RESEARCH ARTICLE

Spectrophotometric Determination of Trimethoprim in Pharmaceutical Formulation via Schiff base Reaction using Prepared Organic Reagents

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ABSTRACT

This study dealt with the preparation of new organic reagents and used them in the spectrophotometric determination of trimethoprim (TMP), where a simple, sensitive, and rapid spectrophotometric method was developed for the determination of trimethoprim in aqueous solutions. The method is based on Schiff's base formation, which is achieved by coupling of the drug with a prepared organic reagent in an acidic medium to yield a color product exhibiting maximum absorbance at 573 nm. Beer's law is obeyed in the concentration range 2–24 µg/mL, with a molar absorptivity 1.437 x 10^4 $L.mol^{-1}.cm^{-1}$ and Sandell's index of 0.0202 $\mu g.cm^{-2}$. The average recovery is 100.82, and the relative standard deviation is less than 5%, with a detection limit of 0.0497 µg/mL. This method has been applied successfully to the determination of trimethoprim in pharmaceutical preparations (tablets), and included the use of trimethoprim as the basic nucleus to prepare a number of new derivatives (Schiff's base) by reaction with these organic reagents. Also contain characterization of the prepared compounds by multiple spectral methods, including the fourier transform infrared spectroscopy (FTIR), the ^{1}H-NMR nuclear spectrum, and the carbon magnetic resonance spectrum (^{13}C-NMR).

Keyword: Organic reagents, Spectrophotometric Determination, Schiff base, Trimethoprim.

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INTRODUCTION

Schiff bases are one of the well-known and important organic chemical compounds in organic synthesis which contains the Azomethine Group (C = N) as a functional group. It was prepared from a simple condensing reaction Between aldehydes or ketones with primary amines.[1] Likewise, Schiff bases are one of the most important ligands, which are many coordination complexes by their association with metals. They are colored complexes used to determine metals in selective and sensitive ways.[2]

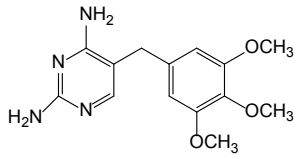
The reaction of the formation of Schiff bases was used to determine several pharmaceutical compounds in different analytical methods.[3-5] A number of Schiff bases have also been prepared from pharmaceutical compounds (aromatic amines) such as sulfamethoxazole,[6,7] trimethoprim,[8] cefalexin, amoxicillin,[7] procaine,[9] and ampicillin,[10] by reacting with various aldehydes using ethanol or methanol as a solvent.

Trimethoprim (TMP) is an organic compound first described by (Roth and CO-workers).[11] It is in the form of a white or yellowish-white powder, odorless and very slowly dissolves in water and is also slightly soluble in alcohol with a bitter taste.[12-14]

Trimethoprim has several scientific designations such as: 5-[(3,4,5-trimethoxyphenyl)methyl]pyrimidine-2,4-diamine (2,4-diamino-5-(3,4,5- trimethoxybenzyl) pyrimidine).[15,16]

And its structural formula is shown below :

Its molecular formula is $C_{14}H_{18}N_4O_3$ and molecular weight is 290.32 g/mol[12,13] and fused at a temperature of 199–203°C,[14] which is highly effective against a wide range of bacterial organisms such as, gram-positive and gram-negative, and has a half-life of about 8 to 11 hours. As for its pharmaceutical preparations, it is in the form of tablets. And a syrup.[12,16]



Scheme 1: Structure of Trimethoprim

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Sulfamethoxazole and TMP may be used in drugs usually together to form co-trimoxazole[17] because they together affect the prevention of folic acid synthesis and thus result in stopping the synthesis of nucleic acids and proteins needed for germ cells, as the mixture slows the growth of bacterial strains and also because Both have the same half-life, and they also inhibit two steps in the metabolic pathway. Sulfonamide inhibits the biosynthesis of dihydropteroate acid while tri trimethoprim inhibits the conversion of dihydrofolic acid to tetrahydrofolic acid.[18.]

One of the reactions that occur for TMP is the condensation of amine groups present in it with the carbonyl groups in the aldehydes, or ketones to form Schiff bases.[19,20]

Trimethoprim has been determined by various analytical methods, including the spectral method.[21-26] high-performance liquid chromatography (HPLC) method,[27] liquid chromatography method,[28] and the electrical method.[29,30]

PRACTICAL PART

Apparatus

The following instruments were used for the measurements :

- Fourier-transform infrared (FT-IR) Spectrophotometer NICOLT 100
- NMR Spectrophotometer : Bruker Analyische Messtechnik GmbH (500 MHz)
- Vis spectrophotometer 722 and UV-Vis double-beam spectrophotometer – PG – 92.
- Electrothermal Melting Point Apparatus Stuart –SMP11.
- HANNA pH 211, Microprocessor pH meter.

Reagents and Chemicals Used

All the analytical chemicals and reagents used were of high purity, and the following solutions were used:

- *Standard trimethoprim solution 500 µg/mL:* This solution is prepared by dissolving 0.5000 g of pure trimethoprim (supplied by the Private Middle East Pharmaceutical Industry Company, Baghdad, Iraq) in 10 mL of ethyl alcohol, then complete the volume with distilled water using a 100 mL volumetric flask. From this solution, the necessary dilute solutions were prepared.
- *Hydrochloric acid solution of 2 molars:* This solution was prepared by diluting 16.9 mL of concentrated hydrochloric acid solution (11.8 molars) in a 100 mL volumetric flask, and the volume was completed with distilled water up to the mark.
- *Solutions of prepared organic reagents (2%) w/v:* These solutions were prepared by dissolving 2 g of each of the prepared organic reagents in 30 mL of methyl alcohol, then complete the volume to 100 mL using distilled water.
- *Tablet solutions 500 µg/mL of trimethoprim (200 mg) and (100 mg):* Each trimethoprim tablet (supplied by Actavis, Barnstapie, EX32 8NS, UK) contains 200 mg of TMP, and TMP tablet supplied by Bristol Laboratories Ltd., Berkhamsted, Herts, HP4 1EG, UK contains 100 mg of the same drug) Grain solutions weighing 10 tablets are brought together and then crushed. The tablets are crushed well and an equivalent weight ratio of one tablet is taken (0.2537 g) and (0.1546 g) of TMP (200 mg and 100 mg, respectively); weights were dissolved separately by the same method in which the standard solution was dissolved. The solutions were filtered and the residue was washed several times. The volume was supplemented in a volumetric flask 100 mL, then 25 mL and 50 mL are taken from the filtrate, respectively, and complete the volume to 100 mL with distilled water using a volumetric flask to obtain solutions at a concentration of 500 µg/mL. From these solutions, prepared solutions at a concentration of 100 µg/mL by taking 20 mL of each solution and completing the volume to the mark in a volume bottle of 100 mL.

Preparation of Organic Reagents (AZO compounds)

The compounds (1,2) were prepared by dissolving 0.01 mole of P-amino acetophenone in a dilute hydrochloric acid solution (1 mL HCl: 2 mL of distilled water) in a suitable baker and cooled to 0°C with its contents constantly stirred, then added 5 mL of sodium nitrite solution, as well, and the addition is in small and slow batches while maintaining continuous stirring and the temperature at (0–5)°C to obtain diazonium salt. Add the solution of the prepared diazonium salt in batches slowly and continuously stir to another solution which was cooled to 0°C, and prepared from dissolving 0.01 mol of each of 1-naphthol and pyrocatechol each separately in (10 mL, 1-molar) of sodium hydroxide while maintaining the heat at the range 0–5°C, until the solution becomes basic by examining it with pH paper. Then, stir the mixture using an ice bath for 30 minutes, filter the precipitate, wash with distilled water several times to obtain the neutral medium, and recrystallize it with ethanol.

Characterization of Prepared Organic Reagents

The reaction was confirmed by the change in the physical properties such as melting point and color, and the prepared compounds were charactered by infrared spectra as shown below:

- **(Z)-1-(4-((1-hydroxynaphthalen-2-yl)diazenyl)phenyl) ethan-1-one (Comp. 1)**
  Molecular Formula $C_{18}H_{14}N_2O_2$, Black crystals, Yield 78 %, m.p. (197-200) C°, IR (KBr) : ύ = 3404 cm^{-1} (phenolic group OH), 1674 cm^{-1} (C = O), 1404 cm^{-1} (azo group -N = N-) and 1534-1597 cm^{-1} (C = C aromatic).
- **(E)-1-(4-((2,3-dihydroxyphenyl)diazenyl)phenyl)ethan-1-one (Comp. 2)**
  Molecular Formula $C_{14}H_{12}N_2O_3$, Brown crystals, Yield 82 %, m.p. (151-154) C°, IR (KBr) : ύ = 3378 cm^{-1} (phenolic group OH), 1677 cm^{-1} (C = O), 1425 cm^{-1} (azo group -N = N-) and 1518-1600 cm^{-1} (C = C aromatic)**.**

RESULTS AND DISCUSSION

200 µg (2 mL of solution at a concentration of 100 µg/mL) of TMP at a final volume of 25 mL was used for subsequent experiences, and absorbances were measured against the blank solution.

The General Principle of the Method

When adding the solution of the organic reagent to the trimethoprim in an acidic medium, it immediately produces a colored product (Schiff base) because of the coupling of the drug with the organic reagent.

Preliminary Study

A total of 3 mL of organic reagent solutions with a concentration of 2% (w/v) are added to 2 mL of TMP with a concentration of 100 µg/mL in a 25 mL volumetric flask, as it forms a color product once additions are completed, and the volume is completed up to the mark limit. Then, the absorption of the colored product was measured against its blank solution, and it was found that the highest absorption of the colored solution was at the wavelength of 573 nm, while the blank solution showed a very small absorption in this region.

Table 1: Choosing the best coupling detector

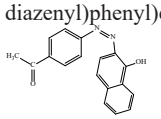
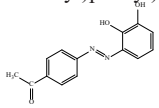
Reagent symbol	*Reagent 2%*	*λ_{max} (nm)*	*Abs.*
R_1	(Z)-1-(4-((1-hydroxynaphthalen-2-yl) diazenyl)phenyl)ethan-1-one 	409	0.197
R_2	(E)-1-(4-((2,3-dihydroxyphenyl) diazenyl)phenyl)ethan-1-one 	579	0.604

Table 2: Studying the effect of the amount of acid on absorption

Amount of 2M HCl solution, (mL)	*Absorbance*	*pH*
0.5	0.463	2.4
1.0	0.528	2.1
1.5	0.567	1.9
2.0	0.602	1.6
2.5	0.581	1.4
3.0	0.570	1.2

Table 3: Effect of coupling reagent amount

Amount of 2 % Reagent solution, (mL)	*Absorbance*
1.0	0.541
1.5	0.557
2.0	0.570
2.5	0.581
3.0	0.594
3.5	0.610
4.0	0.586

Table 4: Effect of coupling time on absorption

	Absorbance/standing time (minutes)					
Drug	*0*	*2*	*5*	*10*	*15*	*20*
TMP	0.577	0.582	0.606	0.601	0.597	0.582

Optimum Conditions for the Reaction

Choosing the Coupling Reagent

The volume of 2 mL of solutions with a concentration of 2% was used for the prepared reagents and used in the reactions of the formation of the Schiff base of the trimethoprim and the spectra were measured from 250–700 nm against its blank solutions and the results are listed in Tables 1.

From Table 1, it was found that the R_2 gave the highest absorption value when used with TMP, so this reagent was used as the best coupling reagent in subsequent experiments.

The Effect of the Acid

This study was carried out by adding different volumes (0.5–3.0 mL) of hydrochloric acid. The results showed that adding the acid leads to an increase in the absorption and then decreasing as the highest absorption of the colored output was given at pH 1.6 and with a volume of 2 mL of hydrochloric acid of 2 molar concentration. This volume was adopted in subsequent experiences. The results are listed in Table 2.

Effect of the Coupling Reagent Amount

The effect of the coupling reagent amount on the final absorption spectrum was studied by taking different volumes (1.0–4.0 mL) of the organic reagent solution with a concentration of 2%; the absorption of the solutions was measured at the wavelength of 573 nm versus the blank solution. It was found that the volume 3.5 mL of coupling reagent gave the highest absorption value and the results are listed in Table 3.

Effect of Coupling Reaction Time

The effect of coupling time, which gives the highest absorption of the colored output, was determined by studying the effect of the reagent's coupling time with the drug at different periods (0–20 minutes). The results in Table 4 show that 5 minutes are enough to complete the coupling process for trimethoprim and obtain the colored output.

THE STABILITY

The stability of the colored reaction product with different concentrations has been studied. The results in Table 5 show the appearance of color after the addition, and the color absorption

Table 5: The stability

	Absorbance/standing time (minutes)						
µg/mL of drug	*0*	*5*	*10*	*20*	*30*	*40*	*50*
4	0.478	0.491	0.492	0.490	0.482	0.471	0.459
8	0.569	0.580	0.581	0.580	0.572	0.561	0.552
12	0.711	0.725	0.725	0.723	0.717	0.710	0.701
16	0.826	0.839	0.838	0.836	0.832	0.820	0.812

of the colored product proved after 5 minutes. It was found that the solutions remain stable for 30 minutes after dilution at least.

FINAL ABSORPTION SPECTRUM

When adding the coupling reagent to a solution containing trimethoprim and then adding hydrochloric acid under optimal conditions, experimentally, a colored product (Schiff base) is formed whose absorption spectrum is measured after 5 minutes of completion of additions and dilution. The final absorption spectrum is shown in Figure 1 shows maximum absorption at 573 nm against the blank solution.

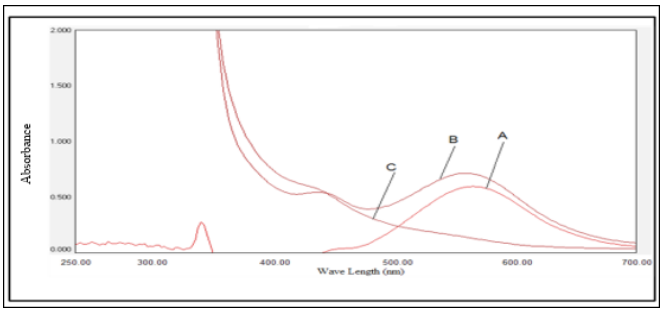


Figure 1: Absorption spectrum of 8 µg/mL of trimethoprim as measured:
(A) vs. blank solution, (B) vs. distilled water, (C) blank solution vs. distilled water

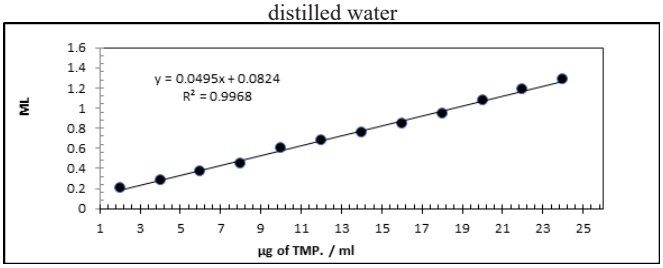


Figure 2: Standard curve for determination of trimethoprim using a schiff base formation reaction

Table 6: Accuracy and precision

Drug Conc., µg/mL	*RE*%*	*Recovery*%*	*Average recovery%*	*RSD*%*
4	1.47+	101.47		2.375
12	0.61+	100.61	100.82	1.063
20	0.38+	100.38		0.517

*Average of five determinations

Table 7: Detection limit

Concentration µg/mL	*Absorbance*	*(S.d)*	*D.L. µg/mL*
2	0.205	0.0116	0.3395

ANALYTICAL DATA AND THE CALIBRATION CURVE

Different volumes (0.5–6.0 mL) of trimethoprim solution with a concentration of 100 µg/mL (2–24 µg/mL) were added To a series of volumetric flask 25 mL, then add 2 mL of dilute hydrochloric acid solution of 2 molars and 3.5 mL molar of R_2 (coupling reagent solution) with 2% concentration (w/v), then leave the solutions for 5 minutes, then dilute with distilled water to the mark and leave the solutions for 5 minutes to complete the reaction, then the absorption of the solutions was measured at 573 nm versus the blank solution, as shown in Figure 2.

This method follows Beer's law for a range of concentrations between 2–24 µg/mL of trimethoprim with a standard coefficient of 0.9968. The molar absorptivity of the colored product was 1.437 x 10^4 $L.Mol^{-1}.cm^{-1}$ and sandell's index 0.0202 $\mu g.cm^{-2}$, which indicates that the method is highly sensitive.

METHOD ACCURACY AND PRECISION

The optimum conditions were used in the procedure to test the accuracy of the calibration curve and its precision for the determination of trimethoprim, as five readings were taken for three different concentrations of trimethoprim within the limits of Bear's law, where it was found that the method has high accuracy and good precision, and the results are listed in Table 6.

DETECTION LIMIT

The detection limit for calculating the trimethoprim at 573 nm was calculated, and the results are shown in Table 7:

PHARMACEUTICAL APPLICATIONS

The method could be applied to pharmaceutical preparations containing trimethoprim, which are TMP tablets 100 mg and TMP tablets 200 mg. The results showed accuracy in determination each drug, as shown below:

Direct Method

In this method, three different concentrations were prepared 4, 12, 20 µg/mL of pharmaceutical solutions with a concentration of 100 µg/mL, and the same procedural steps were applied when preparing the standard curve. The absorption of these solutions was measured against the blank solutions at 573 nm. The results obtained are listed in Table 8, which shows the

Table 8: Determination of trimethoprim in pharmaceutical preparations by direct method

Drug	*Name of drug*	*Type of pharmaceutical*	*Conc., of drug (µg/mL)*	*RE*,%*	*Recovery*%*	*Average recovery*,%*
TMP	Trimethoprim (100 mg)	Tablet	4.0	2.29+	102.29	
			12.0	0.95+	100.95	101.24
			20.0	0.48+	100.48	
	Trimethoprim (200 mg)	Tablet	4.0	1.66+	101.66	
			12.0	0.80+	100.80	100.99
			20.0	0.53+	100.53	

*Average of six determinations

Table 9: Determination of trimethoprim in pharmaceutical preparations by standard addition method

Drug	*Pharmaceutical preparation*	*µg/ mL Present*	*µg/ mL measured*	*Recovery,%*
Trimethoprim (100 mg)	Tablet	4	4.12	103

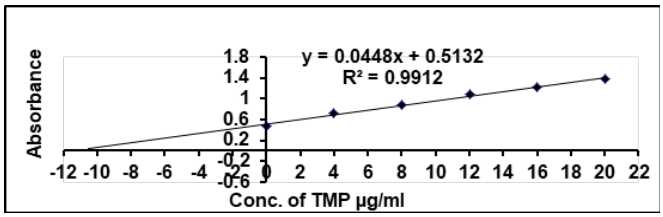
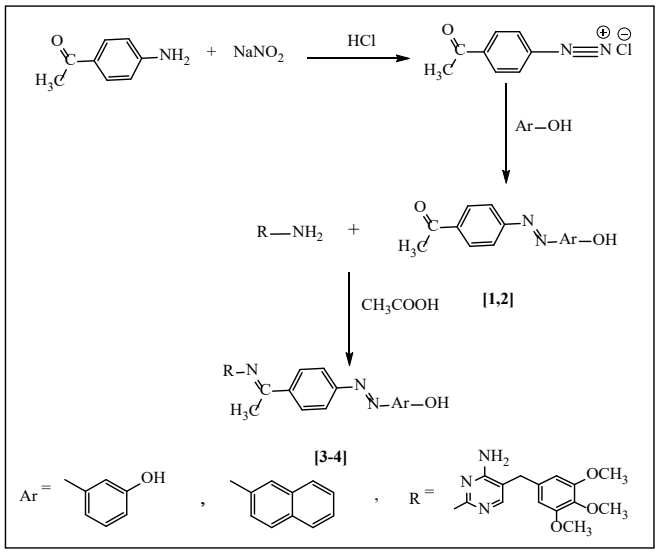


Figure 3: Curves of the standard additions method for the determination of trimethoprim in trimethoprim tablet solution (100 mg) at a concentration of 4 µg/ mL.



Scheme 2: Preparation of Azo-Schiff base compounds derived from trimethoprim

efficiency and success of the method developed for application to pharmaceutical preparations containing trimethoprim.

Standard Additions Method

In order to prove that the developed method is free from interference, therefore the standard additions method has been applied. This method was applied to determine trimethoprim, and the method relied on concentrations of the drug. The solutions were then treated with the same approved procedure when preparing the calibration curve, and their absorptions were recorded at the wavelength of 573 nm. Figure 3 and Table 9 show the results obtained when applying the standard additions method. From the results, it is clear that the method of standard additions is in good agreement with the proposed method for the determination of trimethoprim in its pharmaceutical preparations.

PREPARATION AND CHARACTERIZATION OF AZO-SCHIFF BASES COMPOUNDS DERIVED FROM THE REACTION OF TRIMETHOPRIM WITH PREPARED ORGANIC REAGENTS

The compounds (3-4) shown in Scheme 2 were prepared by grinding a mixture consisting of 0.001 mol of trimethoprim mixture with a 0.001 mol of the two compounds (1–2) separately in a ceramic basin. Put the mixture in a beaker and add 1–2 mL of absolute ethanol and 3-4 drops of glacial acetic acid; the mixture was irradiated in a microwave oven at medium temperature for 5 minutes. The solid product was washed by petroleum ether several times, followed by washing it with ethanol.

Characterization of Prepared Compounds (3,4)

The change in the physical properties, such as color and melting point, was adopted as a first indication of the success of the preparation, supported by studying the infrared and NMR spectra.

- **2-((Z)-(4-((Z)-1-((4-amino-5-(3,4,5-trimethoxybenzyl) pyrimidin-2-yl)imino)ethyl)phenyl)diazenyl) naphthalen-1-ol (Comp. 3)**

Molecular Formula $C_{32}H_{30}N_6O_4$, Reddish Brown crystals, Yield 67%, m.p. (158-161) C°, IR (KBr) : ύ = 1656 cm^{-1} (C=N, azomethine bond).

- **3-((4-((Z)-1-((4-amino-5-(3,4,5-trimethoxybenzyl) pyrimidin-2-yl)imino)ethyl)phenyl)diazenyl)benzene-1,2-diol (Comp. 4)**

Molecular Formula $C_{28}H_{28}N_6O_5$, Dark Brown crystals, Yield 67%, m.p. (149-152) C°, IR (KBr) : ύ = 1658 cm^{-1} (C=N, azomethine bond), ^{1}H-NMR (500 MHz, D_2O) : δ = 3.6-3.8 ppm (OCH_3 protons), 6.2 ppm (NH_2 protons), 10.93 and 11.33 ppm (OH protons), 6.59-8.30 ppm (aromatic ring protons). ^{13}C-NMR (500 MHz, DMSO) : δ = 169.53 ppm (C=N), 44.69 and 65.39 ppm (OCH_3), (142.32,142.80,157.16) ppm (Pyrimiden ring in trimethoprim), 96.23–141.60 ppm (aromatic rings).

CONCLUSIONS

The proposed method offers clear advantages for the fast determination of TMP in pure form and in pharmaceutical preparation. The method was found to be very simple, rapid, low cost, and fairly selective than some of the reported colorimetric methods. They had the advantage of being accurate. The proposed method offers good linearity and precision. It was applied to the analysis of TMP in tablets, and new compounds were synthesized from the reaction of TMP with prepared organic reagents to form Schiff's base and were characterized of the prepared compounds by multiple spectral methods.

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