-Dimer imp	1.49	1.53	1.57
Name of Impurity	Observed RRT With Buffer pH		
	pH-2.2	pH-2.4	pH-2.6
Methyl analog imp	0.76	0.77	0.76
VXZ-3D imp	0.96	0.96	0.96
VXZ-Dimer-A imp	1.51	1.51	1.56
VXZ-4D-Dimer imp	1.55	1.53	1.58

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Table-11: Solution stability of test and spiked solution

Name of the Impurities	Initial % of Impurity		After 42 hours (%)		Difference from Initial (%)	
	Test	Spiked test	Test	Spiked test	Test	Spiked test
Methyl analog	ND	0.155	ND	0.157	0	1.2
VXZ-3D imp	ND	0.153	ND	0.154	0	0.6
VXZ-Dimer-A	0.231	0.388	0.230	0.388	0.3	0
VXZ-4D-Dimer	0.133	0.287	0.136	0.291	2.4	1.4
Total Impurities	0.56	1.33	0.56	1.34	0	0.4

4. Stress Studies of Viloxazine hydrochloride

Stability studies were conducted to demonstrate how the quality of the drug changes when exposed to various environmental factors such as hydrolysis, oxidation, temperature, and others. Dry heat and photolytic degradation were performed for Viloxazine Hydrochloride API.

4.1 Acid hydrolysis

For stress degradation study, the solutions were prepared using API of Viloxazine Hydrochloride. Accurately weighed 500 mg of Viloxazine Hydrochloride was transferred into a 50 mL volumetric flask and added 30 mL of 1N HCl solution and made upto the mark with water, this solution was kept in water bath at 70°C for 7 days. After 7days diluted 4 mL this solution to 50 mL of diluent composed of 80:20 (%v/v) water: methanol.

4.2 Alkaline hydrolysis

500 mg of Viloxazine Hydrochloride was transferred into a 50 mL volumetric flask and added 30 mL of 1N NaOH solution and made upto the mark with water, this solution was kept in water bath at 70°C for 7 days. After 7days diluted 4 mL this solution to 50 mL of diluent composed of 80:20 (%v/v) water: methanol.

4.3 Oxidative degradation

500 mg of Viloxazine Hydrochloride was transferred into a 50 mL volumetric flask and added 30 mL of 3%H2O2 solution and made upto the mark with same solution, this solution was kept in a dark place at room temperature for 7 days. After 7days diluted 4 mL this solution to 50 mL of diluent composed of 80:20 (%v/v) water: methanol.

4.4 Thermal degradation

40 mg of Viloxazine Hydrochloride API was kept in an oven at 105°C for 7 days and then transferred into a 50 mL volumetric flask and made upto the mark with diluent composed of 80:20 (%v/v) water: methanol.

4.5 Photolytic degradation

40 mg of Viloxazine Hydrochloride API was exposed to 200 Watt-hours/square meter and 1.2 Million lux hours and then transferred into a 50 mL volumetric flask and made upto the mark with diluent composed of 80:20 (%v/v) water: methanol.

Table-12: Percentage of degradation for each stress condition

Stress conditions	% of impurities					
	Methyl analog	VXZ-3D	VXZ-Dimer-A	VXZ-4D-Dimer	Any unspecified imp's	Total Imp's
Acid	0.10	ND	ND	ND	0.07	0.26
Base	0.10	ND	ND	ND	0.17	0.51
Peroxide	0.09	ND	ND	ND	0.18	0.39

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Thermal	0.10	ND	ND	ND	ND	0.10
Photolytic	0.10	ND	ND	ND	ND	0.10
As such	0.10	ND	ND	ND	ND	0.10

BDL=Below detection limit, ND=Not detected, imp's=Impurities

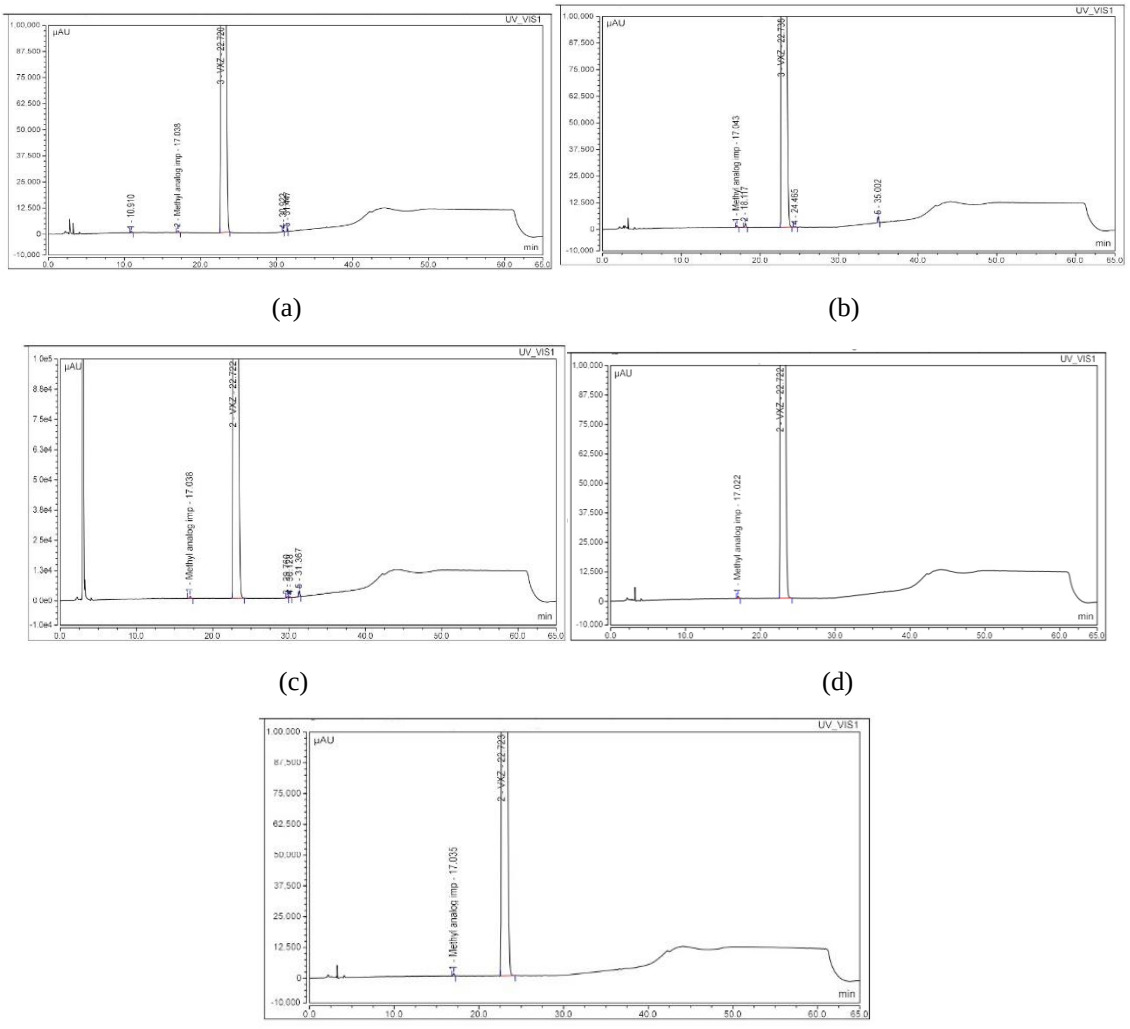

Fig 14: Chromatogrames of Viloxazine Hydrochloride after (a) Acid degradation, (b) Alkaline degradation, (c) Oxidative degradation, (d) Thermal degradation and (e) photolytic degradation.

5. Conclusion

The developed method demonstrates accuracy, precision, and reliability in analyzing Viloxazine Hydrochloride Active Pharmaceutical Ingredient (API) form. Validation encompassed linearity, accuracy, precision, robustness, forced degradation, and stability of Viloxazine Hydrochloride. The % RSD, Accuracy values confirming the method's validity, and the results obtained

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exhibit fair agreement.

Consequently, this method stands as a proficient means for analyzing Viloxazine Hydrochloride and its related substance. Hence it can be used for routine analysis in plant scale level.

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