RESEARCH PAPER

UPLC Method for Analysis of Troxipide and Its Impurities with Stress Degradation Studies Using LC-ESI-MS

Arti Swami[1*], Akanksha Lonkar[1] and Vishnu Choudhari[1]

[1]*Department of Pharmaceutical Sciences, School of Health Sciences and Technology, Dr. Vishwanath Karad MIT World Peace University, Pune, India*

[*]***Corresponding author:*** *arti.swami@mitwpu.edu.in*

Received: 16th Feb, 2025; Revised: 8th Mar 2026; Accepted: 28th Mar, 2026; Available Online: 30th Mar, 2026

ABSTRACT

Troxipide (TRP) is an antiulcer agent that acts by reducing the inflammation in the stomach. The present work describes the development and validation of a rapid, stability-indicating UPLC method for impurity profiling and degradation studies with minimal time and resource utilization. The UPLC method was developed on an ACQUITY UPLC BEH (2.1 x 100 mm, 1.7 µm) column at 400C. The mobile phase optimized was Ammonium Acetate buffer pH 3: Acetonitrile (75:25 % v/v) with wavelength of detection 260 nm. Further stress degradation studies (0.5 M HCl at 60 °C/4 h, 0.5 M NaOH at 60 °C/4 h, 6% H2O2 (w/w) at 60ºc/4h, UV light 24 h, and refluxed for 40ºc/4h) were performed, and the method was validated according to ICH guidelines. For Troxipide and its two impurities, the quantification limits, linearity, and recoveries were found in the range of 0.03-0.7 µg/mL, 20-120 µg/mL ($R2 > 0.999$), and 99-101%, respectively. The stress degradation study data were analysed using LC-MS, and three degradation products were identified with m/z ratios 251.03, 393.39, and 218.25 amu, and their probable structure were elucidated. The proposed method is suitable for routine quality control and stability testing of Troxipide in pharmaceutical formulations.

Keywords: Impurity profiling, method development and validation, UPLC, LC-MS, forced degradation.

How to cite this article: Swami A, Lonkar A, Choudhari V. UPLC Method for Analysis of Troxipide and Its Impurities with Stress Degradation Studies Using LC-ESI-MS. Int J Drug Deliv Technol. 2026;16(25s): 518-526. DOI: 10.25258/ijddt.16.25s.64

Source of support: Nil.

Conflict of interest: None

1 INTRODUCTION

Troxipide (TRP) is an antiulcer agent used to mitigate peptic ulcer, gastroesophageal reflux disease, and acute and chronic gastritis. The prescribed dose of TRP is 100 mg thrice a day after every meal. TRP reduces the inflammation in the stomach and increases the secretion of mucus in the stomach, which forms a protective layer over the damaged stomach lining, which in turn further prevents damage to the stomach lining and induces natural healing.[1-2] Chemically, Troxipide is [3, 4, 5-trimethoxy-N-3-piperidylbenzamide] (Figure 1 (a)), and its weight is 294.4, and molecular formula is $C_{15}H_{22}N_2O_4$.

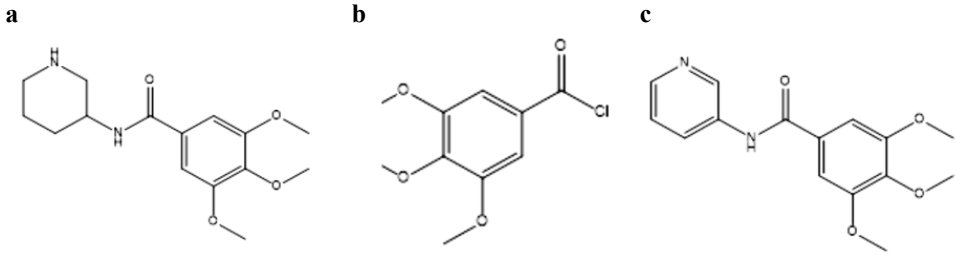

Figure 1: Molecular structure of (a) Troxipide, (b) Impurity A, and (c) Impurity B

Preliminary literature survey revealed that for quantitative estimation of Troxipide, various analytical methods were found to be reported, which include the Spectrophotometric Method, Reverse phase High-Performance liquid chromatography (HPLC) method,[3] stability indicating HPLC and HPTLC method. [4-8] Comprehensive evaluation of existing literature revealed the absence of any reported UPLC method capable of determining Troxipide together with its associated impurities and degradation products. In recent times, the pharmaceutical industry is more focused on minimum economy and time expenditure; hence, instead of HPLC, nowadays UPLC is the most preferred technique in all industries as it enhances mainly in three areas: speed, resolution, and sensitivity. Compared to HPLC, UPLC achieves separation and quantification under very high pressure (up to 100 MPa) using smaller particle sizes, which results in earlier elution of analytes, lower solvent

**Author for Correspondence: arti.swami@mitwpu.edu.in*

consumption, and improved resolution due to sharper peaks. Hence, the present manuscript describes an optimized and validated UPLC method (ICHQ2(R1)1995) for quantitative estimation of Troxipide, its impurities, as well as stress degradation behavior of Troxipide when subjected to thermal, photolytic, oxidative, and hydrolytic conditions.

2 MATERIALS AND METHODS

2.1 Chemicals and Solutions

Emcure Pharmaceuticals Ltd., Jammu, India, gifted a sample of standard Troxipide. All reagents and chemicals employed for the experiment were of AR grade. Troxipide tablet (TROXIP, 100 mg/tablet with batch number 16A13004) was procured from the local market, which is marketed by Zuventus Healthcare Ltd., Mumbai, India. Research Lab Fine Chem Industries in Mumbai, India, supplied AR-grade chemicals like ammonium acetate and glacial acetic acid, as well as HPLC-grade solvents like methanol and acetonitrile.

Impurity A [3,4,5 trimethoxy benzoyl chloride] (Figure 1(b)) was procured from Sigma Aldrich, and Impurity B [3-(3,4,5-trimethoxybenzamido) pyridine] (Figure 1 (c)) was synthesized in-house, purified, and characterized by NMR, Mass, and FTIR techniques.

2.2 Instrumentation and Conditions

WATERS UPLC^{TM} system, consisting of a quaternary solvent delivery pump, autosampler, and PDA detector, facilitated through Empower 2 software, utilized for chromatographic analysis.

Dionex Ultimate 3000 UHPLC system integrated with Impact II UHR-TOF mass spectrophotometer (Bruker Daltonics) fitted with ESI-TOF Analyser was employed for UPLC-MS studies.

Table 1 represents the chromatographic parameters as follows:

Table 1: Chromatographic conditions

Parameters	Description
Mobile phase	Ammonium acetate buffer: acetonitrile (75:25 v/v)
Column	ACQUITY UPLC BEH (2.1 x 100 mm, 1.7 µm) maintained at 40 % throughout the analysis
Flow rate (mL/min)	0.2
Detection wavelength (nm)	260
Injection volume (µL)	2

2.2.1 Preparation of buffer, standard, and sample solution

Ammonium acetate (0.7708 g) was dissolved in water (800 mL), and glacial acetic acid was added to adjust the pH of the solution to 3.0, followed by volume makeup to 1 L using water to make a buffer solution.

The standard stock solution was made by dissolving 10 mg of TRP separately in methanol and diluting suitably to produce a concentration of 1000 and 100 µg/mL of TRP and impurities. A final dilution was performed to obtain a concentration of TRP (100 µg/ml) and for TRP impurities (10 µg/ml).

In 10 mL volumetric flask, amounts of precisely weighed tablet powder equal to 10 mg (i.e., 26.6 mg) was added. To this, 6 mL of methanol was added, thoroughly combined, then sonicated for fifteen min. After sonication, the flask was completely filled with methanol and properly mixed. After that, a 0.45 µm membrane filter was used to filter the mixture. [9-11]

2.3 Stress degradation studies

In order to check the form of degradation, 20 tablets were carefully weighed and ground into a fine powder. The same was then transferred to 10 mL volumetric flask, equivalent in portion to 10 mg of TRP, and 6ml of methanol added. The solution was stirred both thoroughly and sonicated after 15 min intermittently with shaking. The necessary volume of methanol was added, and the solution was thoroughly mixed and filtered using the nylon membrane filter (0.45 µm); the first portion of the filtrate was discarded. To obtain forced degradation, the formulation was subjected to the acidic, alkaline, and oxidative stress conditions by adding hydrochloric acid (0.5 M), sodium hydroxide (0.5 M), and 6% hydrogen peroxide, respectively, and letting each react under the condition of 60 °C in the presence of a water bath for 4 h. The formulation in solid form was thermally stressed by keeping it in an oven at 60 °C for a period of up to 24 h. The drugs were subjected to photostability test by 1.2 million lux hours near UV (200-watt h/m2). The stress conditions were designed to achieve nearly 20% degradation under at least one condition. Both the drug product and the placebo were subjected to the same conditions in order to be compared.[13-15]

2.4 Mass Spectrometry

To determine the probable TRP degradation products, mass spectrometric analysis was used. The mass range chosen was 50-600 amu in positive ionization mode. Nitrogen was employed for nebulization, with the gas flow adjusted to 7 L/min, while the ion source was operated at capillary potential of 4500 V and a temperature of 200^0c.

2.5 Method validation

It was carried out according to ICH Q2 (R1) on the established chromatographic parameters, assessing system suitability, accuracy, specificity, range, limit of detection (LOD), precision, linearity, limit of quantification (LOQ), and robustness.

2.5.1 Specificity

To evaluate the method's specificity, forced degradation studies were conducted under variety of circumstances, such as exposure to acid and base, oxidation, hydrolysis,

photolysis, and heat. To ensure that there was no interference from placebo peaks or other degradation products, the purity of the peaks was assessed.

2.5.2 Linearity and Range

The method's linearity was evaluated across various concentrations, scanning an approximate range of 20 to 120 µg/mL of the target concentrations for TRP, TRP impurity A and B, 0.2 to 1.2 µg/mL. The linearity graph, which shows the relationship between the area and the concentration of TRP, TRP impurity A, and B, was plotted using a linear regression model.

2.6.3 Limit of Detection and Limit of Quantification

Various methodologies exist for determining LOD and LOQ values in accordance with ICH guidelines, including the visual method, signal-to-noise ratio, and slope technique. The present study has opted for the slope method to establish the LOD and LOQ standards.

2.6.4 Precision

Reproducibility was ensured by analyzing six individual samples drawn from a homogeneous mixture. Percentage relative standard deviation (% RSD) indicates the precision of the method.. Additionally, analyses performed on different days were used to assess the method's ruggedness, also termed intermediate precision.

2.6.5 Accuracy

The evaluation was conducted using a spiking test solution at the LOQ as well as at concentrations of 80%, 100%, and 120% of the limit. Each concentration level, including the LOQ, 80%, 100%, and 120%, was prepared in triplicate to ensure consistency in the results.

2.6.6 Method's Robustness

In order to assess the method's robustness, the chromatographic conditions were purposefully changed within and around the ideal range. This approach assesses if the method is capable of consistently producing reliable findings despite purposeful alterations in the parameters. Robustness assessments enhance control and comprehension of the method's performance by identifying key method parameters and their acceptable ranges.

3 RESULTS AND DISCUSSION

3.1 Development of Method

In optimizing the method, several mobile phase compositions were evaluated, including methanol-water, acetonitrile-water, and buffer-methanol systems with varying pH levels under isocratic conditions. After a series of experiments, the best mobile phase was chosen to be 10 mM ammonium acetate buffer with an adjusted pH of 3 and acetonitrile in a 75:25 (% v/v) ratio, with detection done at 260 nm. This method achieved well-resolved peaks for TRP and both the impurities, which fulfill all the parameters of system suitability mentioned in Table 2 and Figure 2. Thus, the mentioned method was proved to be efficient for the separation of degradation products from Troxipide.

Table 2: System suitability parameters of Troxipide and its impurities

Parameter	Troxipide	Imp A	Imp B
Peak Area (% RSD)	1332411,(0.41)	51894,(1.19)	79582,(0.71)
No of theoretical plates (%RSD)	9956.6,(1.35)	3394.3,(1.42)	8660.6,(1.16)
USP Tailing Factor (±SD)	1.7, (0.03)	0.84,(0.01)	1.3,(0.02)
USP Resolution (R)(±SD)	3.6,(0.06)	---	18.2,(0.2)
Capacity Factor(k)(±SD)	2.37,(0.03)	1.53,(0.01)	3.98,(0.16)

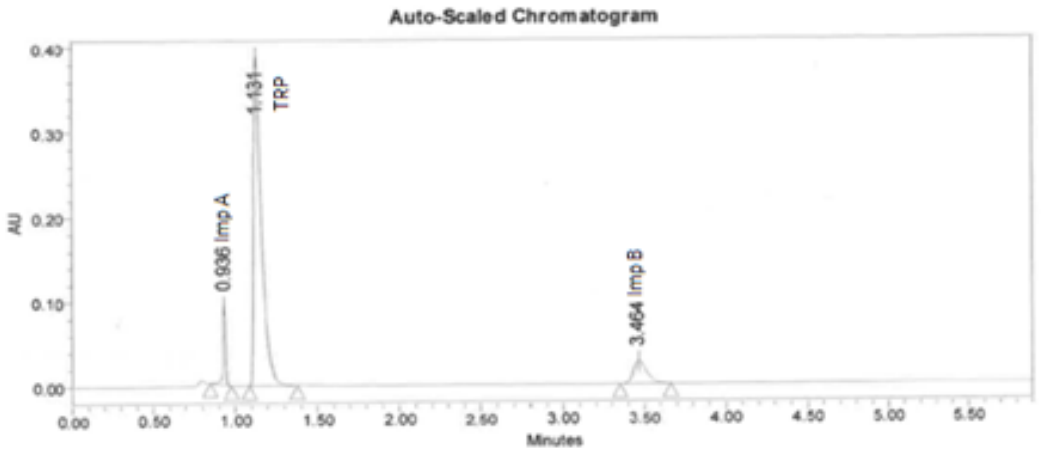

Figure 2: UPLC chromatogram of Troxipide with Impurities

3.2 Method Validation

3.2.1 Linearity

In accordance with ICH requirements, the linearity of TRP and its related impurities was evaluated by generating calibration curves over defined concentration intervals. The concentration range considered was 20-120 µg/mL in

TRP, 0.2-1.2 µg/mL for impurities A and B, respectively. Calibration plots were drawn to solve the equation, analyte concentration versus peak area, and a statistical analysis with impurities A and B was done, which proved the equation to be linear. The coefficients of determination obtained were 0.999, 0.999, and 0.998 for TRP, impurity A, and B, respectively, since there was great linearity within the chosen range. Table 3 shows the linearity results of TRP and impurities.

3.2.2 Limit of Detection and Limit of Quantification

LOD and LOQ values for TRP were found to be 0.243 µg/mL and 0.736 µg/mL, respectively, and for Impurity A and B were 0.038 µg/mL, 0.11 µg/mL, 0.055 µg/mL, and 0.16 µg/mL, respectively. These results demonstrate the high sensitivity of the method, enabling reliable detection and quantification of TRP and its impurities at very low concentration levels.

Table 3: Linearity of TRP and its impurities

Parameter		TRP	Imp A	Imp B
Linearity equation (Y=mX+c)	**Linearity range** (µg/ml)	20 to 120	0.2 to 1.2	0.2 to 1.2
	m	13431	51259	78652
	C	4952.6	1084.5	1173.1
	R^2	0.999	0.999	0.998
Method Sensitivity (µg/ml)	**LOD**	0.243	0.038	0.055
	LOQ	0.736	0.11	0.16

3.2.3 Recovery study (Accuracy)

Accuracy was evaluated by performing recovery studies from the LOQ level up to 120 % through the addition of known quantities of impurities. For each concentration level, six replicate analyses were carried out, and the percentage recovery was determined. The recoveries obtained for TRP as well as impurities A and B ranged between 99 % and 101 %, as summarized in Table 4.

Table 4: Troxipide and its impurities accuracy data (n=6)

Mean % Recovery				% RSD		
Spiking Levels	TRP	Imp-A	Imp-B	TRP	Imp-A	Imp-B
LOQ Level	99.08	103.26	104.67	1.06	5.28	6.39
80%	99.21	100.25	101.52	0.39	0.25	0.57
100%	99.10	99.96	99.85	0.47	0.18	0.48
120%	99.78	101.14	101.24	0.52	0.105	0.47

3.2.4 Precision

% RSD is used to determine method variability through repeatability and intermediate precision studies. Three levels of concentration were used to assess the precision of TRP (80, 100, and 120 µg/mL) and for impurities A and B (0.8, 1.0, and 1.2 µg/mL). All measurements were performed in six replicates. After samples were analyzed on the same day, the intra-day precision was computed, and inter-day accuracy was assessed, three days in a row. The developed method's satisfactory precision was demonstrated by the % RSD values obtained for both studies being less than 2%. Table 5 displays the percentage peak areas and retention time of TRP and its impurities.

Table 5: Troxipide and its impurities precision data

Parameters	Intra-day (% RSD)			Intra-day (% RSD)		
Number of samples	**TRP**	**Imp-A**	**Imp-B**	**TRP**	**Imp-A**	**Imp-B**
1	0.896	0.821	0.985	0.285	0.148	0.457
2	0.958	0.121	1.108	0.425	0.159	1.102
3	1.021	1.105	0.862	0.632	0.251	0.985

3.2.5 Robustness

The resilience of the analytical method to intentional variations in chromatographic conditions was assessed by robustness. The study results indicated that such deliberate changes did not produce any significant impact on the UPLC performance. As presented in Table 6, the percentage recovery and % RSD values remained within the acceptable limits of 100±1.5% and <2%, respectively.

Table 6: Troxipide and its impurities robustness data (n=3)

Parameters and optimized levels	Changed Level	TRP		Imp A		Imp B	
		%Assay	**%RSD**	**%Assay**	**%RSD**	**%Assay**	**%RSD**
Composition of mobile phase(±1mL) and 75:25 %v/v	74:26 %v/v	99.65	0.31	100.32	1.2	99.78	0.62
	76:24 %v/v	100.35	0.82	101.08	1.38	100.71	1.54

Wavelength (±1nm) and 260 nm	259 nm	101.25	0.85	101.57	0.98	99.85	0.58
	261 nm	100.98	0.69	99.85	0.72	99.71	1.02
Column Temp(±1ºC) and 40ºC	39ºC	100.42	0.45	99.63	0.95	99.92	0.77
	41ºC	99.25	1.29	99.29	1.45	100.09	1.06
Injection Volume (±1µL) and 2 µL	1µL	100.32	1.12	99.21	0.42	100.08	1.102
	3µL	99.75	0.72	99.81	0.91	100.12	1.23

3.2.6 Specificity

The absence of interference from excipients and degradation products was confirmed by specificity. A homogeneous drug peak was indicated by the purity angle being lower than the purity threshold, according to peak purity analysis. This finding confirms that the formulation excipients do not affect the analyte response, thereby establishing the specificity of the method.

3.3 Forced Degradation study

The analysis of stress degradation samples was carried out using previously optimized UPLC conditions with a PDA detector. Under circumstances of thermal, oxidative, photolytic, acidic, and alkaline hydrolysis, TRP showed significant degradation. The chromatograms obtained for the stress-degraded samples are illustrated in Figure 3[a] acid hydrolysis, Figure 3[b] alkaline hydrolysis, Figure 3[c] oxidative, Figure 3[d] photolytic, and Figure 3[e] thermal degradation. The overall degradation behavior of TRP under different stress conditions is summarized in Table 7.

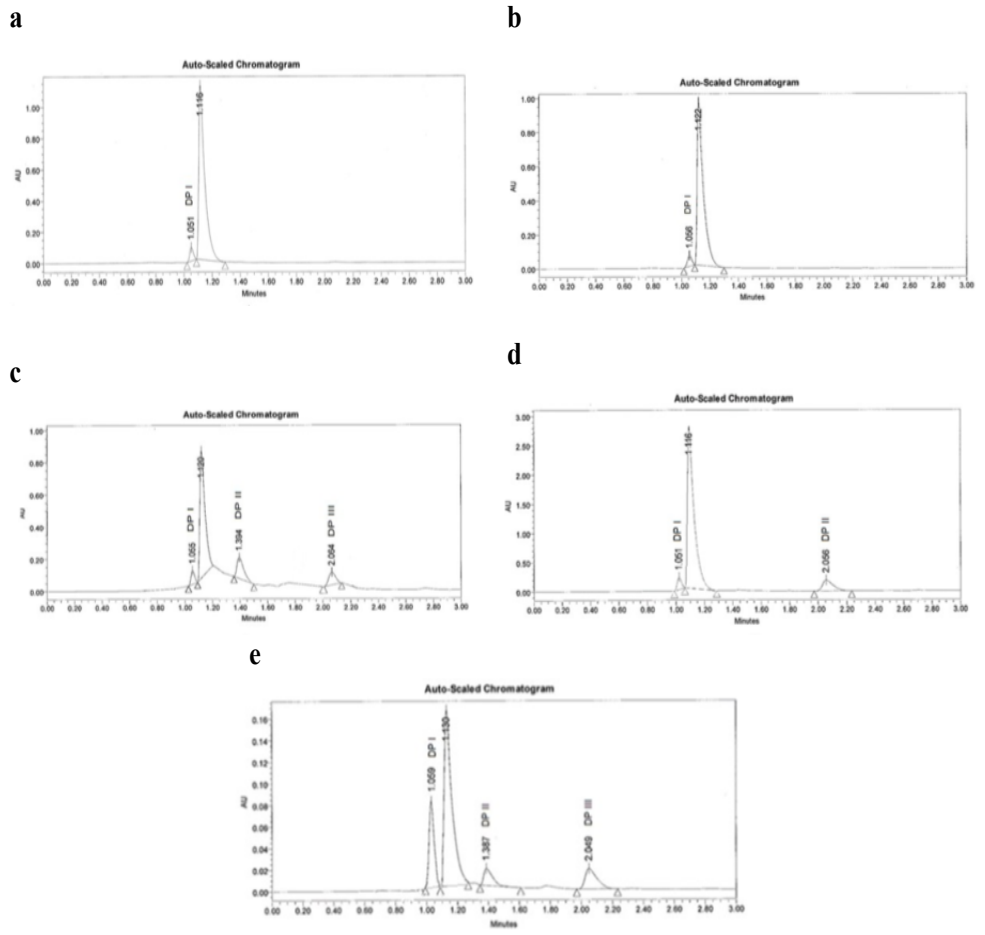

Figure 3: Forced degradation chromatograms [a] Acid hydrolysis, [b] Alkaline hydrolysis, [c] Oxidative degradation, [d] Photolytic degradation, [e] Thermal degradation

Table 7: Summary of stress degradation

Stress Conditions	t_R of Troxipide	t_R of degradation products	% Degradation	Peak Purity Pk Ang, Pk Th.
Acidic hydrolysis	1.116	1.051	15.37	0.038,0.345
Alkaline hydrolysis	1.122	1.056	17.33	0.036,0.324

Oxidative degradation	1.120	1.055,1.394,2.064	18.25	0.041,0.361
Photolytic degradation	1.116	1.051,2.056	10.94	0.032,0.315
Thermal Degradation	1.130	1.059,1.387,2.049	16.74	0.034,0.318

DP I was found to be eluted at Retention time 1.051 min, which exhibited molecular ion peak at 253.13 amu $(M+H)^+$ (Figure 4 (a)). The degradation product DP II was found to be eluted at Retention Time 1.387 min, which exhibited molecular ion peak at 393 amu (Figure 4 (b)). Similarly, degradation product DP III was found to be eluted at RT 2.049 min, which exhibited its molecular ion peak at 218 amu (Figure 4 (c)).

From this LC/MS data, the probable structures with the fragmentation pattern of these DP I (Figure 5), DP II (Figure 6), and DP III (Figure 7) were proposed.

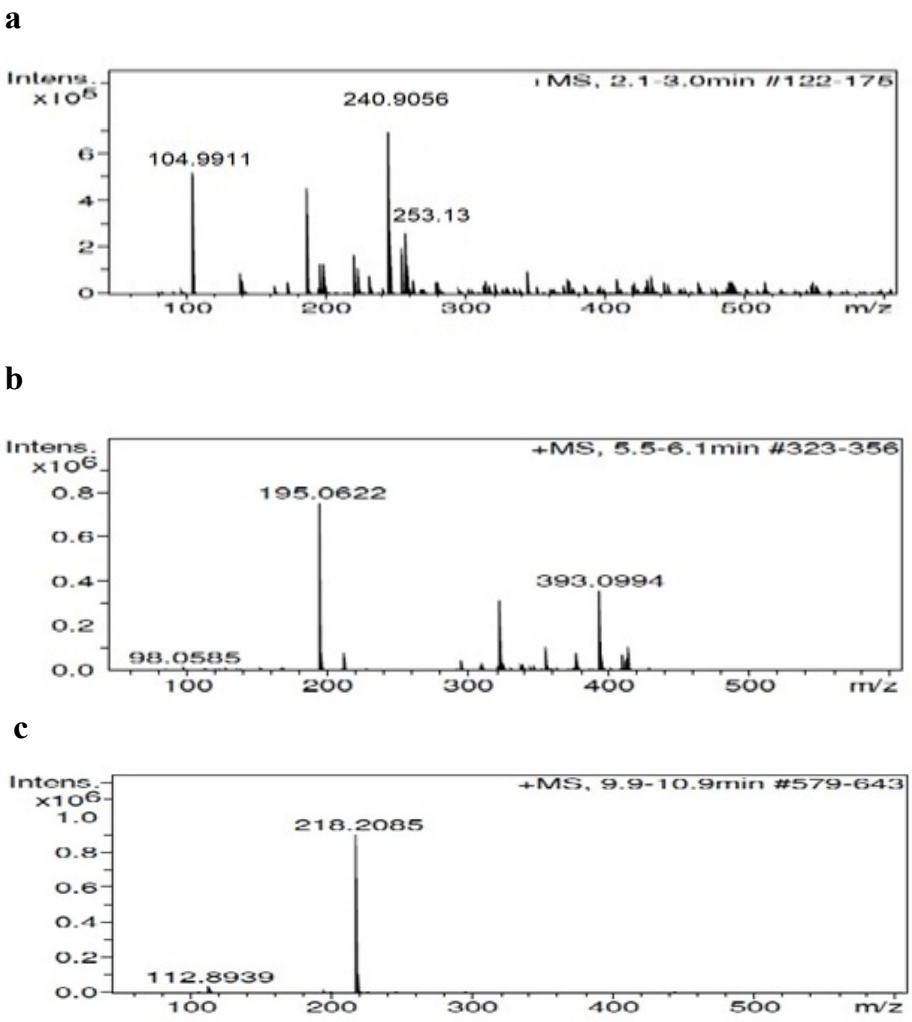

Figure 4: DP's (a) DPI (b) DP II (c) DP III MS/TOF spectra

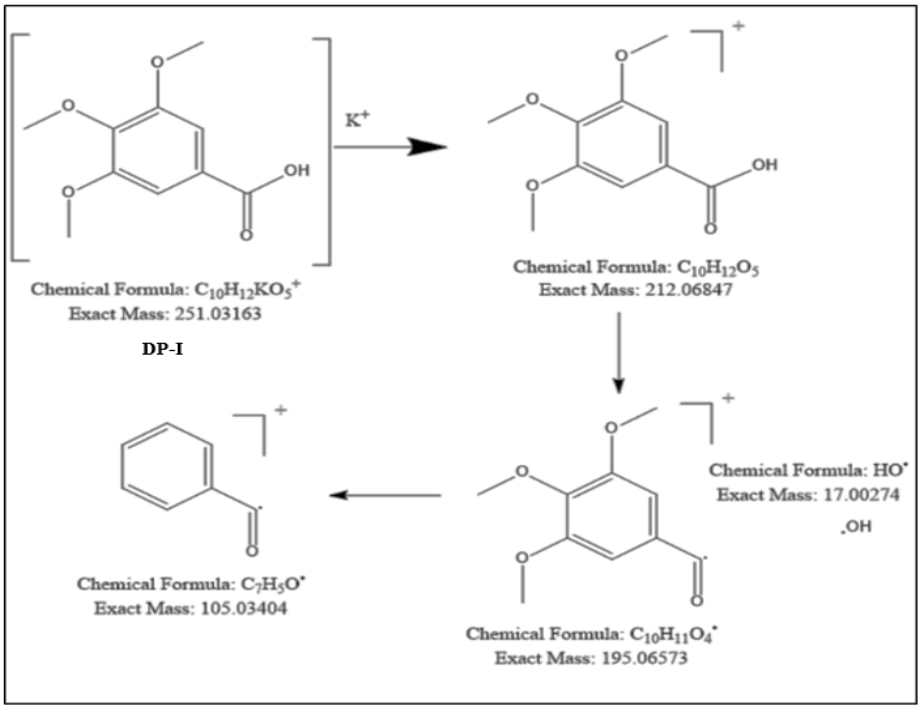

Figure 5: Acid hydrolysis of DP I proposed fragmentation pattern with m/z=251.03

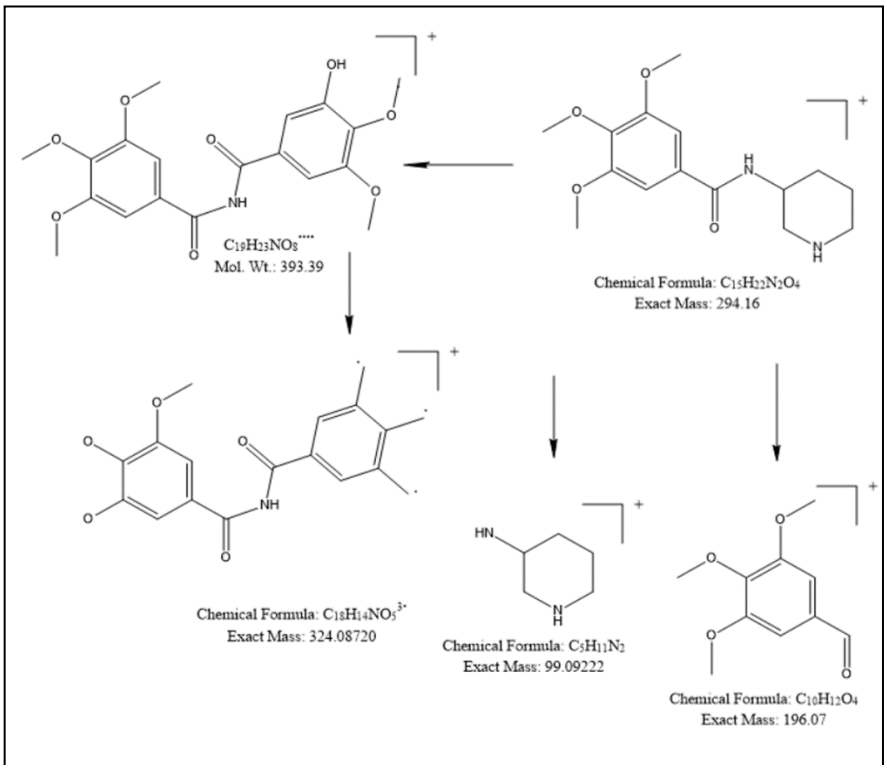

Figure 6: Oxidative proposed fragmentation scheme of DP II (m/z=393.39)

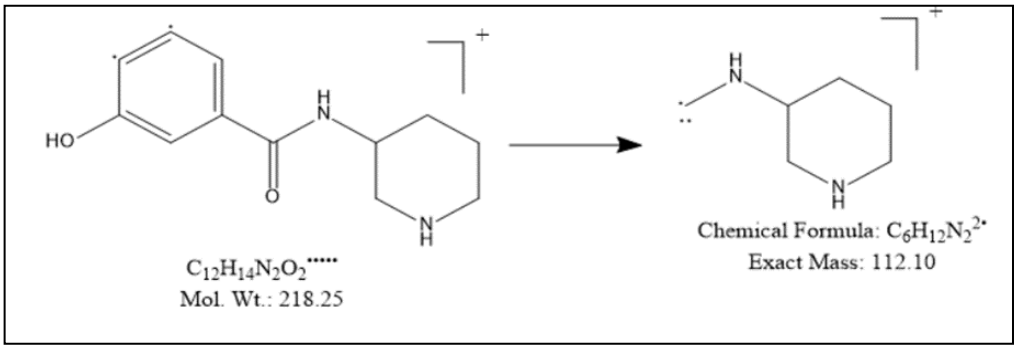

Figure 7: Oxidative proposed fragmentation scheme of DP III (m/z=218.25)

3.4 Evaluation utilizing green analytical metric tools

Using green assessment tools such as Analytical Eco-Scale, Green Analytical Procedure Index (GAPI), and AGREEprep, the environmental impact of the developed analytical method was evaluated by comparing it with previously reported methods. The chromatographic method employed ammonium acetate buffer (pH 3) and acetonitrile as the mobile phase, using a column of 2.1 mm × 100 mm with a particle size of 1.7 µm. Under optimized conditions, TRP, along with Impurity A and Impurity B, was analyzed within a total runtime of 5 minutes. Greenness evaluation using AGREEprep yielded a score of 0.6 (Figure 8a). The GAPI assessment provided a pictorial representation of the environmental impact of each analytical step, where the red zone is due to solvents/reagents, waste generation, and waste collection, while the remaining parameters ranged from yellow to green. The GAPI index obtained was 81 (Figure 8b). Further evaluation using the Analytical Eco-Scale considered factors such as reagent consumption, instrument energy use, and waste generation. The method resulted in a penalty score of 18, corresponding to an Eco-Scale score of 82, indicating excellent greenness.

(a)

(b)

Figure 8. Greenness assessment of the developed analytical method using (a) AGREEprep and (b) Complex GAPI metrics

4 CONCLUSION

For Troxipide impurity profiling and the detection and quantification of degradation-related impurities, the developed UPLC approach was demonstrated to be simple, specific, accurate, and precise. Troxipide is vulnerable to deterioration under oxidative, hydrolytic, thermal, and photolytic conditions, with the highest extent of degradation observed under oxidative stress.

To further characterize the degradation products formed under these conditions, the method was successfully extended to LC-MS analysis. Based on the obtained mass fragmentation patterns and m/z values, the probable structures of three major degradation products were elucidated. Overall, the optimized method proved to be robust and reliable and can be effectively applied as a quality control tool for routine analysis and stability assessment of Troxipide.

ACKNOWLEDGEMENTS

The authors are thankful to “Serum Institute of India, Pune” for providing the facility of UPLC and also to “central Instrumentation Facility (CIF), Savitribai Phule Pune University for providing UPLC-MS facility”.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Japanese Pharmacopoeia. 15th ed. Tokyo: Ministry of Health, Labour and Welfare; 2009. 2208–10.
2. Troxip OD prescribing information [Internet]. Available from: https://troxip.wordpress.com/prescribing-information/troxip-od/
3. Aparajitha K., Shankarananth V., Nagamallika L., Jyosthna P., Narmada D., Padmavathamma M. Spectrophotometric And RP-HPLC Methods For the Estimation of Troxipide, J. Pharm. Res. 2012;5:2814-7.
4. Labana SM, Hamrapurkar PD, Jain AP, Madrewar DP, Sonandkar AA. Development And Validation of Stability Indicating RP-HPLC Method For Troxipide from Bulk Drug. Int. j. res. pharm. biomed. sci. 2013;4: 1179-86.
5. Gupta M. and Mallah V. A Stability Indicating RP-HPLC Method for the determination of Troxipide in Bulk and Commercial Formulation. Der Pharm. Lett. 2012; 4:1376-85.
6. Pulla RP, Sastry BS, Prasad RE, Raju NA. Estimation of troxipide in tablet dosage form by RP-HPLC. Int. J. Pharm. Pharm. Sci. 2011;3: 316-8.
7. Gowrisankar D, Rao NM. Development and Validation of an Improved Stability-Indicating RP-HPLC Method for the estimation of Troxipide in Bulk and Tablet Dosage Forms. Indian Drugs. 2015 ;52(07):07.
8. Gajbhar AV, Kuchekar BS. Development and Validation of Stability Indicating HPTLC Method For Determination of Troxipide. Int. J. Pharm. Sci. 2016;7(1):300-6. DOI: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (1).300-06
9. International Conference on Harmonisation (ICH). Q3A(R2): Impurities in new drug substances; 2006.
10. International Conference on Harmonisation (ICH). Q3B(R2): Impurities in new drug products; 2006.
11. Görög S, editor. Identification and determination of impurities in drugs. Amsterdam: Elsevier; 2000.
12. International Conference on Harmonisation (ICH). Q2(R1): Validation of analytical procedures: text and methodology; 2005.
13. International Conference on Harmonisation (ICH). Q1A(R2): Stability testing of new drug substances and products; 2003.
14. International Conference on Harmonisation (ICH). Q1B: Photostability testing of new drug substances and products; 1996
15. Thakker NM, Choudhari VP, Kuchekar BS, Panchal HB, Rakholiya DR, Murugan R. Development and validation of a stability indicating RP-HPLC method for simultaneous estimation of Olmesartan Medoxomil and Metoprolol Succinate in pharmaceutical dosage form. Pharm. Methods. 2012 ;3(2):84-9. DOI: 10.4103/2229-4708.103880.