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ORIGINAL RESEARCH ARTICLE

Method Development and Validation of Sulphadiazine in Bulk and Pharmaceutical Dosage Form by UV-Spectrophotometric Method

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ABSTRACT:

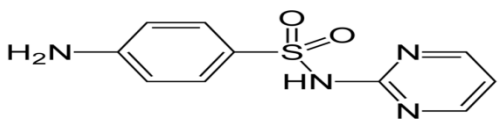
A new, simple, precise and accurate method for the estimation of Sulphadiazine in bulk and pharmaceutical dosage form has been developed. A mixture of methanol and water (90:10) was chosen as the solvent system. The λmax was found to be 270nm. The responses were linear in the range of 05-30µg/ml. The regression equation of the calibration graph and correlation coefficient were found to be y = 0.099x + 0.031 and 0.999 respectively. Validation of the method was done in order to demonstrate accuracy, precision, interday and intraday assay, robustness and ruggedness of the proposed method. The %RSD values for both intraday and interday precision were less than 1%. The recovery of the drug from the sample was ranged between 99.48% and 100.90%. Commercial tablets containing 500mg of Sulphadiazine (SULPHADIAZINE manufactured by Abbot Healthcare Pvt. Ltd. Mumbai) were analyzed by the proposed method and the results were well within the claimed limits.

Key words: Sulphadiazine, Intraday Assay, Robustness, Validation

INTRODUCTION

Sulphadiazine (**Fig.1**) is a sulphonamide group of antibiotic drug which acts by inhibiting the production of folic acid inside the bacterial cell. Chemically, it is 4-amino-*N*-pyrimidin-2-yl-benzenesulfonamide. It is used for the treatment of urinary tract infections. Literature surveys reveal different spectrophotometric methods [1-2], HPLC [3-7] and LCMS [8-9] methods for its determination. There was no simple method for estimation of Sulphadiazine, so a new, simple, accurate and validated method for determination of Sulphadiazine was developed by UV spectrophotometric method.

Fig 1: Chemical structure of Sulphadiazine



MATERIALS AND METHODS

Chemicals & Reagents: Analytically pure Sulphadiazine was obtained as a gift sample from Abbot Healthcare Pvt. Ltd., Mumbai (India). Commercial tablet formulations were purchased from the local market. All chemicals and reagents used were of Analytical Grade, unless otherwise stated.

Instrument: A SHIMADZU double beam UV/Visible recording spectrophotometer (Model: 1700) with 2 nm spectral bandwidth was employed for all spectrophotometric measurement using 10mm matched quartz cell and Borosil glass wares were used for the study. Calibrated electronic single pan balances Sartorius CP 225 D, pH Meter (LABINDIA), Enertech Fast Clean Ultrasonicator were also used during the analysis.

Standard Stock Solution and Working Standard Solutions: The standard stock solution of Sulphadiazine was prepared by transferring accurately weighed 10 mg of drug to 10 ml volumetric flask and dissolving it with mixture of methanol and water (90:10) to get a concentration of 1000 µg/ml. The solution was diluted accordingly to get a concentration of 100µg/ml and was kept as the stock solution. The prepared stock solution was diluted with the solvent system to get working standard solutions of concentrations 10-80 µg/ml.

Determination of λmax: The standard solution of Sulphadiazine (10 µg/ml) was scanned in the

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wavelength region of 200-400 nm and the λmax was found to be 270 nm. (**Fig.2**)

Preparation of calibration curve: The working standard solutions of Sulphadiazine were scanned in the UV region and the absorbances were observed against mixture of methanol and water (90:10) solution as blank at 270nm. Finally the calibration curve was plotted between concentration (x-axis) and absorbance (y-axis) shown in (**Fig 3**).

Assay of tablet dosage form: 10 tablets of brand SULPHADIAZINE (manufactured by Abbot Healthcare Pvt. Ltd., Mumbai) containing 500mg of Sulphadiazine were weighed, average weight determined and finely crushed to powder. An accurate weight equivalent to 10mg of the drug was transferred to 100ml volumetric flask. The drug was extracted 4 times by adding solvent in potions, 20 ml each time and the volume was made upto the mark by using solvent. It was then diluted (within the linearity range), absorbances of the sample solution were recorded at determined λmax and the concentration of the drug in sample was found out.

VALIDATION

Accuracy: The accuracy of the proposed method was tested by recovery studies at 80%, 100%, and 120% by adding a known amount of pure drug to the pre-analyzed formulation of concentration 10µg/ml.

Precision: The precision of the proposed method was ascertained by actual determination of 6 replicates of a fixed concentration of the drug within the Beer's range and finding out the absorbance by the proposed method.

Intraday Assay: The intraday assay of the proposed method was ascertained by actual determination of 6 replicates of a fixed concentration of the drug within the Beer's range and finding out the absorbance by the proposed method at 3 different time period of the same day.

Interday Assay: The interday assay of the proposed method was ascertained by actual determination of 6 replicates of a fixed concentration of the drug within the Beer's range and finding out the absorbance by the proposed method on 3 different days.

Robustness: The robustness of the method was carried out by changing the solvent system to mixture of methanol and water from 90:10 to 85:15.

Ruggedness: In order to determine the ruggedness of the proposed method, the method was carried out simultaneously by two analysts.

RESULTS AND DISCUSSION

The regression equation of the calibration curve was found to be y=0.099x+0.0311. The calibration curve is shown in figure 3.and represented in (**Table 1**)**.** The assay results of the commercial formulations are shown in (**Table 2**). The method was found to be accurate and precise which was evident from its low %RSD values.(**Table 3 & 4**). Similarly the %RSD for Intraday and Interday Assay were found to be 0.1244 and 0.1277 respectively.(**Table 5 & 6**). The %RSD for Robustness was found to be 0.0273 and 0.0158 for the proposed method by taking mixture of methanol and water in the ratio of 90:10 and 85:15 respectively (**Table 7**) while the %RSD for Ruggedness was found to be 0.0265 and 0.0345 respectively when performed by two analysts separately. (**Table 8**).

Fig 2: Overlay Spectra of Sulphadiazine showing λmax at 270nm

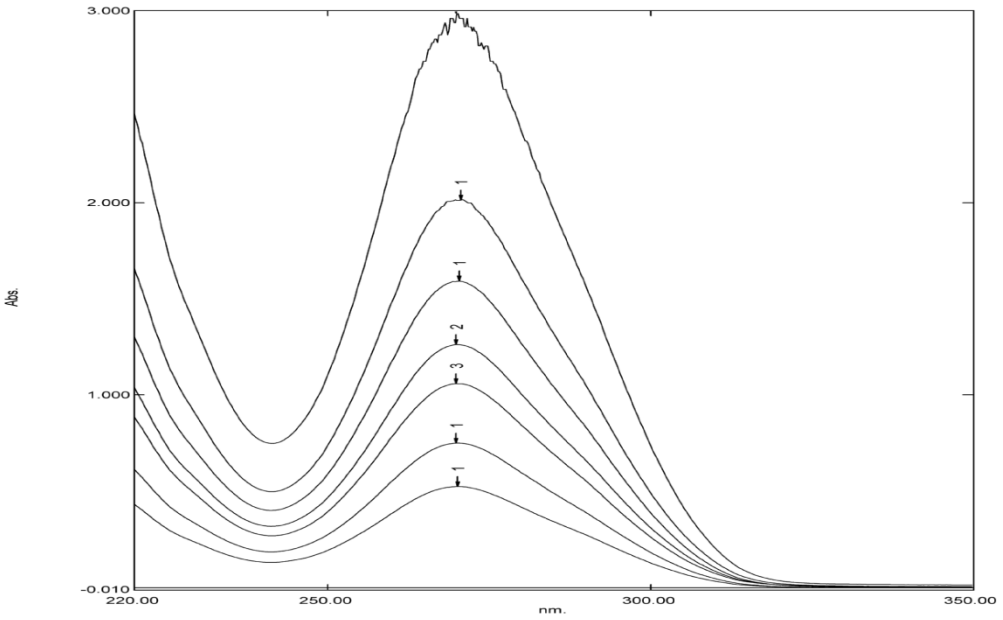

Fig 3: Calibration curve of Sulphadiazine at 270nm

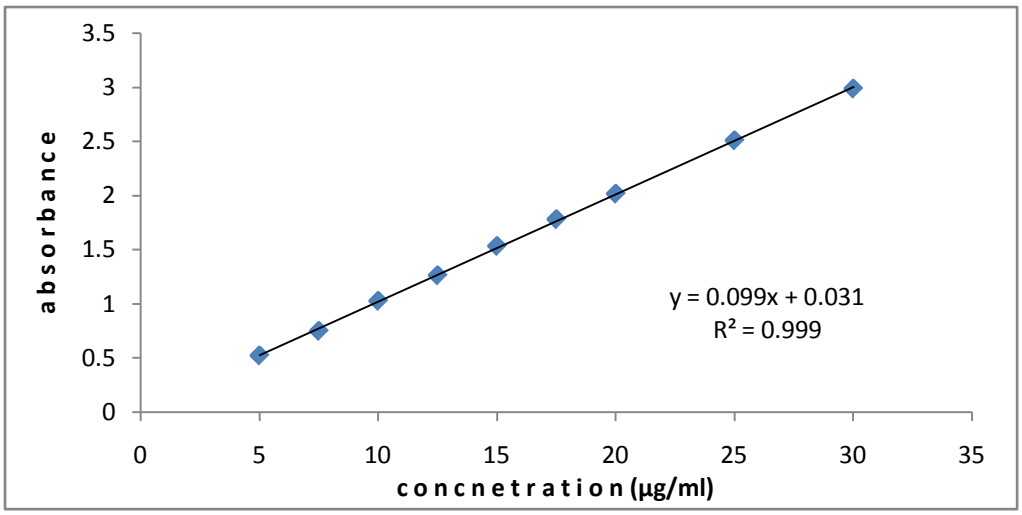

Table 1: Linearity table of Sulphadiazine in 0.1N HCl solution

Concentration (µg/ml)	Absorbance
5.0	0.525
7.5	0.751
10.0	1.028
12.5	1.264
15.0	1.534
17.5	1.781
20.0	2.016
25.0	2.508
30.0	2.985

Table 2: Assay results of the marketed formulation

Formulation	Label Claimed(mg)	Observed amount(mg)	Assay Result (%)
SULPHADIAZINE (Abbot Healthcare Pvt. Ltd., Mumbai)	500	491.476	98. 29

Table 3: Statistical analysis for ACCURACY of the proposed method

Samples	Concentration (µg/ml)		%Recovery	Statistical Analysis
	Pure	Formulation		
S1: 80%	8	15	99.89	Mean: 099.48
S1: 80%	8	15	99.73	S.D:0.5657
S1: 80%	8	15	98.84	%RS.D:0.4619
S2: 100%	10	15	101.15	Mean:100.90
S2: 100%	10	15	100.89	S.D:0.2402
S2: 100%	10	15	100.67	%RS.D:0.1961
S3: 120%	12	15	100.26	Mean:100.69
S3: 120%	12	15	101.15	S.D: 0.4454
S3: 120%	12	15	100.67	%RSD:0.3637

Table 4: Statistical analysis for PRECISION of the proposed method

Concentration (µg/ml)	Absorbance	Amount Present	Statistical Analysis
15	1.451	14.9697	
15	1.458	15.0404	
15	1.455	15.0101	Mean:15.02146
15	1.458	15.0404	S.D:0.027229
5	1.456	15.0202	%RSD:0.02547
15	1.456	15.0202	
15	1.455	15.0101	
15	1.460	15.0606	

Table 5: Statistical analysis for INTRADAY ASSAY of the proposed method

S. No	Concentration (µg/ml)	Absorbance			Statistical Analysis
		Sampling 1	Sampling 2	Sampling 3	
01	15	1.452	1.456	1.452	Mean:15.0202 S.D:0.012528 %RSD:0.1243
02	15	1.459	1.456	1.459	
03	15	1.456	1.458	1.454	
04	15	1.458	1.455	1.451	
05	15	1.456	1.457	1.458	
06	15	1.455	1.458	1.459	

Table 6: Statistical analysis for INTERDAY ASSAY of the proposed method

S. No	Concentration (µg/ml)	Absorbance			Statistical Analysis
		DAY 1	DAY 2	DAY 3	
01	15	1.456	1.455	1.452	Mean: 15.0139 S.D: 0.01287 %RSD: 0.1277
02	15	1.454	1.451	1.451	
03	15	1.458	1.459	1.456	
04	15	1.451	1.456	1.459	
05	15	1.456	1.454	1.454	
06	15	1.459	1.459	1.457	

Table 7: Statistical analysis for ROBUSTNESS of the proposed method

METHANOL: WATER (90:10)				METHANOL: WATER (85:15)			
Conc. (µg/ml)	Abs	Calculated amount (mg)	Statistical Analysis	Conc. (µg/ml)	Abs	Calculated amount (mg)	Statistical Analysis
15	1.459	15.0505	Mean:15.0101 S.D: 0.02996 %RSD:0.0273	15	1.461	15.0707	Mean:15.0589 S.D: 0.01739 %RSD:0.0158
15	1.451	14.9697		15	1.461	15.0707	
15	1.454	15.0		15	1.459	15.0505	
15	1.454	15.0		15	1.462	15.0808	
15	1.454	15.0		15	1.458	15.0404	
15	1.458	15.0404		15	1.458	15.0404	

Table 8: Statistical analysis for RUGGEDNESS of the proposed method

ANALYST-I				ANALYST-II			
Conc. (µg/ml)	Abs	Calculated amount (mg)	Statistical Analysis	Conc. (µg/ml)	Abs	Calculated amount (mg)	Statistical Analysis
15	1.456	15.0202	Mean:15.0168 S.D: 0.02903 %RSD:0.0265	15	1.458	15.0404	Mean:15.0622 S.D: 0.03796 %RSD: 0.0345
15	1.454	15.000		15	1.455	15.111	
15	1.458	15.0404		15	1.457	15.0303	
15	1.451	14.9697		15	1.458	15.0404	
15	1.456	15.0202		15	1.458	15.0404	
15	1.459	15.0505		15	1.455	15.111	

CONCLUSION

The proposed method was found to be simple, sensitive, precise and rapid for the determination of Sulphadiazine from pure and its dosage forms. The sample recoveries in all formulations were in good agreement with their respective label claims without interference of excipient and the other additives. Thus the proposed method can be used as an alternative method to the reported ones for the routine analysis of the drug in bulk and pharmaceutical dosage forms and can also be used for dissolution or similar studies.

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