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Indirect Spectrophotometric Determination of Amoxicillin by oxidative with Cerium ammonium nitrate using Arsenazo III as a reagent

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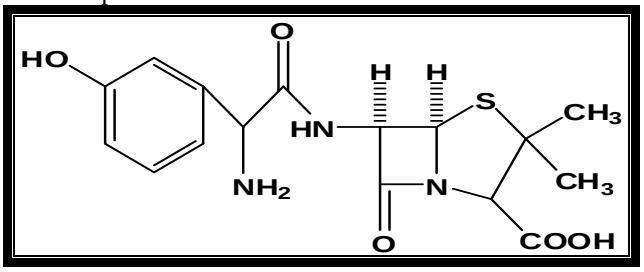
Abstract

A simple, accurate and sensitive spectrophotometric way used to preparation. The suggested method depends on using Arsenazo III as reagent. The current method was depending on oxidative coupling reaction of amoxicillin by cerium ammoniumnitrate with ArsenazoIIIin the presence of sulfuric acid as Acidic media. then reaction CeIII with arsenazo III to form pink water-soluble dye product, that has a maximum absorption at max 500 nm. Beers law is obeyed in the concentration of (1 – 10 µg.ml -1).The molar absorptivity is (3 * 10 4)L.mol -1.cm-1, a sandal sensitivity of (0.012) µg.cm-2. Limit of detection (LOD) and, Limit of Quantitation are (0.00874)µg.ml-1, (LOQ)(0.0291)µg.ml-1 respectively . The analytical recovery of the new method was good (100.72%). The method successfully applied for the determination of Amoxicillin in pharmaceutical preparations

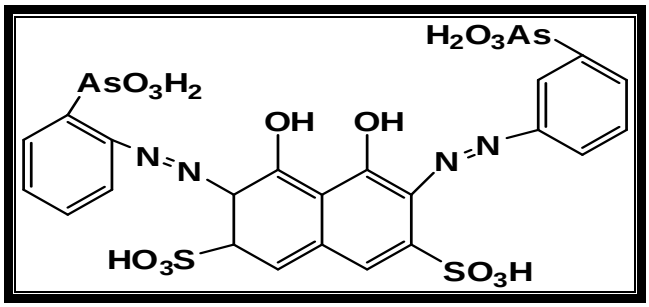
Keywords: Amoxicillin, Spectrophotometry, Arsenazo III

INTRODUCTION

Amoxicillin Scheme (1) mild - spetrum (3-lactam antibiotic used to entertainment bacterial contagion occasion by sensitive microorganisms. Amoxicillin is an antibiotic active against a wide large of Gram-positive, and a limited range of Gram-negative organisms. Amoxicillin work by control the synthesis of bacterial cell walls. It controls cross-linkage between the linear peptidoglycan polymer chains that industrialize a prime component of the cell wall of Gram-positive bacteria. It is ordinarily the Medicine of choice within the group because it is better absorbed, following oral direction, than other beta-lactam antibiotics. Amoxicillin is oversensitive to degeneracy by (3-lactamase-producing bacteria, and so may be given with clavulanic acid to decrease its oversensitive in prior. In past studies; the estimation of amoxicillin in medicinal preparations containing only amoxicillin was made by using several methods including spectrophotometry [1-8], HPLC [9-11], spectrofluorimetry [12], flow-injection analysis [13-18], voltammetry and polarography [19,20] and titrimetry [21]. Estimation of amoxicillin in the existence of sulbactam sodium, clavulanic acid, fluxacilline and metronidazole was realized by using HPLC [22-28], TLC [29], CE [30] and chemometry [31]. In the present Research, selective spectrophotometric estimation of amoxicillin based on the oxidative drug by Ce IV and coupling the product CeIII with Arsenazo III scheme(2), which results to formation coloured products in Acidic medium.



Scheme (1): The chemical structure of Amoxicillin.



Scheme (2): The chemical structure of Arsen azo III

MATERIALS AND METHODS:

Apparatus

AUV / Visible Spectrophotometer(1800 Shimadzu) , Spectrophotometric UV1100 with 1 cm matched quartz cells was used for absorption measurements , WTW 720 PH meter Germany , Electronic balance (Sartorius AG Germany).

Reagents:

- Stock Solution from drug (1000 µg.ml^{-1}) of Amoxicillin (SDI) samara – Iraq were prepared by dissolving (0.1 gm) of Amoxicillin in (20 ml) absolute methanol and then diluted with distilled water to 100 ml.
- Stock Solution of cerium ammonium nitrate was prepared by dissolving (0.1gm) in distilled water and diluting to mark in 100 ml volumetric flask.
- Stock Solution of Arsenazo III was prepared by dissolving (0.1gm) in distilled water and diluting to mark in 100 ml volumetric flask.
- Sulfuric acid H_2SO_4 (0.5 M) was prepared by take (2.72 ml) from concentration sulfuric acid and diluting with distilled water to mark in 100 ml volumetric flask.

Procedure for assay of Amoxicillin in pharmaceutical preparations Capsules:

Amoxicillin capsule:

The proposed method was applied successfully to the determination of AMXT in different pharmaceutical formulations, which commercially available in market. The state company for drug industrial and medical application Global pharma, UAE and used for the analysis: Each capsule contains 500 mg amoxicillin. (10) Capsules were powdered was then dissolved in 20 ml methanol and complete to 100 ml with distill water. This solution was diluted quantitatively to form a concentration in the range of calibration curve.

Recommended procedures:

A working standard solutions were prepared by serial dilution of 100 µg.ml -1 Amoxicillin to the final volume of 25 ml volumetric flask to cover the range of calibration curve (1 – 10) µg.ml -1, then (3ml) cerium ammonium nitrate (100 µg.ml-1) was added and shacked well. After that (3ml) ArsenazoIII(100 µg.ml -1)and (0.3ml) of H 2SO4 (0.5 M)were added to the mixture then diluted to the mark with distilled water, and permit the flasks to stand for 30 min at room temperature (25Ċ).The absorption was monitored at (500) nm against the blank.

RESULTS AND DISCUSSION:

Amoxicillin drug react with cerium ammonium nitrate and Arsenazo III in the presence of sulfuric acid as acidic media to form an intense pink colour product that can be measured spectrophotometrically at 500 nm **Figure (1)**.

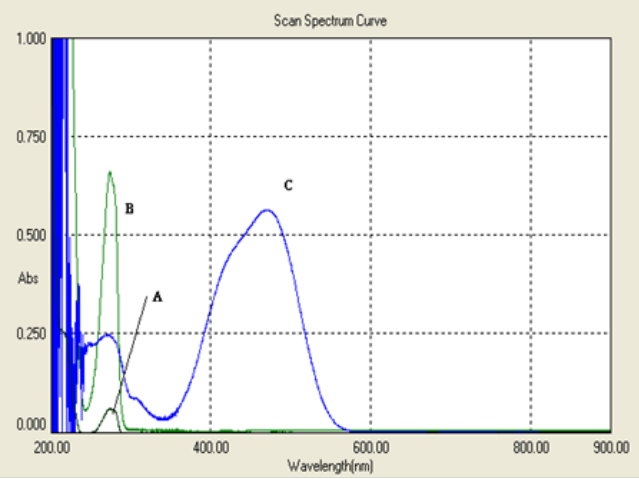


Figure (1) :(B) Arsenazo III Vs. distilled water. (A): Amoxicillin Vs. Distilled water. (C): Complex product Vs. Blank

Optimization of the reaction conditions*:*

The main consideration with the optimization to study the effect of deferent parameters on the absorption intensity of the colourcomplex formed. All optimization for the reaction conditions were studied. The factors affecting color development, reproducibility, sensitivity and conformity with Beers law were investigated with Amoxicillin.

Effect of cerium ammonium nitrate volume:

The optimum concentration of cerium ammonium nitrate solution was found to be 3 ml of cerium nitrate ammonium (100 µg.ml^{-1}) for Amoxicillin figure 2.

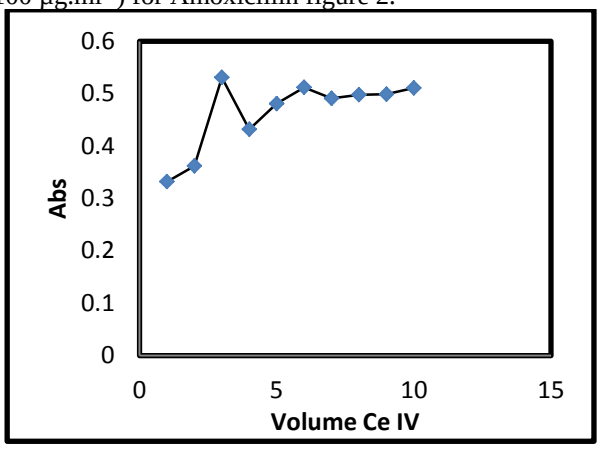


Figure (2): Effect of cerium ammonium nitrate volume

Effect of Arsenazo III volume*:*

The amount of coupling agent was optimized by adding 1 – 10 ml 0f (100 µg.ml^{-1}) of Arsenazo III. It was found that maximum and stable Colors were formed with 3 ml of Arsenazo III solution for Amoxicillin in final volume of 25 ml. Figure3.

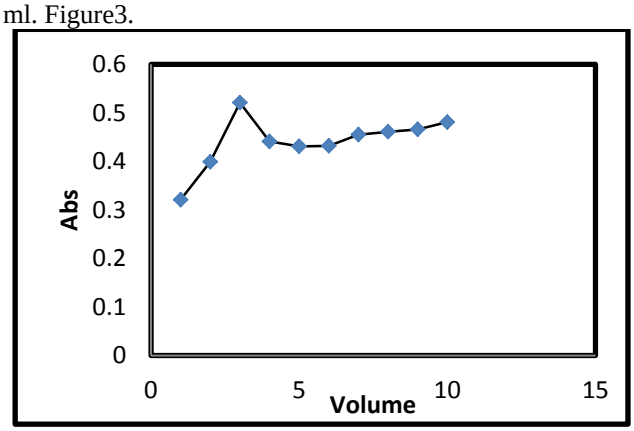


Figure (3): Effect of Arsenazo III volume

Effect of different temperature:

Optimization of different temperatures on oxidative coupling of Amoxicillin led to obtain a high absorption at room temperature (25) °C. Figure 4.

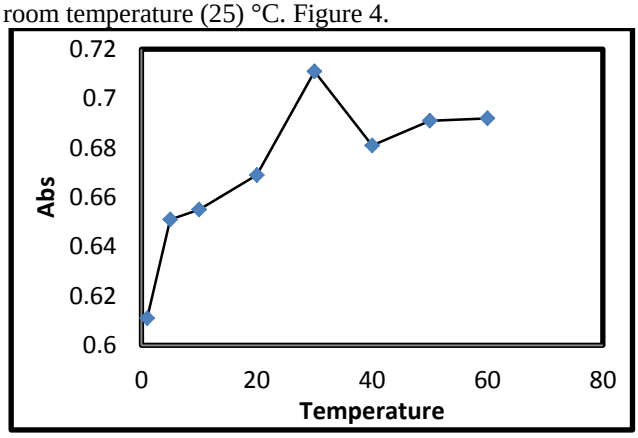


Figure (4): Effect of different temperature

Effect of sulfuric acid volume:
The maximum absorption was observed at 0.3 ml of sulfuric acid (0.5 M) for Amoxicillin so, 0.3 ml of sulfuric acid was selected.

Effect of order of addition:
The absorption of pink product dye was monitored via changing the order of addition, which led to the following order: (Drug: cerium ammonium nitrate: ArsenazoIII: sulfuric acid). Due to show the highest absorption and thus was selected for further use. See table (1)

Table (1): Effect of order of addition

No.	Order of addition	Absorbance at 500 nm
1	Drug : Cerium ammonium nitrate :Arsenazo III :sulfuric acid	0.392
2	Drug :Sulfuric acid: cerium ammonium nitrate :Arsenazo III	0.362
3	Drug :Arsenazo III: cerium ammonium nitrate :sulfuric acid	0.371
4	Drug : Cerium ammonium nitrate : sulfuric acid: Arsenazo II	0.388
5	Sulfuric acid: Drug: cerium ammonium nitrate :Arsenazo III	0.381

Calibration Curve:
Table 2 summarize the optical characteristics and the analytical figures of merit of determination of Amoxicillin based on optimization condition. The excellent correlation coefficient 0.9997 confirm linear correlation between absorption signal and Amoxicillin concentration. The concentration range is (1-10) µg.ml-1, and the linear regression equation is (Y= 0.0803x + 0.0208). Figure (2) shows the calibration curve

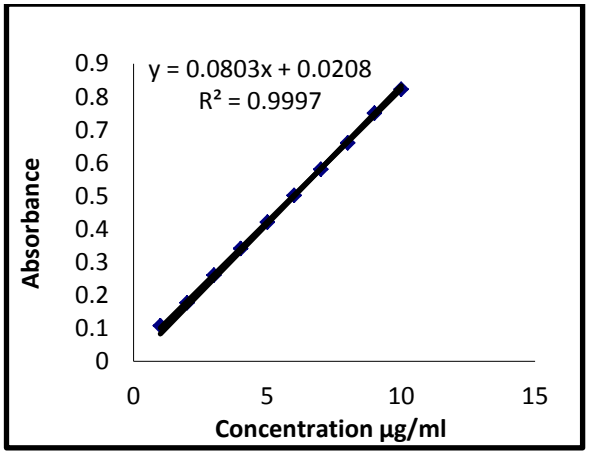
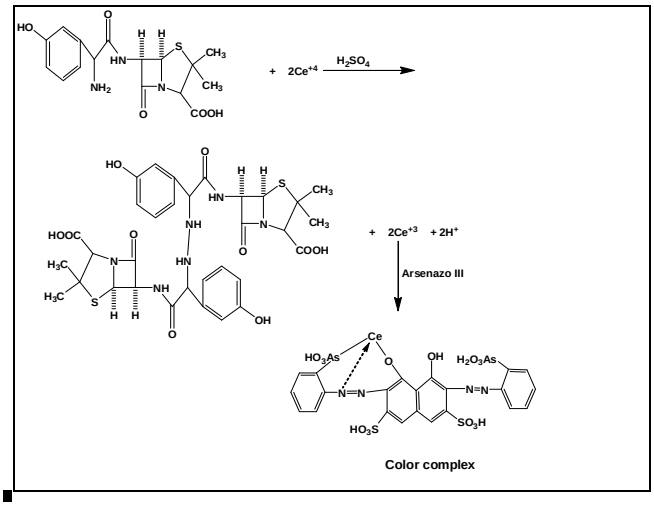


Figure (2): Calibration Curve for the determination of Amoxicillin.

Nature of the dye product:
Table 2 summarize the optical characteristics and the analytical figures of merit of determination of Amoxicillin based on optimization condition. The excellent correlation coefficient 0.9997 confirm linear correlation between absorption signal and Amoxicillin concentration. The concentration range is (1-10) µg.ml-1, and the linear regression equation is (Y= 0.0803x + 0.0208). Figure (2) shows the calibration curve

Table (2): Optical characteristics and statistical data for the determination of Amoxicillin.

Color	Pink
Linearity range µg.ml^{-1}	1 – 10
Regression equation	Y= 0.0803x + 0.0208
Calibration sensitivity ml .µg $^{-1}$	0.0803
Correlation of linearity	0.9997
Molar absorptivity L.mol^{-1}.cm^{-1}	3 * 10^{4}
Sandals sensitivity µg.cm^{-2}	0.012



Scheme (3): The mechanism of oxidation of Amoxicillin

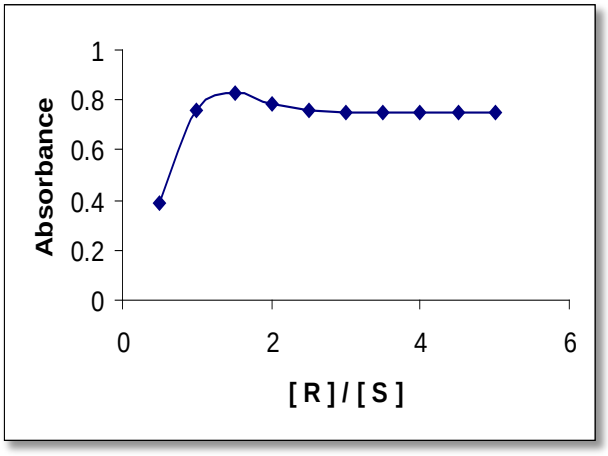


Figure (4): Molar ratio of drug to reagent

Analysis of Amoxicillin in pharmaceutical preparations:
The feasibility of monitoring Amoxicillin in pharmaceutical preparations via proposed method was successfully applied with the relative standard deviation demonstrate excellent accuracy and precision. Table (3) confirm the new suggested method procedure was compared with standard procedure table (2).

Table (3): Result application of pharmaceutical for capsule amoxicillin

3	*Found	Recovery %	Average Recovery	Er %	Average Er %	RSD%
8	8.6	107.5	100.72	7.5	4.2	0.956
9	8.7	96.66		3.3		0.858
10	9.8	98		2		1.26

* Average of five determinations

CONCLUSION:

A simple, accurate and excellent spectrophotometric method was investigated for the determination of amoxicillin in pure and in pharmaceutical preparations. The proposed method can be carried out with no need for further steps such as solvent extraction step. PH. or temperature control.

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