# Indirect Spectrophotometric Method for Determination of Amoxicillin Trihydrate in Aqueous Solution and Pharametrical Samples

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## Abstract

A spectrophotometric method for determination of amoxicillin trihydrate (AT) in microgram level depending on its ability to reduce the yellow absorption of ($I^-_3$) at $\lambda_{max}$: 351 nm. The optimum conditions such as concentration of reactants, time of sitting, order of addition, were studied to get a high sensitivity ($\varepsilon = ٥,٨\times 10^{٣}$ L. $mol^{-1}$. $cm^{-1}$, with wide range of calibration curve ( 2- 40) μg.$ml^{-1}$, good stability ( more than 24 hr.) , repeatability (RSD%: 0.356-0.505%) and recovery ( 99.8 – 100.3%). This method was used for quantity assessment of five AT drug products with good precision and accuracy using standard addition method.

**Key Words:** Spectrophotometric method, amoxicillin trihydrate, standard addition method.

## Introduction

Amoxicillin Trihydrate (AT) is (2S, 5R, 6R) -6- [(R) -2- Amino -2- (*p*-hydroxyphenyl) acetamido] -3,3-dimethyl -7- oxo -4- thia -1- azabicyclo [3.2.0] heptane -2-carboxylic acid trihydrate [$C_{16}H_{19}N_3O_5S.3H_2O$, MW: 419.46 g/mol], and the structure as in (Fig. 1):

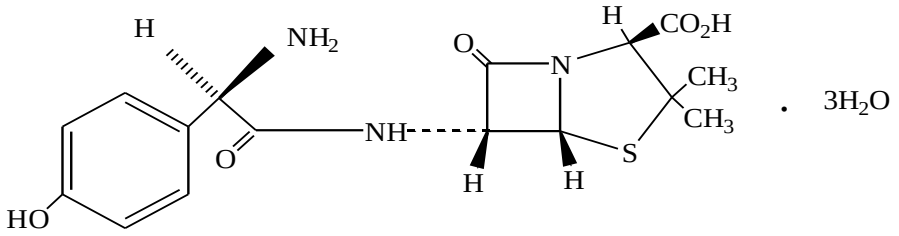


**Fig. 1: Structure of Amoxicillin Trihydrate.**

Amoxicillin Trihydrate is semi synthetic antibiotic with a broad spectrum of bactericidal activity against many gram-positive and gram- negative microorganisms.[1,2]

It has been determined quantitatively by different techniques including UV spectrophotometer[3-7], HPLC[8],HPTLC[9], RP-HPLC[10,11], fluorimetric method[12], In-vitro evaluation pH-sensitive[13] and using diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) and partial least squares (PLS)[14].

This paper reports a simple, sensitive and accurate spectrophotometric method for the simultaneous determination of amoxicillin trihydrate. This method is based on the indirect measurement of absorbance of drug with $I_3^-$ complex at 351 nm against the reagent blank for amoxicillin. The equation of reducing reaction is:

$$I_3^- + 2e^- \rightleftharpoons 3I^-$$

## Experimental

### Apparatus:

UV/VIS spectrophotometer (Shimadzo, 1650 pc, Japan) with 1 cm matched quartz cell.

### Chemicals and reagents:

All chemicals used were of analytical reagent grade and amoxicilline trihydrate standard material was provided from state company for drug industries and medical appliance - (SDI) Samaraa – Iraq. Commercial drugs from the markets are:

Amoxicillin trihydrate 500 mg (Akai Ltd, Amman, Jordan)

Amoxicillin trihydrate 500 mg (Nainawa Industrial drug/ Nainawa, Iraq)

Amoxicillin trihydrate 500 mg (Micro Labs Limited, Bangalore SCo-100, India).

Amoxicillin trihydrate 500 mg (Athlone Ltd, England).

Amoxicillin trihydrate 500 mg (Samara industrial drug, Samaraa, Iraq).

Iodine of (98 % purity) was obtained from (BDH).

Potassium iodide (99 % purity), from (BDH).

### Preparation of solutions:

1. A standard solution of 100 μg . $ml^{-1}$ Amoxicilline trihydrate was freshly prepared by dissolving 0.01 g of AT in distilled water and diluted to 100 ml. The solution was stable at least for 10 days at room temp.

2. A standard solution of 250 μg . $ml^{-1}$ was prepared by dissolving 0.063g of $I_2$ in water and then diluted with distilled water to 250 ml.

3. 5.05% Potassium iodide (99% purity, from (BDH) was prepared by dissolving 5.05 g of potassium iodide in distilled water and diluted to 100 ml.

### General procedure:

The solutions of AT of concentrations (2-40 μg. $ml^{-1}$) were prepared by using the mixture (5 ml of 5.05% potassium iodide with 10 ml of 250 μg. $ml^{-1}$ of Iodine) in 25ml volumetric flask and diluted with

distilled water to the mark, and then absorbance was measured for each solution at $\lambda_{max}$ = 351 nm after 5 min.[15]

**Procedure for assay of amoxicillin trihydrate in pharmaceutical preparation:**

Capsules: ten capsules were weighed, 100 mg of Amoxicillin trihydrate was weighed and dissolved in distilled water in 100 ml of volumetric flasks to get 100 µg. $ml^{-1}$,thereafter a solution of 50 µg. $ml^{-1}$ was prepared from the solution of 100 µg. $ml^{-1}$.

**Procedure for standard addition method:**

A series of solutions were prepared by adding constant volume of unknown solutions of capsules to (2 -10 µg. $ml^{-1}$**)** of standard solutions of AT, and a constant volume of ($KI+I_2$) was added in a 25 ml volumetric flask. The same procedure was preformed for all kinds of capsules.

## Result and Discussion*:*

**Procedure of appropriate wave length:**

A wavelength of yellow mixture solution of (Iodine with potassium iodide) was found to be at $\lambda_{max}$ = 351 nm due to the formation of $I^-_3$ complex, then drugs with different microgram concentrations were added, (Fig. 2) shows the ability of drug to reduce the yellow absorption of $I^-_3$ at $\lambda_{max}$ = 351 nm.

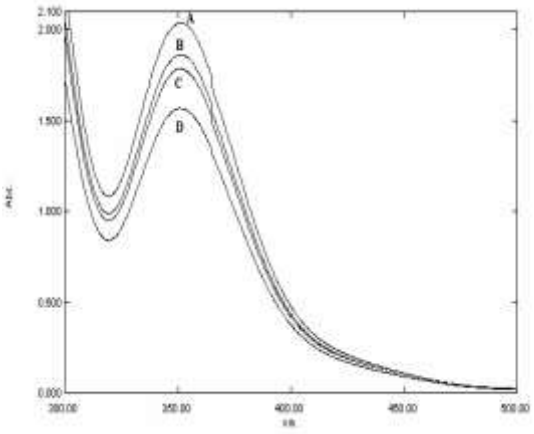


**Fig. 2: Spectrum of $I_3^-$ solution A:$I_3^-$ only ,B, C and D in the presence of 10, 20 and 30 µg $ml^{-1}$ of amoxicillin tri-hydrate respectively.**

**Procedure to optimum conditions.**

1. The best mixture of $I^-_3$ complex from ($KI+I_2$) to form highest absorbance at $\lambda_{max}$ = 351 nm .(Fig. 3) shows the addition (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 ml) of 250 µg. $ml^{-1}$ iodine solution to (1 ml) 5.05% potassium iodide solution effected the absorbance of $I^-_3$ and the highest absorbance resulted by adding 10 ml from 250 µg. $ml^{-1}$ of iodine. While (Fig. 4) shows the effect of addition 10 ml from 250 µg. $ml^{-1}$ of iodine to (1, 2, 3, 4, 5, 6, 7ml) 0f 5.05% KI.

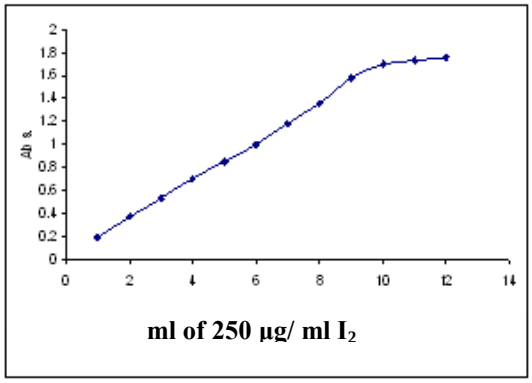


**Fig. 3: Effect of adding different volume of iodine solution (250 µg.$ml^{-1}$) to the absorption of $I^-_3$ complex at $\lambda_{max}$ = 351 nm**

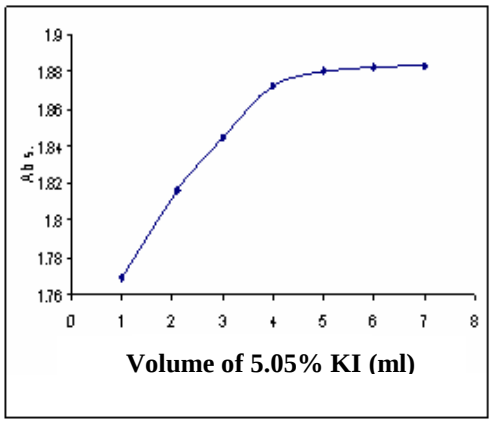


**Fig. 4: Addition of 10 ml from250 µg. $ml^{-1}$ of iodine to(1,2,3,4,5,6,7ml) of 5.05%KI at $\lambda_{max}$ = 351 nm**

2. Effect of time sitting.

It was found that the solution of $I^-_3$ was stable in dark place for 24 hours at room temperature when tested at $\lambda_{max}$ 351 nm .

3. Effect order of addition.

In order to obtain high sensitivity of the calibration curve for standard solution the order of addition of iodine was varied as it was added first to potassium iodide solution and then the standard solution (AT) was added to the resulted mixture .This solution was found to give a higher absorbance value in compares to that mixture obtained from adding iodine to a mixture of potassium iodide and standard solution(AT), Table (1) shows order no. I and II .This might a rise from the formation of $I^-_3$ in I . Therefore the order of addition thus taken for the subsequent in this work was taken as in order no. I .

**Table 1: The order of addition of reactants.**

| Order no. | Reaction component | Abs. |
|---|---|---|
| I | KI +$I_2$ +AT | 0.670 |
| II | KI +AT +$I_2$ | 0.508 |

4. When procedure for optimum conditions was employed a linear calibration curve was obtained for ( 2 – 40 µg.ml$^{-1}$) Amoxicillin trihydrate added in portions to blank solution of (10 ml from 250 µg. ml$^{-1}$ of $I_2$+5ml of 5.05%KI). The absorbance after adding AT was subtracted from that of blank solution, Fig(5).This linear curve was found to obey Beer's Law with correlation coefficient of 0.9991. The conditional molar absorptivity was found to be 5.8 × 10$^3$ L.mol$^{-1}$.cm$^{-1}$.

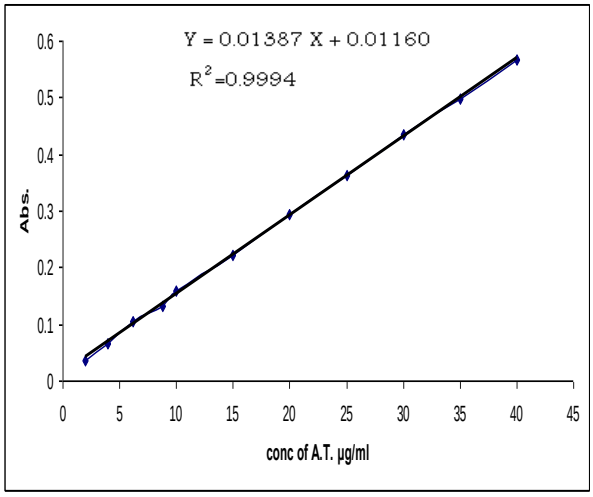


**Fig. 5: Calibration curve of amoxicillin tri-hydrate from (2-40) µg.ml$^{-1}$**

Linear regression analysis of calibration data gave the regression equation sited in Table (2) with correlation coefficients close to unity.

**Table 2: Summary of linearity studies in pure form.**

| Parameters | Value |
|---|---|
| Linearty range (µg.ml$^{-1}$) | 2- 40 |
| Regression equation | Y = 0.01387 X + 0.01160 |
| Slope (a) | 0.01387 |
| Intercept (b) | 0.01160 |
| Correlation coefficient ( r) | 0.9996 |
| Linearity (%r$^2$) | 99.94 |
| Molar absorptivity (L. mol$^{-1}$. cm$^{-1}$) | 5.8x10$^3$ |

With respect Y = aX + b, where X is the concentration in µg. ml$^{-1}$, Y is absorbance.

The results shown in Table( 3) were obtained for an average of three experiments for each 2, 6 and 10 µg. ml$^{-1}$ solutions of AT the results of % recovery and %RSD values were in the range 99.82% - 100.3% and 0.356%-0.505% respectively .

**Table (3): Summary of accuracy and precision results of the method.**

| Amount (µg. ml$^{-1}$) | | RSD% | Rec.% | $E_{re}$.% | C.L. |
|---|---|---|---|---|---|
| Taken | Found ±SD | | | | |
| 2 | 1.997±0.010 | 0.505 | 99.8 | 0.15 | 1.997 ± 2.5×10$^{-8}$ |
| 6 | 5.989±0.021 | 0.356 | 99.8 | 0.15 | 5.989 ± 5.2×10$^{-2}$ |
| 10 | 10.031±0.042 | 0.424 | 100.3 | -0.3 | 10.031 ± 1.04×10$^{-1}$ |

Mean for three independent analysis, standard deviation SD, relative standard deviation RSD%, Ere.%,Confidence limit C.L at 95% confidence level and 2 degrees of freedom (t = 4.30).

The linearity studies of AT in capsules using standard addition can be show in (table4).

**Table (4): Summary of linearity studies of Amoxicillin trihydrate incommercial capsules using standard addition method.**

| Manufacturing compouny | Regression eq. | Slope (a) | Intercept (b) | Correlation (r) | Len. | Molar absor |
|---|---|---|---|---|---|---|
| Alkei 500 mg | Y = 0.09345 X + 0.21490 | 0.09345 | 0.21490 | 0.9991 | 99.89 | $3.9 \times 10^4$ |
| India 500 mg | Y = 0.0795 X + 0.27030 | 0.0795 | 0.27030 | 0.9994 | 99.9 | $3.3 \times 10^4$ |
| Engiland 500 mg | Y = 0.097 X + 0.14780 | 0.097 | 0.14780 | 0.9993 | 99.89 | $4.0 \times 10^4$ |
| Samaraa 500 mg | Y = 0.09840 X + 0.18420 | 0.09840 | 0.18420 | 0.9998 | 99.96 | $4.1 \times 10^4$ |
| Nainawa250mg | Y = 0.07870 X + 0.33960 | 0.07870 | 0.33960 | 0.9995 | 99.90 | $3.3 \times 10^4$ |

The precision and accuracy were carried out through replicate analysis (n= 3) of different kind of Amoxicillin.

The results are reported in table (5) . As can be seen from it that the %recovery and% RSD values were in the ranges 99 – 100.5 % and 0.681 – 1.336% respectivly.

**Table (5): Summary of data for the determination of Amoxicilline trihydrate in pharmaceutical preparations by standard addition method.**

| Drug | Amount added ($\mu g.\ ml^{-1}$) | Amount recovered ($\mu g.ml^{-1}$)±SD (n = 3) | RSD% | Recovery% | $E_{re.}$% | C.L. |
|---|---|---|---|---|---|---|
| Akai 500 mg | 2 | 1.980±0.026 | 99.0 | 1.336 | -1 | $1.980\pm6.5\times10^{-2}$ |
| Samaraa 500 mg | 2 | 2.011±0.018 | 100.5 | 0.890 | -0.55 | $2.011\pm4.5\times10^{-2}$ |
| Engiland 500 mg | 2 | 1.995±0.0136 | 99.75 | 0.681 | 0.25 | $1.995\pm3.4\times10^{-2}$ |
| India 500 mg | 4 | 4.021±0.036 | 100.5 | 0.896 | -0.52 | $4.021\pm8.9\times10^{-2}$ |
| Nainawa 250mg | 4 | 4.048±0.031 | 100.5 | 0.775 | -0.3 | $4.048\pm7.7\times10^{-2}$ |

Mean for three independent analysis, $\%E_{rec}$ error, relative standard deviation RSD.

## Conclusion:

The method has wider linear dynamic range from 2 – 40 $\mu g.\ ml^{-1}$ with good accuracy and precision. This method is simple, coast affective fast and efficient. In addition the proposed method is simple, sensitive and rapid for the determination of amoxicillin trihydrate in bulk and pharmaceutical.

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# حساب الاموكسيسين ثلاثي الهايدريت بطريقة طيفية غير مباشرة في المحاليل القياسية المستحضرات الصيدلانية

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**( تاريخ الاستلام:** ١٩ / ٥ / ٢٠١١ ---- **تاريخ القبول:** ٢٨ / ٦ / ٢٠١١ )

**الملخص:**

تم استخدام طريقة طيفية لتقدير الاموكسيسلين ثلاثي الهيدريت بتركيز (مايكروجرام/مل) بالاعتماد على الصفة الاختزالية للاموكسيسلين من خلال أخماده لطيف معقد اليوديد الثلاثي($I_3^-$) ذي اللون الاصفر والذي له أعظم امتصاص عند الطول الموجي ٣٥١ نانوميترذُرست الظروف العملية الفضلى لاخماد هذا التفاعل بواسطة الاموكسيسلين (لأجل الحصول على حساسية عالية وأفضل أستقرارية ومدى واسع لمنحني المعايرة) والمتضمنة تأثير تراكيز المواد المتفاعلة، تأثير الزمن في أستقرارية المعقد، تأثير ترتيب الاضافة للوصول الى بناء منحني المعايرة. أعطت الطريقة حساسية عالية حيث بلغت قيمة معامل الممتصية المولارية $5.8 \times 10^3$ لتر.مول $^{-1}$. سم $^{-1}$. ووجد ان المدى التركيزي الخاضع لقانون بير من (٢- ٤٠) مايكروغرام. مل $^{-1}$.واستقرارية عالية للمحلول للاكثر من ٢٤ ساعة. واما التكرارية للنتائج المحصلة فكانت جيدة تراوحت قيم RSD% بين - 0.505 0.356 % اما قيم الاسترداد المئوي فتراوحت بين ٩٩,٨ – ١٠٠,٣ %. تم تطبيق هذه الدراسة على مناشئ مختلفة الاموكسيسلسن بأستخدام طريقة الاضافة القياسية.