Spectrophotometric Determination of Trimethoprim in Pure Form and Pharmaceutical Formulations with Metol and potassium hexacyanoferrate (III)

***Ayad M.R. Raauf, **Huda Mohammed Ali, ***Halah Hameid**
,**,Department of pharmaceutical Chemistry and pharmacognacy, Collage of Pharmacy, University of Al-Mustansriya, Baghdad, Iraq**

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Abstract

A simple, sensitive and accurate spectrophotometric method of determination of trimethoprim (TMP) in pure form and pharmaceutical formulation. The method is based on the formation of (TMP) complex. The reaction between of the trimethoprim with the mixture of metol and potassium hexacyanoferrate (III) was evaluated for the spectrophotometric determination of the trimethoprim. The maximum absorbance of the colored complex occurred at λ=540nm. Reaction conditions have been optimized to obtain (TMP) complex of high sensitivity and longer stability. Under optimum conditions the absorbance of the (TMP) complex where found to increase linearly with increase in concentration of the trimethoprim, which corroborated with correlation coefficient value. The concentration ranges are 10-100 µg mL^{-1} with detection limit 0.0286 µg mL^{-1} and relative standard deviation 0.74 % and relative error of prediction for drug were lower . The proposed method was successfully applied to determine of the selected trimethoprim in pure form and pharmaceutical formulations with good precision and accuracy compared to standard method as revealed by t- and F- values and the results obtained agree well with the labeled contents.

Key word: trimethoprim, spectrophotometric, oxidation–reduction reaction.

الخلاصة

طريقة بسيطة، عالية الحساسية وذات دقة عالية لتقدير الميثوبرين بشكل نقي او في المستحضرات الصيدلانية من خلال تكوين معقد .تضمنت الطريقة تفاعل الميثوبرين مع مزيج الميتول وبوتاسيوم سداسي سيانيد الحديديك وتكوين معقد ملون لتقدير الميثوبرين طيفيا عند اقصى ط ول موجي 540 نانوميتر .تم تحديد الظروف الفضلى للتفاعل للحصول على اعلى حساسية واطول استقرارية . عند الظروف الفضلى للامتصاصية لمعقد الملون وجد ازدياد بالخطية مع ازدياد تركيز الميثوبرين والموثق من خلال قيمة معامل الارتباط .مدى التركيز المستخدم 10-100 مايكروغرام مل$^{-1}$ وبحدود كشف 0,0286 مايكروغرام مل$^{-1}$ وبانحراف قياسي نسبي **0.0074**% . طبقت الطريقة المقترحة بدقة وضبط عالي وبنجاح لتقدير نسبة الميثوبرين بشكل نقي او في المستحضرات الصيدلانية وتم مقارنة النتائج الاحصائية باستخدام اختباري وقد وجد ان ان قيمهما اقل من قيمهما الواردة بالطريقة المستخدمة في تقديره بالدستور البريطاني .

Introduction

Trimethoprim, 2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine (TMP) (Fig. 1)is a synthetic (man-made) antibiotic that interferes with the production of tetrahydrofolic acid, a chemical that is necessary in order for bacteria and human cells to produce proteins. Trimethoprim inhibits production of tetrahydrofolic acid by inhibiting the enzyme responsible for making tetrahydrofolic acid from dihydrofolic acid. Trimethoprim inhibits the bacterial enzyme more than the corresponding human enzyme. Therefore, trimethoprim has less effect on the production of tetrahydrofolic acid by humans. Trimethoprim is effective against a wide variety of bacteria.[1]

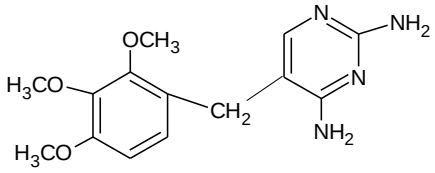

Fig. 1

There are various analytical procedures for the assay of TMP, the most important being ion-selective piezoelectric sensor,[2] Spectrofluorometry,[3] differential pulse voltammetry,[4] Visible and UV Spectrophotometry,[5,6,7] spectrofluorometric,[8] Continuous Wavelet Transforms,[9] Micellar Electrokinetic Capillary Chromatography ,[10] SPE-HPLC/DAD ,[11,12]non-aqueous titrimetry, ion-selective electrode (ISE),[13] and polarography and voltammetry[14].

Experimental

Apparatus:

Spectral and absorbance measurements were made on UV 1650 Shimadzu spectrophotometer by using 1 cm quartz cell.

Materials and Reagents

All reagents used were analytical grade and water was always double distilled.

Pure samples

Trimethoprim pure grade was provided by SID- Samara factory.

Standard stock solution

Stock solutions of trimethoprim were prepared by dissolved (0.01g) of standard trimethoprim in 5ml ethanol, then transferred into 100 ml volumetric flasks and diluted to the mark with bidistilled water.

Market samples

Trimethoprim tablets, labeled to contain (80mg). They were obtained from commercial sources in the local market.

Reagents

Metol solution (13 and 20mM) were freshly prepared by dissolved (0.225g) and (0.68889g) of metol respectively, and diluting to 100ml with bidistilled water in volumetric flasks. (20mM) potassium hexacyanoferrate (III) were prepared by dissolved (0.332 g) $K_3[Fe(CN)_6]$ and diluting to 100 ml with bidistilled water in volumetric flask.

Recommended analytical procedure

Method

Different aliquots of trimethoprim standard stock (100 µg ml^{-1}) were transferred into a series of 10 ml volumetric flasks, equivalent to (1-10) µg ml^{-1} to each these were added 1ml of buffer (pH 4.5) and metol (1ml) and potassium hexacyanoferrate (III) (1ml) were diluted to the mark with bidistilled

water. The absorbance was measured at λ=540nm against a reagent blank prepared similarly. A calibration graphs were drawn by plotting the absorbance against the drug concentration.

Analytical of pharmaceutical formulation

Ten tablets were accurately weighted and finally powdered. An amount of the powder equivalent (50mg) TMP was dissolved in 5ml of ethanol and transferred to 100 ml calibrated flask. The contents of the flask were shaken and then make up to the mark with bidistilled water to obtain (500 µg.ml$^{-1)}$ of TMP.

Results and Discussion

Absorption spectra

Throughout the preliminary investigation on the reaction[15], between drugs (TMP) with metol in the presence of potassium hexa cyanoferrate (III), colored (red purple) products obtained with a maximum absorption at λ=540nm (Fig. 2). The absorbance of the colored products measured against reagent blank which has minimum absorbance at the same wavelength from the results obtained, appeared that it is possible to determine a microgram of this drug.

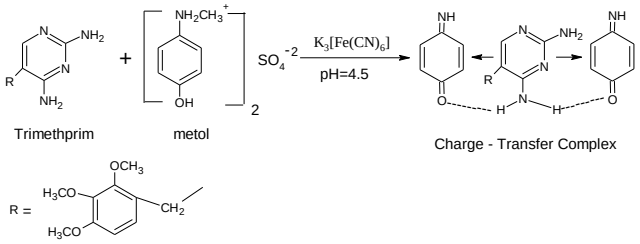

Fig.(2):- Reaction scheme

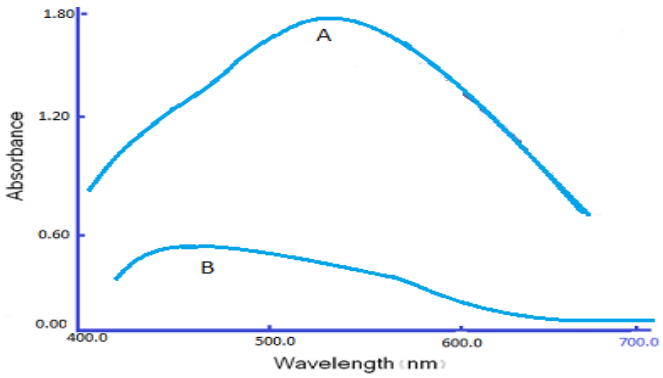

Fig. (3):- Absorption spectra of A= drug Methoprim complex, B=metol/ potassium hexacyanoferrate (III) reagent

Optimization of Experimental Conditions

The effect of various variables on the color development was tested to establish the optimum conditions for the determination of trimethoprim by using metol and potassium hexacyanoferrate (III).

Effect of pH

The optimum pH for complete color development is 4.5. The buffer solution is added to give the required pH.

Effect of volume of potassium hexacyanoferrate (III)

The effect of the different volumes of (20mM) of $k_3[Fe(CN)_6]$ solution was examined on the maximum absorbance of the colored product in the presence of (1ml) metol (20mM). Fig.3 shows that 1ml of the solution of potassium hexacyanoferrate (III) was enough to obtain the maximum absorbance.

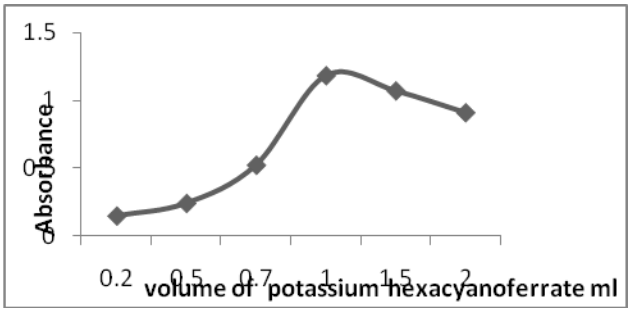

Fig. (4):-Effect of volume of (20mM) potassium hexacyanoferrate (III) on the absorbance intensity of Trimethoprim drug at 540nm

Effect of volume of metol reagent

Metol was found to be a useful for charge transfers reaction, because it was produced a stable charge transfer complexes rapidly with drugs in presence of potassium hexacyanoferrate (III). More over this reagent is easily obtainable and is soluble in water. The effects of the different volumes of (20mM) metol solution were examined on the maximum formation of the colored product. Fig. 4 shows that (1ml) of the solution was enough to obtain the maximum absorbance, and it was used in the subsequent experiments.

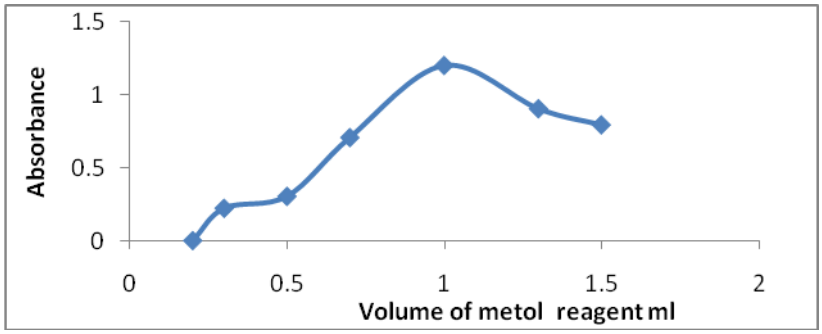

Fig.(5):-Effect of volume of metol reagent (20 mM) on the absorbance intensity of trimethoprim drug using (1ml) of potassium hexacyanoferrate at 540nm

Effect of addition orders

Seven orders of addition were examined as shown below.

ON.	Addition order	Absorbance (nm)
1	B+D+M+F	0.234
2	M+D+B+F	0.259
3	D+B+M+F	1.169
4	F+M+D+B	0.545
5	D+B+F+M	0.444
6	F+D+B+M	0.522
7	F+M+B+D	1.789

D= Drug, R= Reagent (metol), O= Oxidant $k_3[Fe(CN)_6]$, B=buffer

Effect of temperature

The effect of temperature on the color intensity of the product was studied Fig. 5 in practice a maximum absorbance was obtained when the color was developed at room temperature (25^0C), but when the color was developed in an ice bath (5^0C) or in a water bath(45^0C) a loss in color intensity and stability were observed. It is therefore recommended that the color reaction should be carried out at room temperature (25^0C) after 20min.

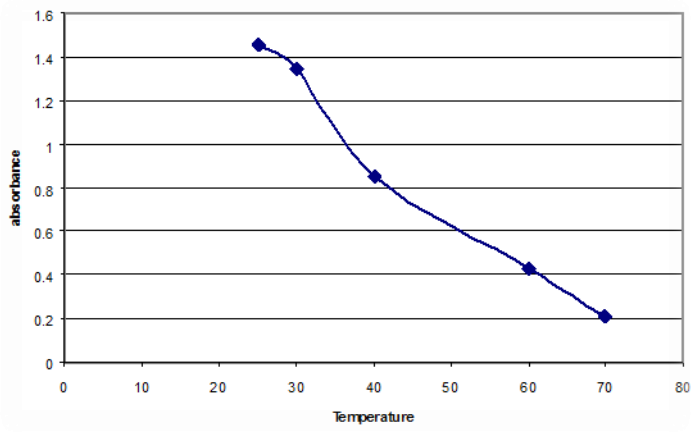

Fig.(6):- Effect of Temperature

Effect of time on stability

The color intensity reached a maximum after drug solution had been reacted immediately with metol and potassium hexacyanoferrate (III) in aqueous medium and became stable after (15min), remained stable for at least 3h (Fig. 6).

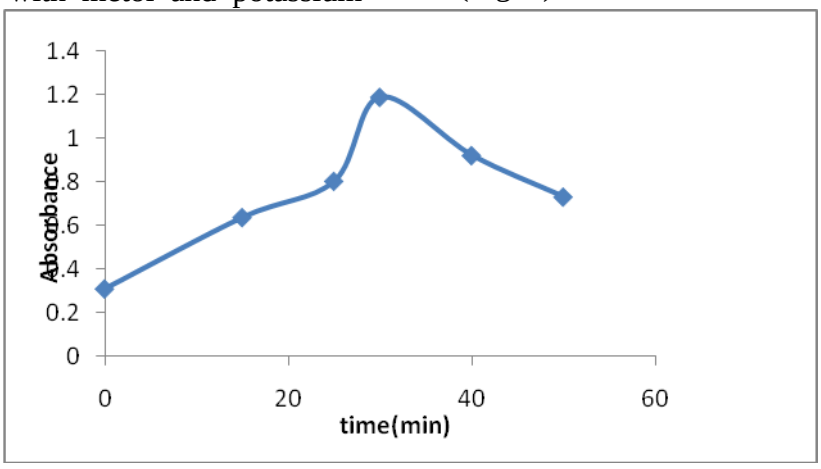

Fig.(7):-Effect of time (min) on the absorbance of the formed complexes of Trimethoprim drug

Calibration Graph

After the optimizing reaction conditions describe above, calibration curve (Fig. 7) for TMP was constructed by plotting absorbance of TMP complex and the concentration of the TMP drug. The calibration curve was linear. The analytical values of statistical treatments for the calibration curve are summarized in Table 1.

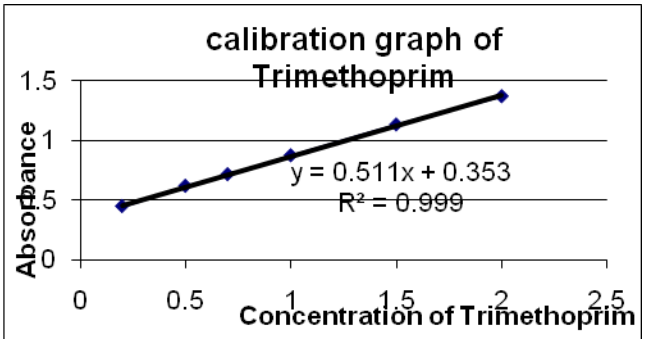

Fig. 8. Calibration curve of TMP

Accuracy and precision

The accuracy and precision of the determination of TMP were studied depending upon the value percentage of the relative error (E%), recovery (REC%) and relative standard deviation (RSD%) respectively. For five replicates of each concentration of TMP (0.3, 0.8, 1.3) $\mu g.ml^{-1}$. The results in Table 2 show a good accuracy and precision.

Table (1):- Performance data of the proposed method

Parameter	Value
Correlation coefficient ®	0.99968
Linearity percentage (r^2 %)	99.94
Test for a significant correlation (t)	132
Regression equation	y=0.5112x+0.3537
Slope (ml μg^{-1})	0.5112
Intercept	0.3537
Standard deviation of the residuals,$S_{y/x}$	**0.00953749443**
Standard deviation of the slope, S_b	0.0063892
Standard deviation	(0.00740)
Linearity range (µg ml^{-1})	10_100
Molar absorptivity, ε (l mol^{-1} $cm^{-2)}$	$1.498x10^4$
Sandell's sensitivity, S(µg cm^{-2})	$1.938x10^{-2}$
Limit of detection LOD (µg ml^{-1})	0.028604832
Limit of quantification LOQ (µg ml^{-1})	0.095374944

Table(2):- Accuracy and precision of the proposed method.

ON.	Conc µg/ml		**E**%*	**CER**%*	**R**SD%*
	present	found			
1	**0.3**	**0.302**	**0.667**	**100.667**	**0.748**
2	**0.8**	**0.795**	**-0.625**	**99.375**	**0.274**
3	**1.3**	**1.294**	**-0.642**	**99.538**	**0.397**

***Average of five determinations**

Stoichiometry of the formed product

The stoichiometry of the formed product was investigated by mole ratio. Continuous variation (job's method), and slope ratio methods. In the mole ratio method increased volumes of (20 mM) metol were added to a (1ml) of (20mM) TMP in a series of (10ml) volume flasks, followed by 1ml of 20mM potassium hexacyanoferrate (III), the volumes were made up to the mark with distill water, allowed to stand to 15min. and the absorbenc were measured at 540nm versus the reagent blanks. The results were plotted as shown in (Fig.9-10) which indicated the existence of 1:1 metol:TMP

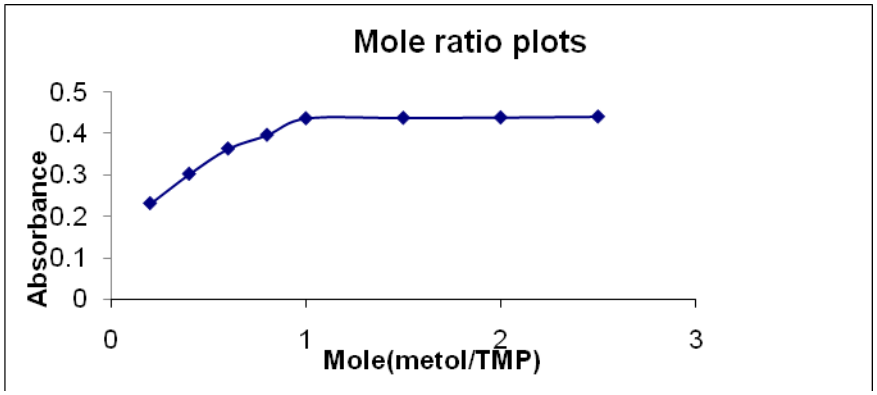

Fig.(9):- Mole ratio plots

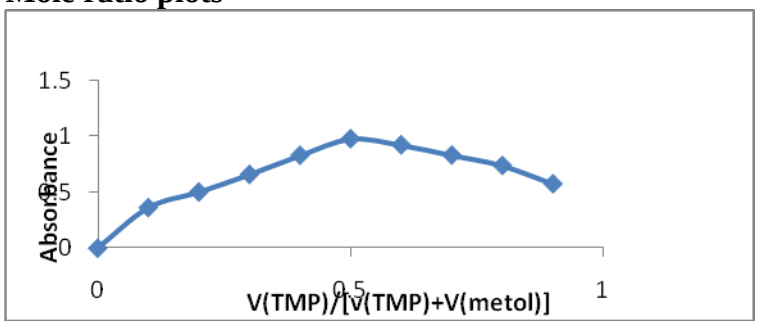

Fig. (10):- Continuous variation plots

The job method was applied by placing 1 to 9ml of 20mM trimetheprim solutions in to a series of 25ml volume flasks; this was followed by placing 9 to 1ml of 20mM reagent metol and 7ml of potassium hexacyanoferrate (III). The solutions were diluted to the mark with distilled water, allowed to stand for 15 min, versus reagent blank. The results were plotted as shown in figure which indicated the existence of 1:1 (metol:trimethoprim)

Sulfamethaxazole (SMZ)

An investigation was carried out to determine the effect of sulfamethaxazole which was found with pharmaceutical preparations of trimethoprim (TMP) as binary mixtures at the level found in pharmaceutical preparations and different levels Table 3. No interference were observed in the determination of trimethoprim in the presence of the SMZ at these levels

Table (3):-Effect of SMZ on the recoudy of TMP (10 µg.ml^{-1}) at different levels

TMP:SMZ	Concentration of TMP.µg ml^{-1}found	E%	REC%
15:1	10.055	0.55	100.55
10:1	10.079	0.79	100.79
5:1	10.099	0.99	100.99
5:3	9.871	-1.29	98.71
1:1	10.112	1.12	101.1
1:3	10.161	1.16	101.61
1:*5*	*9.988*	*-0.12*	*99.88*

Pharmaceutical application

Solutions of pharmaceutical preparations were prepared as given under solutions of pharmaceutical preparations the proposed method was applied for the determination of trimethoprim in tablets by the analysis by the analysis of two concentrations of sample using the recommended procedure. The results obtained are summarized in Table 4.

Table (4):- Application of the proposed method for determination of Trimethoprim in pharmaceutical preparations

SMZ	Pharmaceutical preparation		E%	REC%	RSD%
	Con.µg/ml present	Con.µg/ml Found			
METHEPRIM TABLETS	0.2	0.201	0.5	100.5	0.075
	0.7	0.698	-0.2	99.714	0.274
	1.2	1.204	0.333	100.333	0.397
SULFAPRIM TABLETS	0.2	0.199	-0.5	99.5	0.073
	0.7	0.701	0.143	100.143	0.314
	1.2	1.2O2	0.1667	100.167	0.333
COTRIMOXAZOLE SUPSPENSIONS	0.2	0.202	1	101	0.066
	0.7	0.702	0.286	100.286	0.249
	1.2	1.196	-0.333	99.667	0.331

- **Average of five determinations.**

Evaluation of the proposed method

For evaluation the competence and the success of the proposed method the result obtained were compared with those obtained by British pharmacopeia. The results obtained by the different method stable were statistically compared using the student t- test and variance ration F-test at 95% confidence level in all cases, the calculated t- test and F-values Table-5 did not exceed the theoretical values which indicated that there is no significance difference between either methods in accuracy and precision in determination of trimethoprim in pharmaceutical preparation
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Table (5):- Comparison of the proposed method with standard method using t- and F- statistical tests.

No.	Proposed method		Standard method			Value	
Pharmaceutical preparation	Rec%* Xi	$(X_i - \overline{X})^2$	Rec% $(Xi)_2$*	$(X_i - \overline{X})^2$	S**	t (theo.)	F (theo.)
TMP Pure	100.0	0.0625	100.00	0.008300	0.240	2.003 (2.447)	6.524 (9.277)
Metheprim tablet	100.5	0.0625	99.70	0.040000			
Sulfaprim tablet	99.5	0.5625	99.71	0.040000			
Cortimoxazole suspension	101.0	0.5625	100.00	0.099225			
	$\overline{X}$ =100.25	$\sum$=1.25	$\overline{X}$ =99.91	$\sum$=0.191625	$n_1+n_2-2=6$		$n_1-1=3$ $n_2-1=3$

*** Average of five determinations, S ** Pooled standard deviation**

Conclusion

Despite the great number of methods described in the literature for analysis of trimethoprim, the proposed method for the determination of trimethoprim in pharmaceutical samples have the advantage to be simple, sensitive, accurate and inexpensive. The method represented good accuracy and precision so that the respective relative standard deviation and relative error of prediction for drug were lower. The proposed method was applied successfully to analysis of drugs in tablets and thus is very appropriate for routine quality control analysis of drug.

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