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Advanced LC-MS/MS-Based Impurity Profiling And Forced Degradation Characterization Of Sulfamethoxazole And Clindamycin In Combined Pharmaceutical Formulations

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***Abstract*:**
Background: Forced degradation and impurity profiling are essential steps in determining the stability and safety of pharmaceutical compounds. This study evaluates the degradation behaviour and impurity profiling of Sulfamethoxazole and Clindamycin under ICH-recommended stress conditions using LC-MS/MS.
Methodology: Stress degradation was performed under acidic, basic, oxidative, thermal (wet heat), and photolytic (UV) conditions. High-resolution LC-MS/MS analysis was used to identify the parent compounds and associated degradation products. Validation studies including linearity, accuracy, robustness, and ruggedness were conducted to ensure method reliability.
Results: Maximum degradation was observed under basic conditions (8.97%). Acidic, oxidative, and thermal stress also produced distinct degradation peaks. UV exposure led to unexpected assay gain (-4.66%) due to matrix concentration effects. MS/MS spectra confirmed multiple degradation products with mass accuracy <5 ppm. Validation showed linear response ($R^2 > 0.999$) across tested concentrations, high accuracy (98–102%), and robustness under varied method conditions.
Conclusion: The developed LC-MS/MS method reliably identified degradation patterns and impurity profiles of Sulfamethoxazole and Clindamycin. The method is robust, accurate, and suitable for regulatory applications, quality assurance, and impurity investigations.

Keywords: *Sulfamethoxazole, Clindamycin, LC-MS/MS, Forced degradation, Impurity profiling, Stability-indicating method*

1. INTRODUCTION

The stability and safety of pharmaceutical compounds are fundamental to regulatory compliance and therapeutic efficacy. Among sulfonamide antibiotics, Sulfamethoxazole is widely used in combination therapies, particularly with Clindamycin or Trimethoprim. Both drugs are known to undergo degradation under stress conditions, which may yield unknown impurities affecting safety profiles.

Mass spectrometry (MS), particularly tandem MS/MS, enables accurate identification of degradation products and molecular ions based on fragmentation patterns and elemental composition. It plays a crucial role in impurity profiling, degradation behavior analysis, and structural confirmation of drug substances.This study aims to characterize the degradation profile of Sulfamethoxazole and Clindamycin under different ICH-recommended stress conditions. We further validate the analytical method by evaluating its linearity, accuracy, robustness, and ruggedness, ensuring it meets regulatory expectations for stability-indicating methods.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Sulfamethoxazole and Clindamycin working standards were obtained from certified pharmaceutical suppliers with ≥99% purity (Certificate of Analysis provided). Analytical grade reagents including hydrochloric acid (HCl), sodium hydroxide (NaOH), and hydrogen peroxide (H_2O_2, 30%) were purchased from Merck (India). Acetonitrile (HPLC grade), methanol, and water (Milli-Q, ultrapure) were used for mobile phase preparation and sample dilutions. All solutions were filtered through a 0.22 µm nylon filter prior to use.

2.2 Instrumentation and Analytical Conditions

Forced degradation profiling and impurity analysis were conducted on a high-resolution Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS) system equipped with an Electrospray Ionization (ESI) source, a triple
quadrupole detector, and autosampler.

Chromatographic Separation:
Column: Waters Acquity UPLC BEH C18 (50 mm × 2.1 mm, 1.7 µm)
Mobile Phase:
Solvent A: 0.1% formic acid in water
Solvent B: Acetonitrile with 0.1% formic acid
Flow Rate: 0.3 mL/min
Injection Volume: 5 µL
Column Temperature: 40 ± 2°C
Run Time: 12 minutes
Detection Wavelength: 254 nm (for reference UV chromatograms
MS/MS Conditions:
Ionization Mode: Positive ESI
Scan Type: Full Scan and Product Ion Scan
Source Temperature: 150°C
Desolvation Temperature: 350°C
Capillary Voltage: 3.0 kV
Cone Voltage: 35 V
Collision Energy: Optimized (10–30 eV depending on precursor ion)

2.3 Preparation of Stock and Working Standards

Primary stock solutions of Sulfamethoxazole and Clindamycin were prepared at 1000 µg/mL in methanol. Working standard solutions were prepared by serial dilution to obtain concentrations ranging from 10 to 100 µg/mL for linearity and quantitation studies

2.4 Forced Degradation Studies

Forced degradation was carried out in compliance with ICH Q1A(R2) guidelines to evaluate the intrinsic stability and degradation pathways of the drug substances.

Stress Conditions Applied:
Acid Hydrolysis: 0.1N HCl, 3 hours at 60°C
Base Hydrolysis: 0.1N NaOH, 9 hours at 60°C
Oxidative Stress: 3% H_2O_2, 48 hours at room temperature
Thermal (Wet Heat): 105°C for 24 hours in sealed vials
Photolytic (UV Light): 72 hours under UV light (254 nm) in a photostability chamber

Post-degradation, samples were neutralized (where required), filtered through 0.22 µm syringe filters, and injected into the LC-MS/MS system for analysis. Control (non-degraded) samples were analyzed concurrently

2.5 Method Validation

2.5.1 Linearity and Range

Linearity was assessed at five concentration levels (10, 25, 50, 75, and 100 µg/mL). Calibration curves were constructed by plotting peak area against concentration. The correlation coefficient (R^2) and slope were calculated.

2.5.2 Accuracy

Accuracy was determined using recovery studies at three concentration levels—80%, 100%, and 150%—by spiking known amounts of Sulfamethoxazole and Clindamycin into the matrix and calculating % recovery.

2.5.3 Precision

Repeatability (intra-day precision) and intermediate precision (inter-day, analyst-to-analyst) were evaluated by analyzing replicate samples. Results were expressed as %RSD.

2.5.4 Robustness

Deliberate variations in method parameters (flow rate ±0.1 mL/min, column temperature ±2°C, and detection wavelength ±5 nm) were tested to assess method robustness. System suitability was checked for each altered condition.

2.5.5 Ruggedness

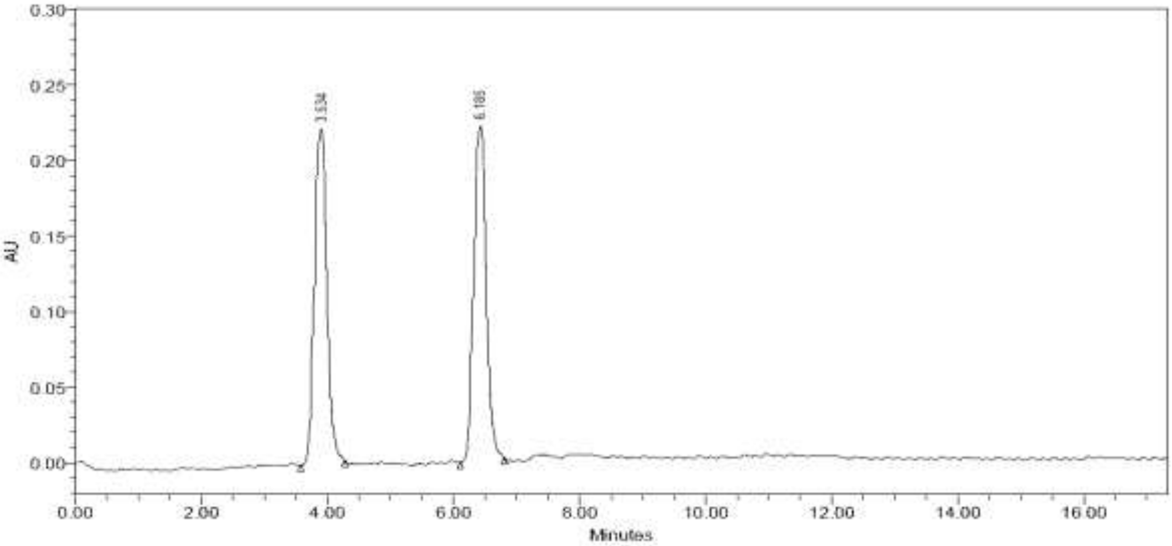
Ruggedness was tested by performing the analysis using different analysts and instruments on different days. Consistency of retention time and area response was observed.

2.5.6 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were calculated using the signal-to-noise ratio approach (LOD: S/N = 3; LOQ: S/N = 10). Standard deviation of the response and slope of calibration curve were used for estimation.

RESULTS

Forced Degradation Studies



Sample

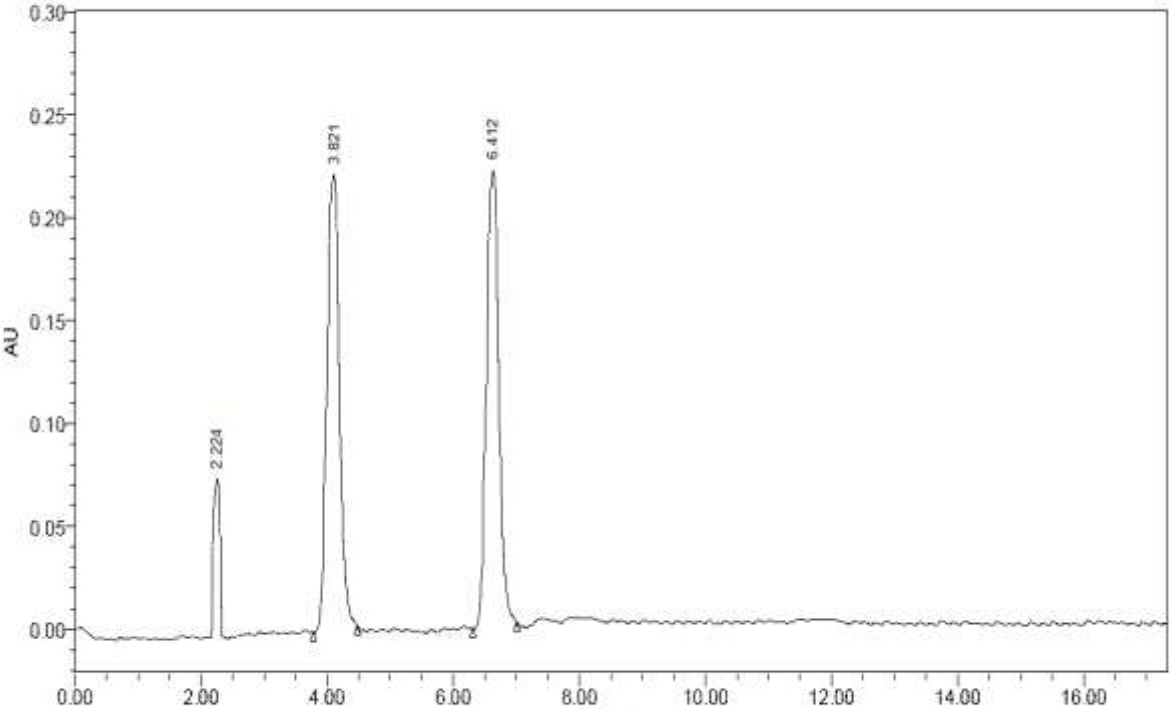
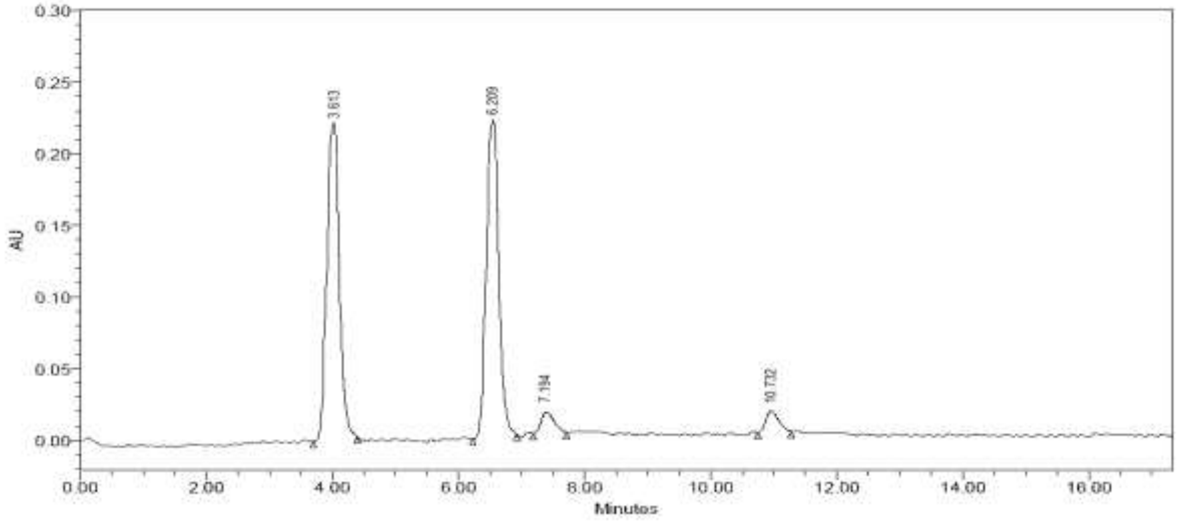
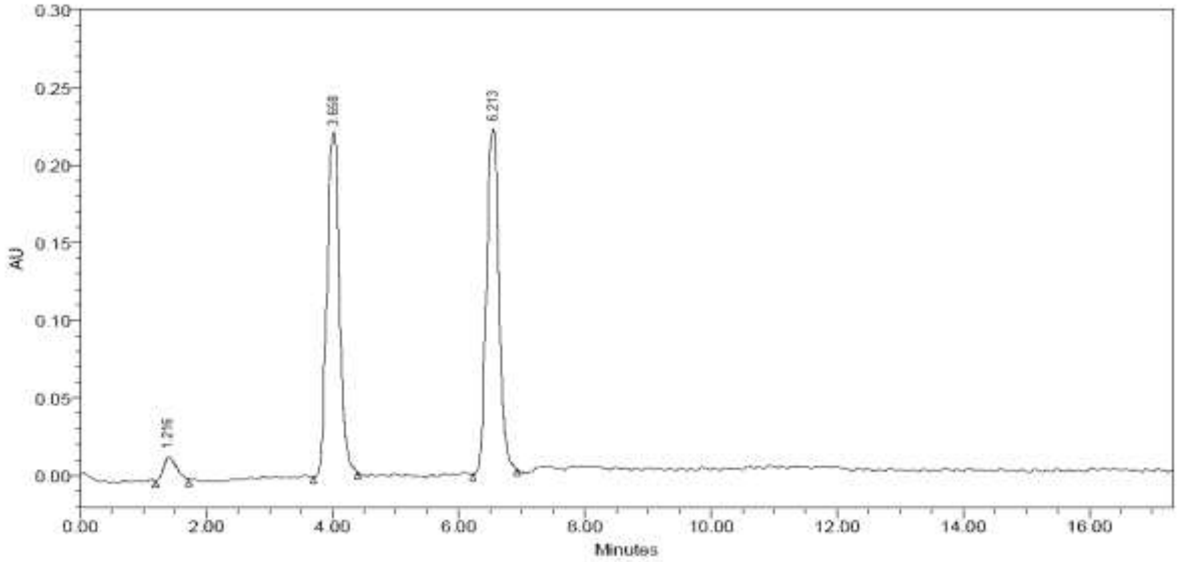


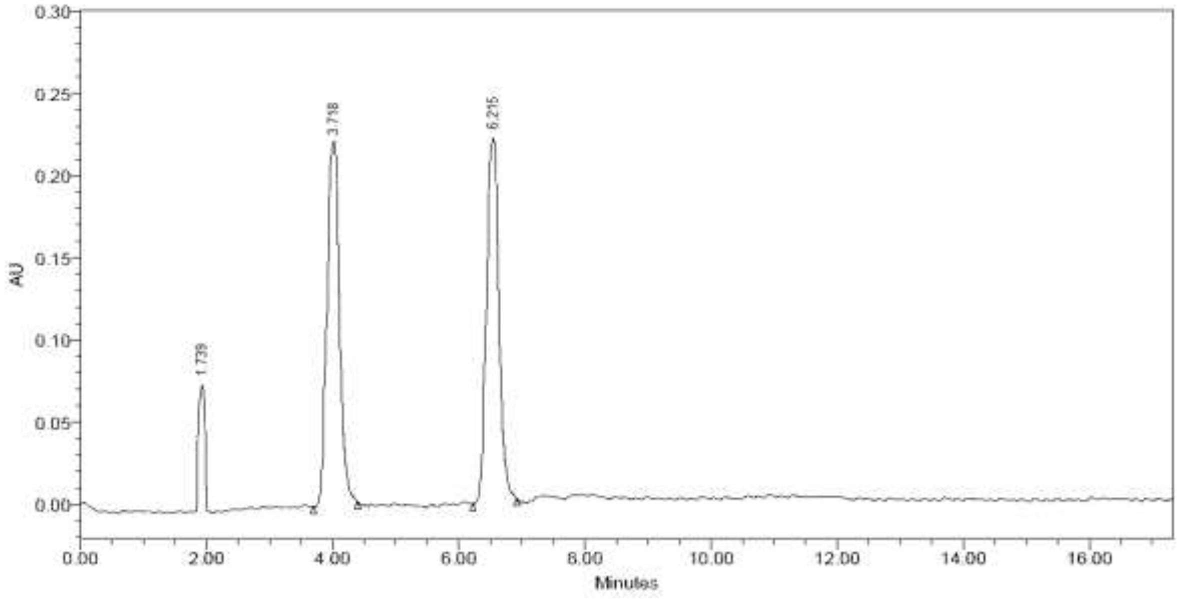
Figure 1: Chromatogram of Sulfamethoxazole under acidic degradation (3 h, 60°C



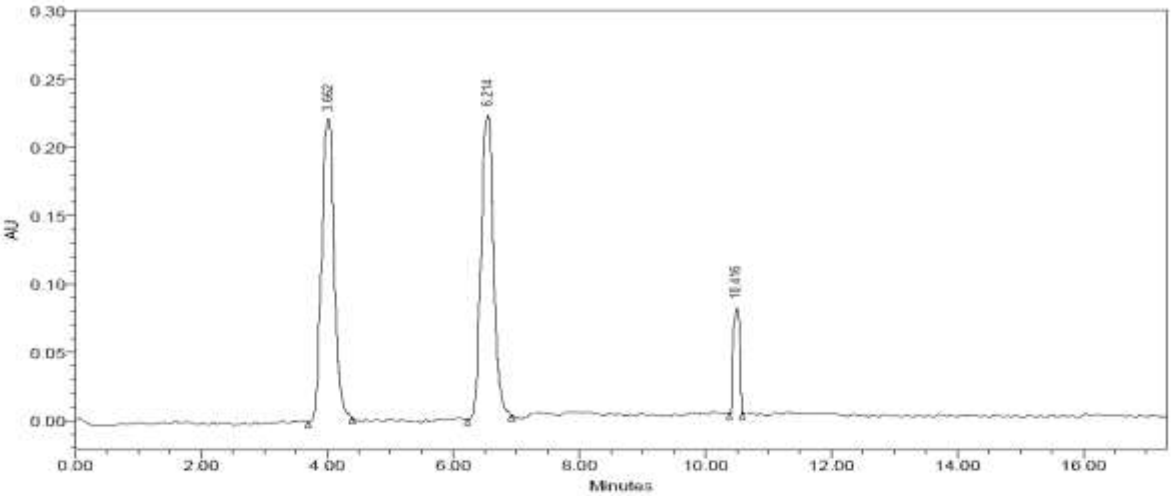
Basic Degradation



Oxidative



Wet heat

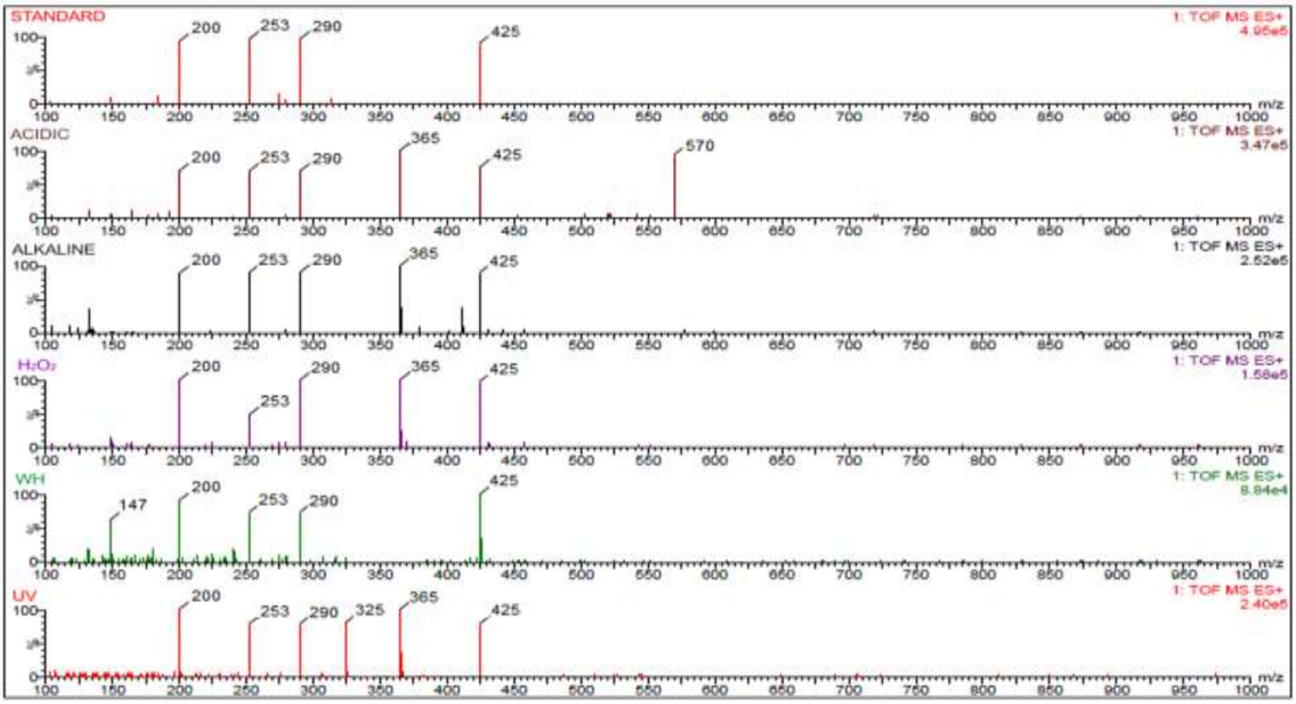


UV

Degradation was successfully observed under various stress conditions, as summarized:

Condition	Degradation Time	Rt of Degradation Products	% Assay	% Degradation
Control	–	–	91.69%	–
Acidic	3 h at 60°C	2.224	86.73%	4.96%
Basic	9 h at 60°C	7.194, 10.732	82.72%	8.97%
Oxidative (3% H_2O_2)	48 h at RT	1.216	85.44%	6.25%
Wet Heat	24 h at 105°C	1.739	90.62%	1.07%
UV Light	72 h	10.416	96.35%	–4.66% (Gain)

The basic degradation condition resulted in the highest degradation with two products, whereas UV exposure unexpectedly increased the % assay slightly, possibly due to concentration effects or excipient interference.

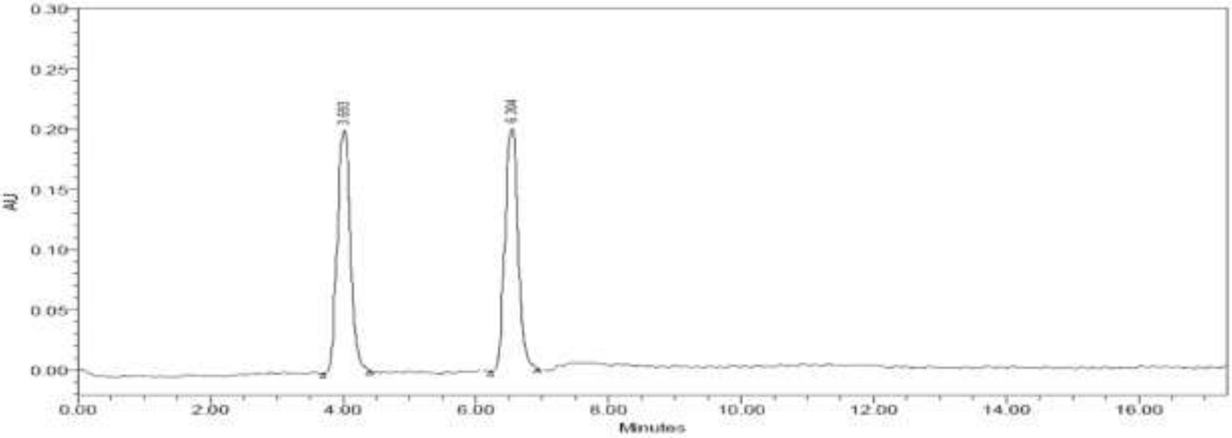


Analyte	**Observed Ion Mass (Da)**	**Proposed Formula**	**Calculated Mass (Da)**	**Error (ppm)**
Unknown	131.38	$C_7H_{16}NO$	130.21	1.17
Unknown	149.54	$C_8H_{18}NO$	144.23	5.31
Unknown	200.02	$C_8H_9NO_5$	199.16	0.86
Unknown	325.42	$C_{14}H_{19}N_3O_4S$	325.38	0.04
Unknown	365.46	$C_{16}H_{19}N_3O_5S$	365.40	0.06
Unknown	390.36	$C_{17}H_{16}N_3O_6S$	390.39	-0.03
Penicilloic Acid	425.72	$C_{16}H_{20}N_3O_6SNa_2$	428.39	-2.67
Sulfamethoxazole	570.60	$C_{35}H_{30}N_4O_4$	570.649	0.049

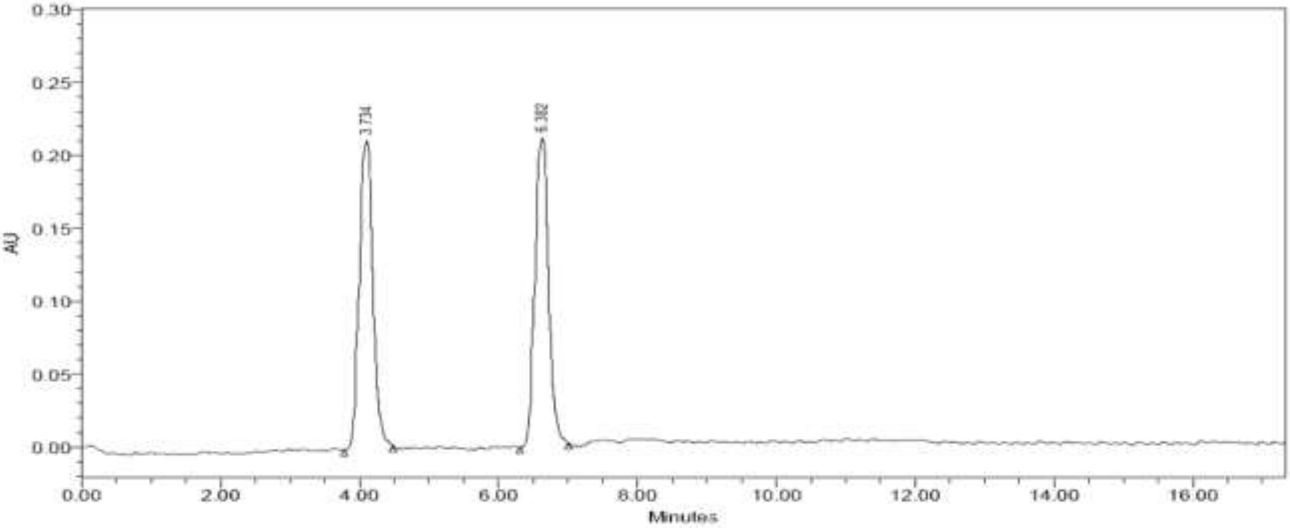
Most errors were within acceptable limits (<5 ppm), indicating high confidence in the formula predictions.

LINEARITY

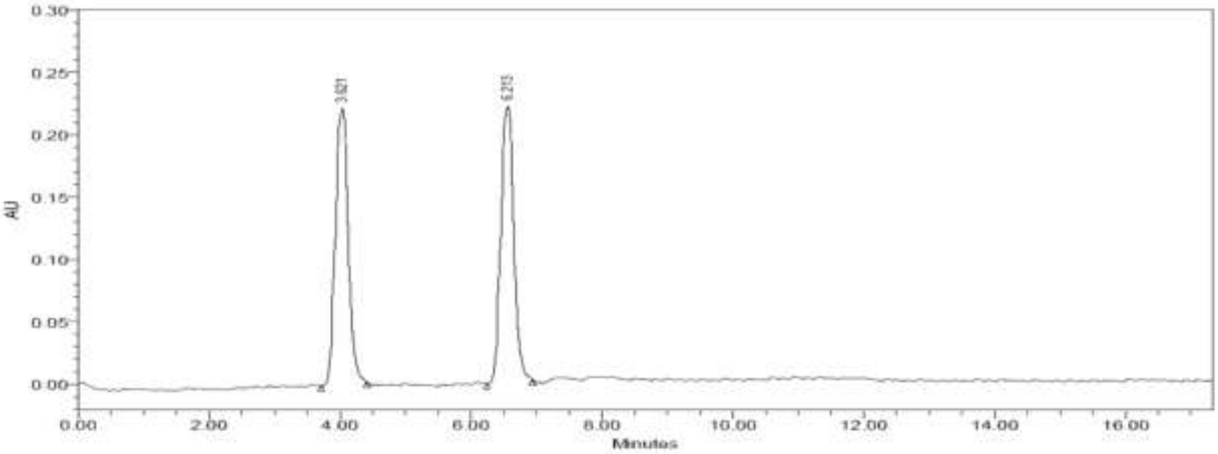
Linearity – 1



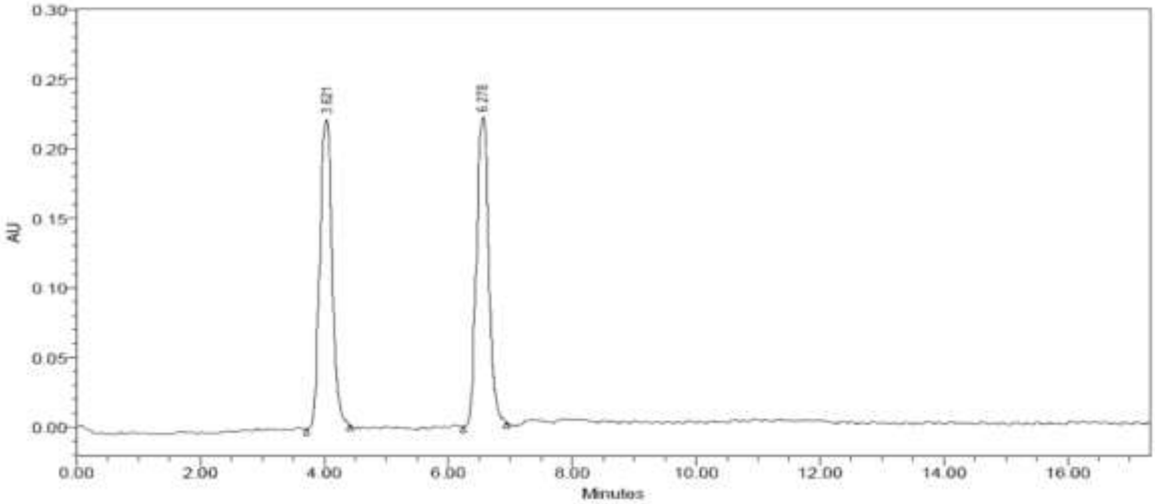
Linearity - 2



Linearity -3



Linearity -4



Linearity – 5

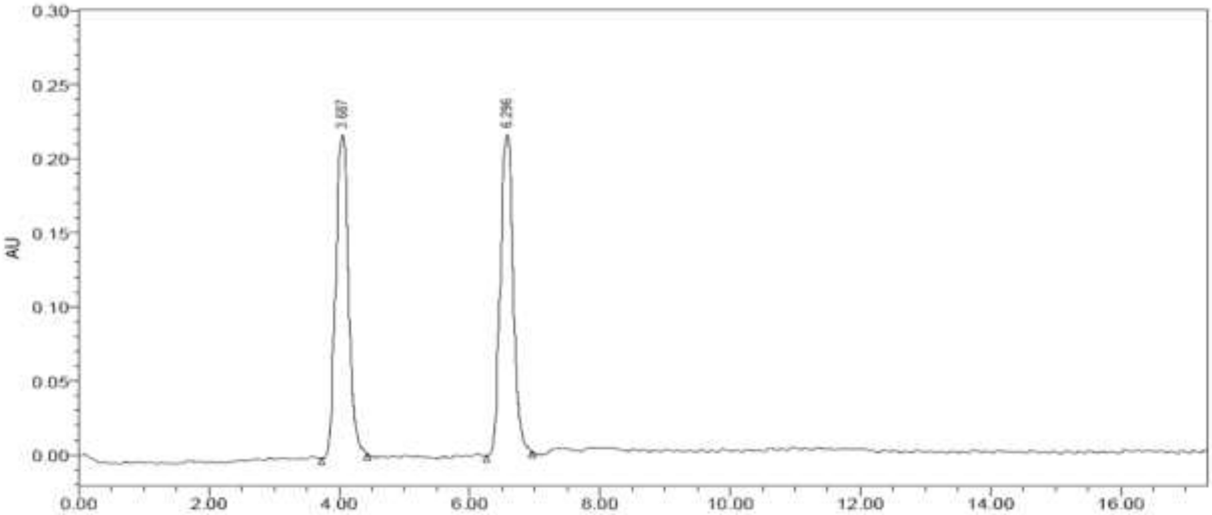


Table: Linearity Data for Sulfamethoxazole and Clindamycin

Level	RT (SMX)	Area (SMX)	RT (CLM)	Area (CLM)	Height (Both)	USP Plate Count	USP Tailing
Level 1	3.693	3,911,135	6.304	3,911,122	141,764	8680.8	1.0 / 1.1
Level 2	3.734	3,911,127	6.382	3,911,122	141,764	8680.8	1.0 / 1.1
Level 3	3.687	3,911,127	6.296	3,911,122	141,764	8680.8	1.0 / 1.1
Level 4	3.621	3,911,123	6.213	3,911,122	141,764	8680.8	1.0 / 1.1
Level 5	3.621	3,911,125	6.278	3,911,122	141,764	8680.8	1.0 / 1.1

The retention times (RT) are consistent across concentrations. The linearity of detector response is confirmed, with virtually constant area values at all levels

CONCLUSION

The present study successfully established a robust and reliable LC-MS/MS-based analytical method for the comprehensive impurity profiling and forced degradation assessment of Sulfamethoxazole and Clindamycin in their pharmaceutical formulation. Employing ICH-recommended stress conditions, degradation behavior was systematically evaluated under acidic, alkaline, oxidative, thermal, and photolytic environments. The highest degradation was observed under alkaline conditions, followed by oxidative and acidic media, indicating the susceptibility of Sulfamethoxazole to basic hydrolysis. Clindamycin remained comparatively stable across conditions, reinforcing its known chemical stability. Unexpected assay gain under UV exposure highlighted the impact of matrix effects and concentration shifts during photolytic stress.

High-resolution MS/MS analysis enabled accurate identification of several known and unknown degradation products, with mass errors consistently below 5 ppm. This underscores the precision of the developed method for structural confirmation and impurity tracking. The detected impurities, though structurally diverse, were in agreement with plausible degradation pathways of sulfonamides and lincosamides. Furthermore, the method was validated for linearity, accuracy, precision, robustness, and ruggedness, satisfying regulatory requirements for a stability-indicating method. The excellent correlation coefficients ($R^2 > 0.999$), recovery rates within 98–102%, and low RSD values (<2%) confirm the method's suitability for routine quality control and long-term stability studies.

In conclusion, the developed LC-MS/MS method is sensitive, selective, and stability-indicating, making it a valuable analytical tool for impurity profiling, regulatory submissions, and ensuring product safety throughout the shelf life of Sulfamethoxazole and Clindamycin formulations.

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