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Development and validation RP-HPLC method for simultaneous estimation of Sulfamethoxazole and Trimethoprim

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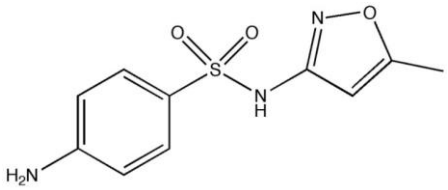
ABSTRACT

A simple, economic, selective, precise, and accurate Reverse phase High Performance Liquid Chromatography method for analysis of Sulfamethoxazole and Trimethoprim, was developed and validated according to ICH guidelines. The quantification of drug was carried out using Agilent C18 250mm× 4.6mm × 5µm column or its equivalent in isocratic mode, with mobile phase compressing of Triethylamine: Acetonitrile (30:70) the flow rate was 1.0ml/min and the detection was carried at 260nm. The retention time and percentage assay for Sulfamethoxazole and Trimethoprim was found to be 2.688 and 4.388min, 100.23% and 99.30% respectively. Proposed method was validated for precision, accuracy, linearity range, specificity and robustness.

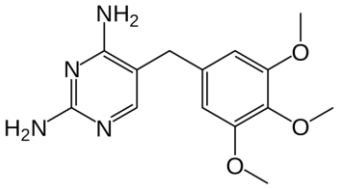
1. INTRODUCTION

Sulfamethoxazole is chemically 4-amino –N (5-Methel-1,2-oxazol-3-yl)benzene-1-sulfonamide.
The mechanism of sulfamethoxazole is inhibit conversion of pteridine and p-aminobenzoic acid to dihydropteroic acid by competing with PABA for binding to dihydrofolate synthetase an intermediate of tetrahydrofolic acid synthesis (THF). THF is required for the synthesis of purines and dTMP and inhibition of bacterial growth.

Trimethoprim is chemically 5(3,4,5 trimethoxy phenyl) methyl phrimidine-2,4-di amine. The mechanism of trimethoprim is, it binds to dihydrofolate reductase and inhibits dihydrofolic acid to tetrahydrofolic acid. THF is an essential precursor in the thymidine synthesis pathway and interference with this pathway inhibits bacterial DNA synthesis.



Sulfamethoxazole



Trimethoprim

2. MATERIAL AND METHODS

Chemicals and Reagents: Sulfamethoxazole and Trimethoprim were obtained as gift samples from AURUBINDO labs Pvt. ltd, Hyderabad. We used HPLC grade acetonitrile, water and GR grade triethylamine and ortho phosphoric acid.

Instrumentation: A HPLC (Alliance,Water2695) with UV/VIS Detector/PDA detector, UV (lab India ,UV 3000 series) and Agilent C18 250mm × 4.6mm × 5µm column was used. The HPLC system was equipped with Empower software for data processing.

Chromatographic Condition: The mobile phase containing Buffer: Acetonitrile (30:70) was found to resolve Sulfamethoxazole and Trimethoprim. Ortho phosphoric acid was used for pH adjustment of buffer to 4 .0. The mobile phase was filtered through 0.45 nylon filter and then ultrasonicated for 30 min. The flow rate was set to 1.0ml/min. The drug shows good absorbance at 260nm, which was selected as wavelength for further analysis.

Buffer Preparation: Accurately weighed and transferred 1.143grams of ortho phosphoric acid into 1000ml of distilled water and adjust pH with tri

ethylamine to 4.0. Filter the solution through 0.45µm nylon filter.

Preparation of Mobile Phase: Prepare, filtered and degassed mixture of buffer and Acetonitrile in the ratio of 30:70 v/v.

Preparation of Standard solution: Accurately weighed and transferred about 50mg of sufamethoxazole and 10mg of Trimethoprim working standard into a 10ml volumetric flask add 2ml of mobile phase, sonicated for 15min and make up to the mark with mobile phase.

Preparation of Sample solution: Crush 20 tablets and transferred accurately weighed powder equivalent to 50mg of Sulfamethoxazole and 10mg of Trimethoprim into 10ml volumetric flask add 7ml of mobile phase sonicate for 20min to dissolve and make up to the volume with mobile phase. Filter the solution through 0.45µm nylon filter. Transfer 0.1ml of above solution into 10ml volumetric flask and make up to the volume with mobile phase.(50ppm of sulphamethoxazole &10ppm of trimethoprim).

Method validation:

i) System Suitability: System Suitability was performed by injecting five replicate injections of standard solutions of Sulfamethoxazole and Trimethoprim at 100% & measured retention time, theoretical plates and tailing factor.

ii) System Precision: To assess the system precise for conducting validation inject five replicates of standard preparations of 100% level for Sulfamethoxazole and Trimethoprime and expressed as %RSD of peak area.

iii) Specificity: To demonstrate that diluents and placebo are not interfering with analytic peak. Solutions of Standard and Sample were prepared as per test procedure and injected into the HPLC system.

iv) Method Precision: Method Precision was measured in terms of repeatability of application and measurement. Repeatability of sample application was carried out using five replicates of the same sample concentration.

v) Linearity: The linearity of the HPLC method was demonstrated for Sulfamethoxazole and Trimethoprim solutions ranging from 50% to 250% and 10% to 50% of standard concentrations.

vi) Accuracy (%Recovery): %Recovery studies were carried out at three different levels of 50%, 100% and 150% of standard solution (i.e. Sulfamethoxazole and Trimethoprim API spiked to the placebo) in triplicate in each level.

vii) Robustness: The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate and wave length which may differ but the responses were still within the specified limits.

viii) Ruggedness: The variability of the results obtained with the analysis of Sulfamethoxazole and Trimethoprim sample five times by two different analysts, two different reagents, two different columns, two different instruments on two different days to assess the method ruggedness.

3. RESULTS AND DISCUSSION

Optimization of the mobile phase was performed based on resolution, asymmetric factor and peak area. The mobile phase Acetonitrile: buffer (70:30) was found to be satisfactory and gave symmetric and well resolved peak for Sulfamethoxazole and Trimethoprim. Results were summarized in Table.1.

Table.1.System suitability data

Parameter	Sulfamethoxazole	Trimethoprim
Tailing Factor	1.28	1.13
Theoretical Plates	2842	5525
%RSD of Peak area	0.2	0.8

The percentage relative standard deviation for peak area of Sulfamethoxazole and Trimethoprim in system precision was found to be 0.2 and 0.2 which indicate that the test method meets the acceptance criteria. Results were summarized in Table.2.

Table.2.System Precision Data

Parameter	Sulphamethoxazole	Trimethoprim
Mean peak area	4744773.8	1148228.6
SD	3732.3	7654.0
%RSD of Peak area	0.8	0.7

Good resolution obtained between analytes of Sulfamethoxazole and trimethoprim peaks and no interference of blank and placebo observed at the retention times of sulphamethoxazole and trimethoprim and chromatograms were shown in Fig.1 & 2.

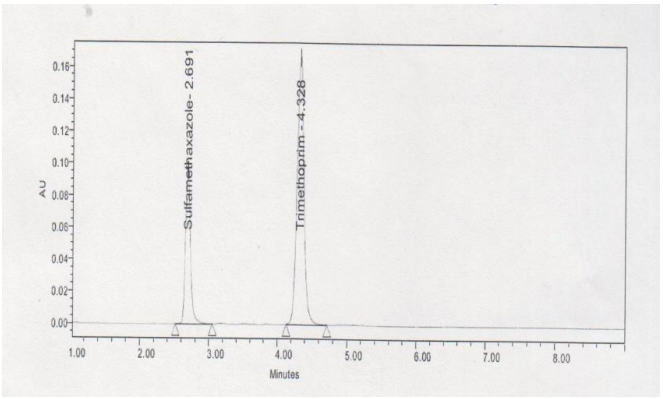


Figure.1.Chromatogram of Standard

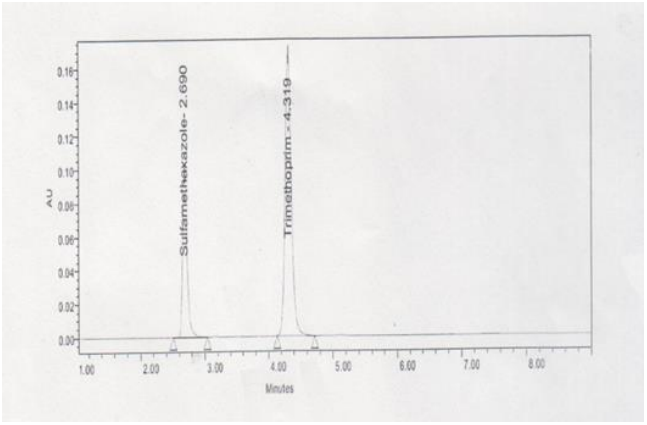


Figure.2.Chromatogram of Sample

Precision: Precision was determined & the results are represented in the form of %RSD for peak area of Sulfamethoxazole and Trimethoprim were found to be below 2% & shows that the test method was highly precise and results are given in Table.3.

Table.3.Repeatability data

Parameter	Sulphamethoxazole	Trimethoprim
Mean peak area	480040.6	1156687.4
SD	30002.7	4080.8
%RSD	0.6	0.4

Linearity: The correlation coefficient (r2) for Sulphamethoxazole and Trimethoprim was found to be 0.999 & 0.999 and shows good linearity. Data of the calibration curve was given in Table.4.

Table.4.Linearity Data

Level	Concentration (µg/ml)		Peak Area	
	Sulfamethoxazle	Trimethoprim	Sulfamethoxazole	Trimethoprim
1	50	10	399310	967642
2	100	20	474677	1145547
3	150	30	505907	1259209
4	200	40	590529	1417945
5	250	50	640393	1679025
Correlation Coefficient	-	-	**0.999**	**0.999**

The mean recovery for Sulphamethoxazole and Trimethoprim were found to be in range of 99.51%-99.52%and99.14%-99.23% the result summarized in Table.5.

Table.5.Accuracy data for Sulphamethoxazole and Trimethoprim

Injection	50%	100%	150%	50%	100%	150%
Inj-1	681496	912240	1475725	1609146	2213573	1664652
Inj-2	682147	900935	1472830	1611190	2187730	1659574
Inj-3	678341	897878	1476143	1603831	2178547	1664048
AVG	680661.3	903684.3	1474899.3	1608055.6	2193283.3	1662758
S.D	2035.66	7565.44	1804.24	3798.72	18161..35	2773.91
%RSD	0.2990	0.8371	0.1223	0.2362	0.8280	0.1668

Robustness: As part of the robustness, deliberate changes in the flow rate and wave lengt was made to impact on the method. Retention time and Peak area were significantly changed but within the acceptance limit and results given in Table.6.

Table.6.Robustness data

Parameter	Retention Time		Peak Area	
	Sulfamethoxazol	Trimethoprim	Sulfamethoxazo	Trimethopri
Actual	2.688	4.388	467354	112147
More Flow Rate	2.544	3.997	422704	1016303
Low Flow Rate	2.867	4.621	518500	1253302
More organic	2.674	3.302	473756	1093816
Less organic phase	2.731	4.595	465830	1158297

4. CONCLUSION

It can be concluded that the proposed RP-HPLC method is accurate, precise, sensitive, specific, robust and reproducible for the simultaneous analysis of sulphamethoxazole and trimethoprim with less tailing factor and is also economical.

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