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DEVELOPMENT AND VALIDATION OF AN ENVIRONMENTALLY SUSTAINABLE LIQUID CHROMATOGRAPHY METHOD FOR QUANTIFICATION OF LOXAPINE AND CHARACTERIZATION OF ITS DEGRADATION PRODUCTS BY LCMS/MS

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ABSTRACT

The green analytical UPLC methods are highly effective for quantifying impurities because these methods reduce the usage of harmful chemicals and generate less waste leading to minimal environmental influence. Currently, there are no green methods reported for quantifying impurities in Loxapine and hence this study plans to fill the gap noticed in the literature. In method optimization, various compositions of green solvents including ethanol, and water with buffers were utilized as mobile phase. The conditions that produce acceptable results were studied for valuation in terms of linearity, accuracy, precision, and sensitivity. This method was applied for the resolution and structural evaluation of degradation products (DPs) of Loxapine using LCMS/MS. The method comprises Waters Acquity UPLC® HSS C18 (2.1mm × 100mm, 1.8µm) column along with ethanol, 0.1M Formic acid in water, and water in 50:20:30 (v/v) at pH 4.8 at 0.75 mL/min flow and 265 nm. All validation parameters were tested, and acceptable results were obtained for Loxapine and its impurities. The stress study identified three separate degradation products in the acid and base degradation chromatograms, labeled DP 1, DP 2, and DP 3. MS^n studies and mass fragmentation analysis confirmed DP 1 as *2-chlorodibenzo[b,f][1,4]oxazepin-11(10H)-imine,* DP 2 as *3-chloro-N-phenylbenzene carboximidamide* and DP 3 as *N-(2-chlorodibenzo [b,f][1,4]oxazepin-11-yl) -N-methylethane-1,2-diamine*. The Greenness of the method was evaluated using GAPI (Green Analytical Procedure Index) and AGREE (Analytical GREEnness) tools, confirming that this technique can greatly cut down the use of harmful solvents while maintaining good chromatographic results. Hence, this method is effective for evaluating impurities of Loxapine and also characterizing its DPs.

Keywords: Loxapine, Impurities, LCMS, Degradation Products, Green Analytical Method.
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INTRODUCTION

Loxapine is a conventional antipsychotic medical drug used to treat schizophrenia. It represents the dibenzoxazepine class that works by blocking dopamine receptors to reduce symptoms like hallucinations and agitation. Loxapine can cause mild liver enzyme elevations and, rarely, acute liver injury.[1] Loxapine's side effects include drowsiness, dry mouth, dizziness, and extrapyramidal symptoms such as tremors and muscle stiffness. It can also cause weight gain, constipation, and orthostatic hypotension.[2] Loxapine chemically designated as *2-chloro-11-(4-methylpiperazin-1-yl)dibenzo[b,f][1,4]oxazepine*. The empirical formula is $C_{18}H_{18}ClN_3O$ and the molecular weight is 327.81 g/mol its structure is represented in Fig.-1A. There is insufficient information published on a thorough analysis of DPs of loxapine. Some studies reported for estimation of loxapine using HPLC[3,4], HPLC-UV[5], HPLC-MS[6], and LCMS with a combination of other drugs.[7,8] However, other methods were not published on loxapine with its degradation products using UPLC. Unfortunately, reported studies do not comprehensively cover the topic, leaving significant gaps in characterizing DPs that result from various stress conditions. Green analytical methods follow green chemistry principles that reduce the use of harmful chemicals, cut down on waste, and conserve resources. Additionally, these methods can lower costs and improve efficiency, making them a practical choice for accurately and reliably measuring impurities in pharmaceutical compounds. With this in mind, the goal of

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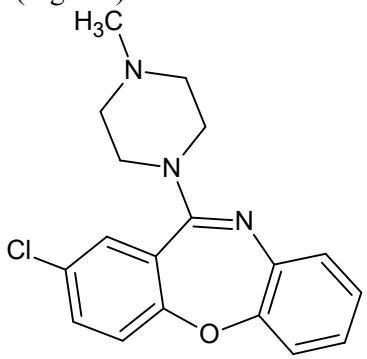
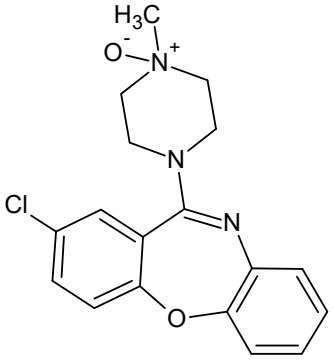
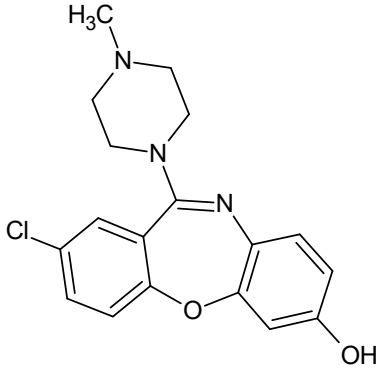
this research was to develop an eco-friendly analytical method for quantifying Loxapine and its impurities using HPLC, as well as to study its degradation behavior through LC-MS/MS analysis. The impurities of Loxapine such as N-oxide and 7-hydroxy impurity were selected for this study. N-oxide impurity has scientific name of *2-chloro-11-(4-methyl-4-oxidopiperazin-1-yl)dibenzo[b,f][1,4]oxazepane* with molecular formula of $C_{18}H_{18}ClN_3O_2$ and mass of 343.81 g/mol (Fig.-1B) whereas *2-chloro-11-(4-methylpiperazin-1-yl)dibenzo[b,f][1,4]oxazepin-7-ol* is the name of 7-hydroxy impurity with 343.81 g/mol as mass and $C_{18}H_{18}ClN_3O_2$ as formula (Fig.-1C).



(A) Loxapine



(B) N-Oxide impurity



(C) 7-hydroxy impurity

Fig.-1: Molecular Structure of Loxapine and Studied Impurities

EXPERIMENTAL

Chemicals and Reagents

The Loxapine standard drugs with purity greater than 99.15%, along with its N-Oxide and 7-hydroxy impurities were generously provided by Simson Pharma Limited, located in Hyderabad, India. The chemicals employed in this study were all of analytical grade and were sourced from Merck Chemicals, Mumbai. Additionally, high-purity solvents required for HPLC analysis including ethanol, and water were obtained from Merck Chemicals, Mumbai. The Loxacon® capsules having 50 mg of Loxapine were purchased from Consern Pharma Limited, Ludhiana, Punjab.

Instrumentation

The analytical experiments were conducted using an Agilent 2695 series HPLC-UV system (USA) with a binary pump and degasser. This system also features an auto-sampler and a compartment that controls the temperature of the column, ensuring consistent conditions throughout the analysis. The data collected from these experiments were integrated through Empower 3 software. The detailed characterization of DPs was performed on an Agilent 1290 series LC-MS/MS system (USA) paired with a Q-TOF mass detector. The ionization of analytes including the DPs was performed using Electrospray Ionization (ESI) in positive mode. The raw data obtained were exported and analyzed through Mass Hunter Workstation software. The pH of the samples was carefully measured using a pH meter from Mettler Toledo (Switzerland). The samples were fully dissolved before analysis with the usage of an ultrasonic sonicator (Oscar Ultrasonic Pvt Ltd, India).

Preparation of Standard Solution

In the process of preparing of stock solution, exactly 10 mg of standard substance was carefully weighed and then transferred into a 10 mL flask. 5 mL of ethanol was added to this flask and then sonicated to achieve complete solubility of standard in ethanol separately. After ensuring complete solubility, it was filtered through a 0.22 µm nylon membrane filter and then the solution was diluted to a 10 mL mark with additional ethanol to reach 1000 µg/mL concentration. The prepared stock solution was then stored for future use as a standard solution.

Forced Degradation Study

The stress degradation was induced by adopting a procedure prescribed by ICH Q1 A (R2)[9] and a procedure available in the literature.[10] The procedure briefly, 100 mg of loxapine was dissolved in 50 mL of diluent in a 100 mL flask, followed by 5 mL of 2 N HCl was added for acid degradation. This solution was refluxed at 80°C for 8 hours, neutralized with 2 N NaOH after 3 days, and the volume was adjusted. A 5 mL aliquot was then diluted to 20 mL with diluent, filtered, and adjusted to 100% concentration for analysis. The base degradation was induced by combining 100 mg of standard loxapine with 5 mL of 3 N NaOH and 45 mL diluent, refluxed at 80°C for 8 hours, neutralized with 3 N HCl, and the volume was adjusted. A 5 mL portion was diluted to 20 mL with diluent, filtered, and adjusted to 100% concentration. The oxidative degradation was performed by mixing 100 mg of the drug with 50 mL of diluent and 5 mL of 30% H_2O_2. This mixture was kept in the dark at room temperature for 3 days before filtering and adjusting to 100% concentration. Thermal and UV degradation study conducted by exposing pure drug in an air oven at 80°C for 2 days and under UV light for 3 days at room temperature. After stress exposure, it was diluted to 100% concentration for analysis.

Method Validation

The validation of the proposed method was conducted according to ICH Q2(R1) guidelines[11] and available literature.[12] System suitability was evaluated by repeatedly injecting known-strength Loxapine solution spiked with 1% impurities. The resultant chromatographic results including resolution, tailing factor, theoretical plate count, and % RSD of the area were summarized to ensure the method's performance. Linearity was determined by analyzing six different concentrations of Loxapine with 1% impurities and the resultant area response was plotted against its concentration. The linear relationship with a correlation coefficient of greater than 0.999 was treated as acceptable. To assess method accuracy, Loxapine and its impurities were spiked at levels ranging from 50% to 150% of the target concentration. These spiked analysis results were correlated with calibration results to confirm method reliability. Precision and ruggedness were examined by analyzing 100% level solution in calibration range under various conditions including same day, day change, and analyst change. The resultant area response was used to evaluate % RSD and the results confirm the method's repeatability and reproducibility. Robustness was validated by introducing minor intentional changes to method parameters such as detector wavelength, composition, and flow of mobile phase. The impact on % area response and system performance in every change was observed to assess method robustness. Sensitivity was evaluated by determining LOD and LOQ for impurities utilizing standard error and slope from a linear curve.

Characterization of DPs

Stressed solutions were analyzed on an HPLC-MS/MS system under proposed optimized conditions. The method allowed the detection of peaks associated with Loxapine, impurities, and DPs. The mass range covered during the analysis was from 10 to 500 m/z. In LC-MS analysis, 25% of column eluents were allowed to pass through the MS system by fixing a splitter between the column and the mass spectrometer detector.

Assessment of Method Greenness

The proposed quantitative method was green-evaluated through GAPI and AGREE on metric tools[12]. The Green evaluation specifically helps to estimate different parameters for ensuring the safety of the drug by reducing the health and environmental impact. This estimation includes aspects such as sample collection, the method used, solvents, and reagents used in the method, energy expenditure, steps of disposal of wastes, and other pertinent considerations. Among them, GAPI is highly effective in examining environmental

sustainability factors. In this evaluation, the figure was prepared with different color combinations like green, red, and yellow. The whole Figure is divided into six different parts, where each part signifies different factors, such as the origin of the sample, the process type that it undergoes, sample preparation, the chemicals and reagents used, the instruments, and most importantly, the type of quantitative analysis, which is signified by the letter "O". The AGREE method was used to determine the extent of environmental sustainability. The method by metric is based on the twelve principles of analytical green chemistry.

RESULTS AND DISCUSSION

The optimization was started with the Acquity C8 column. Many organic modifiers have been tried to get the desired asymmetric peak shape, resolution, and minimum tailing. However, the first set of conditions did not provide an acceptable asymmetric peak shape. Given the above, changes were introduced into the mobile phase, and pH was altered to attempt to yield better results but did not produce permissible results. Hence, an Acquity C18 column (Waters, 150mm × 4.6mm, 5µm) was employed. A mobile phase of ethanol, 0.1M aqueous formic acid, and water in 50:20:30 (v/v) was utilized, which provided adequate resolution with an asymmetric peak shape of low tailing. Therefore, these conditions were optimized given the development of the method for the analysis of Loxapine and its impurities. The wavelength was set to 265 nm, based on the iso-absorption point of Loxapine and its impurities.

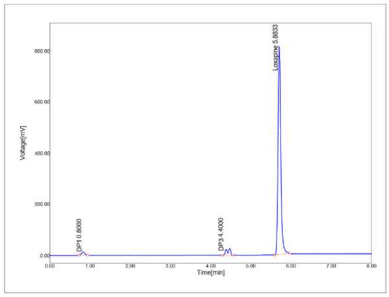
Stress Degradation Study

The degradation behavior of Loxapine was studied under various stress conditions using HPLC analysis. Different stress environments were applied to observe how Loxapine responds to each stress factor. The specific stress conditions used, along with the observed degradation results, are summarized in Table-1. This approach helped in understanding the stability profile of Loxapine and identifying any degradation products formed under each condition.

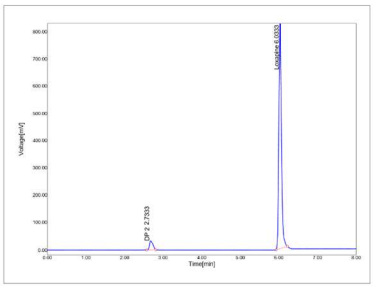
Table-1: Forced Degradation Conditions for Loxapine

Condition	Stressor	Conditions	Duration	% degradation	DPs
Base	3N NaOH	80°C	8 hours	7.93	DP 2
Acid	2N HCl	80°C	3 days	9.01	DP 1 & DP 3
Oxidative	30% H_2O_2	Room temperature	3 days	4.67	---
UV light	200 W h/m^2	Room temperature	3 days	4.85	---
Thermal	Air oven	80°C	2 days	2.09	---

Table-1 displays the data obtained from the stress study indicating that under the oxidation, heat, and light conditions, no degradation compounds were noticed for Loxapine. However, the drug did degrade when subjected to acidic and basic environments (Fig.-2). In these sensitivity tests, three new peaks were found in the chromatograms at 0.80 min, 2.73 min, and 4.40 min, which were considered to be DPs in addition to known impurities. Based on the time they elute, these degradation products were designated as DP 1, 2, and 3 respectively, and were analyzed using the LCMSMS technique.



Acid Stress Chromatogram Shows DP 1 and 3



Base Stress Chromatogram Shows DP 2

Fig.-2: Stress Degradation Chromatograms Noticed in the Study

LC-MS/MS Studies of DPs

The high-resolution fragmentation patterns noticed in the mass spectrum of DPs were utilized to evaluate its accurate mass measurements. The exact mass prediction of Loxapine and its DPs was achieved through the utilization of RDB (Ring and Double Bond) calculations and the nitrogen rule. The elemental

compositions of protonated DPs and their corresponding product ions are summarized in Table-2 whereas Fig.-4, 6, and 8 illustrate the fragmentation pathways for DP 1, DP 2, and DP 3, respectively.

Table-2: HRMS Analysis Results Noticed for Loxapine and its DPs

Compound	MF	m/z Calculated	m/z observed	Error in ppm	Fragmentation(m+1)
Loxapine	$C_{18}H_{18}ClN_3O$	328.8080	328.8081	0.304	279.7567 251.7035 186.2444 95.1134
DP 1	$C_{13}H_9ClN_2O$	245.6763	245.6760	1.221	220.6464 195.6177 166.5990 114.5642
DP 2	$C_{13}H_{11}ClN_2$	231.6928	231.6929	0.431	178.6098 156.6043 141.5896 114.5643
DP 3	$C_{16}H_{16}ClN_3O$	302.7707	302.7705	0.660	286.7476 231.6691 179.6376 153.6003

DP 1

The acid stress chromatogram display peak corresponds to DP 1 at 0.80 min and exhibits a mass value of 245.6763 with a possible molecular formula of $C_{13}H_9ClN_2O$. The mass fragmentation spectra displayed in Fig.-4 and the fragmentation pattern as shown in Fig.-3 further prove the molecular structure of DP 1. From DP 1 total molecular formula loss of C_2H resulted in the formation of the $C_{11}H_8ClN_2O$ production with 219.64 as the molecular weight of the formed production. From this production, another production is formed due to the loss of C_2H forms $C_9H_7ClN_2O$ with a molecular weight of 194.61. From the by-product ion of $C_9H_7ClN_2O$ forms another by-product ion is formed. The formed product ion exhibits a formula of $C_8H_6ClN_2$ with 165.59 of molecular weight. The production of $C_8H_6ClN_2$ also forms C_6H_6Cl by the loss of C_2N_2. Finally formed DP 1 was identified as *2-chlorodibenzo[b,f][1,4]oxazepin-11(10H)-imine.*

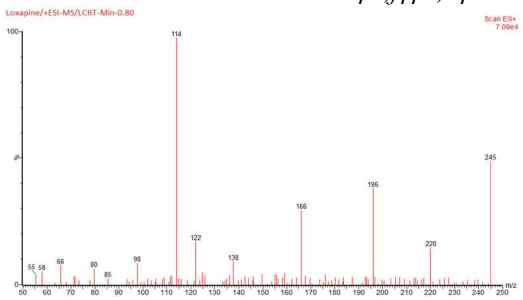


Fig.-3: Mass Spectrum of DP 1

DP 2

DP 2 exhibits 230.69g/mol molecular mass with the formula of $C_{13}H_{11}ClN_2$ and was produced specifically during base degradation at an elution time of 2.73 min. Under these conditions, DP 2 loses a C_4H_5 group, resulting in a fragment ion with a formula of $C_9H_9ClN_2$ and a mass of 177.60 m/z. This fragment further loses a C_2H_2 group and forms fragment ion with the formula of $C_7H_8ClN_2$ and a mass of 155.60 m/z. Another fragment formed by the loss of NH and produces C_7H_7ClN ion at 140.58 m/z. A final fragment ion with a formula of C_6H_6Cl and a mass of 113.56 m/z was formed. The mass spectrum of DP 2 is illustrated in Fig. 5, and its full degradation pathway is presented in Fig.-6. Based on fragmentation data, it was characterized as *3-chloro-N-phenylbenzenecarboximidamide*.

DP 3

The acid degradation chromatogram also displays an additional peak at 4.4 min retention time due to the observation of DP 3. It exhibits a molar mass of 301.77 with the formula of $C_{16}H_{16}ClN_3O$. In the

fragmentation process, DP 3 loses a H_2N group, resulting in a fragment ion with a formula of $C_{16}H_{14}ClN_2O$ and a mass of 285.74 m/z.

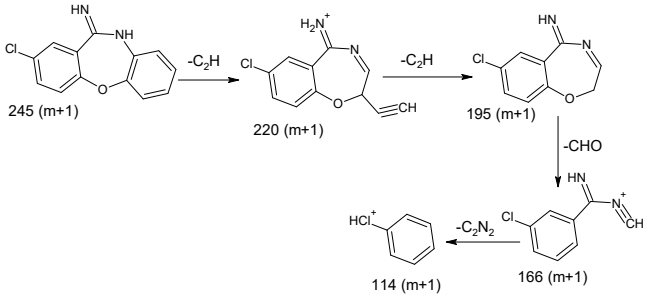


Fig.-4: Degradation Pathway for DP 1

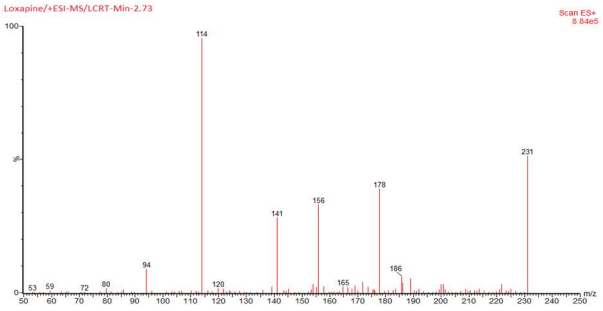


Fig.-5: Mass Spectrum of DP 2

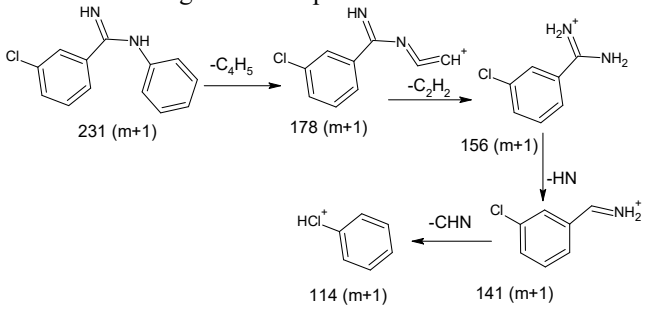


Fig.-6: Degradation Pathway for DP 2

This ion then loses a C_3H_5N group, creating another production with the formula of $C_{13}H_9ClNO$ and a mass of 230.66 m/z. Further fragmentation involves the loss of C_3O (tricarbon monoxide), forming a new ion with the formula of $C_{10}H_9ClN$ and a mass of 178.63 m/z. The mass spectrum also displays a production due to the loss of a C_2H_2 group yielding a fragment with the formula C_8H_7ClN and a mass of 152.60 m/z. The entire degradation pathway of DP 3 is depicted in Fig.-8, while its mass spectrum is presented in Fig.-7. Based on this fragmentation pattern, DP 3 is named *N-(2-chlorodibenzo[b, f][1,4]oxazepin-11-yl)-N-methylethane-1,2-diamine*.

Method Validation

Linearity

In the linearity study, we used the linear method to determine the relation of the concentration of Loxapine and its impurities to their area measurement responses. The linear relationship was seen in from 25 to 150 µg/mL of Loxapine and 0.25 and 1.5 µg/mL for its impurities. Linear equation was noticed to be $y = 7126.3x + 101378$ ($R^2 = 0.9994$) for Loxapine, $y = 73136x + 17324$ ($R^2 = 0.999$) for N-Oxide impurity, and $y = 45745x + 8181.7$ ($R^2 = 0.9987$) for 7-Hydroxy impurity. These results justify that this proposed method is suitable for both qualitative and quantitative determination of Loxapine and its impurities in the concentration ranges studied.

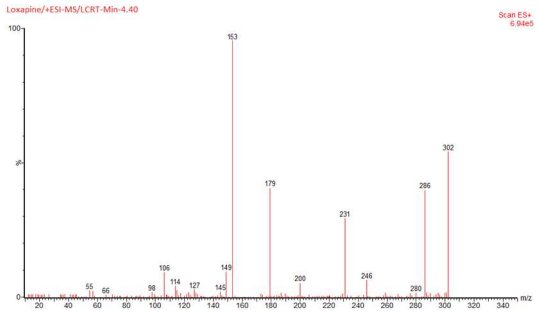


Fig.-7: Mass Spectrum of DP 3

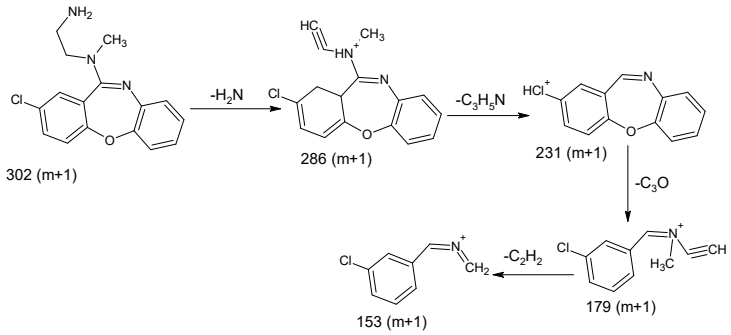


Fig.-8: Degradation Pathway for DP 3

Ruggedness and Precision

The values of the precision and ruggedness assay percentages and the corresponding % RSD of Loxapine and its impurities are precise and reproducible (Table-3). The measured assay value for Loxapine is 99.71 %. As far as the precision is concerned the % RSD of 0.69% for intraday measurements for Loxapine, 0.65%, 1.12% in interday precision days 1 and 2 for Loxapine, and 1.41% RSD for ruggedness studies. In the case of N-Oxide Impurity, the assay value was 100.30%, while 0.64% RSD was observed for intra-day measurements of precision and RSD 0.35% for ruggedness confirming reliable measurements. The 7-Hydroxy Impurity was determined to have an assay of 100.19% with the % RSD of 0.43% on intraday precision. This suggests that daily estimates are quite dependable. Based on the results, the values were all approaching very close to 100% confirming that the analytes were accurately estimated in the proposed method.

Table-3: Ruggedness and Precision Results of Loxapine and its Impurities

S. No.	Analyte	% Assay	% RSD in Intraday precision	% RSD in Interday precision		% RSD in Ruggedness
				Day 1	Day 2	
1	Loxapine	99.71	0.69	0.65	1.12	1.41
2	N-Oxide Impurity	100.30	0.35	0.12	1.42	0.83
3	7-Hydroxy Impurity	100.19	0.43	0.61	1.44	1.64

Robustness

Robustness results shown in Table-4 highlight that the analytical method used for testing Loxapine and its impurities were dependable under different conditions. When the mobile phase was adjusted to a mixture of ethanol, 0.1M aqueous formic acid, and water in a 50:15:35 ratio (v/v), the resolution between 7-hydroxy impurity and Loxapine was 9.07 with an assay percentage of 98.04%. Changing the mobile phase composition to a 50:25:25 ratio (v/v) improved the separation to 9.58 and the assay percentage to 99.53%. Similarly, the method produced reliable results when flow rate and wavelength were altered. These findings indicate that the method consistently performs well, providing reliable resolution, peak shape, plate count, and % assay across different conditions.

Table-4: Robustness test results noticed for Loxapine and Impurities in this Proposed Method

Altered condition	Resolution between and 7-hydroxy impurity Loxapine	Tailing factor	% Change	% Assay
ethanol, 0.1M aqueous formic acid, and water in a 50:15:35 (v/v)	9.12	0.99	0.71	99.29
ethanol, 0.1M aqueous formic acid, and water in a 50:15:35 (v/v)	9.05	1.01	0.47	99.53
0.80 mL/min flow rate	9.82	0.98	0.54	99.46
0.70 mL/min flow rate	9.27	0.99	1.21	98.79
270 nm wavelength	9.51	0.97	0.68	99.32
260 nm wavelength	9.09	1.01	0.90	99.10

Accuracy

Accuracy testing was conducted across three concentration levels such as 50%, 100%, and 150%. At the 50% level, 75 µg/mL of Loxapine and 0.75 µg/mL of each impurity were utilized as test concentrations. At 100% level, Loxapine at 100 µg/mL and 1.0 µg/mL of each impurity solution was tested whereas at the 150% level, Loxapine at 125 µg/mL and 1.25 µg/mL of impurities were used. This approach ensured accurate measurements across a range of concentrations to validate the method's precision and reliability. The results proved that 99.32 % was recovered for Loxapine in 50% accuracy, 99.51 % was recovered in 100%, and 99.31% was recovered in 150% accuracy. For N-Oxide impurity 98.74, 100.24, and 98.48 % recovery was found whereas 7-Hydroxy impurity displays the % recovery of 99.38, 98.77, and 98.64 % in 50, 100, and 150 % respectively. The result of recovery shows that the range of limit suggests method accuracy. The results of accuracy are given in Table-5.

Table-5: Accuracy Test Results Noticed for Loxapine and Impurities in This Proposed Method

Target Amount in µg/mL	Spiked amount in µg/mL	Final amount in µg/mL	% recovery	%RSD
Loxapine				
50	25	75	99.39	1.00
50	50	100	99.51	0.55
50	75	125	99.32	0.52
N-Oxide Impurity				
0.5	0.25	0.75	98.74	0.55
0.5	0.50	1.0	98.17	1.04
0.5	0.25	1.25	99.50	0.34
7-Hydroxy Impurity				
0.5	0.25	0.75	98.38	1.42
0.5	0.50	1.0	98.77	1.43
0.5	0.25	1.25	98.64	1.12

Solution Stability

To assess solution stability, a 100% standard solution of Loxapine was stored at room temperature and tested at various intervals. The stability was evaluated by comparing the % difference between injected samples and standards. The %differences were 0.08% for the sample and 0.17% for the standard indicating minimal degradation and excellent stability of Loxapine solutions over 48 hours. Stability results are presented in Table-6.

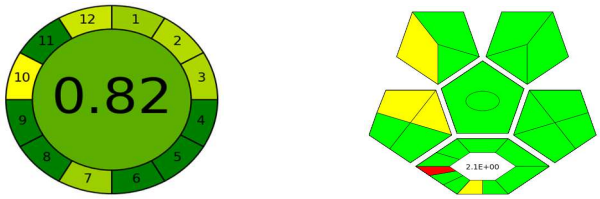
Method Greenness Assessment

This study focused on proposing an environmentally friendly analytical method to analyze Loxapine and its DPs. Traditional analytical methods often utilize hazardous solvents such as acetonitrile and methanol, but this approach replaces them with safer, eco-friendly solvents like ethanol and water. These green solvents were carefully chosen to prepare both the mobile phase and sample solutions, aiming to minimize the environmental impact. Additionally, a 100 mm column was utilized in this study that facilitates shorter analysis time.

Table-6: Solution Stability Test Results Noticed for Loxapine in This Proposed Method

Time Interval	Standard	Sample
0 hour (Initial)	99.87	98.73
24 hours (after)	99.62	98.81
48 hours (after)	99.45	98.73
% difference	0.17	0.08

This choice minimizes solvent use and energy consumption making the method both more sustainable and efficient. The environmental impact of the method was assessed by employing AGREE and GAPI software tools. AGREE tool gave the method a high score of 0.82 (Fig.-9A), indicating strong compliance with green chemistry principles. This method stands out for its minimal use of samples and solvents, reliance on non-toxic chemicals, energy efficiency, and thorough environmental assessment, all contributing to its sustainability. Meanwhile, the GAPI tool offered a detailed visual summary through pictograms and pentagrams that highlighted the method's environmental effects. Most pictograms appeared in green and yellow, showing low environmental risks (Fig.-9B). With a GAPI score of 2.1E+00, the method is confirmed to have a minimal environmental footprint. However, the yellow pictograms, specifically related to sample handling and preparation, suggest areas where the method could be further refined to enhance its eco-friendliness.



(A) AGREE (B) GAPI

Fig.-9: Environmental Assessment Pictograms Observed in the AGREE and GAPI Tools for the Suggested Analytical Method

In the literature, HPLC methods were reported for quantifying loxapine in single and in combination with similar-class drugs. No author Author characterizes the DPs of Ripretinib and this technique represents a notable improvement by integrating principles of green chemistry making it an eco-friendlier option. Hence, this study finding can be the best choice for the analysis of Ripretinib with environmental sustainability

CONCLUSION

In conclusion, this study reports a green analytical HPLC method for quantifying Loxapine and its impurities. This method effectively reduces the use of harmful chemicals and minimizes waste by green chemistry principles. The study addressed a notable gap in the literature by providing an adequate green method which comprises Waters Acquity C18 column with a solvent mixture of ethanol, aqueous formic acid, and water. The technique demonstrated precise performance for quantifying Loxapine and its impurities with validation parameters that meet the required standards. Stress testing identified three distinct DPs under acidic and basic conditions and their structures were confirmed through MS^n studies. The green assessment tools such as GAPI and AGREE verified that this method significantly reduces the use of hazardous solvents with excellent chromatographic performance. Overall, this green HPLC method is well-suited for routine applications in quality control and stability studies that offer a sustainable approach for analyzing Loxapine and its DPs in synthetic samples and formulations.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

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