

Utilizing the H-point Standard Addition Method for the Simultaneous Spectrophotometric Determination of Sulfadiazine and Trimethoprim in Binary Mixtures and Pharmaceutical Preparations

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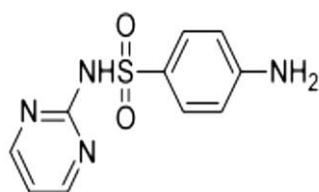
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In this research, the H-point standard addition method was employed for simultaneous spectrophotometric determination of sulfadiazine (SDZ) and trimethoprim (TRM) in binary mixtures and therapeutic doses. The absorbance at 272 and 296 nm was monitored using standard solutions of sulfadiazine and trimethoprim, and the results showed that SDZ and TRM could be simultaneously determined at concentration ratios ranging from 1 -15 and 1-14 $\mu\text{g/mL}$, respectively. The recoveries were found to be 97.78-102.62% for SDZ and 97.7-102.8 % for TRM, with a relative standard deviation (RSD) of $\leq 1.44\%$ and $\leq 2.85\%$, respectively. The method was successfully applied to the determination of sulfadiazine and trimethoprim in their binary formulations of pharmaceutical preparations with acceptable accuracy and good recoveries.

Keywords: Sulfadiazine, trimethoprim, HPSAM, determination, spectrophotometric

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Sulfadiazine (SDZ) is a white, non-crystalline powder that is soluble in alcohols and slightly soluble in water, chemically called [4-amino-N-pyrimidin-2-yl-benzenesulfonamide] [1]. SDZ belongs to the sulfonamide group of compounds, which have been widely used as antibacterial in human and veterinary medicine for nearly 60 years. It is an antibiotic used to treat a variety of infections caused by bacteria. SDZ has been prescribed to treat various types of infections, such as urinary tract infections and eye infections [2]. Chemical structure of SDZ is as follows [3].

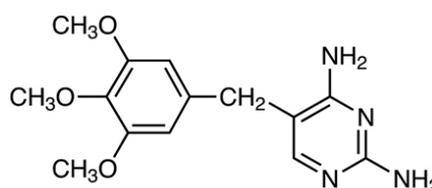


Sulfadiazine ($\text{C}_{10}\text{H}_{10}\text{N}_4\text{O}_2\text{S}$)
 $M_w = 250.3\text{g/mol}$

SDZ works to kill the bacteria that cause the disease by preventing the production of folic acid inside the bacterial cell. [4]. It has been used to treat trachoma and painful ulcers, and more recently, it has been used in the production of ointments that are useful in treating burns and skin ulcers [5].

Trimethoprim (TMP) is a white, crystalline powder that is soluble in water, ethanol, and methanol, but insoluble in ether. Its chemical name is 2,4-

diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine [6], and its structural and molecular formula are as follows [7]:



Trimethoprim ($\text{C}_{14}\text{H}_{18}\text{N}_4\text{O}_3$)
 $M_w = 290.3\text{g/mol}$

TRM is an antibiotic used for the effective treatment of a wide range of infections, such as urinary tract infections [8]. TRM kills bacteria by inhibiting dihydrofolate reductase, which hinders the formation of deoxyribonucleic acid (DNA). TMP is used in combination with sulfamethoxazole (SMX) to treat certain bacterial infections of the urinary tract. This combination can also be used to protect against the risk of infection with resistant strains of bacteria [9]. Through a survey of the scientific literature, several analytical methods and techniques were found for the determination of SDZ and TRM, individually and simultaneously, which include: Spectrophotometric [10, 11, 12], Chromatographic [13, 14, 15, 16,17], Electrochemical [18, 19], for SDZ, and Spectrophotometric [20, 21], Chromatographic [22, 23, 24], Electrochemical [25, 26] for TRM. Several previous studies have used HPSAM technology for the simultaneous determination of

some pharmaceutical mixtures, including: the determination of ciprofloxacin and cephalexin [27], Ciprofloxacin and Ornidazole [28], phenylephrine hydrochloride, chlorpheniramine maleate, and paracetamol [29], captopril and hydrochlorothiazide [30], thiamine and pyridoxine [31]. This manuscript aimed to develop a simple, easy, accurate, selective, and inexpensive spectrophotometric method using the H-point standard addition method for the simultaneous determination of SDZ and TRM and its application to veterinary drugs.

EXPERIMENTAL

Apparatus

A Shimadzu UV-Vis 1900i dual-beam spectrometer, equipped by Shimadzu Corporation (Japan), with 1.0 cm quartz cells, was used for spectral measurements. A sensitive balance ABS 120-4 Kern and Sohn, equipped by GmbH-Germany, was used for sensitive mass measurements. A professional pH meter, model BP3001, from Bante Instruments-China, was used for pH measurements.

Preparation of Stock Solutions

SDZ Stock Solution 100 $\mu\text{g/mL}$: 0.010 g of pure SDZ was dissolved in about 5 mL of ethanol, then the volume was completed to 100 mL with distilled water in a 100 mL calibrated flask. This solution was kept in a dark bottle. A working solution ($40 \mu\text{g/mL} = 1.6 \times 10^{-4} \text{ M}$) of SDZ was freshly prepared by diluting 40 mL of the stock solution with distilled water in a 100 mL calibrated flask.

TRM Stock Solution 100 $\mu\text{g/mL}$: 0.010 g of pure TRM was dissolved in about 15 mL of water, slightly heating, and then the volume was brought up to 100 mL with distilled water in a 100 mL calibrated flask. This solution was kept in a dark bottle. Similarly, a working solution ($40 \mu\text{g/mL} = 1.377 \times 10^{-4} \text{ M}$) of TRM was freshly prepared by diluting 40 mL of the stock solution with distilled water in a 100 mL calibrated flask.

Sodium Carbonate Solution (1 M): This solution was prepared by dissolving 10.6 g of pure

sodium carbonate in 20 mL of distilled water and completing the volume to 100 mL using a volumetric flask.

Pharmaceutical Solutions Preparation

Vapcotrim Injection Solution: 1 mL of the injection solution (each mL of injection contains 200 mg SDZ and 40 mg trimethoprim, TRM) was diluted with distilled water in a 100 mL calibrated flask to prepare a 2000 $\mu\text{g/mL}$ SDZ solution, then prepared the stock solution 10 mL of it was transferred to a 100 mL calibrated flask and filled up by distilled water to the mark. A working solution ($40 \mu\text{g/mL}$) was prepared by diluting 40 mL of stock solution with distilled water to 100 mL using a calibrated flask.

Intertrim-480 Veterinary Powder Solution: The stock solution was prepared by weighing 0.025 g of the drug powder (each gram of powder contains 400 mg of SDZ and 80 mg of TRM), which is equivalent to 0.010 g of pure SDZ, and dissolving it in approximately 10 mL of distilled water under gentle stirring and heating until completely dissolved. The resulting solution was then filtered and transferred to a 100 mL volumetric flask, and the volume was made up to the mark with distilled water. The working solution ($40 \mu\text{g/mL}$) was prepared by dilution.

RESULTS AND DISCUSSION

Optimization of Conditions

All experiments were performed using 4 μg of SDZ, as well as 4 μg of TRM in a final volume of 10 mL, and optimal conditions were determined.

Effect of Solvents

The effect of several known solvents on the λ_{max} value for each pharmaceutical compound was studied individually, as the aim of finding the optimal solvent for successful application of the HPSAM method. Figure 1 shows that water is the optimal solvent for use in the proposed method.

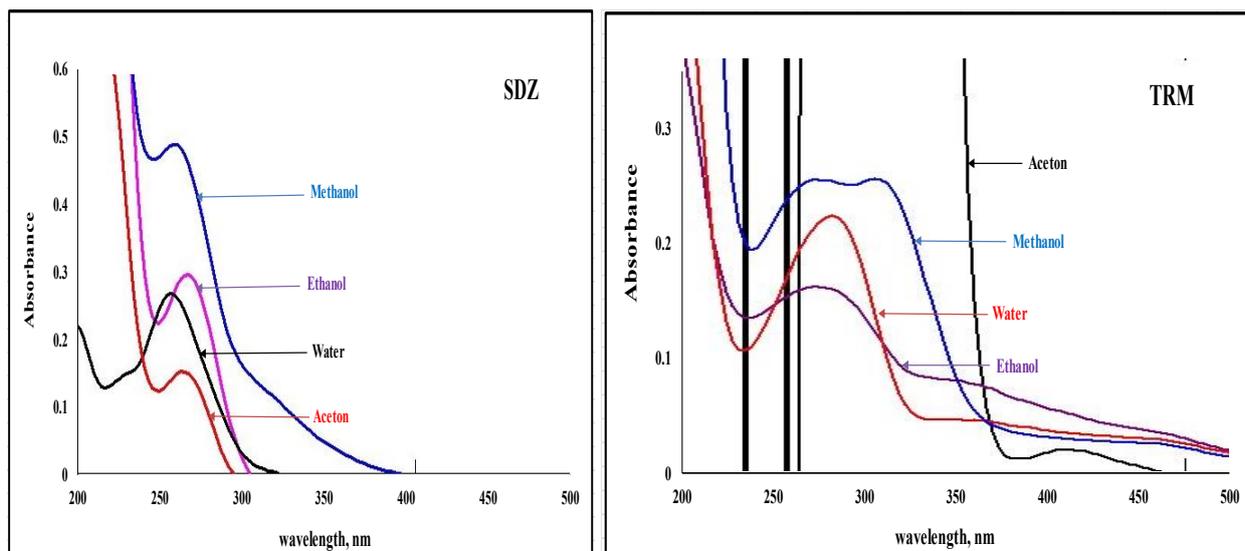


Figure 1. Effect of solvents on λ max of SDZ and TRM.

Effect of pH Value

The effect of pH on the absorption value, as well as the maximum λ value, was studied for a mixture of the two drugs, using a fixed concentration of each, and using several strong and weak acids and bases. The best medium for obtaining the highest absorption value for both drugs was generally alkaline, as a pH above 7 was found to be very

suitable for determining the drug mixture using the HPSAM method. Therefore, the appropriate base was chosen from among the multiple base solutions, namely 1 M sodium carbonate (Na_2CO_3), due to its safety, availability, and low cost. A suitable volume of this base was 0.5 mL for use in subsequent experiments. Figure 2 shows the absorption spectra of both drugs in the presence of the basic medium.

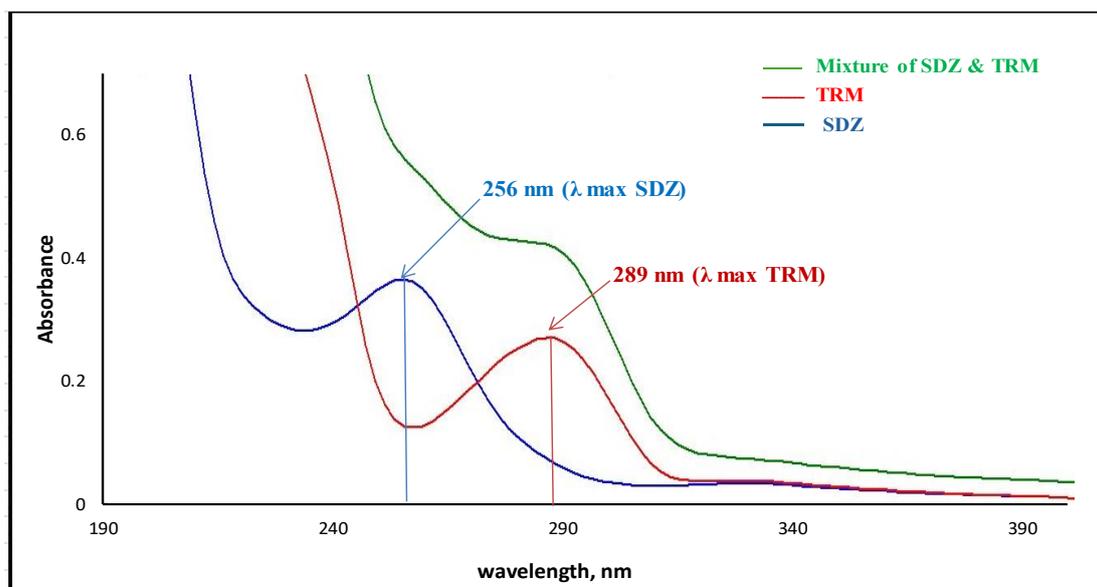


Figure 2. Spectrum of a mix of 4 $\mu\text{g}/\text{mL}$ of SDZ and 4 $\mu\text{g}/\text{mL}$ TRM in basic medium.

Selection of Better Wavelengths for Mixture Analysis

In this study, several pairs of wavelengths were identified for use in the HPSAM method. A series of fixed-concentration solutions (4 $\mu\text{g}/\text{mL}$) of SDZ and TRM, and sodium carbonate (0.5 mL of 1 M), were prepared, and increasing concentrations (0–8 $\mu\text{g}/\text{mL}$) of SDZ were added. The absorbance values of these solutions were then measured against the blank solution at each of the previously

identified wavelength pairs. Based on the recorded absorbance values, a calibration curve was drawn for each series using each wavelength pair. The optimal wavelength pair was selected, possessing the highest correlation coefficient and highest recovery of SDZ; therefore, wavelengths 272 and 296 nm were chosen for the analysis of the mixture. Figure 3, Table 1, and Figure 4 show the calibration curves drawn based on the above absorbance values. Figure 5 shows the wavelengths selected for the method.

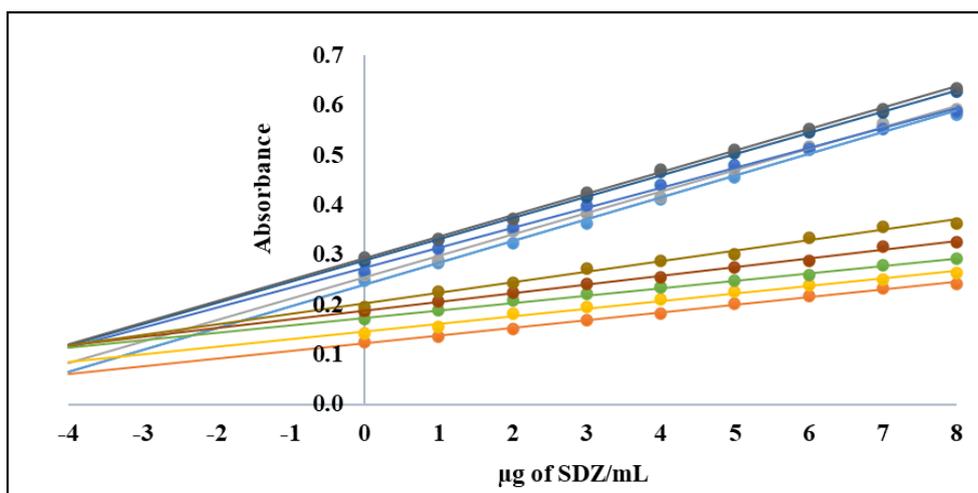


Figure 3. Selection of wavelengths for a mixture of 4 $\mu\text{g}/\text{mL}$ of SDZ and 4 $\mu\text{g}/\text{mL}$ of TRM with increasing amount of SDZ (0 - 8 $\mu\text{g}/\text{mL}$).

Table 1. Selection of a better wavelength for mixture analysis.

Wavelength (nm)	A-C Equations	R^2	Taken ($\mu\text{g}/\text{mL}$)		Found ($\mu\text{g}/\text{mL}$)		Absorbance (A_H)	Recovery (%)
			SDZ	TRM	SDZ	TRM		
258-304	$Y_{258}=0.0438x+0.2399$	0.9962	4	4	4.13	0.124	103.25	
	$Y_{304}=0.0154x+0.1226$	0.9963						
262-302	$Y_{262}=0.0431x+0.2546$	0.9969	4	4	3.892	0.143	97.30	
	$Y_{302}=0.0153x+0.1464$	0.9911						
268-300	$Y_{268}=0.0402x+0.2738$	0.9980	4	4	3.933	0.171	98.32	
	$Y_{300}=0.0149x+0.1734$	0.9962						
272-296	$Y_{272}=0.0428x+0.2885$	0.9991	4	4	3.988	0.187	99.70	
	$Y_{296}=0.0175x+0.1876$	0.9962						
274-294	$Y_{274}=0.0432x+0.2927$	0.9987	4	4	4.068	0.196	101.70	
	$Y_{294}=0.0211x+0.2028$	0.9891						

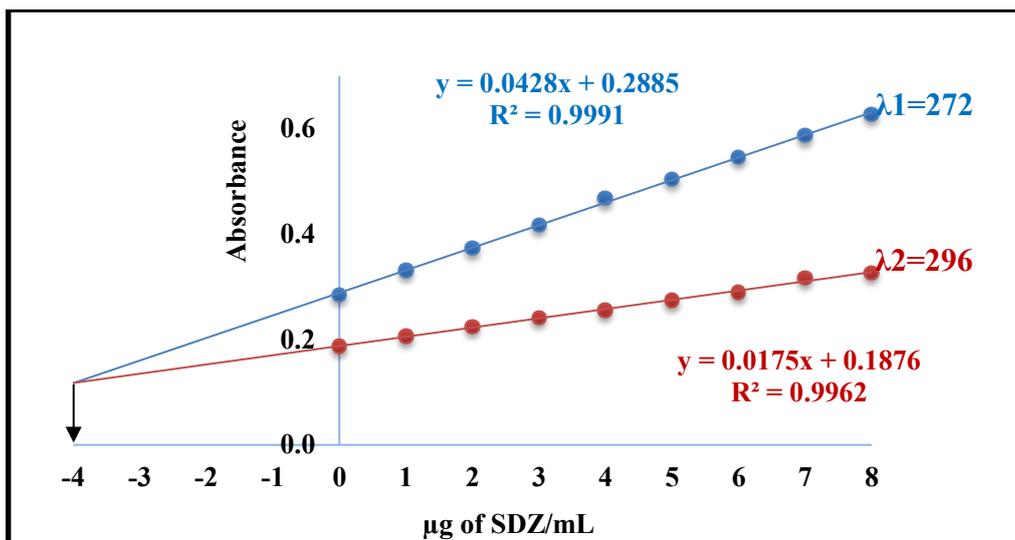


Figure 4. HPSAM standard curve for 4 µg/mL of SDZ at selected wavelengths.

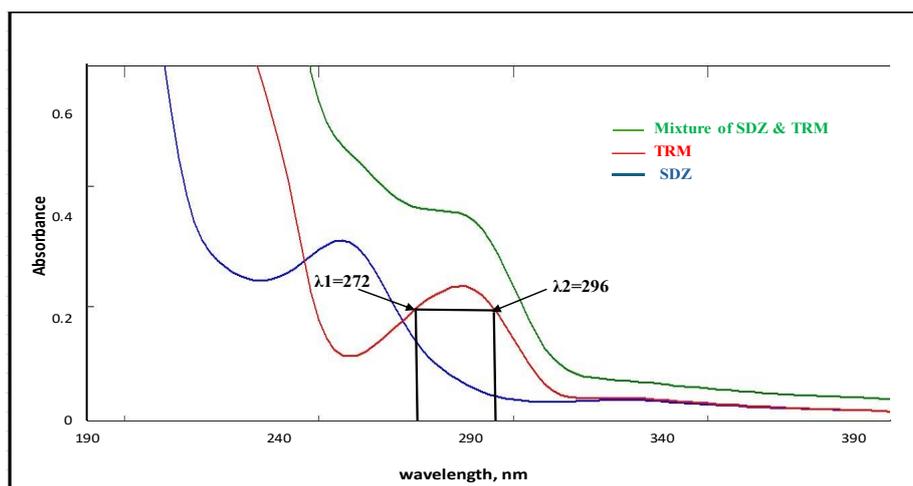


Figure 5. The wavelengths for the mixture of SDZ and TRM analysis.

Figure 5 shows that the absorption value of TRM is equal at wavelengths 272 and 296 and that the absorption value of SDZ changes gradually within the range between these wavelengths. This property is used to estimate SDZ in the presence of TRM.

Calibration Curve for SDZ

A sulfadiazine calibration curve was prepared by adding increasing volumes (0 to 4 mL) of 40 µg/mL SDZ solution (working solution) to several 10-mL volumetric flasks (covering the concentration range of 0 to 18 µg/mL) containing 1 mL of 40 µg/mL trimethoprim solution and 0.5 mL of 1 M sodium

carbonate (each group). Different volumes of sulfadiazine solution with concentrations ranging from 0 to 8 µg/mL were added to each group, and the volumes were then adjusted with distilled water to the mark. The absorbances of the resulting solutions were measured at laboratory temperature at wavelengths of 272 and 296 nm. Linear relationships between absorbance and SDZ concentration were plotted for each group, and the results are shown in Figures 6 and 7, which indicate the standard curve for sulfadiazine that follows the Beer-Lambert law over the concentration range from 0 to 15 µg/mL, while Table 2 shows the straight-line equations for each concentration.

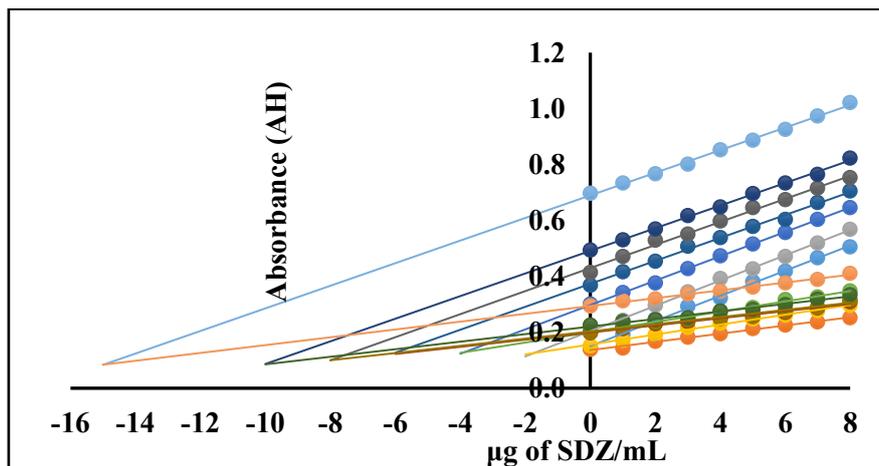


Figure 6. HPSAM curves for the determination of SDZ in a mixture.

Table 2. Results calibration curve of SDZ in the presence of a fixed amount of TRM.

A-C Equations	R ²	Taken (µg/mL)		SDZ	
		SDZ	TRM	Found (µg/mL)	Recovery (%)
Y ₂₇₂ =0.0488x +0.1461	0.9998	0.0	4	0.00	0.00
Y ₂₉₆ =0.0144x +0.1379	0.9976				
Y ₂₇₂ =0.0437x +0.2075	0.9975	2	4	2.04	102.00
Y ₂₉₆ =0.0177x +0.1565	0.9917				
Y ₂₇₂ =0.0452x +0.3052	0.9971	4	4	3.921	98.04
Y ₂₉₆ =0.0184x +0.2001	0.9944				
Y ₂₇₂ =0.0410x +0.3732	0.9957	6	4	6.17	102.84
Y ₂₉₆ =0.0128x +0.1992	0.9848				
Y ₂₇₂ =0.0412x +0.4279	0.9930	8	4	8.017	101.22
Y ₂₉₆ =0.0129x +0.2028	0.9685				
Y ₂₇₂ =0.0405x +0.4912	0.9977	10	4	10.03	100.3
Y ₂₉₆ =0.0135x +0.2204	0.9805				
Y ₂₇₂ =0.0404x +0.6895	0.9967	15	4	14.98	99.87
Y ₂₉₆ =0.0140x +0.2940	0.9935				

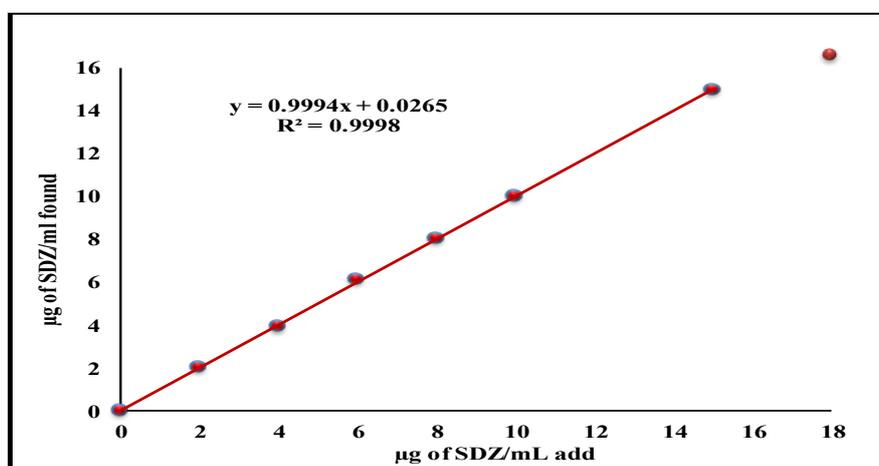


Figure 7. Calibration curve for SDZ according to HPSAM in a mixture.

Calibration Curve for TRM

The calibration curve for trimethoprim was prepared by adding increasing volumes of its working solution at a concentration of 40 $\mu\text{g/mL}$ (covering a range of concentrations from 0 to 16 $\mu\text{g/mL}$) to a series of 10 mL volumetric vials containing 1 mL of the working solution of SDZ (40 $\mu\text{g/mL}$) and 0.5 mL of 1 M sodium carbonate. Then increasing amounts of the working

solution of sulfadiazine were added to each series, and the volume was made up to the mark with distilled water. The absorbance of the resulting solutions was measured at laboratory temperature at wavelengths of 272 and 296 nm. After plotting the standard curve, it was found that it followed Beer–Lambert law over a range of concentrations of 1 to 14 $\mu\text{g/mL}$ for trimethoprim, and there is a positive deviation after that, as shown in Table 3 and Figures 8 and 9.

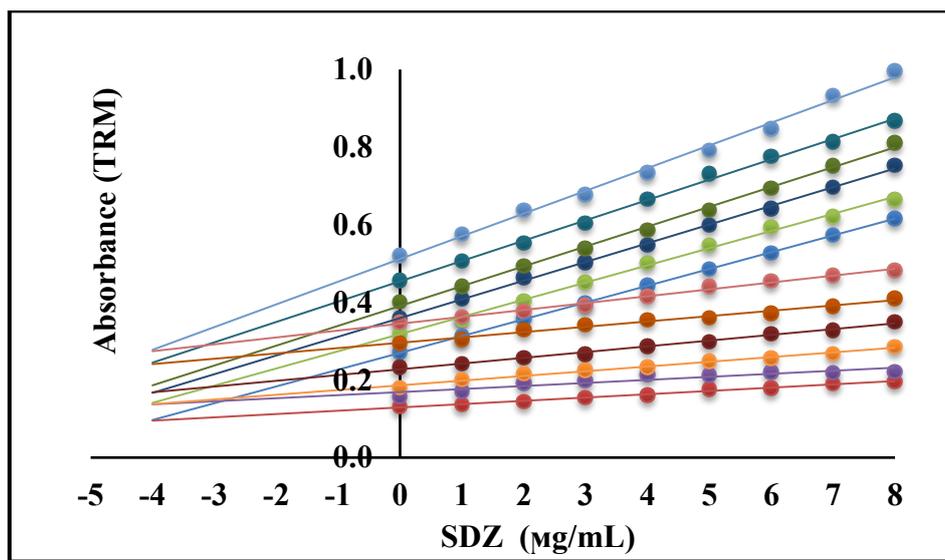


Figure 8. Calibration curves for TRM according to HPSAM in a mixture.

Table 3. Results of the calibration curve for TRM in the presence of a fixed amount of SDZ.

A-C Equation	R^2	Taken ($\mu\text{g/mL}$)		SDZ		TRM	
		SDZ	TRM	Found ($\mu\text{g/mL}$)	Recovery (%)	Found ($\mu\text{g/mL}$)	Recovery (%)
$Y_{272}=0.0443x+0.3182$ $Y_{296}=0.0079x+0.1688$	0.9971 0.8656	4	2	4.068	101.70	2.052	102.61
$Y_{272}=0.0458x+0.3353$ $Y_{296}=0.015x+0.2112$	0.9976 0.9908	4	6	3.933	98.32	6.043	100.73
$Y_{272}=0.0482x+0.3595$ $Y_{296}=0.0148x+0.2275$	0.9986 0.9962	4	8	3.988	99.70	7.904	98.80
$Y_{272}=0.0511x+0.3906$ $Y_{296}=0.0159x+0.2466$	0.9970 0.9826	4	10	4.068	101.70	10.272	102.72
$Y_{272}=0.0524x+0.4542$ $Y_{296}=0.0137x+0.2958$	0.9972 0.9830	4	12	4.068	101.70	12.279	102.33
$Y_{272}=0.0585x+0.5118$ $Y_{296}=0.0177x+0.3450$	0.9942 0.9921	4	14	3.978	99.46	14.57	104.07

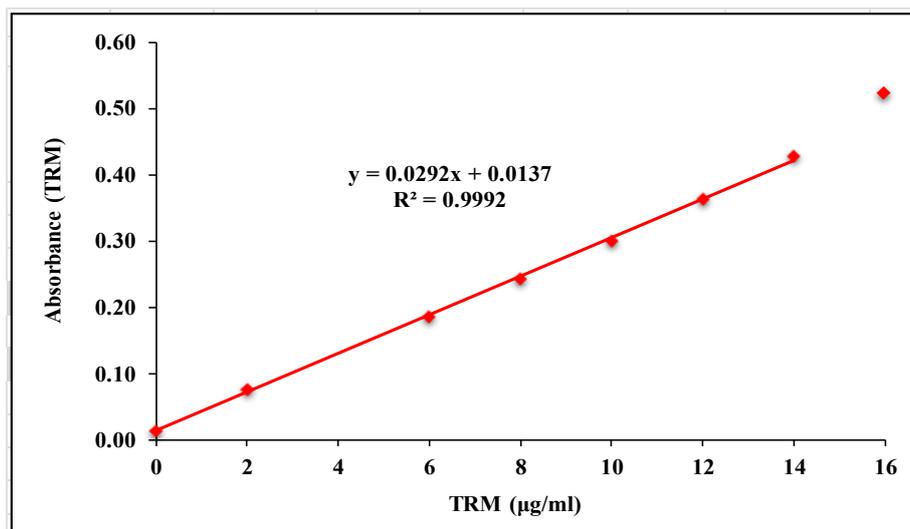


Figure 9. Calibration curves for TRM according to HPSAM in a mixture.

Accuracy and Precision

In this experiment, several samples were prepared for different mixtures of SDZ and TRM containing specific proportions of each compound (three replicates of each mixture), as shown in Table 4. The mixtures were analyzed using the proposed procedure,

and the recovery values, relative error, and relative standard deviation were calculated based on the analysis results. The accuracy of the procedure was evaluated by comparing the analysis results with the actual values. The results in Table 4 indicate that the accuracy of the method in determining SDZ and TRM in the mixtures is good.

Table 4. Results of accuracy and precision for the HPSAM procedure.

A-C Equation	R ²	Taken (µg/mL)		Found (µg/mL)		Recovery (%)		RSD (%)	
		SDZ	TRM	SDZ	TRM	SDZ	TRM	SDZ	TRM
Y ₂₇₂ =0.0406x + 0.467	0.9936	9	4	9.0936	3.967	101.04	99.18	0.53	1.79
Y ₂₉₆ =0.0128x + 0.2142	0.9628								
Y ₂₇₂ =0.0415x + 0.4634	0.9933	9	4	8.7939	4.063	97.71	101.58	0.53	1.79
Y ₂₉₆ =0.0129x + 0.2119	0.9862								
Y ₂₇₂ =0.0402x + 0.467	0.9982	9	4	8.9604	4.109	99.56	102.73	0.85	1.61
Y ₂₉₆ =0.0122x + 0.2161	0.9856								
Y ₂₇₂ =0.0382x + 0.4969	0.9958	12	6	12.128	6.09	101.07	101.50	1.44	2.85
Y ₂₉₆ =0.0149x + 0.2143	0.9856								
Y ₂₇₂ =0.0383x + 0.501	0.9989	12	6	11.961	5.979	99.68	99.65	0.85	1.61
Y ₂₉₆ =0.0146x + 0.2175	0.9797								
Y ₂₇₂ =0.0379x + 0.5013	0.9969	12	6	11.894	5.897	99.12	98.28	1.44	2.85
Y ₂₉₆ =0.0141x + 0.2182	0.9903								
Y ₂₇₂ =0.0412x + 0.307	0.9993	4	10	3.967	10.016	99.17	100.16	1.44	2.85
Y ₂₉₆ =0.017x + 0.211	0.9917								
Y ₂₇₂ =0.0384x + 0.2964	0.9944	4	10	3.930	9.625	98.25	96.25	1.44	2.85
Y ₂₉₆ =0.0171x + 0.2127	0.9842								
Y ₂₇₂ =0.0375x + 0.2934	0.9979	4	10	4.043	10.177	101.08	101.77	1.44	2.85
Y ₂₉₆ =0.0167x + 0.2093	0.9939								

Table 5. Results of application of the HPSAM procedure for the determination of SDZ and TRM.

Pharmaceutical preparation	A-C Equation	R ²	Taken (µg/mL)		Found (µg/mL)		Recovery (%)	
			SDZ	TRM	SDZ	TRM	SDZ	TRM
Vapcotrim injection 200 mg SDZ and 40 mg TRM/1 mL (Jordan)	Y ₂₇₂ =0.0431X +0.3495	0.9988	5	1	5.131	1.028	102.62	102.8
	Y ₂₉₆ =0.0133X +0.1966	0.9815						
	Y ₂₇₂ =0.0418X +0.5015	0.9979	10	2	9.846	1.969	98.46	98.45
	Y ₂₉₆ =0.0133X +0.2209	0.9807						
	Y ₂₇₂ =0.041X +0.7131	0.9959	15	3	14.673	2.931	97.82	97.7
	Y ₂₉₆ =0.0143X +0.3057	0.9942						
Intertrim-480 veterinary powder 400 mg SDZ and 80 mg TRM/1 g (Holland)	Y ₂₇₂ =0.0425X +0.3506	0.9977	5	1	5.065	1.014	101.30	101.40
	Y ₂₉₆ =0.0117X +0.1946	0.9820						
	Y ₂₇₂ =0.0416X +0.5076	0.9979	10	2	9.778	1.955	97.78	97.75
	Y ₂₉₆ =0.0119X +0.2172	0.9947						
	Y ₂₇₂ =0.0416X +0.7164	0.9922	15	3	15.081	3.016	100.54	100.53
	Y ₂₉₆ =0.0142X +0.3032	0.9834						

Table 6: Comparison of the developed method with a previous chromatographic method.

Parameter	Literature method*	Present method [15]
Reagent	With out	EDTA
λ _{max} (nm)	272 and 296	----
Beer's law range (µg/mL)	0 - 15	0.0025–1.00
Cost	inexpensive	expensive
Application of the method	veterinary drugs	medicated animal feeding stuffs

Application of the HPSAM Procedure

The proposed procedure was tested for the determination of TRM and SDZ in available pharmaceutical preparations, using three different concentrations (5, 10, and 15 µg/mL) of SDZ. The recovery percentage for the obtained concentrations was calculated based on the analysis results. Table 5 represents the application results, which confirm the successful use of the procedure for the determination of TRM and SDZ in pharmaceutical preparations containing mixtures of these two compounds simultaneously.

Comparison of Procedure

Some analytical values and practical considerations of the developed method are compared with a previous

chromatographic method, and the results of the comparison are listed in Table 6.

CONCLUSION

This paper aims to develop a simple, easy-to-use, accurate, selective, and inexpensive spectrophotometric method using the H-point-Scale Addition Method (HPSAM) for the simultaneous determination of SDZ and TRM at two wavelengths, 272 and 296 nm, and has been applied to veterinary drugs. This method eliminates the matrix and spectral interference associated with conventional spectrophotometry, offering an alternative to more complex chromatographic techniques (such as HPLC and GC) for routine quality control, with good recovery rates and acceptable relative error.

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