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Development and validation of RP-HPLC method for the simultaneous estimation of sulfadoxine and trimethoprim in bulk and pharmaceutical dosage forms

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ABSTRACT

A simple, fast, accurate, precise, method has been developed for the simultaneous estimation of Sulfadoxine and Trimethoprim in bulk and in pharmaceutical dosage forms by reversed-phase high performance liquid chromatography. The separation was carried out on C_{18} Phenomenex column, using mobile phase consisting of a mixture of acetonitrile: potassium dihydrogen orthophosphate buffer in the ratio 20: 80 and pH adjusted to 3.8 using orthophosphoric acid. The flow rate was adjusted to 1 ml/min. the UV detection was carried out at a wavelength of 248 nm. The retention time of Sulfadoxine and Trimethoprim was found to be 5.2 min and 2.4 min respectively. Linear response obtained for Sulfadoxine was in the concentration range 2-10 µg/ml (r^2 = 0.999) and Trimethoprim in the range 1-5 µg/ml (r^2 = 0.999). LOD for both the drugs were 0.0024 µg/ml and 0.0009 µg/ml respectively and LOQ for both the drugs were found to be 0.0072 µg/ml and 0.0028 µg/ml respectively. The method was validated according to ICH guidelines with respect to linearity, precision, accuracy, reproducibility, LOD, LOQ and robustness. Thus, proposed method can be successfully applicable to the pharmaceutical preparations containing the above mentioned drugs.

Key words: Sulfadoxine, Trimethoprim, RP-HPLC and Method Validation.

INTRODUCTION

Sulfadoxine (SDX) is chemically N¹ - (5, 6- dimethoxypyrimidin-4yl) sulphanilamide is a bacteriostatic agent used in the Suppressive therapy of chronic urinary tract infection, for streptococcal pharyngitis and gum infection [1, 2]. It is official in Indian Pharmacopoeia (IP) and British Pharmacopoeia (BP). Literature survey reveals that HPLC, Ultra Performance Liquid Chromatography (UPLC) Spectrophotometric and HPTLC methods for determination of SDX with other drugs [3, 4].

Trimethoprim is chemically 5-(3, 4, 5-trimethoxybenzyl) pyrimidine-2, 4-diamine is a bacteriostatic agent against most common bacterial pathogens [5]. Trimethoprim is effective as sole therapy in treating urinary and respiratory tract infections due to susceptible organisms and for prophylaxis of urinary tract infections. It is official in Indian Pharmacopoeia (IP) and British Pharmacopoeia (BP). Literature survey reveals that HPLC, tandem mass spectrometry, Spectrophotometric and HPTLC methods for determination of TMP with other drugs [6, 7].

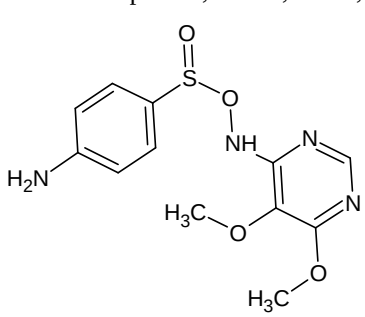
Sulfadoxine and Trimethoprim is a recent combination in the market widely used as aqueous solution for parenteral administration. The injection may be used in the treatment of a wide range of diseases and conditions of bacterial origin in cattle and horses. The management of diseases and disorders are done by using multiple therapeutic agents,

which acts at different sites. Synergistic combination of Sulfadoxine and Trimethoprim provides enhanced antibacterial activity. Active against gram negative and gram positive bacteria, so provides wide species protection [8].

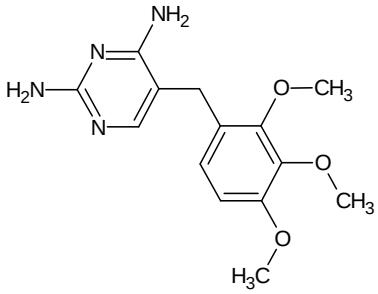
Literature survey reveals that various methods for the estimation of Sulfadoxine and Trimethoprim are reported for individual drug, but no methods have been reported for the simultaneous estimation of the Sulfadoxine and Trimethoprim in combine dosage form and bulk drug. So here an attempt has been made to develop simple, accurate, sensitive, rapid and economic method for simultaneous estimation of Sulfadoxine and Trimethoprim from combined dosage forms using High Performance Liquid Chromatography as per International Conference on Harmonization guidelines (ICH).

HPLC is a modern versatile quantitative tool, representing an instrumental development arising from the old column idea, where the mobile phase is pumped under high pressure through a column at a controlled rate. A sample having mixture of constituents is separated into its components while travelling through the column and the individual solutes are monitored by the detector [9, 10].

The column and mobile phase are the principle factors for achieving the proper separation. The variety of stationary phases is used results in a wide variety of separation modes and each mode demands a particular composition or type of the solvent system to effect separation [11]. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low [12].



Sulphadoxine



Trimethoprim

MATERIALS AND METHODS

Instrumentation:
The liquid chromatographic system consisted of following components: Shimadzu HPLC model containing LC-20AD (VP series) pump, variable wavelength programmable UV - Vis detector SPD-20A (VP series) and Hamilton syringe (705 NR, 20 µL). Chromatographic analysis was performed using Phenomenex- C_{18} column with 250 x 4.6 mm i.d. and 5 µm particle size.

Materials and Reagents:
Active pharmaceutical ingredients of SDX (Sulfadoxine) and TMP (Trimethoprim) were received as gift samples from Shasun Pharmaceuticals Pvt Ltd, Pondicherry, India. Acetonitrile (HPLC grade), potassium dihydrogen orthophosphate (AR), ortho phosphoricacid (AR) and water (HPLC grade) were procured from Merck Chemicals, India. Injection containing SDX and TMP (200:40mg) were procured from local pharmacy retail shop (Manufactured by Vivek Pharmachem India Ltd).

Chromatographic conditions:
The mobile phase consisted of acetonitrile and potassium dihydrogen orthophosphate buffer in the ratio 20: 80, pH adjusted to 3.8 using orthophosphoric acid. The mobile phase was premixed and filtered through a 0.45 µm nylon filter and degassed. The injection volume was 20 µl and eluted at a flow rate of 1 ml/min. The detection wavelength was 248 nm.

Preparation of standard stock solution:
Sulfadoxine standard stock solution:
10 mg of standard SDX was weighed and transferred to a 100 ml volumetric flask and dissolved in 50 ml mobile phase and then content was kept in ultrasonicator for 10 min. Then volume was made up to the mark with mobile phase to obtain final concentration of 100 µg/ml of SDX.

Trimethoprim standard stock solution:
10 mg of standard TMP was weighed and transferred to a 100 ml volumetric flask and dissolved in 50 ml mobile phase and then content was kept in ultrasonicator for 10 min. Then volume was made up to the mark with mobile phase to obtain final concentration of 100 µg/ml of TMP.

Analysis of injection formulations:
Marketed powdered injection formulations (BACTRIDOX & BORGAL) containing 200mg of Sulfadoxine and 40mg of Trimethoprim were analyzed by this method. 1ml injection formulations equivalent to 200mg of SDX and 40mg of TMP was accurately taken and transferred to 100ml volumetric flask and dissolved in 50ml mobile phase and the flask was kept in ultrasonicator for 10 min. The flask was shaken and volume was made up to the mark with mobile phase to give a solution of 2000 µg/ml of SDX and 400 µg/ml of TMP (stock 'A' solution.)

From the above stock 'A' solution 1 ml of the aliquot was pipetted out and transferred to a 100 ml volumetric flask. The volume was made up to the mark with mobile phase to obtain a solution with final concentration of 20 µg/ml of SDX and 4 µg/ml of TMP.

A 20 µl volume of sample mixture was injected into the sample injector of HPLC system and their chromatograms were recorded under the same chromatographic conditions as described above. The area of each peak was determined at 248 nm and the amount of drug present in the sample mixture was determined.

METHOD VALIDATION
Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics [13].

The method was validated for different parameters like Accuracy, Precision, Linearity, Reproducibility, Limit of Detection (LOD), Limit of Quantification (LOQ), System suitability and Robustness [14, 15].

ACCURACY
Procedure for determination of Accuracy:
The procedure for the preparation of solutions for Accuracy determination at 80%, 100% and 120% level were prepared in the same manner as explained in method A. But in this method mobile phase i.e. Acetonitrile: 0.02 M phosphate buffer (20: 80 v/v) was used as solvent. The solutions were filtered through 0.4 µm membrane filter paper and then they were subjected to analysis by RP-HPLC method under the same chromatographic conditions as described above. At each level, three determinations were performed. The results obtained were compared with expected results and were statistically validated.

PRECISION
Procedure for determination of Precision:
The procedure for the preparation of solution for the determination of precision was same as explained in the analysis of injection formulation.

Procedure for determination of Intra-day Precision:
In intraday precision the sample mixture containing 20 µg/ml of SDX and 4 µg/ml of TMP was analyzed six times at different time intervals in the same day. The concentration of the sample mixture was determined as per the procedure given for the injection formulation by determining area under curve at selected analytical wavelength 248 nm. The variation of the results within the same day was analyzed and statistically validated.

Procedure for determination of Inter-day Precision:
In inter-day precision a set of six sample mixtures containing 20 µg/ml of SDX and 4 µg/ml of TMP were prepared and analyzed at same time on different days. The concentration of the sample mixture was determined as per the procedure given for the injection formulation by determining area under curve at selected analytical wavelength 248 nm. The variation of the results on different days was analyzed and statistically validated.

LINEARITY
To establish the linearity, a series of dilutions ranging from 2-10 µg/ml for SDX and 1-5 µg/ml for TMP were prepared separately and calibration graph was plotted between the mean peak area Vs respective concentration and regression equation was derived.

REPRODUCIBILITY

Reproducibility expresses the precision between laboratories. It is assessed by means of inter laboratory trial. It should be considered in case of standardization of an analytical procedure. The area under curve of the sample mixture was measured by another analyst at selected analytical wavelength 248 nm under the same chromatographic condition as described above. The results obtained were evaluated using t-test to verify their reproducibility.

LIMIT OF DETECTION:

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample. The LOD was calculated using the formula involving standard deviation of response and slope of calibration curve as mentioned in Table: I .

LOD= 3.3 ×SD/S

LIMIT OF QUANTIFICATION:

The LOQ is the concentration that can be quantified reliably with a specified level of accuracy and precision. The LOQ was calculated using the formula involving standard deviation of response and slope of calibration curve as mentioned in Table: I .

LOQ=10×SD/S

SYSTEM SUITABILITY

To check the system suitability, six replicate injections of mixed standard solution were injected and parameters such as retention time, resolution factor, capacity factor, tailing factor, theoretical plate, and asymmetry factor of the peaks were calculated.

ROBUTNESS

The evaluation of robustness should be considered during the development phase and depends upon the type of procedure under study. It should show the reliability of analysis with respect to deliberate variations in method parameters. The solution containing 20 µg/ml of SDX and 4 µg/ml of TMP was injected into sample injector of HPLC three times under deliberate variations in flow rate.

RESULTS

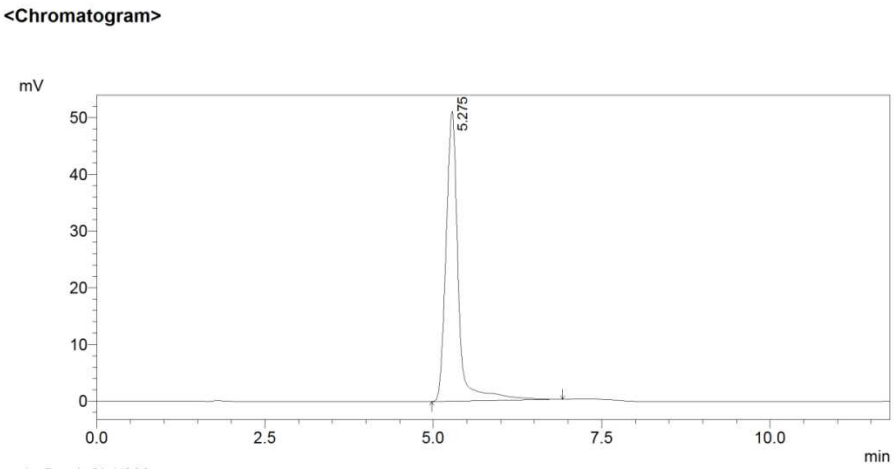


Fig: I. Chromatogram for Sulfadoxine

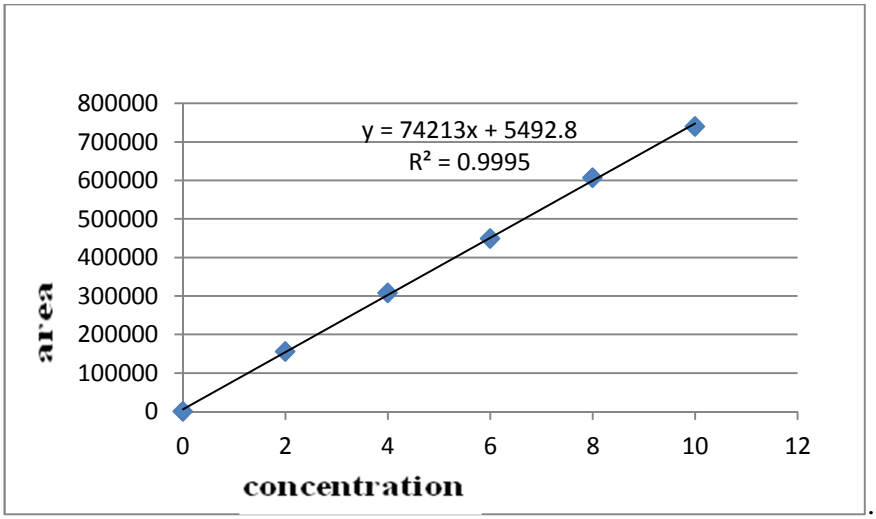


Fig: II. Calibration curve for Sulfadoxine

<Chromatogram>

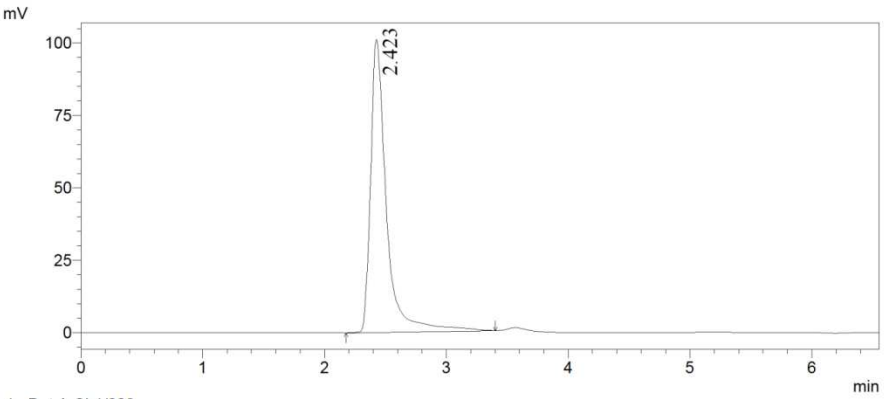


Fig: III. Chromatogram for Trimethoprim

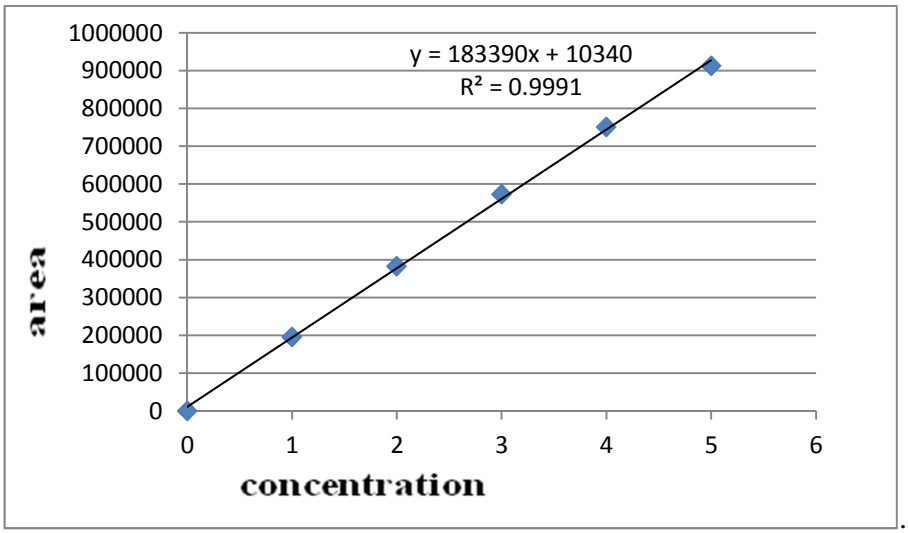


Fig: IV. Calibration curve for Trimethoprim

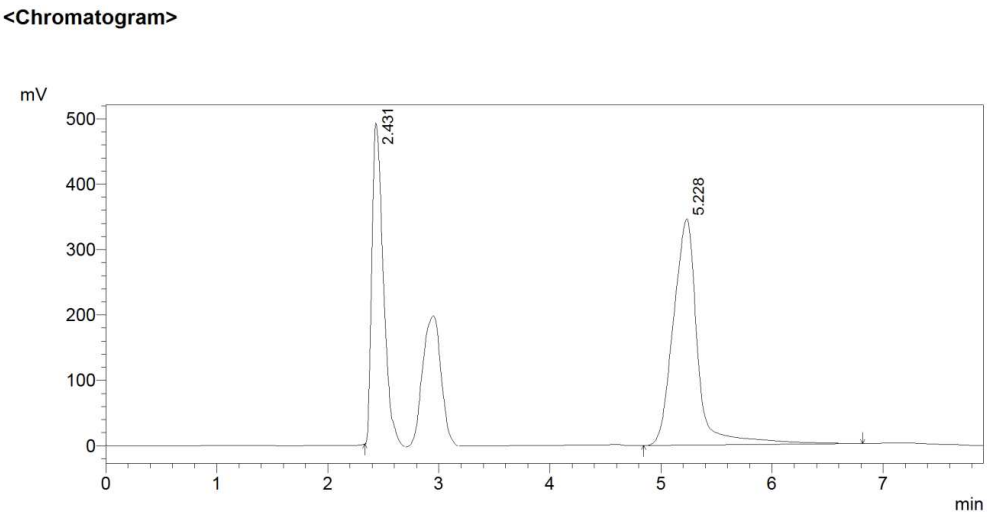


Fig: V. Chromatogram of mixture of Sulfadoxine and Trimethoprim

Table: I. Statistical data of Sulfadoxine and Trimethoprim at 248 nm

Parameter	Sulfadoxine (SDX)	Trimethoprim (TMP)
Linear Range (µg/ml)	2-10	1-5
Slope	74213	18339
Intercept	5492	10340
Correlation coefficient(r^2)	0.999	0.999
Limit of Detection (µg/ml)	0.0024	0.0009
Limit of Quantification (µg/ml)	0.0072	0.0028

Table: II. Assay results of Injection formulations.

Brand name	Components	Mean*	Standard Deviation*	Co-efficient of Variation*
Bactridox	**SDX**	99.86	0.28	0.28
	TMP	100.03	0.54	0.05
Borgal	**SDX**	99.83	0.25	0.25
	TMP	100.07	0.57	0.56

**n = 6*

Table: III. Statistical validation data for accuracy determination

Level of % Recovery	Mean*		Standard Deviation*		Co-efficient of Variation*	
	SDX	TMP	SDX	TMP	SDX	TMP
80%	99.76	98.79	0.35	0.31	0.35	0.31
100%	99.93	99.26	0.15	0.06	0.15	0.06
120%	99.54	99.44	0.36	0.31	0.35	0.31

**n = 6*

Table: IV. Statistical validation data for intra-day precision

Components	Mean*	Standard Deviation*	Co-efficient of Variation*
SDX	40.04	0.15	0.36
TMP	149.92	0.42	0.28

**n = 6*

Table: V. Statistical validation data for inter-day precision

Components	Mean*	Standard Deviation*	Co-efficient of Variation*
SDX	39.99	0.13	0.32
TMP	149.70	0.51	0.34

**n = 6*

Table: VI. Reproducibility results of SDX at 248 nm

Analyst 1	Analyst 2	Result of t-test*	Inference
101265.941 ± 1420	100948.441 ± 1264.5	0.9409	No significant difference

Table: VII. Reproducibility results of Trimethoprim at 248 nm

Analyst 1	Analyst 2	Result of t-test*	Inference
101265.941 ± 1420	100948.441 ± 1264.5	0.9409	No significant difference

*Where, *n = 6 at 95% confidence level*

Table: VIII. Summary of system suitability parameters of SDX and TMP

Parameters	SDX	TMP
Retention time (min)	5.27	2.42
Resolution factor	2.397	2.397
Tailing factor	1.139	1.70
Theoretical plate	4530.733	1900.144
HETP	33.107	78.94
Capacity factor	0	0

Table: IX. Robustness results for variation in flow rate (ml/min)

Flow rate (ml/min)	Level	Retention time		Tailing factor	
		SDX	TMP	SDX	TMP
0.9	-0.1	5.543	2.628	1.299	1.402
1	0	5.228	2.431	1.295	1.385
1.1	+0.1	4.892	1.622	1.309	1.385

DISCUSSION

The objective of the proposed work was to develop simultaneous methods for the determination of SDX and TMP, and to validate the methods according to ICH guidelines and applying the same for its estimation in marketed formulations. There is no official method for the simultaneous estimation of SDX and TMP in combination.

In HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to separate title ingredients. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time, resolution. The system with acetonitrile and buffer (20:80 %v/v) with 1.0ml/min flow rate is quite robust.

The optimum wavelength for detection was 248 nm at which better detector response for both the drugs was obtained. The calibration was linear in the concentration range of 2-10 µg/ml and 1-5 µg/ml, with regression 0.999 and 0.999 for SDX and TMP respectively. The low values of % R.S.D indicate the method is precise and accurate. The mean recoveries were found in the range of 98 – 102 %.

Sample to sample precision and accuracy were evaluated using six samples solution, which were prepared and analyzed on same day. Day to day variability was assessed using six sample solution analyzed on three different days over a period of three days. These results showed the accuracy and reproducibility of the assay. The proposed method was validated in accordance with ICH parameters. High % recovery and low % RSD suggests that the method can be used for the routine analysis of commercial formulations.

CONCLUSION

All these factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive, rugged, and rapid as per the guidelines prescribed by ICH, and can be applied successfully for the estimation of SDX and TMP in pharmaceutical formulations without interference and with good sensitivity; hence it can be used for the routine analysis in quality control department.

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