

Spectrophotometric Determination of Amoxicillin in Pharmaceutical Preparations using In Aqueous Solution Alizarin Red S Reagent

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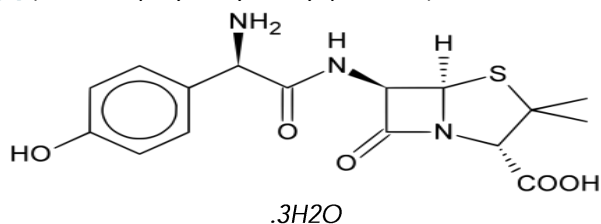
Abstract

A simple, rapid and sensitive spectrophotometric method for the Determination of amoxicillin was developed. The method is based on the Proton transfer reaction with alizarin red sulphonate in aqueous neutral Solution to form a violet product showing a maximum absorbance at 531nm with a molar absorptivity of 8091 L.mol⁻¹.cm⁻¹. Beer's law is obeyed Over the concentration range (1-20) μ g.ml⁻¹ with the accuracy (average Recovery) 100.51% and the precision (RSD) less than 2%. The method Was successfully applied for the determination of amoxicillin in Pharmaceutical formulation as injection and the results were in good Agreement with the standard addition procedure.

Keywords: amoxicillin; alizarin red S reagent; Spectrophotometric

1. Introduction

Amoxicillin is chemically alpha-amino-para-hydroxy benzyl penicillin (It has the following structural formula [1] (α-amino-p-hydroxy benzyl penicillin).



Amoxicillin is practically similar to ampicillin in that it affects both positive and negative bacteria. It is cream and its effect on the bacterial cell wall prevents its division and reproduction. Longer Amoxicillin is a second-generation penicillin, but it differs from penicillin in that More effective against gram-negative bacteria, but inactive against secondary yeasts (beta- lactamase [2] it is more absorbed than ampicillin in the intestine and does not cause diarrhea, as it is It is used in the treatment of middle ear infections [3], but it is considered less effective than ampicillin in treating Because of its high and rapid absorption into the blood, it remains (bacillary dysentery) dysentery (Ampicillin lasts longer in the stomach and intestines and is better in treating dysentery , Several analytical methods were used for the determination of amoxicillin, including liquid chromatography (associated with mass spectrometry [4] differential pulse polarography [5], and capillary electric migration [6].

And HPLC [7], a spectroscopic and volumetric method for the real-time determination of ampicillin and amoxicillin (HPLC) is described. And cloxacillin in their pharmaceutical preparations. The method is based on the hydrolysis of antigens the vitality is in an alkaline medium and then smeared with potassium iodate until the red

color appears Purple at the end point followed by separation of the organic layer by carbon tetrachloride Absorbance measured at 520 nm; I used charge transfer complex formation reactions, in the determination of amoxicillin as an electron donor using electron acceptors such as 2,3bi-Cyano- 5.6dichloro-parabenzoquinone (DDQ) [8] and chloranyl [9] Floranyl [10], bromanyl and tetracyanoethylene (TCNE). A spectrophotometric method was developed to estimate Amoxicillin in pharmaceutical preparations includes method Double ionic composition between Amoxicillin and the inorganic complex Mo (v)-thiocyanate followed by an extraction process using Methylene Chloride [11], nitro enation and conjugation reactions were also used by conjugating amoxicillin with alkaline nitro aniline Oxidative coupling reactions have also been used In combination amoxicillin with N,N- di ethyl – Para phenylene Diamine Hydrochloride In the presence of the oxidizing agent N-BroomSuccinimide An indirect spectroscopic method was also used For the determination of amoxicillin by the reaction of penicillamine acid resulting from acid hydrolysis For amoxicillin with palladium ion [12] In this research, a simple spectrophotometric method was used for the determination of amoxicillin using the reagent Alizarin red sulfonate by proton transfer reaction to form a colored product measured max Absorption at 531 nm in aqueous solution.

2. Experimental

Apparatus

All spectroscopic measurements were made using device Shimadzu UV-210 Double Beam Spectrophotometer and use cells from Silica has a width of 1 cm.

Weighing operations were carried out using a sensitive balance type Mettler H10 the acidity function was measured for solutions using the acidity function device (pH meter) type Philips PW 9420 linked to pole type CE10-

12 pH and conducted heating processes using a water bath type Forst Instruments, LTD.

3. Materials and Chemical Solutions Used

All reagents and chemicals used were of a high degree of purity company production BDH and Fluka.

Red alizarin solution S (ARS) 1X10⁻³ molar

The solution is prepared by dissolving 0.0845gm, From ARS in 250 ml ethanol then it is placed in an opaque bottle and remains stable for at least a month.

Amoxicillin solution 100 μ g. ml⁻¹

A solution has been prepared 100 μ g.ml⁻¹ from amoxicillin by dissolving.

0.01gm in a small amount of ethanol then completes the volume to 100 milliliters with distilled water.

Spectrophotometric determination of amoxicillin in aqueous solution Procedure

To be added to a set of volumetric bottles of 10 ml increased volumes (milliliters) of 100 μ g.ml⁻¹ from amoxicillin to cover concentration rang

(1 – 20) μ g.ml⁻¹ In the final volume, followed by the addition of 3 ml of red alizarin solution S and supplement it up to the mark with distilled water the solutions were left for 5 minutes at laboratory temperature (25°C) The absorbance was measured at the wavelength 531nm Using silica cells with a width of 1 cm .

Method of work for the analysis of amoxicillin injection

Discharge of three injections of amoxicillin Mix well, weighing the same as one injection Which contains 500 mg of amoxicillin and dissolve 0.5 gm in a small amount of ethanol To increase the solubility, then fill the volume with distilled water to 1 liter, filter the solution, and dilute 20 ml of it with distilled water to 100 ml. Enough (10-200) μ g, were added into 10ml volumetric bottles and treated in the manner described by the method of work to find the concentration of amoxicillin in one injection .

4. Results and Discussion

Absorption spectrum

Amoxicillin interacts with alizarin red sulfonate in the neutral medium to form a violet product that gives the highest absorption at a wavelength of 531nm.

Fig (1)

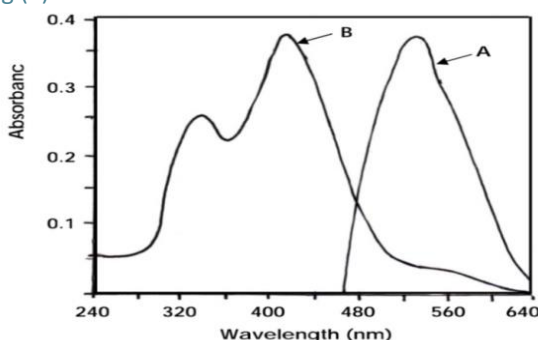


Fig (1): absorption spectrum

(A): alizarin product – amoxicillin concentrated 180 μ g. 10 ml⁻¹ vs. Solution blank, (B): blank solution vs. Distilled water

Study of the optimum conditions for the reaction

The reaction conditions affecting the adsorption of the colored product were studied using 0.5ml from solution 100 μ g.ml⁻¹, amoxicillin In order to obtain the highest sensitivity.

Study the effect of time and temperature on the composition of the product

To find the optimum temperature for the formation of the colored product The composition of the product has been studied red alizarin S With amoxicillin at different temperatures ranged from zero to (50°C), the results showed no noticeable effect on the product was absorbed at these temperatures, so the method was applied at room temperature (25°) with a time of 10 minutes, and Table (1) shows the results obtained.

Table (1): The effect of temperature on the formation time and stability of the colored product								
Temp. (C)	Absorbance							
	Time (min)				Time (hour)			
	10	20	30	40	50	1	1.5	2
0	0.10	0.11	0.10	0.10	0.07	0.07	0.07	
RT*	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
40	0.09	0.10	0.10	0.10	0.09	0.10	0.10	0.10
50	0.10	0.10	0.10	0.10	0.08	0.08	0.08	0.08

* Room temperature (25 C°)

Study the effect of reagent quantity

The effect of the amount of ARS reagent on the absorption of the product was studied, where increasing volumes of ARS solution (1-3) ml at a concentration of 1x 10⁻³ molar were added to equal amounts of amoxicillin in 10 ml volumetric bottles After completing the volume to the mark with distilled water, the bottles were left for 5 minutes at room temperature, where the absorbance was measured at the wavelength of 531 nm versus blanks solutions Where it was found that the highest absorption gives the result when using 3 ml of ARS solution, so it was used in the subsequent studies Table 2.

Table (2) the effect of ARS					
Volume of ARS solution 1× 10 ⁻³ M (ml)	1	1.5	2	2.5	3.0
Absorbance	0.02	0.03	0.05	0.05	0.10

Standard curve

Following the previously described working method, the relationship between the absorption and concentration of amoxicillin was plotted and the standard curve shown in Figure (2) was obtained where the curve appears with good linear characteristics and that it follows Beer's law within, the range

(1-20) μ g.ml⁻¹ and with a molar absorptivity 8091 L.mol⁻¹.cm⁻¹ and Table (3) shows the specifications of the standard curve.

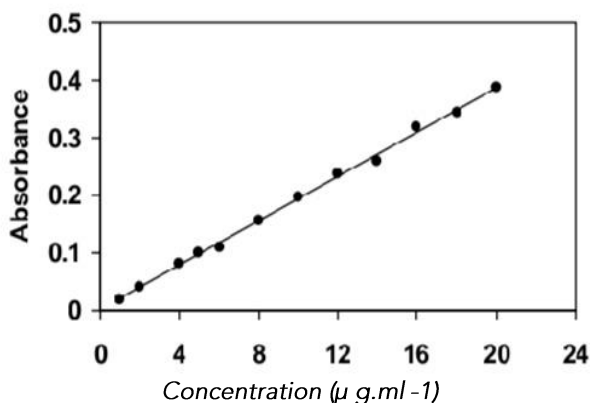


Fig (2): Standard curve for the determination of amoxicillin

Table (3): Estimated range, molar absorbance, slope, section and correlation coefficient for amoxicillin					
Drug	Linearity range (μg.ml ⁻¹)	Molar absorptivity (L.mol ⁻¹ .cm ⁻¹)	Slope	Intercept	Correlation coefficient
Amoxicillin	1-20	8091	0.0193	0.0011	0.9979

Method accuracy and compatibility

The accuracy and compatibility of the method was tested by measuring three different concentrations of amoxicillin in a final volume of 10 mL. Table (4) shows the results obtained.

Table (4): Accuracy and compatibility of the method for the determination of amoxicillin				
Compound	Amount added (μg.ml ⁻¹)	Recovery* (%)	Average Recovery (%)	RSD* (%)
Amoxicillin trihydrate	8	100.64	100.51	1.80
	10	100.50		1.53
	12	100.42		1.39

* Average for six determinations.

From observing the results shown in Table (4), it is clear that the method has good accuracy (recovery rate 100.51%) and good agreement, as the relative standard deviation value is less than 2%.

Studying the nature of the formed colored product and its stability:

According to Job's method of continuous changes it was observed that the resulting compound consisted of 1 mole of amoxicillin to 1 mole of reagent.

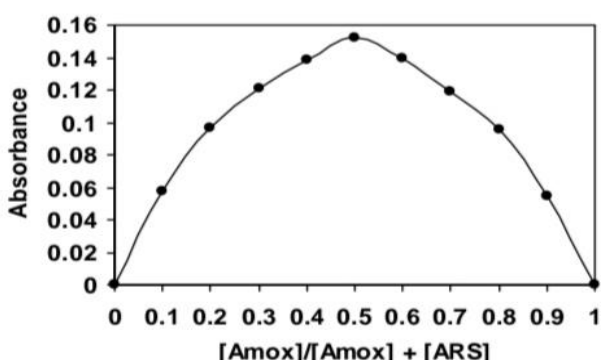
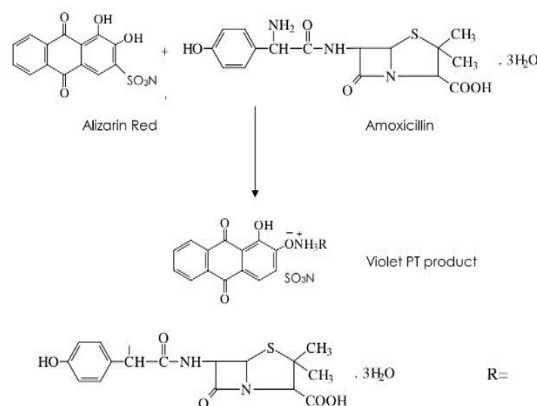


Fig (3): Plotting the continuous change of ARS product

with amoxicillin

Accordingly, the proposed chemical composition of the formed colored compound is as follows [13]



The stability constant was also calculated and found to be equal to $2.90 \times 10^5 \text{ L.mol}^{-1}$.

Analytical applications

The proposed method has been successfully applied for the determination of amoxicillin in its pharmaceutical preparation including amoxicillin injection Table (5).

Table (5): Determination of a drug compound in its pharmaceutical preparation				
Pharmaceutical preparation	Certified	Found	Recovery* (%)	Average Recovery.
Amoxicillin Ampoule SDI- Iraq	500	501.45	100.28	100.31
		502.94	100.58	
		500.42	100.07	

* Average for six determinations

Apply the standard addition method to the proposed method

In order to prove the efficiency of the proposed method for amoxicillin and its freedom from the interference of additives, the standard addition method was applied to amoxicillin injections due to the lack of the requirements of the standard method approved in the British Pharmacopoeia [1] and the American Pharmacopoeia.

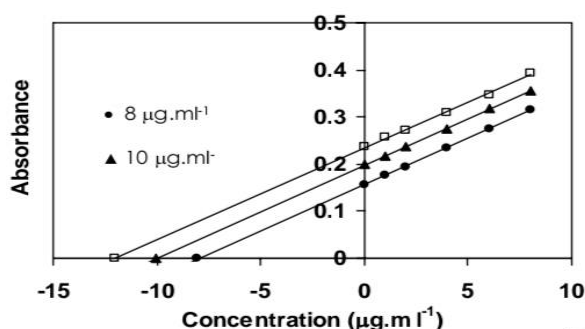


Fig (4): Plotting the standard addition curve for the determination of amoxicillin in an amoxicillin injection

Table (6) Determination of amoxicillin in a pharmaceutical preparation by the standard addition method				
Pharmaceutical preparation	Certified value (mg)	Amount present (μg.ml ⁻¹)	Drug content found (μg)	Recovery* (%)

Amoxicillin Ampoule	500	8	503.15	100.63
		10	501.00	100.20
		12	502.50	100.50
* For three determinations.				

It can be inferred from the results shown in Figure (4) and Table (6) that the results of the standard addition method agree well with the proposed method within the acceptable range of error, which indicates that the method has good selectivity.

5. Conclusion

A simple, rapid and sensitive spectrophotometric method was described for the determination of amoxicillin using the ARS reagent by means of a proton transfer reaction and the formation of a violet-colored product with a maximum absorption measured at 531 nm. The obtained results showed that the method has good accuracy and agreement the method was successfully applied for the determination of amoxicillin in a pharmaceutical preparation that includes an amoxicillin injection, and the results were in good agreement with the original content of pharmaceutical preparations as well as with the standard addition method.

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