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Development and Validation of a Liquid Chromatography Method for the Simultaneous Estimation of Sulfadoxine and Pyrimethamine in Combined Dosage Forms

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Abstract
The final method employed a Platisil C18-ODS column (250×4.6 mm, 5 µm) with a mobile phase of 50% 0.1% formic acid (pH 4.5) and 50% methanol, at a flow rate of 1.0 ml/min, and UV detection at 230 nm. System suitability parameters such as retention time, theoretical plates, and tailing factor were within acceptable limits, confirming the method's efficiency and reliability. Validation was performed according to ICH Q2(R1) guidelines, demonstrating excellent analytical performance. Assay results showed drug content of 99.1% for Sulfadoxine and 98.6% for Pyrimethamine. Linearity was observed over wide concentration ranges with correlation coefficients of 0.9997 and 0.9996, respectively. Precision and intermediate precision studies yielded %RSD values below 2%, indicating high repeatability and ruggedness. Recovery studies confirmed accuracy within the 98–102% range, while LOD and LOQ values (0.02 µg/ml for Sulfadoxine and 0.05µg/ml for Pyrimethamine) highlighted the method's sensitivity. Robustness testing under slight variations in flow rate and mobile phase composition showed consistent results, affirming the method's suitability for routine quality control of combined dosage formulations.

INTRODUCTION

RP-HPLC operates on the principle of hydrophobic interactions, which originates from the high symmetry in the dipolar water structure and plays the most important role in all processes in life science. RP-HPLC allows the measurement of these interactive forces. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand on the stationary phase.

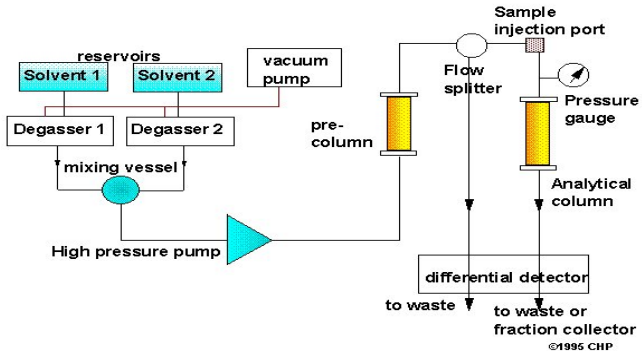

Fig.1: Schematic Diagram of HPLC

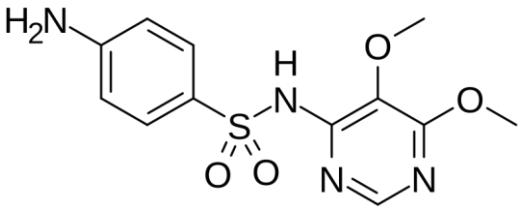

Fig.2: Sulfadoxine

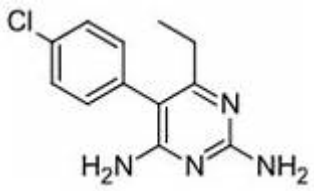

Fig.3: Pyrimethamine

MATERIALS AND METHODS

Table 1: Instruments used

S.No	Instrument	Model
1	HPLC	WATERS, software: Empower, 2695 separation module.2487 UV detector.
2	UV/VIS	LABINDIA UV 3000$^+$
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table 2: Chemicals used

S.No	Chemical	Brand
1	Betamethasone	Supplied by KP LBAS
2	Calcipotriene	Supplied by KP LBAS
3	Ortho phosphoric acid	FINAR chemical LTD
4	Water and Methanol	Standard solutions Ltd
5	Acetonitrile for HPLC	Standard solutions Ltd
6	HCl, H_2O_2, NaOH	MERCK

Wave length selection:

UV spectrum of 10µg/ml Sulfadoxine and Pyrimethamine in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm and the isobestic λmax of both the drugs obtained at 230 nm.

Optimization of Column:

Platisil C18-ODS, (250×4.6mm, 5□m) was found to be ideal as it gave good peak shape and resolution at 1.0 ml/min flow.

Optimized Chromatographic Conditions

Instrument used : High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector

Temperature : Ambient
Column : Platisil C18-ODS, (250×4.6mm, 5µm)
Buffer : 0.1% Formic acid (Ph 4.5)
Mobile phase : 50% 0.1% Formic acid: 50% Methanol
Flow rate : 1.0ml per min
Wavelength : 230nm
Injection volume : 10µl
Run time : 10min.

Preparation of buffer and mobile phase:

Preparation of 0.1% Formic acid PH 4.5:

To prepare 0.1% Formic acid buffer solution by adding 1ml of Formic acid in 100ml HPLC water adjust the solution to PH 4.5 by using NAOH solution.

Preparation of mobile phase:

Mix a mixture of above 0.1% Formic acid (PH 4.5) 500ml (50%) and 500 ml MEOH (50%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

0.1% Formic acid (PH 4.5): MEOH (50:50ml)

System Suitability:

Tailing factor for the peaks due to Sulfadoxine and Pyrimethamine in Standard solution should not be more than 2.0. Theoretical plates for the Sulfadoxine and Pyrimethamine peaks in Standard solution should not be less than 2000

Calculation: (For Sulfadoxine and Pyrimethamine)

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

AT = average area counts of sample preparation.
AS = average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard
LC = Label Claim mg/ml.

Standard Solution Preparation:

Accurately weigh and transfer 20 mg of Sulfadoxine and 12mg Pyrimethamine working standard into a 20 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.(30ppm, 18ppm).

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 20 mg of Sulfadoxine and 12mg Pyrimethamine equivalent weight of the sample into a 20 ml clean dry volumetric flask add about 7ml of Diluents and sonicate to Dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure: Inject 30 µL of the standard, sample into the chromatographic system and measure the areas for the Sulfadoxine and Pyrimethamine peaks and calculate the % Assay by using the formulae.

RESULTS AND DISCUSSION

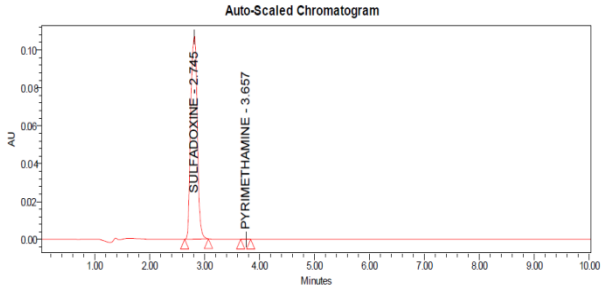

Fig.3: Chromatogram for system suitability

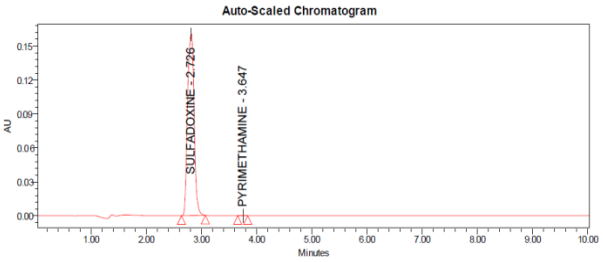

Fig.4: Chromatogram for Sample

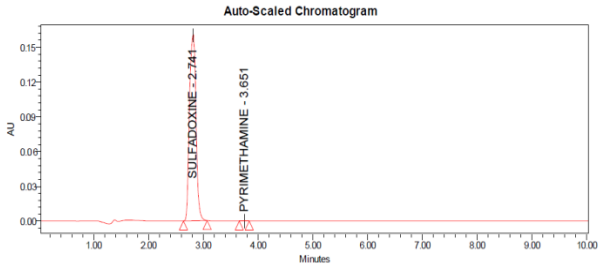

Fig.5: Chromatogram for Standard

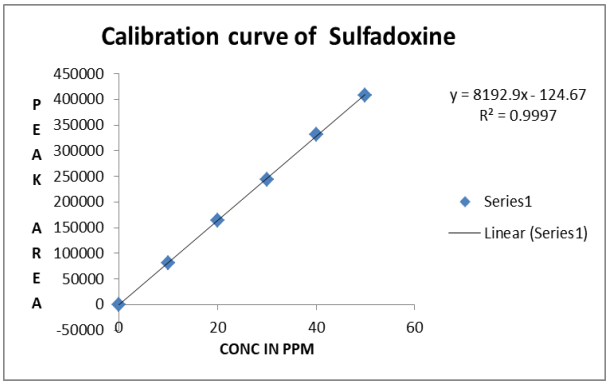

Fig.6: Calibration graph for Sulfadoxine

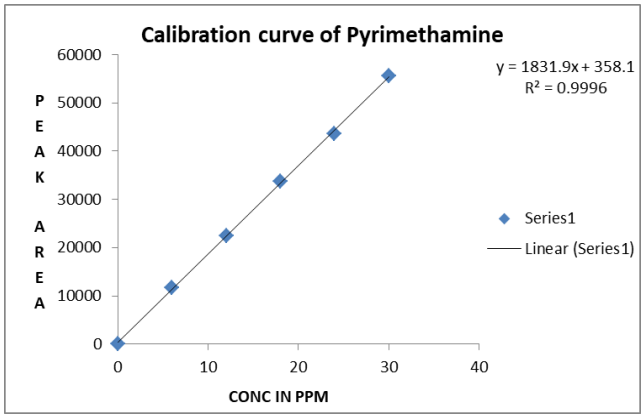

Fig.7: Calibration graph for Pyrimethamine

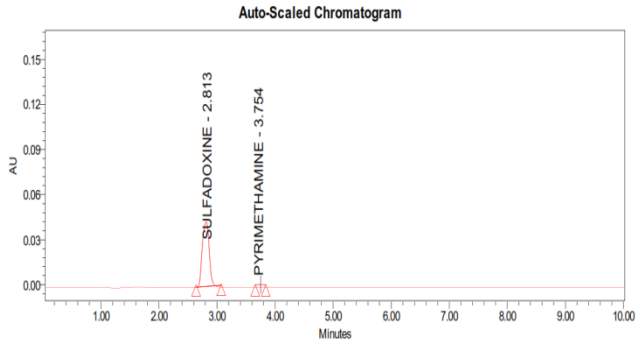

Fig.8: Sulfadoxine and Pyrimethamine showing LOD

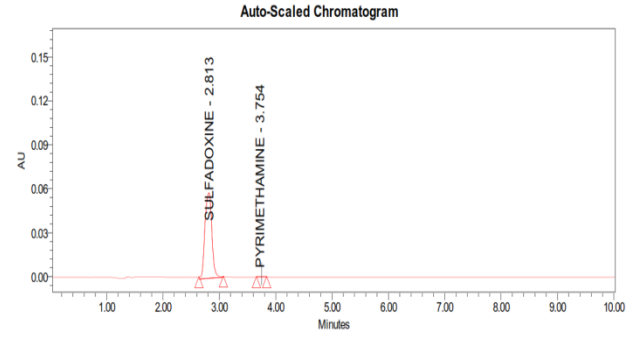

Fig.9: Sulfadoxine and Pyrimethamine showing LOQ

CONCLUSION

The validated method met all ICH Q2(R1) guidelines for analytical performance. Assay results showed high accuracy, with content found to be 99.1% for Sulfadoxine and 98.6% for Pyrimethamine. The method was proven to be linear over a wide concentration range with correlation coefficients of 0.9997 for Sulfadoxine and 0.9996 for Pyrimethamine. Precision and intermediate precision showed %RSD values below 2, indicating excellent repeatability and ruggedness. The accuracy/recovery studies yielded mean recoveries within the acceptable range (98–102%). LOD and LOQ values demonstrated the sensitivity of the method, with limits as low as 0.02 µg/ml for Sulfadoxine and 0.05 µg/ml for Pyrimethamine. Moreover, robustness studies revealed the method's stability under slight variations in flow rate and mobile phase composition. Overall, the method is simple, reliable, precise, and suitable for routine quality control analysis of Sulfadoxine and Pyrimethamine in combined dosage formulations.

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