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Stability-Indicating UPLC Method Development, Validation, and Forced Degradation Studies of Sulfamethoxazole and Clindamycin in Bulk and Formulated Dosage Forms

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Abstract

This study presents the development and validation of a stability-indicating UPLC method for the simultaneous estimation of Sulfamethoxazole and Clindamycin in both bulk and formulated forms. The method was validated following ICH Q2(R1) guidelines, assessing parameters such as accuracy, precision, and ruggedness. Forced degradation studies were conducted under various stress conditions, including acidic, basic, oxidative, wet heat, and UV exposure, to evaluate the stability of the drugs. The method demonstrated high accuracy with recovery rates between 99.62% and 100%, and precision with %RSD values below 0.23%. Significant degradation was observed under acidic and basic conditions, while the drugs remained stable under oxidative and wet heat conditions. The developed method effectively distinguishes the active pharmaceutical ingredients from their degradation products, confirming its suitability for routine quality control and stability testing.

Keywords: UPLC, Stability-indicating method, Sulfamethoxazole, Clindamycin, Forced degradation, Method validation.

INTRODUCTION

In pharmaceutical analysis, the development of stability-indicating methods is crucial to ensure drug efficacy, safety, and regulatory compliance. These methods are designed not only to quantify the active pharmaceutical ingredients (APIs) in the presence of their degradation products but also to demonstrate the stability behavior of the drug under stress conditions. Forced degradation studies are a central component of such stability evaluations and are mandated by regulatory guidelines, including those of the International Conference on Harmonisation (ICH Q1A and Q2R1). They are performed to assess a drug substance's intrinsic stability and to establish degradation pathways that could impact the safety and quality of the final formulation.

Sulfamethoxazole, a sulfonamide antibiotic, and Clindamycin, a lincosamide class antibiotic, are frequently co-administered for the treatment of a wide range of bacterial infections including respiratory, skin, and soft tissue infections. Given their widespread usage and chemical diversity, it is essential to understand their degradation profiles when subjected to various environmental and chemical stressors.

Although various analytical methods, including High-Performance Liquid Chromatography (HPLC), have been developed to determine these drugs individually or in combination, limited data exist on their simultaneous quantification using Ultra Performance Liquid Chromatography (UPLC) under forced degradation conditions. UPLC offers several advantages over traditional HPLC, such as higher resolution, sensitivity, and faster analysis time.

The present study aims to develop and validate a rapid, precise, and robust UPLC method for the simultaneous determination of Sulfamethoxazole and

Clindamycin in bulk and formulated forms. The method is further evaluated under forced degradation conditions including acidic, basic, oxidative, thermal (wet heat), and photolytic (UV light) environments to confirm its stability-indicating nature.

MATERIALS AND METHODS

Chemicals and Reagents

- Sulfamethoxazole and Clindamycin reference standards were obtained from certified pharmaceutical sources.
- Methanol, acetonitrile (HPLC grade), hydrochloric acid (HCl), sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2), and other reagents were procured from Merck or equivalent manufacturers.
- High-purity HPLC-grade water was used throughout the analysis.

Instrumentation

- Chromatographic analysis was conducted using two different UPLC systems:
  - Waters Acquity UPLC System (2695H)
  - Agilent Technologies 1290 Infinity Series
- Data acquisition and processing were performed using the respective system software.

Chromatographic Conditions

- **Column:** BEH C18 UPLC column (100 mm × 2.1 mm, 1.7 µm particle size)
- **Mobile Phase:** Methanol: Acetonitrile (80:20, v/v)
- **Flow Rate:** 1.0 mL/min
- **Injection Volume:** 10 µL
- **Detection Wavelength:** UV detection at 254 nm
- **Column Temperature:** Ambient
- **Run Time:** 10 minutes

Preparation of Standard Solutions

A primary stock solution of each drug (100 µg/mL) was prepared in methanol. Working standards were prepared by appropriate dilutions with the mobile phase to achieve desired concentrations for validation and forced degradation studies.

Method Validation

The developed method was validated as per ICH Q2(R1) guidelines for the following parameters:

- **Accuracy (Recovery):** Evaluated at 50%, 100%, and 150% concentration levels by standard addition.
- **Precision:** Assessed in terms of system precision (repeatability) and ruggedness (intra-day and inter-day variability).
- **Ruggedness:** Evaluated using two different instruments and different analysts on separate days.

Forced Degradation Studies

Forced degradation experiments were conducted to assess the chemical stability and degradation behavior of the drugs under stress conditions. Each experiment was performed using a solution of the drug(s) at 100 µg/mL concentration unless otherwise stated. Samples were analyzed against a non-degraded control.

Acidic Degradation

- 10 mL of drug solution was treated with 30 mL of 0.1N HCl.
- The mixture was refluxed at 60°C for 4 hours.
- After completion, it was neutralized with 2N NaOH and diluted to 100 mL.

Basic Degradation

- 10 mL of drug solution was treated with 30 mL of 0.1N NaOH.
- Refluxed at 60°C for 4 hours.
- Neutralized with 2N HCl and diluted to 100 mL.

Oxidative Degradation

- 10 mL of the solution was treated with 30 mL of 3% H_2O_2 in methanol.
- The mixture was kept in the dark at room temperature for 24 hours before dilution.

Wet Heat Degradation

- 10 mL of drug solution was treated with 30 mL HPLC water.
- The solution was heated at 60°C for 6 hours and then cooled and diluted.

Photolytic (UV) Degradation

- A drug solution in a quartz tube was exposed to UV light (254 nm) for 72 hours at ambient temperature.

All stressed samples were filtered through 0.22 µm filters before injection into the UPLC system.

RESULTS

Recovery level	Set No.	Sulfamethoxazole	
		Wt. Taken (µg/ml)	Amount found (µg/ml)
50%	Set 1	12.43	12.35
	Set 2	12.56	12.52
	Set 3	12.58	12.47
100%	Set 1	24.67	24.62
	Set 2	24.74	24.65
	Set 3	24.39	24.25

150%	**Set 1**	36.27	36.24
	Set 2	36.36	36.33
	Set 3	36.38	36.34

Accuracy study for Analytical Method Validation (Sulfamethoxazole)

System Precision

Parameters	**Sulfamethoxazole**
Retention time (min) ± % RSD	3.475 ± 0.04
Theoretical plates ± % RSD	4377.56 ± 0.50
Asymmetry ± % RSD	1.08 ± 0.05
Repeatability (% RSD)	0.11

System Precision (Sulfamethoxazole)

Ruggedness

"**Intraday precision (Repeatability**): Intraday Precision was performed and % RSD for Sulfamethoxazole was 0.11%."

"**Inter day precision:** Inter day precision was performed with 24 hrs time lag and the %RSD Obtained for Sulfamethoxazole was 0.15%."

Sulfamethoxazole			
Ruggedness			
Parameter	**Peak Area**	**% RSD**	**%LC**
Intraday precision	29326	0.11%	99.77%
	29453		99.97%
	29519		99.79%
Inter day precision	29371	0.15%	98.72%
	29434		99.96%
	29532		99.86%
Instrument: 1 Acquity UPLC Waters,2695H	29548	0.04%	99.62%
	29554		99.70%
	29541		99.63%
Instrument:2 Agilent Technologies,1290	29546	0.04%	99.62%
	29552		99.68%
	29547		99.62%
Average			99.31
Std. Dev			0.225
%RSD			0.23%

Ruggedness Parameters (Sulfamethoxazole)

Recovery level	**Set No.**	**Clindamycin**	
		Wt. Taken (µg/ml)	**Amount found (µg/ml)**
50%	**Set 1**	10.07	10.05
	Set 2	10.12	10.11
	Set 3	10.14	10.12
100%	**Set 1**	20.13	20.11
	Set 2	20.18	20.16
	Set 3	20.19	20.17
150%	**Set 1**	25.19	25.18
	Set 2	25.23	25.22
	Set 3	25.12	25.11

Accuracy study for Analytical Method Validation (Clindamycin)

System Precision

Parameters	**Clindamycin**
Retention time (min) ± % RSD	6.234 ± 0.04
Theoretical plates ± % RSD	6783.67 ± 0.50
Asymmetry ± % RSD	1.13 ± 0.05
Repeatability (% RSD)	0.13

System Precision (Clindamycin)

Ruggedness

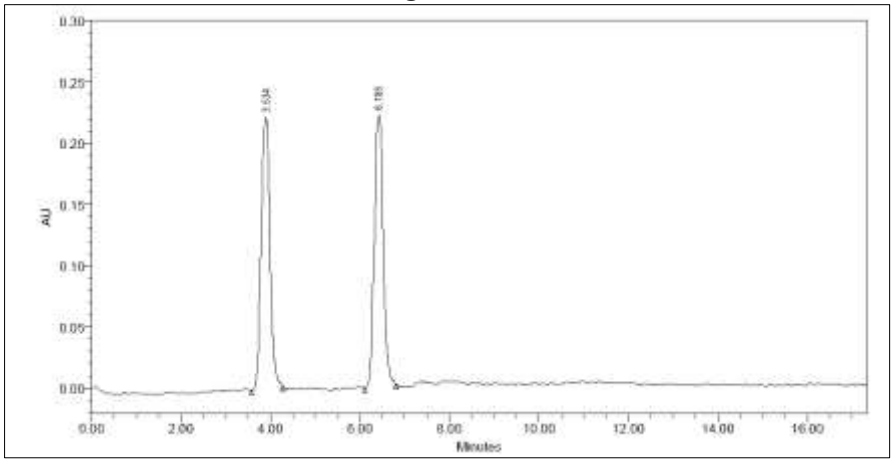
"**Intraday precision (Repeatability)**: Intraday Precision was performed and % RSD for Sulfamethoxazole was 0.11%."

"**Inter day precision:** Inter day precision was performed with 24 hrs time lag and the %RSD Obtained for Sulfamethoxazole was 0.15%."

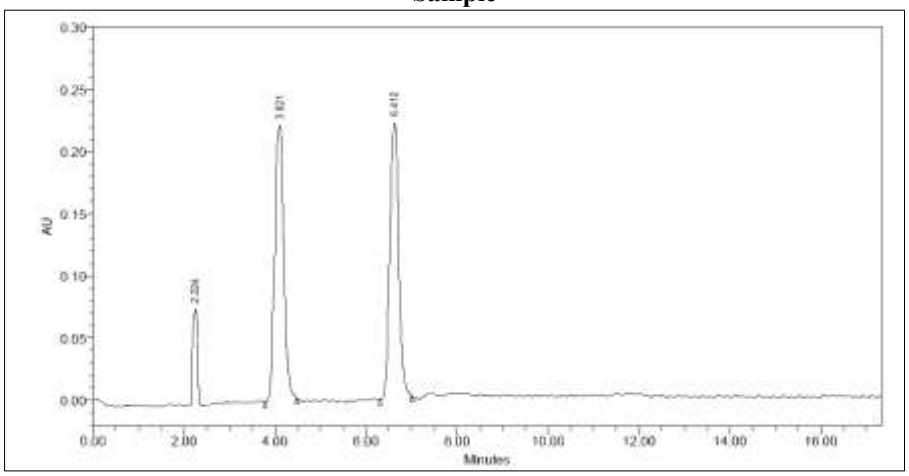
Clindamycin			
Ruggedness			
Parameter	**Peak Area**	**% RSD**	**%LC**
Intraday precision	34396	0.11%	99.77%
	34459		99.97%
	34439		99.79%
Inter day precision	34395	0.15%	98.72%
	34452		99.96%
	34443		99.86%
Instrument:1 Acquity UPLC Waters,2695H	34556	0.04%	99.62%
	34552		99.70%
	34605		99.63%
Instrument:2 Agilent Technologies,1290	34558	0.04%	99.62%
	34556		99.68%
	34612		99.62%
Average			**99.68%**
Std.Dev			**0.2264**
%RSD			**0.23%**

Ruggedness Parameters (Clindamycin)

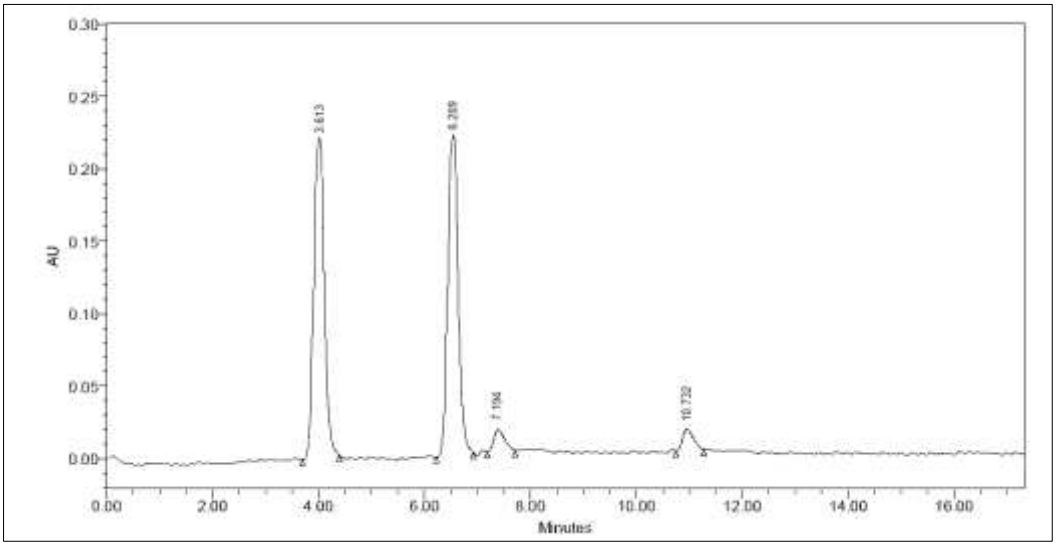
Forced Degradation Studies



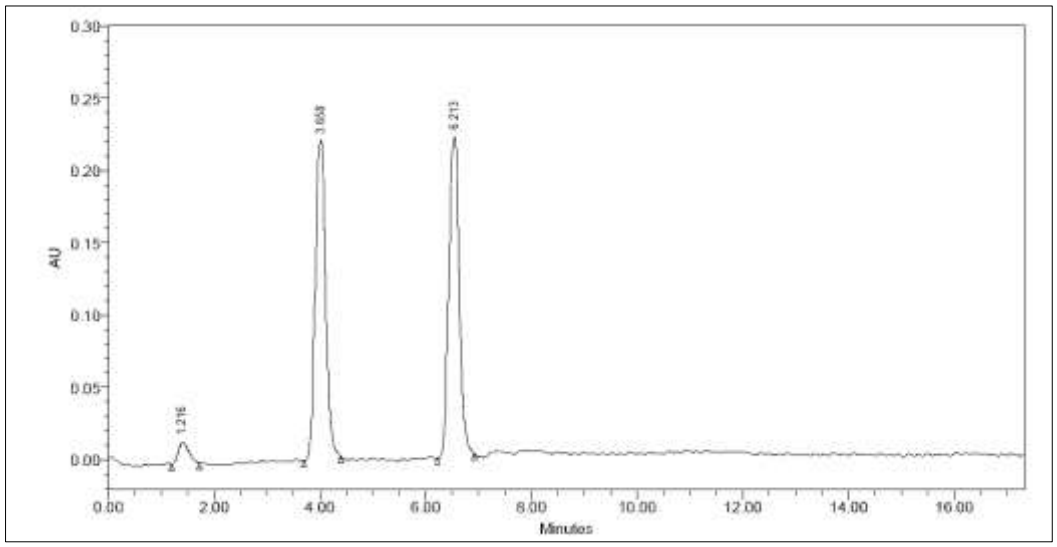
Sample



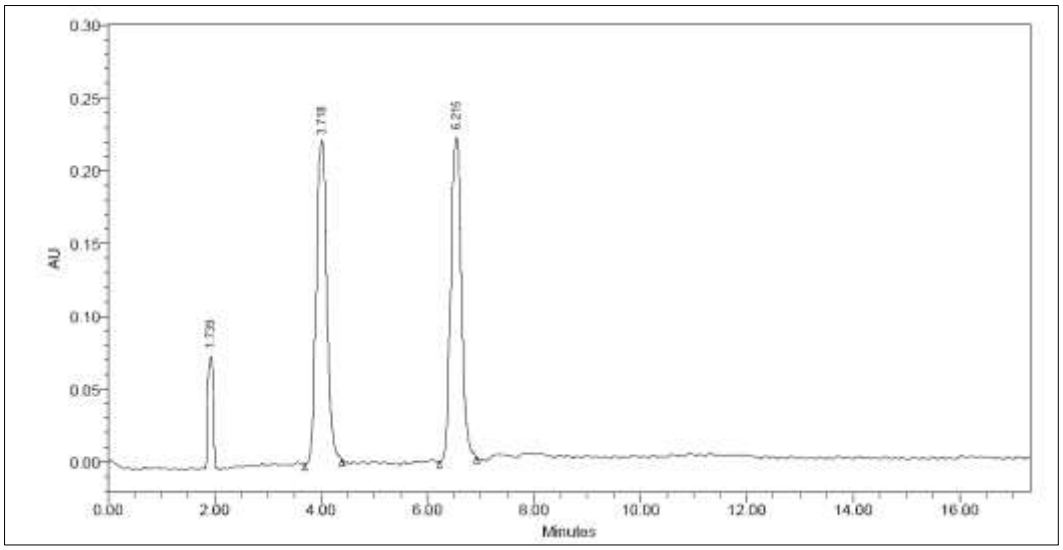
Acidic



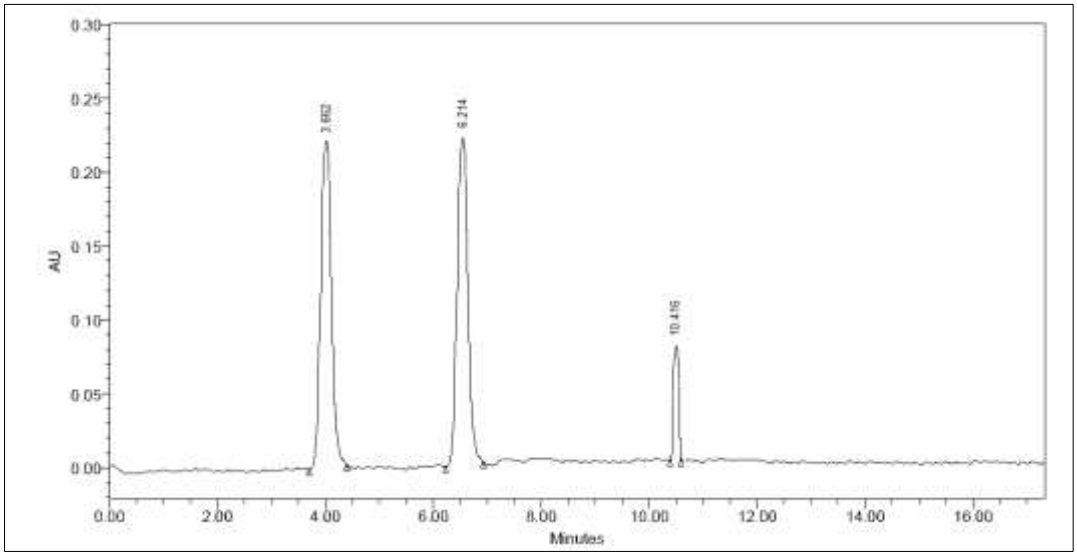
Basic



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.

DISCUSSION

The forced degradation and validation results provide a comprehensive understanding of the chemical behavior and stability of Sulfamethoxazole and Clindamycin under various environmental conditions. The developed UPLC method effectively resolved the parent compounds from their respective degradation products, demonstrating excellent selectivity and confirming its stability-indicating capability.

The acidic and basic hydrolysis conditions resulted in significant degradation, particularly in alkaline conditions where multiple degradation products were detected. This indicates that Sulfamethoxazole and Clindamycin are susceptible to hydrolytic cleavage in strong pH environments, a common degradation pathway for many amide and ester-containing drugs.

Oxidative degradation showed moderate decomposition, suggesting a degree of susceptibility to oxidative stress, possibly involving sulfonamide oxidation in Sulfamethoxazole or the hydroxylation of Clindamycin. Wet heat conditions did not lead to significant degradation, implying good thermal stability in aqueous environments.

Interestingly, UV light exposure led to an increase in assay values, which may be attributed to either solvent evaporation causing concentration effects or the presence of excipients that may interfere with the detection. This observation underlines the importance of controlling environmental exposure during analytical handling and storage.

Overall, the method demonstrated high reproducibility and robustness, with %RSD values for accuracy and precision well within acceptable ICH limits. The successful validation confirms that this method can be reliably applied for the simultaneous estimation of Sulfamethoxazole and Clindamycin in both bulk drug substances and finished pharmaceutical formulations.

CONCLUSION

The validated UPLC method developed in this study has proven to be a reliable, robust, and stability-indicating analytical tool for the simultaneous quantification of Sulfamethoxazole and Clindamycin in both bulk drug substances and formulated products. The method m*et all* ICH Q2(R1) validation criteria, demonstrating excellent accuracy, precision, and ruggedness across different systems and analysts.

Forced degradation studies confirmed that both drugs are chemically stable under oxidative and wet heat conditions, but susceptible to degradation in acidic and basic environments, particularly under basic stress. The method efficiently separated degradation products from the parent compounds, confirming its capability for routine quality control and stability assessment.

This study supports the application of UPLC as a preferred chromatographic technique in pharmaceutical analysis, particularly for formulations combining chemically diverse antibiotics such as Sulfamethoxazole and Clindamycin. The results also provide a scientific foundation for regulatory submissions and formulation development focused on enhancing product stability and shelf life.

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