Spectrophotometric Determination of Sulfamethoxazole with P-N,N-dimethyl amino benzaldehyde as Condensation Reaction

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Abstract

Sulfamethoxazole (SMX) was added to P-N,N-dimethyl amino benzaldehyde (PDAB) by condensation reaction in acidic medium to form, a yellow colored dye compound which exhibits maximum absorption (λ_{max}) at 450.5 nm. The concentration of (SMX) was determined spectrophotometrically. The optimum reaction conditions and other analytical parameters were evaluated. In addition to classical univariate optimization, design of experiment method has been applied in optimization of the variables affecting the color producing reaction.

Beer's law obeyed in the concentration range of 0.1-10 $\mu g.mL^{-1}$ with molar absorptivity of 5.7950×10^{4} $L.mol^{-1}.cm^{-1}$. The limit of detection and Sandell's sensitivity value were 0.078 $\mu g.mL^{-1}$ was 4.3706 $\mu g.cm^{-2}$ respectivily. The proposed method could be successfully applied to the determination of (SMX) in synthetic sample and urine.

Key words: Spectrophotometric determination, Sulfamethoxazole, Condensation reaction, P-N,N-dimethyl amino benzaldehyde

Introduction

Chemically sulfamethoxazole (Figure 1) is 4-Amino-N-(5-methyl-3-isoxazoyl) benzene sulfonamide) antibacterial drug that interferes with folic acid synthesis in susceptible bacteria. Its use has been limited by the development of resistance and it is now used mainly as a mixture with trimethoprim [1].

Sulfamethoxazole and other sulfonamides having similar structures to p-amino benzoic acid, are used in the treatment of urinary tract infections, eye infections and as a prophylaxis of rheumatic fever. It acts as competitive inhibitors of the enzyme dihydropteroate synthetase, DHPS in bacteria by blocking the conversion of p-amino benzoic acid to dihydro pteroate, a reduced form of folic acid [2].

A survey of literature revealed that several analytical methods such as high performance liquid chromatography [3-5], flow injection [6,7], high performance thin layer chromatography[8], solid phase extraction[9], voltammetry[10] and spectrophotometric methods[11-14] have been reported for the determination of sulfamethoxazole.

The present study describes the use of (PDAB) as a chromogenic reagent in the development of simple, sensitive and a rapid spectrophotometric method for the estimation of (SMX) with reasonable precision, accuracy. Experimental conditions have been studied and the method optimized using univariate and multivariate central composite design method.

Experimental

Instruments

The absorption spectra were recorded on a double-beam (shimadzu 1800), and all spectrophotometric measurements were carried out on (CECIL 1011)UV-Visible single beam spectrophotometer with 1cm matched quartz cells.

Materials and Reagents

Pharmaceutical grade sulfacetamide received as gift sample powder in pure form (99.99%) the State Company for Drug Industries and Medical Appliances Samara-Iraq (SDI). All chemicals and reagents used were of analytical grade.

Reagents solution

1- P-N,N-dimethyl amino benzaldehyde [6 % (m/v)]: prepared by dissolving 6 g of P-N,N-dimethyl amino benzaldehyde in 100 mL methanol.
2- Sulfuric acid [7 % (v/v)]: prepared by mixing 7 mL of concentrated sulfuric acid (sp.gr.=1.84) with 50 mL of distilled water, and diluting to the mark in a 100 mL volumetric flask using distilled water.
3- Sulfuric acid [2 % (v/v)]: prepared by mixing 2 mL of concentrated sulfuric acid (sp.gr.=1.84) with 50 mL of distilled water, and diluting to the mark in a 100 mL volumetric flask using distilled water.

Standard Sulfamethoxazole Solution 100 µg.mL^{-1}

Standard solution of SMX was prepared by dissolving accurately weighted 10 mg of pure drug in 1.5 mL of 5 M HCl and further diluted to 100 mL with distilled water.

Preparation of Synthetic Drug Sample

1- 20 mg of the bulk drug was mixed with 5 mg of interfering substance mixture (consisting of 0.01 g of each of glucose, lactose, soluble starch, and vanillin).

2- 12.5 mg of the resulted mixture was dissolved by the same manner as used for the preparation standard drug to obtain 100 $\mu g.mL^{-1}$.

Preparation of Drug Solution in Urine

Solution of drug in urine was prepared by dissolving 10 mg of (SMX) in 1.5 mL of 5 M HCl and complete volume to 100 mL urine in volumetric flask to obtain 100 $\mu g.mL^{-1}$ stock solution.

General Standard Procedures

Univariate Method

Aliquots of the standard solution (100 $\mu g.mL^{-1}$) containing (0.5-50.0) µg of sulfamethoxazole were transferred into a series of 5 mL volumetric flasks. 1.0 mL of 5 % (m/v) PDAB was added to each flask, and then 0.25 mL of 5 % (v/v) sulfuric acid was added. The solutions were shaken thoroughly, and making up to the mark with methanol. After mixing the solution well, the absorbance of yellow colored dye was measured at 450.5 nm against the reagent blank.

Design of Experiment Method

Aliquots of the standard solution (100 $\mu g.mL^{-1}$) containing (0.5-50.0) µg of sulfamethoxazole was transferred into a series of 5 mL volumetric flasks. 1.0 mL of 5.6 % (m/v) PDAB was added to each flask, and then 0.25 mL of 5.7 % (v/v) sulfuric acid was added, the solutions were shaken thoroughly. After 20 min., the solutions were making up to the mark with methanol. After mixing the solution well, the absorbance of yellow colored dye was measured at 450.5 nm against the reagent blank.

Results and Discussion

Absorption Spectra and Reaction Scheme

Primary amines type (RNH_2) or ($ArNH_2$) add to aldehyde and ketone by nucleophilic addition to yield imines (or Schiff 's bases). An imine is a nitrogen analog of an aldehyde or ketone in which the C=O group is replaced by a C=NR group, where R= alkyl, aryl, or H. The investigated method involve the condensation reaction between sulfamethoxazole and p-N,N-dimethyl amino benzaldehyde in acidic medium to yield a yellow colored dye with a maximum absorption at 450.5 nm as shown in Figure 2. The reaction can be represented in Scheme (1).

Optimization of Reaction Variables

Univariate Method

For the development of maximum color intensity of dye, various experimental parameters viz (p-N,N-dimethyl amino benzaldehyde concentration, effect of different acids, sulfuric acid concentration and coupling reaction time) which influence the formation of the colored dye were necessary optimized. The optimization was done by studying one parameter while keeping the others fixed.

Effect of P-N,N-Dimethyl Amino Benzaldehyde Concentration

The studying of p-N,N-dimethyl amino benzaldehyde concentrations were done by taking concentration of (0.25-6.00) % (m/v) and volume of 1.0 mL. The absorbance of the reaction solution is increased as the PDAB concentration increased, and the highest absorption intensity was attained at PDAB concentration of 5 % (m/v). Higher PDAB concentrations up

to 6 % (m/v) had no effect on the absorption values. Figure 3 shows the study of concentration of PDAB.

Effect of Different Acids

When some acids (sulfuric acid, hydrochloric acid, nitric acid and acetic acid) with 2 % v/v were tested for the acidic medium of coupling reaction, it was found that sulfuric acid was the most suitable acidic medium for a maximum absorbance for method and was used in all subsequent experiments (Table 1).

Effect of Sulfuric Acid Concentration

Studies for optimization of sulfuric acid concentration revealed that the optimum amount was 0.25 mL of 5 % (v/v) concentration as shown in Figure 4. (0.5-7.0) % (v/v) solution of sulfuric acid was used in the volume of 0.25 mL was suitable for the color development.

Effect of Coupling Reaction Time

The effect of coupling reaction time on color of chromogen development was studied. The yellow product formed due to the condensation coupling of sulfamethoxazole with p-N,N-dimethyl amino benzaldehyde, attained the maximum color at 0 min. as shown in (Table 2). The color of the chromogen was stable for 1.5 h.

Multivariate Method

Experimental Design and Statistical Analysis

To find the optimal conditions for the estimation of sulfamethoxazole via condensation reaction with p-N,N-dimethyl amino benzaldehyde, a central composite design was used.

The central composite design (CCD) with a quadratic model was employed. Three independent variables namely p-N,N-dimethyl amino benzaldehyde concentration (X_1), sulfuric acid concentration (X_2) and reaction time (X_3) were chosen. Each independent variable had three levels, which were -1, 0 and +1. A total 20 different combinations (including six replicates of center point each signed the coded value 0) were chose in random order according to a CCD configuration for three factors. The coded values of independent variables were found from equations:

$x_1 = (X1-3.125) / 2.875$

$x_2 = (X2-3.250) / 2.750$

$x_3 = (X3-10.000) / 10.000$

and are given in Table 3. Response surface model was applied to study the effect of the three variables, reagent concentration, sulfuric acid concentration and reaction time on the absorbance of SMX-PDAB complex and generate an optimal and robust response surface. A second order polynomial equation was used to express the absorption as a function of independent variables namely, reagent concentration, sulfuric acid concentration and reaction time.

Absorbance = $\beta_0 + \beta_1\times$ (Reg. conc.) + $\beta_2\times$ (H_2SO_4 conc.) + $\beta_3\times$ (reaction time) + $\beta_4\times$ (Reg. conc.$\times$ H_2SO_4 conc.) + $\beta_5\times$ (Reg. conc.$\times$ reaction time) + $\beta_6\times$ (H_2SO_4 conc.$\times$ reaction time) + $\beta_7\times$ (Reg. conc.)^2 + $\beta_8\times$ (H_2SO_4 conc.)^2 + $\beta_9\times$ (reaction time)^2 .

Table 4 shows the study of optimum conditions according to central composite design and the experimental points used according to the design. The coefficients of the response surface equation were determined by **STATISTICA 8.0** software **(StatSoft. Inc, release 2007)**.

Optimum conditions that are developed from central composite design for the determination of sulfamethoxazole via condensation reaction with p-N,N-dimethyl amino benzaldehyde were calculated mathematically and the results are 5.7 % (m/v) for the p-N,N-dimethyl amino benzaldehyde concentration, 5.6 %(v/v) for sulfuric acid concentration and 20 minutes for

reaction time. Figures 5, 6, and 7 give the imagine of response surface model if one of the three variables is remained constant.

Calibration Curves and Analytical Data

Univariate Method

In order to test whether the colored species formed in univariate method adhere to Beer's law, the absorbance at appropriate wavelengths of a set of solutions containing varying amounts of sulfamethoxazole and specified amounts of reagents were recorded against the corresponding reagent blanks.

The Beer's law plots were recorded graphically (Figure 8). A linear correlation was found between absorbance at λmax and concentration ranges (0.1-10.0) $\mu g.mL^{-1}$ of SMX. Sensitivity parameters such as molar absorptivity, correlation coefficient (r), Sandell's sensitivity and detection limit are presented in (Table 5).

Design of Experiment Method

The optical characteristics such as maximum absorption, Beer's law limits, molar absorptivity and Sandell's sensitivity for the proposed method are summarized in (Table 5) and (Figure 9).

Accuracy and Precision

Accuracy of the methods was done by relative error % and precision was evaluated by coefficient of variation (C.V) %. Three different concentration levels of sulfamethoxazole were analyzed in five replicates. Lower values of relative error and coefficient of variation signifies the accuracy and precision of the methods. The results of accuracy, precision for univariate and DOE are listed in (Table 6).

Interference Study

In pharmaceutical analysis, it is important to test the selectivity towards the excipients added to the pharmaceutical preparations. Commonly encountered excipients such as vanillin, glucose, lactose, starch did not interfere in the determination of sulfamethoxazole and did not effect on the reaction between the SMX and PDAB. 5 $\mu g.mL^{-1}$ of SMX was analyzed and design of experiment method was used for analyzing (Table 7).

Application in Synthetic Sample

The quantitative determination of three concentration levels of sulfamethoxazole in its synthetic sample was done by the proposed method. The values of recovery percentage obtained are tabulated in (Table 8) revealed that the common excipients usually present in the dosage form that do not interfere in the proposed procedure .

Application in Spiked Urine

As another application of the proposed method, recovery from human urine samples was carried out on three different concentrations of sulfamethoxazole. The results in (Table 9) show, the percentage recovery values and coefficient of variation values proved that non-interferences from matrices are present in urine.

Application in Spiked Urine by Standard Additions Method (SAM)

For additional application to assay of sulfamethoxazole in spiked urine sample, the standard additions method was applied to eliminate the effect of any species may by present in sample. (Table 10) shows the results of recovery and coefficient of variation (C.V %) for the standard additions method. (Figure 10) shows the plot of assay of sulfamethoxazole in urine by standard additions method.

Conclusions

Condensation reaction of primary amine group with p-N,N-dimethyl amino benzaldehyde in acidic medium was found to be a simple, sensitive, accurate and economic spectrophotometric method for quantitative determination of (SMX) in pure form and synthetic samples. The classical univariate and experimental method have been used for optimizing the different variable affecting the completion of the reaction. The proposed method offers good linearity and precision.

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Table (1): Effect of different acids on absorbance of sulfamethoxazole dye.

Acidic solution (2 % v/v)	Absorbance
Sulfuric acid	0.902
Hydrochloric acid	0.853
Nitric acid	0.881
Acetic acid	0.378

Table (2): Effect of coupling reaction time.

Time (min.)	Absorbance
0	1.136
5	1.097
10	1.094
15	1.082
20	1.072
90	1.072

Table (3): UN coded and coded levels of the independent variables for the determination of sulfamethoxazole.

Drug	Independent variable	Coded unit		
		-1	0	1
SMX	PDAB conc.(%)	0.250	3.125	6.000
	H_2SO_4 conc.(%)	0.500	3.250	6.000
	Reaction time (min.)	0.000	10.000	20.000

Table (4): The central composite design with three independent variables (un coded variables) and their experimental absorption values of SMX-PDAB complex.

Exp. no.	PDAB conc. (% m/v)	H_2SO_4 conc. (% v/v)	Reaction time (min.)	Abs.
1	3.125	6.00	10	1.050
2	6.000	0.50	20	0.582
3	3.125	3.25	20	0.907
4	0.250	6.00	20	0.156
5	3.125	3.25	10	0.927
6	3.125	3.25	10	0.922
7	3.125	3.25	10	0.919
8	6.000	6.00	20	1.132
9	0.250	0.50	0	0.073
10	3.125	3.25	10	0.925
11	3.125	3.25	10	0.932
12	3.125	0.50	10	0.454
13	0.250	6.00	0	0.168
14	3.125	3.25	10	0.927
15	6.000	6.00	10	1.160
16	0.250	0.50	20	0.061
17	0.250	3.25	10	0.193
18	3.125	3.25	0	0.962
19	6.000	0.50	0	0.604
20	6.000	3.25	10	1.105

Table (5): Optical characteristics and statistical data for the determination of sulfamethoxazole by univariate method and DOE.

Parameter	univariate	DOE
λmax (nm)	450.5	450.5
Color	Yellow	Yellow
Linearity range ($\mu g.mL^{-1}$)	0.1-10.0	0.1-10.0
Regression equation	Y=0.2138[SMX. $\mu g.mL^{-1}$] +0.0197	Y=0.2288[SMX. $\mu g.mL^{-1}$] +0.0012
Calibration sensitivity (mL. μg^{-1})	0.2138	0.2288
Correlation coefficient (r)	0.9997	0.9997
Correlation of linearity (r^2)	0.9994	0.9994
Molar absorptivity (ε) (L. $mol^{-1}.cm^{-1}$)	5.4151×10^4	5.7950×10^4
Sandell's sensitivity ($\mu g.cm^{-2}$)	4.6772	4.3706
LOD ($\mu g.mL^{-1}$)	0.081	0.078
LOQ ($\mu g.mL^{-1}$)	0.269	0.257

Table (6): Evaluation of accuracy and precision for the determination of sulfamethoxazole by proposed methods.

	Conc. of SMX (µg.mL^{-1})		Relative error %	C.V %
	Taken	Found*		
For univariate	0.500	0.488	-2.400	0.8930
	5.000	5.040	0.800	0.4870
	8.000	7.966	-0.425	0.3150
For DOE	0.500	0.494	-1.200	1.0280
	5.000	4.959	-0.820	0.6040
	8.000	8.033	0.537	0.2600

*Average of five determinations.

Table (7): Percent recovery for 5 µg.mL^{-1} of sulfamethoxazole in the presence of 500µg.mL^{-1} of excipients.

Excipients	Concentration (µg.mL^{-1})	Sulfamethoxazole conc. taken (5 µg.mL^{-1})	
		Conc. found (µg.mL^{-1})	Recovery %
Vanillin	500	5.086	101.72
Glucose		5.053	101.06
Lactose		4.960	99.20
Starch		5.022	100.44

Table (8): Application of the DOE method to the sulfamethoxazole concentration measurements in synthetic sample.

Sample	Weight* found mg/25mg	Conc. taken µg.mL^{-1}	Conc.* found µg.mL^{-1}	Recovery %	C.V* %
20mg of SMX	19.56	0.500	0.489	97.80	1.5431
	20.35	4.000	4.070	101.75	0.8742
	20.06	8.000	8.025	100.31	0.3260

*Average of three determinations.

Table (9): Application of the DOE method to the sulfamethoxazole concentration measurements in spiked urine.

Sample	Conc. taken (µg.mL^{-1})	Conc.* found (µg.mL^{-1})	Recovery %	C.V* %
SMX in urine	0.500	0.519	103.80	1.6457
	4.000	3.960	99.00	0.7255
	8.000	8.093	101.16	0.4414

*Average of three determinations.

Table (10): Application of the DOE method to the sulfamethoxazole concentration measurements in spiked urine by standard additions method.

Sample	Conc. taken ($\mu g.mL^{-1}$)	Conc.* found ($\mu g.mL^{-1}$)	Recovery %	C.V* %
SMX in urine	100.000	101.966	101.97	1.3320

*Average of three determinations.

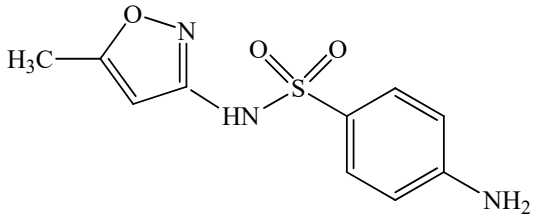


Figure (1): The structure formula of sulfamethoxazole.

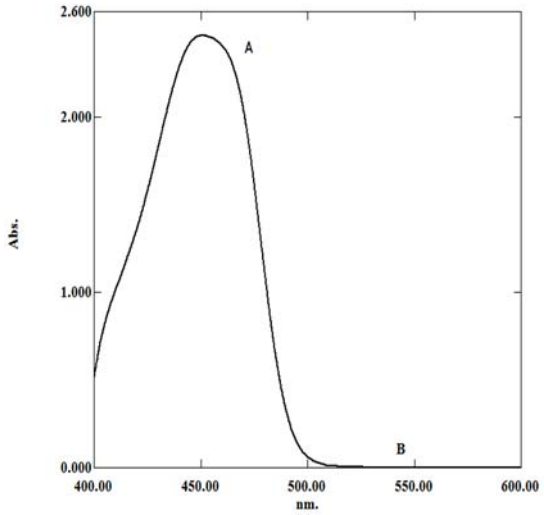


Figure (2): Absorption spectrum of: (A) 5 $\mu g.mL^{-1}$ SMX-PDAB against reagent blank, (B) blank solution against (D.W.) under optimization conditions.

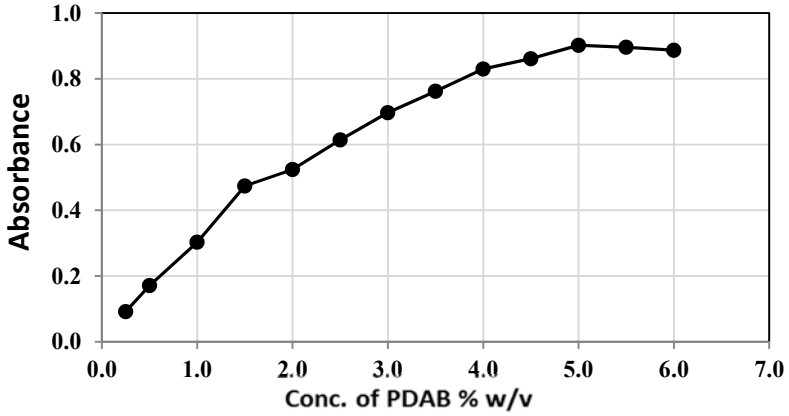


Figure (3): Effect of p-N,N-dimethyl amino benzaldehyde concentration on absorbance of sulfamethoxazole dye.

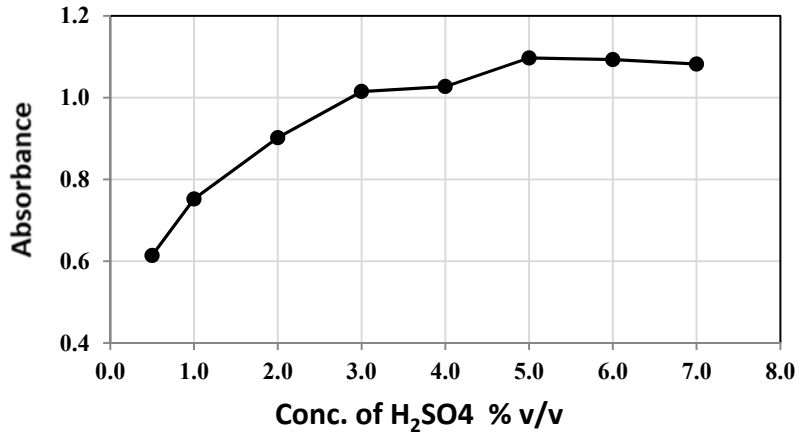


Figure (4): Effect of sulfuric acid concentration on the color development of sulfamethoxazole dye.

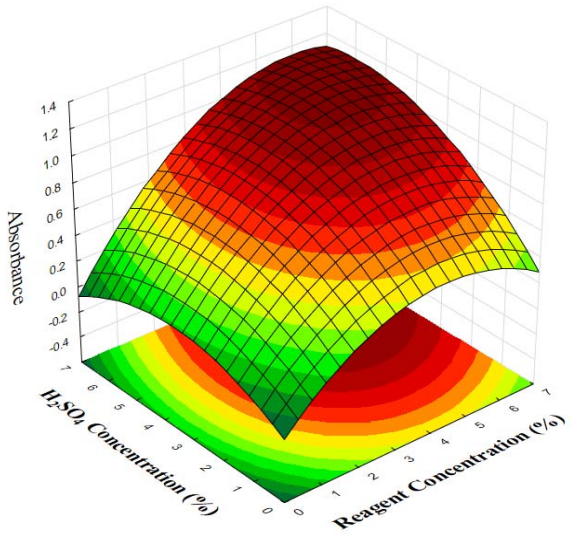


Figure (5): The response surface for the absorbance of SMX-PDAB complex as a function of reagent concentration and H_2SO_4 concentration (at constant optimum value of reaction time, 20 min.).

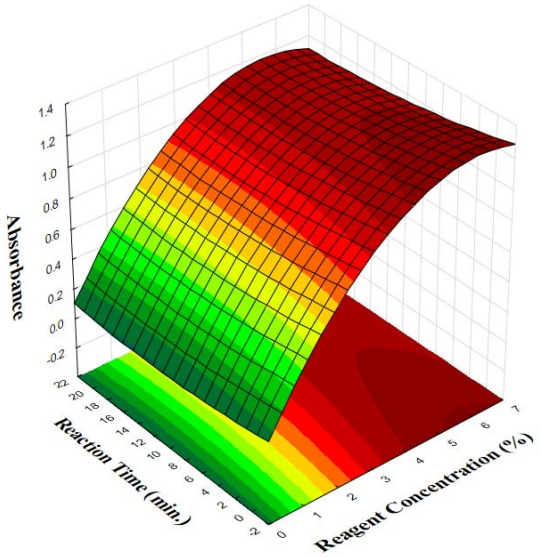


Figure (6): The response surface for the absorbance of SMX-PDAB complex as a function of reagent concentration and reaction time (at constant optimum value of H_2SO_4 concentration, 5.6 % v/v).

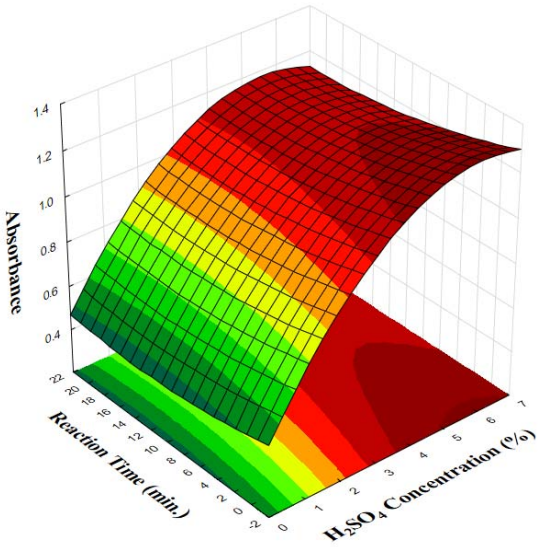


Figure (7): The response surface for the absorbance of SMX-PDAB complex as a function of H_2SO_4 concentration and reaction time (at constant optimum value of reagent concentration, 5.7 % m/v).

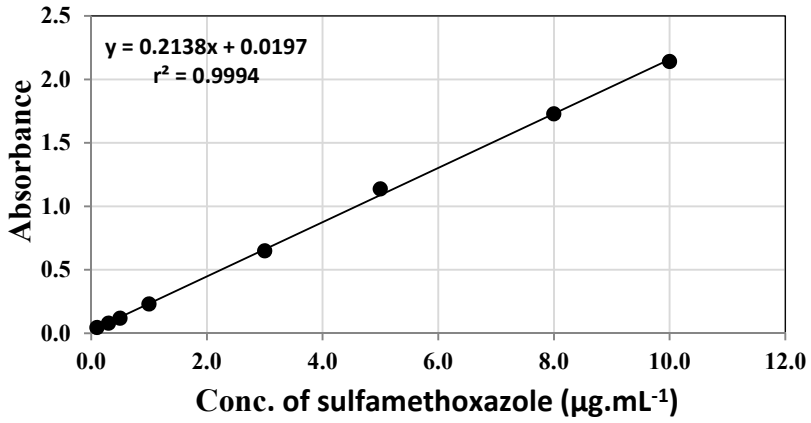


Figure (8): Calibration curve for the determination of sulfamethoxazole by univariate optimal condition.

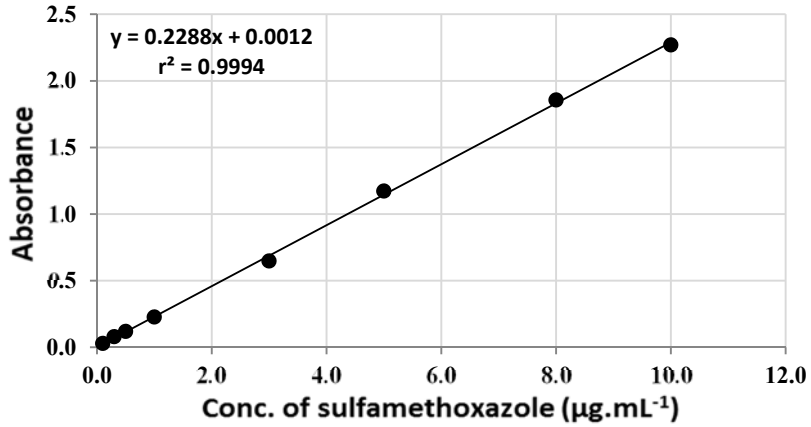


Figure (9): Calibration curve for the determination of sulfamethoxazole by DOE optimal condition.

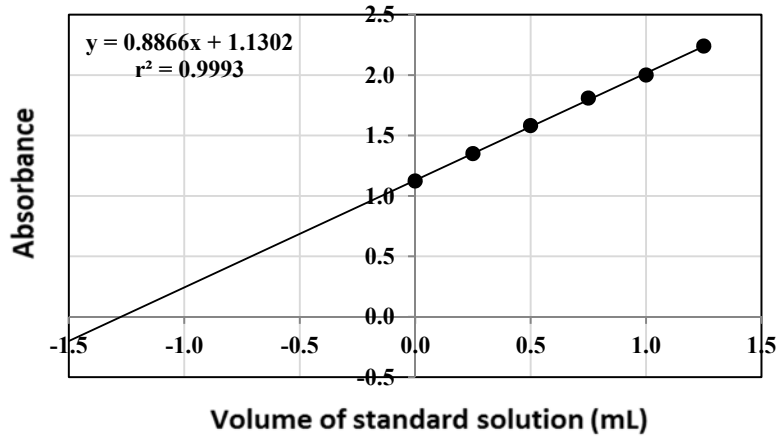
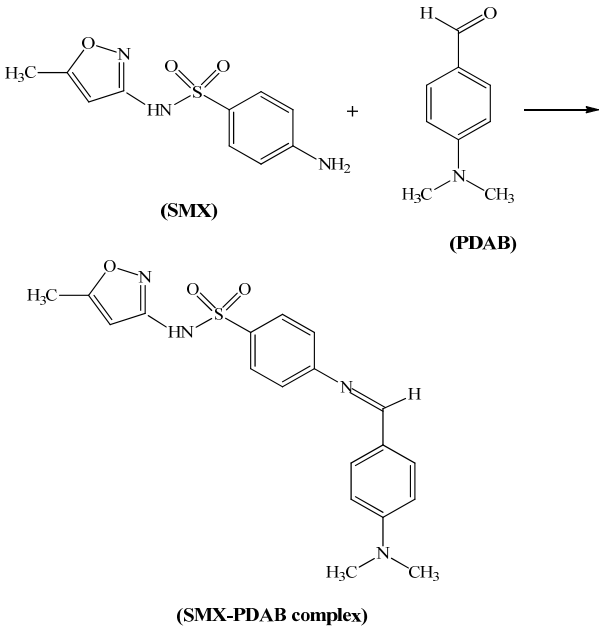


Figure (10): Determination of sulfamethoxazole in spiked urine sample by standard additions method.



Scheme (1): The reaction mechanism for condensation reaction between sulfamethoxazole and p-N,N-dimethyl amino benzaldehyde.

التقدير الطيفي للسلفاميثوكسازول مع بارا داي مثيل أمينو بنزلدهايد كتفاعل تكثيف

سمر أحمد درويش
عبد المحسن عبد الحميد الحيدري
علاء كريم محمد
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الخلاصة

أضيف عقارالسلفاميثوكسازول (SMX) الى بارا داي مثيل أمينو بنزلديهايد بوساطة تفاعل التكثيف في وسط حامضي لتكوين صبغة صفراء اللون التي تظهر أعظم امتصاص (λ_{max}) عند 450.5 نانومتر. قدرتركيزالسلفاميثوكسازول طيفياً. وقد تم تعيين الظروف الفضلى التي تؤثر في التفاعل والعوامل التحليلية الأخرى. فضلا عن الطريقة الكلاسيكية بنمط المتغير الواحد طبقت طريقة تصميم التجربة لتعيين الظروف الفضلى للمتغيرات التي تؤثر في التفاعل اللوني قيد الدراسة.

تم تطبيق قانون بير على مدى من التراكيز يتراوح بين ($0.1\text{-}10\ \mu g.mL^{-1}$) وكانت قيمة معامل الامتصاص المولي مساوية لـ $5.7950\times10^{4}\ L.mol^{-1}.cm^{-1}$ حد الكشف ومعامل ساندل يساوي $0.078\ \mu g.mL^{-1}$ و $4.3706\ \mu g.cm^{-2}$ على التوالي. لقد أمكن تطبيق الطريقة المقترحة بنجاح لتقدير السلفاميثوكسازول في نماذج محضرة وكذلك فى الادرار.

الكلمات المفتاحية: التقدير الطيفي, سلفاميثوكسازول, تفاعل التكثيف, بارا داي مثيل أمينو بنزلديهايد.