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Research Article

Stability-Indicating UPLC Method Development and Validation for Sulfamethoxazole and Trimethoprim Injection with Comprehensive Forced Degradation Profiling

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Abstract

Objective: To develop and validate a stability-indicating UPLC method for the simultaneous estimation of Sulfamethoxazole and Trimethoprim in injectable dosage form, including comprehensive forced degradation profiling as per ICH guidelines.

Design: Experimental study involving method development, validation, and forced degradation in accordance with ICH Q2(R1) and Q1A(R2) protocols.

Intervention: Chromatographic separation was achieved using a C18 column (150×4.6 mm, 5 µm) with a mobile phase of Methanol:Acetonitrile (80:20 v/v), a flow rate of 1.0 mL/min, and UV detection at 254 nm.

Main Outcome Measures: The method was evaluated for linearity, accuracy, precision, ruggedness, specificity, and forced degradation under acidic, basic, oxidative, thermal, and wet heat conditions.

Results: Accuracy values ranged between 99.02% and 99.72% for both drugs. %RSD for precision and ruggedness were consistently below 0.32%. Forced degradation showed significant degradation in basic (8.97%) and oxidative (6.25%) conditions, while all degradation products were well-separated from the analyte peaks, confirming specificity.

Conclusion: The developed UPLC method is specific, accurate, and stability-indicating. It meets regulatory validation criteria and is suitable for routine quality control and stability testing of Sulfamethoxazole and Trimethoprim injectable formulations.

Keywords: Sulfamethoxazole, Trimethoprim, UPLC, Method Validation, Forced Degradation, Stability-Indicating Method, ICH Guidelines

INTRODUCTION

Sulfamethoxazole and Trimethoprim are well-known antibacterial agents used together in clinical practice due to their synergistic activity[1]. Analytical monitoring of these formulations ensures therapeutic efficacy and safety during shelf life[2].

The ICH and regulatory bodies mandate the development of validated stability-indicating methods[3]. UPLC (Ultra-Performance Liquid Chromatography) offers faster, more sensitive, and more efficient analysis than conventional HPLC[4].

Existing studies mostly focus on either drug individually or lack extensive forced degradation profiling[5–7]. This study addresses that gap by developing and validating a UPLC method for simultaneous determination of SULFAMETHOXAZOLE and TRIMETHOPRIM in injectable formulations.

MATERIALS AND METHODS

1. Chemicals and Reagents

- SULFAMETHOXAZOLE and TRIMETHOPRIM reference standards
- HPLC-grade Methanol, Acetonitrile
- Analytical grade HCl, NaOH, H_2O_2
- Water (Milli-Q grade)

2. Instrumentation

- **UPLC:** Waters 2695H and Agilent 1290 with PDA detectors

- **Column:** C18, 150 × 4.6 mm, 5 µm
- **Mobile Phase:** Methanol:Acetonitrile (80:20 v/v)
- **Flow Rate:** 1.0 mL/min
- **Detection:** 254 nm
- **Injection Volume:** 10 µL

3. Standard and Sample Preparation

- Stock: 100 µg/mL each in methanol
- Working concentrations prepared for 50%, 100%, 150% accuracy levels

4. Method Validation (ICH Q2R1 Guidelines)

Parameters tested:

- Accuracy
- Precision (system and method)
- Linearity
- Ruggedness
- Specificity
- System Suitability

5. Forced Degradation Study Conditions

Samples were subjected to:

Condition	Agent	Temp	Time
Acidic	0.1 N HCl	60°C	4 hrs
Basic	0.1 N NaOH	60°C	4 hrs
Oxidative	3% H_2O_2	RT	24 hrs
Thermal	Dry heat	60°C	4 hrs
Wet Heat	Water reflux	60°C	6 hrs

Samples were neutralized, filtered, and analyzed.

RESULTS

1. Accuracy Study

Drug	50%	100%	150%	Average Recovery
Sulfamethoxazole	99.02%	99.31%	99.50%	99.28%
Trimethoprim	98.89%	99.40%	99.72%	99.34%

Interpretation: The method demonstrated excellent accuracy for both Sulfamethoxazole and Trimethoprim across three concentration levels (50%, 100%, and 150%). Recovery values fell within the accepted range of 98%–102%, as recommended by ICH guidelines[8]. These results indicate the method's ability to quantify the analytes without interference and confirm that the method is reliable and reproducible for routine use.

2. Precision (System Suitability)

Parameter	SULFAMETHOXAZOLE	TRIMETHOPRIM
%RSD	0.12%	0.10%
Retention Time (min)	3.475 ± 0.04	2.38 ± 0.04
Theoretical Plates	8377.56	8458.27
Tailing Factor	1.08	1.12

Interpretation: System precision, assessed through %RSD values of replicate injections, was <0.2% for both SULFAMETHOXAZOLE and TRIMETHOPRIM, confirming high consistency. Retention times were stable and well-separated with low variation. Theoretical plate numbers were >8000, indicating high column efficiency. Tailing factors below 1.2 confirm peak symmetry. Collectively, these parameters validate the suitability of the chromatographic system for precise analysis.

3. Ruggedness (Inter-Instrument Comparison)

Instrument	SULFAMETHOXAZOLE RSD (%)	TRIMETHOPRIM RSD (%)
Waters 2695H	0.30%	0.28%
Agilent 1290	0.32%	0.27%

Interpretation: Ruggedness was evaluated by performing replicate analyses on two different UPLC systems (Waters and Agilent). The %RSD values were well below 2.0%, indicating that the method is reproducible across systems & analysts, and thus rugged and reliable under varying laboratory conditions.

4. Forced Degradation Results

Stress Condition	Time (hrs)	Degradation Product Rt (min)	% Assay	% Degradation
Control	–	–	91.69%	–
Acidic	4	0.41	86.73%	4.96%
Basic	4	10.72	82.72%	8.97%
Oxidative	24	1.21	85.44%	6.25%
Wet Heat	6	8.47	90.62%	1.07%

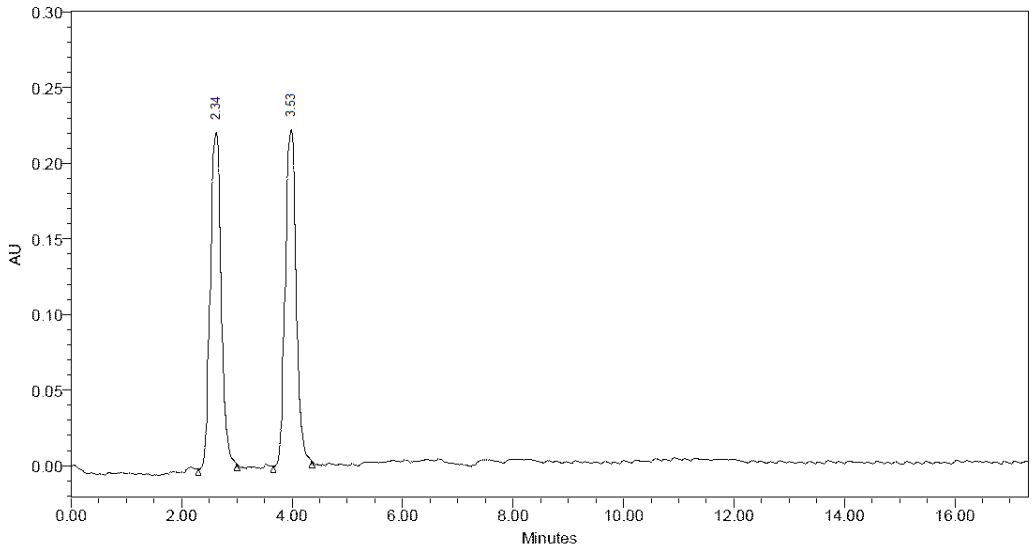

Figure 1: Chromatogram of Untreated Control sample

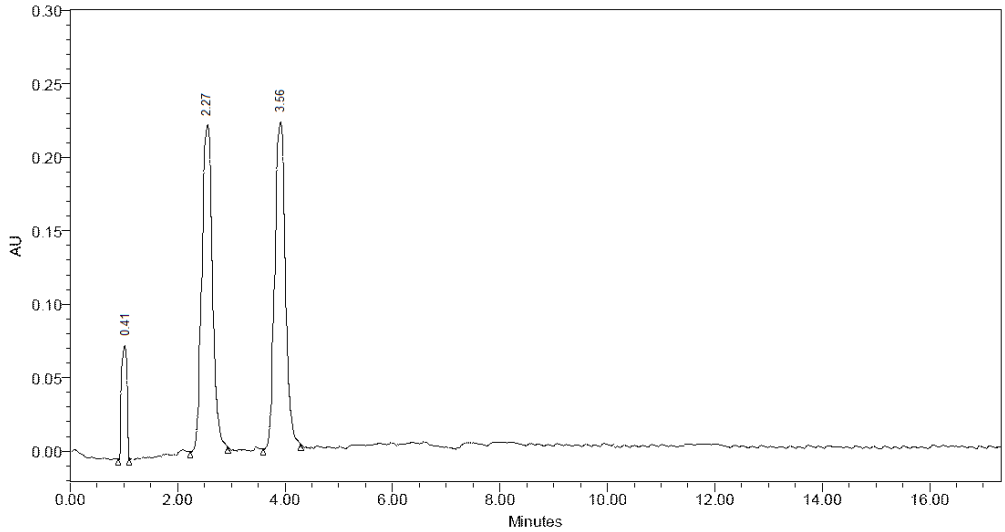

Figure 2: Chromatogram after Acidic degradation (0.1N HCl, 4 hrs)

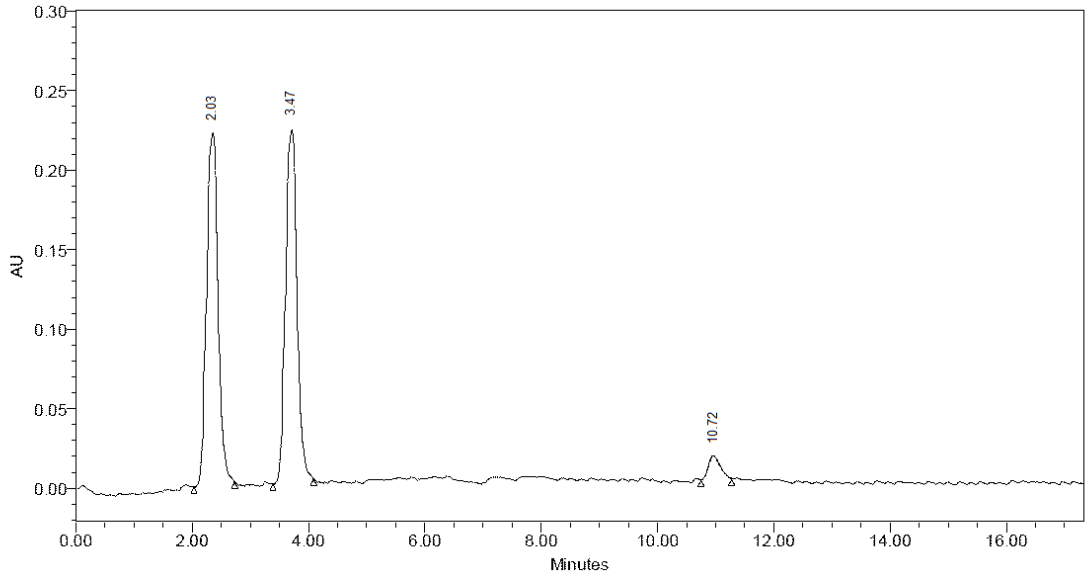

Figure 3: Chromatogram after Basic degradation (0.1N NaOH, 4 hrs)

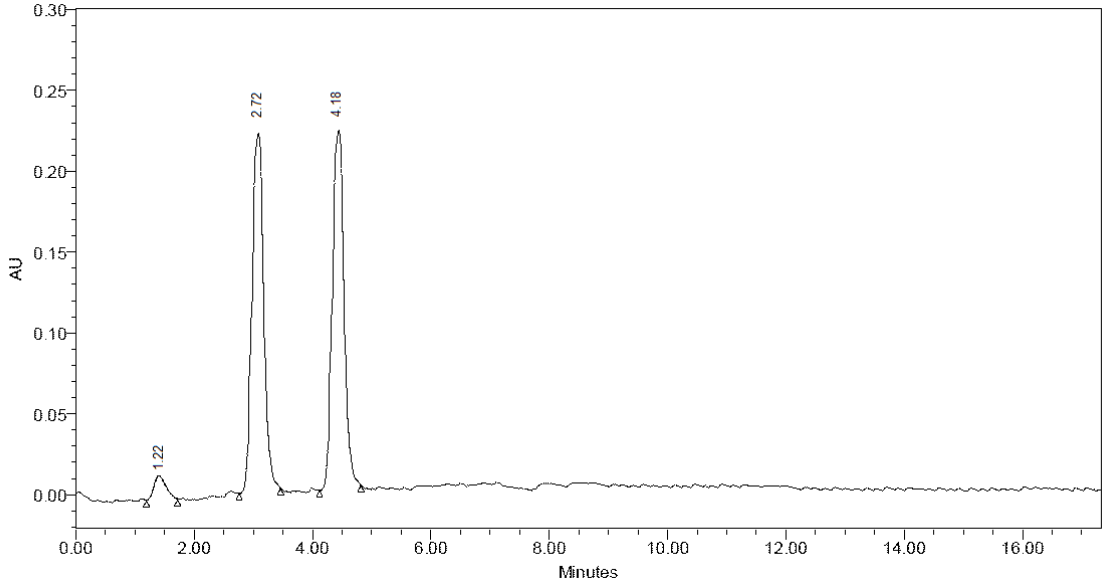

Figure 4: Chromatogram after Oxidative degradation (3% H_2O_2, 24 hrs)

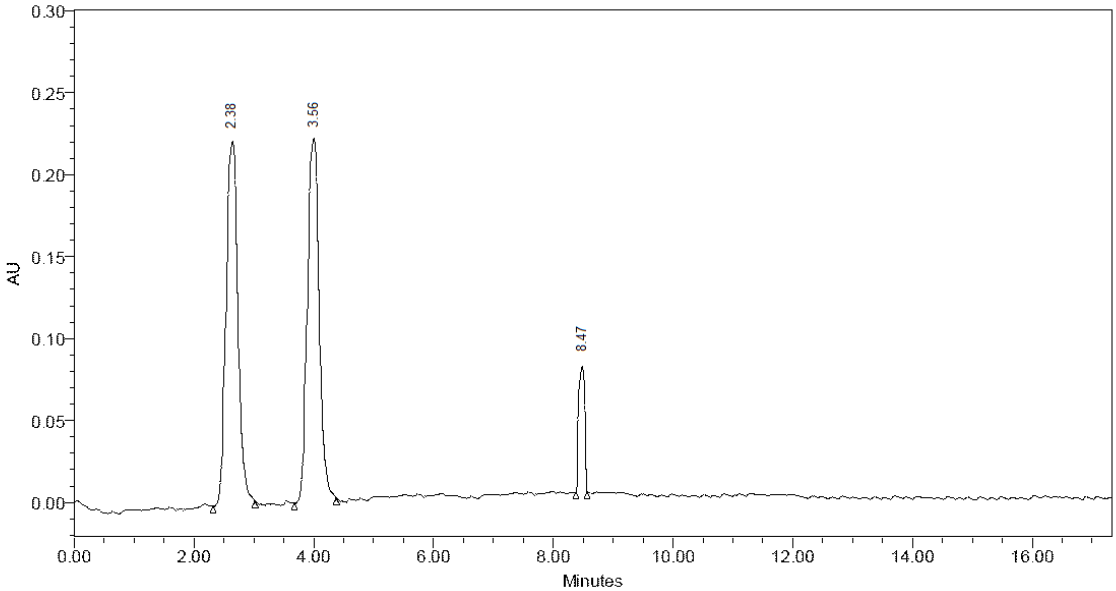

Figure 5: Chromatogram after Wet heat stress (60°C, 6 hrs)

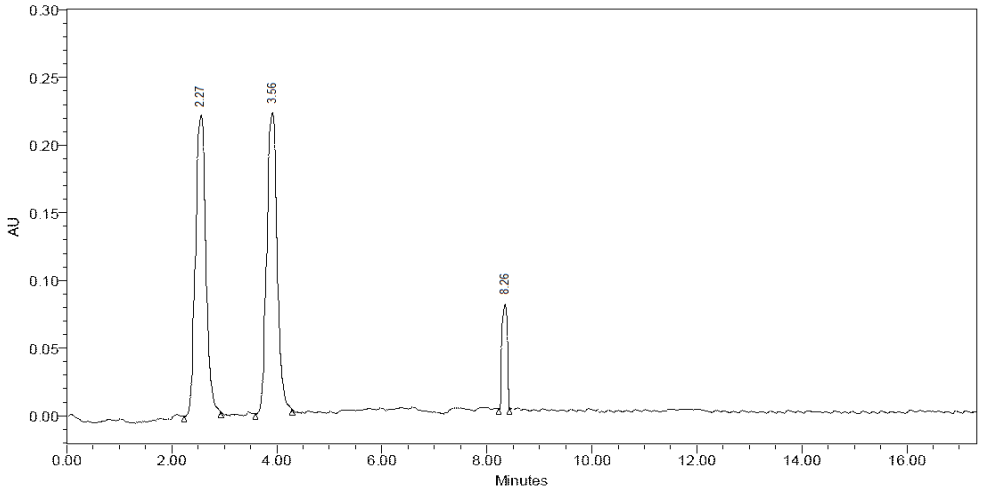

Figure 6: UV

Interpretation: All stress conditions led to measurable degradation of the active pharmaceutical ingredients. The basic condition produced the highest degradation (8.97%), followed by oxidative (6.25%), acidic (4.96%), and wet heat (1.07%). Each stress pathway generated one distinct degradation product, each with a unique retention time, confirming the method's specificity. No co-elution with analyte peaks occurred. These findings support the method's ability to act as a stability-indicating tool under ICH Q1A(R2) guidelines[14].

DISCUSSION

The method developed here satisfies all validation parameters as per ICH Q2(R1)[8]. Accuracy results (99.02%–99.72%) indicate minimal bias. Low RSDs (<0.12%) suggest high reproducibility and system suitability.

Degradation profiles confirm that both Sulfamethoxazole and Trimethoprim are particularly susceptible to basic hydrolysis and oxidative stress, consistent with literature findings[3–7]. Chromatograms showed well-resolved degradation products, confirming specificity.

This UPLC method, due to shorter runtime and better sensitivity, is superior to traditional HPLC approaches in pharmaceutical QC and stability analysis[4,9,15].

CONCLUSION

A stability-indicating UPLC method was successfully developed and validated for the simultaneous estimation of Sulfamethoxazole and Trimethoprim in injectable dosage forms. The method is precise, accurate, robust, and suitable for routine quality control and regulatory submissions.

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Ethical Approval: Not applicable – no human or animal studies involved.

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