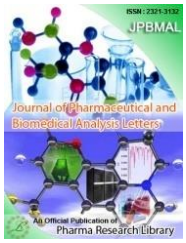

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Integrated Analytical Quality by Design Approach for the Development and Validation of Liquid Chromatography Method for Simulataneous Estimation of Sulfadoxane and Pyrimethamine

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A b s t r a c t
This research evaluates a validated chromatographic method for analyzing sulfadoxine and pyrimethamine. A quadratic model was most effective for retention time analysis ($p < 0.0001$). System suitability met regulatory standards for resolution, theoretical plates, and tailing. The method was validated for assay, linearity, precision, intermediate precision, accuracy, LOD, and LOQ. Assay values were 99.79% (sulfadoxine) and 99.52% (pyrimethamine). Linearity showed strong correlation ($r = 0.9996$ and 0.9993, respectively). Precision was excellent (%RSD<1), and accuracy fell within 98–102%. LODs were 0.5µg/mL and 0.2µg/mL, while LOQs were 1.9 µg/mL and 0.8µg/mL. The method proved robust under varied conditions and is suitable for concurrent formulation analysis.

Keywords: Chromatographic resolution, Sulfadoxine, Pyrimethamine, Validated method, Quadratic model, Retention time analysis, System suitability parameters, Assay and linearity

A r t i c l e I n f o

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C o n t e n t s

1. Introduction

Analytical chemistry helps understand matter's composition through qualitative and quantitative methods. It plays a key role in food safety, environmental monitoring, pharmaceuticals, forensics, and diagnostics. Chromatography is a vital separation technique used to identify individual components in complex mixtures. Invented by Tswett, modern chromatography resolves colored and colorless. Substances efficiently. HPLC is preferred for its speed, precision, accuracy, and ability to automate complex analyses. Reversed-phase HPLC separates compounds based on polarity and hydrophobic interactions. Effective method development ensures proper identification and quantification of drugs. Selection of HPLC conditions depends on the compound's molecular weight, pKa, and solubility. Chromatographic selectivity is influenced by mobile phase, pH, temperature, and solvent composition. Method development focuses on specificity, resolution, and speed of analysis. Method validation confirms the method's reliability, accuracy, and performance consistency. It involves parameters like

linearity, precision, detection limits, and robustness. ISO defines validation as verifying that a method meets its intended use.

ICH and USP guidelines offer standardized steps for method validation. Modern challenges demand new, cost-effective and accurate analytical methods. Excipients and delays in pharmacopoeial inclusion necessitate fresh method development. This study adopts AQbD (Analytical Quality by Design) principles for method optimization. DOE and statistical tools aid in identifying critical method variables. AQbD ensures robustness, lifecycle management, and resource-efficient method design. Overall, the study supports improved, consistent drug analysis with regulatory compliance.

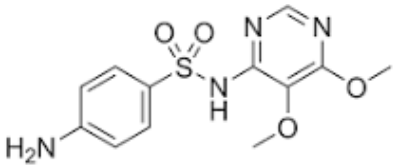

Fig.1: Chemical structure of Sulfadoxine

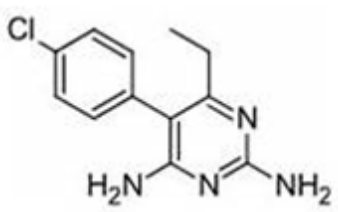

Fig.2: Chemical structure of Pyrimethamine

2. Materials and Methods

List of Proposed Materials:

The analytical estimation of sulfadoxine and pyrimethamine was carried out using high-purity reagents and standards. Potassium dihydrogen phosphate (KH_2PO_4) and formic acid, both of HPLC grade and procured from Qualigens, were specifically utilized for the analysis of sulfadoxine and pyrimethamine. Additionally, HPLC grade water and acetonitrile from Qualigens, along with methanol of HPLC grade from Rankem, were employed universally for the estimation of all drug samples to ensure accuracy and consistency in chromatographic performance.

Equipments and instruments used in the study:

The analytical procedures were carried out using a range of calibrated laboratory equipment. Weighing of samples was performed using a SAB2032 electronic balance by Scaletec, ensuring precision in mass measurement. Sample preparation involved the use of an SE60US ultra-sonicator from Labman Scientific India. Thermal stability and drying processes were conducted using an i-THERM A17782 thermal oven manufactured by Dwaraka Scientific. pH adjustments were made with the help of an ORION STAR A111 pH meter from Thermo Scientific. Filtration of mobile phases and sample solutions was achieved using 0.45-micron filter paper supplied by Millipore. The core chromatographic analysis was conducted using the WATERS 2690 separation module HPLC system from Waters, ensuring high-resolution performance and reproducibility.

HPLC Method Development:

Choosing λmax:

UV spectrum of 10µg per ml Sulfadoxine & Pyrimethamine in diluents (MP ratio) was examined by scanning in the scale of 200 to 400nm and the isobestic λmax of both the drugs acquired at 230 nm.

Method Development by QBD Optimization

Column Optimization:

Phenomenex Zorbax C_{18}-SB, (150×4.6mm, 3µm) uncovered to be perfect as it gave excellent Guassian shape peak & R_s at flow 1 ml per min.

Chromatographic Optimization Conditions

Instrument used : RP-HPLC having Auto Sampler and PDA or UV detector

Temperature : Ambient

Column : Phenomenex ZorbaxC_{18}-SB, (150×4.6mm, 3µm)

Buffer : Triehylamine (pH-5)

MP ratio: 50% Triehylamine: 50% Methanol

Flow : 1.0 ml per min

Λmax : 230 nanometers

Volume Injected : 10 µl

Run time : 10 min

Buffer & mobile phase making:

KH2PO4 PH 3.5Preparation:

By adding 6.4g of KH2PO4 in 1L HPLC water adjust the solution to PH 3.5 by using 0.1% of OPA solution.

Preparation MP:

Combine 500 ml of KH2PO4 solution (pH 3.5) with 500 ml of ACN, each at 50% concentration. Degas the mixture in an ultrasonic water bath for 5 min's, then filter it using a 0.45 µm filter using vacuum filtration.

Diluent: KH2PO4 (PH 3.5): ACN (50:50ml)

System Suitability: Tailing factor for Sulfadoxine &Pyrimethamine in Standard solution shouldn't >2.0 For Standard solution Theoretical plates for the Sulfadoxine& Pyrimethamine peaks shouldn't <2000

Calculation: **(For Sulfadoxine and Pyrimethamine)**

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Acceptance criteria of System Suitability:

Tailing factor should be < 2

Theoretical Plates should be >2000

Validation Method Parameters

Assay Method (HPLC)

Standard Solution Preparation:

- Accurately weigh 25 mg of Sulfadoxine and 15 mg of Pyrimethamine into a 25 mL volumetric flask.
- Add diluent, sonicate to dissolve, and make up the volume.
- Pipette 0.6 mL of this solution into a 10 mL volumetric flask and dilute to volume (final conc.: Sulfadoxine 60 ppm, Pyrimethamine 36 ppm).

Sample Solution Preparation:

- Accurately weigh a quantity equivalent to 25 mg Sulfadoxine and 15 mg Pyrimethamine into a 25 mL flask.
- Add diluent, sonicate to dissolve, and make up the volume.

- Pipette 0.6 mL of this into a 10 mL flask and dilute to volume.

Procedure:

- Inject 10 µL of standard and sample into the HPLC.
- Record peak areas and calculate % Assay using standard formula.

Linearity Study

Stock Solution:

- Prepare as above (25mg Sulfadoxine + 15mg Pyrimethamine in 25 mL with diluent).

Linearity Levels:

To establish linearity for Sulfadoxine and Pyrimethamine, five concentration levels were prepared from a common stock solution. For Level I, 0.2 mL of the stock solution was diluted to 10 mL with diluent, yielding a final concentration of 20 ppm Sulfadoxine and 12 ppm Pyrimethamine. In Level II, 0.4 mL of the stock was diluted to 10 mL, resulting in 40 ppm Sulfadoxine and 24 ppm Pyrimethamine. Similarly, Level III was prepared using 0.6 mL of stock diluted to 10 mL, giving 60 ppm Sulfadoxine and 36 ppm Pyrimethamine. For Level IV, 0.8 mL of stock was used to achieve 80 ppm Sulfadoxine and 48 ppm Pyrimethamine. Finally, Level V involved diluting 1.0 mL of stock to 10mL to obtain 100ppm Sulfadoxine and 60 ppm Pyrimethamine. These solutions were injected into the HPLC to construct a calibration curve and evaluate linearity.

Procedure:

- Inject each concentration into the HPLC.
- Plot Calibration Curve: Peak Area (Y-axis) vs. Concentration (X-axis).
- Determine correlation coefficient (R^2) for linearity.

Precision

Preparation: 25 mg of Sulfadoxine and 15 mg of Pyrimethamine were dissolved in 25 mL diluent, sonicated, and diluted to volume. From this, 0.6 mL was transferred into a 10 mL volumetric flask and diluted (60 ppm/36 ppm).

Procedure:

Standard solution was injected six times**;** %RSD of peak areas was calculated and found within acceptable limits.

Intermediate Precision (Ruggedness)

Procedure repeated on a different day using the same preparation as above. Six replicate injections were performed and %RSD calculated, confirming method consistency.

Accuracy:

Prepared 50%, 100%, and 150% concentrations using proportional weights of the analytes.

Procedure: Inject standard, and three accuracy levels. Calculate recovery for each level; mean recovery found within ICH acceptance criteria.

Limit of Detection (LOD)

Sulfadoxine LOD: Prepared a 0.5 µg/mL solution by serial dilution.

Pyrimethamine LOD:

Prepared a 0.2 µg/mL solution by stepwise dilution. Both analytes showed detectable peaks at respective concentrations.

Limit of Quantification (LOQ)

Sulfadoxine LOQ: 1.9 µg/mL solution prepared via serial dilution.

Pyrimethamine LOQ:

0.8 µg/mL solution prepared similarly. Both analytes showed quantifiable and reproducible peaks.

Robustness

- Changing flow rate (0.8–1.2 mL/min)
- Altering mobile phase organic ratio (40%–60%)
- Standard solutions (30µg/mL) were analyzed. No significant change in retention time or peak area observed, confirming robustness.

3. Results and Discussion

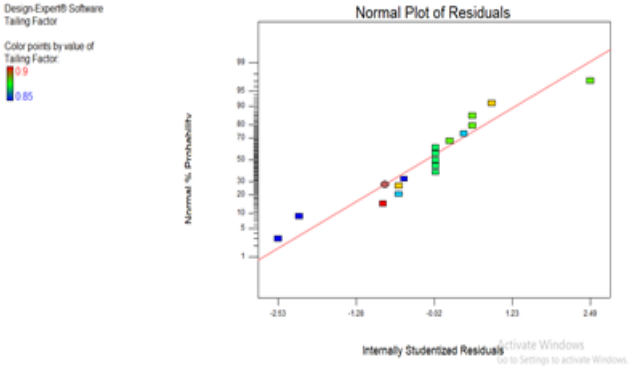

Fig.3: Normal plot of Residuals for Sulfadoxine and Pyrimethamine

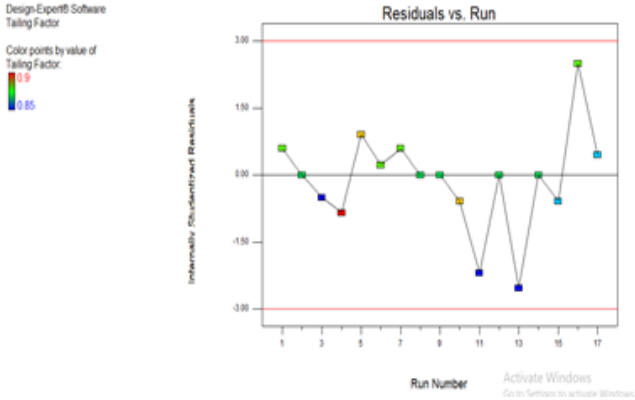

Fig.4: Residuals vs. Run for Sulfadoxine and Pyrimethamine

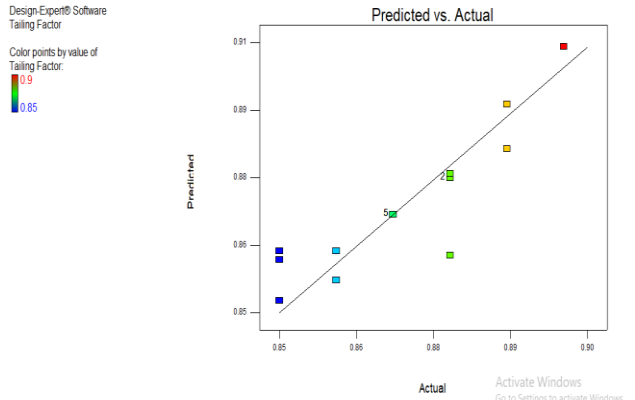

Fig.5: Predicted vs. Actual for Sulfadoxine and Pyrimethamine

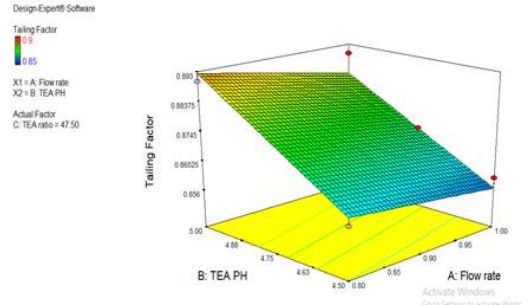

Fig.6: 3D Surface for Sulfadoxine and Pyrimethamine

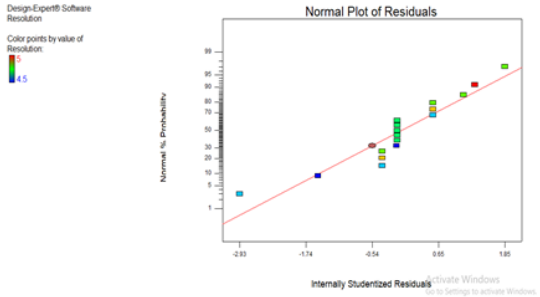

Fig.7: Normal plot of Residuals for Sulfadoxine and Pyrimethamine

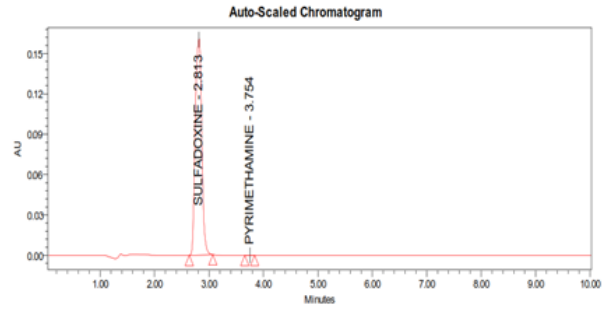

Fig.8: System suitability Chromatogram

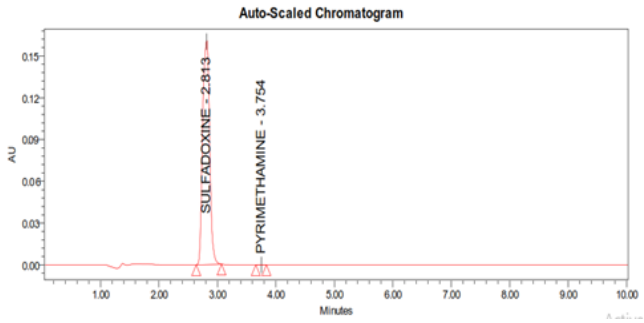

Fig.9: Chromatograph for Standard

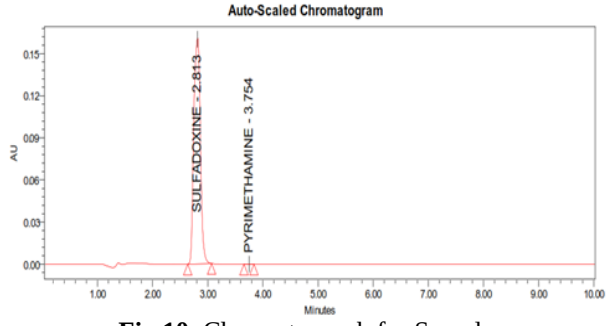

Fig.10: Chromatograph for Sample

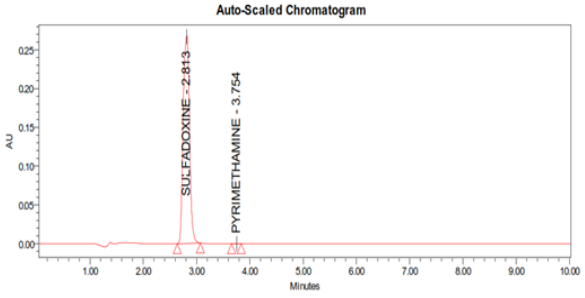

Fig.11. linearity-5 Chromatogram

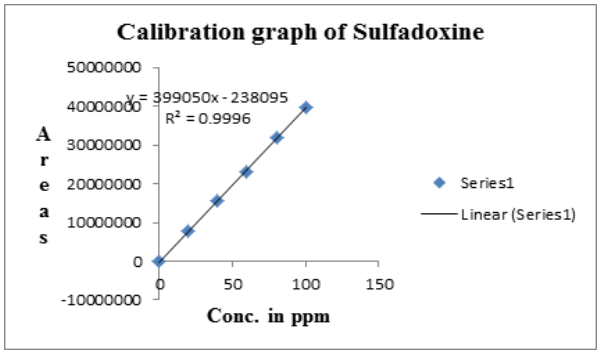

Fig.12: Sulfadoxine Calibration graph

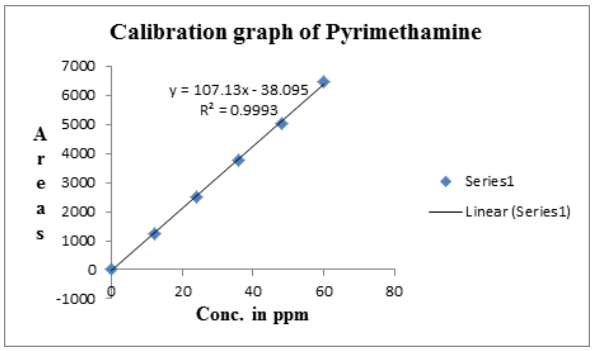

Fig.13: Pyrimethamine Calibration graph

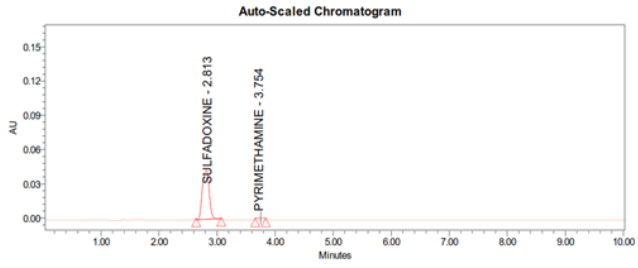

Fig.14: Sulfadoxine and Pyrimethamine depicting LOD Chromatogram

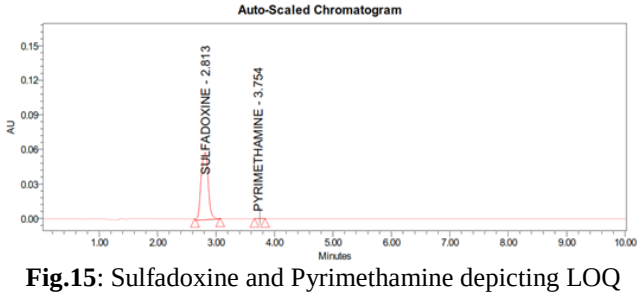

Fig.15: Sulfadoxine and Pyrimethamine depicting LOQ Chromatogram

Table 1: Software Information

		1 Factor	2 Factor	3 Factor	1 Response	2 Response
Std	**Run**	**A:Flow Speed**	**B:TEA PH**	**C:TEA ratio**	**Tailing factor**	**Resolution**
5	1	0.80	4.75	45.00	0.88	4.8
16	2	1.00	4.75	47.50	0.87	4.7
11	3	1.00	4.50	50.00	0.85	4.5
12	4	1.00	5.00	50.00	0.9	5
4	5	1.00	5.00	47.50	0.89	4.9
8	6	1.00	4.75	50.00	0.88	4.8
7	7	0.80	4.75	50.00	0.88	4.8
14	8	1.00	4.75	47.50	0.87	4.7
15	9	1.00	4.75	47.50	0.87	4.7
3	10	0.80	5.00	47.50	0.89	4.9
9	11	1.00	4.50	45.00	0.85	4.5
17	12	1.00	4.75	47.50	0.87	4.7
10	13	1.00	5.00	45.00	0.85	4.6
13	14	1.00	4.75	47.50	0.87	4.7
1	15	0.80	4.50	47.50	0.86	4.6
6	16	1.00	4.75	45.00	0.88	4.8
2	17	1.00	4.50	47.50	0.86	4.6

Table 2: Response 2: tailing factor

Souces	**Sum of square**	**df**	**Mean square**	**F value**	**P value Prob>F**	
Model	0.21	3	0.071	9.58	0.0013	significant
A-Flow rate	0.014	1	0.014	1.86	0.1955	
B-TEA PH	0.18	1	0.18	24.19	0.0003	
C-TEA ratio	0.020	1	0.020	2.69	0.1251	
Residual	0.097	13	7.441E-003			
Lack of Fit	0.097	9	0.011			
Pure Error	0.000	4	0.000			

Table 3: SST parameters outcomes

S.No	**Name's**	**RT's(min)**	**Area(µV sec)**	**Height (µV)**	**tailing USP**	**plate count USP**
1	Sulfadoxine	2.813	23257290	2812779	1.06	2990
2.	Pyrimethamine	2.754	3771	632	1.04	7488

Table 4: Assay Outcomes for Sulfadoxine & Pyrimethamine

	Label Claim (mg)	**% Assay**
Sulfadoxine	500mg	99.79
Pyrimethamine	25mg	99.52

Table 5: Regression equation parameters of Sulfadoxine & Pyrimethamine

Parameters	**Sulfadoxine**	**Pyrimethamine**
Slope (m)	399050	107.13
Intercept (c)	238095	38.095
Coefficient of Correlation (R^2)	0.9996	0.9993

Table 6: LOD Outcomes

Drug's	**Baseline noise(µV)**	**Signal attained(µV)**	**Signal/Noise ratio**	**Conc.**
Sulfadoxine	88	262	2.97	0.5µg/ml
Pyrimethamine	88	253	2.87	0.2µg/ml

Table 7: Sulfadoxine and Pyrimethamine depicting LOQ Outcomes

Drug's	Baseline noise(µV)	Signal attained(µV)	Signal/Noise ratio	Conc.
Sulfadoxine	88	875	9.94	1.9µg/ml
Pyrimethamine	88	870	9.88	0.8µg/ml

Figure 8: Sulfadoxine and Pyrimethamine depicting LOD Chromatogram

Drug's	Baseline noise(µV)	Signal attained(µV)	Signal/Noise ratio	Conc.
Sulfadoxine	88	262	2.97	0.5µg/ml
Pyrimethamine	88	253	2.87	0.2µg/ml

4. Conclusion

An HPLC method was developed for the simultaneous estimation of Sulfadoxine and Pyrimethamine using a Phenomenex Zorbax C18-SB column (150 × 4.6 mm, 3 µm) with a flow rate of 1.0 mL/min. The mobile phase, a 1:1 mixture of Triethylamine buffer (pH 5) and Methanol, provided sharp peaks with retention times of 2.189 min (Sulfadoxine) and 3.754 min (Pyrimethamine). Detection was at 230 nm with a 10 µL injection volume. System suitability was confirmed with tailing factors <2.0 and theoretical plates >2000. Validation included assay and linearity (20–100 ppm), confirming the method's reliability. The developed HPLC method is simple, precise, and robust for simultaneous estimation of Sulfadoxine and Pyrimethamine. It meets ICH validation criteria and is suitable for routine pharmaceutical analysis.

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