Spectrophotometric assay for determination of sulfamethoxazole in pharmaceutical preparations via diazotization coupling reaction with catechol

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Keywords: Sulfamethoxazole, Spectrophotometric, Diazotization-Coupling reaction, Catechol.

Received (October), **Accepted** (December)

ABSTRACT
A simple, sensitive and accurate spectrophotometric method for the determination of sulfamethoxazole is developed. The method is based on diazotization reaction of sulfamethoxazole and coupling with catechol in alkaline medium to form an orange-reddish water soluble azo dye, that has a maximum absorption at 495nm. A graph of absorbance versus concentration shows that Beer law is obeyed in the range of 0.5-5ppm of sulfamethoxazole in a final volume 25ml with a molar absorptivity of 3.03x104l.mol-1.cm-1, a sandal sensitivity of 8.32x10-6µg.cm-2, a relative error of 0.18-0.78% and a relative standard deviation of 0.32-0.65%, depending on the concentration. The optimum conditions for full color development were optimized and the proposed method was applied satisfactorily to pharmaceutical preparations containing sulfamethoxazole. Statistical treatments of the obtained results was carried out using F-test and t-test, the results shows there are no significant differences between the proposed method and Bratton-Marshall method, therefore the developed method can be used as an alternative method for the determination of sulfamethoxazole in different pharmaceutical preparations.

التقدير الطيفي للسلفاميثوكسوزول في المستحضرات الصيدلانية من خلال تفاعل الازوتة والازدواج مع الكيتيكول

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الكلمات المفتاحية: السيلفاميثوكسيزول، التقدير الطيفي، تفاعل الازوتة والازدواج، الكيتيكول.

الخلاصة
يتضمن البحث تطوير طريقة طيفية بسيطة، حساسة ودقيقة للتقدير الكمي للسلفاميثوكسيزول. تعتمد الطريقة على تفاعل الازوتة للدواء ومن ثم الازدواج مع الكيتيكول في محيط قاعدي لتكوين صبغة الازو ذات اللون البرتقالي المحمر والتي تمتلك اعظم امتصاص عند الطول الموجي 495 نانوميتر. ويشير الرسم البياني للامتصاصية مقابل التركيز ان حدود قانون بير تنطبق ضمن مدى التركيز 0.5-5 جزء لكل جزء بالمليون في حجم نهائي 25مل وكانت قيمة الامتصاصية المولارية مساوية $3.03x10^4$ لتر.مول$^{-1}$.سم$^{-1}$ وقيمة حساسية ساندل $8.32x10^{-6}$ مايكروغرام.سم$^{-2}$ مع خطا نسبي مقدارة 0.18-0.78 % وانحراف قياسي نسبي 0.32-0.65% اعتمادا على مستوى التركيز المراد تقديرة. تم دراسة الظروف المثلى للتفاعل وتطبيق الطريقة المقترحة على المستحضرات الصيدلانية التي تحتوي السليفاميثوكسيزول. كما تم اجراء المعالجات الاحصائية للنتائج التي تم الحصول عليها باستخدام اختبارات F و t وبينت النتائج انة لايوجد فروقات جوهرية بين الطريقة المقترحة وطريقة براتون-مارشل المعتمدة وعلى هذا الاساس يمكن استعمال الطريقة المطورة في تقدير السليفاميثوكسيزول في مختلف المستحضرات الصيدلانية.

1. INTRODUCTION

Sulphonamides are an important class of antibacterial drugs used in medicine and veterinary practice. Sulpha drugs are widely used in the treatment of infections They are bacteriostatc against a wide range of gram-negative and gram-positive organisms [1-3].
Sulfamethoxazole (SMZ), 4-amino-(5-methyl-3-isoazolyl) benzensulfonamide as shown in figure(1), is a white crystalline powder.

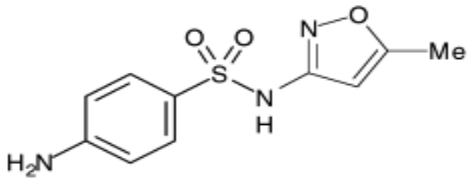


Figure 1: The chemical structure of sulfamethoxazole.

It's has been principally employed in the treatment of respiratory and urinary-tract infections[4]. Numerous methods have been developed for the determination of sulphamethoxazole in pharmaceuticals preparations and biological fluids, These methods have been summarized as spectrophotometric[5-26], Chromatographic[27-31], Electrchemical sensors[32-34], Flow injection[35-37], Voltameteric[38] and NMR spectrophotometric method[39].
The present work describes the development of simple, and rapid spctrophotometric method for the quantitative determination of suflamethoxazole (SMX) in pure and different pharmaceutical preparations based on diazotized sulfa drug is coupled with catechol in aqueous medium to give an orange-reddish product which have a maximum absorption at λ_{max}(495nm). The obtained results indicated that the developed method can be applied successfully for determination of mentioned drug.

2. MATERIALS AND METHODS

Apparatus:

-The spectral and absorbance measurements were performed on a Shimadzu UV- Vis 1800(Japan) digital double beam using 1cm quartz cells.
- Four digital balance type Denever instrument (Germany).
- PH meter Hanna microprocessor.
- Labtech digital water bath.
- Ice bath type Charisma.

Reagents:

All chemicals used were of analytical reagents grade.

-Sulfamethoxazole(SMX) stock standard solution(100ppm) was prepared by dissolving 0.01gm into 5ml ethanol and diluted with distilled water in 100ml volumetric flask.

-Catechol (0.1gm%) was prepared by dissolving 0.1gm in 100ml ethanol.

-Sodium nitrite(5x10-3M) was prepared by dissolving 0.0690gm in 200ml distilled water. Then 3.95x10-3M was prepared by diluting 19.75ml from 5x10-3M with distilled water in 250ml volumetric flask.

-Sodium hydroxide solution(4M) was prepared by dissolving 16gm in 100ml distilled water. Then standardization was carried out with standard hydrochloric acid.

-Hydrochloric acid(1M) was prepared by diluting 43ml of concentrated hydrochloric acid in 500ml distilled water, then standardized with sodium carbonate.

Recommended Procedure:

Into a series of 25ml calibrated flask, transfer increasing volumes from 100ppm of sulfamethoxazole solutions to cover the range of the calibration curve(0.5-5ppm) in a final volume of 25ml. Add 0.5ml of 1M hydrochloric acid and 1ml of $3.95x10^{-3}$M of sodium nitrite then shake well. Followed by 0.5ml of 0.1% catechol and 0.5ml of 4M sodium hydroxide under swirling, dilute the mixture to the mark with distilled water and allow the reaction components to stand for 10min at room temperature. Measure the absorbance at 495nm against reagent blank prepared in the same way but containing no SMX.

3. RESULTS AND DISSCUSION

Preliminary Studies:

Throughout the preliminary study on the diazotization reaction of SMX with sodium nitrite in acidic medium; the formed diazotization product is then coupled with different chromogenic reagents(catechol, Hydroquinone, Toludine p-sulphanilic acid) in sodium hydroxide medium, an orange-reddish color azo dye was obtained when only catechol used as chromogenic reagent. Therefore, based on this observations catechol was used in all the subsequent experiments.

Absorption Spectra:

When a diluted aqueous solution of SMX was diazotized with hydrochloric acid and sodium nitrite, followed by coupling with catechol in sodium hydroxide medium, an intense orange-reddish azo product was formed which became stable after 5min. The azo color product has a maximum absorption at 495nm, in contrast to the reagent blank over the range of 350-750nm. Figure(2) shows the spectra of the orange-reddish color product and the reagent blank. Therefore, 495nm was used in all subsequent experiments.

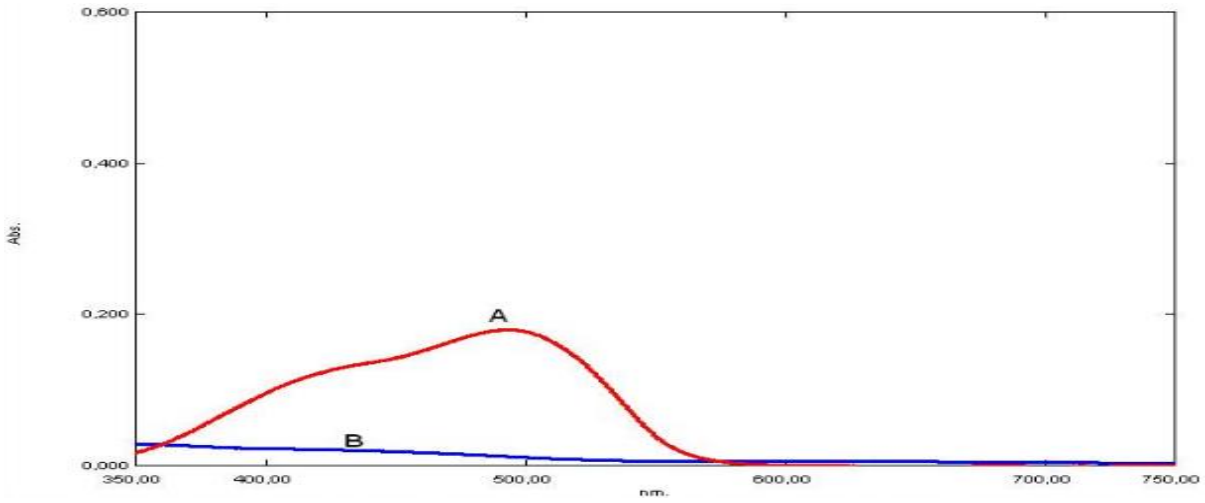


Figure 2: Absorption spectra of A (2ppm) of SMX treated as described under recommended Procedure and measured against reagent blank and B the reagent blank measured Against distilled water.

Optimization of the reaction conditions:

The effect of various parameters on the absorption intensity of the azo color product were optimized. For the optimization of conditions and in all subsequent experiments, 2ppm solution of SMX(100ppm stock solution) were used and the final volume was 25ml. The effect of different volumes (0.1-2.2ml) of HCl, (0.1-2.5ml) of $3.95x10^{-4}$M $NaNO_2$, (0.1-2.5ml) of 0.1% catechol and (0.1-2ml) of 4M NaOH were studied on the maximum absorbance of the orange-reddish azo product. Figure(3A,B) shows that 0.5ml of 1M HCl and 1ml of $3.95x10^{-4}$M $NaNO_2$, While figure(3C,D) indicated that 0.5ml of 0.1% catechol and 0.5ml of 4M NaOH were enough in order to obtain a maximum absorbance.

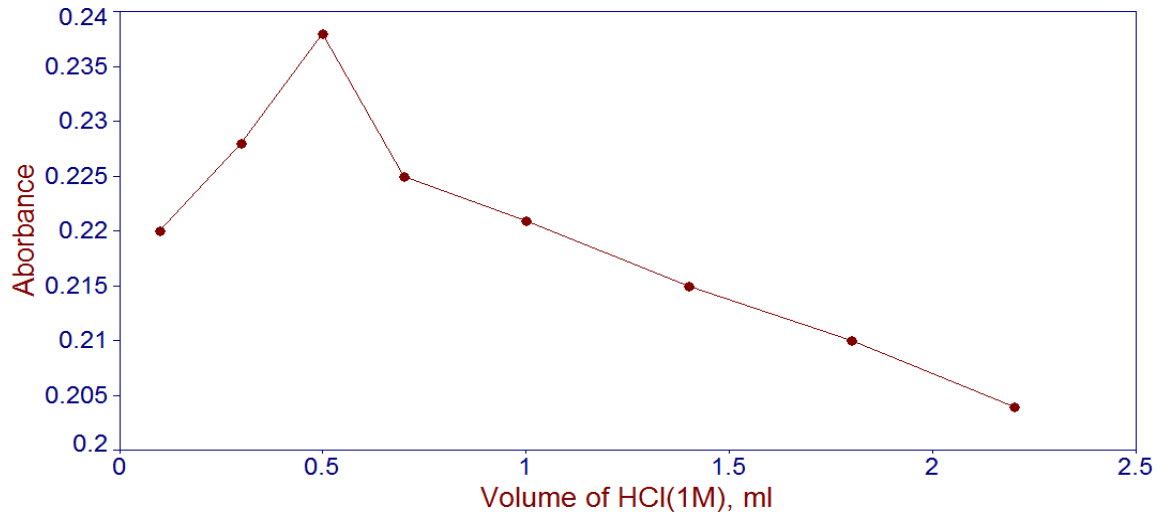


Figure 3A: Effect of various volumes of 1M of HCl on the absorbance of colored product.

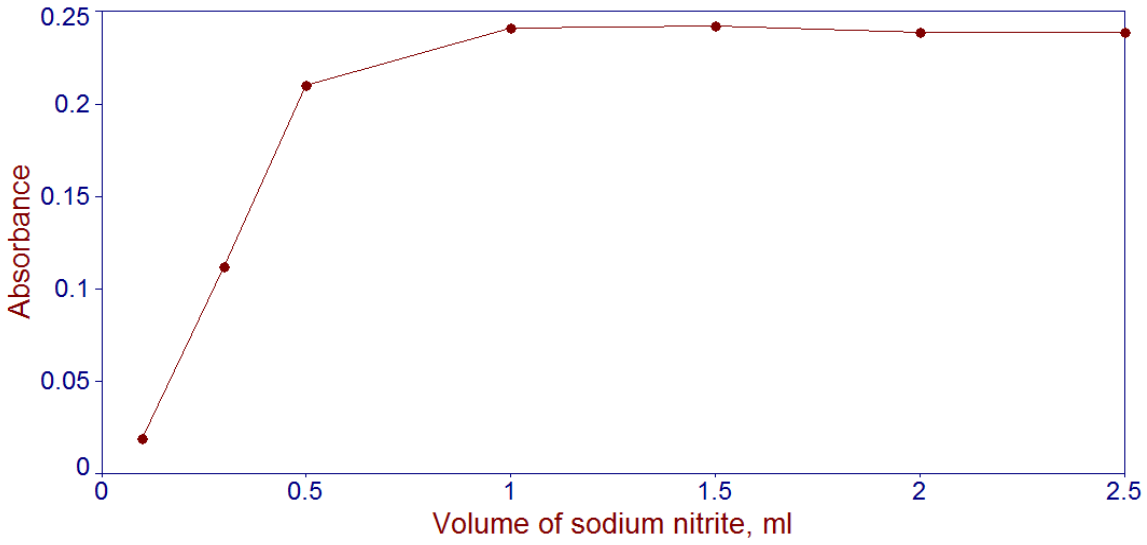


Figure 3B: Effect of different volumes of 3.95x10-4M of NaNO2 on the absorbance of azo colored product.

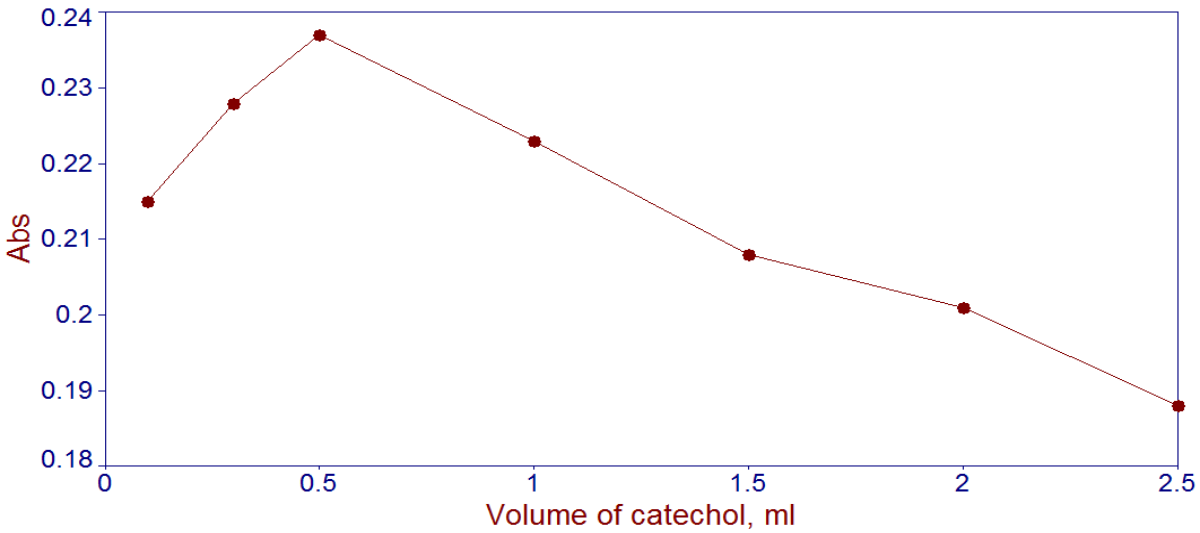
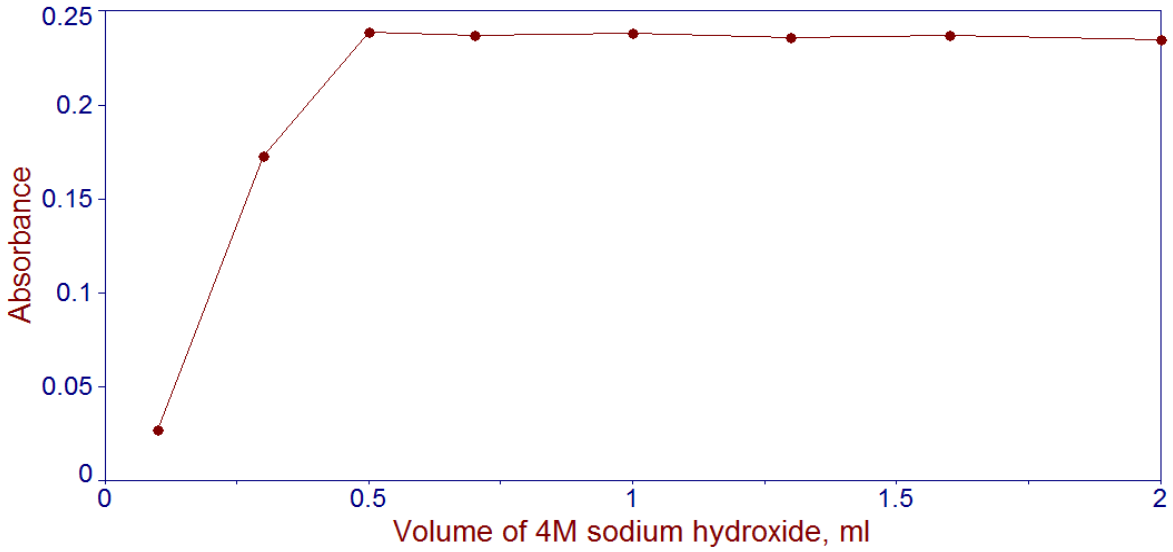


Figure 3C: Effect of variable volumes of 0.1% catechol on the absorbance of orange-reddish product.



Figur 3D: Effect of volumes of 4M NaOH on the absorbance of azo dye product.

The effect of temperature on the maximum absorbance of azo color product was also studied. The obtained results as shown in table(1) indicated that the color reaction should be carried out at room temperature($25C^0$).

Table 1: Effect of various temperature on the absorbance of azo color product.

Temperature(C^0)	Absorbance
10	0.235
25	0.237
50	0.206

The colored azo dye product developed immediately after mixing and reach maximum absorbance about 5min at room temperature. The color was stable for a period of 2h, therefore 10min was selected as an optimum in general procedure.

Construction of calibration curve:

For the proposed spectrophotometric method, employing the optimum reaction conditions described in previous section; a series of standard solutions were analyzed in triplicate to investigate the linearity which shows that Beers law is obeyed over the concentrations range of 0.5-5ppm of SMX as shown in figure(4), with correlation coefficient of 0.9998 and intercept of $-4.72x10^{-5}$.
The molar absorptivity(ε) of the azo color product was found to be $3.03x10^{4}$ l.mol^{-1}.cm^{-1} and sandell sensitivity(S) was $8.32x10^{-6}$µg.cm^{-2}.

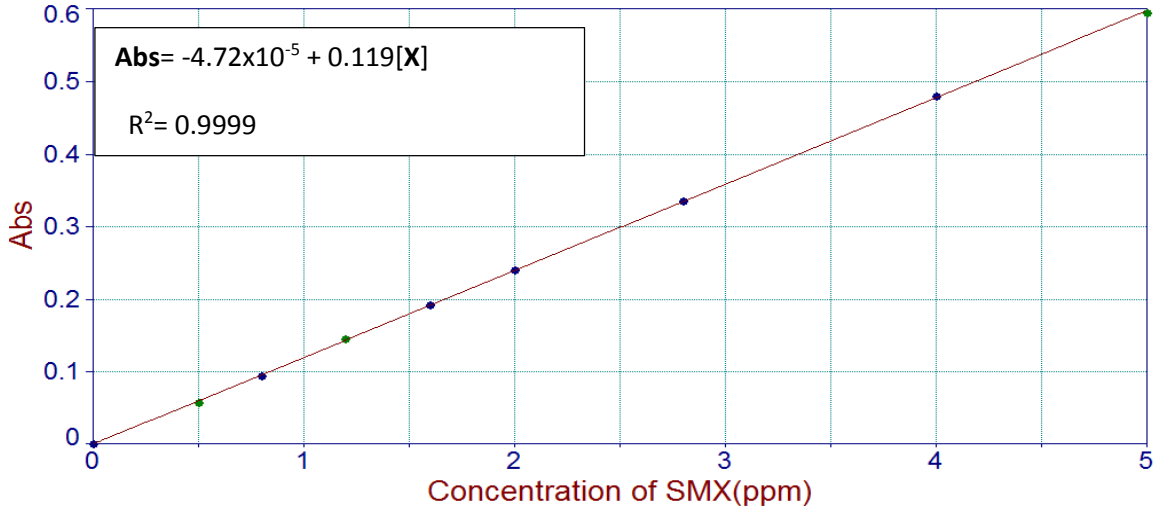


Figure 4: Calibration curve of SMX.

Statistical parameters of the calibration curve at 95% confidence limit were shown in table (2).

Table 2: Statistical values of calibration curve of SMX using proposed method.

Parameters	Value
Linearity range(ppm)	0.5-5
r	0.9998
r^2	0.9999
ε, l.mol^{-1}.cm^{-1}	$3.03x10^{4}$
S, µg.cm^{-2}	$8.32x10^{-6}$
a, ml.µg^{-1}	$-4.72x10^{-5}$
b	0.119
S_a	0.00109
S_b	0.00043
T_{cal}	264.58

S_a: Standard deviation of intercept.
S_b: Standard deviation of slope.
T_{cal}: Calculated t-value.

Accuracy and Precision of the proposed spectrophotometric method:

The accuracy and precision of the proposed method were determined by analyzing five replicated of SMX for two different concentrations. The obtained results were shown in table(3), indicated the high accuracy and precision of the proposed method.

Table 3: Accuracy and precision of the proposed method.

Conc of SMX (PPM)		%E	%Rec	%RSD
Present	Found			
1.6	1.603	0.187	100.18	0.650
2.8	2.822	0.788	100.78	0.326

*Average of five measurements.

Suggested structure of Azo product:

The stoichiometric of the azo product was studied using the mole ratio and continuous variation methods. In mole ratio method an increased volumes(0.1-1.2ml)of $3.94x10^{-4}$M of catechol were added to 0.6ml of $3.94x10^{-4}$M of SMX followed the recommended procedure. The obtained results is shown in figure(5).

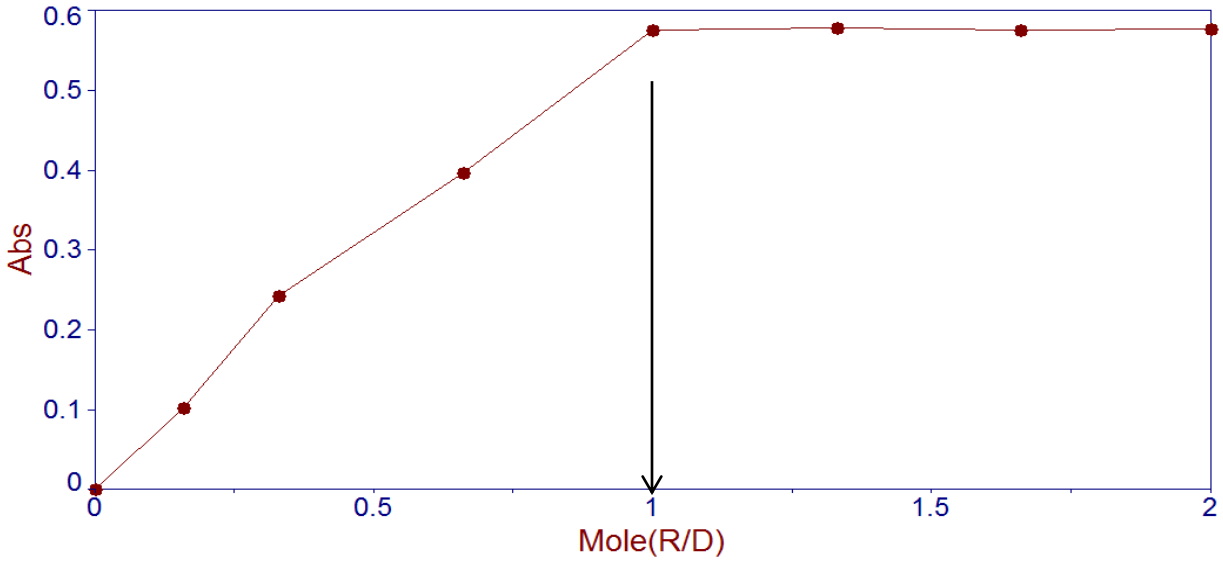


Figure 5: Mole ratio of reagent to sample.

While in continuous variation method(Jobs method), volumes

of (0-2ml) of $3.94x10^{-4}$M of SMX (Vd) were added under recommended procedure to the complementary volumes of $3.94x10^{-4}$M of catechol (Vr) to give a total volume of 2ml(Vd+Vr). The obtained results were plotted as shown in figure (6). Based on the results obtained from both methods, 1:1 Azo dye was formed between SMX and catechol.

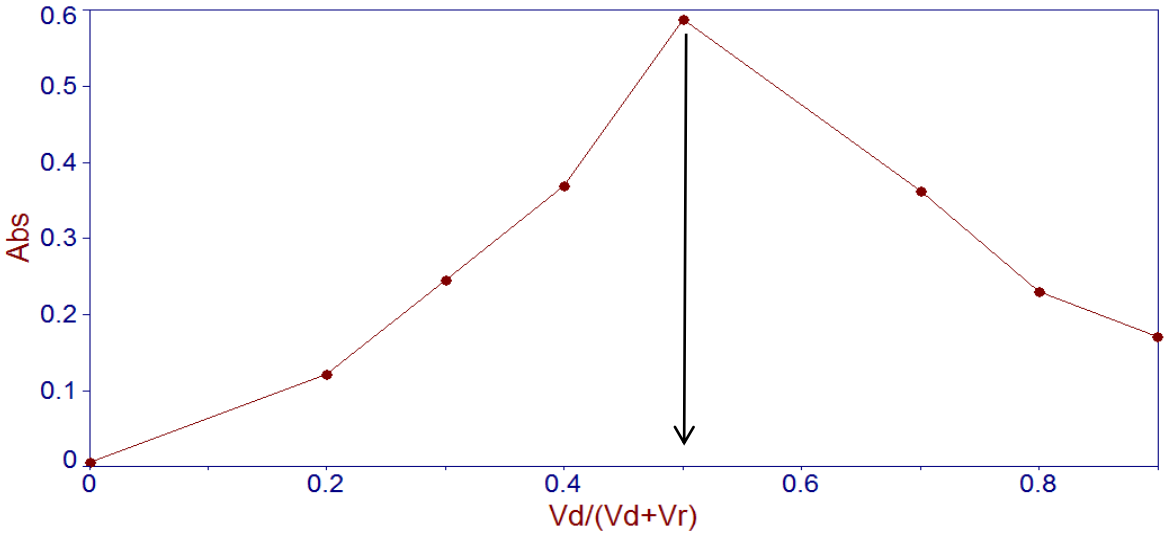
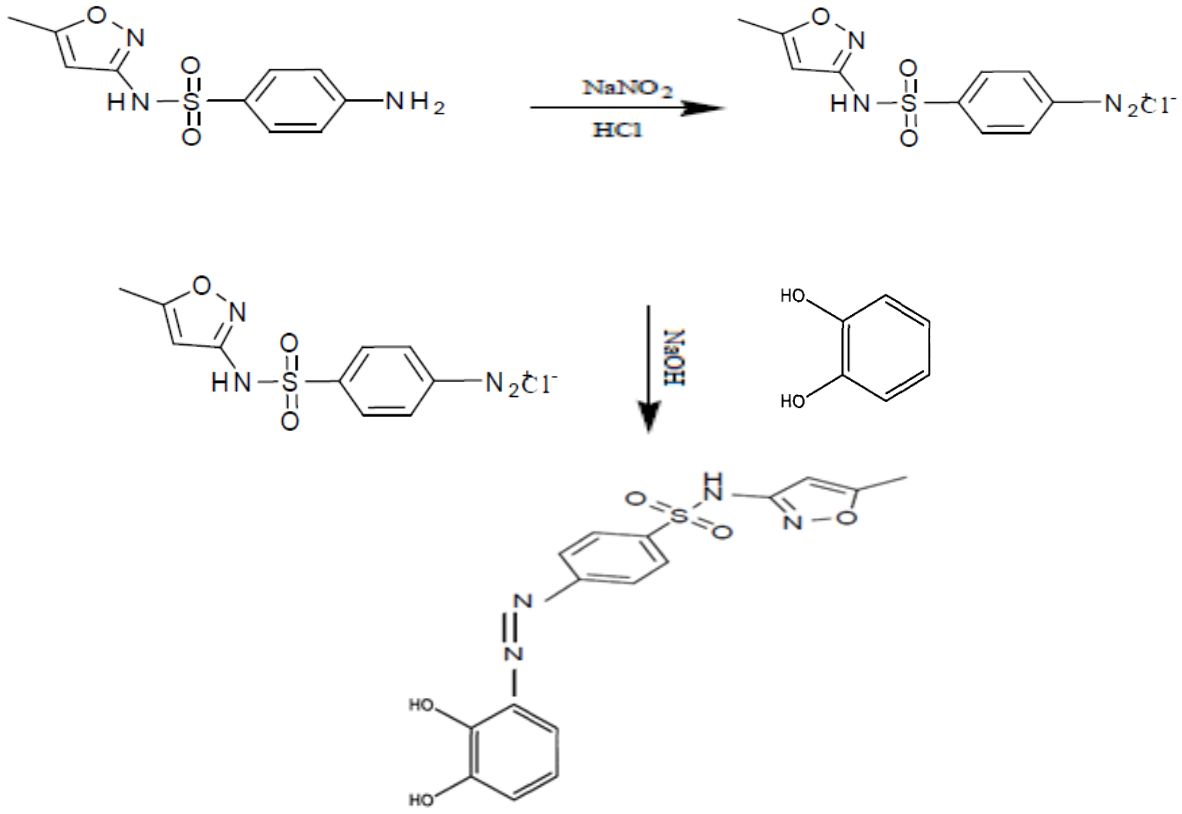


Figure 6: Continuous variation method(Jobs method) for SMX.

And due to the diazotization amino group of SMX, it can readily coupled with catechol according to the following scheme(Figure(7)).



Azo color product

Figure 7: Suggested mechanism between sulfamethoxazol and catechol.

Limit of detection (LOD):

The limit of detection of SMX using proposed spectrophotometric method was calculated using two methods, first was based on gradual dilution of minimum concentration in standard curve(0.5ppm), while the second method is based on the value of slope. The obtained results are tabulated in table(4).

Table 4: Limit of detection(LOD) of SMX using proposed method.

Based on gradual dilution of minimum concentration	Based on the value of slope $X=3S_B$ + Slope
0.15ppm	0.05ppm

S_B: Standard deviation of blank solution.

Analysis of commercial dosage forms:

The proposed spectrophotometric method was used for the analysis of sulfamethoxazol (SMX) in different pharmaceutical preparations and was compared with the official method. Teen tablets from each dosage form were accurately weighed and crushed to fine powder. An amount equivalent to 10mg of pure sulfamethoxazol from each kind of pharmaceutical preparations was dissolved in 5ml of ethanol and transferred into a 100ml calibrated flask and completed to the mark with distilled water. The flasks with it is contents were shaked well and filtered. 0.75ml from each filtrate was taken to the measurements as described under general procedure. The obtained results were tabulated in table (5), which confirms the applicability of the proposed method.

Table 5: Application of the proposed method for determination of SMX in pharmaceutical preparations.

SMX Sample	SMX(ppm)		%R.S.D*	%Rec*	%Er*
	Taken	Found			
TMS forte(800mg) Germany	3	2.96	0.119	98.78	-1.23
Septrin(800mg) Germany	3	3.02	0.277	100.85	0.82
Metheprim(400mg) Iraq	3	2.92	0.44	97.38	-2.70
Lagatrim(400mg) Switzerland	3	2.94	0.45	98.13	-1.90

***Average of three determinations.

The obtained results from the proposed spectrophotometric method were compared with Bratton – Marshall method [40] as shown in table(6) using F-test and t-test at 95% confidence level. The calculated values of t=0.527 and $F_{(4,4)}$=1.795 for the proposed method were less than tabulated t=2.31 and $F_{(4,4)}$=6.39 , showed that there are no significant differences between the proposed spectrophotometric method and Bratton-Marshall method.

Table 6: Comparison of the proposed method with Bratton-Marshall method for determination of SMX in pharmaceutical preparations.

Pharmaceutical sample	%Recovery*	
	Proposed method	Bratton-Marshall method
Pure SMX	99.25	100.03
TMS forte	98.78	101.13
Septrin	100.85	98.68
Metheprim	97.38	98.87
Lagatrim	98.13	102.09

***Average of three determinations.

CONCLUSION

The proposed spectrophotometric method was found to be simple, rapid, selective and more sensitive than most of the spectrophotometric methods available in literature. It is also accurate and precise enough to be successfully adopted as an alternative method and estimation of drugs containing sulfamethaxzol(SMX) to assure a high standard of quality control. The Azo color product was stable for a sufficient interval of time making the adopting method useful in practice.

ACKNOWLEDGEMENTS

Author is thankful to all whom sharing their knowledge, and all who participates in this work.

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