Paper ID: 029_ISCPS
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LETTER OF ACCEPTANCE

Dear Authors,

On behalf of the ISCPS -20 Committee, we are pleased to inform you that your paper entitled

"RP – HPLC method for the determination of removal phenol as a pollutant in aqueous solution"

Written by

Ahmed Mahdi Saeed, Dheyaa H. Ibrahim , Hind A.W. A. Al – latief

Has been accepted and will be proceeded to publication in the IOP Journal of Physics (Online ISSN: 1742-6596, Print ISSN: 1742-6588, Volume JPCS_1660, ISCPS 2020). We congratulate you for your achievement, the technical details about the publication will be informed later. The publication of the accepted paper will be provided by the end of November 2020.

We Will encourage more quality submissions from you and your colleagues in future

Regards,

Shubham Sharma

Prof. Dr. Shubham Sh. Sharma

Special Issue (Guest Editor), ISCPS-20

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Acceptance Letter

2 March 2019

Dear Author(s)

Dr./ Mr(s). H.ABDULWHAAB ABDULATEEF /Department of chemistry, college of education for pure sciences, University of Diyala, Iraq

Greetings, With reference to your article titled:

Spectrophotometric method for determination of Trimethoprim by using NQS

We wish to bring to your kind notice the following

√ **We acknowledge the receipt of the above mentioned article**

√ **The above mentioned article(s) has been sent to the reviewer of expert comments**

√ **The above mentioned article(s) (*Biochemical & Cel. Arch. Alk 41*) have been accepted and it will be published in the April-June-Special issue/ 2019 V19/No. (1) Journal (Biochemical and Cellular Archives ISSN: 0972-5075).**

IOP Publishing

Toward investing in scientific research for social betterment

2nd International Scientific Conference
College of Science, University of Al-Qadisiyah
24th-25th April 2019

No. 168
Date: 14/03/2019

Acceptance Letter

We are pleased to inform you that your manuscript ID: JPCSJ12945011 entitled " **Spectrophotometric method for designation of nitrobenzene by using o-chloranil so application in Diyala River and waterproof case**" has been accepted for online publication in the 2nd International Scientific Conference that held in College of Science, University of Al-Qadisiyah, Iraq, *Which will be publishing at the IOP publishing, Journal of Physics: Conference Series.*

Authors and Affiliation Details:

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Thank you for your contribution to 2nd ISC 2019

Sincerely

Prof. Dr. Nabeel Abed Adul-Reda
Chief of Conference

Copy to:
- Conference Secretary

For more details, please visit the conference website and do not hesitate to contact us if you want further info http://qu.edu.iq/2isc2019 (+964781١٢٨٧٠٧٨)

Spectrophotometric method for determination of Trimethoprim by using NQS

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Abstract

Asensitive and simple spectrophotometric method to assay the Trimethoprim was in Vestigated the method based on Schiff base formation reaction of Trimethoprim with 1, 2-naphthoquinone sulphonate (NQS), in the buffer solution of carbonate and sodium carbonate in an aqueous solution to give coloured interaction product with maximal absorption at 478nm, As well Beer s Law was obeyed in the range of (5-16) µg/mL. The limt of detection and quantitation were 0.0436 and 0.135 µg/mL respectively, the average recovery was 100.38% and the relative standard deviation was 0.380. Also it was found that the product formed in ratio of 2:1 product. The stability constant of 6.21×10^{7}L.mol^{-1}. Therefore, the placebo method was successfully applied to the assay of Trimethoprim in pharmaceutical for mulations.

Keywords: Trimethoprim; NQS; Schiff's base

Introduction :- Trimethoprim is a drug contains the active element methoprim which is an antibiotic used to treat the infections alony with bacteria [1], where bacterial cell need to product DNA in order to grow and reproduce, This drug prohibits the bacteria producing the folic acid therefore the bacteria cannot reproduce and increase [2]. Various analytical method have been recorded for determination it in pure or dosage forms including chromatographic methods [3, 4], Voltammetry [5, 6], titrimetry [7], atomic absorption [8], flow injection [9, 10], and ion selective electrode [11,12]. The above techinques are critical but expensive method several spectrophotometric procedures have been applied for determination of trimethoprim using different reagents including p-chloranilic acid reagent, [B] p-Benzoquinone reagent [14], Blue prothaimol so green promocresol and the red alizarin in different organic medium [15], p-Nitrophenol reagent[16], 2,4 (Dinitro-1-floro benzene) reagent in acetone medium[17], Bromocresd purple reagent by extraction of double ionic yellow[18], also spectrophotometric methods for determination of Trimethoprim with sulfamethoxazole in Dual mix [19-20-21].

Experimental Apparatus: - UV- VIS spectrophotometer 1650 shimadzu cell 1cm path length silica cell. Pw 9421-pH meter with acommon glass electrode was used for pH measurements. The reagent is supplied by BDH, Fluka and Molekula companies; a standard solution of 100 µg/ml of trimethoprim was prepared by dissolving 0.01g in 2ml of water and diluting to the mark with distilled water in 100ml volumetric flask. 5×10-3M of NQS reagent was maked by dissolving 0.065g in distilled water and mating the Volume up to 50ml in a volumetric flask, this Solution was maked fresh as adaily procedure.

0.1M sodium bicarbonate was prepared by dissolving 2.65g in distilled water. and making the volume up to 250 ml in avolumetric flask 0.1M sodium hydroxide was prepared by dissolving 1gm in 250mLin distilled water, 0.1 g m % of CPC was prepared in warm distilled water.

Result and Discussion:

Study of the optimum reaction conditions to prove the sensitivity of proposed, the reagent concentrations must be optimized the parameters were optimized by settling all parameters constant and optimizing one each time at 55 for 20 min.

Test of types bases:

To obtain high sensitivity for the product the test of some bases such as sodium hydroxide sodium bicarbonate sodium carbonate, potassium hydroxide and ammonium hydroxide, AS shown in figure 1. Sodium carbonate gave maximum absorption at 478 nm.

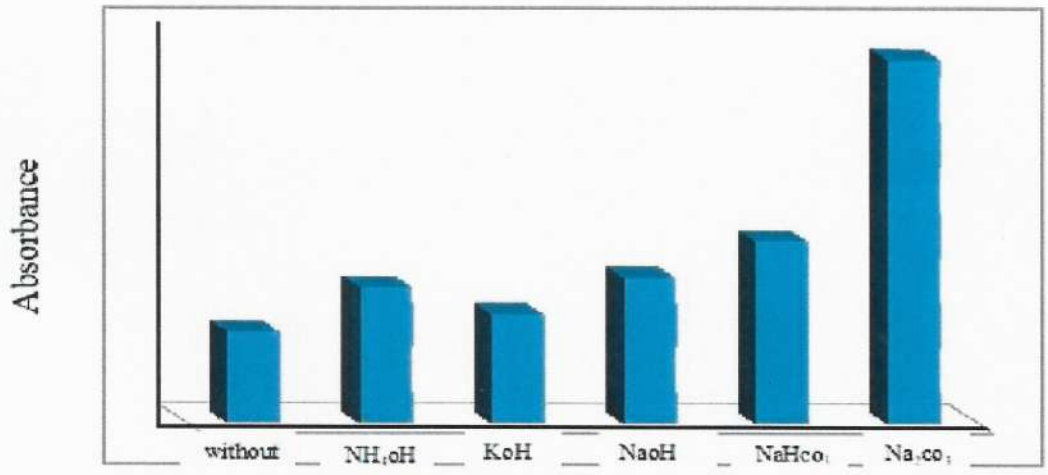


Fig 1: Effect of bases

As well the of effect of the sodium carbonate amount and pH were studied and found that 1.5 ml gave maximum absorbance at pH 10.5. (figure2) shown this

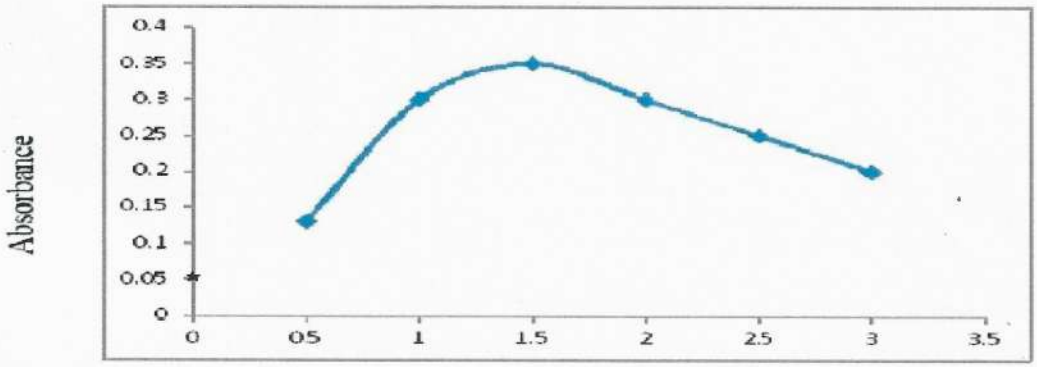


Fig 2: Effect of Na_2Co_3 amount and pH on absorption of reaction mixture of 4µg/ml trimethoprim

Effect of buffer Solution: - The effect of buffer solution with pH 10.5 was examined such as ammonium, borate, carbonate and phosphate buffers, but decrease in the absorbance of the product was observed.

Effect of NQS reagent:- The absorbance increase with increasing NQS concentration and reached maximum on using 0.8 ml of 5×10^{-3} M NQS. Table[2] which is recommended this work.

Table 1: Effect NQS amount

NQS(5×10^{-3}m) ml	Without	0.1	0.2	0.4	0.6	0.8	1.0	1.2	1.4
Absorbance	0.03	0.19	0.25	0.28	0.34	0.37	0.35	0.31	0.21

Effect of temperature and developing time:-

The reaction time was determined by following the colour development at room temperature and at different temperature up to 60 C°. It was observed that the absorbance reached maximum after 25 min at 40 C° and remained constant for 35 min, Table 3 show the results . 25 min at 40C° was used in this work.

Table 2: Effect of temperature and developing time

Temp	Absorbance at time (min)								
	5	10	15	20	25	30	35	40	45
RT*	0.194	0.198	0.221	0.234	0.318	0.335	0.310	0.261	0.121
30	0.260	0.278	0.284	0.347	0.353	0.351	0.312	0.231	0.135
40	0.261	0.291	0.321	0.383	0.393	0.389	0.361	0.290	0.180
50	0.293	0.298	0.342	0.353	0.372	0.342	0.311	0.211	0.124
60	0.274	0.295	0.241	0.235	0.283	0.270	0.161	0.113	-

Effect of Surfactants:-

The impact of surfactants such as (CTAB), (CPC), (TW 80) and (TX–100) of 0.1% concentration. the (TX -100) increased the absorbance of trimethoprim – NQS product but other surfactant showed negative impact as shown in table (2) therefore (TX-100) was selected in this method. The absorbance increase with an increased (TX -100) concentration up to 1ml (Fig 3) shows this.

Table3: Effect of Surfactants on the absorption

Surfactant	Absorbance
Without	0.390
CPC	0.321
SDS	0.383
CTAB	0.351
TX – 100	0.401
TW 80	0.371

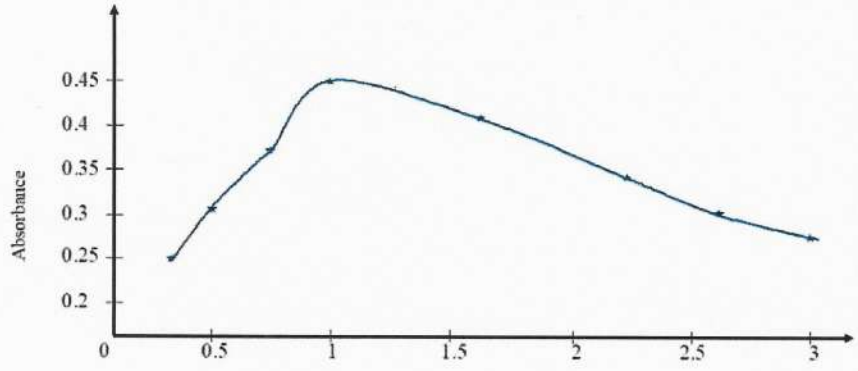


Fig5: Effect of (TX-100) concentration on the absorption of reaction mixture of 4µg/ml for Trimethoprim

Effect of order addition:-

The order of addition of addition of reagent was tested under the optimum conditions.

Table 4: shows that order no. I is the best order.

Order of addition	Order no	Abs
Drug +NQS +base+ (TX-100)	I	0.456
Drug +base + NQS +(TX-100)	II	0.432
Drug +(TX-100) + NQS+ base	III	0.421
Drug +(TX-100) + base + NQS	IV	0.426
Drug + base +(TX-100) + NQS	V	0.394
Drug +NQS+(TX-100)+ base	VI	0.367
NQS+ base+ Drug+ (TX-100)	VII	0.245
NQS+ base+ (TX-100)+ Drug	VIII	0.264

Absorption Spectra:-

The final absorption spectra of NQS- Trimethoprim product are plotted under the best condition reached above. Figure [6] establish that Schiff base product has maximum absorption at 478 nm versus reagent blank, while the reagent blank has low absorbance at this wavelength and has a maximum absorption at 370 nm versus distilled water.

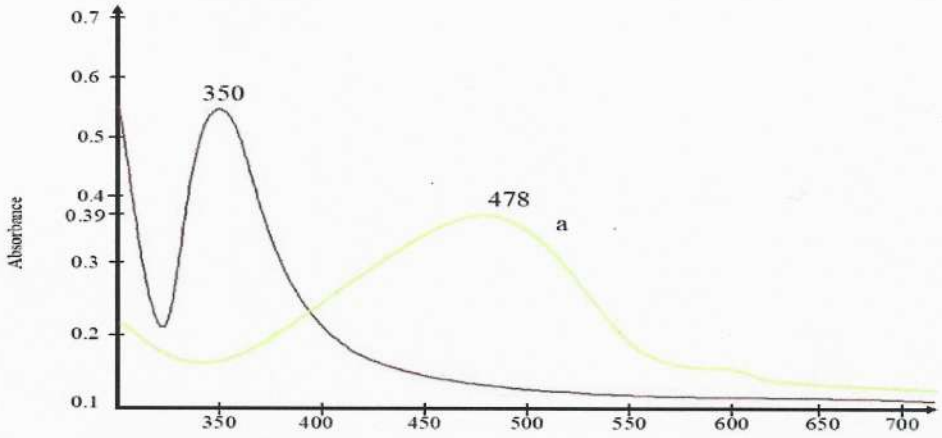


Fig 6: Absorption of Spectra of (a) trimethoprim (8µg/ml) with NQS against reagent blank and (b) reagent blank versus distilled water

Quantification: - The absorbance of the product was measured at 478 nm after developing the color by following the general procedure calibration graph for aseries of solutions containing increasing amount of trimethoprim. The molar absorptivity values and Beer's law limits were evaluated and given in Table [5] the linearity was represented by the regression equation and the correspording correlation coefficient for the studied, the relative standard deviation and accuracy for the analysis of six replicates of each three different concentration Limit of detection is in accepted range.

Table5: summary of optical characteristics and statistical data for the proposed method.

Parameter	Trimethoprim
Beer's law limits ($\mu g.m^{-1}$)	(0 – 15)
LoD ($\mu g.m^{-1}$)	0.0436
LoQ ($\mu g.m^{-1}$)	0.135
Average recovery%	100.38
Correlation Coefficient	0.999
Slop , a	0.1143
Intercept , b	0.0173
RSD	≤ 0.44

Inteference :-

It was found that the studied excipients did not interfere seriously (Table 6) show it.

Table 6: Effect of excipients for assay of Trimethoprim.

Excipient	Recovery % of 8 µg/ml Trimethoprim Per µg/ml excipient added			
	200	300	400	500
Glucose	101.8	103.2	100.4	102.5
Starch	96.91	100.5	102.1	101.12
NaCl	98.99	98.7	100.10	103.5
Lactose	101.2	100.3	103.5	104.19
Acacia	101.8	101.7	99.4	98.2

The molar ratio and Job's methods: The stoichiometry of the reaction was studied by the molar ratio and Job method [22], as shown in Figure [7] a&b the results indicate that 1:2. The apparent stability constant was estimated by comparing the absorbance of solution containing stoichiometric amounts of the trimethoprim and NQs (As) to one containing an excessive amount of NQs reagent (Am), The average stability constant of the product was calculated by following equation:- $K_C=1-\alpha/\alpha^2 c$ and α=Am-As/Am The average Kc is (2.895×10^{-4}) $l.mol^{-1}$

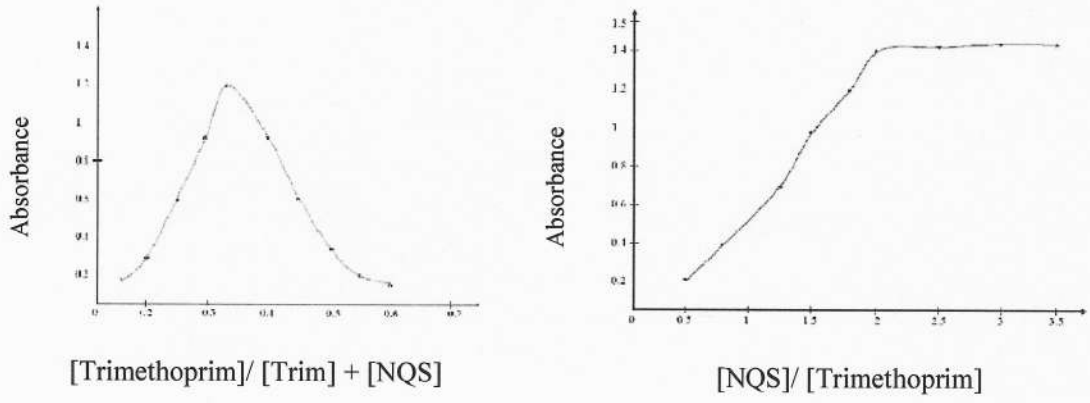
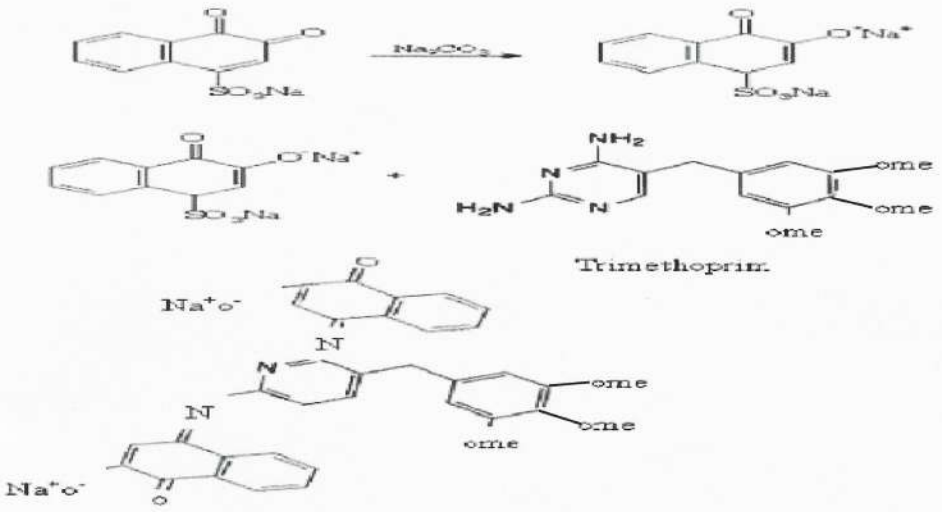


Fig 7: (a) continuous Variations (b) mole ratio for product of Trimethoprim (1×10^{-2}M) and NQs (1×10^{-2}M) under the optimum conditions.

Reaction Mechanisms:-

Acharacteristic prduct of λmax 478nm for Trimethoprim was formed when it allowable to react with NQS in the presence of Na_2Co_3 in aqueous medium under the experiential conditions.



Scheme1: shows the probable product formation mechanism.

Applying the proposed method on the pharmaceutical formulations:-

Due to the incapability of having the pharmaceutical composition of the trimethoprim alone without mixture, it can found mixed with asulfonamide medications that contain amine groups with the capability of interaction with the reagent NQs which cause spectrum interference with the complex, therefore, the pharmaceutical composition has been prepared by using placebo's method [23]. Table [7] shows that the studied excipients did not interfere seriously in pharmaceutical products of trimethoprim.

Table 7: Effect of exciepients for assay 30 µg/ml of Trimethoprim

No, of placebo	Recovery %	RSD
1	103.8	2.01
2	100.75	1.32
3	102.21	1.98

Also, the proposed method was successfully applied to determin the trimedthoprim in pharmaceutical preparations. The results showed that the experimental t-test and f- test were less than the theoretical value (t= 1.20, f=4.10), indicating that there was no significant difference between the proposed method and official method

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