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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF SULFAMETHOXAZOLE AND TRIMETHOPRIM BY RP-HPLC

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Abstract:

Objective: *A simple, Accurate, precise method was developed for the simultaneous estimation of the Sulfamethoxazole and Trimethoprim in pharmaceutical dosage form.*

Methods: *Chromatogram was run through Symmetry C18 (4.6 x 150mm, 5µm). Mobile phase containing 70% MeOH: 30% phosphate buffer pH 4.6 was pumped through column at a flow rate of 1 ml/min. Buffer used at pH 4.6. Temperature was maintained at Ambient. Optimized wavelength for Sulfamethoxazole and Trimethoprim was 273 nm.*

Results: *Retention time of Sulfamethoxazole and Trimethoprim were found to be 2.003 min and 5.067 min. The % purity of Sulfamethoxazole and Trimethoprim was found to be 100.84 % and 100.29 % respectively. The system suitability parameters for Sulfamethoxazole and Trimethoprim such as theoretical plates and tailing factor were found to be 2711,1.6 and 3428, 1.3. the resolution was found to be 10.0. The linearity study for Sulfamethoxazole and Trimethoprim was found in concentration range of 25µg-125 µg and 25 µg-125 µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100.17 % and 100.24 %, %RSD for repeatability was 0.54 and 0.27 %. The precision study was precise, robust and repeatable. LOD value was 3.14 and 2.75, and LOQ value was 10.05 and 9.96 respectively.*

Conclusion: *The results of study showed that the proposed RP-HPLC method is a simple, accurate, precise, rugged, robust, fast and reproducible, which may be useful for the routine estimation of Sulfamethoxazole and Trimethoprim in pharmaceutical dosage form.*

Keywords: *Sulfamethoxazole, Trimethoprim, RP-HPLC, Simultaneous estimation.*

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INTRODUCTION:

Sulfamethoxazole is a bacteriostatic sulfonamide antibiotic that interferes with folic acid synthesis in susceptible bacteria. [1] It is generally given in combination with trimethoprim, which inhibits a sequential step in bacterial folic acid synthesis - these agents work synergistically to block two consecutive steps in the biosynthesis of nucleic acids and proteins which are necessary for bacterial growth and division, and using them in conjunction helps to slow the development of bacterial resistance. [1] In this combination, sulfamethoxazole is useful for the treatment of a variety of bacterial infections, including those of the urinary, respiratory, and gastrointestinal tracts. Sulfamethoxazole is indicated in combination with trimethoprim, in various formulations, for the following infections caused by bacteria with documented susceptibility: urinary tract infections, acute otitis media in pediatric patients (when clinically indicated), acute exacerbations of chronic bronchitis in adults, enteritis caused by susceptible Shigella, prophylaxis and treatment of Pneumocystis jiroveci pneumonia, and travelers' diarrhea caused by enterotoxigenic E. coli. [2] IUPAC name 4-amino-N-(5-methyl-1,2-oxazol-3-yl) benzene-1-sulfonamide. Molecular formula $C_{10}H_{11}N_3O_3S$. Molecular Weight 253.7.

Trimethoprim is an antifolate antibacterial agent that inhibits bacterial dihydrofolate reductase (DHFR), a critical enzyme that catalyzes the formation of tetrahydrofolic acid (THF) - in doing so, it prevents the synthesis of bacterial DNA and ultimately continued bacterial survival. [3] Trimethoprim is often used in combination with sulfamethoxazole due to their complementary and synergistic mechanisms but may be used as a monotherapy in the treatment and/or prophylaxis of urinary tract infections.[4] It is structurally and chemically related to pyrimethamine, another antifolate antimicrobial used in the treatment of plasmodial infections IUPAC Name 5-[(3,4,5-trimethoxyphenyl)methyl]pyrimidine-2,4-diamine hydrochloride. Molecular Formula $C_{14}H_{19}ClN_4O_3$. Molecular Weight 326.7

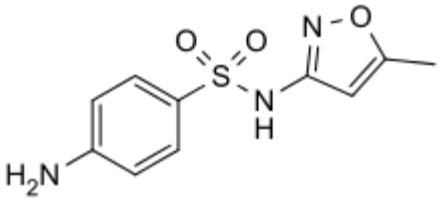

Figure 1: Structure of Sulfamethoxazole

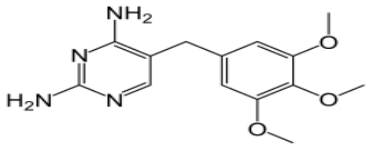

Figure 2: Structure of Trimethoprim

The literature survey revealed that There are very few methods reported in the literature for analysis of Sulfamethoxazole and Trimethoprim alone or in combination with other drugs in the pure form and pharmaceuticals formulations by RP-HPLC.[5-9] In view of the need for a suitable, cost-effective RP-HPLC method for routine analysis of Sulfamethoxazole and Trimethoprim Simultaneous estimation of in pharmaceutical dosage form. Attempts were made to develop simple, precise, accurate and cost-effective analytical method for the estimation of Sulfamethoxazole and Trimethoprim. The proposed method will be validated as per ICH guidelines. The objective of the proposed work is to develop a new, simple, sensitive, accurate and economical analytical method and validation for the Simultaneous estimation of Sulfamethoxazol eand Trimethoprim in pharmaceutical dosage form by using RP-HPLC. To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form.

MATERIALS AND METHODS:

Chemicals and Reagents: Sulfamethoxazole and Trimethoprim were Purchased from market. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 273 nm with column Symmetry C18 (4.6 x 150mm, 5µm), dimensions at 25^0C temperature. The optimized mobile phase consists of70% MeOH: 30% phosphate buffer pH 4.6. Flow rate was maintained at 1 ml/min.

Preparation of solutions:

Preparation of buffer:

Weighed 6.8 grams of KH2PO4 was taken into a 1000ml recepticle, isolated and incapacitated to

1000ml with HPLC water, changed the pH to 4.6 with ortho phosphoric disastrous.

Preparation of mobile phase:
A blend of pH 4.6 Phosphate support 300 mL (30%), 700 mL of MEOH (70%) are taken and aired in ultrasonic water shower meant for 5 minutes. Then, at that point, this strategy is disengaged through 0.45 µ channel under vacuum filtration.

The diluents:
The Mobile phase was used as the diluent.

Preparation of the individual Trimethoprim standard preparation:
80mg of Trimethoprim working standard was unequivocally selected and moved with a 10ml clean dry volumetric cup and around 2ml of diluent is added. Then, at that point, it is sonicated to disengage it totally and made volume upto the scratching with the diluant. (Stock course of action). Further 1.0 ml from the above stock course of action is pipette into a 10 ml volumetric carafe and was harmed upto the scratching with diluant.

Preparation of the individual Sulfamethoxazole standard preparation:
400 mg of Sulfamethoxazole working standard was everything thought of as enlisted and moved with a 10ml clean dry volumetric carafe and around 2ml of diluent is added. Then, at that point, it is sonicated to separate it totally and made volume upto the scratching with the diluant. (Stock method). Further 10.0 ml from the above stock method is pipette into a 100 ml volumetric compartment and was weakened upto the drawing with diluant.

Preparation of Sample Solution:
Unequivocally 10 tablets are checked and squashed in mortar and pestle and weight indistinct from 10 mg of Trimethoprim and Sulfamethoxazole (progressed choosing) test into a 10mL clean dry volumetric carafe and around 7mL of Diluents is added and sonicated to spoil it totally and made volume upto the drawing with a relative dissolvable. (Stock strategy) Further 3 ml of above stock methodology was pipetted into a10ml volumetric cup and debilitated upto the scratching with diluant.

Procedure:
10µL of the standard, sample are injected into the chromatographic system and the areas for Trimethoprim and Sulfamethoxazole peaks are measured and the %Assay are calculated by using the formulae.

METHOD:
The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters:
To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 12 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 10 µL of standard into Symmetry C18 (4.6 x 150mm, 5µm), the mobile phase of composition 70% MeOH: 30% phosphate buffer pH 4.6 was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation:
The proposed validated method was successfully applied to determine Sulfamethoxazole and Trimethoprim in their tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method:
Linearity:
The linearity study was performed for the concentration of 25 ppm to 125 ppm and 25 ppm to 125 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3.

Accuracy studies:
The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Sulfamethoxazoleand Trimethoprim and calculate the individual recovery and mean recovery values. The results are shown in table 4.

Precision Studies:
precision was caliculated from Coefficient of variance for five replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The

%RSD for the area of six replicate injections was found. The results are shown in table 5,6.

Ruggedness:
To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found. The resulte are shown in table 7,8.

Robustness:
As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.7 ml/min to 0.9 ml/min. The resulte are shown in table 9,10.

LOD and LOQ:
The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The resulte are shown in table 11.

LOD = 3.3σ/S and
LOQ = 10 σ/S, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

RESULTS AND DISCUSSION:

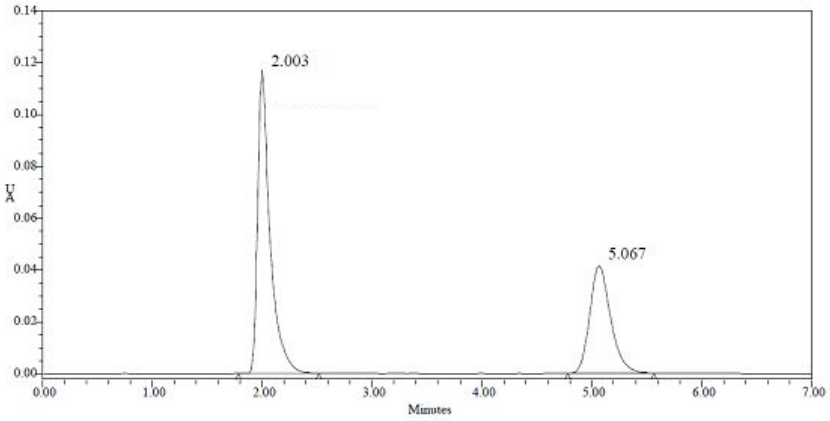

Figure 3: Standard chromatogram

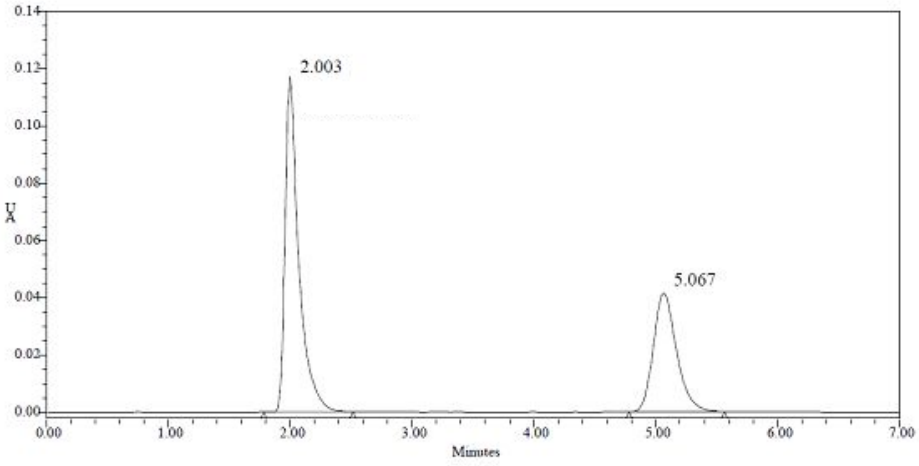

Figure 4: Sample chromatogram

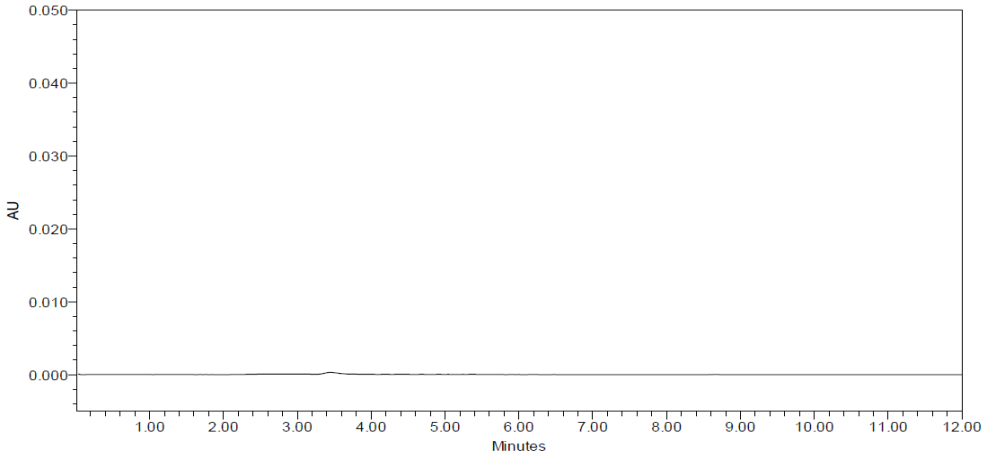

Figure 5: Blank chromatogram

Table 1: System suitability parameters

Parameters	Sulfamethoxazole	Trimethoprim
Retention time	2.003	5.066
USP Plate count	2711.8	3428.2
USP Tailing	1.6	1.3

Table 2: Assay results for Sulfamethoxazole and Trimethoprim

	Label Claim (mg)	% Assay
Sulfamethoxazole	400	101.3
Trimethoprim	80	100.6

Table 3: Linearity results of Trimethoprim and Sulfamethoxazole

Concentration (µg/ml)	Peak area of Sulfamethoxazole	Peak area of Trimethoprim
25	295700	179761
50	653819	386681
75	983775	598908
100	1342535	799619
125	1694286	1019614

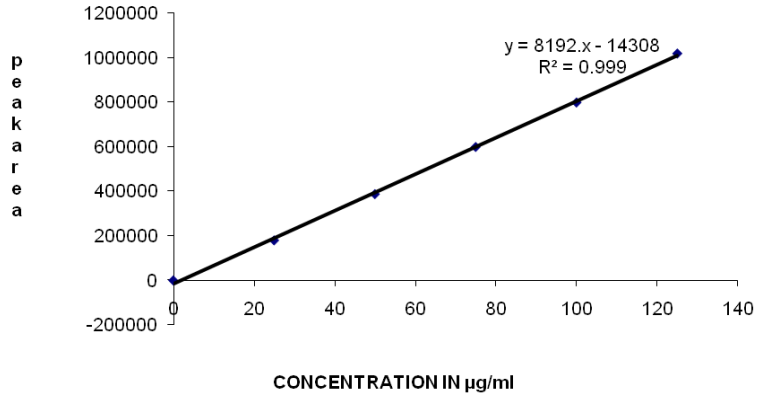

Figure 6: Linearity graph for Trimethoprim

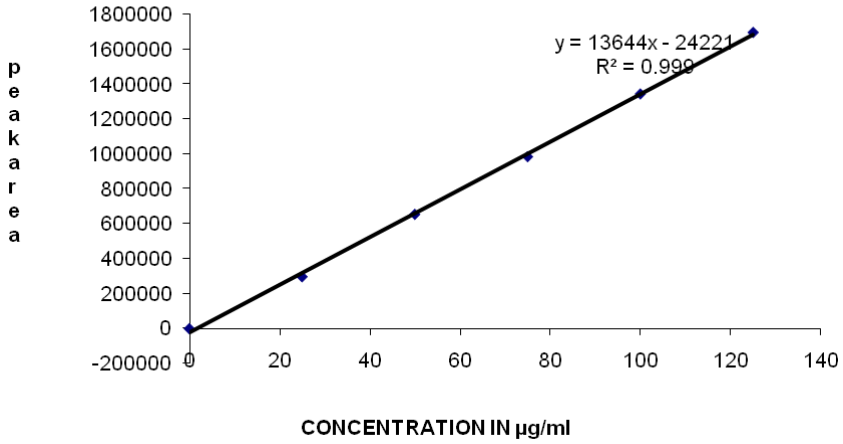

Figure 7: Linearity graph for Sulfamethoxazole

Table 4: Showing accuracy results for Sulfamethoxazole and Trimethoprim

Sample concentration	Sample set no	Sample area		Assay		% Recovery	
		Sulfa	Trim	Sulfa	Trim	Sulfa	Trim
50%	**1**	460064	276931	23.9	24.0	98.8	100
	2	460124	276684	23.6	23.9	98.6	98.6
	3	460196	276781	24.7	24.7	99.7	99.5
	Average Recovery					**99.6%**	**99.6%**
100%	**1**	923429	553256	49.9	50.0	99.8	100
	2	923654	552497	49.8	49.9	99.6	99.8
	3	923742	552571	49.8	49.9	99.6	99.8
	Average recovery					**99.6%**	**99.8%**
150%	**1**	1382601	849113	73.8	75.0	98.8	99.9
	2	1385760	827894	73.9	74.9	98.8	99.7
	3	1386984	828349	74.6	74.8	99.5	99.7
	Average recovery					**99.7%**	**99.8%**

Table 5: Precision results for Sulfamethoxazole

S. No	Sample area	Standard area	Percentage purity
1	983375	971536	101.04
2	985049	973007	101.03
3	982956	975717	100.54
4	985219	978909	100.44
5	994135	982422	105.08
Avg			**100.84**
%RSD			**0.304**

Table 6: Precision results for Trimethoprim

S.NO	Name	RT	Area	Height
1	Trimethoprim	2.320	2267519	196958
2	Trimethoprim	2.341	2208588	197584
3	Trimethoprim	2.356	2275569	195874
4	Trimethoprim	2.344	2258841	194583
5	Trimethoprim	2.325	2257967	194587
Mean			2254401	
Std.dev			6535.5	
%RSD			0.31	

Table 7. Ruggedness results of Sulfamethoxazole

S. No	**Sample area**	**Standard area**	**Percentage purity**
1	979556	984395	99.30
2	982467	984039	99.64
3	979717	983976	99.36
4	978909	984278	99.28
5	981432	973915	100.57
Average			**99.63**
%RSD			**0.54**

Table 8. Ruggedness results of Trimethoprim

S. No	**Sample area**	**Standard area**	**Percentage purity**
1	583416	593403	99.12
2	583657	594352	99.01
3	584731	593357	99.52
4	583594	592673	99.61
5	597649	593671	99.12
Average			**99.27**
%RSD			**0.27**

Robustness results:

Table 9: Flow variation results for Sulfamethoxazole and Trimethoprim

S. No	**Less flow (0.7 ml/min)**		**More flow (0.9 ml/min)**	
	Sulfamethoxazole	**Trimethoprim**	**Sulfamethoxazole**	**Trimethoprim**
1	982165	574651	971563	592641
2	985134	580381	973021	592352
3	983467	587724	975674	595471
4	985217	583190	978974	594416
5	994245	584468	984542	583453
Mean	**986306**	**582223**	**976755**	**591667**
%RSD	**0.45**	**0.80**	**0.53**	**0.80**

Table 10: Mobile phase composition variation effect for Sulfamethoxazole and Trimethoprim

S. No	Less organic(70%)		More organic (90%)	
	Sulfamethoxazole	**Trimethoprim**	**Sulfamethoxazole**	**Trimethoprim**
1	984455	574481	982965	593761
2	986214	585161	982427	592462
3	984268	587627	985489	594491
4	986216	585362	987954	596316
5	995247	585448	994672	587353
Mean	**987286**	**583658**	**986641**	**592877**
%RSD	**0.45**	**0.90**	**0.51**	**0.57**

Table 11: LOD, LOQ of Sulfamethoxazole and Trimethoprim

Drug	LOD	LOQ
Sulfamethoxazole	3.14	10.05
Trimethoprim	2.75	9.96

CONCLUSION:

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Sulfamethoxazole and Trimethoprim in its pure form and in its pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Trimethoprim and Sulfamethoxazole in pure and its pharmaceutical dosage forms.

REFERENCES:

1. Sharma BK., "Instrumental Method of Analysis"; 27th Edn; Goel Publishing House, Meerut, 2011; 96-113.
2. "Quality Assurance of Pharmaceuticals", (A summary of rules and related materials), Volume I, WHO, Geneva, 1997; 119-124.
3. Mendham J., Denny R.C., Barnes J.D., Thomas M. Vogels Text Book of Quantitative Chemeical Analysis; Pearson schooling, 2002; sixth Edn; 123-136.
4. ayar E, Sahin S, Cevheroglu S, Hincal AA. Development and validation of an HPLC method for simultaneous determination of trimethoprim and sulfamethoxazole in human plasma. Eur J Drug Metab Pharmacokinet. 2010 Sep;35(1-2):41-6.
5. Rønn AM, Mutabingwa TK, Kreisby S, Angelo HR, Fuursted K, Bygbjerg IC. A reversed-phase high-performance liquid chromatography method for the determination of cotrimoxazole (trimethoprim/ sulphamethoxazole) in children treated for malaria. Ther Drug Monit. 1999 Dec;21(6):609-14.
6. Gurumurthy.T*, Monika.M, Ashwini.V, Development and validation RP-HPLC method for simultaneous estimation of Sulfamethoxazole and Trimethoprim, Indian Journal of Research in Pharmacy and Biotechnology, 2017, 5(3): 235-238
7. Rajapandi, Raju & Resmi, V.R. & Venkateshan, N. & Babu, G.. (2016). Development and validation of RP-HPLC method for the simultaneous estimation of sulfadoxine and trimethoprim in bulk and pharmaceutical dosage forms. 8. 316-323.
8. Sayar, E., Sahin, S., Cevheroglu, S. et al. Development and validation of an HPLC method for simultaneous determination of trimethoprim and sulfamethoxazole in human plasma. Eur J Drug Metab Pharmacokinet 35, 41–46 (2010).