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# Spectrophotometric Determination of Amoxicillin in Pharmaceutical Preparations

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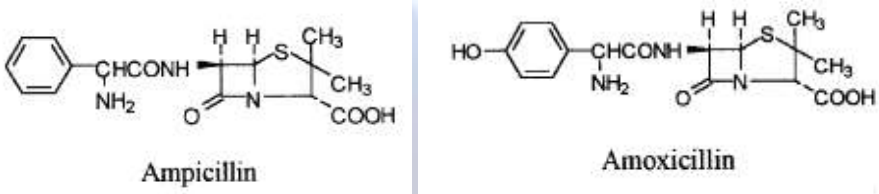
**Abstract: A simple and sensitive spectrophotometric method was developed for the determination of amoxicillin (Am) in pharmaceutical preparations. The method based on oxidation of amoxicillin with a known excess amount of N-bromosuccinamid (NBS) in an acidic medium and then the residual NBS was determined by bleaching the color of methylene blue dye, then measuring the absorbance of residual dye at 663 nm. The amount of NBS react corresponds to the Am concentration in sample solution. Beer's law was obeyed in the range of 5-50 µg/10ml with good molar absorptivity of $8.3051x10^4$ l. $mol^{-1}$ .$cm^{-1}$ and Sandell's sensitivity index of $5.0505\ x10^{-3}$µg.$cm^{-2}$ with a relative error of +0.1 to+1.2 and relative standard deviation of ±0.27 to ±0.72% depending on the concentration. The method has been successfully applied to the determination of Am in its pharmaceutical preparations.**

**Keywords: amoxcillin, methylen blue, bleaching color, indirect, spectrophotometric determination, N-bromosuccinamid.**

## Introduction

Amoxicillin is [[2S[2α,5α,6β,(S*)]]-6-[[Amino(4-hydroxyphenyl) acetyl]amino]-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxilic acid]. (1) is an oral semi-synthetic penicillin structurally related to ampicillin (2). (Schem1)




**Scheme 1: Chemical structures of ampicillin and amoxicillin**

Amoxicillin is β -lactam antibiotic that belong to the group of penicillin-it is semi-synthetic , broad spectrum, acid stable, orally absorbed antibiotics that inhibit bacterial cell ,it is normally used for the treatment of common bacterial infection.(3).

Several techniques have been used in determination amoxicillin , the major methods in chromatography techniques included HPLC(4-6) solid-phase extraction-liquid chromatography (SPE-LC)(7), LC-MS(8), SPE-LC.MS(9),SPE-LC.MS.MS(10-11), SPE-LC-ES-MS-MS(12) capillary electrophoresis (13-14), micelllar electro kinetic capillary chromatography(15). Also atomic absorption spectroscopy (indirect method) has been used in determination of amoxicillin (16). Different spectrophotometric methods have been used in the determination of amoxicillin by using various reagent such as : bromocresol green (17), 4-bromobenzaldehyde (18), folin-Clocalten (19), diazotized p-aminobenzioc acid and diazotized procain (20), sodium 1,2-naphthoquinone-4-sulfonate (21) ,N,N-dimethyl-p-phenylendiamine and potassium hexacyanoferrate (22), formaldehyde (23), 2,4-dinitrophenylhydrazine(24), hematoxyline (25), chloramine-T and iodide (26), N-bromosuccinimide or N-chlorosuccinimide (27), The derivative and uv spectrophotometry methods also used (28-30) .

The present paper , for the first time is the description of a simple accurate and precise visible spectrophotometric method for the determination of amoxicillin in bulk and in it's pharmaceutical preparations based on oxidation of amoxicillin by N- bromosuccinamid, then bleaching the color of methylene blue dye by the unreacted N-

bromosuccinamid. The method has been proved successfully for the determination of amoxicillin in pure form and in it's pharmaceutical preparations.

## Experimental

**Apparatus:**
All measurement were performed using CE7200 recording spectrophotometer, with 1cm quartz cells.

**Reagents and solutions:**
All chemical used are of analytical grade. amoxicillin trihydrate was purchased from the State Company for Drug Industries and Medical Appliances (SDI),.

**Amoxicillin solution , 10 µg.ml$^{-1}$**
This solution was prepared by dissolving 0.0100 g of amoxicillin trihydrate in 100 ml distilled water in a volumetric flask, 10 ml of above solution was diluted to 100 ml with distilled water in a volumetric flask.

**Methylene blue dye solution, 1.6×10$^{-3}$M**
This solution was prepared by dissolving 0.0050 g in 100 ml distilled water in a volumetric flask, 10 ml of above solution was diluted to 100 ml with distilled water in a volumetric flask.

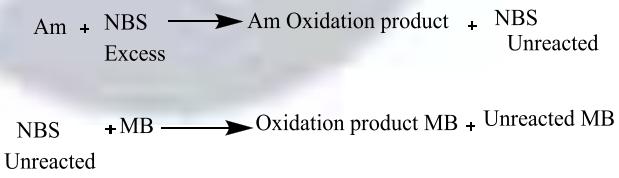
**N-Bromosuccinamid solution, 1×10$^{-3}$M**
This solution was prepared by dissolving 0. 0177g of N-bromosuccinamid (Fluke) in 100 ml distilled water in a volumetric flask**.**

**Hydrochloric acid solution, 2M**
This solution was prepared by diluting 16.4 ml of concentrated hydrochloric acid to 100 ml distilled water in a volumetric flask**.**

**Analytical procedure:**
Into a series of 10 ml volumetric flasks an increasing volume of amoxicillin trihydrate solution (10 µg ml$^{-1}$) were transferred to cover the range of the calibration graph (5 -50µg.10 ml$^{-1}$). Then 0.6 ml of HCl (2 M) and 1 ml of N-bromosuccinamid (0.001M) were added the solutions were lifted for 15 min. at room temperature (25˚C), finally adding 1.25 ml of methylene blue, after 5 min. then the flasks diluted to the mark with distilled water .The absorbance was measured at 663 nm versus the reagent blank, prepared in the same manner but containing no drug.

## Results and Discussion:

The method included oxidation of amoxicillin by adding an excess of N-bromosuccinamid, then the unreacted of N-bromosuccinamid was used in bleaching the color of methylene blue (schem 2). Then the absorbance measured at 663 nm , which is increased linearly with increasing concentration of amoxicillin.




**Scheme (2): Reactions of indirect determination of amoxicillin**

The effect of various variables on the color development was tested to establish the optimum conditions for the determination of amoxicillin in pharmaceutical preparations.

**The chosen of dye and concentration:**

The preliminary experiments were performed to optimize the useful and optimum concentration of dye (methylene blue, match it green and crystal violate) that can be determined spectrophotometrically. The results indicated that methylene blue was found to be a useful agent for reaction .

Figure (1) shows that 1.25 ml of methylene blue dye was the best volume , it gave highest intensity.

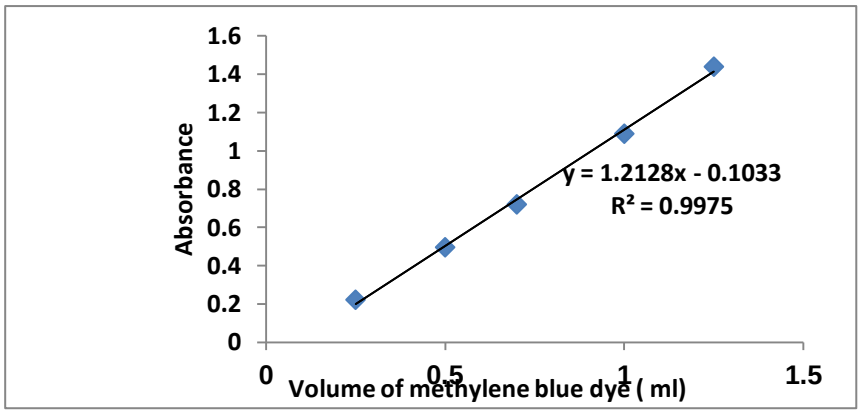



**Fig. (1): The effect of methylene blue amount on absorbance**

**The effect of oxidant reagents:**

N-bromosuccinamid was found to be a useful oxidizing agent, other oxidizing agents such as (NCS and $KIO_4$) have also been tested, but none offered real advantages over N-bromosuccinamid. Fig. (2).

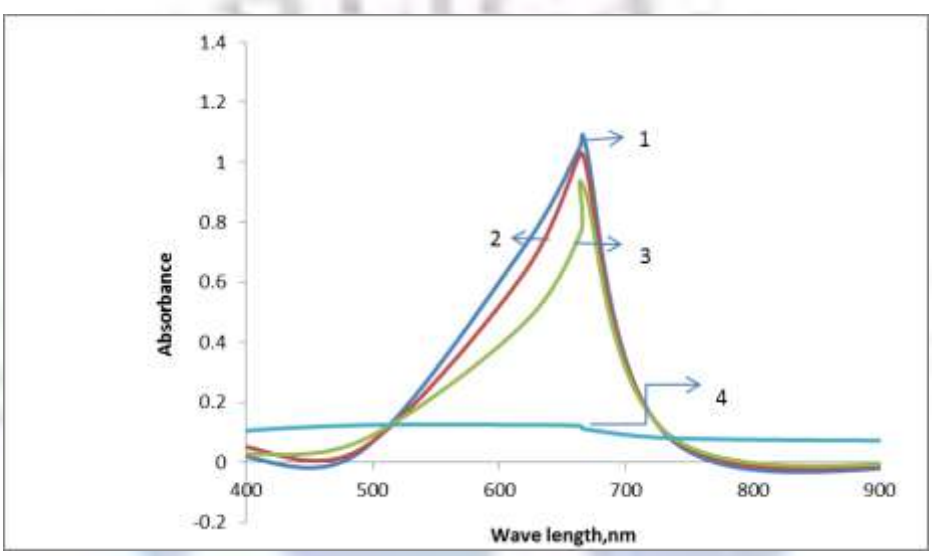



**Fig. (2): The effect of oxidant on bleaching the dye when number 1 is methylene blue dye (MB), 2 is (MB) with potassium per iodide, 3 is (MB) with N-chlorosuccinamid and 4 is (MB) with N-bromosuccinamid**

**Effect of oxidant amount:**

The effect of different volumes (0.1 – 2 ) ml of 0.001M of N-bromosuccinamid on the color of methylene blue dye was studied without amoxicillin. Figure (3) shows that 1ml of N-bromosuccinamid solution was enough to obtain a maximum bleaching of the color of methylene blue dye therefore it was recommended in the subsequent experiments.

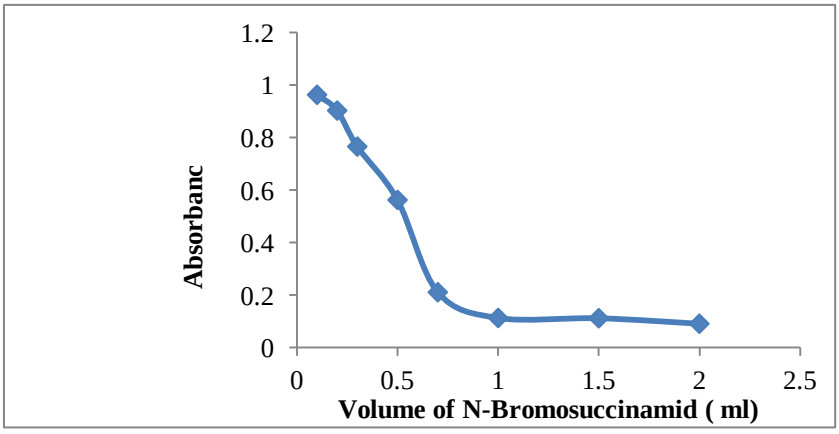



**Fig. (3): The effect of oxidant amount**

**The effect of acid:**
The effect of acid on oxidation of amoxicillin was studied, the obtained results by adding different amounts of various type of acids (HCl,$HNO_3$,$H_2SO_4$ and $CH_3COOH$) showed that $CH_3COOH$ gave the highest absorbance with low stability of color, while HCl gave good absorbance with a highest stability of color so that it has been recommended with 0.6 ml in the subsequence experiments.

**Effect of temperature:**
The effect of temperature on the color intensity of methylene blue was studied. In practice a maximum absorbance was obtained when the color was developed at room temperature (25˚C) , loss in color intensity and stability were observed in low or high temperature, therefore room temperature is recommended for subsequent experiments.

**Order of addition:**
To obtain optimum results the order of addition of oxidant reagent (NBS) should be followed as given under the analytical procedure, otherwise a loss in color intensity and less stability were observed.

**The effect of time on oxidation and bleaching the dye**
The effect of time on oxidation of amoxicillin trihydrate by N-bromosuccinamid and the time needed to optimum bleaching color of methylen blue were studied (Table 1).

**Table (1): The effect of time on oxidation and bleaching of the dye.**

| Addition of oxidant, min. | Standing time before dilution, min. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 30 | 40 | 50 | 60 |
| 5 | 0.690 | 0.666 | 0.666 | 0.669 | 0.665 | 0.666 | 0.662 | 0.665 |
| 10 | 0.716 | 0.716 | 0.710 | 0.705 | 0.701 | 0.699 | 0.662 | 0.665 |
| 15 | 0.722 | 0.722 | 0.720 | 0.719 | 0.721 | 0.720 | 0.724 | 0.720 |
| 20 | 0.676 | 0.675 | 0.665 | 0.662 | 0.665 | 0.665 | 0.661 | 0.662 |

The results in Table (1) show that the standing time of 15 minutes was necessary for the complete oxidation of amoxicillin and 5 minutes was necessary for bleaching of the color of methylene blue dye and the color of methylene blue dye was stable for at least another 55 minutes.

**Method validation:**
Employing the conditions described under the analytical procedure, a calibration graph for amoxicillin trihydrate was studied. The linearity of calibration graph, molar absorpitivity, Sandell's sensitivity, limit of detection, and limit of quantitative are summarized in Table(2).

**Table (2): Analytical parameters of the method**

| Parameter | Value | Parameter | Value |
|---|---|---|---|
| Temperature (°C) | 25 | Sandell's sensitivity | 5.0505 x$10^{-3}$µg.$cm^{-2}$ |
| $\lambda_{max}$ (nm) | 663 | Stability | 50 minutes |
| Beer's law range | (5-50) µg/10ml | Molar absorptivity | (8.30511x$10^4$ l. $mol^{-1}$.$cm^{-1}$ ) |

**Calibration curve:**

Following the general procedure linear relationship was obtained between the absorbance and the concentration of amoxicillin trihydrate within the range (5-50)µg/10ml (0.5-5) µg.$ml^{-1}$,with good value of determination coefficient ($R^2$)Figure (4) .

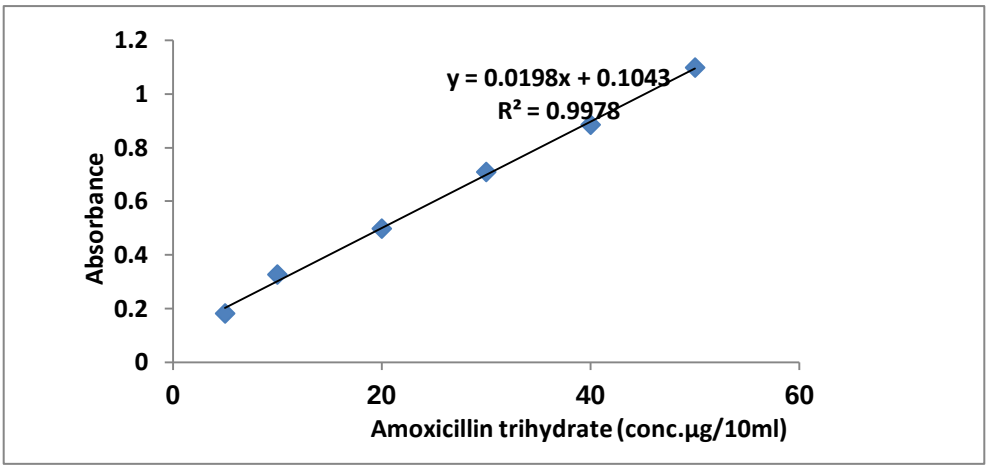



**Fig. 4: Calibration curve of amoxicillin trihydrate determination.**

**Accuracy and precision:**
The accuracy and precision of the method was evaluated by performing five replicate analysis on pure drug solution at two different concentration of amoxicillin. The results in Table (3) show a good accuracy and precision.

**Table (3) : The accuracy and precision.**

| Amoxicillin. µg./10ml | Relative error,%* | Relative standard deviation,%* |
|---|---|---|
| 30 | 0.1 | ±0.274 |
| 50 | 1.2 | ±2.722 |

**Effect of interference:**
The effect of some excipients usually present in the pharmaceutical preparations were investigated by carrying out the determination of amoxicillin in the presence of different excipients (Glucose, Starch, Arabic gum and lactose). Experimental results showed that there was no interference from excipients for the experimental method.

**Determination of amoxicillin trihydrate in a pharmaceutical formulations.**
The validity of the proposed method for spectrophotometric determination of amoxicillin was checked by the analysis of amoxicillin in different pharmaceutical preparations. The results obtained were given in Table(4).

**Table (4): The results of application**

| Drug | Amount taken µg/ml | Recovery% | RSD% |
|---|---|---|---|
| Julphamox- capsul, 500mg (Julphar, com.) | A | 99.5 | ±0.529 |
| | B | 99.8 | ±1.04 |
| Almox-capsul, 500mg , (ALKEM- U.A.E) | A | 100.4 | ±0.318 |
| | B | 99.8 | ±0.431 |
| GlomoX-capsul, 500mg (globalpharma-U.A.E) | A | 100.4 | ±0.346 |
| | B | 99.5 | ±1.42 |
| AugmentinTm capsul-625mg m, (GlaxoSmithkline-gSK) | A | 99.2 | ±1.20 |
| | B | 97.4 | ±1.05 |

| | | | |
|---|---|---|---|
| Augmentin solution- 250mg/5ml | A | 100.5 | ±4.9 |
| | B | 100.4 | ±2.8 |
| Amoxynin-capsul,500mg,(IRAQ-H.D.I) | A | 100.1 | ± 0.673 |
| | B | 99.5 | ±4.1 |

When A= 30 µg/10ml , B=50 µg/10ml

**Valuation:**

The validity of the method was confirmed by applying the standard addition procedure. The results in Table (5) are in agreement with certified values.

**Table (5): Determination of amoxicillin for many company by standard addition method .**

| Amoxicillin10 µg/ml | Amount taken µg/ml | Recovery% |
|---|---|---|
| Julphamox- capsul, 500mg (Julphar, com.) | A | 103.2 |
| | B | 105.2 |
| Almox- capsul, 500mg , (ALKEM-U.A.E) | A | 101.9 |
| | B | 103.5 |
| Glomo X-capsul, 500mg (globalpharma-U.A.E) | A | 99.3 |
| | B | 97.61 |
| Augmentin Tm capsul-625mg/ ml, (GlaxoSmithkline-gSK) | A | 101.4 |
| | B | 96.6 |
| Augmentin solution- 250mg/5ml | A | 105 |
| | B | 98.1 |
| Amoxynin-capsul,500mg,(IRAQ-H.D.I) | A | 100 |
| | B | 96.2 |

When A = 0.5 µg / ml B = 1 µg / ml

## Conclusion

The suggested method described the successful development of simple ,sensitive and accurate spectrophotometric method for the determination of amoxicillin using N-bromosuccinamid as oxidant agent of amoxicillin and the un-reacted N-bromosuccinamid bleached the methylene blue dye .The method has been applied successfully to determination amoxicillin in various pharmaceutical preparations.

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