Identification and Structural Elucidation of Forced Degradation Impurities in Agrochemical Technical by LC–MS

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Abstract

Understanding degradation pathways and impurity formation is critical for maintaining the quality, safety, and regulatory compliance of agrochemical active ingredients. In the present study, the forced degradation behavior of chlorantraniliprole technical material was systematically investigated under acidic, alkaline, oxidative, thermal, UV, and photolytic stress conditions using a UPLC–single quadrupole liquid chromatography–mass spectrometry (LC–MS) approach. Chromatographic separation was achieved using a short C18 column with a low-flow gradient system, enabling rapid analysis with minimal solvent consumption LC–MS analysis of the unstressed sample revealed only two trace-level impurities, indicating good inherent stability of the technical material. Significant degradation was observed exclusively under alkaline conditions, resulting in the formation of a major degradation product with an observed molecular ion at m/z 469.12 Based on accurate mass measurements and comparison with reported impurity data, this product was tentatively identified as impurity-3, suggesting a base-catalyzed degradation pathway. Comparative evaluation of LC–UV and LC–MS detection demonstrated the superior sensitivity of mass spectrometric analysis for identifying low-level degradation products with limited UV response.

The environmental performance of the analytical method was evaluated using the Green Analytical Procedure Index (GAPI), revealing a high degree of greenness due to low solvent consumption, short run time, ambient temperature operation, minimal sample preparation, and complete solvent waste recycling. Overall, the study provides a robust and environmentally sustainable LC–MS-based strategy for impurity profiling and forced degradation assessment of chlorantraniliprole technical material.

Keywords: Chlorantraniliprole; Forced degradation; Degradation impurities; LC–MS; Structural elucidation; Green analytical chemistry; GAPI

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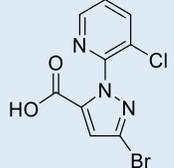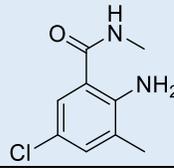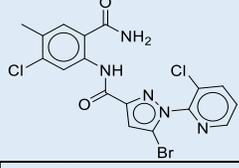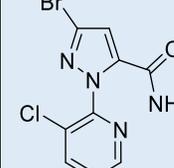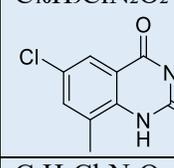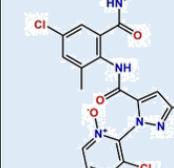Email: drgopalarora.chem@sanskriti.edu.in

Introduction

Chlorantraniliprole is a widely used anthranilic diamide insecticide recognized for its high efficacy against lepidopteran pests and comparatively favorable toxicological profile [1, 2]. Due to its extensive agricultural application, detailed understanding of its chemical stability, degradation behavior and impurity profile is essential to ensure product quality, environmental safety, and regulatory compliance [3].

During manufacture, storage, and application, chlorantraniliprole may undergo chemical or photochemical transformations, leading to the formation of degradation products and related impurities. Several studies have reported its environmental dissipation and transformation pathways in soil, water, and crop matrices, demonstrating the formation of multiple transformation products under different stress conditions [4-6] However, these studies primarily focus on environmental fate and residue analysis rather than systematic impurity profiling of the technical material.

The main purpose of these experiments is to understand how the drug breaks down under stressful conditions and which degradation products are formed from it. In this process, active substances are subjected to various types of stress conditions, such as: Hydrolytic condition – reaction with water Oxidative condition – oxidation Thermal condition – high temperature Photolytic condition – effect of light These tests are conducted according to regulatory and analytical guidelines. LC–MS (Liquid Chromatography–Mass Spectrometry) is an advanced analytical technique that works by combining two techniques. In this, various compounds are separated with the help of liquid chromatography. After this, the identification and structural analysis of these compounds are done using mass spectrometry. This technique is considered very effective due to its high sensitivity, greater selectivity, and ability to identify the structure of unknown compounds [7]. The degradation products formed under all these conditions were analyzed using UPLC–Single Quadrupole LC–MS technique. The main objective of this study is to examine the stability of the technical substance chlorantraniliprole. For this, the forced degradation behaviour of the drug was systematically studied. In the study, the

Table 1 Chlorantraniliprole Impurities

No.	Compound / Impurity Name	CAS	Molecular Formula and Chemical Structure	Average molecular weight (g/mol)
1	Chlorantraniliprole – 3-bromo-N-[4-chloro-2-methyl-6-(methylcarbamoyl)phenyl]-1-(3-chloro-pyridin-2-yl)-1H-pyrazole-5-carboxamide	500008-45-7 [10]	$C_{18}H_{14}BrCl_2N_5O_2$	483.15
2	Impurity 1 – 3-Bromo-1-(3-chloro-pyridin-2-yl)-1H-pyrazole-5-carboxylic acid	500011-86-9 [10]	$C_9H_5BrClN_3O_2$	300.93
3	Impurity 2 – 2-Amino-5-chloro-N,3-dimethylbenzamide	89007-28-5 [11]	$C_9H_{11}ClN_2O$	198.65
4	Impurity 3 – 3-Bromo-N-(2-carbamoyl-4-chloro-6-methylphenyl)-1-(3-chloropyridin-2-yl)-1H-pyrazole-5-carboxamide	1006621-50-6 [14]	$C_{17}H_{12}BrCl_2N_5O_2$	469.12
5	Impurity 4 – Des-[N-(4-chloro-2-methyl-6[((methylamino)carboyl))phenyl]] Chlorantraniliprole	1438853-57-6 [10]	$C_9H_6BrClN_4O$	301.52
6	Impurity 5 – 6-Chloro-3,8-dimethylquinazoline-2,4(1H,3H)-dione	2309733-25-1 [11]	$C_{10}H_9ClN_2O_2$	224.64
7	Impurity 6 – 3-Chloro-1-(3-chloropyridin-2-yl)-1H-pyrazole-5-carboxylic acid	458543-79-8 [12]	$C_9H_5Cl_2N_3O_2$	256.98
8	Impurity-7-N-oxide – Pyridine N-oxide derivative	[13]	$C_{18}H_{14}BrCl_2N_5O_3$	499.00

9	Impurity-8-Dechlorinated Pyrazole Fragment – bromopyrazole amide variant	[14]	$C_9H_5BrClN_3O$	283.00
10	Impurity-9-Halogen-exchanged Synthetic Residual – related intermediate	[15]	$C_{16}H_{12}Cl_2N_4O_2$	350.00
11	Impurity-10-Benzo[d][1,3]oxazin-4-one derivative (2-(3-Bromo-1-(3-chloropyridin-2-yl)-1H-pyrazol-5-yl)-6-chloro-8-methyl-4H-benzo[d][1,3]oxazin-4-one)	500011-87-0* [16]	$C_{17}H_9BrCl_2N_4O_2$	452.09
*CAS entry for benzo[d][1,3]oxazin-one derivative is listed in supplier impurity catalogs.				

substance was subjected to various stress conditions, such as: Acidic., Alkaline. Oxidative. Thermal UV light Normal light or photolytic conditions The degradation products formed under all these conditions were analyzed using UPLC–Single Quadrupole LC–MS technique. The objective of the study was also to sensitively identify the degradation impurities and determine their initial structure. Along with this, a comparative evaluation of the products formed under different conditions was also conducted. In the study, the Green Analytical Procedure Index (GAPI) [8,9] was also used to examine how safe and sustainable (eco-friendly) the analytical method used in the experiment is for the environment. The objective of the study was also to sensitively identify the degradation impurities and determine their initial structure. Along with this, a comparative evaluation of the products formed under different conditions was also conducted. In the study, the Green Analytical Procedure Index (GAPI) was also used to examine how safe and sustainable (eco-friendly) the analytical method used in the experiment is for the environment.

The main objective of this study is to examine the stability of the technical substance chlorantraniliprole. For this, the forced degradation behavior of the drug was systematically studied. In the study, the substance was subjected to various stress conditions, such as: Acidic., Alkaline. Oxidativ. Thermal UV light Normal light or photolytic conditions The degradation products formed under all these conditions were analyzed using UPLC–Single Quadrupole LC–MS technique. The objective of the study was also to sensitively identify the degradation impurities and determine their initial structure. Along with this, a comparative evaluation of the products formed under different conditions was also conducted.

In the study, the Green Analytical Procedure Index (GAPI) was also used to examine how safe and sustainable (eco-friendly) the analytical method used in the experiment is for the environment.

The novelty of the present work lies in the application of a UPLC–single quadrupole LC–MS approach for systematic impurity profiling and forced degradation assessment of chlorantraniliprole technical material. Unlike previously reported LC–UV methods, the developed method enables sensitive detection and tentative structural identification of degradation products through molecular ion information without requiring impurity reference standards. Additionally, the method combines rapid UPLC separation with low solvent consumption, providing a practical, cost-effective, and environmentally sustainable strategy for routine agrochemical impurity analysis.

Research Methodology
Materials and Reagents
Chemicals and Reagents

Chlorantraniliprole technical material (purity ≥ 98.0%) was sourced from Crystal Crop Protection Limited (India) and used without further purification. All chemicals and reagents used in the study, including hydrochloric acid, sodium hydroxide, and hydrogen peroxide (30% w/v), were of analytical reagent grade and supplied by S.D. Fine Chem Limited (India).

HPLC-grade acetonitrile and water were used for chromatographic analysis, and formic acid (LC–MS grade) was employed as a mobile-phase additive. Purified water was prepared using a laboratory water purification system and used throughout the study. (Prepared by using PURELAB Quest water purification system)

Instrumentation

Chromatographic analysis was performed using a Waters UPLC system coupled with a single quadrupole mass spectrometer (Waters SQD2). The system consisted of a binary pump, autosampler, column oven, and PDA detector. Mass spectrometric detection was carried out using an electrospray ionization (ESI) source operated in both positive and negative ionization modes.

Chromatographic and Mass Spectrometric Conditions

Separation was achieved using an Acquity BEH C18 column (50 mm × 2.1 mm, 1.7 μm). Mobile phase A consisted of 0.1% formic acid in water and mobile phase B consisted of 0.1% formic acid in acetonitrile. A gradient elution was employed at a flow rate of 0.25 mL min^{-1}. The column temperature was maintained at 35 °C and the injection volume was 5 μL.

Mass spectra were acquired over an m/z range of 80–800 with a cone voltage of 20 V. Data acquisition was performed in full-scan mode, and MS/MS experiments were conducted where necessary for structural interpretation.

Forced Degradation Conditions

Forced degradation studies were conducted to evaluate the stability of chlorantraniliprole under different stress conditions:

    Acidic   : 0.1 N HCl
    Alkaline   : 0.1 N NaOH
    Oxidative   : 3% H_2O_2
    Thermal   : 105 °C for 24 Hrs
    UV   : 254 nm for 200 W·h/m²
    Photolytic   : Sunlight exposure for 1.2 million lux·h

All samples were maintained for 24 h at 25 degrees Celsius and analysed at 0 h (as a control sample) and 24 h (as a degraded sample). The respective solutions from acidic and alkaline degradation were neutralized to quench the reactions before final dilution.

Sample Preparation

Degraded as well as control samples were dissolved in 100% acetonitrile, sonicated to ensure complete dissolution (100 ppm sample concentration), and filtered through 0.22 μm PTFE filters prior to injection. No impurity standards were used; identification of known impurities was performed using reported molecular weights and molecular formulas as references in LC–MS analysis.

### Green Analytical Procedure Index (GAPI) Evaluation

The environmental sustainability of the analytical procedure for forced degradation and impurity profiling of chlorantraniliprole was evaluated using the Green Analytical Procedure Index (GAPI). The method employs UPLC coupled with a single quadrupole mass spectrometer using a short column (50 mm), a low flow rate (0.25 mL min^{-1}), and a short run time (12 min), resulting in low solvent consumption of approximately 3.0 mL per analysis. Sample preparation involved only simple dissolution without derivatization or clean-up, and chromatographic separation was performed at ambient temperature, minimizing energy demand. The mobile phase consisted of water and acetonitrile with 0.1% formic acid; although acetonitrile is a hazardous solvent, its impact was mitigated by low usage and complete collection and recycling of all liquid waste. The use of a single quadrupole detector resulted in reduced energy consumption. Furthermore, due to the unavailability of reference standards for impurities, the usage of chemicals also had to be minimized. Consequently, this approach is considered to be more environmentally friendly. Overall, this method demonstrates good environmental compatibility and, in accordance with GAPI principles, can be classified as a green analytical process.

Result and Discussion

LC–MS analysis revealed that in the unstressed chlorantraniliprole technical material, apart from the parent compound, only two very low-level impurities (trace impurities) were present. These impurities were tentatively identified as Pyridine N-oxide derivative Impurity-7. This indicates that the intrinsic stability of chlorantraniliprole is good. In LC–UV analysis, these impurities were not clearly visible or well-separated. However, they were easily identified by LC–MS technique, demonstrating the high sensitivity of this technique. Forced degradation study showed that chlorantraniliprole remains stable under acidic, oxidative,thermal, UV, and photolytic conditions. A significant degradation product was formed under alkaline conditions. The molecular ion (m/z) of this degradation product was found to be 469.12, and it was observed as the major degradation product. This degradation product with m/z 469.12 was tentatively identified as impurity

Table 2 Combined GAPI and AGREE Evaluation of the UPLC–MS Method [8.9]

Analytical Aspect	Method Characteristic	GAPI Assessment	AGREE Principal Alignment
Sample collection	Solid technical material; no field sampling	◉ Green	Waste prevention
Sample preparation	Simple dissolution; no extraction or derivatization	◉ Green	Minimal sample handling
Solvent consumption	0.25 mL min^{-1}, 12 min run (≈3.0 mL per run)	◉ Green	Reduced solvent use
Solvent type	Water–acetonitrile with 0.1% formic acid	◉ Acceptable	Safer solvents (partial)
Buffer salts	No non-volatile buffers used	◉ Green	MS-compatible reagents
Instrumentation	UPLC with single quadrupole MS	◉ Acceptable	Energy-efficient instrumentation
Run time	12 minutes per injection	◉ Green	High throughput
Energy consumption	Ambient temperature operation	◉ Green	Energy efficiency
Waste generation	Only liquid waste generated	◉ Acceptable	Waste minimization
Waste management	100% solvent waste collected and recycled	◉ Green	Waste recycling
Reference standards	No impurity standards used	◉ Green	Reduced chemical usage
Operator safety	Fully enclosed LC–MS system	◉ Green	Safer working conditions
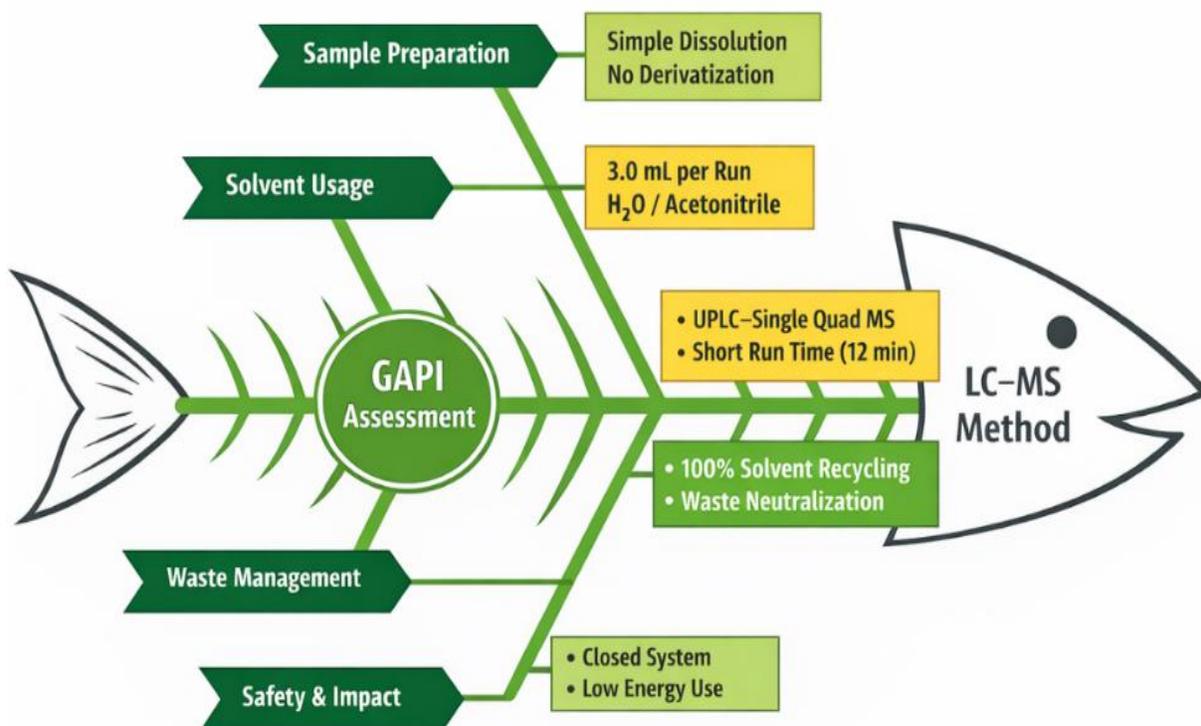
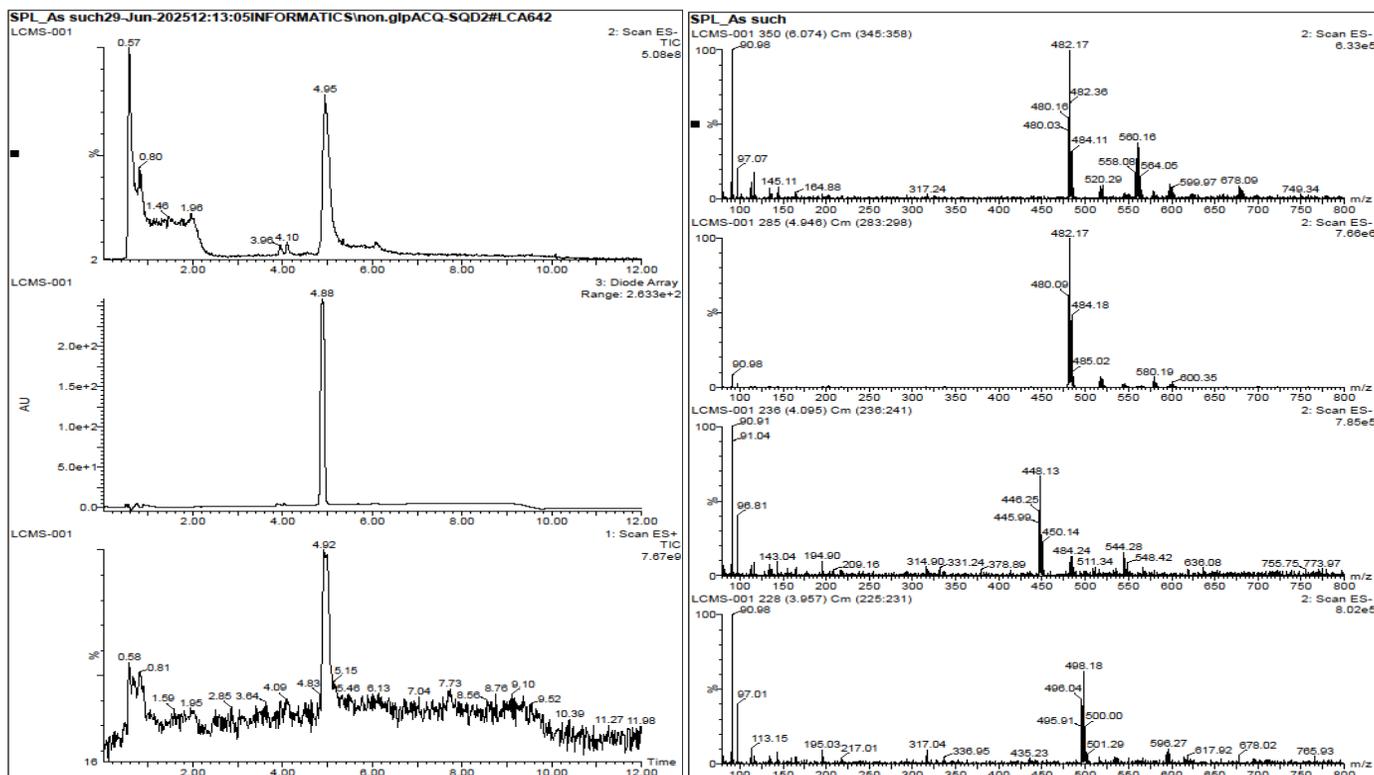**Figure 1** LC-MS GAPI assessment diagram

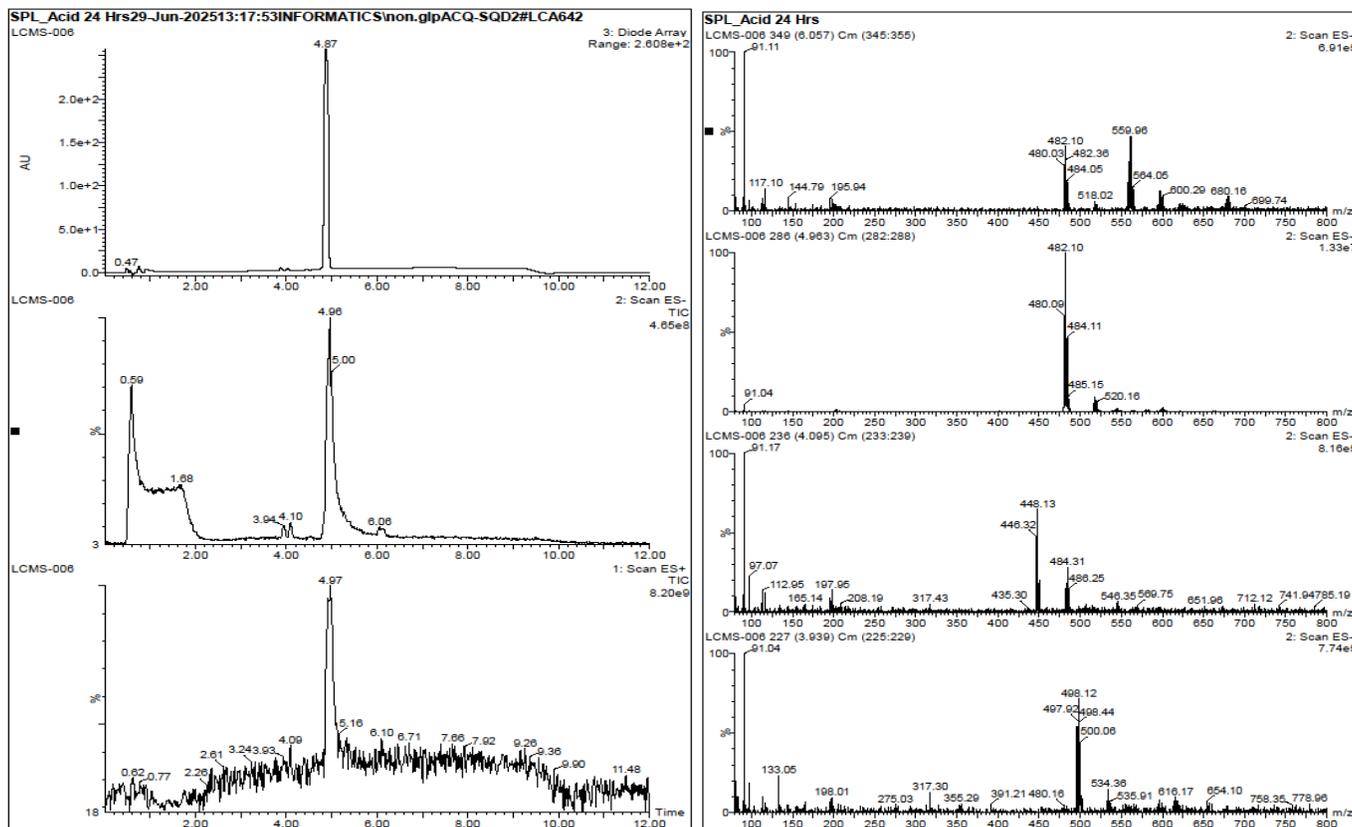**Figure 2** LC–UV and MS Spectrum of Control (Unstressed) Chlorantraniliprole Sample

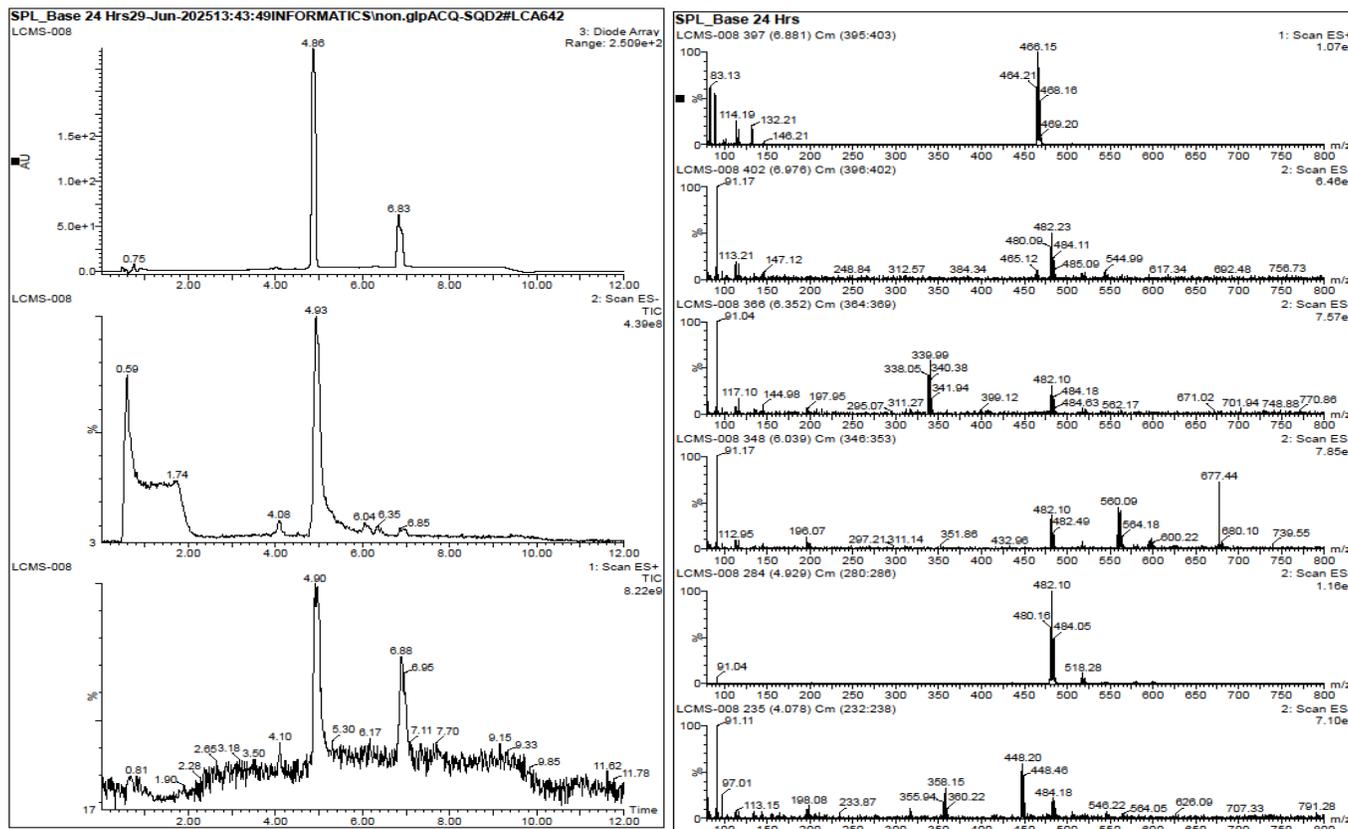**Figure 3** LC–UV and MS Spectrum after Acidic Degradation (24 h)

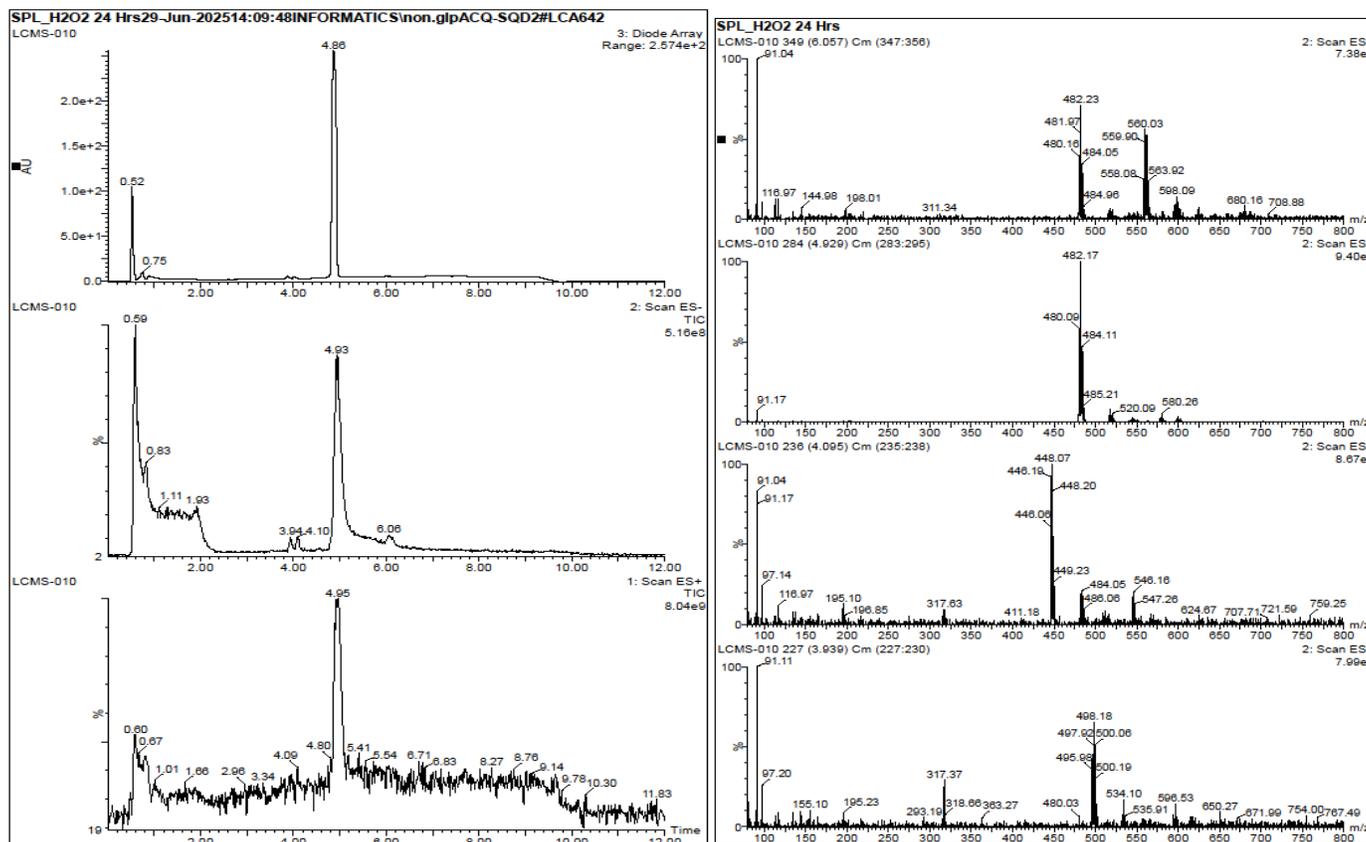**Figure 4** LC–UV and MS Spectrum after Alkaline Degradation (24 h)

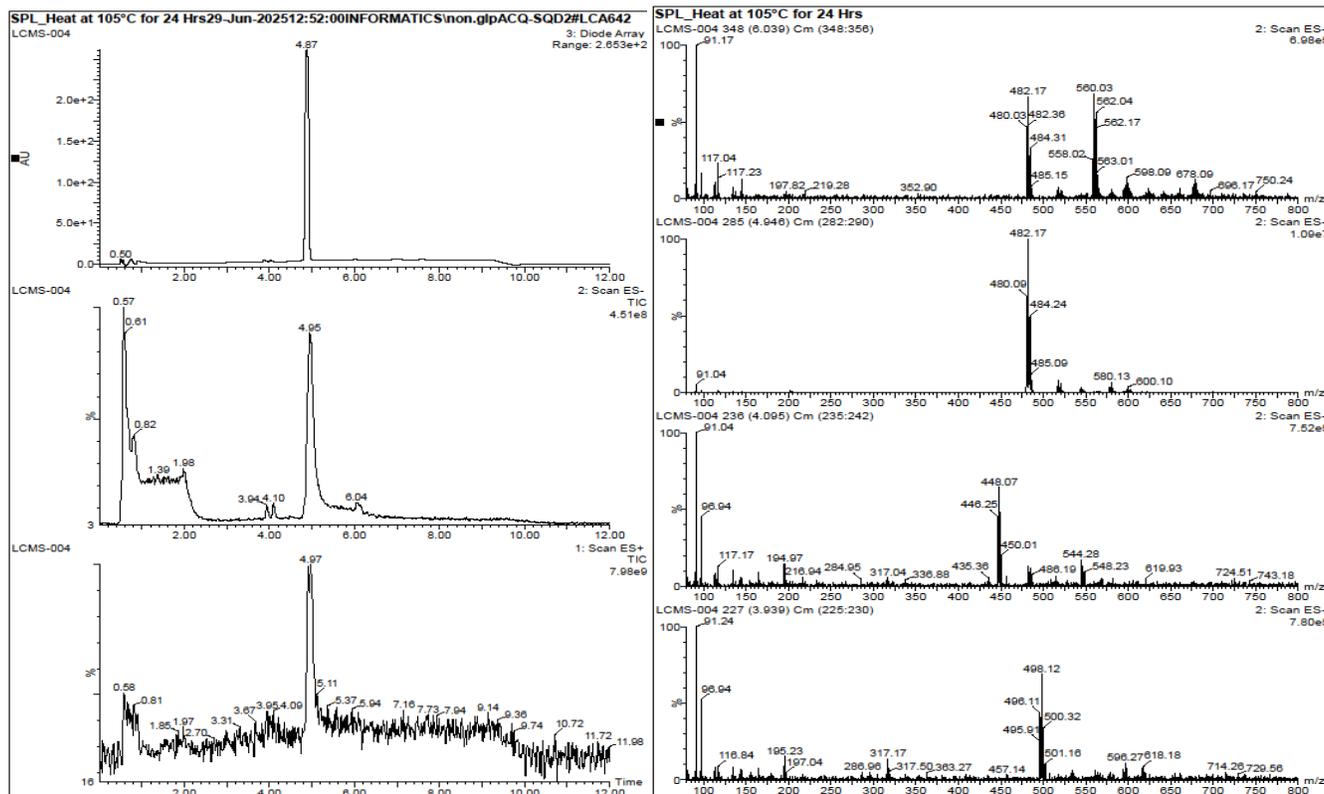**Figure 5** LC–UV and MS Spectrum after Oxidative Degradation (24 h)

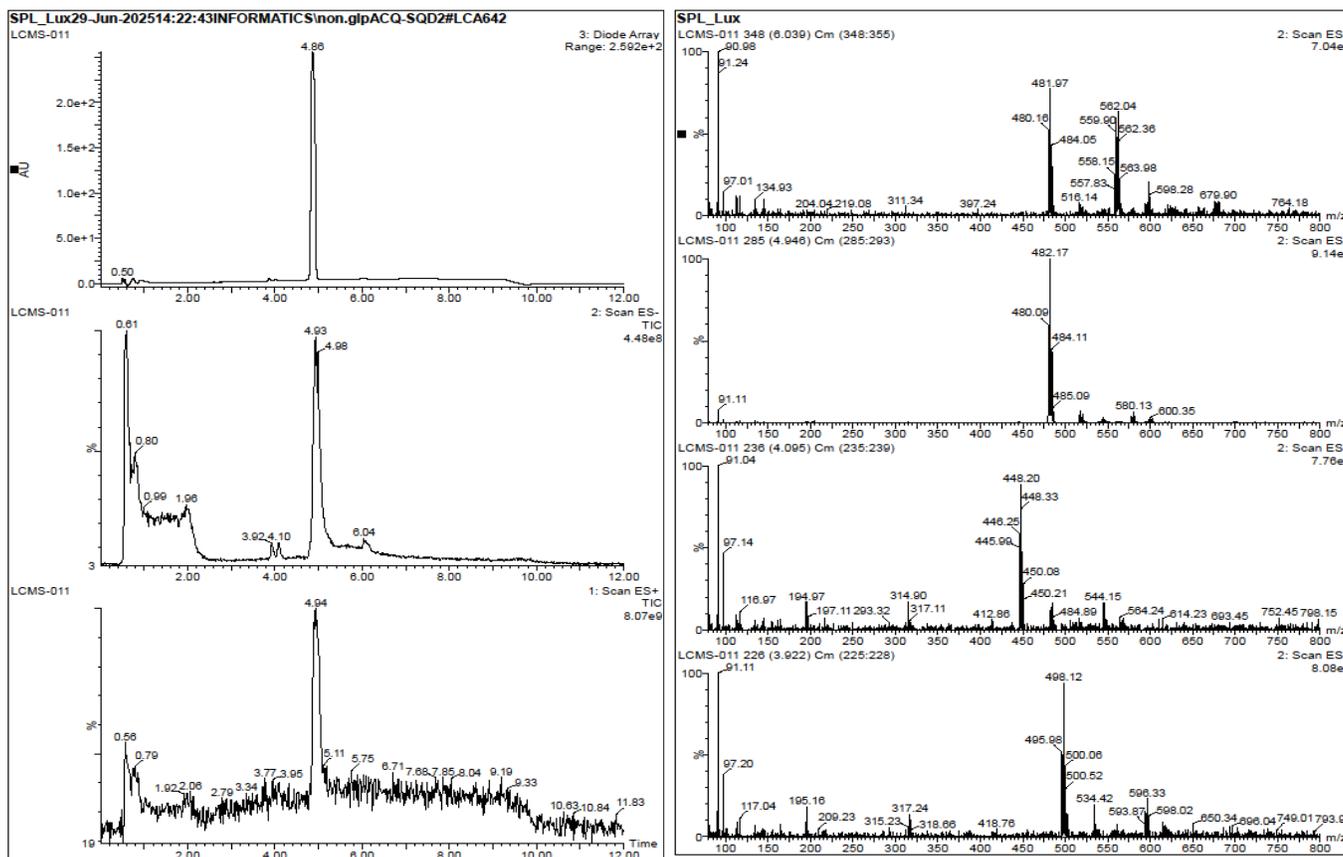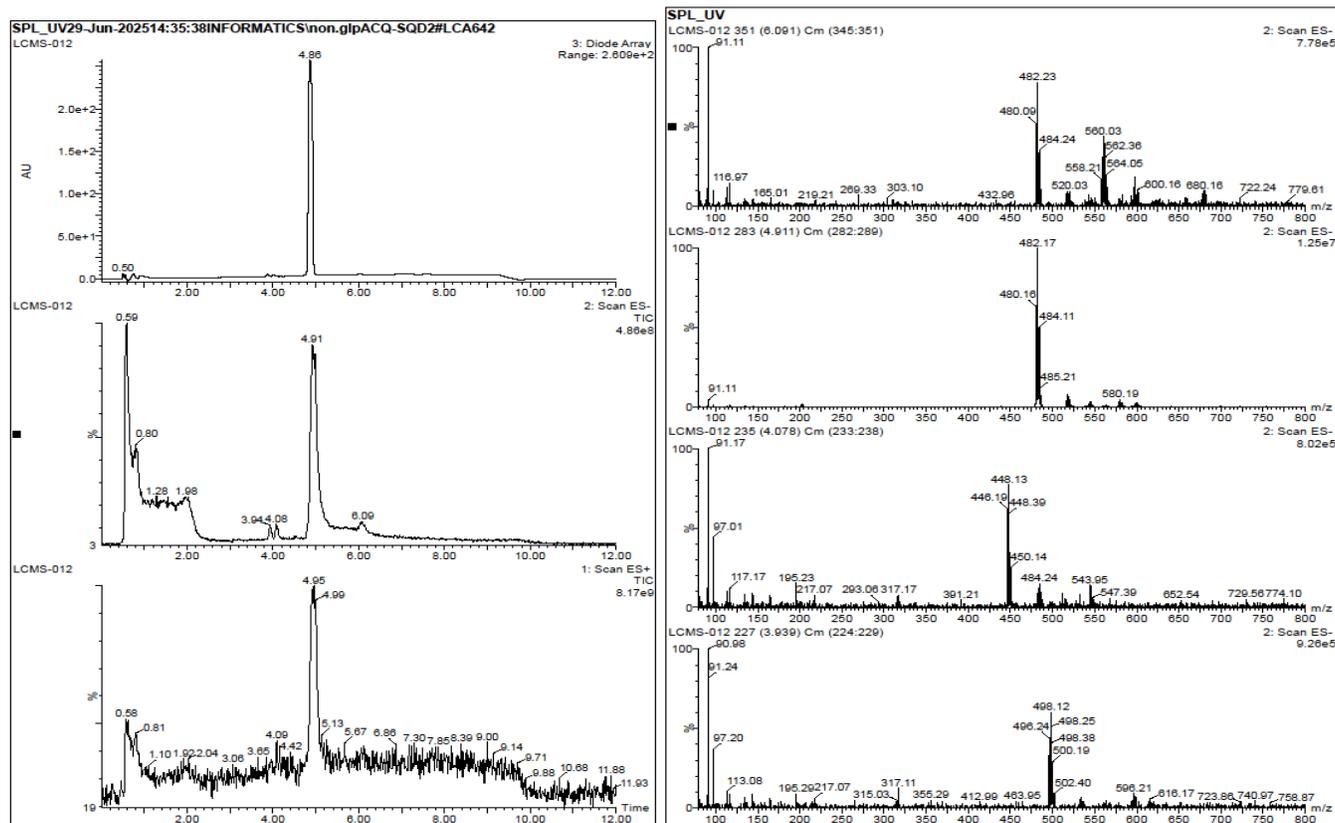**Figure 6** LC–UV and MS Spectrum after Thermal Degradation (24 h)

Figure 7 LC–UV and MS Spectrum after Photolytic Degradation (24 h)

Figure 8 LC–UV and MS Spectrum after UV Degradation (24 h)

Conclusion

LC–MS analysis revealed that in the unstressed chlorantraniliprole technical material, apart from the parent compound [17], only two very low-level impurities (trace impurities) were present. These impurities were tentatively identified as Pyridine N-oxide derivative Impurity-7. This indicates that the intrinsic stability of chlorantraniliprole is good. In LC–UV analysis, these impurities were not clearly visible or well-separated. However, they were easily identified by LC–MS technique [18-20], demonstrating the high sensitivity of this technique. Forced degradation study showed that chlorantraniliprole remains stable under acidic, oxidative, thermal, UV, and photolytic conditions. A significant degradation product was formed under alkaline conditions. The molecular ion (m/z) of this degradation product was found to be 469.12, and it was observed as the major degradation product. This degradation product with m/z 469.12 was tentatively identified as impurity

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