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LC-MS/MS-Based Selective Degradant Separation and Mass Spectral Characterization of Viloxazine

Shivaprasad [1],Adithyamathur [2],Dr.Ratnasree [3],Nabab Ali Molla [4,]
Assistant professor [1,2,3,4],
Department of Pharmacy,
Samskruti College of Pharmacy,
Kondapur (V), Ghatkesar (M) Medchal Dist, Telangana, India.

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ABSTRACT
This study presents a unique method for the selective extraction of degradants from API using HPLC and online connection of a SCIEX QTRAP 5500 mass spectrometer with a triple quadrupole mass analyzer and PDA detector. Chromatography was employed using mobile phase ACN: 0.1% TEA (40:60) %v/v to separate all degradants on the Agilent Eclipse XDB (150 mm x 4.6 mm, 3.5 µ) column. It was discovered that the maximum absorption occurs at 220 nm, allowing for simultaneous detection unaffected by the placebo matrix. It was decided to approve the recommended RP-HPLC technique in accordance with the general ICH guidelines. The parameters that were considered adequate were specificity, linearity, LoD, LoQ, accuracy, precision, and robustness of validation. The suggested approach demonstrates excellent linearity and robust correlation over the range of 12.5–75 µg/mL. The precision tests' percent RSD was less than 2%, whilst the accuracy trials yielded consistent recoveries (95–105%). It may be possible to determine the inherent stability of the drug molecules in the present formulation by doing forced degradation tests and evaluating the degradation products generated under different stress conditions. By using MS/MS analyses, the generated degradants were further described and effectively isolated. Validation studies showed that the recently developed method is stable and sensitive to all degradants. Validation studies show that within the required operating range, the newly created approach was also linear, accurate, precise, robust, and selective.

Introduction

Chemically speaking, viloxazine is 2-[(2-ethoxyphenoxy) methyl] morpholine, an antidepressant.[1] In Fig. 1, the construction was shown. ADHD, or attention deficit hyperactivity disorder, is treated with it. ADHD is a common neurodevelopmental disorder in children that is characterized by hyperactivity and inattention. An imbalance of neurotransmitters, namely dopamine (DA) and norepinephrine (NE), is the cause of this pathogenesis. It is thought that the medication works by changing the monoaminergic neurotransmitter systems. By attaching to the norepinephrine transporter, it is a moderate and selective norepinephrine reuptake inhibitor that prevents norepinephrine reuptake. As a result, it raises the amounts of extracellular norepinephrine in several brain regions.[2,3] In April 2021, the FDA authorized QELBREE, an extended-release form of viloxazine, for the treatment of ADHD.[4] Major depressive illness was treated with it as an antidepressant prescription. It was believed to be beneficial for both severe depression and mild to moderate depression, whether or not co-morbid symptoms were present.[5]

Material and Methods
Equipment

During the investigation of viloxazine, the Waters, Alliance e 2695 HPLC system—which is outfitted with a triple quadrupole mass analyzer, column oven, degasser, and high-speed autosampler—was used. It is linked to the SCIEX QTRAP 5500 mass spectrometer. The information gathering was

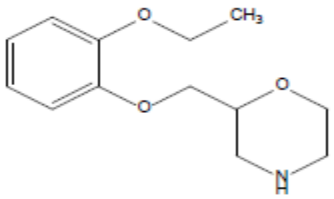

Fig. 1: Chemical structure of viloxazine[1]

controlled by use of the Class Empowerment-2 Program. ChemDraw 20.1.1. Ink Software was used to produce all of the structures and IUPAC names.

Setting Up the Mobile Phase

One milliliter (mL) of triethylamine (TEA) was dissolved in one thousand milliliters (mL) of water to create the 0.1% TEA buffer. ACN and 0.1% TEA were then combined in a 40:60%v/v ratio. Prior to use, it was run through a 0.45 µ membrane filter. The diluent used was the same mobile phase.

Getting the Standard Solution Ready

Viloxazine, precisely weighed at 50 mg, was ingested and diluted with diluent to the necessary amount of 100 mL. The 5 mL solution mentioned above was further diluted to 50 mL, which had a 50 µg/mL concentration.

Getting the Sample Solution Ready

Weighing ten pills precisely was done. They were ground into a powder using a mortar and pestle. An equal quantity of powder was then removed and put into a 100 mL volumetric flask, along with 70 mL of diluents. Dilution with diluent after sonication for dissolution. After diluting 5 mL of the stock solution above to 20 mL, it was filtered using a 0.45 µ nylon syringe filter.

Studies on Forced Degradation

In compliance with ICH requirements, investigations on acid, base, oxidation, reduction, heat, and hydrolysis degradation were conducted. The alkali degradation was carried out by exposing the solution to 0.1 N NaOH for 15 minutes, and the acid degradation was carried out by adding 1 mL of 1N HCl and neutralizing the solutions thereafter. A 30% hydrogen peroxide solution (0.3 mL) was used to help in the peroxide therapy. Thermal deterioration caused by heating the stock solution to 105°C for six hours. In order to do reduction degradation, one milliliter (30 percent sodium bisulfate solution) was used. To look for photodegradation, the solution was exposed to UV light for six hours.

Table 1: LC and Mass parameters optimized

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

In accordance with ICH Q2 (R1) criteria, the analytical technique had been verified for characteristics such system appropriateness, accuracy, specificity, precision, linearity, robustness, LoD and LoQ, forced deterioration, and stability.[6]

System Appropriateness

System performance was assessed using system suitability metrics. By injecting a standard solution containing 50 µg/mL of viloxazine six times, the system suitability was obtained. The drug's findings showed that the system suitability parameter was within permissible bounds.

Particulars

By looking at the chromatograms of the blank and viloxazine-spiked samples, it was verified. In order to detect interference from representative peaks, 100 µg/mL concentration solutions of the diluent (blank), placebo, working standard, and sample solution were injected into each other to assess the specificity of the analytical procedure. The overlay chromatogram in Figure 2 shows that no co-eluting peaks appeared throughout the viloxazine retention period, suggesting that the analyte peak was pure and that the formulation's excipients had no effect on the analyte of interest.

Consistency

By calculating the calibration curve of the peak response at each concentration, linearity was ascertained. injected

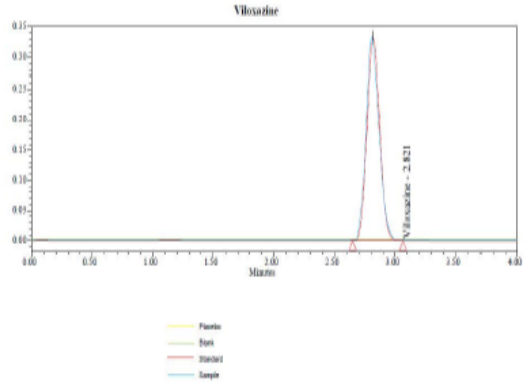

Fig. 2: Using the chromatographic procedure, the overlay chromatogram of the blank, placebo, standard, and sample at each concentration solution level was assessed to determine the peak area. peak area vs concentration (X and Y-axis peak areas) on a graph, with the correlation coefficient calculated. The calibration curve demonstrated that the curve in the range of 12.5 to 75 µg/mL was straight. Y=48681x + 6239.1 (R2-0.9992) for viloxazine was the regression equation for the calibration curves, and Fig. 3's overlay showed the linear concentration.

By adjusting the mobile phase, flow rate, and other parameters, the robustness of the approach was verified, and it was determined to be effective. The method's accuracy was shown by the results of the intraday and interday precision tests. The drug's mean percentage recovery demonstrated the accuracy of the approach. Limits were identified for the %mean and %assay. Table 2 contains a tabulation of all the outcomes from the aforementioned parametric studies.

Studies on Forced Degradation

Chromatographic resolution of a mixture of stress response solutions, especially those in which notable deterioration was seen, was used to examine the established HPLC technique used here. It showed that the medication and all of the main degradation products could be separated using the approach. The drug was prone to degradation, as shown by the overlay chromatogram in given Fig. 4, where distinct degradants were produced and recognized at respective retention times under acid (DP I - 1.671), alkali (DP II - 2.183), peroxide (DP III - 3.794), and reduction degradation (DP IV - 4.028). Table 3 contains tabular data on the studies' mass balance and deterioration percentages.

LC-MS/TOF analyses of samples under stress

Using the optimum mass, LC-MS/TOF investigations were conducted on the drug and combination of stressed samples.

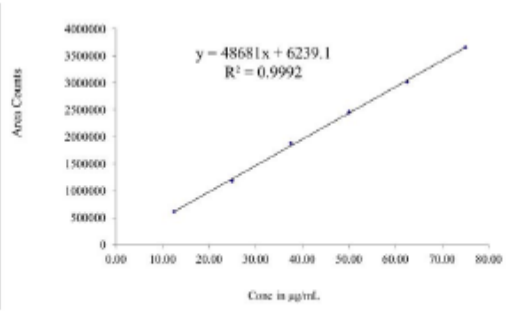

Fig. 3: A calibration plot of Viloxazine

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

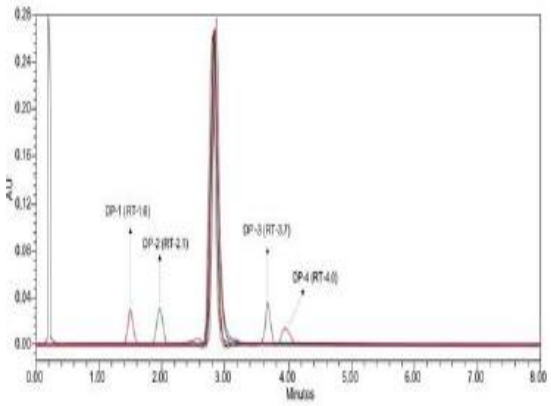

Fig. 4: An overlay chromatogram of degradation products of viloxazine
Table 3: Forced degradation results

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

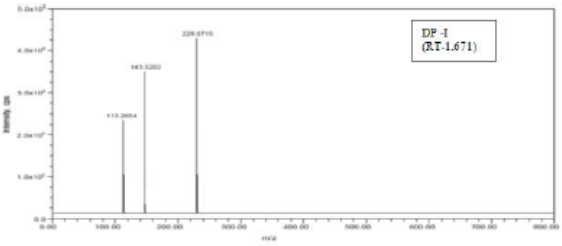

Fig. 6: Mass spectra of DP-I (1.671 RT)

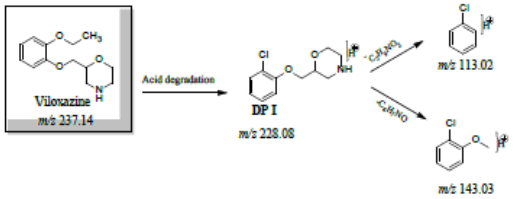

Fig. 6a: DP-1 fragmentation pathway

was seen under circumstances of alkali degradation. There are a lot of [M+H]+ product ions in the spectra at m/z-125.36 and m/z-95.42. The expected scheme in Fig. 7a has been established by the MS/MS experiments in conjunction with precise mass measurements derived from spectra in Fig. 7.

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

demonstrates the degradation product's fragmentation process, which was isolated at 3.794 RT of m/z-226.49.

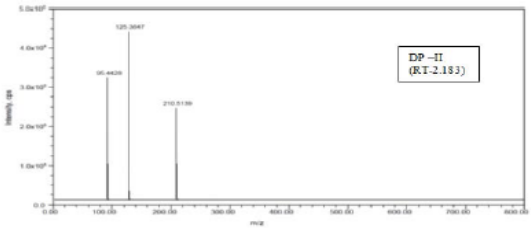

Fig. 7: Mass spectra of DP-II (2.183 RT)

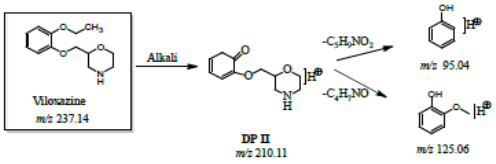

Fig. 7a: DP-II fragmentation pathway

detected under conditions of peroxide decomposition. There are a lot of [M+H]+ product ions in the spectra at m/z-124.53 and m/z-95.76. The suggested approach in Fig. 8a has been validated by the MS/MS experiments in conjunction with precise mass measurements derived from spectra in Fig. 8.

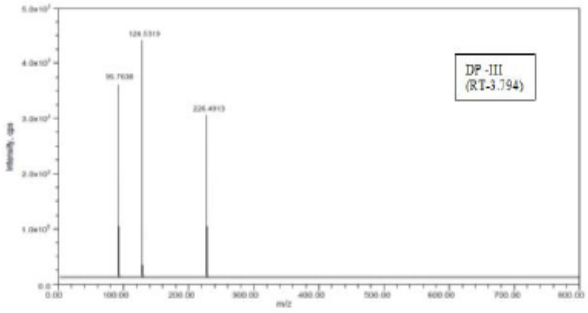

Fig. 8: Mass spectra of DP-III (3.794 RT)

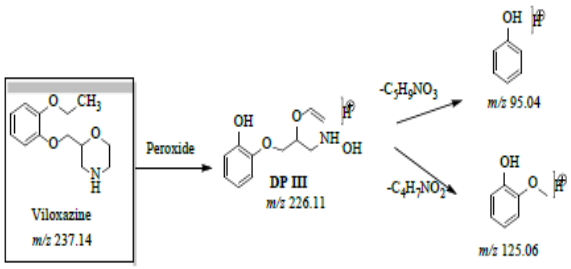

Fig. 8a: DP-III fragmentation pathway

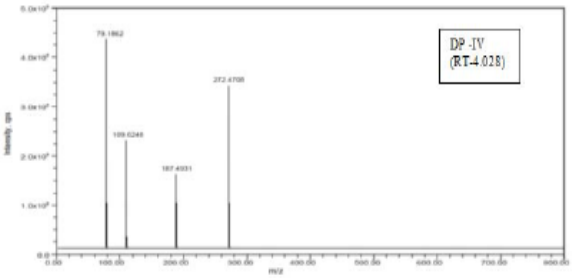

Fig. 9: Mass spectra of DP-IV (4.028 RT)

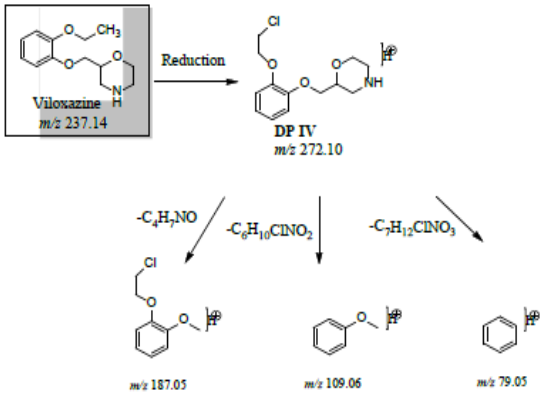

Fig. 9a: DP IV fragmentation pathway

Conclusion

The intended aim and goals were met by the obtained results. The degradants from the main medicine were effectively isolated thanks to this investigation. Furthermore, a systematic investigation was conducted using LC-MS/MS methods to identify the degradation products (DP-I to IV) in the stability assessment. The degradation products (DPs) were suggested based on the m/z measurements, which was helpful in determining the need for more characterisation. The drug was more vulnerable to reduced degradation with a high percentage of degradation, acid, alkali, and peroxide than it was to other stressful situations. Among the benefits this method offered over more traditional ones were the ability to assess identification and quantification concurrently in a same run, which boosts analytical confidence, prevents occurrence of co-elution, simplifies identification, and prevents repeat analysis. Thus, the recommended approach proved appropriate for tracking and measuring the degradation products throughout manufacturing and stability studies.

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