369 *Egypt. J. Chem.* **Vol. 63,** No. 12 pp. 4681 - 4693 (2020)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Spectrophotometric Determination of Paracetamol using a Newly Synthesized Chromogenic Reagent 4-[(2-amino-1, 3thiazol-4-yl)amino]nitro benzene

Ruba Fahmi Abbas[a*], Amjad Gali Allawi[a], Nagham Majid Abdulhassan[a], Nawal Hamdan Mahmoud[a]

[a]*Department of chemistry, Collage of Science Al-Mustansiryiah University, Baghdad-Iraq.*

Abstract

This study describes the new simple and accurate spectrophotometric method for the determination of paracetamol after the formation of azo dye with a new chromogenic reagent 4 [(2-amino-1,3thiazol-4-yl)amino] nitrobenzene to form an orange-coloured product measured at 425 nm. The molecular structure of the newly synthesized compound was confirmed by several spectroscopic techniques such as UV-visible, FTIR, 1HNMR, and mass spectroscopy. Newly synthesized compound was in vitro screened against several bacterial species. The experimental conditions that affect the reaction were carefully optimized and under the optimized conditions, a linear relationship was obtained in the concentration range of 5–25mg.L^{-1} of paracetamol. A reaction with a new chromogenic reagent has been occurred at a stoichiometric ratio of 1:1. This method has a limit of detection of 1.334 mg.L^{-1} and Sandell's sensitivity of 0.0235 mg.cm^{-1} for new azo dye product.

Keywords: *Paracetamol; Azo dye; Antibacterial activity; Thiazol; ^{1}HNMR; Spectrophotometric*

1. Introduction

Paracetamol (PCT) is a common antipyretic analgesic drug. It is used in the treatment of a headache, colds, joint pain, fever and pain after surgery in the clinic[1]. Different methods have been employed for the determination of the paracetamol which includes electrochemical sensor[2], voltammetric using poly(2,2′-(1,4-phenylenedivinylene)bis-8-hydroxyquinaldine) modified glassy carbon electrode[3], voltammetric using a carbon paste electrode modified with CdO nanoparticles[4], HPLC[5], spectrophotometry with chemometric methods[6, 7], spectrophotometry using continuous wavelet and derivative transform[8], spectrophotometry using different derivative methods[9]. Most of these methods consume solvent, time and the high cost of method development, and require the highly trained staff to operate the apparatus. On the other hand, spectrophotometers are available in most labs and easier to operate. Also, spectrophotometric methods are considered cheaper and faster. Thiazole derivatives are the most important in medicinal chemistry and have a number of characteristics, such as

1. built-in biocidal unit

2. easy metabolism of compounds

3. Enhanced lipid solubility with hydrophilicity[10].

Thiazole is widely used as anti-inflammatory drugs[11], sulfathiazole as the antimicrobial agent[12, 13], penicillin that has a thiazole ring in their structure as Antibiotics[14], Antioxidant[15], Antiviral like Ritonavir drug[16], and anti-cancer properties[17, 18]. They are also used in the treatment of Alzheimers disease, hypertension[19], Anti-allergies[20], cytotoxicity[21], and Anti-HIV[22]. Antimicrobial activities of some substituted thiazole derivatives due

*Corresponding author e-mail: ***rubaf1983@uomustansiriyah.edu.iq*** ; (Ruba Fahmi Abbas).
Receive Date: 20 September 2019, Revise Date: 11 October 2019, Accept Date: 02 June 2020
DOI: 10.21608/EJCHEM.2020.17136.2053

to the toxophoric unit (S-C=N)[23]. The aim of this study is to develop rapid, accurate, precise, and simple spectrophotometric methods for the determination of paracetamol in pharmaceuticals tablets in Iraqi markets.

.

2. Experimental

2.1. Apparatus and Chemical

Infrared spectra were recorded on a Shimadzu model FTIR-8400. 1HNMR spectra were obtained with Bruker spectrometer model at 300 MHZ Ultra-shield in DMSO-d6 solution with the TMS as an internal standard. Mass spectra were recorded on a Shimadzu GCMS-QD 1000EX, The melting point was measured by using Hot-stage Gallen Kamp melting point apparatus uncorrected, UV-visible spectrophotometer (Shimadzu, Japan) with 1 cm quartz cells, Paracetamol pharmaceutical was a sample gift from the state company for Samarra drugs factory, Iraq (SDI), sodium nitrite (Merck), sodium hydroxide (BDH, UK) and Doliprane® tablets which were labelled to contain 500 mg of paracetamol per tablet.

2.2. General Procedure for the Synthesis of Compounds 1-3

2.2.1. Synthesis of 4-nitro[(chloro acetyl) amino] benzene (1)[24]

A mixture of 4-nitroaniline (1 gm, 10 mmol) and chloro acetyl chloride (10 mmol) in dimethylformamide (20 ml), and anhydrous potassium carbonate was heated under reflux for 5-8 hrs. The product was poured into ice water (200 ml). The solid mass was filtered and recrystallized from ethanol. Green crystals, yield 70%,m.p 148-150 ºC; IR (KBr, cm^{-1}): 3228(N-H), 3072 (C-Har), 2941 (C-Hal), 1685 (C=O), 1597, 1502(C=C) 1HNMR(300 MHZ, DMSO-d6): δ(ppm) 7.6-8.5(4H, M, aromatic), 11.0(1H, s, NH), 4.5(2H,s, CH_2), GCMS m/z: 214.5(M+H)+ for $C_8H_7ClN_2O_3$.

2.2.2. Synthesis of 4[(2-amino-1,3 thiazole-4-yl) amino] nitro benzene(2)[25]

4-nitro[(chloro acetyl) amino] benzene(0.01 mol) and thiourea (0.01mol) have been dissolved in (30ml) ethanol and refluxed for 10 hrs. The mass obtained was filtered and recrystallized from methanol, yellow crystals, yield 50%; m.p 207-208 ^{0}C; IR(KBr, cm^{-1}): 3481, 3358 (NH_2), 3066(C-Har), 1627(C=N), 1595, 1491(C=C),1HNMR(300 MHZ, DMSO-d6): δ(ppm) 7.2-7.8(4H,m, H aromatic), 7.3 (1H, d, Hthiazol), 7.5(2H, s, NH_2), GCMS m/z: 236 (M+H)+ for $C_9H_8SN_4O_2$.

2.2.3. Synthesis of 4[(2-amino-1,3 thiazole-4-yl)] nitro benzene diazenylN-acetyl –para amino phenol(4)

A well-stirred solution of 4[(2-amino-1,3 thiazole-4-yl) amino] nitrobenzene(0.5gm, 2 mmoles) in 5ml of concentrated HCl was cooled in an ice-salt bath and diazotized with a cold solution of sodium nitrite (0.23gm, 2mmol) in 2ml HCl. The above reaction mixture was stirred for two hours at the same temperature. The cold diazonium salt solution obtained was added to the well-stirred solution of coupling compound in dilute NaOH solution. The resulting solution was stirred for additional three hours at 0-5ºC. This coupling is associated immediately a yellow-orange colour of the reaction mixture. The pH of the reaction mixture was adjusted to 6-7 by adding a solution of sodium bicarbonate. All reactions were monitored by a thin-layer chromatography (TLC) using silica gel. The precipitate formed is collected by filtration and washing with water and ethanol. The precipitate crude azo product was recrystallized from ethanol. Yellow crystals, yield 75%, m.p128-130-0C; IR(KBr, cm^{-1}): 3137 (N-H), 3090(C-Har), 2921 (C-Hal), 1665 (C=O), 1521(N=N) 1HNMR(300 MHZ, DMSO-d6): δ(ppm) 7.6-8.5(4H , M, H aromatic), 14.6(1H, s, NH,Hydrazone), 4.1-3.6(2H, s, $-OCH_3$).

2.3. Materials and Methods for the Diazotization Reaction

2.3.1. HCl (1 $mol.L^{-1}$) was prepared by dilution (15.4 ml) of concentrated hydrochloric acid in distilled water in 250 ml volumetric flask.

2.3.2. $NaNO_2$ (0.02 $mol.L^{-1}$) stock solution was prepared by dissolving (1.38 gm) of the sodium nitrite in distilled water in 25 ml volumetric flask.

2.3.3. NaOH (3 $mol.L^{-1}$) stock solution was obtained by dissolving (12 gm) of the sodium hydroxide in distilled water in 100 ml volumetric flask.

2.3.4. Paracetamol (1000 $mg.L^{-1}$) stock solution was prepared in distilled water in 250 ml volumetric flask; working solutions at a concentration (100 $mg.L^{-1}$) were prepared by aqueous dilution in 250ml volumetric flask.

2.3.5. Preparation of diazonium salt (chromogenic reagent): (0.02 mol.L^{-1}) solution of 4-[(2-amino-1,3thiazol-4-yl) amino] nitro benzene, used as a chromogenic reagent, was prepared by dissolving (0.118 gm) of 4-[(2-amino-1,3thiazol-4-yl)amino] nitro benzene in (10 ml) of (1 mol.L^{-1}) HCl in 25 ml beaker and transferred to 25 ml volumetric flask. Finally, (8 ml) of (0.02 mol.L^{-1}) $NaNO_2$ was added, in an ice bath at 0-5^{0}C was used for the completion of the reaction, and the flask was completed with distilled water.

2.3.6. Preparation of sample: one tablet market pharmaceutical containing paracetamol was obtained from local pharmacies in Baghdad City. (0.025 gm) of Doliprane® tablets was weighed out and dissolved in distilled water, and then transferred to 250ml volumetric flask to get a concentration equals to 100 mg.L^{-1}. Sample solutions at two concentrations of 15 mg.L^{-1} and 20 mg.L^{-1} were then prepared by dilution (3 ml) and (4 ml) in 20 ml volumetric flask.

2.3.7. Preparation of calibration graph: (1-5 ml) of the standard solutions (100 mg.L^{-1}) containing (5-25 mg.L^{-1}) of paracetamol were transferred into a series of 20ml volumetric flasks and cooled at 0-5ºC in an ice bath, and then 2 ml of 3 mol.L^{-1}NaOH was added with shaking. Then (1 ml) of the diazonium salt (chromogenic reagent) was added, the mixtures were allowed to stand in an ice bath for 5 min., and then were made up to the mark with distilled water. The absorbance of the orange product was measured against a reagent blank at 425 nm.

2.3.8. Job's method: an aliquot (2, 3, 4, 5, 7, and 8 ml) of (100 mg.L^{-1}) of paracetamol was added to a series of 20ml volumetric flask, to each flask, 2 ml of 3 mol.L^{-1}NaOH solution and (6,5, 4, 3, 1, and 0 ml) of (0.02 mol.L^{-1}) diazonium salt reagent were added, and then diluted to the mark with the distilled water and after 5 min., the absorbance was measured at 425 nm.

3. Results and Discussion

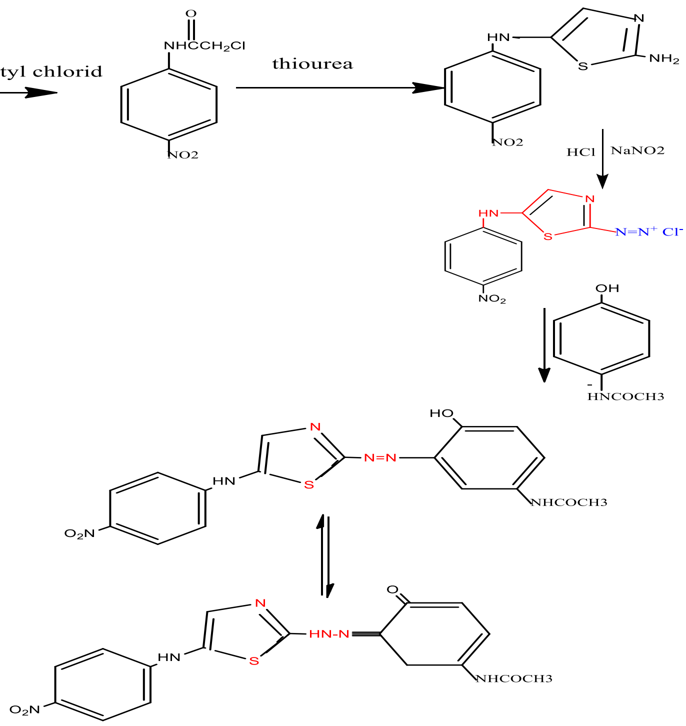
3.1. Chemistry

The synthesis of all new derivatives is shown in Scheme 1.

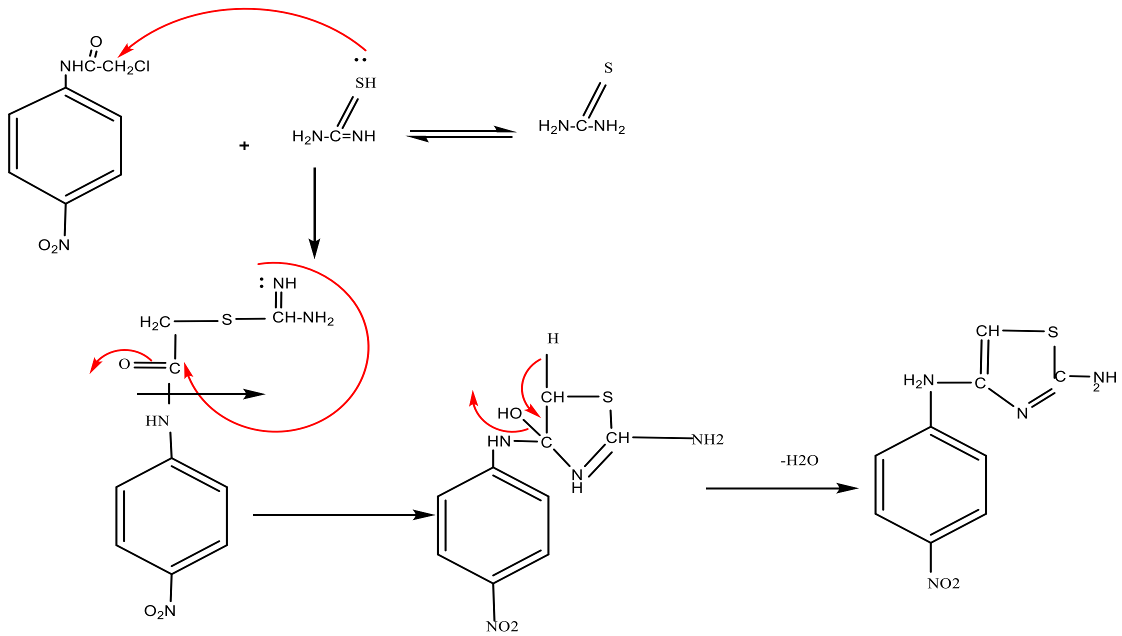
The 4-nitro [(chloro acetyl) amino] benzene(1) was prepared by reaction of equivalates moles of 4-nitrobenzene with chloro acetyl chloride using dry DMF as a solvent in K_2CO_3 medium. The FTIR spectrum of compound(1)shows the disappearance of NH_2 stretching peak with the appearance of C=O acyl absorption bands at 1685 cm^{-1} and absorption band the C-H aliphatic absorption band at 2941 cm^{-1}, and a new band in the region 710-775 cm^{-1}. The derivative 2- amino -1,3thiazol -4-yl (2) was synthesized by reaction of 1 with thiourea in good yield. The proposed mechanism of this reaction is shown in scheme 2.

This compound characterized by FTIR, IHNMR and mass spectroscopy. The mass spectrum of compound 2 (Figure 1) and 1HNMR for the same compound (Figure 2), appeared the peaks a singlet at 7.5 ppm due to NH_2 protons; the hydrogen atom of the thiazol ring appeared as a doublet at δ 7.3ppm, and multiplet signals in the region δ 7.2-7.8 ppm may be attributed to the fourth aromatic protons.

The azo dye was synthesized by coupling of 4[(2-amino-1,3thiazole-4-yl)amino] nitrobenzene with diazotized 4-hydroxyphenyl acetamide at 0-5ºC.The structure of this dye was characterized by UV-visible, FTIR and 1HNMR spectroscopy.IR spectra of the compound were recorded as KBr pellets in the region 4000-400cm^{-1}. Strong absorption bands appeared in the area 3300-3100cm^{-1} and 3480-3300cm^{-1} were assigned to NH and phenolic OH groups, respectively. Weak bands were observed at 1552-1512cm^{-1} and 1643-1610cm^{-1} due to the presence of (N=N)and (C=N) groups, respectively. 1HNMR spectra confirm the structure of azo dye (Figure 3).The proton –NH hydrazone or OH phenolic proton, according to resonance state shown in scheme1, appeared as a singlet at 14.6 ppm, and aromatic protons were resonated as multiplet in the region 7.01-8.61 ppm. The protons of –OCH3 group were observed in the region 4.1-3.6ppm.



Scheme 1: Synthesis of azo dye derivative



Scheme 2: The cyclization mechanism of synthesized amine

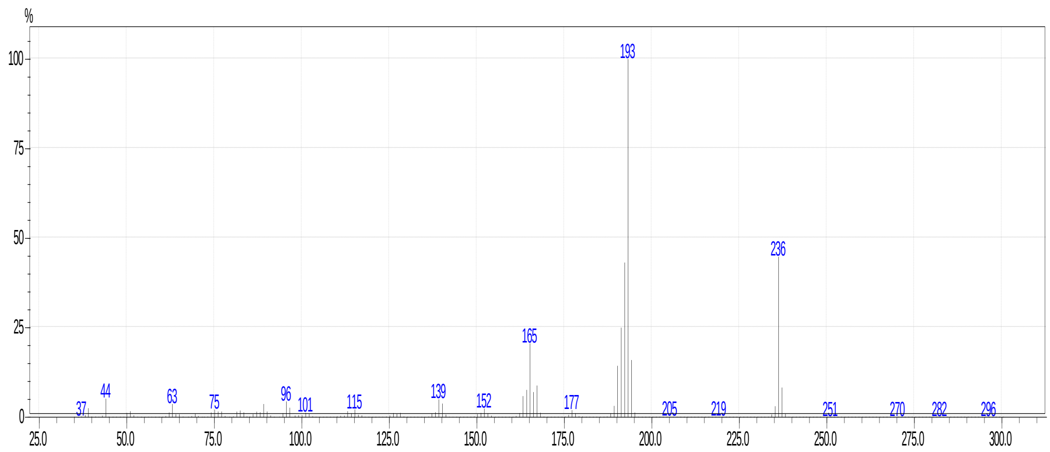


Figure1: GC-mass spectrum of compound no.2

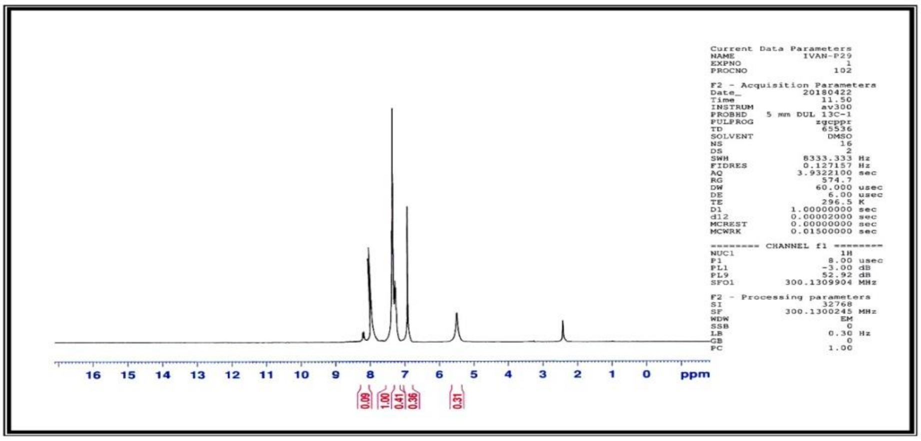


Figure2: [1]HNMR spectrum of compound no.2

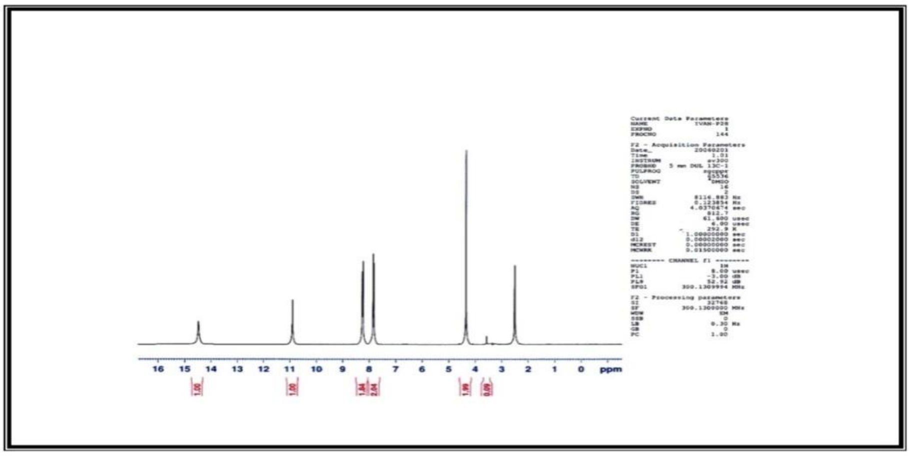


Figure3: [1]HNMR spectrum of compound no. 4

3.2. Antimicrobial Activity

The activity of antibacterial and antifungal was studied by using cup-plate agar diffusion method in a different concentrations (1000, 500, 100 and 50 $mg.L^{-1}$) [26, 27]. The inhibition zones were measured in mm; amoxicillin (500 $mg.ml^{-1}$) was used as a standard drug for antimicrobial activity. The compound was screened for antibacterial activity against *Escherichia coli*, *Psreudomonasaeruginosa*, *streptococcussp*, and *Staphylococcus aureus* in Muller Hinton agar, and the results shown in table1 indicate that azo dye derivative shows a very good activity against *condid fungi* more than the activity of an amoxicillinin the same concentration, then comparing these results with the standard drug (amoxicillin) for antibacterial activity, it is observed that when the concentration of azo dye is high, the antibacterial activity is increased except when the antibacterial activity is against *Psreudomonasaeruginosa*

Table1. Antimicrobaial activity of azo dye derivative represented by% inhabitation against different bacterial and fungl species

Conc. $mg.L^{-1}$	Inhibition Zone against(in mm)				Candida albicans
	Gram negative		Gram positive		
	E.Coil	*Psed. aerugi*	*S. aureus*	*Streptococcus*	
1000	15	-	15	16	25
500	13	-	14	14	22
100	12	-	10	12	20
50	10	-	7	10	12
Amoxicillin	36	-	15	28	12

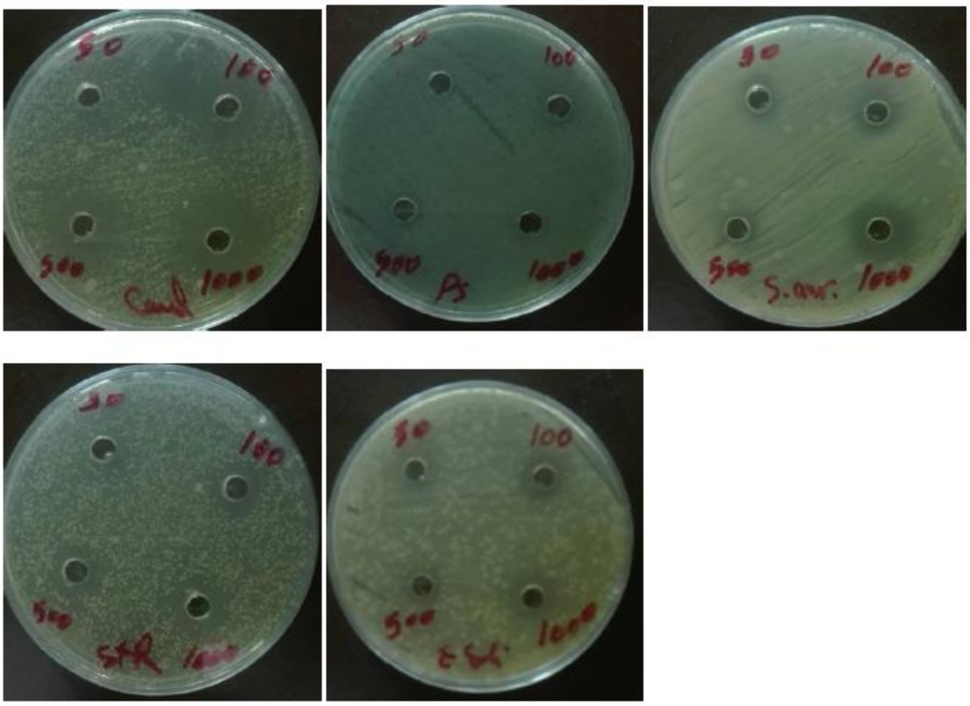


Figure 4: Activity of *Escherichia coli, Psreudomonasaeruginosa, streptococcussp, Staphylococcus aureus* andcondid fungiat four different concentrations (1000, 500, 100, and 50 $mg.L^{-1}$).

3.3. Spectrophotometric study

3.3.1. Effect of Experimental Conditions

The experimental conditions for the determination of paracetamol have been studied. The diazotization coupling reaction occurred in an acidic medium and HCl of concentration 1 mol.L^{-1} is selected. The effect of different volumes (6-12 ml) of 1mol.L^{-1}HCl solution has been studied and 10 ml volume seems to be optimum for an intense azo dye colour (Figure 5a). Effects of the volumes of 3 mol.L^{-1}NaOH have been studied between (1-4 ml), 2 ml volume of 3 mol.L^{-1} NaOH is fixed for obtaining a stable diazonium ion(Figure 5b). The volume of sodium nitrite is varied between (4-10 ml) of 0.02 mol.L^{-1} sodium nitrite in distilled water. The results (Figure 5c) show that 8 ml of 0.02 mol.L^{-1} sodium nitrite gives a maximum absorbance at 425 nm. When various volumes of diazonium salt (chromogenic reagent) solution were added to 10 mg.L^{-1} paracetamol solution, 1ml of 0.02 mol.L^{-1}diazonium salt is found enough to get a stable orange-colour for the preparation of azo dye (Figure 5d). Finally, different periods of time from 0-30 min., have been observed for the diazotization reaction. The results (Figure 5e) show that the reaction is completed in 5 min.

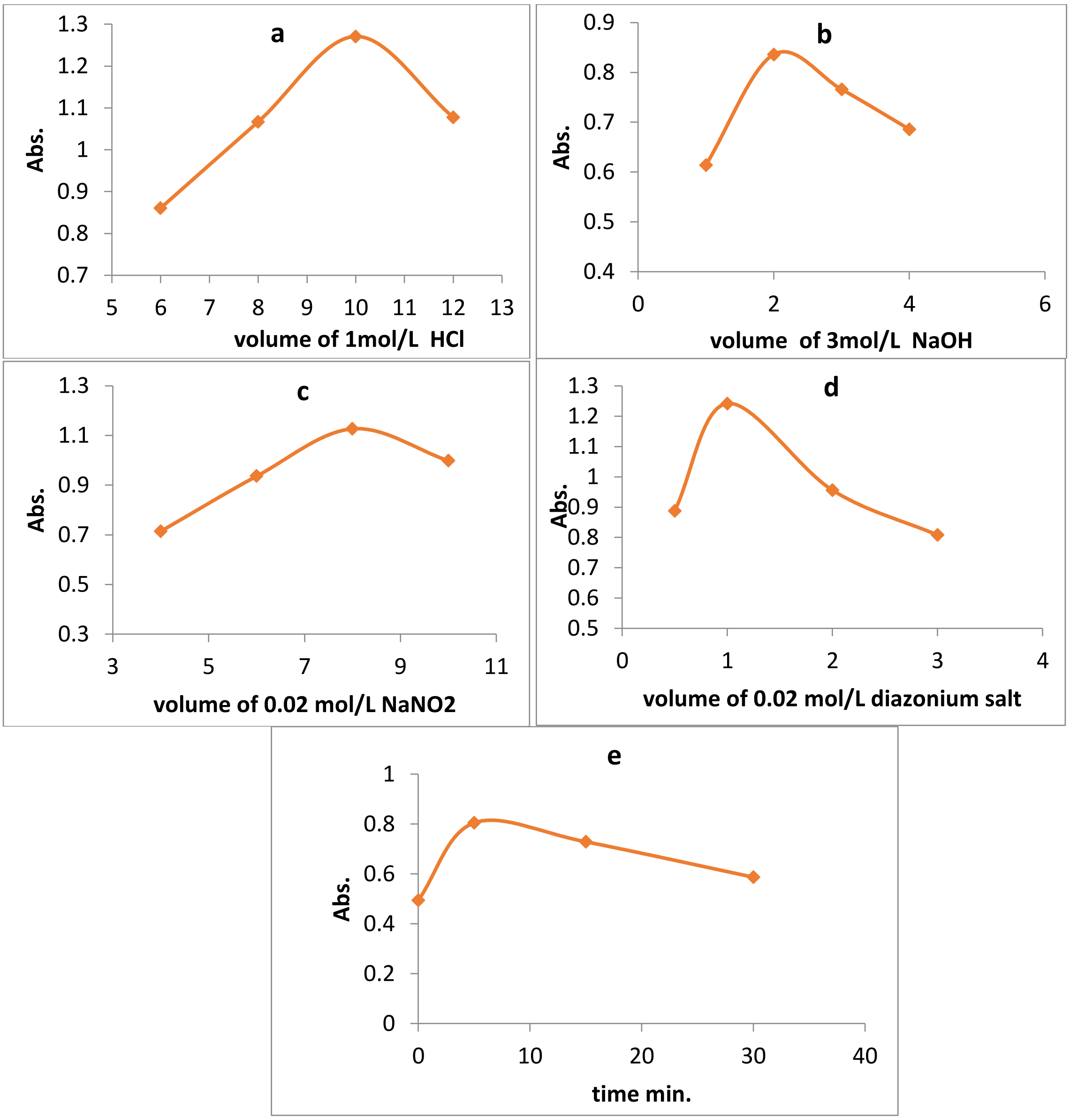


Figure 5: The Effect of experimental conditions of coupling reaction; (a) acid, (b) base, (c) sodium nitrite, (d) diazonium salt, and (e) time.

3.3.2. Absorbance Spectra and Calibration Curves

4-[(2-amino-1,3thiazol-4-yl)amino]nitro benzene is primary an aromatic amine which is reacted with sodium nitrite in acidic medium at low temperatures 0-5ºc to form diazonium salts[28], then a reaction with paracetamol (phenolic drug) in the alkaline medium to obtain an orange product.

Figure 6 (a and b) shows that the ʎ max is recorded at wavelength 425 nm and overly absorption spectra of a new azo dye product at the concentration range from 5 $mg.L^{-1}$ to 25 $mg.L^{-1}$.

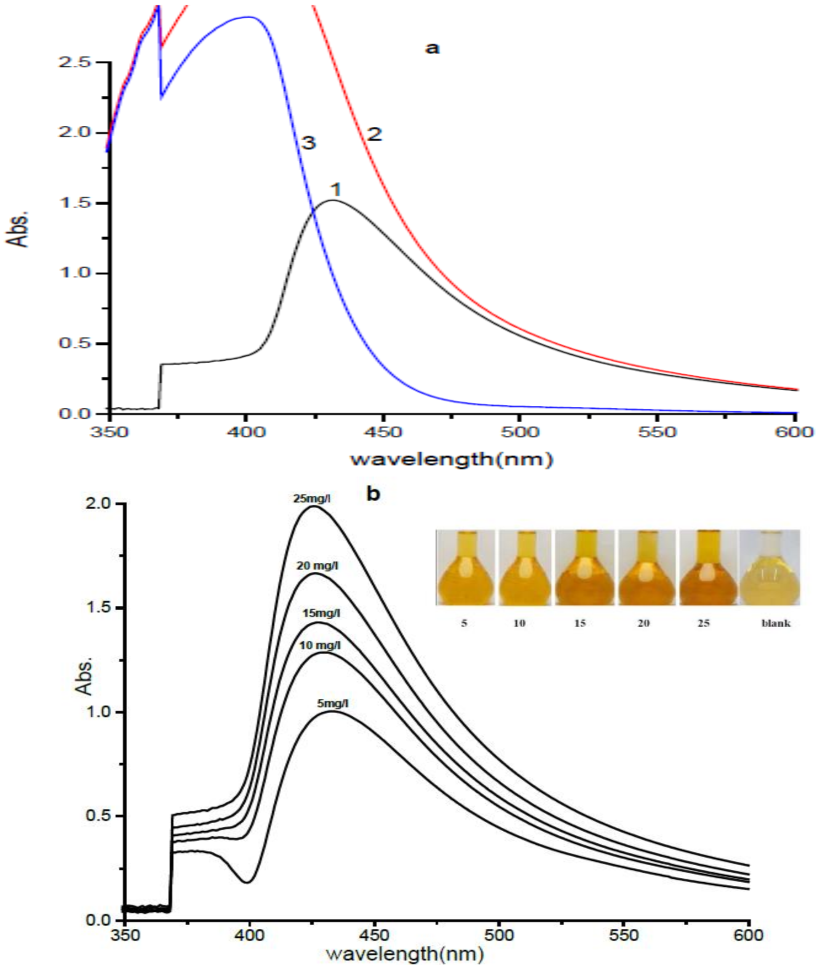


Figure6: Absorbance spectra of (a)1- the azo dye coloured product measured against blank, 2- the blank against distilled water, and 3-the azo dye product against distilled water, (b) Overly absorption spectra of a new azo dye formed between the paracetamol and4[(2-amino-1,3 thiazole-4-yl) amino] nitro benzene

A calibration curve was constructed at the wavelength 425 nm and the regression equations were calculated and found to be:

$D0 = 0.047C+0.715$ at ʎ=425 nm,

where D0 is the peak amplitude for the spectra of a new azo dye, and c is the paracetamol drug concentration in $mg.L^{-1}$. The validation data were obtained from the calibration curves for the proposed methods as shown in Table 2. The small values for the most parameters such as slope, intercept and Sandell's sensitivity that referred to the high reliable precision of the proposed methods in this study.

Table 2. The validation parameters obtained from the regression equations.

Parameter	Value
Linearity $mg.l^{-1}$	5 - 25
Regression equation(y)	Y= 0.047 × +0.715
Correlation of determination (r^2)	0.998
Slope (b)	0.047
Intercept (a)	0.715
Conf. limit for slope b± t_{sb}	0.047 ± 41.890
Conf. limit for Intercept a ± t_{sa}	0.715 ± 37.701
Standard deviation of Intercept S_a	0.019
Molar absorptivity Ɛ($L.mol^{-1}.cm^{-1}$)	16464.646
Sandell's sensitivity ($mg.cm^{-1}$)	0.0235
Limit of detection LOD ($mg.L^{-1}$)	1.334

LOD = 3.3×SD_b/S, SD_b= the standard deviation of intercepts of regression lines

3.3.3. Stoichiometric Ratio Determination

The stoichemistry of the diazotization reaction between the chromogenic agent 4-[(2-amino-1,3thiazol-4-yl)amino]nitro benzene and paracetamol was investigated using job's method. (Figure 7) shows that the orange azo dye is formed in the ratio1:1(chromogenic reagent{R}:}: paracetmol{D})

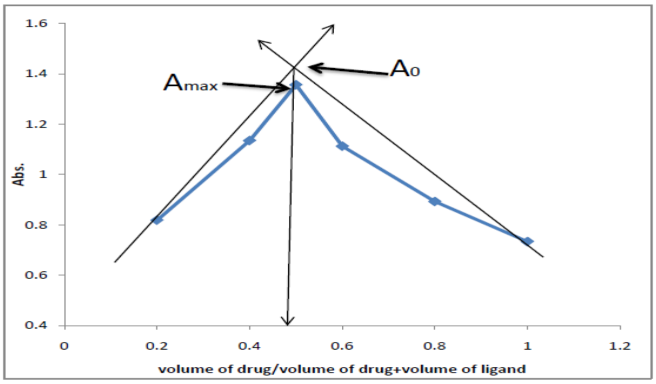


Figure7: Job plot for diazotized the new reagent 4-[(2-amino-1,3thiazol-4-yl)amino]nitrobenzene coupled with paracetamol drug.

Vargas method is used for the calculation of the stability constant K, as in the following equation[29, 30]:

$$K = \frac{c - \frac{A\alpha}{\epsilon}}{n^n (\frac{A\alpha}{\epsilon})^{n+1}}$$

where Є is a molar absorpitivity (16464.646), n is the no. of the chromogenic reagent 4-[(2-amino-1,3thiazol-4-yl)amino] nitro benzene (0.000099), and Aα is the absorbance of the part of dissociated constant of the orange azo dye product (0.07).The following equation is used to find the value of the Aα:

$A\alpha = A_0 - A_{max}$

where A0 is a value of the theoretical absorbance which is obtained from job plot (1.45), and Aα is a maximum absorbance which is obtained from job's experimental method (1.38). The stability constant K is found to be 52.45674 × 10^{+5} $L.mol^{-1}$, which indicates that the azo dye product is stable.

3.3.4. Accuracy and Precision

The accuracy and precision of the new azo dye product have been calculated at two concentration levels of paracetamol by analyzing three replicate samples of each concentration. The obtained high percentage recoveries with low relative standard deviation have indicated good accuracy and precision of the proposed spectrophotometric method as shown in table3.

Table 3. Accuracy and Precision for the Proposed Method.

Method	Amount of paracetamol $mg.l^{-1}$		E*%	Rec*% = E%+100	Average of Rec.%	RSD*%
	Taken	Found				
Suggest method	20	19.940	-0.003	99.997	99.9945	0.742
	15	14.880	-0.008	99.992		0.691

* Average of three times, E% = relative error = $=\frac{found-taken}{taken}\times 100$, Rec% =recovery, and RSD%=relative standard deviation.

3.3.5. Application

A new method has been applied for the analysis of paracetamol in commercial Doliprane® tablets (500 mg paracetamol per tablet). Three replicate determinations were made. The results obtained are in good agreement with label claims as shown in Table4. The comparison of a limit of detection LOD of the proposed method with different methods reported in the literature is given in table 5. The proposed method shows a lower or comparable detection limit. For these reasons, the proposed method can be generally applied for routine analytical laboratories.

Table 4: The relative error, recovery and relative standard deviations of the commercial Doliprane® tablets at 20and 15 $mg.L^{-1}$

Pharmaceutical commercial tablet	Amount of paracetamol $mg.L^{-1}$		E*%	Rec*%= E%+100	Average of Rec.%	RSD*%
	Taken	Found				
Doliprane® tablets- Farance.	20	20.16	0.008	100.008	100.001	0.440
	15	14.920	-0.005	99.994		0.922

* Average of three time, E% = relative error = $=\frac{found-taken}{taken}\times 100$, Rec% =recovery, and RSD%=relative standard deviation.

Table 5: The comparison of the values of Limit of detection of the proposed method with different methods reported in literature.

method	LOD	Ref.
HPLC	0.08 $mg.L^{-1}$	31
Ratio subtraction coupled with ratio difference (RSDM)	0.152 $mg.L^{-1}$	32
Spectrophotometric using 2,5-Dihydroxy Benzyldehyde	0.0479 $mg.L^{-1}$	33
Continuous wavelet transformation (CWT)	0.247 $mg.L^{-1}$	34
Spectrophotometric using new synthesis chromogenic reagent 4-[(2-amino-1, 3thiazol-4-yl)amino]nitro benzene	1.334 $mg.L^{-1}$	present work

4. Conclusion

In this paper, paracetamol reacted as azo-coupling with a new chromogenic reagent 4-[(2-amino-1, 3thiazol-4-yl)amino]nitro benzene in alkaline medium and has spectrophotometric characteristics suitable for application for the determination of the drug by a spectrophotometric method. The present method of the azo dyes formation has the advantage of being fast, novel, simple and very sensitive, and applicable over a wide concentration range with good precision and accuracy.

5. Acknowledgements

This study is supported by the Chemistry Department, College of Science, Al-Mustansiriyah University, in Baghdad, Iraq.

References

1. Athersuch T. J., Antoine D. J., Boobis A. R., Coen M., Daly A. K., Possamai L., Nicholson J. K. and Wilson I. D., Paracetamol metabolism, hepatotoxicity, biomarkers and therapeutic interventions: a perspective. Toxicology research. 7(3): 347-57(2018).
2. Zhu A., Xu G., Li L., Yang L., Zhou H. and Kan X., Sol-gel imprinted polymers based electrochemical sensor for paracetamol recognition and detection. Analytical Letters. 46(7): 1132-44(2013)
3. Filik H., Aydar S. and Avan A. A., Poly (2, 2′-(1, 4-phenylenedivinylene) Bis-8-hydroxyquinaldine) Modified Glassy Carbon Electrode for the Simultaneous Determination of Paracetamol and p-Aminophenol. Analytical Letters. 48(16): 2581-96(2015)
4. Cheraghi S., Taher M. A. and Karimi-Maleh H., A novel strategy for determination of paracetamol in the presence of morphine using a carbon paste electrode modified with CdO nanoparticles and ionic liquids. Electroanalysis. 28(2): 366-71(2016)
5. Dewani A. P., Dabhade S. M., Bakal R. L., Gadewar C. K., Chandewar A. V. and Patra S., Development and validation of a novel RP-HPLC method for simultaneous determination of paracetamol, phenylephrine hydrochloride, caffeine, cetirizine and nimesulide in tablet formulation. Arabian journal of chemistry. 8(4): 591-8(2015)
6. Abed S.S. and Sinan R. Nitroso-R-salt as a sensitive spectrophotometric reagent for the determination of paracetamol in pharmaceutical preparations. Baghdad Science Journal. 6(3): 570-7(2009).
7. Ahmed R. K., Muhammad S.S. and Khodaer E.A., Spectrophotometric Determination of Paracetamol in bulk and Pharmaceutical Preparations. Baghdad Science Journal. 12(2): 317-23(2015).
8. Ashour A., Hegazy M. A., Abdel-Kawy M. and ElZeiny M. B., Simultaneous spectrophotometric determination of overlapping spectra of paracetamol and caffeine in laboratory prepared mixtures and pharmaceutical preparations using continuous wavelet and derivative transform. Journal of Saudi Chemical Society. 19(2): 186-92(2015).
9. Youssef S. H., Hegazy M. A., Mohamed D. and Badawey A. M., Analysis of paracetamol, pseudoephedrine and cetirizine in Allercet Cold® capsules using spectrophotometric techniques. Chemistry Central Journal. 12(1): 67 (2018).
10. Mishra R., Sharma P. K., Verma P. K., Tomer I., Mathur G. and Dhakad P. K. Biological Potential of Thiazole Derivatives of Synthetic Origin. Journal of Heterocyclic Chemistry. 54(4): 2103-16(2017).
11. Poojari S. and Naik S., Anti-inflammatory, antibacterial and molecular docking studies of novel spiro-piperidine quinazolinone derivatives. Journal of Taibah University for Science. 11(3): 497-511(2017).
12. Salem M. A., Synthesis of new thiazole, bithiazolidinone and pyrano [2, 3-d] thiazole derivatives as potential antimicrobial Agents. Croatica Chemica Acta. 90(1): 7-15(2017).
13. Bikobo D. S., Vodnar D. C., Stana A., Tiperciuc B., Nastasă C., Douchet M. and Oniga O., Synthesis of 2-phenylamino-thiazole derivatives as antimicrobial agents. Journal of Saudi Chemical Society. 21(7): 861-8(2017) .
14. Sekhri L., Kadri M. L., Samira C. and Senigra M., Synthesis of penicillin derivatives and study of their biological antibacterial activities. Biomedical and Pharmacology Journal. 1(2): 257-64(2015).
15. Jaishree V., Ramdas N., Sachin J. and Ramesh B., In vitro antioxidant properties of new thiazole

derivatives. Journal of Saudi Chemical Society. 16(4): 371-6(2013).

16. Rashad A. E., Hegab M. I., Abdel-Megeid R. E., Micky J. A. and Abdel-Megeid F. M., Synthesis and antiviral evaluation of some new pyrazole and fused pyrazolopyrimidine derivatives. Bioorganic and medicinal chemistry. 16(15): 7102-6(2008).
17. Ozen C., Unlusoy M. C., Aliary N., Ozturk M. and Dundar O. B., Thiazolidinedione or Rhodanine: A Study on Synthesis and Anticancer Activity Comparison of Novel Thiazole Derivatives. Journal of Pharmacy and Pharmaceutical Sciences. 20(1): 415-27(2018).
18. Kayagil I. and Demirayak S., Synthesis and anticancer activities of some thiazole derivatives. Phosphorus, Sulfur, and Silicon. 184(9): 2197-207(2009).
19. Sowjanya C., Swamy S., Gomathi, S. and Ashok Babu K., Synthesis, Chemistry and Anti-Hypertensive Activity of Some New Thiazole-Thiadiazole Derivatives. International Journal Of Advanced Research In Medical and Pharmaceutical Sciences. 1(1): 6-10(2016).
20. Yurttaş L., Özkay Y., Karaca Gençer H. and Acar U., Synthesis of some new thiazole derivatives and their biological activity evaluation. Journal of Chemistry. 2015: 1-7(2015).
21. Rahman F. S., Yusufzai S. K., Osman H. and Mohamad D., Synthesis, characterisation and cytotoxicity activity of thiazole substitution of coumarin derivatives (characterisation of coumarin derivatives). Journal of Physical Science. 27(1):77(2016).
22. Bhavsar D., Trivedi J., Parekh S., Savant M., Thakrar S., Bavishi A., Radadiya A., Vala H., Lunagariya J., Parmar M. and Paresh L., Synthesis and in vitro anti-HIV activity of N-1, 3-benzo [d] thiazol-2-yl-2-(2-oxo-2H-chromen-4-yl) acetamide derivatives using MTT method. Bioorganic and medicinal chemistry letters. 21(11): 3443-6(2011).
23. Abdel-Sattar N. E., El-Naggar A. M. and Abdel-Mottaleb M. S., Novel Thiazole Derivatives of Medicinal Potential: Synthesis and Modeling. Journal of Chemistry. 2017:1-11(2017).
24. Raju G. B., Mahesh M., Manjunath G. and Ramana P.V., Synthesis of New Pyrazole Derivatives Containing 2-Methylquinoline Ring System: A Novel Class of Potential Antimicrobial Agents. Chemical Science. 2016(5): 1(2016).
25. Gouda M. A., Eldien H. F., Girges M. M.and Berghot M. A., Synthesis and antitumor evaluation of thiophene based azo dyes incorporating pyrazolone moiety. Journal of Saudi Chemical Society. 20(2): 151-7(2016).
26. Sonia Jas M. J., Marimuthu G. and Prithivirajan B., Hydrazone Analogues: Molecular Modeling, Synthesis, In- vivo Anti-Nociceptive Activity and in-vitro Antimicrobial Activity. Egyptian Journal of Chemistry. 62(8): 1441 – 1450(2019)
27. Abdelshafeek K. A., Elgendy H. A., ElMissiry M. M., Seif ElNasr M. M., Isolation and Identification of Some Chemical Constituents and Antimicrobial Activity of Two Lamiaceae Plants Growing in Saini. Egyptian Journal of Chemistry. 59(1): 21-31(2016).
28. Abbas R. F., Wheeb A. A. and jasim A. A., Spectrophotometric determination of doxycycline hyclate in pure and capsule using diazotization reaction. Al-Mustansiriyah Journal of Science. 27(5): 50-4(2017).
29. Garcia-Vargas M., Bautista J. M. and De Toro P., Analytical possibilities of pyridine-2-acetaldehyde benzoylhydrazone as a chromogenic reagent. Microchemical Journal. 26(4): 557-68(1981).
30. Abbas R. F., Spectrophotometric Determination Stability Constant by Classical and Modified Varagas Equations for Procaine Penicillin G using Diazotization Reaction Depending On Stoichiometric Curves. International Journal of ChemTech Research. 10(3): 485-496(2017).
31. Borahan T., Unutkan T., Şahin A. and Bakırdere S., A rapid and sensitive reversed phase-HPLC method for simultaneous determination of ibuprofen and paracetamol in drug samples and their behaviors in simulated gastric conditions. Journal of separation science. 42(3): 678-683(2019).
32. Youssef S. H., Hegazy M. A. M., Mohamed D., and Badawey A. M., Analysis of paracetamol, pseudoephedrine and cetirizine in Allercet

Cold® capsules using spectrophotometric techniques. Chemistry Central Journal. 12(1): 67(2018).

33. Younis M. S. and Othman N. S., Using of 2, 5-Dihydroxy Benzyldehyde in Spectrophotometric Assay of Paracetamol in Pharmaceutical Preparations. International Journal of Recent Research and Review. XI(2): 10-17(2018).

34. Hegazy M. A., Elshahed M. S., Toubar S. S. and Helmy M. I., Efficient processing of Single and Multiple Spectral Variables for Resolution and Quantitation of Paracetamol, Chlorzoxazone and Diclofenac. Journal of Advanced Pharmacy Research. 2(4): 269-282(2018).