New spectrophotometric determination sulfamethaxazole drug via various analytical techniques in pharmaceutical formulation

[1]Dalia M. Jameel and [2]Nisreen Kais Abood

[1] Department of Chemistry, College of Science, Al-Nahrain University ,Baghdad Iraq
[2]Department of Chemistry, College of Science, Mustansiriyah University Baghdad, Iraq
Daliamahmood34@yahoo.com

Abstract

The first method on the oxidative coupling Amoxixilin reaction with 2,4-dinitrophenyl hydrazine and Sodium Iodate diluted to produce a soluble red-Orange dye product was devised to estimate the Amoxicillin in bulk and pharmaceutical preparation. The concentration range is between 1 and 50 g/mL. Beer's law is met by the product in terms. With a molar absorptivity of 6.2×10^{3}L.mol-1.cm-1 and a correlation coefficient of 0.9995, Sandell's sensitivity is 0.059g.cm^{-2} at 475 nm. A second technique for determining a trace amount in an aqueous solution product is called cloud point extraction (CPE), and it is based on measurements made with a UV-visible spectrophotometer at a wavelength of 480 nm. The correlation coefficient was 0.9996 and the Beer's law concentration range was 0.25–6 g/mL The molar absorptivity was 103623.79 L.mol-1.cm-1. Pre-concentration 25, detection of limit 0.023 µg/mL. The distribution coefficient (D) was 158.6, and enrichment factor was 28.35. The last method is flow injection analysis, which is straightforward to estimate. The Beer's law concentration range was 2-150 µg / mL, Sandell's sensitivity is 0.13 µg.cm-2 at 475 nm, with a molar absorptivity of 2.8×10^{3} L.mol-1.cm-1 and correlation coefficient was 0.9996, it is successfully used for drug estimation. Qualities in a straightforward situation or pharmacological formulations.

Key words: Amoxicillin, Batch method, Cloud point extraction, Flow injection analysis.

Introduction

The most popular -lactam antibiotic is amoxicillin trihydrate, also known chemically as [2S-[2a,5a,6a(S)]]-6- [[Amino(4-hydroxyphenyl)acetyl]amino]-3,3

dimethyl-7-oxo-4-thia1-azabicyclo, for treating bacterial infections of the ear, nose, throat, skin, and lower respiratory tract caused by susceptible microorgan [1-4].

Antibiotic resistance is increasing as a result of widespread antibiotic us decreasing the efficacy of antibiotics and increasing bacterial populations. However, most of the Antibiotics that have been administered reach the environment through direct runoff and excretion of feces or urine as active metabolites, breakdown products, or unmodified original medicines. One of the most expensive and often used medicines is amoxicillin. Therefore, removing amoxicillin from pharmaceutical wastes has numerous positive effects on the economy and the environment. Consequently, the objective of the current study was to establish a method for identifying amoxicillin and to investigate the impacts of various the base type, complex, temperature, and impact of concentration. A For the determination, a spectrophotometric, sensitive, and selective approach has been proposed [5].

Amoxicillin, a penicillin-family -lactam antibiotic, is regarded as a necessary drug by the World Health Organization because of its pharmacological qualities that can be used to treat bacterial meningitis, sinusitis, pneumonia, pharyngitis, sepsis, and other conditions [6].

A p-hydroxyl group was added to the side chain of ampicillin, another aminopenicillin, in 1964 to create amoxicillin, which is a member of the aminopenicillins subgroup. As a result, when compared to ampicillin, Pediatricians and parents are concerned as a nationwide shortage of amoxicillin, an antibiotic frequently prescribed for kids with ear infections, bronchitis, and strep throat, strains hospitals, many of which are still suffering from the coronavirus pandemic [7-8].

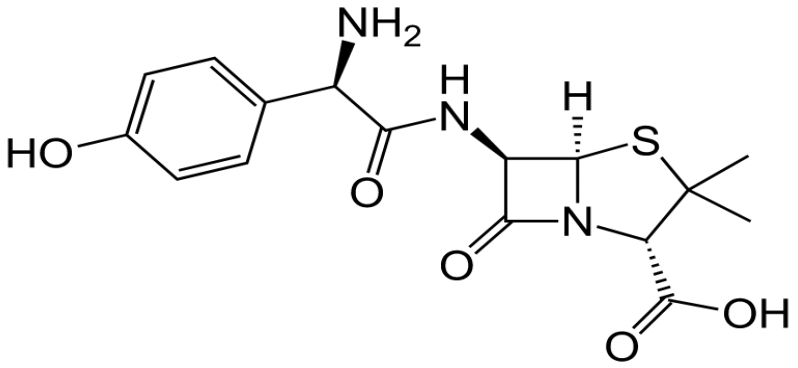


Figure 1-1: The Chemical structure of Amoxicillin tri hydrate [9]

Material sand Methods

Instrumentation

Single-beam Spectrophotometric UV -Visible 295 (Lasany-India) Quartz spectroscopic quivet in sizes of 1 cm and 0.5 cm, along with thermostatic with ultrasonic instrument Elma's water for bathing Additionally, Hans Schmidbauer Gmbh. Co.KG is used in conjunction and solute extraction. Manifold for two-channel flow injection as shown in figure2.

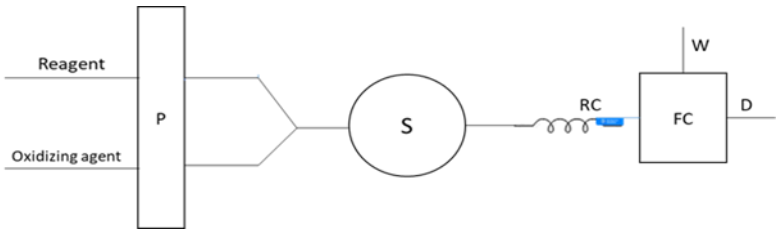


Figure 2: Scheme of the employed flow system, P: peristaltic pump, R.C: reaction coil, S: sample injection, W: waste, FC: flow cell

Chemicals and Reagents:

Amoxicillin can be provided from Samarra in Iraq (SDI) as purity 99.8%, which were of analytical purity. Amoxicillin was delivered to the quality control laboratory. (The general company for the manufacture of medicines and medical supplies India and Iraq).

Standard solution preparation with Standard Reagents Pharmaceutical

Amoxicillin solutions from the India and Iraq (1g) were carefully weighed, and the average weight was taken from each capsule. That prepares 100 mL of D.W. with 1000 ppm for each capsule.

Reagents:

Prepare the standard solution of 1000 ppm by dissolving 0. 1 mL of pure antibiotic in water then adding volumetrically to the mark, Amoxicillin was created. 0.1 g of 2, 4- di nitro phenyl hydrazine (1000 ppm) by dissolve in 100 mL

of distilled water to make a standard solution then diluted to the appropriate concentration. Preparation 5% w/v Na2SO4, 10% CTAB, 10% Triton X-114, and 4% sodium hydroxide (cetyle trimethy ammonium bromide) D.W. : 0.3644g in 100 ml 0.1g in 100ml Sodium iodate hydrate.

Making a standard solution and the necessary Reagents Standard

The solutions of pharmaceutical Amoxicillin supplied from the India and Atabay (1g) were carefully weighed; the average weight was extracted from the individual powder. That prepares 1000ppm from all powder in 100 mL D.W.A general.

Basic Technique Oxidative Coupling

The best procedure to prepare oxidative coupling reaction of Amoxicillin adding (1ml 2,4-di nitro phenyl hydrazine) to the Oxidizing agent (1000ppm, 1mL) potassium ferric cyanide with Amoxicillin drug to form the reddish- Orange solution that give absorbance at λmax 475nm.

Extraction of cloud points: a general process (CPE)

Various ranging of 0.25 to 6 µg/ mL with dye 2mL with 1mL of triton x-114, 1mL CTBA with 2mL of Na2SO4 with complete 12.5 mL distilled water. Test tube placed in the heating until formed cloudy and separation two phasas is content then transfer in the centerfuge for 2 min untile 4000m2/sec. then put in the ice water to produce the clear cloud. Add 0.5 mL ethanol with shack the tube and determined the spectrum to determine the wavelength.1000 mg/m-1 stander solutions Amoxicillin was made by dissolving 0. 1 g of pure medication dissolved in water and filling volumetrically to the mark. 100 mL flask with distilled water a standard solution of by dissolving 2,4- di nitro phenyl hydrazine (1000 g/mL) 2,4-di nitro phenyl hydrazine, 0.1 g in distilled water in a 100ml volumetric flask, dilute to the actual concentration. Preparation 4%, sodium hydroxide, 10% Triton X-114, 5% w/v Na2SO4, and 10% CTAB (cetyle trimethy ammonium bromide) 0.3644g in 100ml D.W.

General flow injection technique

0.1g in 100mL Potassium ferric cyanide.100 liters of pharmaceutical-grade Amo. were injected into the system carrier; There are three channels in this reaction's manifold.. By employing T-shaped channels, the first channel carries the Drug (Amo.), the second channel carries the reagent (2,4-di nitro phenyl hydrazine), and the third channel carries the Oxidizing agent (Potassium ferric cyanide) after which the medicine is injected. The reaction in coil (50 cm) and produced a reddish-Orange color with a maximum absorbance of 480 nm.

Result and Discussion for flow injection technique

An oxidative coupling reagent (2, 4- di nitro phenyl hydrazine) with an oxidizing agent are used to produce the reddish-Orange color with a wavelength of 475 nm (Sodium Nitrate). This technique is to increase Amo.'s sensitivity. The oxidative coupling spectra are displayed in Figure 3: 100 liters of pure or pharmaceutical Amo. were added to them results were as follows: The suggested conversion technique

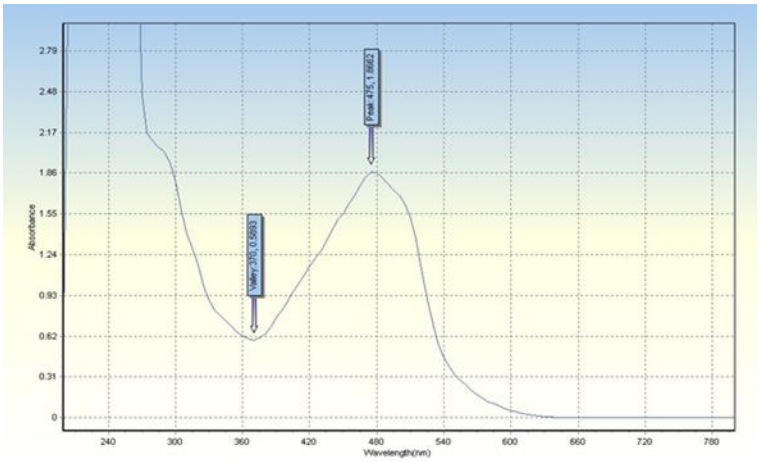
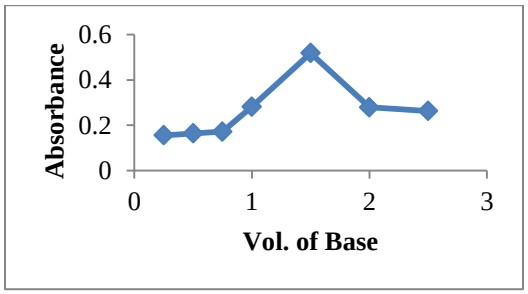


Figure 3: Absorption spectrum for 100 µg/mL Amoxicillin with the reagent against the reagent blank under optimum Conditions

The best oxidative medication pairing was studied.

Some factors, including the order of addition, have been studied in relation to colored dye absorbance. Due to the high absorption at this wave length, the

addition of reagent + oxidizing agent + base + Drug has the best effects. The best absorption occurs in base media, was indicated in figure 4, Then effected the character of the media. And the coupling reagent's volume, the best reagent volume in 1.5 mL is show in figure 5. The best volume of the oxidize was 1.5 mL at the conclusion of the oxidizing agent's impact in base medium (1.5) mL, as showed in figure 6. Effect of Temperature; best temperature is 25^0C.



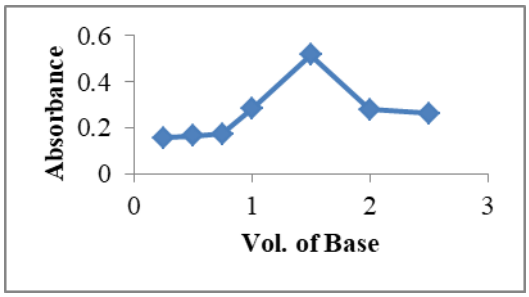
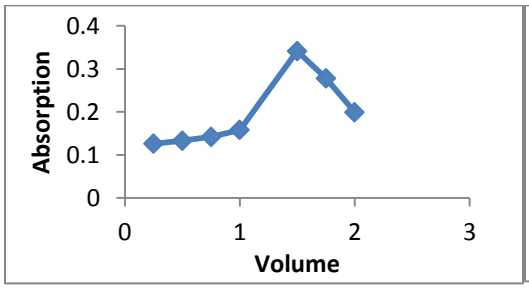


Figure 4: Effect the base Volume **Figure 5: Effect the reagent Volume**



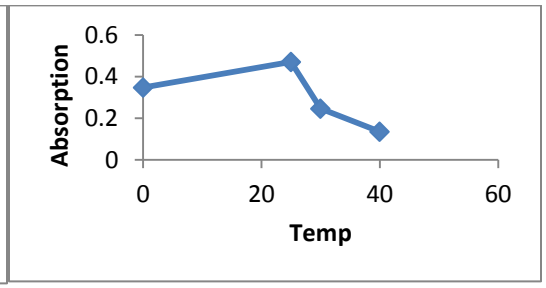


Figure6: Effect Oxidizing Volume of agent **Figure 7: Effect the Temperature**

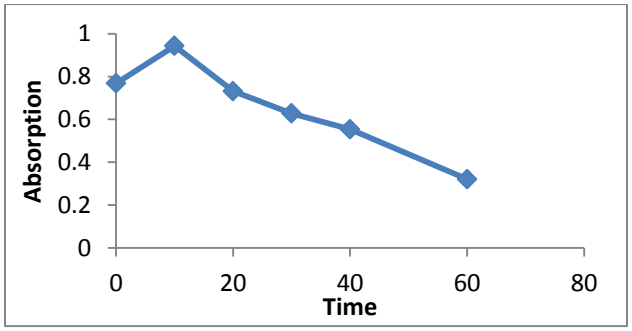


Figure8: Effect of Time

To determine the reaction of Amo. with 4-AAP,mole ratio method was used to indicates that the formed dye that have composition ratio 1:1 as shown in figure 9.

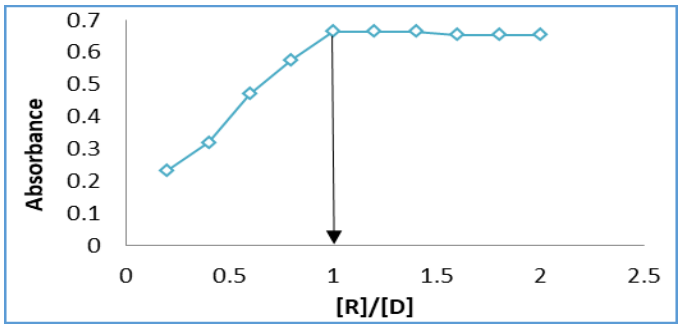


Figure 9: Mole ratio of Amo. with 2,4- di nitro phenyl hydrazine

Due to the medication and reagent reaction in the current oxidizing agent, dye is created that is colored in line with that reaction. shown in figure 10.

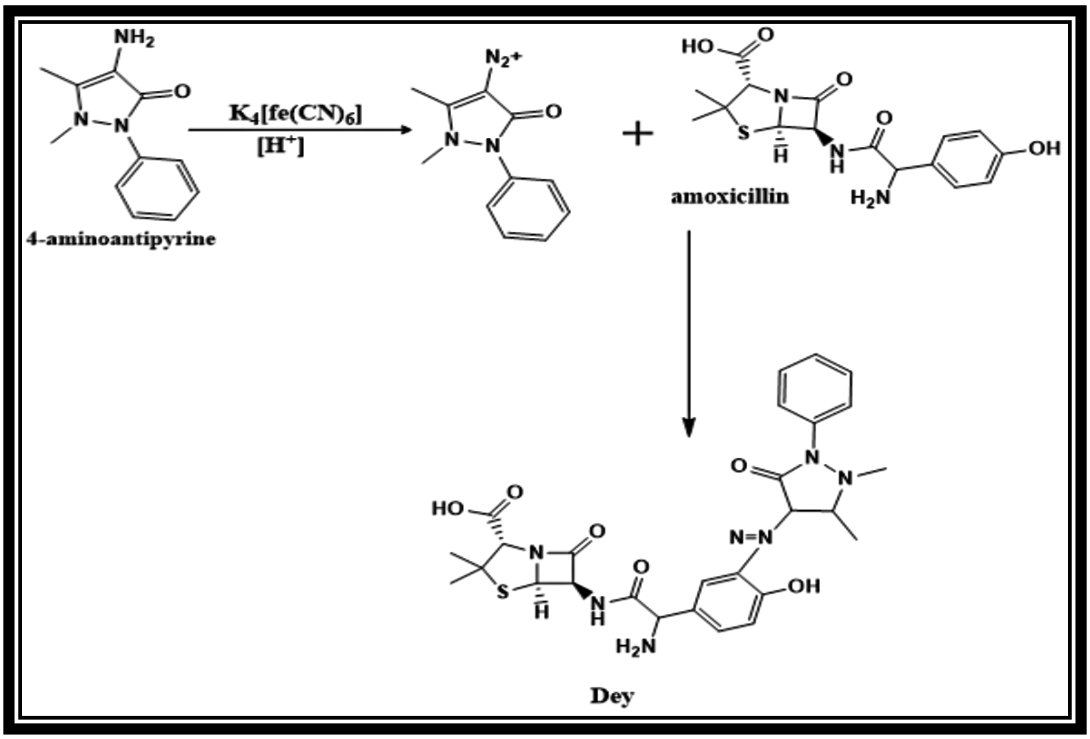


Figure 10: Mechanisms of reaction of Amoxicillin with 4- Amino antpyrine

After the optimization of the Amo oxidizing coupling, the calibration graph using different Amo. concentrations (1–50 Ug/mL), and the correlation coefficient (R), slope (a), and intercept (P) of the linear regression equation were determined as shown in figure 11.

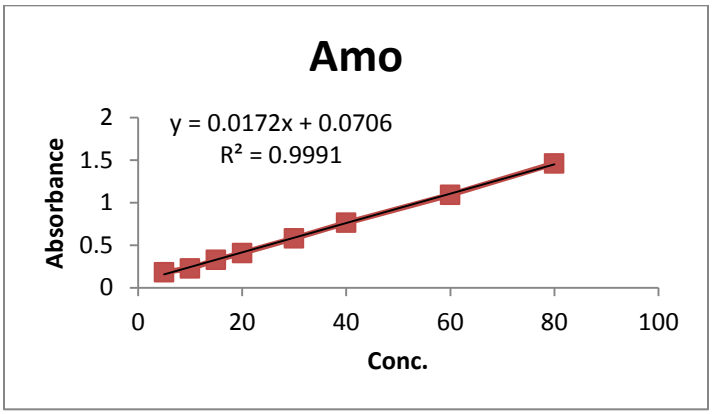


Figure 11: Calibration Curve of the Oxidative Coupling of Amoxicillin

Cloud Point for Amoxicillin Oxidative Coupling

Triton X-114 (0-2.5) mL amounts used to determine their effects. When to enhancing your income, TritonX-114 up to 0.5 mL boosted the process's absorption while decreasing it at higher doses. In this instance, 1 mL of TritonX-114 was selected, as seen in figure 12. The nonionic of the CMC of the surfactant decreased as the temperature increased, showing how temperature affects the effectiveness of Amo extraction. Figures 13 and 14 showed the results of a study using various CTAB and salt volumes.

The hydrophobic micelles increased with temperature, because Triton has increased in the Surfactant Process Due to the extraction capacity and X-114 spacing Dehydration of the external micelle layer. Because of the viscosity, Amo absorption reduced after 40 ^{0}C whereas it rose at 400C. Increasing extraction the aqueous and rich phases of the surface must be in equilibrium before extraction via the cloud point may take place. Higher micelle concentration in the material. The amount of heat that had collected in the solution during this period allowed Micelles to lose water molecules and form a small amount of hydrophobic material. Time to incubate (10-60 minutes) Viscosity is important because it traps dye quickly, and temperature It was agreed to cook the food for 20 minutes at a temperature of 40 °C. Figures 15 and 16 show the results, respectively.

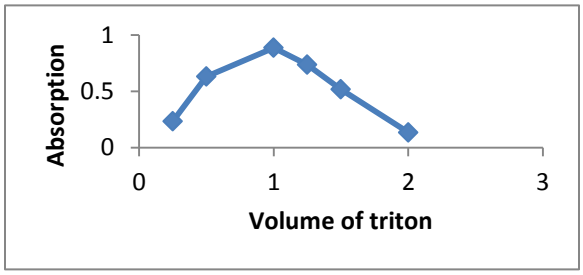


Figure 12: effect Triton- x114 Volume

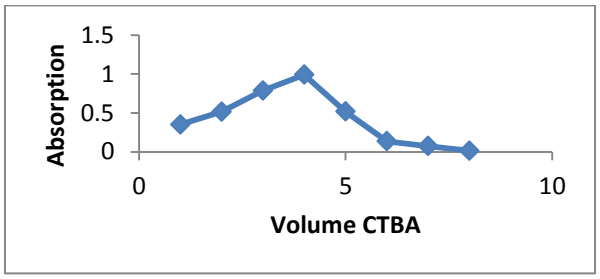


Figure 13: Effect the Volume CTAB

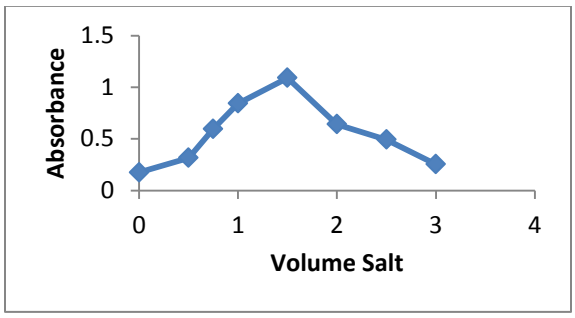


Figure 14: Effect the Volume Salt

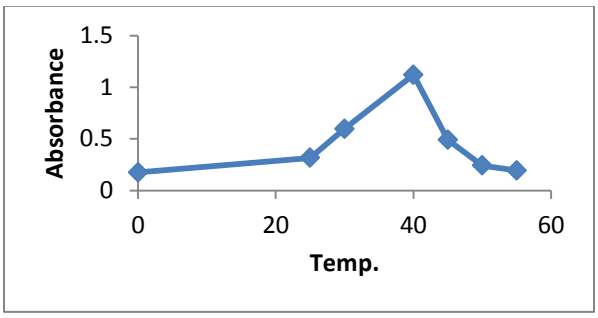


Figure 15: Effect Temperature

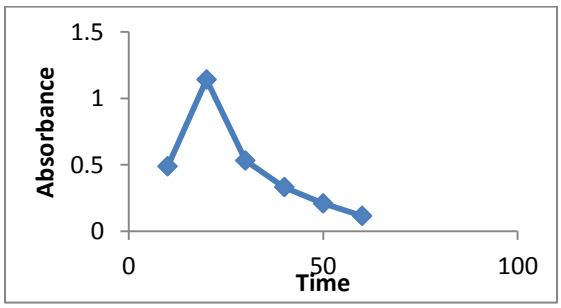


Figure 16: Effect the Time

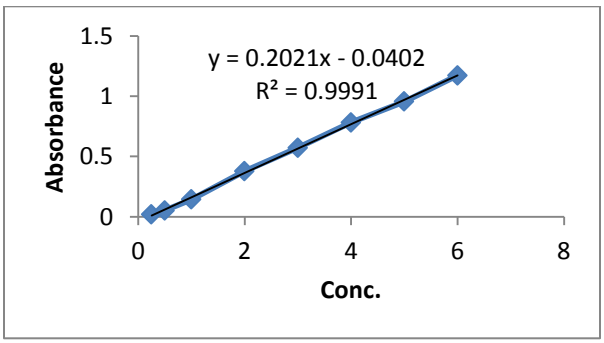


Figure 17: Calibration Curve of the Cloud Point extraction of Amoxicillin

Optimum reaction conditions of flow injection analysis technique

Figures 18, 19, 20, and 21 demonstrate the results of some parameter chemical with physical studies, such as reagent concentration, oxidizing agent concentration, reaction coil, and flow rate.

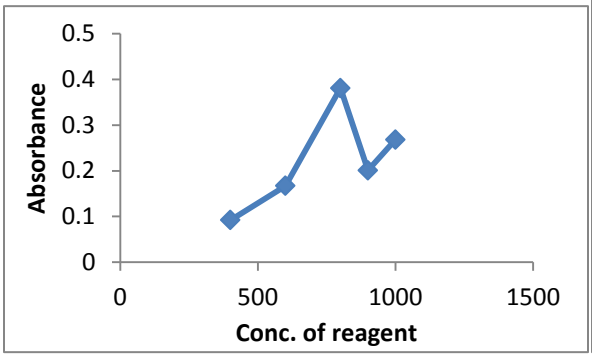


Figure 18: different Reagent Concentration oxidizing

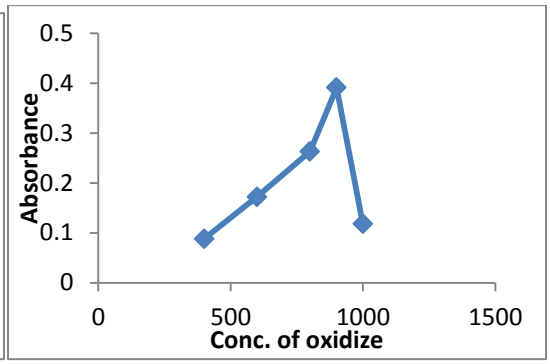


Figure 19: different concentration of Agent

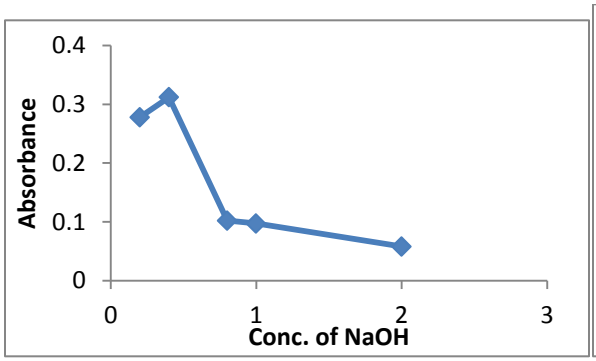


Figure 20: different concentration of base

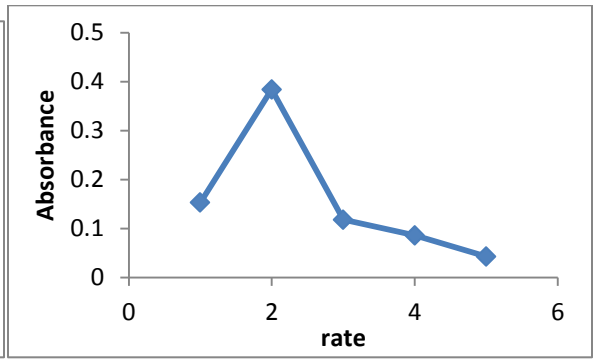


Figure21: Effect the rate

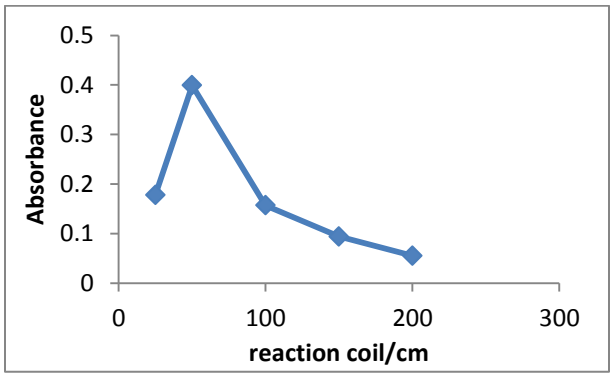


Figure 22: Change in reaction coil

After studing an optimal flow injection study of Amo, discuss a calibration graph for Amo. at various concentrations (2-150 g/mL), and calculating the correlation coefficient (R), slope (a), and intercept (P) of the linear regression equation, the results are displayed in figure 23. The regression equation's characteristic parameter for the proposed oxidative coupling CPE and FIA methods is provided in Table 1.

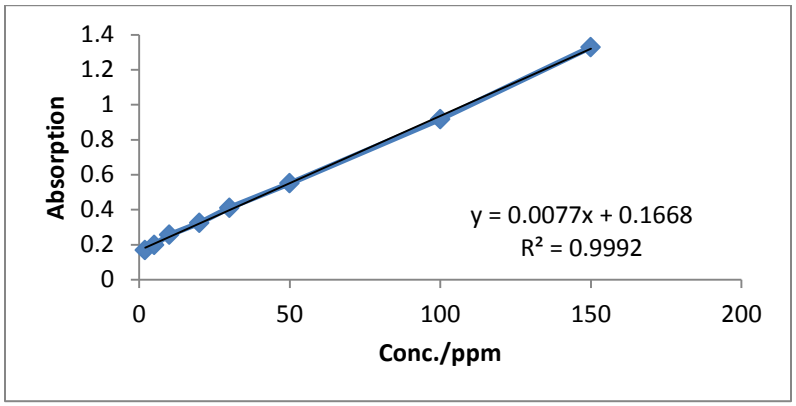


Figure 23: Calibration Curve of the Flow Injection of Amo.

Table 1. Characteristic parameter for the regression equation of the proposed oxidative coupling CPE and FIA methods.

Parameter	Oxidative coupling	Cloud point extraction	Flow injection analysis
λ max(nm)	475	480	475
Color	Red- Orange	Deep Reddish-orange	Red-orange
linearity rangeμg/mL	1-50	0.25-6	2-150
Molar absorptivity (L.mol^{1}.cm-1) Ɛ	6.2×10^{3}	103623.79	2.8×10^{3}
Sandell's sensitivity (μg/cm2)	0.059	3.53×10^{-3}	0.13
Correlation coefficient(r)	0.9995	0.9996	0.9996
Regression equation	Y=0.0172x+0.0706	Y=0.28356x+1.2844	Y=0.0077x+ 0.1668
Slope(b)	0.017231	0.283559	0.0077

Intercept(a)	**0.0706**	**1.2844**	**0.1668**
Analytical sensitivity µg/mL	**0.038**	**0.182**	**0.019**
Limit of detection µg/mLLOD	**0.192**	**0.023**	**0.43**
Limit quantification μ g/mL LOQ	**0.580**	**0.07**	**1.298**
C.L. for the slope(b±tsb)at 95%	**0.0263± 0.000971**	**28.35**	**0.0077± 0.00034**
C.L. for the intercept(a±tsa) at 95%	**-0.0253± 0.01897**	**25**	**0.1688±0.02017**

Accuracy and Precision

Study the accuracy and precision for the proposed methods Oxidative, cloud point and flow injection, under optimum conditions using different concentrations and measured absorbance at a minimum for five readings per concentration. precision and accuracy determination by RE (%), R(%) and RSD (%), as shown in Table 2 &3.

Table 2: Data the accuracy and precision of proposed methods for estimation of pure samples

Drug	Amount of drugs µg /ml		Relative Error %	Recovery %	Average Recovery%	RSD% (n=5)
	Taken	Found				
	Oxidative coupling					
	5	5.12	2.45	102.45		1.21
PEH	20	19.27	-3.65	96.35	99.74	1.85
	50	50.22	0.44	100.44		0.22
	Cloud point					
	0.5	0.46	-8.0	92.0		0.04
PEH	2	2.05	2.5	102. 5	98.16	0.01
	5	4.923	-0.015	99.98		0.19
	Flow injection system					
	5	4.97	-0.6	99.4		0.09
PEH	20	19.42	2.9	102.9	100.43	0.69
	50	49.5	-1.0	99.0		0.79

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

Table 3: The accuracy and precision of proposed method for estimation of commercial pharmaceuticals

Type of Drugs	Amount of drugs µg /ml		Relative Error %	Recovery %	Average Recovery %	RSD% (n=5)
	Taken	Found				
Oxidative coupling						
PEH Jordan 10% w/v	5	5.06	1.2	101.2		1.2
	20	19.95	-0.25	99.75	100.29	0.99
	50	49.96	-0.08	99.92		0.87
PEH European union 10%w/v	5	5.05	1.0	99.5		0.48
	20	19.96	-0.2	99.8	99.79	1.32
	50	50.04	0.08	100.083		0.98
Cloud point						
PEH Jordan 10% w/v	0.5	0.51	1.0	101		0.05
	2	1.89	-5.45	94.55	98.46	0.03
	5	4.99	-0.16	99.84		0.99
PEH European union 10%w/v	0.5	0.507	1.4	101.4		0.12
	2	2.01	0.8	100.8	100.8	0.98
	5	5.01	0.2	100.2		0.76
Flow injection						
PEH Jordan 10% w/v	5	4.93	-1.4	98.6		1.3
	20	20.56	2.8	102.8	101.0	0.06
	50	50.82	1.6	101.6		0.01
PEH European union 10%w/v	5	5.02	0.4	100.4		0.78
	20	19.22	-3.9	96.1	99.3	0.01
	50	50.7	1.4	101.4		0.08

Average of five repeats, E% = relative error Found-taken /taken ×100 , Rec% =recovery, and RSD%=relative standard deviation.

Conclusions

The suggested approach to Amo. For determin the advantages of low cost, high sensitivity streamlined, recurrent and reproducible Amo drug evaluation techniques in pharmaceutical preparedness that can be applied to actual samples. The surfactant was used in pharmaceutical preparations for the isolation and pre-concentration of the Amo compound. For this procedure, a comparison between

the methods already documented using different instrumental techniques appears to be more sensitive and stable, simple, fast, quick and cheap. An FIA method was used to semi-automate the batch spectrophotometric method for the estimation of PEH drug. The proposed methods were successfully applied for the estimation of pure Amo and in pharmaceutical dosage.

References

1) Aseel M Aljeboree and Abbas Noor Alshirifi ; Colorimetric determination of Amoxicillin using 4- Am; Journal of Physics: Conf. Series 1294 (2019) 052067; doi:10.1088/1742-6596/1294/5/052067.

2) Aseel M. Aljeboree , A.N.A., Adsorption of Pharmaceuticals as emerging contaminants from aqueous solutions on to friendly surfaces such as activated carbon: A review J. Pharm. Sci. & Res.; 10(9): p. 2252-2257; 2018.
3) World health Organization; Model list of Essential Medicine; 21st list; 2019.

4) Holten, K. B.; Onsusko, E. M. Appropriate Prescribing of Oral b-Lactam Antibiotics. Am. Fam. Physi. ; 63, 1557–1565; 2000.

5) Rolinson, G. N.; Geddes, A. M. The 50th Anniversary of the Discovery of 6-Aminopenicillanic Acid (6-APA). Interna. J. Antimicro; 29, 3–8; Agents 2007.
6) Kaur, S. P.; Rao, R.; Nanda, S. Amoxicillin: A Broad Spectrum Antibiotic. Interna. J. Pharm. Pharm. Sci. ; 3, 30–37; 2011.

7) WHO. Revised WHO Classification and Treatment of Childhood Pneumonia at Health Facilities. Geneva: WHO; Available at http://www.who.int/maternal_child_adolescent/documents/child-pneumoniatreatment/en/; 2014.

8) A.N. Cammidge, R. Bushby, J. Am.Chem Soc. 1995, 117, 924-927.

9) A.Russel, J.Biol. Chem.,156,457 ,1944