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Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Sulfadiazine and Trimethoprim In Pharmaceutical Formulations

M. Kusuma Kumari[2*], P. Rama krishnaveni[1], Jyothi K Kasthuri[1], B. Hari babu[1], P.V.V.Satyanarayana[1]

1.Department of Chemistry, Acharya Nagarjuna University, Guntur-522 510, AP-India.
2.Department of Chemistry, A.C. College, Guntur- 522 002, AP-India

ABSTRACT

An accurate, simple and precise RP-HPLC method for the simultaneous estimation sulfadiazine and trimethoprim in pharmaceutical formulations was developed and validated. Chromatographic separation of two drugs was achieved on PEAK 7000 isocratic HPLC with rheodyne manual sample injector by using the mobile phase consisting of methanol, water and aceticacid in the ratio 70:25:05 (v/v/v) at a flow rate of 1mL/min and the wavelength of detection was at 237 nm. The retention time for sulfadiazine and trimethoprim were found to be 4.24 and 7.25 min respectively. The linearity of the method was tested over a concentration range of 41-287 µg/mL for sulfadiazine and 9-63 µg/mL for trimethoprim and the correlation coefficient was 0.999 for sulfadiazine and 0.998 for trimethoprim which is almost equal to 1. The limit of quantification was 3.5 µg/mL for sulfadiazine and 1 µg/mL for trimethoprim and the limit of detection was 1 µg/mL for sulfadiazine and 0.3 µg/mL for trimethoprim. The percentage recoveries were ranged from 98.64-101.64 for sulfadiazine and 98.56-100.86 for trimethoprim.

Keywords*:* Sulfadiazine, trimethoprim an RP-HPLC.

*Corresponding Author Email: kmadasu7@gmail.com
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INTRODUCTION

Chemically Sulfadiazine (SUL) (MF: $C_{10}H_{10}N_4O_2S$; MW: 250.278) (Figure.1) is 4-amino-N-pyrimidin-2-yl-benzene sulfonamide. Chemically Trimethoprim (TMP) (MF: $C_{14}H_{18}N_4O_3$; MW: 290.32) (Figure.2) is 5-(3, 4, 5-trimethoxybenzyl) pyrimidine-2, 4-diamine. Sulfadiazine is a short-acting sulphonamide derivative with bacteriostatic action through competitive inhibition of bacterial synthesis of folic acid. Trimethoprim inhibits enzymes folic acid pathway, preventing the reaction of the dihydrolic acid to tetrahydrofolate. Thus this combination antibiotic is used in the treatment of urinary tract infections.

Literature survey reveals that only few selected HPLC, HPLC-LC-MS methods were reported for the estimation of SUL[1] and TMP[2-6] individually and HPLC[7-8] HPLC-LC-MS[9] and LC-MS[10-11] as combination in bulk and biological samples. There are only two methods for the estimation in dosage forms-UV[12] spectroscopic method for SUL and UV-HPLC[13] for SUL and TMP in combination. However to the best of the knowledge of the author no precise or accurate RP-HPLC method was developed for the estimation of this combination. Hence in the present investigation an attempt was made to develop a simple, precise and accurate RP-HPLC method for the simultaneous estimation of SUL and TMP in pharmaceutical dosage forms.

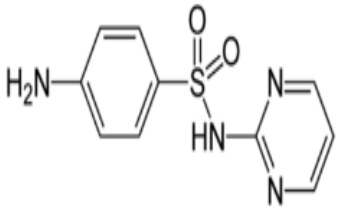


Figure.1: Sulfadiazine

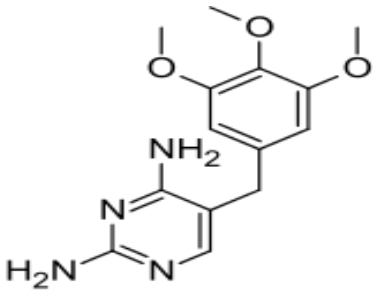


Figure.2: Trimethoprim

MATERIALS AND METHODS

Chemicals and reagents

The reference sample of sulfadiazine and trimethoprim was a kind gift of Hetro Pharma, Hyderabad and Lupin Ltd, Mumbai respectively. The formulation AUBRIL was purchased from local market. The chemicals used were methanol, acetonitrile and water of HPLC grade, acetic acid and reaming buffer solutions of AR grade, were purchased from Merck Specialties Private Limited, Mumbai, India.

Equipment

Chromatographic separation was performed by using PEAK 7000 isocratic HPLC with rheodyne manual sample injector of 20 µL fixed volume, variable wavelength programmable UV detector,

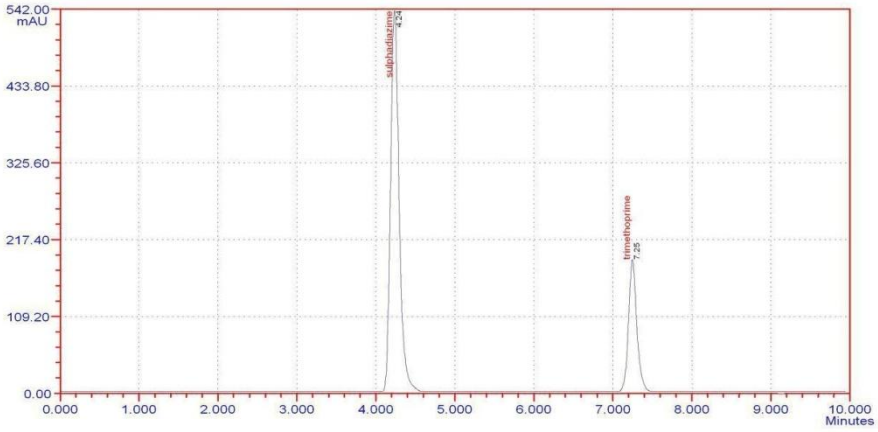
UV7000 and the output signal was monitored and integrated by PEAK Chromatographic Software version 1.06. Teccomp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Sonicator (1.5L) Ultrasonicator was used to sonicate the mobile phase and samples. Denver electronic analytical balance (SI-234) was used for weighing and Systronics digital pH meter for pH measurements.

Year of Experiment: 2

014: Department of chemistry, Acharya Nagarjuna University, Nagarjunanagar, Guntur 522510, Andhra Pradesh (INDIA).

Chromatographic conditions

Selection of the stationary phase depends upon the nature of the sample, molecular weight and solubility. SUL and TMP were analyzed by RP- C18 Column, using an isocratic elution mode with a mobile phase comprised of methanol: water: acetic acid in the ratio 70:25:05 (v/v/v) and the final pH was ajusted to 5.6 with acetate buffer. Composition of mobile phase on the retention time of SUL and TMP were thoroughly investigated. The concentration of methanol: water: acetic acid (70:25:05) (v/v/v) were optimized to give symmetric peak with short runtime. UV detection wavelength was 237 nm, flow rate was 1.0 mL/min and the resulting chromatogram was shown in Figure.3.



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	sulphadiazime	4.235	54075	458594.3	66.075	1.71	5111
2	trimethoprime	7.247	20643	235456.3	33.925	1.03	8046
	Sum:		75478	694050.6	100.0000		

Figure.3: Standard chromatogram of SUL and TMP

Preparation of standard solution

10 mg of the standard drugs were weighed accurately and was dissolved in 10 mL methanol separately to get a concentration of 1000µg/mL of both SUL and TMP separately. It was sonicated to dissolve completely. Then it was filtered through membrane filter paper. This standard stock

solution was used to prepare necessary concentrations to construct calibration curve by proper dilution.

Preparation of sample solution

Twenty tablets of AUBRIL, containing SUL (410 mg) and TMP (90 mg) were powdered and weighed accurately. A quantity of powder equivalent to 10 mg was transferred into 100 mL light resistant flask and made up the required volume by using mobile phase. 1mL of resulting solution is pipetted out into the 10 mL volumetric flask and made up the required volume by using mobile phase. It was sonicated for 15 min and finally filtered through 0.45 µm filter. From this sample a solution contain SUL-164 µg/mL TMP-36 µg/mL was prepared and injected.

Method development

Different mobile phases containing methanol, water and acetic acid in different proportions were tried and finally, methanol, water and acetic acid in the ratio 75:25:05 (v/v/v) was found to be an appropriate mobile phase, which gave acceptable peak parameters for both SUL and TMP.

Method validation

The validation of the method was carried out in terms of parameters like linearity, precision, accuracy, limit of detection, limit of quantification, specificity and robustness.

Linearity

The linearity of the method was tested over a concentration range of 6 to 42µg/mL of PHB and 10-70 µg/mL PHT. 20µL of each concentration was injected in duplicate into the HPLC system. The response was read at 238nm and the corresponding chromatograms were recorded. The regression value of the plots was computed by least square regression method.

Precision

The precision of the method was determined by inter day and intraday studies, solutions of standard and sample were repeated thrice in a day and percentage relative standard deviation (%RSD) was calculated.

Accuracy

The accuracy of the method was determined by recovery experiments. The recovery studies were performed by the regular addition method. At 50%, 100%, 150% level, the percentage recovery was calculated. For both the drugs, recovery was performed in the same way. The recovery studies were performed in triplicate.

Robustness

Robustness of the method was studied by making slight changes in chromatographic conditions, such as mobile phase ratio, pH and wavelength of detection.

System suitability and Specificity

The system suitability and specificity was established by analyzing the number of theoretical plates, retention time and tailing factor.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula:

LOD = 3.3 X Standard Deviation/ Slope of calibration curve

The LOD was found to be 1 µg/mL for SUL and 0.3 µg/mL for TMP.

The limit of quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified.

LOQ = 10Xstandard deviation / Slope of calibration curve

RESULTS AND DISCUSSION

To obtain suitable mobile phase for the analysis of the selected drug combination various mixtures of methanol, water and acetic acid were tested. After some systematic trials it was found that the mixture of methanol, water and acetic acid in the ratio 70:25:05 (v/v/v) as mobile phase was given symmetric peak at 237 nm. The separation of two drugs for the selected mobile phase is much prominent (Fig. 3). The system suitability parameters like retention time, resolution, tailing factor and theoretical plate count were shown uniformity and %RSD was less than 2, so the developed system is suitable for the routine analysis. The absence of interfering peaks near the drug peak in the standard chromatogram, confirms the specificity of the proposed method. The pH was found to be at 5.6 and the chromatogram (Fig. 4) obtained for the mobile phase has been showed good affinity towards SUL (t_R= 4.24min) instead of TMP (t_R=7.25 min).

In order to test the validity of the method several parameters like linearity, precision, accuracy, system suitability, robustness, LOD and LOQ were studied.

Linearity

The linearity of the method was tested over a concentration range of 41-287 µg/mL for SUL and 9-63 µg/mL for TMP with seven concentration levels each. 20 µL of each concentration was injected in duplicate into the HPLC system. The response was read at 237 nm. The regression values of the plots were computed by least square regression method. The correlation coefficient (r^2=0.999 for SUL and r^2=0.998 for TMP) of regression was found almost equal to 1. Linearity results are presented in table 1 and graphs in Figure.4 and Figure.5.

Table 1: Results of linearity

S.No	SUL Concentration (µg/mL)	Peak Area	TMP Concentration(µg/mL)	Peak Area
1	41	289625	9	132934
2	82	341006	18	161720
3	123	402849	27	197582
4	164	458594	36	235456
5	205	517589	45	264368
6	246	580491	54	308375
7	287	643688	63	339113
	Correlation coefficient	0.999	Correlation coefficient	0.998
	Intercept	22542	Intercept	94416
	Slope	1442	Slope	3883

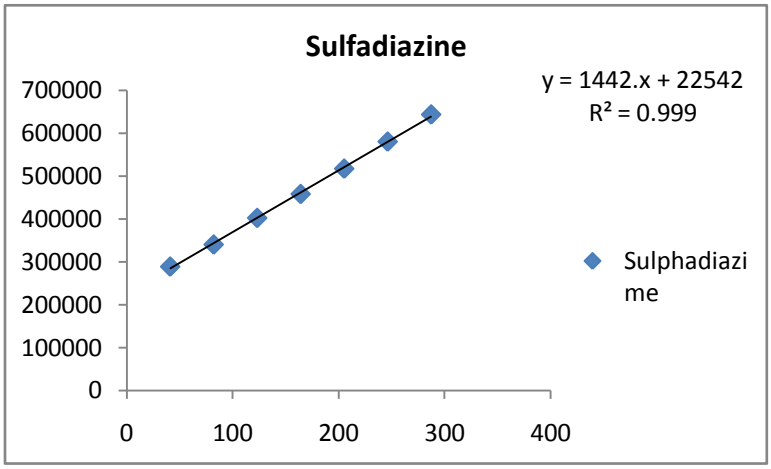


Figure.4: Linearity graph of SUL

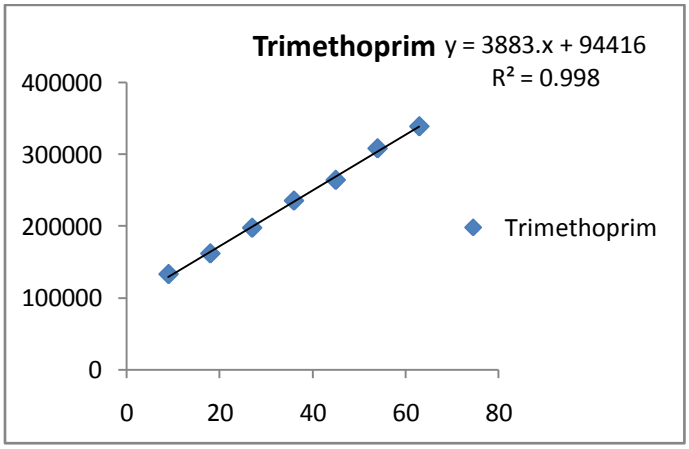


Figure.5: Linearity graph of TMP

The seven point graphs constructed covering a concentration range of 41-287 µg/mL for sulfadiazine and 9-63 µg/mL for trimethoprim were found to be linear. From the data of regression analysis of the calibration curves shown in Table 1; low values of standard deviation denoted very good repeatability of the measurement. Thus it was shown that the equipment used for the study

and the developed analytical method was consistent.

System suitability studies

The resolution, numbers of theoretical plates were calculated for the working standard solutions and are shown in Table 2. The values obtained demonstrated the suitability of the system for the analysis of these drugs in combination.

Table 2: System suitability studies

S.No	Parameter	Sulfadiazine	Trimethoprim
1	Theoritical plates	5111.41	8045.56
2	Tailing factor	1.71	1.03
3	L.O.Q	3.5µg/mL	1µg/mL
4	L.O.D	1µg/mL	0.3µg/mL

Precision

Precision of the method was determined by inter-day and intraday studies, solutions of standard and sample were repeated thrice in a day and percentage relative standard deviation (%RSD) was calculated. Results of system precision studies were shown in Table 3 and 4. The %RSD found were less than 2%. The intraday %RSD of SUL and TMP were found to be 1.68 and 1.31, respectively. The inter-day %RSD of SUL and TMP were found to be 1.35 and 1.24 respectively. The values of %RSD within a day, day to day variation <2% proves that the method is precise.

Table 3: Results of intraday precision

S.No	SUL	TMP
1	449541.4	234954.8
2	457609.8	234901
3	440431.9	238606.1
4	456742.7	231094.4
5	453523.8	231646.3
6	456321.6	237844.6
7	449541.4	234954.8
%RSD	1.68	1.31

Table 4: Results of inter-day precision

S.No	SUL	TMP
1	451362.6	234636
2	458484.2	230486.7
3	463766.1	234230.5
4	465874.4	239184.9
5	454700.3	233542.4
6	451490.6	236411.8
7	451362.6	234636
%RSD	1.35	1.24

Accuracy

The accuracy of the method was determined by recovery experiments. The recovery studies were performed by the standard addition method at 50%, 100%, 150% level and the percentage recovery was calculated. For both the drugs, recovery was performed in the same way. A recovery ranged from 98.64 to 101.64% (Table 5) for SUL and 98.56 to 100.86% (Table 6) for TMP have been obtained. The recovery studies were performed in triplicate and the results were found to be satisfactory.

Table 5: Recovery results of SUL

%Recovery	Concentration in µg/mL			Amount Found	% recovery	RSD
	Target	Spiked	Total			
	82	41	123	122.02	99.21	
50%	82	41	123	124.03	100.841	0.816
	82	41	123	122.97	99.97	
	82	82	164	162.8	100.95	
100%	82	82	164	162.27	100.03	0.477
	82	82	164	164.42	100.26	
	82	123	205	202.199	98.64	
150%	82	123	205	205.70	101.64	1.50
	82	123	205	203.83	100.29	

Table 6: Recovery results of TMP

%Recovery	Concentration in µg/mL			Amount Found	% recovery	RSD
	Target	Spiked	Total			
	18	9	27	26.79	99.25	
50%	18	9	27	26.91	99.67	0.835
	18	9	27	27.23	100.86	
	18	18	36	35.48	98.56	
100%	18	18	36	35.7	99.16	1.05
	18	18	36	36.21	100.60	
	18	27	45	44.98	99.96	
150%	18	27	45	44.79	99.53	0.483
	18	27	45	44.55	99	

Robustness

Table 7: Results of Robustness

S.No	Condition	Change	SUL		TMP	
			Area	% Change	Area	% Change
1	Standard	…….	458594.3	…….	235456.3	…….
2	MP 1	Methanol: Water: Acetic acid 75:20:5	458083.7	0.11	234436.8	0.43
3	MP 2	Methanol: Water: Acetic acid 65:30:5	451812.2	1.48	231982.6	1.48
4	WL 1	232nm	456163.1	0.53	238487.7	1.29
5	WL 2	242nm	456269.5	0.50	235544.2	0.04

6	pH 1	5.7	459306.1	0.152	239488.8	1.71
7	pH 2	5.5	464678.8	1.32	239510.8	1.72

Robustness of the method was studied by making slight changes in chromatographic conditions, such as mobile phase ratio, pH and wavelength of detection (Table 7). It was observed that there were no marked changes in the chromatograms obtained. So the developed RP-HPLC method was robust.

Limit of Detection and Limit of Quantification

The LOD and LOQ values of SUL and TMP have shown in table 8 supports the sensitivity of the developed method.

CONCLUSION

The developed RP-HPLC method for the simultaneous estimation of SUL and TMP in combined dosage form is more precise, robust and accurate in respect of validation and the quality of separation achieved for the two drugs is also good in terms of retention times and tailing factor. Hence, the proposed method is applicable to the determination of SUL and TMP in pharmaceutical formulations.

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