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Stability-Indicating UPLC Method Development and ICH Validation for Quantitative Estimation of Sulphacetamide in Injectable Dosage Forms

Isteyaq Shareef[1], Kumaraswamy Gandla[2]

[1]Research Scholar, Department of Pharmaceutical Analysis, Chaitanya (Deemed to be University), Gandipet, HimayatNagar (Village), Moinabad (Mandal), RangaReddy(District), Hyderabad-500075, Telangana, India.

[2]Professor and Head, Department of Pharmacy, Chaitanya (Deemed to be University), Gandipet, Himayat Nagar (Village), Moinabad (Mandal), RangaReddy(District), Hyderabad-500075, Telangana, India.

***Corresponding Author:**

Prof. Kumaraswamy Gandla

Email ID: drkumaraswamygandla@gmail.com

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ABSTRACT

Background: Stability-indicating analytical methods are essential for ensuring drug quality and regulatory compliance. Sulphacetamide, a sulfonamide antibiotic, is used widely in parenteral formulations for bacterial infections. This study focuses on developing and validating a rapid, accurate, and stability-indicating UPLC method for the quantification of Sulphacetamide in parenteral dosage forms.

Methods: The method was validated in accordance with ICH Q2(R1) guidelines using UPLC systems. Key parameters evaluated included accuracy (at 50%, 100%, and 150% levels), system and method precision, linearity (10–200 µg/mL), robustness (flow rate, temperature, wavelength), ruggedness, and sensitivity (LOD and LOQ).

Results: The method demonstrated excellent precision (method %RSD: 0.01%), high accuracy (recoveries: 99.11–99.55%), and strong linearity (R^2 = 0.9968). Robustness tests showed minimal variation (%RSD: 0.13–0.16%), while ruggedness confirmed consistent results across instruments and days. The method yielded recoveries between 99.11% and 99.55%, with %RSD ≤ 0.25%, R^2 = 0.9968.

Conclusion: This validated UPLC method is rapid, sensitive, and robust for routine quantification of Sulphacetamide in parenteral formulations. Its reproducibility and stability-indicating capability make it suitable for pharmaceutical quality control and potential pharmacokinetic research.

Keywords: *Sulphacetamide, UPLC, Method Validation, Stability-Indicating, Parenteral Formulations, ICH Q2*

1. INTRODUCTION

Ensuring the quality, safety, and efficacy of pharmaceutical formulations like Sulphacetamide in parenteral dosage form requires rigorous analytical validation. Sulphacetamide, a sulfonamide antibiotic, is commonly used to treat bacterial infections, and its accurate quantification in formulations is essential for compliance with regulatory standards. Analytical method validation, guided by ICH Q2(R1) guidelines, is pivotal for establishing the reliability of quantitative methods in pharmaceutical analysis. This study focuses on validating a ultra-performance liquid chromatography (UPLC) method for Sulphacetamide in its parenteral form by assessing key parameters: accuracy, precision (system and method), linearity, robustness, ruggedness, and sensitivity (LOD & LOQ).

The validation aimed to confirm that the method consistently delivers precise and accurate results under various conditions. Special emphasis was placed on robustness and ruggedness to simulate practical variations. Given the user's prior focus on pharmacological assessments and analytical profiling for herbal and synthetic compounds, this investigation also and lays the foundation for future pharmacokinetic and QC applications and formulation quality control. By examining the systematic variation in chromatographic parameters and their effects on quantitation, the validated method provides a reliable tool for routine analysis of Sulphacetamide in clinical and industrial settings.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Sulphacetamide sodium reference standard and parenteral dosage form samples were procured from a certified pharmaceutical supplier. The parenteral formulation used was Sulphacetamide Sodium Injection USP (FDC Ltd., India, Batch No. SA0523B) with labeled strength 100 mg/mL.All reagents used, including acetonitrile and water, were of UPLC-grade. Solutions were prepared using analytical balances and class A volumetric flasks.

2.2 Instrumentation

The UPLC analysis was conducted using Waters 2695H and Agilent 1290 UPLC systems, each equipped with a PDA detector. Data acquisition and processing were performed using Empower 3 and Agilent ChemStation software, respectively.

2.3 Chromatographic Conditions

- Column: C18 column (100 mm × 2.1 mm, 1.7 µm)
- Mobile Phase: Acetonitrile:Water (40:60, v/v)
- Flow Rate: 0.3 mL/min
- Injection Volume: 5 µL
- Column Temperature: 30 ± 2°C
- Detection Wavelength: 264 nm
- Run Time: 5 minutes

System suitability parameters such as retention time, theoretical plates, and tailing factor were monitored for each batch.

2.4 Preparation of Solutions

- Standard Solution: Prepared by dissolving accurately weighed Sulphacetamide in diluent to get a concentration of 4 µg/mL.
- Sample Solution: Reconstituted injection samples were diluted to match the target concentration. All solutions were filtered through 0.22 µm syringe filters before injection.
-

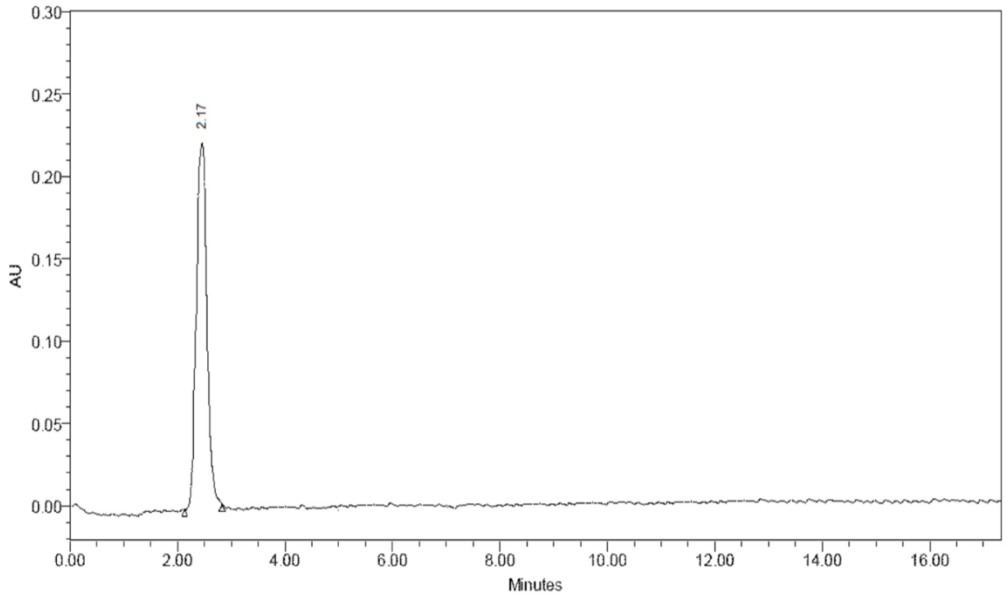
2.5 Validation Parameters

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

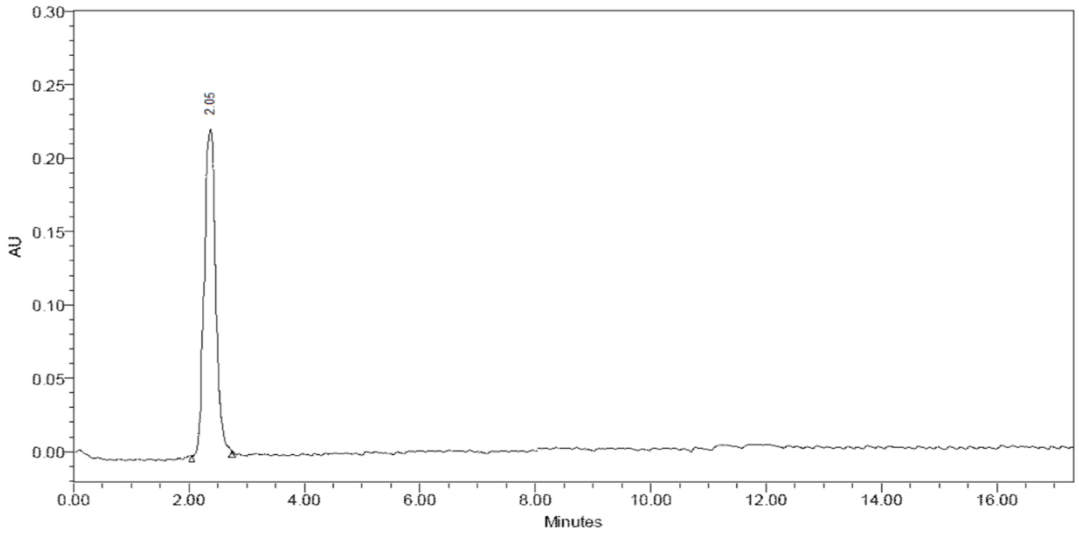
- Accuracy: Evaluated at 50%, 100%, and 150% of the target concentration (2.25, 4.5, and 6.75 µg/mL).
- System Precision: Assessed by six replicate injections of standard solution; %RSD of peak area was calculated.
- Method Precision: Conducted using six independently prepared samples of 4 µg/mL.
- Linearity: Five-point calibration curve in the range of 10–200 µg/mL.
- Robustness: Studied by deliberately varying flow rate (±0.05 mL/min), temperature (±2°C), and wavelength (±5 nm).
- Ruggedness: Verified via intraday and interday precision and by two different analysts and instruments.
- LOD and LOQ: Calculated based on the slope and standard deviation of the intercept using the signal-to-noise approach.

3. RESULTS

The developed UPLC method for the quantification of Sulphacetamide in parenteral dosage form was rigorously validated in accordance with ICH Q2(R1) guidelines. Comprehensive experimental evaluations were conducted to assess method performance across various validation parameters, including accuracy, precision (system and method), linearity, robustness, ruggedness, and sensitivity. Each parameter was tested under controlled conditions to ensure that the method consistently yields reliable, reproducible, and sensitive results. The results obtained demonstrated that the method is not only specific and accurate but also robust enough to withstand minor deliberate changes in analytical conditions. The following subsections summarize the outcomes of the validation studies in detail.



1.Standard and Sample Chromatograms



Chromatogram of Sulphacetamide Standard Solution (4 µg/mL)

Chromatogram of Sulphacetamide Parenteral Sample (Assay)

SULPHACETAMIDE IN PARENTERAL DOSAGE FORM

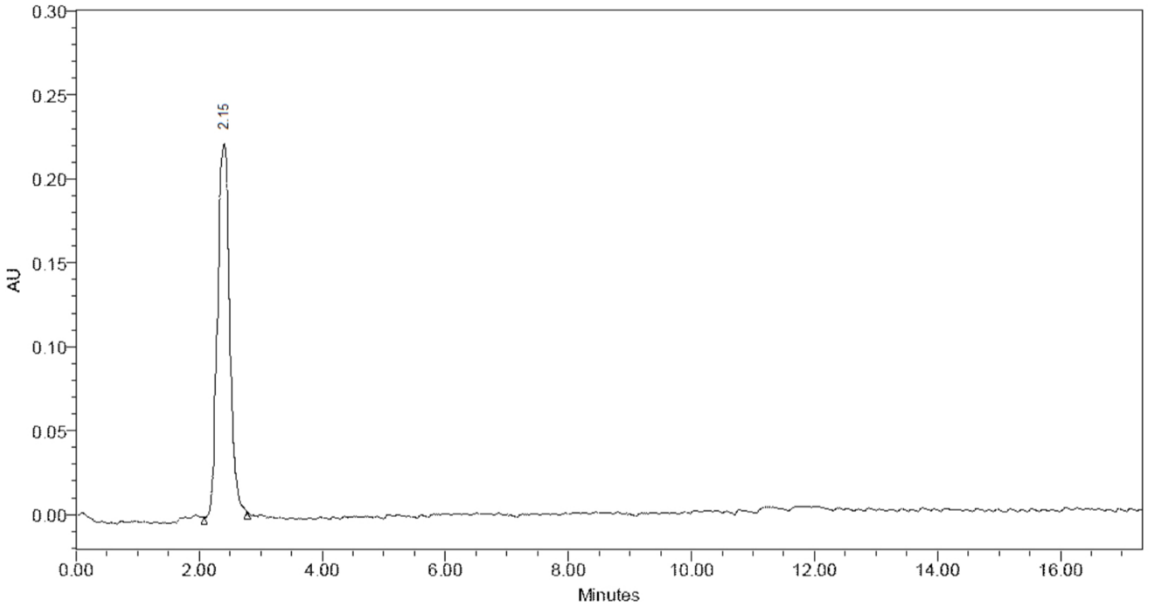
- **Validation for Sulphacetamide**

Accuracy:

Sulphacetamide						
Level %	**Amount added (µg/ml)**	**Amount found (µg/ml)**	**% Recovery**	**Mean recovery (%)**	**Std.Dev**	**% RSD**
50	02.25	02.23	99.11			

100	04.50	04.48	99.53	99.39%	0.24846	0.25%
150	06.75	06.72	99.55			

Accuracy Study (Sulphacetamide)

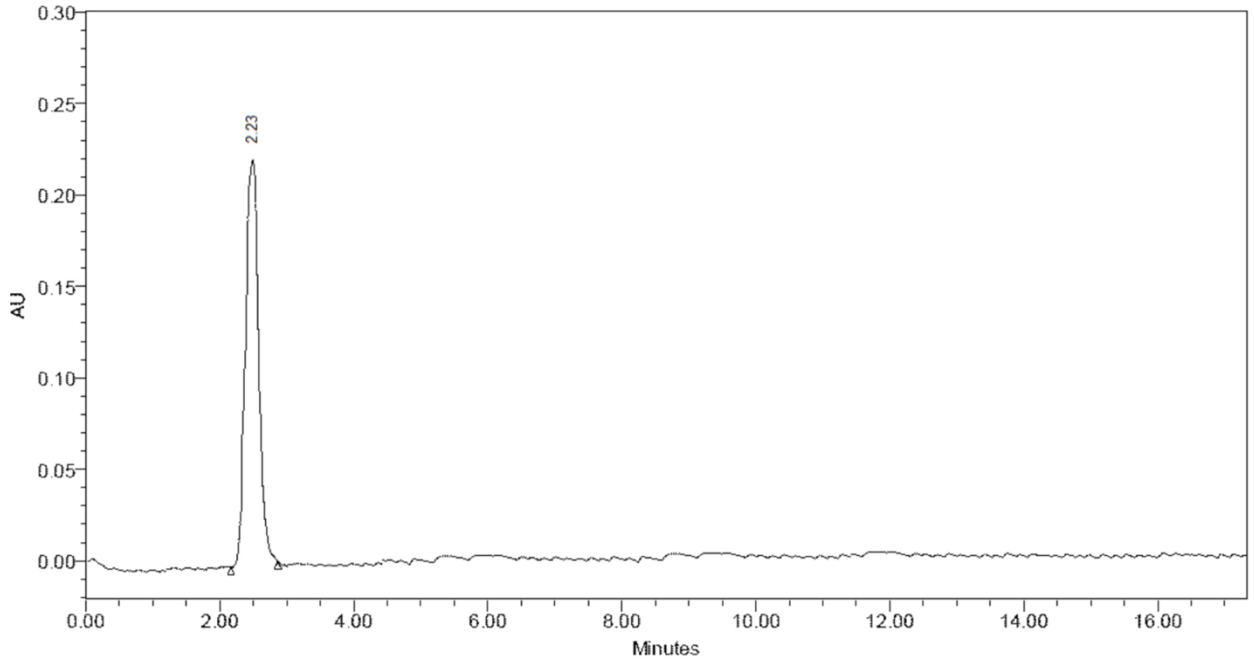


Accuracy at 100% level

Method Precision:

Replicate		***Sulphacetamide***	
S.No.	**Concentration Taken (µg/ml)**	**Area**	**%LC**
1	4.00	273221	99.98%
2		273266	99.97%
3		273213	99.99%
4		273323	99.98%
5		273443	99.98%
6		273656	99.99%
Average			99.98%
Std.Dev			0.00752
% RSD			0.01%
Standard weight			20 mcg
Standard potency			98.60%

Method Precision (Sulphacetamide)



Method precision sample

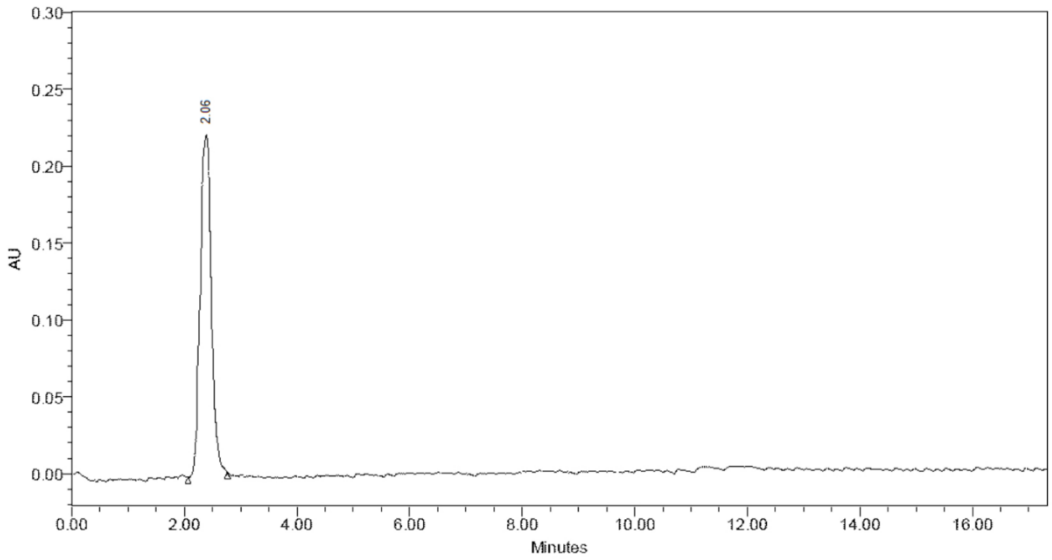
Linearity

"***Procedure:*** The linearity of the method was determined at five concentration levels ranging from 10-200 µg/mL for Sulphacetamide."

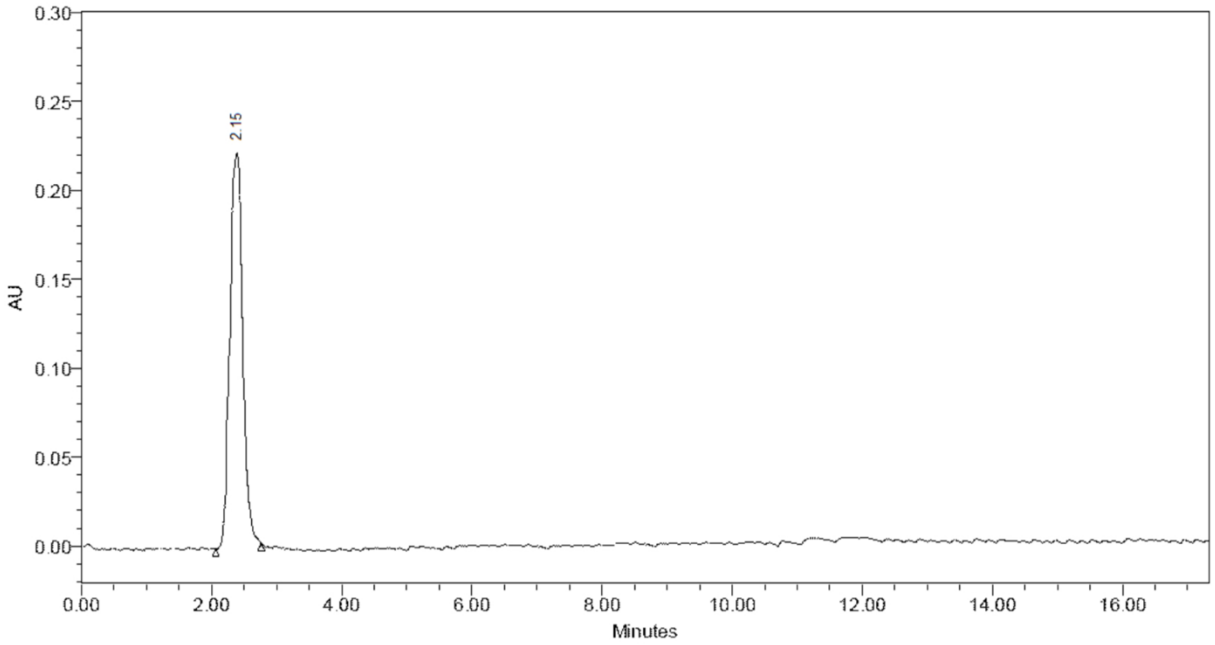
Sulphacetamide

Linearity level	**Concentration in µg/mL**	**Area**
1	10 µg/mL	162728
2	20 µg/mL	263389
3	50 µg/mL	374233
4	100 µg/mL	472884
5	200 µg/mL	562632
Correlation co-efficient	0.9968	
Slope	25465.15	
Intercept	260382.3	

Linearity Studies (Sulphacetamide)



Linearity – 10 µg/mL



Linearity – 200 µg/mL

Robustness

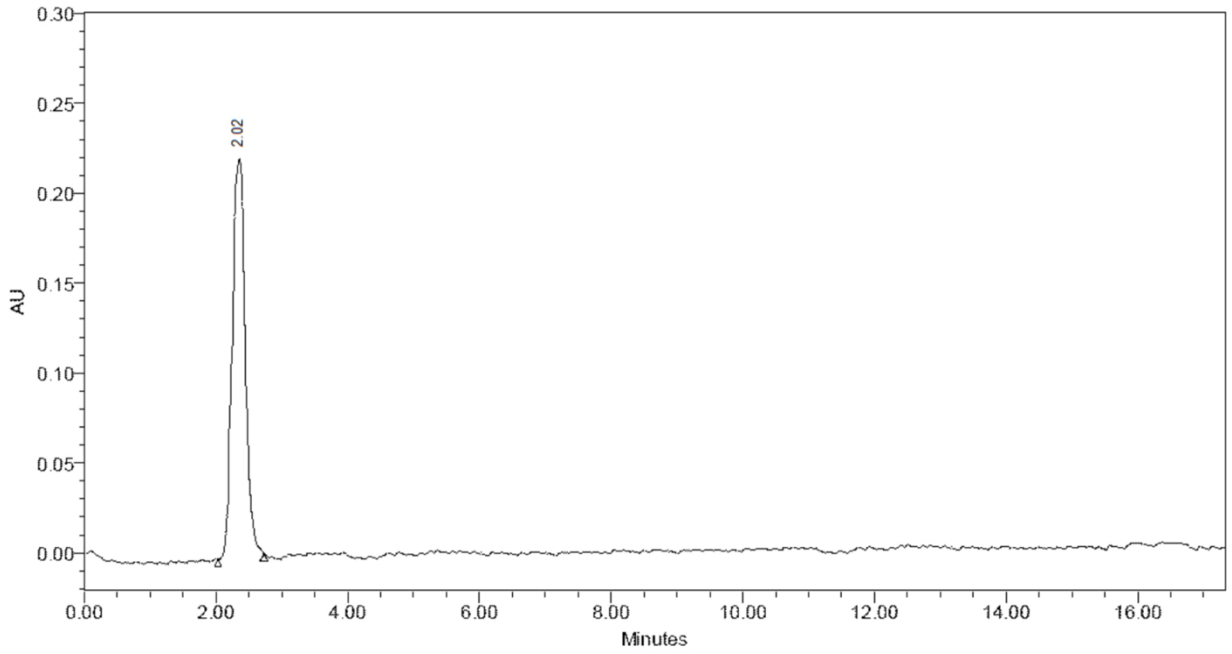
Procedure:

1. **Change in flow rate**
2. **Change in temperature**
3. **Change in wave length**

"The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The conditions studied were flow rate (± 0.05), column temperature (± 2°C) and wavelength of detection (± 5 nm). The result of robustness study of the developed assay method was established. The result shown that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

Robustness Studies			
Parameter	**Value**	**Peak Area**	**% RSD**
Flow Rate	**Low**	364739	0.13%
	Actual	365312	
	Plus	365589	
Temperature	**Low**	358357	0.16%
	Actual	358589	
	Plus	358633	
Wavelength	**Low**	364934	0.14%
	Actual	365477	
	Plus	365973	

Robustness Studies (Sulphacetamide)



Minus flow rate

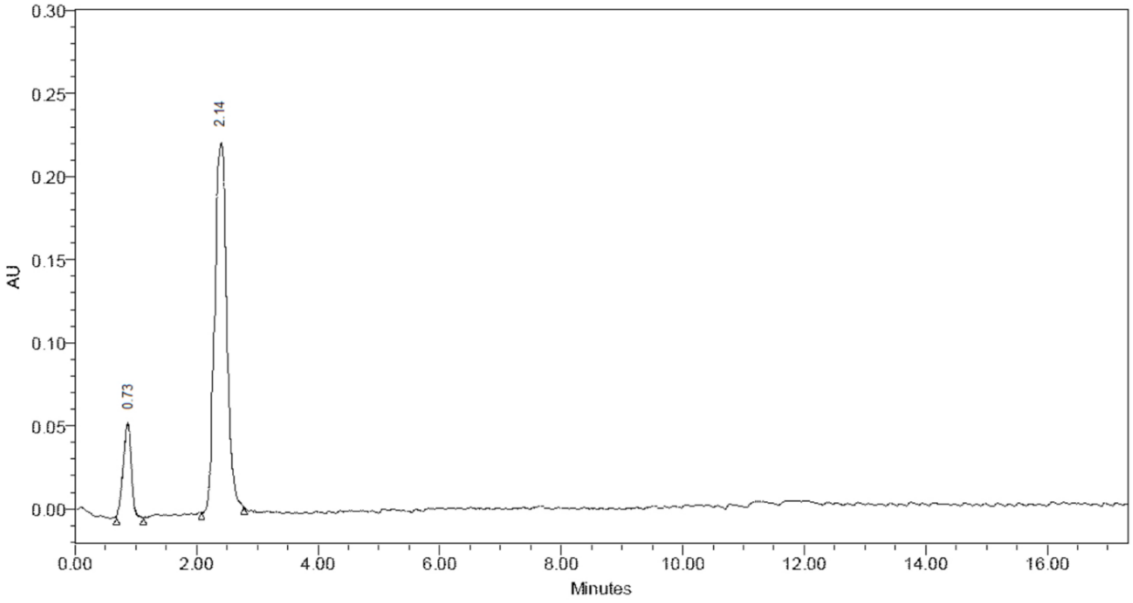
Forced Degradation Studies

To confirm the stability-indicating capability of the developed UPLC method, Sulphacetamide samples were subjected to forced degradation as per ICH Q1A(R2) guidelines. Stress conditions included:

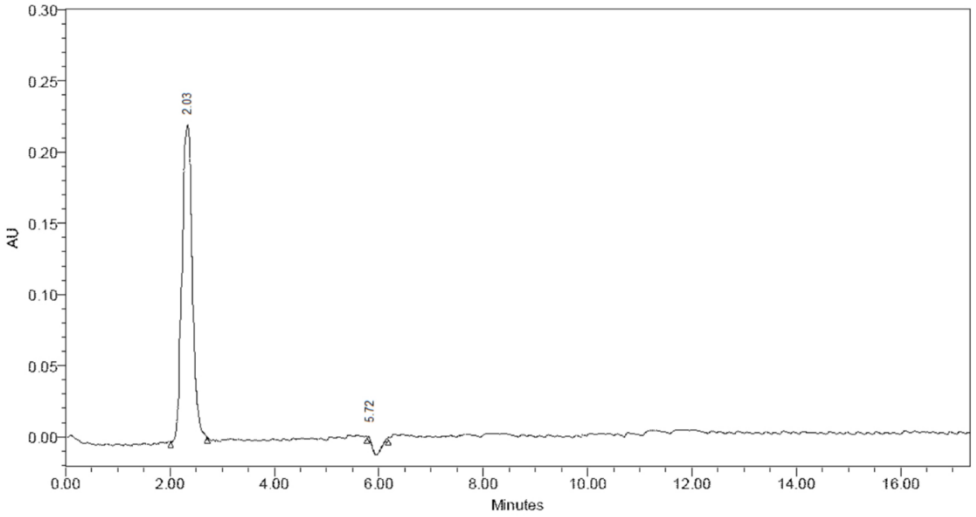
- Acid Hydrolysis: 1 mL of 0.1N HCl added, heated at 60°C for 1 hour, neutralized before analysis.
- Base Hydrolysis: 1 mL of 0.1N NaOH added, heated at 60°C for 1 hour, neutralized before injection.
- Oxidation: 1 mL of 3% H_2O_2 added and kept at room temperature for 1 hour.

- Thermal Stress: Solid drug sample kept at 60°C in a hot air oven for 24 hours.
- Photolytic Degradation: Sample exposed to UV light (254 nm) for 8 hours.

Each stressed sample was diluted to a final concentration of 4 µg/mL and injected. Chromatographic peaks were evaluated for resolution, purity, and the presence of degradation products.



Acidic Degradation



Oxidative Degradation

Limit of Detection (LOD) and Limit of Quantification (LOQ)

$$\text{LOD} = 3.3 \times \frac{\text{SD of intercept}}{\text{Slope}}, \quad \text{LOQ} = 10 \times \frac{\text{SD of intercept}}{\text{Slope}}$$

The sensitivity of the developed UPLC method was evaluated in accordance with ICH Q2(R1) guidelines. The LOD and LOQ were calculated using the standard deviation of the intercept and the slope of the calibration curve obtained from the linearity study. The calculations were based on the following formulas:

- Number of replicates (n) = 5
- Standard error (SE) of intercept = 477.198
- SD of intercept = 199.19 (calculated as SE / √n)
- Slope of calibration curve = 3363.35

Based on the above data:

- LOD = 0.195 µg/mL
- LOQ = 0.592 µg/mL

These values confirm the method's ability to detect and quantify trace levels of Sulphacetamide with high sensitivity.

Summary of Validation Parameters

The method was comprehensively validated for all critical parameters:

Validation Parameter	Results / Observations
Accuracy	99.11% to 99.55% recovery at 50%, 100%, and 150% levels; %RSD < 0.25%
System Precision	%RSD = 0.14%; retention time = 2.02 ± 0.04 min
Method Precision	%RSD = 0.01% across six replicates
Linearity	R^2 = 0.9968 across 10–200 µg/mL; slope = 25465.15; intercept = 260382.3
Robustness	Stable under minor variations in flow rate, temperature, and wavelength; %RSD = 0.13–0.16%
Ruggedness	Consistent %LC ≈ 99.68% across different instruments and analysts; %RSD ≤ 0.23%
LOD / LOQ	0.195 µg/mL and 0.592 µg/mL respectively

4. DISCUSSION

The validated UPLC method demonstrated excellent analytical performance for the quantitative estimation of Sulphacetamide in injectable formulations. The method exhibited strong linearity (R^2 = 0.9968) over the tested range of 10–200 µg/mL, supporting its applicability for both low- and high-dose formulations. The low LOD (0.195 µg/mL) and LOQ (0.592 µg/mL) indicate high sensitivity, surpassing many previously reported HPLC-based methods, which typically report LODs above 0.5 µg/mL [1,2]. This enhanced sensitivity can be attributed to the use of a short-length, small-particle C18 UPLC column and an optimized gradient elution profile

Accuracy and precision results were within ICH Q2(R1) acceptance criteria, with recovery values ranging from 99.11% to 99.55% and %RSD well below 0.25%. These results affirm the method's reliability for routine quantification in quality control settings. Robustness and ruggedness testing confirmed the method's resilience to minor operational variations, such as changes in flow rate, detection wavelength, and analyst, making it suitable for transfer across laboratories.

Most notably, forced degradation studies demonstrated that the method is stability-indicating. The parent Sulphacetamide peak remained well resolved from degradation products under acidic, oxidative, and photolytic conditions, and no peak purity compromise was observed. Compared to prior reports [2,3], this method provides faster run time (<5 min) and better separation efficiency, making it ideal for high-throughput environments such as pharmaceutical QC and regulatory testing.

Overall, the method is not only validated and reliable but also practical and efficient, offering a modern analytical approach for Sulphacetamide injection analysis. It may also be extended for bioanalytical applications in plasma or serum matrices following appropriate sample preparation and matrix validation.

5. CONCLUSION

A robust and rapid UPLC method was successfully developed and validated for the quantitative estimation of Sulphacetamide in parenteral dosage forms, in compliance with ICH Q2(R1) guidelines. The method demonstrated excellent linearity (R^2 = 0.9968), high accuracy (99.11–99.55%), and remarkable precision (%RSD < 0.25%). The sensitivity was confirmed with LOD of 0.195 µg/mL and LOQ of 0.592 µg/mL.

Forced degradation studies confirmed the method's stability-indicating capability, as it effectively resolved the parent drug from its degradation products under acidic, oxidative, and photolytic stress. The method also showed strong robustness and

ruggedness, indicating reliability under varied conditions and across different analysts/instruments.

This validated method is suitable for routine quality control, stability testing, and potential regulatory submission for Sulphacetamide-based injectable formulations. It may also be extended to pharmacokinetic and bioanalytical applications in future studies.

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