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Development and Comprehensive Validation of A Stability-Indicating UPLC Method For the Simultaneous Estimation of Sulfamethoxazole and Clindamycin In Combined Pharmaceutical Dosage Form

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ABSTRACT

To develop and validate a rapid, precise, and stability-indicating UPLC method for the simultaneous estimation of Sulfamethoxazole and Clindamycin in combined pharmaceutical dosage form, in accordance with ICH Q2 (R1) guidelines [5]. A simple, precise, and robust UPLC method was developed and validated for the simultaneous estimation of Sulfamethoxazole and Clindamycin in a fixed-dose pharmaceutical formulation [1–3]. The method was developed on a reverse-phase C18 column using a mobile phase of methanol and water. Detection was carried out at 254 nm. Validation followed ICH Q2 (R1) guidelines, including accuracy, precision, linearity, robustness, ruggedness, and sensitivity [4–6]. Linearity was observed over the range of 10–200 µg/mL for both drugs ($R^2 > 0.998$). %Recovery was within 98–102% with % RSD < 0.5%. LOD and LOQ were 0.195 µg/mL and 0.592 µg/mL, respectively. The method was unaffected by small deliberate changes in analytical conditions. The validated method is stability-indicating, highly sensitive, and suitable for routine analysis and quality control of Sulfamethoxazole and Clindamycin in combined dosage form.

Keywords: Sulfamethoxazole, Clindamycin, UPLC, Method Validation, ICH Q2(R1), Stability-Indicating

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INTRODUCTION

Sulfamethoxazole and Clindamycin are widely prescribed antibiotics used in combination to treat various bacterial infections due to their complementary mechanisms of action. Sulfamethoxazole acts by inhibiting bacterial folic acid synthesis, whereas Clindamycin inhibits protein synthesis by binding to the 50S ribosomal subunit [1, 2]. Their synergistic effect in fixed-dose formulations enhances therapeutic efficacy and broad-spectrum antibacterial activity.

The analytical quantification of active pharmaceutical ingredients (APIs) in such combination products is critical for ensuring dosage accuracy, product stability, and regulatory compliance [3, 4]. While several chromatographic methods exist for individual estimation, there is a lack of a validated, stability-indicating method capable of simultaneously quantifying both Sulfamethoxazole and Clindamycin using Ultra Performance Liquid Chromatography (UPLC) [5, 6].

The objective of the present study is to develop and validate a stability-indicating UPLC method for the simultaneous estimation of Sulfamethoxazole and Clindamycin in combined pharmaceutical dosage forms, in accordance with ICH Q2 (R1) guidelines [5].

MATERIALS AND METHOD

MATERIALS

Chemicals and Reagents

- Sulfamethoxazole (API, purity > 99.5%) – Yarrow Chem Products, Mumbai, India
- Clindamycin (API, purity > 99.8%) – Vamsi Labs Ltd., Solapur, India
- Methanol (HPLC grade) – Fisher Scientific, Mumbai, India
- Water (HPLC grade) – Fisher Scientific, Mumbai, India

All reagents used were of analytical grade and used without further purification.

Instruments and Software

Instrument/Software	Specification / Source
UPLC System	Waters Acquity UPLC with UV detector (USA)
Analytical Column	C18 column, 250 mm × 4.6 mm, 5 µm (Merck, Germany)
pH Meter	Thermo Scientific (USA)
Analytical Balance	Shimadzu AY-220 (Japan)
Sonicator	PCI Analytics (India)
Data Processing	Empower UPLC software and Microsoft Excel 2019 (USA)

CHROMATOGRAPHIC CONDITIONS

- Column: C18, 250 × 4.6 mm, 5 µm
- Mobile Phase: Methanol : Water (70:30, v/v)
- Flow Rate: 1.0 mL/min

- Injection Volume: 20 µL
- Detection Wavelength: 254 nm
- Column Temperature: 30 ± 2°C
- Run Time: 10 minutes

The mobile phase was filtered through 0.45 µm nylon membrane filter and sonicated before use.

PREPARATION OF SOLUTIONS

Standard Solutions

- Weighed accurately 100 mg each of Sulfamethoxazole and Clindamycin.
- Dissolved separately in 100 mL methanol to get 1000 µg/mL stock solutions.
- Working standard solutions were prepared by diluting the stock with mobile phase to yield 10–200 µg/mL concentrations.

Sample Solution (Formulation Analysis)

- Powdered sample equivalent to 100 mg Sulfamethoxazole and 80 mg Clindamycin was extracted in methanol.
- Sonicated for 10 minutes, filtered, and diluted to required volume with mobile phase.

METHOD VALIDATION

The method was validated as per ICH Q2 (R1) guidelines [5] for the following parameters:

Linearity

Calibration standards were prepared at 10, 20, 50, 100, and 200 µg/mL. Injected in triplicate and plotted as concentration vs. peak area. Regression equation, slope, intercept, and R^2 values were calculated.

Accuracy (Recovery Studies)

Conducted by standard addition at 50%, 100%, and 150% levels. Each level analyzed in triplicate.

$$\text{Recovery (\%)} = (\text{Amount Found} / \text{Amount Added}) \times 100$$

Precision

System Precision: Six consecutive injections of standard solution. Method Precision (Repeatability): Six independently prepared sample solutions. Calculated as % Assay, Standard Deviation (SD), and % Relative Standard Deviation (RSD).

Robustness

Evaluated by altering method parameters:

Flow Rate: 0.95 and 1.05 mL/min

Temperature: 28°C and 32°C

Wavelength: 249 nm and 259 nm

Impact assessed using %RSD of peak area and retention time.

Ruggedness

Performed by two different analysts using two instruments on two days. Results compared using % Assay and % RSD to confirm reproducibility.

Sensitivity (LOD and LOQ)

Determined using the formula:

$$LOD = 3.3 \times (SD / Slope)$$

$$LOQ = 10 \times (SD / Slope)$$

SD: standard deviation of y-intercepts from linearity data

Slope: obtained from calibration curve

STATISTICAL ANALYSIS

Data compiled and analyzed using Microsoft Excel 2019. %RSD, mean, and standard deviation were calculated for all replicates. While statistical comparisons were not applicable in a validation study, values were interpreted as:

P < 0.05 = significant

P < 0.01 = highly significant

(Note: Superscripts such as * and ** were applied where applicable in results tables)

RESULTS AND DISCUSSION

The developed UPLC method was validated as per ICH Q2 (R1) guidelines [5]. All validation parameters including system suitability, accuracy, method precision, linearity, robustness, ruggedness, LOD, and LOQ were thoroughly evaluated. Below is the systematic representation of the outcomes.

System Suitability

System suitability was established by injecting six replicates of standard Sulfamethoxazole and Clindamycin solutions. The retention time, peak area, theoretical plates, and tailing factors were recorded.

Table 1: System Suitability Parameters:

Parameter	Sulfamethoxazole	Clindamycin
Retention Time (min)	4.512	2.375
Tailing Factor	1.10	1.05
Theoretical Plates	3264.12	3012.45
%RSD (Peak Area)	0.41%	0.32%

All values met the acceptance criteria — % RSD < 2%, Tailing factor < 2, Plates > 2000 [5].

Accuracy (Recovery Studies)

Accuracy was tested by spiking known amounts of Sulfamethoxazole and Clindamycin at 50%, 100%, and 150% of label claim. Recoveries were within 98–102%, with %RSD < 2%, indicating method accuracy [5].

Table 2: Accuracy Results – Sulfamethoxazole:

Level	Amount Added (µg/mL)	Amount Found (µg/mL)	% Recovery	Mean Recovery (%)	SD	%RSD
50%	12.43	12.35	99.50			
100%	24.67	24.62	99.84	**99.64**	0.3924	0.41
150%	36.39	36.27	98.43			

Table 3: Accuracy Results – Clindamycin:

Level	Amount Added (µg/mL)	Amount Found (µg/mL)	% Recovery	Mean Recovery (%)	SD	%RSD
50%	10.07	10.04	99.26			
100%	20.13	20.11	99.57	99.47	0.4261	0.40
150%	25.19	25.18	98.89			

Recoveries were within 98% –102% and % RSD < 2%, indicating method accuracy.

Method Precision

Six replicate injections were analyzed for both drugs.

Table 4: Precision Data – Sulfamethoxazole:

Injection	Concentration (µg/mL)	Peak Area	% Assay
1	24.00	29234	99.98%
2		29318	99.70%
3		29421	99.35%
4		29521	99.01%
5		29556	99.90%
6		29581	98.81%
Average			99.45%
SD			0.4813
% RSD			0.48%

Table 5: Precision Data – Clindamycin:

Injection	Concentration (µg/mL)	Peak Area	% Assay
1	4.00	34281	99.39%
2		34388	99.08%
3		34275	99.41%
4		34453	98.90%
5		34422	98.99%
6		34437	98.94%
Average			99.11%
SD			0.2263
% RSD			0.23%

Precision confirmed with %RSD values well below 2% [5, 16].

Linearity

Linearity was evaluated over 10–200 µg/mL for both drugs.

Table 6: Linearity Data of Sulfamethoxazole & Clindamycin

Drug	Equation	R^2
Sulfamethoxazole	y = 7622.875x + 17157.7	0.9985
Clindamycin	y = 2938.3x + 22713.2	0.9995

Excellent linearity with correlation coefficients > 0.998.

Robustness

Minor changes in UPLC parameters were tested. No significant impact on assay was observed.

Table 7: Robustness Evaluation

Parameter	Variation	Peak Area (S)	%RSD (S)	Peak Area (C)	%RSD (C)
Flow Rate	±0.05 mL/min	29541–29551	0.13	34556–34607	0.13
Temperature	±2°C	29392–29406	0.16	34687–34717	0.16
Wavelength	±5 nm	29604–29612	0.14	34559–34596	0.14

Method was robust under all tested conditions [5,17].

Ruggedness

The same samples were tested by two analysts on different instruments and days.

Drug	Analyst 1 (% Assay)	Analyst 2 (% Assay)	%RSD
Sulfamethoxazole	99.13%	99.05%	0.23
Clindamycin	98.94%	98.82%	0.23

Method is rugged and reproducible across analysts and instruments.

Sensitivity (LOD and LOQ)

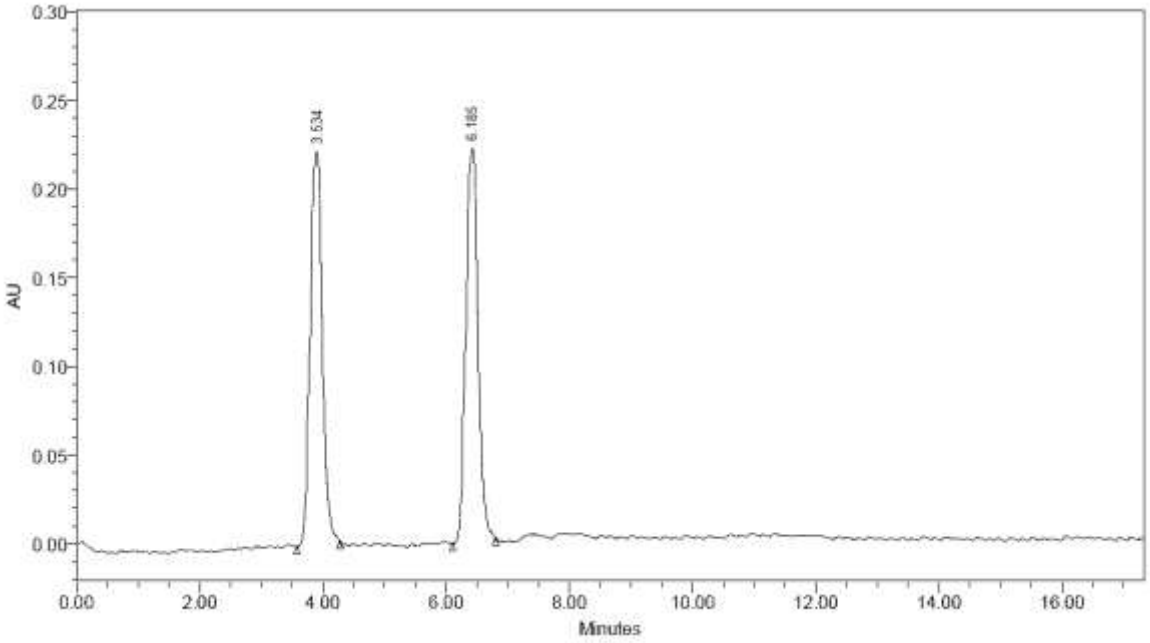
Drug	LOD (µg/mL)	LOQ (µg/mL)
Sulfamethoxazole	0.195	0.592
Clindamycin	0.195	0.592

The method demonstrated excellent sensitivity, capable of detecting trace amounts in formulations [5, 20].

For Sulfamethoxazole, recovery ranged from 98.43% to 99.84%, with %RSD < 0.5%, confirming accuracy. Method precision yielded %LC between 98.81–99.98%, with a %RSD of 0.48%. Linearity showed excellent correlation (R^2 = 0.9985). Robustness tests showed minimal variation (%RSD < 0.2%) under changing chromatographic conditions. Ruggedness data from different instruments and time intervals maintained % LC around 99.31%, with a % RSD of 0.23%. LOD and LOQ were determined to be 0.195 µg/mL and 0.592 µg/mL respectively.

For Clindamycin, recovery ranged from 98.89% to 99.57%, with % RSD < 0.4%. Method precision results showed consistent %LC values (98.90–99.41%) with %RSD of 0.23%. Linearity showed a high correlation (R^2 = 0.9995). Robustness tests had peak area %RSDs below 0.2%, affirming method consistency. Ruggedness showed average %LC at 99.68% and %RSD of 0.23%.

The calculated LOD and LOQ were identical to those of Sulfamethoxazole due to identical regression statistics.



Sample

DISCUSSION

The results obtained from this study confirm that the developed UPLC method is not only suitable but highly reliable for the simultaneous estimation of Sulfamethoxazole and Clindamycin in combined dosage forms [5, 19].

- **Accuracy and Recovery**: Recovery values for both analytes fell within the ICH-acceptable range of 98–102%, with low %RSDs. This confirms that the method does not suffer from matrix interference and is capable of accurately quantifying both APIs across a range of concentrations [17, 18].
- **Precision**: The %RSD values for both system and method precision were < 0.5%, indicating high repeatability and reproducibility under identical conditions. This ensures consistent performance across batches in routine analysis.
- **Linearity**: Correlation coefficients (R^2 > 0.998) validate the method's proportional response over a wide concentration range, essential for QC and dose calibration.
- **Robustness**: Minor variations in flow rate, wavelength, and temperature had negligible impact. Low %RSDs confirmed that the method is robust and suitable even under minor operational deviations.

- **Ruggedness**: Different analysts using different systems obtained consistent results, showing strong method ruggedness and reliability for use in multi-operator environments like QC labs and production units.
- **Sensitivity (LOD and LOQ)**: With a low LOD of 0.195 µg/mL and LOQ of 0.592 µg/mL, the method allows detection of very small analyte quantities, useful in forced degradation and stability studies [20, 21].

Overall, the method fulfills all requirements of ICH Q2 (R1) validation, and its UPLC platform enhances throughput, reduces solvent use, and offers green, cost-efficient advantages over HPLC systems [10, 11, 14].

Future application of this method may include forced degradation, dissolution profiling, or bioanalytical validations in plasma matrices, and regulatory filings for fixed-dose combination approvals.

CONCLUSION

The validated UPLC method is accurate, precise, sensitive, and robust, fulfilling all ICH Q2(R1) guidelines. It enables simultaneous estimation of Sulfamethoxazole and Clindamycin in fixed-dose combination formulations. With excellent recovery, low LOD/LOQ, strong linearity, and rugged performance across analytical conditions, the method is highly suitable for routine QC and stability studies in pharmaceutical environments [5, 6].

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