Original Article

UV SPECTROPHOTOMETRY METHOD DEVELOPMENT AND VALIDATION OF SULFADIAZINE AND TRIMETHOPRIM IN COMBINED DOSAGE FORM

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ABSTRACT

Objective: A new, simple, sensitive and economical UV spectrophotometric method was developed for the simultaneous analysis of Sulfadiazine [SDA] and Trimethoprim [TMP] in pharmaceutical formulations.

Methods: This UV method was developed with methanol as solvent. The wavelengths selected for analysis in the present method were 265 nm for TMP and 289 nm for SDA. Teccomp UV-2301 double beam UV/Vis spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software.

Results: Linearity was found to be within the concentration range of 2-9 µg/ml TMP and 9.08-41 µg/ml of SDA. Accuracy of the method was determined by recovery studies. Percentage recovery was found to be 98.20-99.25 for TMP with a % RSD of 0.338, 0.506 and 0.510 for three spiked levels. % RSD was found to be 0.229 and 0.380; 0.212 and 0.328 for SDA, TMP in intra and inter-day precision respectively. The % RSD value in ruggedness was found to be 0.440 for SDA and 0.569 for TMP.

Conclusion: The advantages of this method for analytical purposes lie in the rapid determination, its cost-effectiveness, easy preparation of the sample, good reproducibility. In addition to this, the present method can be recommended for simultaneous determination of SDA and TMP in routine quality control analysis in combined drug formulations.

Keywords: Sulfadiazine, Trimethoprim, UV-Method and Methanol

INTRODUCTION

SDA is a sulfonamide antibiotic and synthetic bacteriostatic with a wide spectrum against most gram-positive and many gram-negative organisms. This compound belongs to the aminobenzene sulfonamides [1]. The medication was used to treat and prevent a wide variety of infections. It works by stopping the growth of bacteria and other organisms. SDA was used as a competitive inhibitor of the bacterial enzyme dihydropteroate synthetase [2]. This enzyme is needed for the proper processing of para-aminobenzoic acid which is essential for folic acid synthesis. The inhibited reaction is necessary in these organisms for the synthesis of folic acid [3]. This antibiotic treats only certain types of infections. It will not work for viral infections e. g., common cold, flu, nausea, vomiting, diarrhea, loss of appetite, and headaches are the side effects of SDA.
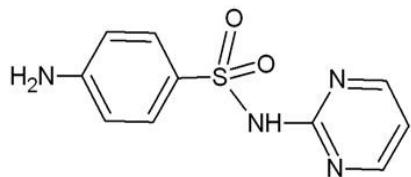
Fig. 1: Chemical structure of SDA [6]

TMP is on the World Health Organization's List of Essential Medicines [4]. TMP is a bacteriostatic antibiotic used mainly in the prevention and treatment of urinary tract infections. The drug belongs to the class of chemotherapeutic agents known as dihydrofolate reductase inhibitors. It is primarily used in the treatment of urinary tract infections, although it may be used against any susceptible aerobic bacterial species [5]. It was also used to treat and prevent *Pneumocystis jiroveci* pneumonia. TMP inhibits production of tetrahydrofolic acid by inhibiting the enzyme responsible for making tetrahydrofolic acid from dihydrofolic acid. TMP has an affinity for bacterial dihydrofolate reductase and has several thousand times greater than its affinity for human dihydrofolate reductase. TMP inhibits the bacterial enzyme more than the human enzyme.
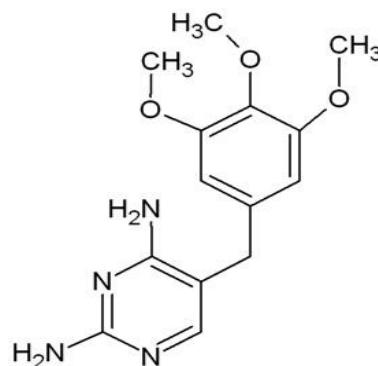
Fig. 2: Chemical structure of TMP [16]

Literature survey reveals that only a few selected spectrophotometric [6-8], HPLC [10-11], and HPLC-LC-MS [18], methods were reported for the estimation of SDA and TMP individually, UV-Visible [9], HPLC [12-17], as a combination with other drugs in bulk and biological samples. There are two HPLC [19-20], one stability indicating HPLC [21], and four LC-MS [22-25] methods for the estimation of SDA and TMP as a combination in bulk and biological

samples. However to the best of the knowledge of the author no spectrophotometric method was developed for the estimation of this combination. Hence in the present investigation, an attempt was made to develop a simple, sensitive and economical UV/Vis spectrophotometric method for the simultaneous analysis of SDA and TMP in pharmaceutical formulations.

MATERIALS AND METHODS

Instrumentation

Teccomp UV-2301 double beam UV/Vis spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Standard cuvettes of 10 mm path length are used for analysis. Sonicator (1.3 L) ultrasonicator was used to sonicate the standard and formulation sample. Standard and sample drugs were weighed by using Denver electronic analytical balance (SI-234).

Chemicals and reagents

The reference sample of SDA and TMP was a kind gift of Hetro Pharma, Hyderabad and Lupin Ltd, Mumbai, India, respectively. The formulation AUBRIL (SDA–410 mg and TMP–90 mg) was purchased from local market. Methanol (solvent) of analytical grade was purchased from Merck specialties Private Limited, Mumbai, India.

Preparation of standard drug solution

10 mg of standard drug SDA and TMP was accurately weighed separately and dissolved in 5 ml diluent (methanol), then transferred into a 10 ml volumetric flask, sonicated it for 5 min, finally, volume was made up to the mark with the same solvent to make 1000 µg/ml stock solution. From this 1 ml was again diluted to 10 ml to get a concentration of 100 µg/ml solution of TMP and SDA, separately. Then the required concentration was prepared separately, 1 ml from each of these solutions were mixed to obtain a combined solution for the simultaneous estimation of TMP and SDA.

Selection of method and wavelength

The standard stock solution was further diluted with milli-Q water to obtain the concentration of 10 µg/ml, each solution was scanned in UV range [200-400 nm] in 1.0 cm cell against solvent blank. The overlain spectrum of drugs was recorded. The study of spectrum

revealed that SDA and TMP show a well-defined λmax individually. The spectra of overlay for the two drugs confirmed that TMP at 265 nm and SDA at 289 nm were found to be suitable λmax for the selected drugs. The obtained wavelength maxima for the two drugs were used for the simultaneous estimation of SDA and TMP using simultaneous equation method. The wavelength scanning overlay spectrum was given in fig. 3.
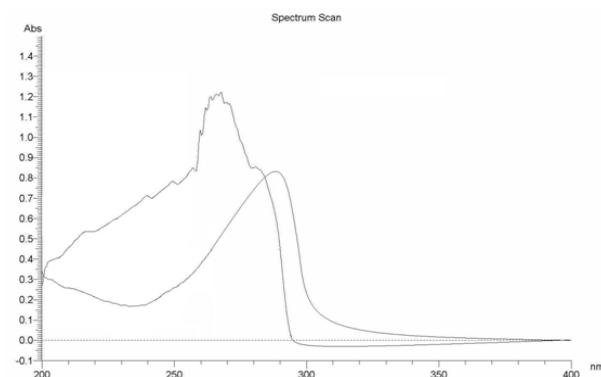
Fig. 3: Overlay spectrum of SDA and TMP

RESULTS

The method was validated as per ICH guidelines [26].

Linearity

Linear relationship between absorbance and concentration of the drugs was evaluated over the concentration range expressed in µg/ml by making three replicate measurements in the concentration range of 2-9 µg/ml TMP and 9.08-41 µg/ml of SDA at the wavelength maxima of TMP at 265 nm and SDA at 289 nm for each of the drugs. The calibration curve was drawn using absorbance obtained against concentration prepared separately for both the drugs. A well correlated linear fit graph was observed for both the drugs in the concentration range studied. Linearity results were shown in table 1 and graphs were given in fig. 4 and fig. 5 for TMP and SDA, respectively.

Table 1: Linearity results for SDA and TMP

S. No.	TMP		SDA	
	Concentration	Absorbance	Concentration	Absorbance
1	2 µg/ml	0.229±0.002	9.08 µg/ml	0.362±0.002
2	3 µg/ml	0.325±0.004	13.64 µg/ml	0.487±0.005
3	4 µg/ml	0.451±0.002	18.2 µg/ml	0.639±0.003
4	5 µg/ml	0.566±0.002	22.76 µg/ml	0.787±0.001
5	6 µg/ml	0.665±0.001	27.32 µg/ml	0.925±0.003
6	7 µg/ml	0.751±0.001	31.88 µg/ml	1.058±0.002
7	8 µg/ml	0.869±0.004	36.44 µg/ml	1.221±0.002
8	9 µg/ml	0.988±0.004	41.00 µg/ml	1.345±0.005
	Slope	0.1075	Slope	0.0312
	Intercept	0.0141	Intercept	0.0721
	r^2	0.9985	r^2	0.9995

*values given in table are the average±standard deviation of three replicate experiments
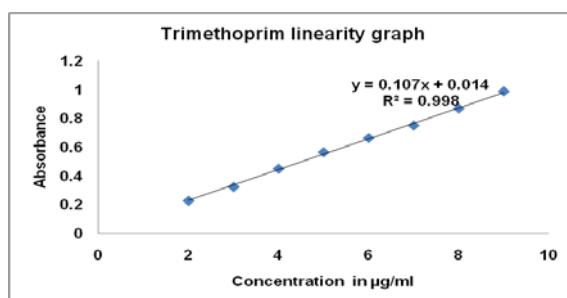
Fig. 4: Linearity graph for TMP
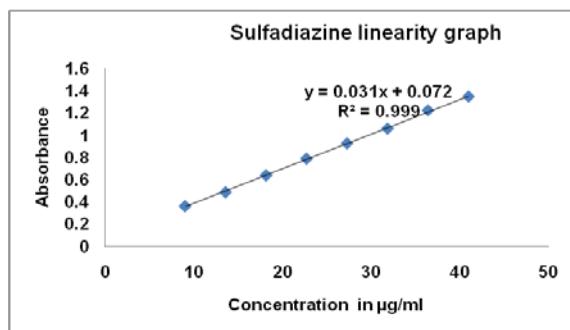
Fig. 5: Linearity graph for SDA

Recovery

Recovery studies were performed by standard addition method. The accuracy of the method was ascertained by carrying out recovery studies at three different levels (50 %, 100 % and 150 %). The resultant solution was analyzed in their corresponding wavelength.

Using the absorbance values obtained, percentage recovery and RSD in each spiked level were calculated. Results were found to be within the acceptance limit of 98-102 and percentage RSD of less than 2.

This confirms that the proposed method was found to be accurate. Results of the recovery were given in *table* 2 for SDA and TMP.

Table 2: Recovery results for SDA and TMP

Drug	% of recovery	Target conc., [µg/ml]	Spiked conc., [µg/ml]	Final conc., [µg/ml]	Conc., obtained [µg/ml]	% recovery	RSD of recovery
SDA	50%	9.08	4.54	13.62	13.553±0.031	99.510±0.229	0.225
	100%	9.08	9.08	18.16	18.113±0.035	99.740±0.191	0.194
	150%	9.08	13.62	31.78	22.533±0.078	99.267±0.343	0.345
TMP	50%	2	1	3	2.960±0.010	98.667±0.335	0.338
	100%	2	2	4	3.950±0.020	98.750±0.500	0.506
	150%	2	3	5	4.937±0.025	98.733±0.503	0.510

*values given in table are the average±standard deviation of three replicate experiments

Precision

Repeatability and intermediate precision of the developed method were expressed in terms of relative standard deviation of the absorbance. The sample application and measurement of absorbance were determined by performing six replicates measurement of the same band using a sample solution containing SDA at 41 µg/ml and TMP at 9 µg/ml. The solution was analyzed six

replicates in the same day for intra-day precision and three successive days for inter-day precision. Percentage RSD was found to be 0.229 and 0.380 for SDA; 0.212 and 0.328 for TMP in intra and inter-day precision, respectively.

Results confirmed that the precision of the method was found to be accepted. Precision results were given in *table* 3 and *table* 4 for intra and inter-day precision respectively.

Table 3: Intra-day precision results for SDA and TMP

S. No.	TMP at 9 µg/ml	SDA at 41 µg/ml
1	0.988	1.345
2	0.986	1.341
3	0.987	1.349
4	0.987	1.341
5	0.986	1.344
6	0.982	1.342
RSD	0.212	0.229

Table 4: Inter-day precision results for SDA and TMP

S. No.	TMP at 9 µg/ml	SDA at 41 µg/ml
1	0.989	1.338
2	0.987	1.334
3	0.989	1.334
4	0.991	1.339
5	0.996	1.346
6	0.993	1.332
RSD	0.328	0.380

Ruggedness

The ruggedness was performed by analyzing the drug solution using the same system but with different analysts. The difference between two analysts was compared using percentage RSD values. The

percentage RSD value was found to be 0.440 for SDA and 0.569 for TMP in six replicates of absorbance.

The low percentage RSD value illustrates the ruggedness of the method. Table 5 gives the results of the Ruggedness.

Table 5: Ruggedness results for SDA and TMP

S. No.	TMP at 9 μg/ml	SDA at 41 μg/ml
1	0.986	1.344
2	0.999	1.349
3	0.997	1.332
4	0.988	1.339
5	0.986	1.345
6	0.992	1.344
RSD	0.569	0.440

Sensitivity of the method

The sensitivity of the developed method was expressed in terms of the limit of detection [LOD] and limit of quantification [LOQ] values. The combined standard solution was prepared and the absorbance of the prepared solution was measured, at decreasing concentrations. The LOD values were found to be 0.04 μg/ml for TMP and 0.13 μg/ml for SDA in the optimized method.

This confirmed that the method can be applicable at lowest concentration for both the drugs. The LOQ values were found to be 0.82 μg/ml for SDA and 0.25 μg/ml for TMP. Results were given in *table 6*.

Table 6: Sensitivity results for SDA and TMP

Drug	LOQ	LOD
Trimethoprim	0.04 μg/ml	0.25 μg/ml
Sulfadiazine	0.13 μg/ml	0.82 μg/ml

Formulation analysis

The tablet sample solution was also subjected to analysis by simultaneous equation method. The absorbance of sample solutions were recorded at 265 nm and 289 nm and concentration of two drugs in the sample were determined by using equations 1 and 2.

Equation 1: Simultaneous equation for the estimation of TMP

$C_x = A_2 a_{y1} - A_1 a_{y2} / a_{x2} a_{y1} - a_{x1} a_{y2}$

Equation 2: Simultaneous equation for the estimation of SDA

$C_y = A_1 a_{x2} - A_2 a_{x1} / a_{x2} a_{y1} - a_{x1} a_{y2}$

Where:

a_{x1} = Absorptivity of TMP at 265 nm

a_{x2} = Absorptivity of TMP at 289 nm

a_{y1} = Absorptivity of SDA at 265 nm

a_{y2} = Absorptivity of SDA at 289 nm

A_1 and A_2 are the absorbances of the diluted sample at 265 nm and 289 nm respectively.

Results of the formulation analysis confirmed that the method can estimate more than 98 % accurately and the results were found to be in good agreement to the label claim values. The % assay was found to be 98.96 for SDA and 99.40 for TMP.

This confirmed that the method was found to be suitable for the routine analysis of SDA and TMP in pharmaceutical formulations. The results of the formulation analysis were given in table 7.

Table7: Formulation results for SDA and TMP

S. No.	Drug	Brand name	Label claim	Amount prepared	Amount found [μg/ml]	% assay
1	TMP	AUBRIL	90 mg	9 μg/ml	8.946±0.021	99.40
2	SDA		410 mg	41 μg/ml	40.573±0.094	98.96

*values given in table are the average±standard deviation of three replicate experiments

DISCUSSION

A simultaneous equation UV spectrophotometric method was developed and validated as per ICH guidelines [26] for the simultaneous estimation of SDA and TMP in tablet dosage form. The solvent used was 50 % *v/v* aqueous methanol. The absorbance was recorded at 265 nm and 289 nm. The overlain spectrum was shown in fig. 3.

The absorbance at TMP at 265 nm and SDA at 289 nm were measured and calibration curves were plotted. The absorptive values were determined with both the wavelengths in the mixture. The absorbance and absorptive values at particular wavelength were calculated and substituted in the equation to obtain the concentration. Linearity was found to be within the concentration range of 2-9 μg/ml TMP and 9.08-41 μg/ml of SDA.

The accuracy of the method was determined by recovery studies. Percentage recovery was found to be 98.20-99.25 for TMP with a % RSD of 0.338, 0.506 and 0.510 for three spiked levels. Results were found to be within the accepted limit of 98-102 and percentage RSD of less than 2. This confirmed that the proposed method was found to be accurate. Repeatability of the method was studied by precision experiments. % RSD was found to be 0.229 and 0.380; 0.212 and 0.328 for SDA, TMP in intra and inter-day precision respectively. The % RSD value in ruggedness was found to be 0.440 for SDA and 0.569 [table 5] for TMP in six replicates of absorbance. The low percentage RSD value illustrates the ruggedness of the method.

The proposed methods were found to be simple, accurate and rapid for the routine determination of SDA and TMP in tablet formulation. To study the validity and reproducibility of proposed methods, recovery studies were carried out. The methods were validated in terms of linearity, accuracy, precision, specificity and reproducibility.

CONCLUSION

A simultaneous equation UV spectrophotometry method was developed and validated as per ICH (International Conference on Harmonization) guidelines for the determination of SDA and TMP in combined dosage forms using methanol as solvent. The advantages of the proposed method for analytical purposes lie in the rapid determination, cost-effectiveness, easy preparation of the sample,

good reproducibility, simple, economic, accurate and practical. Hence, the proposed method can be recommended for simultaneous determination of SDA and TMP in routine quality control analysis in combined drug formulations.

AUTHOR'S CONTRIBUTION

The present work was done in collaboration with the three authors, Rama Krishna Veni, Kusuma Kumari and Hari Babu. Author Rama Krishna Veni is involved in conducting the experiments, collection of literature and preparation of the manuscript. Data analyzing and manuscript corrections were done by authors Kusuma Kumari and Hari Babu.

CONFLICT OF INTERESTS

Declared none

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