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Development And Validation Of HPLC Stability Indicating Assay Method For Trimethoprim In Bulk Drug And Tablets

[1] S. PULLA REDDY, [2] Dr. M. BALARAJU, [3] Dr. G. VIMALA, [4] Dr. V. SYAMALA, [5] Dr. J. Sai Chandra, [6] Dr. K.A. EMMANUEL, [7]K.V.L.N Murthy

[1] Dept. of Chemistry, Anurag Engg. College, Kodad, Telangana, INDIA- 508206
[2] Dept. of Chemistry, Silver Jubilee Government College, Cluster University, Kurnool, Andhra Pradesh INDIA-518002.
[3] Dept. of Chemistry, Chellammal Women's College, Guindy, Chennai- 600032
[4] Dept. of Chemistry, Bapatla Engineering College, Bapatla, A.P. INDIA- 522101.
[5] Dept. of Chemistry, JNTUH University College of Engg. Sultanpur, Telangana, INDIA-502273.
[6] Dept. of Chemistry Y.V.N.R. Government Degree College, Eluru Dist., A.P. India-521333.
[7] Dept. of Chemistry, SVR Degree college, Macherla, A.P, INDIA- 522426

Abstract: A Simple, picky, accurate, provident reverse phase high performance liquid chromatography (RP-HPLC) was developed for estimation of Trimethoprim in pharmaceutical phrasings. Chromatographic separation achieved on a Inertsil C_8 (100 x 4.6μ, 5μ) 250×4.6 mm i.d.) with mobile phase containing acetonitrile and potassium dihydrogen phosphate buffer [pH~ 6.5] in the ratio 35:65 v/v. The flow rate was 1.0 mL/min and effluent was covered at 250 nm. The retention time was 5.70 min. The tactic was validated in terms of linearity, delicacy and perfection. The linearity plant to be direct over 2.5 – 12.5 μg/mL. The limit of discovery and limit of quantification were plant to be 0.0066μg/mL and 0.022μg/mL respectively. The proposed method was successfully used to determine the medicine content of retailed phrasings.
Key words: Trimethoprim, HPLC, linearity, confirmation.

INTRODUCTION

DRUG PROFILE:

Trimethoprim is 5-[(3,4,5-Trimethoxy-benzyl) pyrimidine-2,4-diyldiamine] is an antibiotic used mainly in the treatment of urinary tract infections. Other uses include for middle ear infections and traveler's diarrhea[1,2].

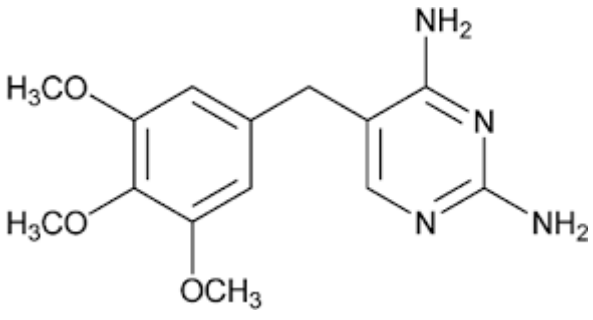


FIG.1.01: MOLECULAR STRUCTURE OF TRIMETHOPRIM

The empirical formula is $C_{14}H_{18}N_4O_3$, representing a molecular weight of 290.3 g/mole. The molecular structure was presented in **Fig.1.01**. Trimethoprim (trimethoprim tablet) is available as 100 mg tablets in the brand name **TRIMPEX** for oral administration.

Few analytical methods have been employed for the assay trimethoprim (β-form) in reported in combination forms [3-9]. In this accord the author made an attempt for the development and validation of

a new stability indicating RP-HPLC method for the assay of trimethoprim in pure and formulations was described. Literature survey revealed few chromatographic methods [6-9] were reported for estimation of trimethoprim individually or in combination with other drugs.In the present investigation a new RP-HPLC method which has been reported for the assay oftrimethoprim in market formulations with good repeatability and reproducibility.

A. EXPERIMENTAL:

a. INSTRUMENTATION: The present HPLC analysis of trimethoprim was performed on Waters liquid chromatographic system [Model 2695] equipped with quaternary pump, UV-Visible sensor, and column roaster and auto sampler. The output signal was covered and integrated using Waters (Alliance) Empower 2 software. An Inertsil C_8 (100 x 4.6µ, 5µ) column was used as stationary phase in the present study. An electronic logical importing balance (0.1mg perceptivity, Shimadzu AY 220), digital pH meter (DELUX model 101) and a sonicator (sonica, model 2200 MH) were also used in
this study**.** The glassware used were of 'A' grade, and were cleaned thoroughly with chromic acid and double distilled water and later dried in hot air oven prior to use.

b. MATERIALS AND REAGENTS: Trimethoprim drug was made available from Merck Ltd. India (purity 99.8) as gratis sample and its market phrasings available in the brand name of TRIMPEX (market claim:100mg of Trimethoprim) was purchased from Local Apollo Pharmacy store. Potassium dihydrogen phosphate, dipotasssium hydrogen phosphates were obtained from Qualigens fine chemicals, India Ltd. Ortho phosphoric acid and Acetonitrile were obtained from Rankem laboratories, India. All chemicals and reagent were used as HPLC grade and Milli-Q-water was used throughout the trail.

c. MOBILE PHASE: The mobile phase used in this study was a mixture of acetonitrile and potassium dihydrogen phosphate buffer[pH~ 6.5] in the ratio of 35:65 v/v respectively. The buffer is prepared by dissolving 1.2gm potassium dihydrogen phosphate and 0.25gms of dipotasssium hydrogen phosphate in 1000mL water and mixed. Adjust the pH 6.5 with dilute orthophosphoric acid.

d. MEDICATION OF STANDARD STOCK SOLUTION: Weigh and transfer 100mg of trimethoprim working standard into 100mL volumetric flask add 60mL of mobile phase and sonicate to dissolve and adulterate to volume with diluent (Stock solution).

e. MEDICATION OF WORKING STANDARD: Transfer aliquots of the above standard stock solution into series of different 100mL volumetric steins and adulterate to volume with mobile phase to gain concentration range of 25- 125µg/m independently.

f. PREPARATION OF PHARMACEUTICAL PHRASINGS: For analysis of market phrasings twenty tablets of **TRIMPEX** (market claim: 100mg of trimethoprim) carried from Local Apollo Pharmacy were counted and finely powderd. An accurately weighed quantity of fine powder equivalent to 100mg of trimethoprim was transferred into 100mL volumetric flask containing 60mL of diluent, sonicated for 10mins to dissolve and eventually adulterated to volume with diluent (mobile phase). The solution (sample stock) was then filtered through 0.45µfilter. From this aliquots were transferred and adulterated with mobile phase; so as to gain a concentration in the range of linearity previously determined (3.0-15.0µg/mL). Laterally, these solutions were then injected in triplicate into the above mentioned HPLC system.

RESULTS AND DISCUSSION:

i. METHOD DEVELOPMENT: The results of optimization trails had revealed that Inertsil C_8 (100 x 4.6µ, 5µ) column maintained at ambient temperature was suitable for the separation of trimethoprim. The stylish results were attained by use of admixture of acetonitrile and potassium dihydrogen phosphate buffer [pH~ 6.5] in the ratio of 35:65 %v/v as mobile phase. Isocratic elution at inflow rate of 1.0mL/min has been employed in the present study. Wavelength of 250nm was selected in the present assay. Under these defined

optimum chromatographic conditions, trimethoprim eluted from the column with a retention time of 5.70min and the validated chromatogram of trimethoprim is shown in **Fig.1.01.**

ii. CHROMATOGRAPHIC CONDITIONS: The mobile phase used in this study was a admixture of acetonitrile and potassium dihydrogen phosphate buffer [pH~ 6.5] in the ratio of 35:65 %v/v. Before use, it was sonicated for 30 min and also filtered through 0.45µ (pore diameter) Whatman filter paper. The mobile phase was pumped from the solvent force to the column at a flow rate of 1.0mL/min. The eluent was covered at 250nm and the column temperature was maintained ambient throughout the trail. \

METHOD VALIDATION:

a) SYSTEM FELICITY: The system felicity was studied by performing the trail and montering for changes in retention time, peak area and peak asymmetry. Five injections of the standard result of trimethoprim (100µg/mL) were fitted for this purpose. The system felicity was verified by calculating the relative standard deviation values for parameters like retention time, peak area, peak asymmetry, theoretical plates, plates per meter and height equivalent to theoretical plate. It was observed that all the values are within the limits (**Table.1.06**).

b) SPECIFICITY: Stress studies were performed for trimethoprim to study the stability indicating property and selectivity of the proposed method. For this purposeful degradation was tried to produce stress condition by exposing trimethoprim with acid (0.1N Hydrochloric acid), alkali (0.01N NaOH) and 5%H_2O_2 and thereby assessing the capability of the proposed method to separate trimethoprim from its degraded products. From the above investigation, it was revealed that in all the above stress studies no degradation products were observed in the chromatograms (**Figs.1.02.a-c**) confirming that the proposed method is selective and stability-indicating.

c) LINEARITY: The linearity of the present method was analyzed by injecting six different concentrations of working standard solutions prepared from trimethoprim stock solution ranged from 50% to 150% o in triplicate into the HPLC system. The corresponding chromatograms at each concentration level were recorded . A calibration curve was constructed by plotting the area recorded against each concentration and a regression equation was computed (**FIG.1.17**) and the results of this study were represented in **Table.1.07**. The calculated correlation coefficient was 0.9995 where in the slope is 424.344 and y-intercept was 127.086. The correlation coefficient indicated the excellent linearity of the proposed method.

d) SENSITIVITY: The LOD and LOQ for trimethoprim were found to be 0.0066µg/mL and 0.022µg/mL, respectively (**Table.1.07**) indicating the adequate sensitivity of the method.

e) PERFECTION: The precision of the proposed method was studied by determining the one fixed concentration of the trimethoprim in the formulation for six times on the same day at different intervals and calculated the value in terms of %RSD. The results of the precision study (**Table.1.08**) indicated the reliability of the method (RSD %< 2).

f) ACCURACY [RECOVERY STUDIES]: The accuracy of the proposed method, were carried out with a known quantity of the pure drug was added to the placebo sample at the level of 50% to 150% of the test concentration and the contents were determined from the respective chromatograms . The mean recoveries were in range of 99.96-99.97% which revealed that there is no interference from excipients of the method (**Table.1.09**).

g) ROBUSTNESS: The robustness of the developed RP-HPLC method was made by making slight changes in chromatographic conditions that include the change in inflow rate and change in column temperature. Each of the above mentioned factor were changed at two situations (-2, +2) at one time with respect to optimized parameters and the results of these studies reported negligible effect on the chromatographic parameters by slight variations in chromatographic conditions (**Table.1.10)**. The

chromatograms obtained during this robustness study were shown in **Fig.1.04(a-d)**.

h) RUGGEDNESS: A 0.02µl aliquot of concentration 50µg/mL was injected to study the ruggedness of trimethoprim by two different cogent chemists (Analyst-1and Analyst -2) and the results were recorded in **Table.1.11** and are in the acceptable range for trimethoprim. The results showed the % R.S.D. was less than 2% respectively. The chromatograms obtained during this robustness study were shown in **Fig.1.05a&b.**

i) ASSAY OF TRIMETHOPRIM IN TABLET PHRASINGS: The assay for the dosage forms of trimethoprim [TRIMPEX-100mg] was established by injecting solution of sample formulation [discussed in the experimental part] with the present chromatographic condition developed so as to obtain concentration in the range of linearity preliminarily determined. All determinations were carried out in six replicates and it was found to be more accurate and reliable. The average drug content was found to be 99.93% of the labeled claim. The results were shown in **Tab.1.12**. The chromatograms attained during this study were shown in **Fig.1.06.**

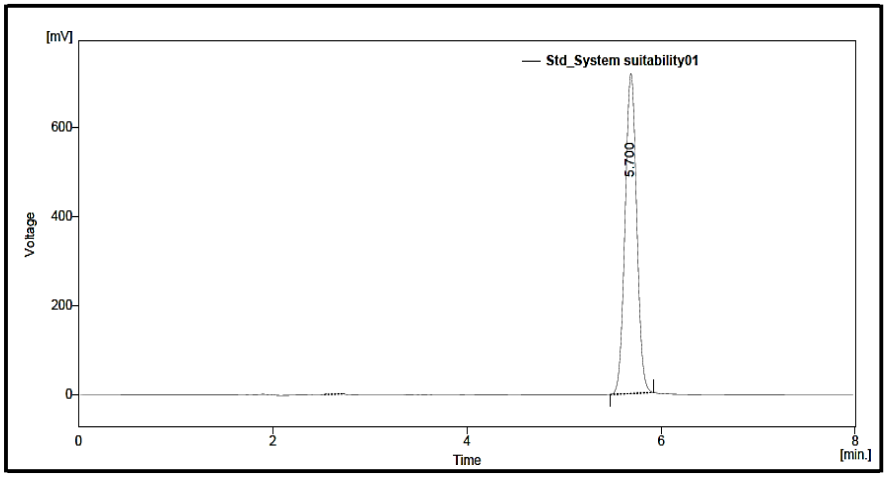


FIG.1.01.a- TYPICAL HPLC CHROMATOGRAM SHOWING THE PEAK OF TRIMETHOPRIM

FIG: 1.02.a - HPLC CHROMATOGRAM OF TRIMETHOPRIM IN ACIDIC HYDROLYSIS

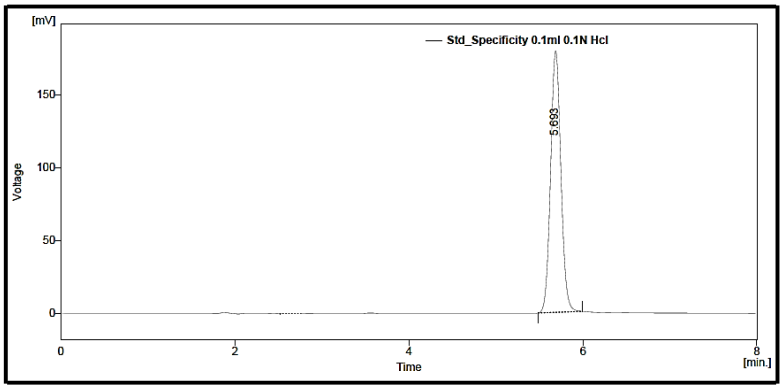


FIG: 1.02.b - HPLC CHROMATOGRAM OF TRIMETHOPRIM IN BASICHYDROLYSIS

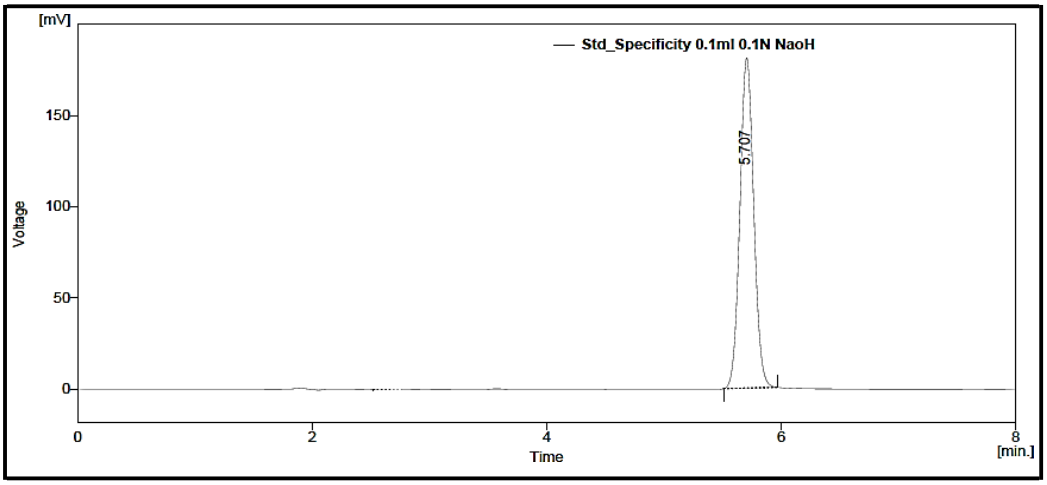


FIG: 1.02.c - HPLC CHROMATOGRAM OF TRIMETHOPRIM IN THERMAL DEGRADATION

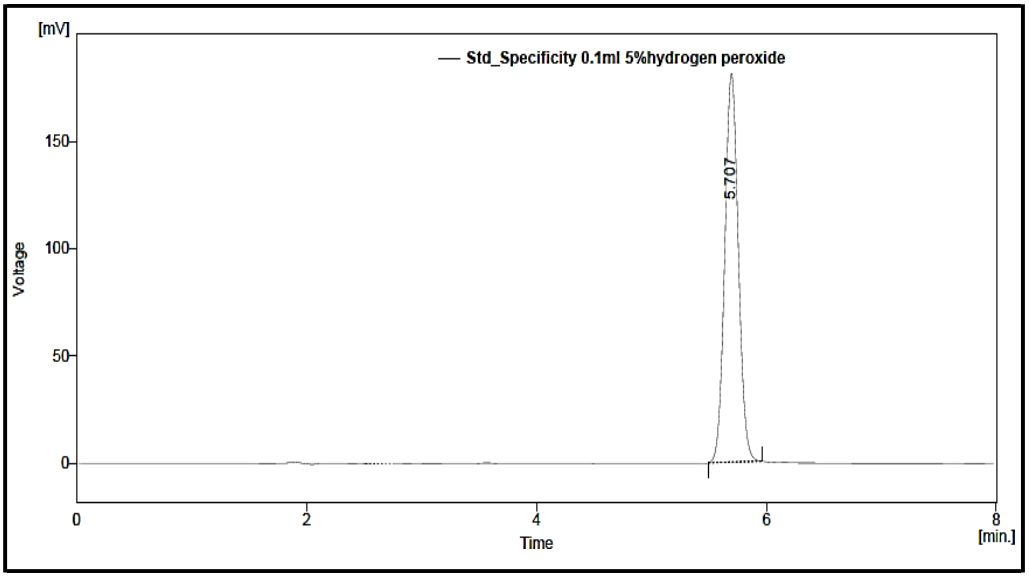


FIG: 1.03 - LINEARITY CURVE FOR TRIMETHOPRIM

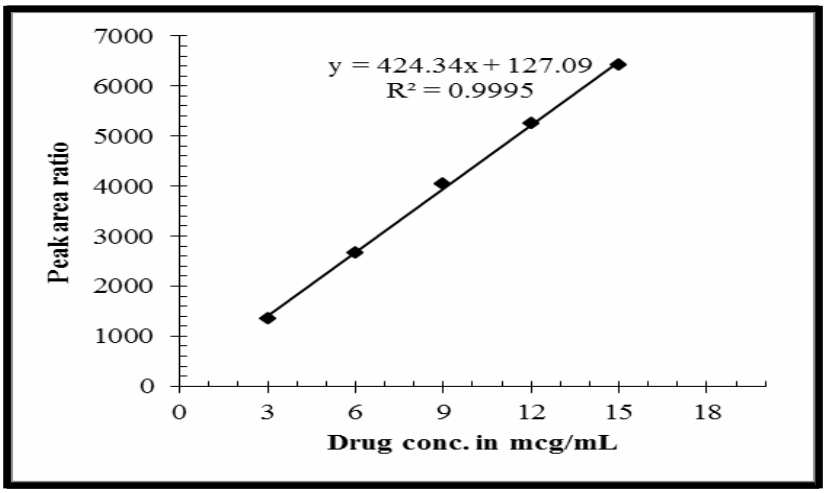


FIG: 1.04.a - ROBUSTNESS CHROMATOGRAM OF TRIMETHOPRIM (FLOWRATE 0.8mL/min)

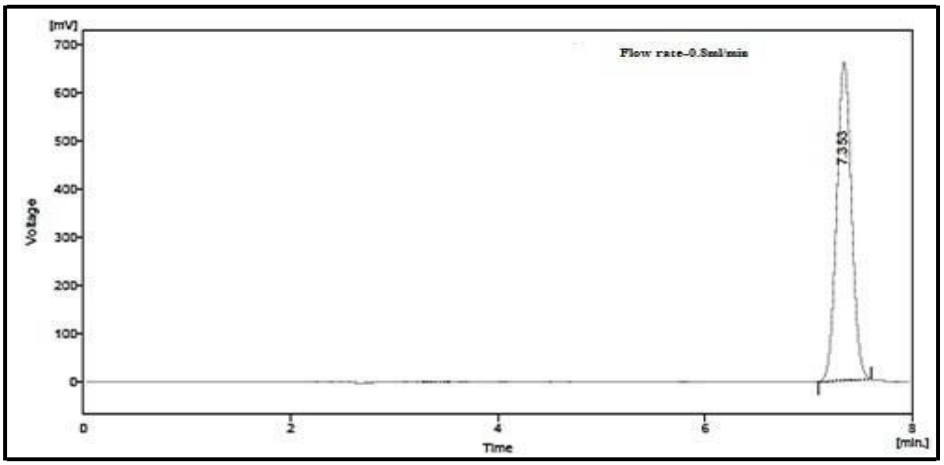


FIG: 1.04.b - ROBUSTNESS CHROMATOGRAM OF TRIMETHOPRIM (FLOW RATE 1.2mL/min)

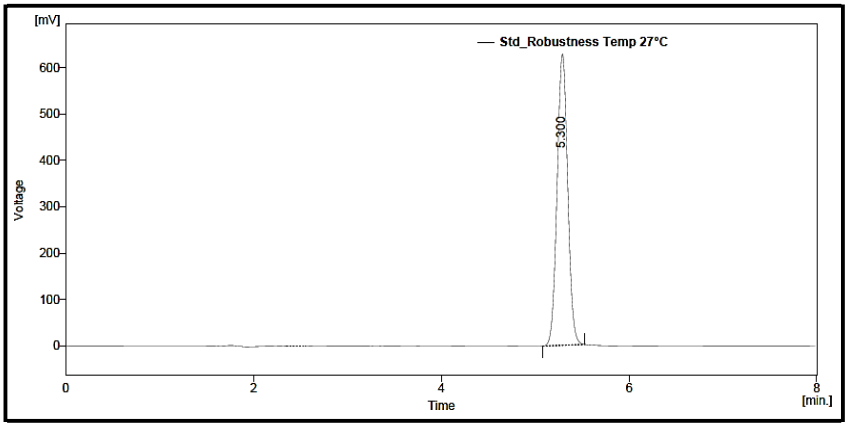


FIG: 1.04.c - ROBUSTNESS CHROMATOGRAM OF TRIMETHOPRIM (TEMP 33ºC)

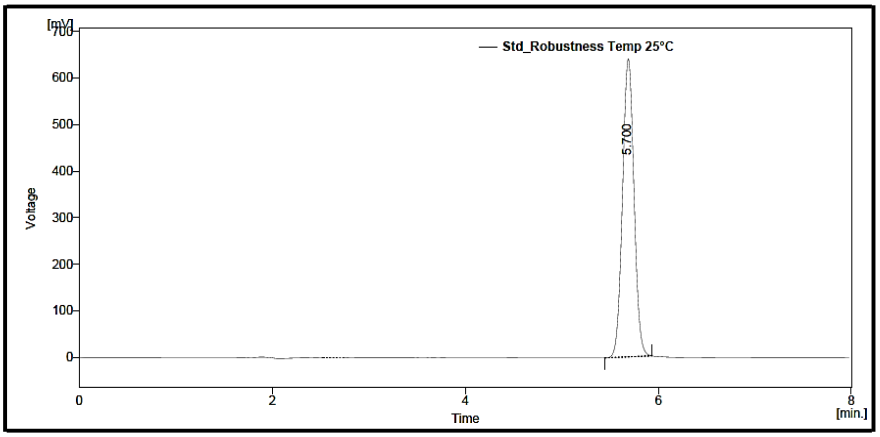


FIG: 1.04.d - ROBUSTNESS CHROMATOGRAM OF TRIMETHOPRIM (TEMP 37ºC)

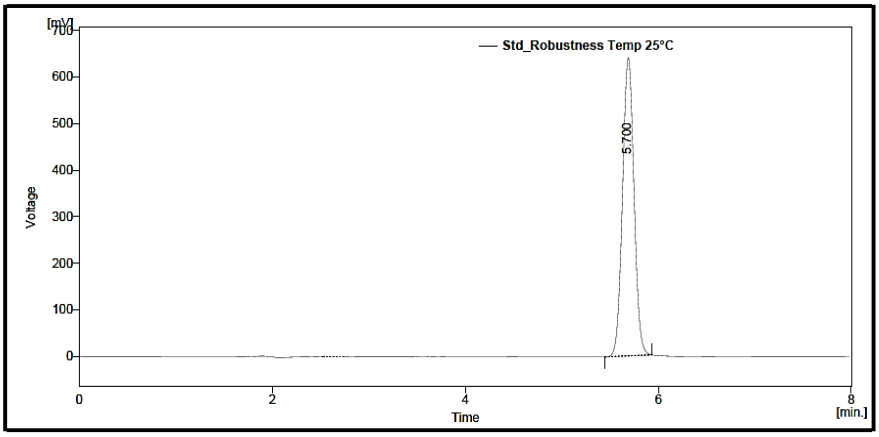


FIG: 1.05.a - RUGGEDNESS CHROMATOGRAM OF TRIMETHOPRIM (ANALYST-1)

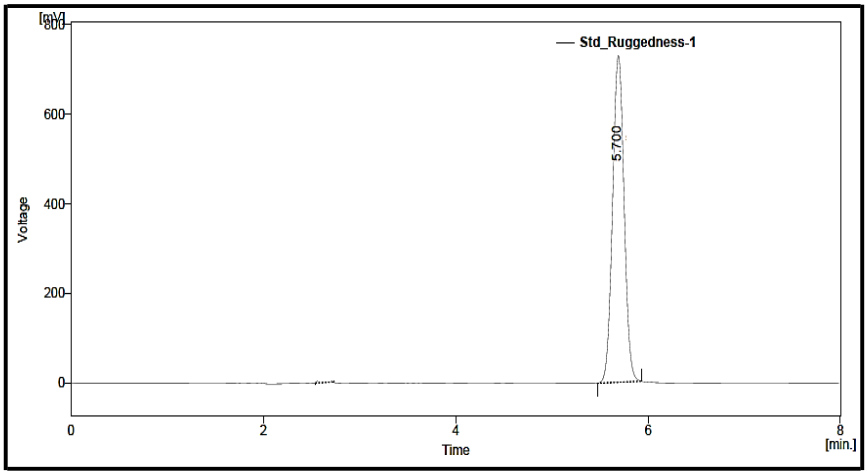
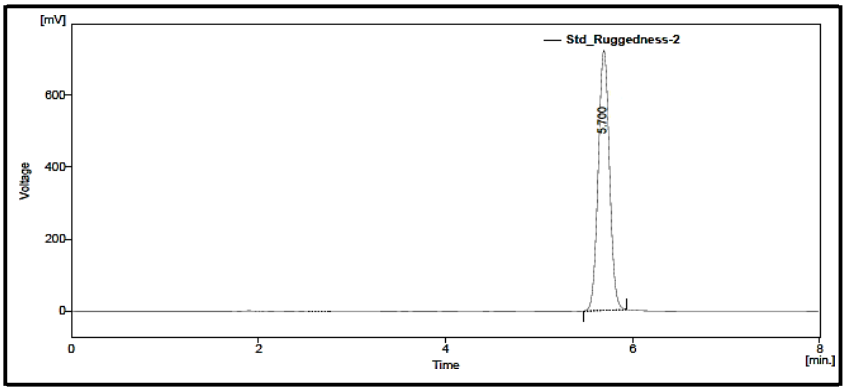


FIG: 1.05.b - RUGGEDNESS CHROMATOGRAM OF TRIMETHOPRIM (ANALYST-2)



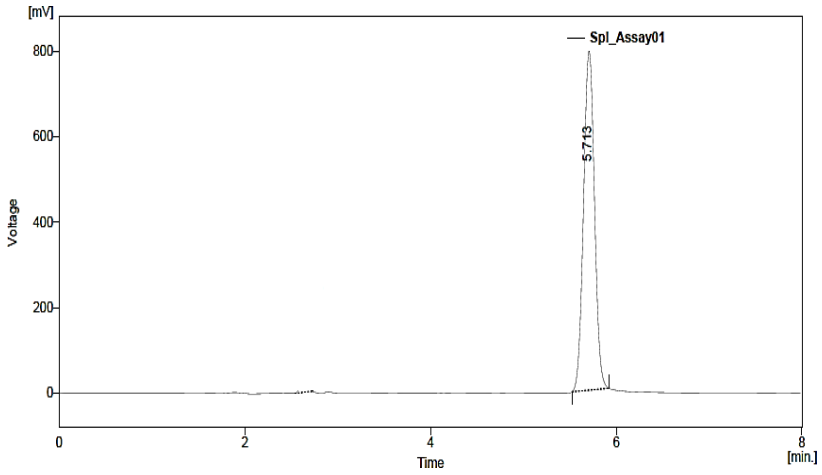


FIG: 1.06. - CHROMATOGRAM OF TRIMETHOPRIM IN PHRASINGS

TABLE.1.05: OPTIMIZED CHROMATOGRAPHIC CONDITIONS

PARAMETERS	**PEAK HPLC**
Elution	Isocratic
Mobile phase	Acetonitrile and potassium dihydrogen phosphate buffer [pH~ 6.5] in the ratio of 35:65 v/v
Column	Inertsil C8 (100 x 4.6 mm) column
Flow rate	1.0 mL.min^{-1}
Detection	UV at 250nm
Injection volume	20µL
Temperature	Ambient
Retention time	5.70 minutes
Run time	8 minutes
Area	3465056 mAU

TABLE:1.06: SYSTEM SUITABILITY PARAMETERS FOR TRIMETHOPRIM BY THE PROPOSED RP-HPLC METHOD

NAME OF THE COMPOUND	**THEORETICAL PLATES**	**'ENTION TIME**	**TAILING FACTOR**
TRIMETHOPRIM	9183	5.700	1.000

TABLE: 1.07: RESULTS OF LINEARITY STUDIES OF TRIMETHOPRIM BY THE PROPOSED METHOD

REGRESSION PARAMETERS	**RESULTS**
Regression equation; Slope (b)	424.344
Intercept (a)	127.09
Correlation coefficient	0.9995
Standard deviation on intercept(S_a)	4.4339
Standard deviation on slope (S_b)	7.6859
Standard error on estimation(S_e)	72.915
Limits of Detection (LOD)[µg/mL]	0.0313
Limits of Quantification [LOQ)[µg/mL]	0.104

TABLE: 1.08: RESULTS OF METHOD PRECISION(PERFECTION) BY THE PROPOSED METHOD

S.NO	RETENTION TIME	PEAK AREA
1	5.693	6005.515
2	5.700	6005.271
3	5.700	6001.974
4	5.700	6005.235
5	5.700	6005.271
Avg*	5.6986	6004.653
Std Dev	0.00313	1.501887
% RSD	0.0549	0.025

*Average of five determinations

TABLE:1.09: RESULTS OF RECOVERY STUDIES(ACCURACY) OF TRIMETHOPRIM

ACCURACY LEVELS IN %	AMOUNT OF SAMPLE(FORMULATION) ADDED IN mcg	AMOUNT RECOVERED*	%RECOVERY*
50	50	49.98	99.96
100	100	99.96	99.96
150	150	149.96	99.97

*Average of three determinations

TABLE: 1.10: RESULTS OF ROBUSTNESS STUDY

ROBUST CONDITIONS	TRIMETHOPRIM				
	THEORETICAL PLATES		ASYMMETRY	RT	PEAK AREA
FLOW RATE	0.8 mL/min	10784	1.095	7.353	6607.153
	1.2 mL/min	9437	1.063	4.953	4640.247
COLUMN TEMPERATURE	AT 25ºC	10125	1.056	5.700	5297.437
	AT 27ºC	8754	1.059	5.300	4936.815

TABLE: 1.11: RESULTS OF RUGGEDNESS STUDIES OF TRIMETHOPRIM

S NO	NAME	ANALYST-1		ANALYST -2	
		RT	AREA	RT	AREA
1	INJECTION-1	5.693	6005.515	5.7	6004.595
2	INJECTION-2	5.700	6005.271	5.645	6005.125
3	INJECTION-3	5.700	6001.974	5.789	6004.567
4	INJECTION-4	5.700	6005.235	5.667	6005.235
5	INJECTION-5	5.700	6005.271	5.5	6005.271
AVG*		5.6986	6004.653	5.6602	6004.959
STD DEV*		0.00313	1.501887	0.105018	0.349011
% RSD*		0.0549	0.025	1.85	0.005

*Average of five determinations

TABLE.1.12: ANALYSIS OF MARKETED TABLETS

IRASINGS IN MARKET	LABELED AMOUNT(mg)	RECOVERED AMOUNT(mg)*	%RECOVERY
TRIMPEX	100	99.95	99.95

*Average of six determinations

CONCLUSIONS:

The RP-HPLC method developed for quantitative determination of trimethoprim in both pure and pharmaceutical dosage forms was precise, accurate, specific and stability indicating. The method was completely validated showing satisfactory data results in accordance to ICH norms. The developed RP-HPLC method is stability indicating and can be used for the routine analysis of production samples and also to check the stability of trimethoprim samples.

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