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Research Article

# Selective Separation and Mass Spectral Characterization of Degradants in Viloxazine by LC-MS/MS

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ABSTRACT

This research describes a novel technique for the selective separation of degradants from API employing HPLC and online coupling of a triple quadrupole mass analyzer and PDA detector with a SCIEX QTRAP 5500 mass spectrometer. Chromatography was used to separate all degradants on the column Agilent eclipse XDB (150 mm x 4.6 mm, 3.5 µ) with mobile phase

ACN: 0.1% TEA (40:60) %v/v. The highest absorption was found to occur at 220 nm, which allows for simultaneous detection without being impacted by the placebo matrix. According to the general ICH recommendations, the suggested RP-HPLC method was accepted. All of the metrics- specificity, linearity, LoD, LoQ, accuracy, precision and robustness of validation were deemed sufficient. The proposed method exhibits strong correlation and great linearity over the range of (12.5–75 µg/mL). The accuracy trials produced consistent recoveries (95–105%), while the precision experiments' percent RSD was less than 2%. The intrinsic stability of the drug molecules in the current formulation could be ascertained by conducting forced degradation studies to assess the degradation products produced under various stress settings. The degradants produced were well separated and further characterized by MS/MS studies. The newly devised approach was demonstrated to be stable and sensitive to all degradants during validation tests. Validation trials demonstrate that the newly developed method was also accurate, precise, resilient, selective, and linear within the necessary operating range.

## Introduction

Viloxazine is an antidepressant that is chemically 2-[(2-ethoxyphenoxy) methyl] morpholine.[1] The structure was depicted in Fig. 1. It is used to treat attention deficit hyperactivity disorder (ADHD). ADHD is a common neurodevelopmental disease characterized by inattention and hyperactivity in children. This etiology involves an imbalance of neurotransmitters, particularly dopamine (DA) and norepinephrine (NE). The drug is assumed to function via altering the monoaminergic neurotransmitter systems. It is a selective and mild norepinephrine reuptake inhibitor that inhibits norepinephrine reuptake by binding to the norepinephrine transporter. Hence, it elevates extracellular norepinephrine levels in multiple brain areas.[2,3] The FDA approved an extended-release version of viloxazine under the brand name QELBREE for the treatment of ADHD in April 2021.[4] It was prescribed as an antidepressant for the treatment of major depressive disorder. It was thought to be helpful in mild to moderate depression and severe depression with or without co-morbid symptoms.[5]

## Material and Methods

### Equipment

The HPLC system Waters, alliance e 2695 HPLC equipped with a high-speed autosampler, column oven, degasser and connected to the SCIEX QTRAP 5500 mass spectrometer equipped with triple quadrupole mass analyzer was used during the analysis of viloxazine. The data collection was

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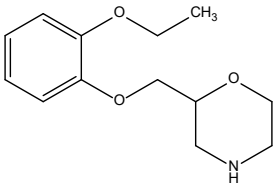

**Fig. 1:** Chemical structure of viloxazine[1]

managed using the class empower- 2 Programme. All the structures and IUPAC names were derived with the help of ChemDraw 20.1.1. Ink Software.

### Preparation of Mobile Phase

The buffer preparation of 0.1% TEA was achieved by dissolving 1-mL Triethylamine (*TEA*) in 1000 mL of water. Later ACN and 0.1% TEA were mixed in the ratio of 40:60 %v/v. It was passed through a 0.45 µ membrane filter before use. The same mobile phase was employed as diluent.

### Preparation of Standard Solution

An accurately weighed quantity of about 50 mg of viloxazine was taken and diluted to the required volume of 100 mL with diluent. The above solution of 5 mL was further diluted to 50 mL which was of concentration 50 µg/mL.

### Preparation of Sample Solution

Ten tablets were accurately weighed. They were powdered by mortar and pestle and an equilibrium amount of powder to one tablet was taken and transferred into a 100 mL volumetric flask and 70 mL of diluents were added. Sonicated for dissolving and diluted with diluent. From the above stock solution, 5 mL was taken, diluted to 20 mL, and filtered through 0.45 µ nylon syringe filter.

### Forced Degradation Studies

Acid, base, oxidation, reduction, thermal, and hydrolysis degradation experiments were carried out in accordance with ICH guidelines. The acid degradation was performed by adding 1-mL of 1N HCl for 15 minutes, alkali degradation by subjecting the solution to 0.1 N NaOH for 15 minutes and later neutralizing the solutions. The peroxide treatment was with aid of 0.3 mL 30% hydrogen peroxide. Thermal degradation by subjecting stock solution to an oven temperature at 105°C for 6 hours. Reduction degradation was conducted by subjecting to 1-mL of 30% sodium bisulfate solution. The solution was exposed to UV radiation for 6 hours to check for photodegradation.

All these samples were injected at the optimized chromatographic and mass parameter conditions which were confirmed after many trails with various mobile phase compositions and different conditions. The optimized chromatographic conditions of LC and mass spectrometry are given in Table 1.

**Table 1:** Optimized LC and Mass parameters

| *S.NO* | *Chromatographic conditions* | | *Mass parameters* | |
|---|---|---|---|---|
| | *Parameter* | *Optimized condition* | *Parameter* | *Optimized condition* |
| 1. | Mobile phase | ACN: 0.1% TEA (40:60 %v/v) | Collision energy (CE) | 15 V |
| 2. | Column | Agilent eclipse XDB 150 mm x 4.6 mm,3.5µ | Ion spray voltage | 5500 V |
| 3. | Flow rate | 1 mL/min | Source temperature | 550 °C |
| 4. | Column temperature | 25°C | Drying gas temperature | 120-250° C |
| 5. | Sample temperature | 25°C | Nebulizing gas | Nitrogen |
| 6. | Wavelength | 220 nm | Drying gas flow stream | 5 L/min |
| 7. | Volume of injection | 10 µL | Declustering potential | 40 V |
| 8. | Period of run | 6 minutes | Entrance potential | 10V |
| 9. | Retention time | 2.821 minutes | Exit potential | 7 V |

## Results and Discussion

### Validation of Method

The analytical method had been validated for parameters such as system suitability, accuracy, specificity, precision, linearity, robustness, LoD and LoQ, forced degradation, and stability in compliance with ICH $Q_2$ ($R_1$) guidelines.[6]

### System Suitability

System suitability parameters were performed to evaluate the system performance. The system suitability was achieved by injecting a standard solution comprising 50 µg/mL of viloxazine in six repeats. The results obtained for the drug indicated that the system suitability parameter was within the limit.

### Specificity

It was checked by examining the blank sample chromatograms and sample spiked with a viloxazine. The analytical method's specificity was tested by injecting 100 µg/mL concentration solutions of diluent (blank), placebo, working standard, and sample solution into each other to look for interference from representative peaks. According to the overlay chromatogram in Fig. 2, there were no co-eluting peaks during the retention time of viloxazine, indicating that the analyte peak was pure and the excipients in the formulation did not interfere with the analyte of interest.

### Linearity

Linearity was determined by drawing the peak response's calibration curve at the respective concentration. Injected

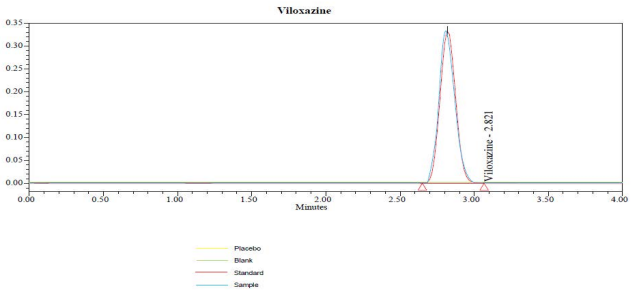


**Fig. 2:** An overlay chromatogram of blank, placebo, standard, sample

each level of concentration solution and measured the peak area using the chromatography process. Graph plot of peak area versus concentration (X and Y-axis peak areas) and estimated the correlation coefficient. The calibration curve showed that the curve was straight in the 12.5 to 75 µg/mL range. The regression equations for the calibration curves were Y=48681x + 6239.1 ($R^2$-0.9992) for viloxazine and the overlay of linear concentration were depicted in an overlay in Fig. 3.

The method was validated for other parameters such as robustness by altering the conditions of mobile phase, flow rate etc. and found to be robust. The intraday and inter-day precision were conducted and the results exhibited proved the method to be precise. The mean %recovery of the drug proved the method to be accurate. The %mean and %assay found to be within limits. All the results obtained in the above parametric experiments were tabulated in Table 2.

## Forced Degradation Studies

The developed HPLC method implemented here was investigated by chromatographic resolution of a blend of stress reaction solutions, particularly those in which significant degradation was observed. It indicated that the method was able to separate the drug and all the major degradation products. The drug was prone to degradation and identifiable degradants were produced and recognized at respective retention time under acid (DP I - 1.671), alkali (DP II - 2.183), peroxide (DP III - 3.794) and reduction degradation (DP IV - 4.028) which were depicted in an overlay chromatogram in given Fig. 4. The %mass balance and %degradation of studies were tabulated in Table 3.

## LC-MS/TOF studies on stressed samples

The drug and mixture of stressed samples were subjected to LC-MS/TOF studies using the optimized mass

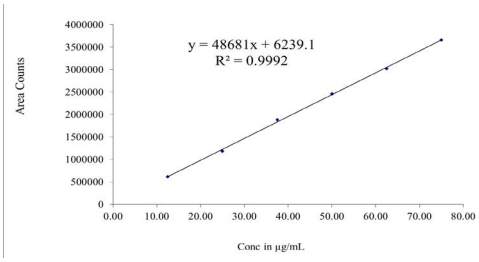


**Fig. 3:** A calibration plot of Viloxazine

**Table 2:** Summary of validation parameters

| *Parameter of validation* | *Results* |
|---|---|
| Theoretical plate count | 3251 |
| Tailing factor | 1.07 |
| Linearity (µg/mL) | 12.50 – 75.00 |
| Regression equation | Y = 48681x + 6239.1 |
| Regression coefficient ($R^2$) | 0.9992 |
| LOD (µg/mL) | 0.5 |
| LOQ (µg/mL) | 1.5 |
| Accuracy (Mean % Recovery) | 100.26 |
| Precision (Intra - day) %RSD | 1.410 |
| %Assay (Mean) | 100.2 |

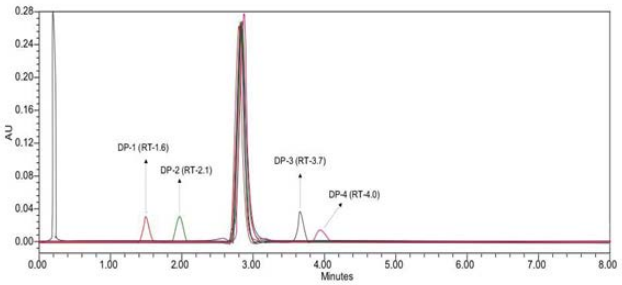


**Fig. 4:** An overlay chromatogram of degradation products of viloxazine

spectrometric conditions. The mass spectra of the drug (Fig. 5) and all the degradation products are shown below (Figs. 6-9). The experimental masses, possible molecular formulae and major fragments of degradation products were enlisted in Table 4. The complete fragmentation pattern was depicted in Fig. 10 below.

## Scheme of DP-I ([M+H]$^+$, m/z 228.07)

Shows the fragmentation mechanism of degradation product separated at 1.671 RT of *m/z*-228.07 which was observed under acid degradation conditions. The spectrum demonstrates abundant [M+H]$^+$ product ions at *m/z*- 143.52 and *m/z* – 113.26. The MS/MS experiments combined with accurate mass measurements obtained from spectra in Fig. 6. have confirmed the proposed scheme in Fig. 6a.

## Scheme of DP-II ([M+H]$^+$, m/z 210.51)

Shows the fragmentation mechanism of degradation product separated at 2.183 RT of *m/z*-210.51 which

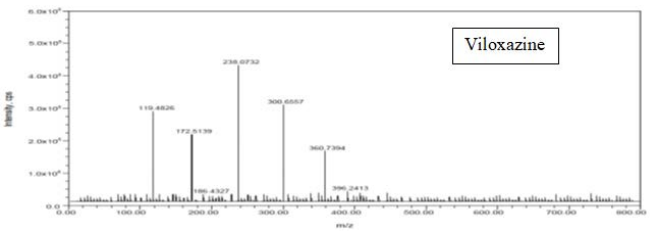


**Fig. 5:** Line spectrum of viloxazine obtained in MS/TOF study

**Table 3:** Forced degradation results

| *Degradation condition* | *Peak Area* | *%Assay* | *%Degradation* | *%Mass balance* | *Purity Angle* | *Purity Threshold* | *Pass/Fail* |
|---|---|---|---|---|---|---|---|
| Acid degradation | 1854763 | 75.7 | 24.3 | 99.8 | 1.269 | 10.767 | Pass |
| Alkali degradation | 1887954 | 76.1 | 23 | 98.9 | 1.214 | 10.722 | Pass |
| Peroxide degradation | 1852013 | 73.4 | 24.4 | 97.6 | 1.276 | 10.747 | Pass |
| Reduction degradation | 2311023 | 93.4 | 5.7 | 98.9 | 1.274 | 10.739 | Pass |
| Thermal degradation | 2315264 | 93.7 | 5.5 | 99.0 | 1.216 | 10.732 | Pass |
| Hydrolysis degradation | 2340125 | 94.2 | 4.5 | 98.5 | 1.269 | 10.741 | Pass |
| Photolytic degradation | 2133157 | 86.3 | 12.9 | 99.0 | 1.234 | 10.708 | Pass |

**Table 4:** LC-MS/TOF data of DPs (I-IV) along with their molecular formulae and major fragments

| *DPs* | *Experimentalmass* | *Best possiblemolecular formula* | *Theoretical mass* | *Major fragments* | |
|---|---|---|---|---|---|
| | | | | *Experimentalmass* | *Theoretical mass (chemical formula)* |
| I | 228.0715 | $C_{11}H_{15}ClNO_2^+$ | 228.0786 | 143. 5202 113. 2654 | 143.0258 ($C_7H_7ClO^+$)<br>113.0153 ($C_6H_6Cl^+$) |
| II | 210.5139 | $C_{11}H_{16}NO_3^+$ | 210.1125 | 125. 3647<br>95. 428 | 125.0597 ($C_7H_9O_2^+$)<br>95.0491 ($C_6H_7O^+$) |
| III | 226.4913 | $C_{11}H_{16}NO_4^+$ | 226.1074 | 124.5319<br>95.4428 | 125.0597 ($C_7H_9O_2^+$)<br>95.0491 ($C_6H_7O^+$) |
| IV | 272.4708 | $C_{13}H_{19}ClNO_3^+$ | 272.1048 | 187. 4931<br>109. 6248<br>79. 1862 | 187.0520 ($C_9H_{12}ClO_2^+$)<br>109.0648 ($C_7H_9O^+$)<br>79.03 ($C_6H_7^+$) |

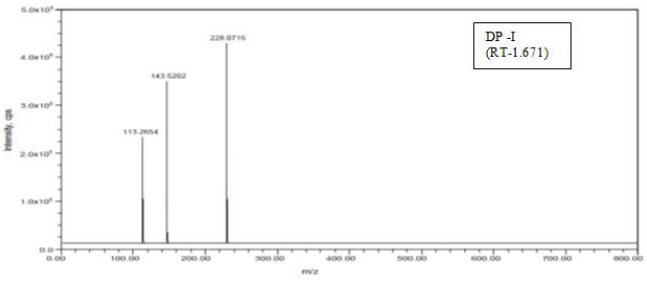


**Fig. 6:** Mass spectra of DP-I (1.671 RT)

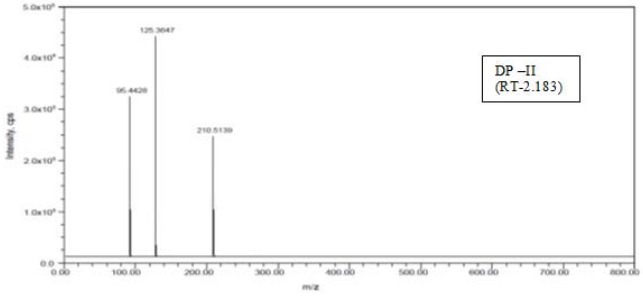


**Fig. 7:** Mass spectra of DP-II (2.183 RT)

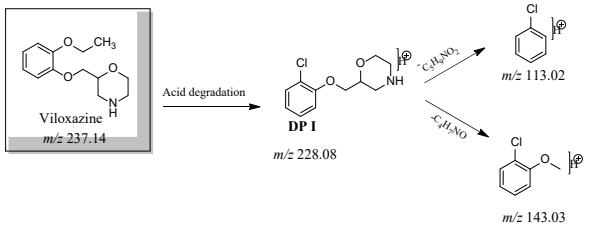


**Fig. 6a:** DP-1 fragmentation pathway

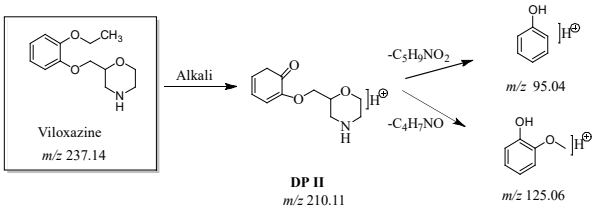


**Fig. 7a:** DP-II fragmentation pathway

was observed under alkali degradation conditions. The spectrum displays abundant $[M+H]^+$ product ions at *m/z*-125.36 and *m/z*-95.42 The MS/MS experiments combined with accurate mass measurements obtained from spectra in Fig. 7. have established the anticipated scheme in Fig. 7a.

## Scheme of DP-III ($[M+H]^+$, m/z 226.49)

Shows the fragmentation mechanism of degradation product separated at 3.794 RT of *m/z*-226.49 which was observed under peroxide degradation condition. The spectrum displays abundant $[M+H]^+$ product ions at *m/z*-124.53 and m/z-95.76. The MS/MS experiments combined with accurate mass measurements obtained from spectra in Fig. 8. have confirmed the proposed scheme in Fig. 8a.

## Scheme of DP-IV ($[M+H]^+$, m/z 272.47)

**S**hows the fragmentation mechanism of degradation product separated at 4.028 RT of *m/z*-272.47, which was observed under reduction degradation condition. The

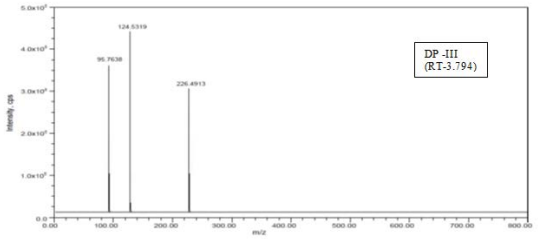


**Fig. 8:** Mass spectra of DP-III (3.794 RT)

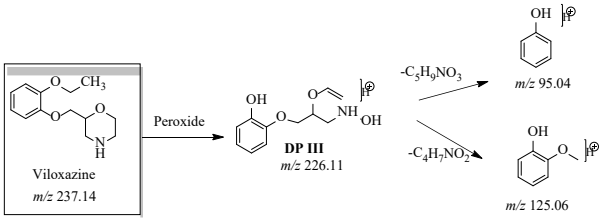


**Fig. 8a:** DP-III fragmentation pathway

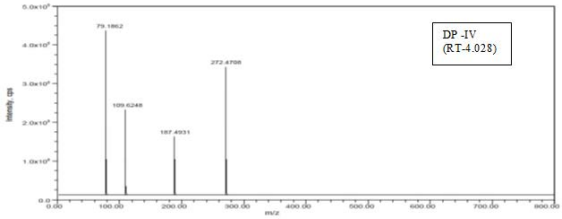


**Fig. 9:** Mass spectra of DP-IV (4.028 RT)

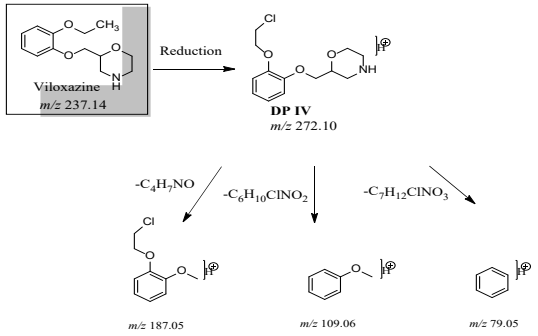


**Fig. 9a:** DP IV fragmentation pathway

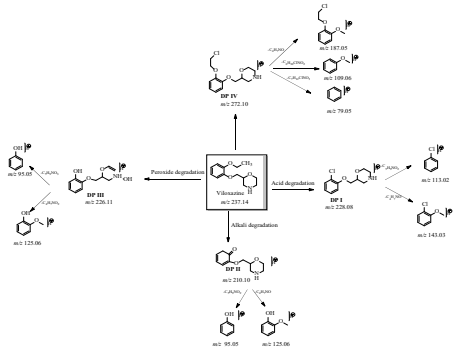

**Fig. 10:** A total representation of degradation pathway of viloxazine

spectrum displays abundant $[M+H]^+$ product ions at *m/z*-187.49 m/z-109.62 and *m/z*-79.18. The MS/MS experiments combined with accurate mass measurements obtained from spectra in Fig. 9. have confirmed the proposed scheme in Fig. 9a.

## Conclusion

The outcomes attained satisfied the proposed goal and objectives. Through this inquiry, the degradants from the primary medication were successfully separated. Additionally, using LC-MS/MS techniques, a systematic study was done to locate the degradation products (DP-I to IV) in the stability evaluation. Based on the m/z readings, the degradation products (DPs) were proposed, which was useful to assess further characterization. When compared to other stressful conditions, the medication was more susceptible to acid, alkali, peroxide, and reduction degradation with a high percentage of degradation.

The capacity to evaluate identification and quantification simultaneously in a single run, which increases analytical confidence, keeps track of co-elution, simplifies identification, and avoids repeating analysis, were just a few of the advantages this approach had over more conventional ones. The suggested method was thus suitable for quantifying and monitoring the degradants during production and stability investigations.

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## Conflicts of Interest

The authors declare no conflicts of interest.

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