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The indirect colorimetric determination of Amoxicillin in its commercial pharmaceutical formulations using methyl red dye

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Abstract:

A simple and sensitive colorimetric method was developed for the indirect determination of amoxicillin in pharmaceutical formulations. The method is based on the oxidation of amoxicillin by potassium permanganate ($KMnO_4$), where the excess oxidizing agent reacts with methyl red dye, leading to an increase in its light absorption with increasing drug concentration. The unoxidized dye showed maximum absorbance at a wavelength of 516 nm in an acidic medium. The optimal reaction conditions were established in terms of oxidation time, temperature, and the concentrations of oxidant, dye, and acid. The method's linearity was up to 1–35 µg/mL with an $R^2 = 0.9997$, a molar absorptivity of 26272.26 L/mol.cm, and a Sandell's sensitivity of 0.0139 µg/cm². The limits of detection and quantification were 0.0245 µg/mL and 0.0743 µg/mL, respectively. The method also showed good recovery values (97.20–103.09%) and low relative standard deviation (%RSD < 1.5%), confirming its accuracy and sensitivity. It was successfully applied for the determination of AMX in pharmaceutical formulations.

Keywords: Amoxicillin, colorimetric determination, potassium permanganate, methyl red dye, indirect determination, pharmaceutical formulations.

التقدير اللوني غير المباشر للأموكسيسيلين في مستحضراته الصيدلانية التجارية مع صبغة المثيل الأحمر

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مستخلص:

طُوّرت طريقة قياس لونية بسيطة وحساسة لتقدير الأموكسيسيلين بشكل غير مباشر في المستحضرات الصيدلانية. تعتمد الطريقة على أكسدة الأموكسيسيلين بواسطة برمنجنات البوتاسيوم ($KMnO_4$)، حيث يتفاعل عامل الأكسدة الزائد مع صبغة الميثيل الأحمر، مما يؤدي إلى زيادة امتصاصه للضوء مع زيادة تركيز الدواء. أظهرت الصبغة غير المؤكسدة أقصى امتصاص عند طول موجي 516 نانومتر في وسط حمضي. تم تحديد ظروف التفاعل المثلى من حيث زمن الأكسدة، ودرجة الحرارة، وتركيزات المؤكسد، والصبغة، والحمض. تراوحت خطية الطريقة بين 35-1 مايكروغرام/ مل، مع معامل امتصاص مولاري قدره 0.9997، وامتصاص مولاري قدره 26272.26 لتر/ مول·سم، وحساسية ساندل قدرها 0.0139 ميكروغرام/ سم². بلغت حدود الكشف والقياس الكمي 0.0245 ميكروغرام/ مل و0.0743 ميكروغرام/ مل، على التوالي. كما أظهرت الطريقة قيم استرداد جيدة (97.20-103.09٪) وانحرافًا معياريًا نسبيًا منخفضًا (RSD < 1.5٪)، مما يؤكد دقتها وحساسيتها. وقد طُبقت بنجاح لتقدير الاموكسيسيلين في المستحضرات الصيدلانية. الكلمات المفتاحية: أموكسيسيلين، التحديد اللوني، برمنجنات البوتاسيوم، صبغة الميثيل الأحمر، التحديد غير المباشر، الصيدلة

الكلمات المفتاحية: أموكسيسيلين، التحديد اللوني، برمنغنات البوتاسيوم، صبغة الميثيل الحمراء، التحديد غير المباشر، المستحضرات الصيدلانية.

Introduction

Amoxicillin is a broad-spectrum antibiotic that belongs to the penicillin group[5]. It is a semisynthetic derivative of natural penicillin. The drug acts by inhibiting the synthesis of the bacterial cell wall, leading to the elimination of bacteria. It is used to treat various bacterial infections, including otitis media, pneumonia and bronchitis, endocarditis caused by enterococci, meningitis, urinary tract infections, and Lyme disease. Amoxicillin is usually administered orally[6].The systematic (IUPAC) name of the drug is:(2S,5R,6R)-6-{[(2R)-2-amino-2-(4-hydroxyphenyl)-acetyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid. It has the molecular formula $C_{16}H_{19}N_3O_5S$ and a molecular weight of 365.4 g/mol[7]. The structural formula is illustrated in Figure (1).

The dye bleaching method is considered one of the important indirect colorimetric techniques for the determination of pharmaceutical compounds[15]. This method is based on the addition of an excess amount of an oxidizing agent to the sample, where part of it reacts with the drug compound, while the remaining unreacted portion interacts with the dye, leading to the fading (bleaching) of its color in either an acidic or alkaline medium. Consequently, the higher the concentration of the drug compound, the lower the amount of oxidizing agent remaining, which in turn reduces the bleaching of the dye and increases the concentration of its unbleached form. Therefore, the absorbance of the dye is directly proportional to the concentration of the drug compound in the sample.

Drug + excess oxidizing reagent (H^+) → Oxidized Drug + unreacted oxidizing agent

Excess of oxidizing agent + dye → bleaching dye

Amoxicillin has previously been determined using several analytical methods, including spectrophotometric techniques with ultraviolet radiation [1,2], colorimetric methods [3,10,20-21], which have been applied to the determination of many pharmaceutical compounds in their pure form and pharmaceutical formulations [16,17], as well as chromatographic methods [4,8,9],

and chromatography coupled with LC/MS[11,12]. The present study aims to develop a spectrophotometric colorimetric method based on the bleaching reactions of methyl red dye for the determination of amoxicillin in its pure form as well as in commercial pharmaceutical formulations.

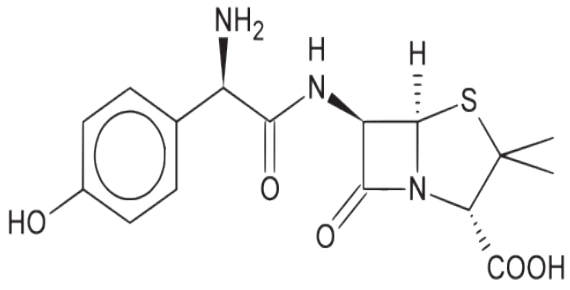

Figure 1. Structure of Amoxicillin

Results and Discussion

Preliminary Observations

When 1 mL of amoxicillin solution (100 µg/mL) was mixed with 1 mL of potassium permanganate solution (0.001 M) and left for 13 minutes, followed by the addition of 0.5 mL of hydrochloric acid (1 M) and 1 mL of methyl red solution (100 µg/mL), a water-soluble pink-colored product was formed. The maximum absorbance was observed at a wavelength of 516 nm.

Optimal Conditions

The study of optimal conditions in analytical methods aims to enhance the sensitivity and accuracy of the method, improve repeatability, reduce errors, lower detection limits, and ensure the reliability of the results. Without investigating these conditions, the method would be inefficient and unreliable for analysis.

Selection of Blank Solution

Selection of Dye Type

Solutions of several dyes (100 µg/mL) were prepared, including crystal violet, phenol red,

eosin, methyl orange, methyl red, and bromophenol blue. One milliliter of each solution was analyzed using UV–Vis spectrophotometry at the appropriate wavelength for each dye. The results showed that methyl red exhibited the highest absorbance, making it the most suitable dye for this study.

Table (1). Selection of Dye Type

Dye	(nm) λ	A
Crystill Violet	542	0.992
Phenol Red	423	0.476
Eosin	512	0.842
Methyl orange	460	0.913
Bromophenol Blue	590	0.575
Methyl Red	**524**	**1.024**

Selection of Dye Volume

The optimal volume of methyl red (100 µg/mL) was investigated using volumes ranging from 0.2 to 1.2 mL, while adjusting the total volume to 10 mL with ethanol as the solvent. The absorbance was recorded and a calibration curve was plotted. The best linearity was observed within the range of 0.4–1.0 mL at 524 nm, with a correlation coefficient of $R^2 = 0.9983$. Beyond this range, the calibration curve began to deviate. Therefore, the volume that provided the highest absorbance at a concentration of 10 µg/mL was selected.

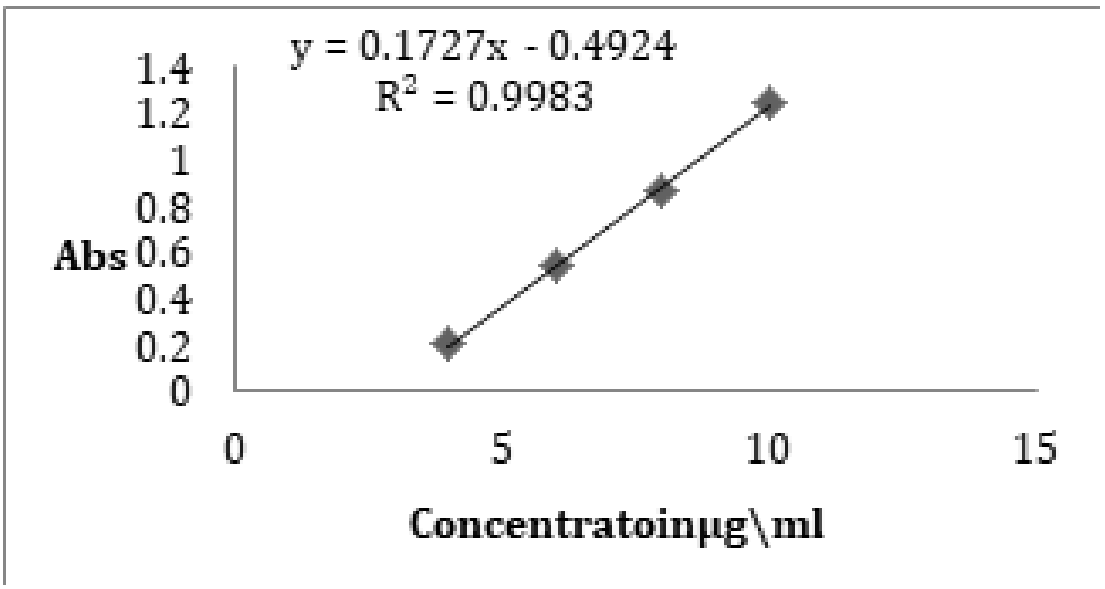

Figure (2). Calibration curve for selecting the optimal volume of methyl red dye

Selection of the Best Oxidizing Agent

Several oxidizing agents were tested (potassium permanganate, cerium sulfate, potassium iodate, ferric sulfate, potassium iodide, and ferric chloride) for their ability to bleach the dye. Potassium permanganate proved to be the most effective, as it removed the color of methyl red within less than one minute and produced a clear solution with the lowest absorbance at 336 nm, as shown in Table (2).

Table (2). Selection of the Best Oxidizing Agent

Oxidizing Agent	Absorbance	Wavelength (nm)
Ferric sulfate	2.198	520
Potassium iodate	2.071	520
Potassium permanganate	**0.205**	**336**
Ferric chloride	0.000	000
Cerium sulfate	1.949	520
Potassium iodide	2.071	520

Selection of Oxidizing Agent Volume ($KMnO_4$)

Different volumes of 0.001 M potassium permanganate ($KMnO_4$) were tested to determine the optimal volume required for complete reaction with methyl red dye and for bleaching its color. The tested volumes ranged from 0.25 to 1.25 mL, added to 0.5 mL of 1 M HCl and 1 mL of methyl red (100 µg/mL), with the total volume adjusted to 10 mL using deionized water. The optimal volume of the oxidizing agent was found to be **1 mL**, at which the dye color completely disappeared, as shown in **Table (3)**.

Table (3). Effect of $KMnO_4$ Volume on the Color of the Blank

Volume of $KMnO_4$ (mL)	Color of Blank Solution
0.25	Purple
0.50	Purple
0.75	Purple
1	Transparent
1.25	Transparent

Selection of Reaction Medium

The effect of the reaction medium was studied using a 10 mL volumetric flask containing 1 mL of $KMnO_4$ and 0.5 mL of acid or base (1 M), followed by the addition of 1 mL of methyl red dye, with the total volume adjusted to 10 mL. The acidic medium provided better contrast between the sample and the blank, while the alkaline medium produced a yellow solution. Hydrochloric acid (HCl) was found to be the most effective, giving the highest absorbance at336 nm, as shown in Table (4).

An acidic medium is preferred when using potassium permanganate for the oxidation of amoxicillin, since the permanganate ion (MnO_4^-) exhibits its strongest oxidizing power in acidic conditions, where it is reduced to Mn^{2+} with a standard reduction potential of +1.51 V. In neutral or alkaline media,

permanganate is reduced to MnO_2 or other species with lower reduction potentials, which decreases its effectiveness as an oxidizing agent. Moreover, the acidic medium ensures the stability of the reaction and prevents the formation of undesirable precipitates such as MnO_2, thereby enabling complete and consistent oxidation of amoxicillin molecules and yielding accurate analytical results[16]. The observed differences in color in acidic media depending on the acid type are likely due to variations in acid strength and the pH of the solution.

Table (4). Effect of Reaction Medium on the Color of the Blank

Acid/Base	Blank Color	Sample Color	Wavelength (nm)	Absorbance at 516 nm
NaOH	Yellow	Yellow	310	0.000
NH_4OH	Yellow	Yellow	310	0.000
HCl	**Transparent**	**Pink**	**336**	**0.196**
H_2SO_4	Transparent	Pink	310	0.131
CH_3COOH	Brown	Pink	340	0.000

Effect of Hydrochloric Acid Volume

The effect of the volume of 1 M hydrochloric acid (HCl) on the reaction efficiency was studied using volumes ranging from 0.25 to 1.25 mL. The absorbance values of the resulting solution were recorded at each acid volume, as shown in **Table (5)**.

Table (5). Effect of HCl Volume on the Absorbance of the Resulting Solution

HCl Volume (mL)	Solution Color	Absorbance
0.25	Brown	0.000
0.50	**Transparent**	**0.196**
0.75	Transparent	0.169
1.00	Transparent	0.147
1.25	Transparent	0.136

The results indicate that 0.5 mL of HCl is the optimal volume to achieve the highest absorbance value (0.196). This behavior is attributed to the role of HCl in providing the acidic medium necessary for the reaction to proceed effectively. At the lower volume (0.25 mL), the solution appeared brown with zero absorbance, indicating that the amount of acid was insufficient to initiate or complete the reaction, and therefore, the product responsible for absorbance was not formed.

Conversely, increasing the acid volume beyond the optimal point (0.5 mL) resulted in a gradual decrease in absorbance. This decrease can be explained according to Beer–Lambert law, where absorbance is directly proportional to the concentration of the absorbing species. Adding larger volumes of HCl dilutes the solution, reducing the concentration of the final product and consequently lowering the measured absorbance.

Effect of Time on Blank Preparation

The stability of the blank solution (1 mL $KMnO_4$ + 0.5 mL 1 M HCl + 1 mL methyl red, with the total volume adjusted to 10 mL) was studied over a period of 1–60 minutes. The absorbance at 336 nm remained nearly constant, indicating that the blank is stable after the addition of the dye, as shown in Table (6).

Table (6). Effect of Time on Blank Stability

Absorbance	Time (min)	Absorbance	Time (min)
0.212	0	0.212	35
0.213	5	0.213	40
0.213	10	0.213	45
0.212	15	0.212	50
0.212	20	0.212	55
0.211	25	0.212	60
0.212	30		

Time plays a crucial role in the reaction, initially driving the color change as methyl red reacts with permanganate until the reaction is complete, af-

ter which the solution reaches a stable phase where absorbance remains constant, confirming the blank's stability.

Oxidation of Amoxicillin

Effect of Time on Amoxicillin Oxidation

One milliliter of amoxicillin solution (100 µg/mL) was mixed with 1 mL of $KMnO_4$ (0.001 M) and allowed to react for different time intervals (1–15 minutes), followed by the addition of 0.5 mL HCl and 1 mL methyl red. The absorbance at 516 nm gradually increased, reaching a maximum at 13 minutes. Therefore, 13 minutes was selected as the optimal oxidation time (Table 7).

Table (7). Effect of Oxidation Time

Absorbance	Time (min)	Absorbance	Time (min)
0.281	Start	0.604	9
0.293	2	0.613	10
0.423	3	0.623	11
0.419	4	0.641	12
0.409	5	**0.683**	**13**
0.475	6	**0.683**	**14**
0.532	7	0.651	15
0.581	8		

The effect of time on the reaction involves an initial phase where amoxicillin is oxidized by permanganate ions, gradually consuming the oxidizing agent, followed by a completion phase in which absorbance at 516 nm stabilizes, indicating full reaction and the attainment of chemical equilibrium.

Effect of Waiting Time Before Dilution

After 13 minutes of oxidation, HCl and methyl red dye were added, and the mixture was allowed to stand for different time intervals before dilution. The highest absorbance was observed after 6 minutes, which was selected as the optimal waiting time before dilution, as shown in Table (8).

Table (8). Effect of Waiting Time Before Dilution

Absorbance	Time (min)	Absorbance	Time (min)
0.494	1	0.763	5
0.497	2	0.819	6
0.530	3	0.756	7
0.610	4	0.651	8

The optimal waiting period of 6 minutes corresponds to the point at which the main reaction is effectively completed, yielding the highest absorbance. Beyond this period, other factors begin to influence the solution, leading to a decrease in absorbance.

Effect of Temperature on Amoxicillin Oxidation

Heating the mixture after 13 minutes of oxidation and before the addition of acid and dye led to an increase in absorbance, reaching a maximum at 50°C, which was selected as the optimal temperature, as shown in Table (9).

Table (9). Effect of Temperature on AMOX Oxidation

Absorbance	Temperature (°C)	Absorbance	Temperature (°C)
0.375	20	0.541	45
0.426	25	0.860	50
0.443	30	0.586	55
0.433	35	0.569	60
0.479	40		

The results show a gradual increase in absorbance with rising temperature up to 50°C. This behavior can be attributed to the increased reaction rate with higher temperatures. According to reaction kinetics principles, an increase in temperature raises the kinetic energy of the reacting molecules. As a result, amoxicillin and permanganate molecules possess higher kinetic energy, increasing the probability of effective collisions. More molecules acquire the energy required to overcome the activation energy barrier, accelerating

the reaction and ensuring its completion in a shorter time. This leads to a greater production of oxidation products within a given period, raising the absorbance to a maximum at 50°C.

Beyond 50°C, the absorbance begins to decrease at 55 and 60°C. This may be due to the thermal instability of the colored oxidation products, leading to their decomposition into simpler compounds that absorb light less efficiently. Excessively high temperatures may also cause unwanted side reactions, such as the degradation of other solution components, affecting the final products and reducing overall absorbance. Additionally, overheating may partially decompose amoxicillin through pathways other than the desired reaction with permanganate, decreasing the amount of reactant available for the intended oxidation.

Effect of Temperature on the Colored Product After Dye Bleaching

When the product of the excess oxidizing agent reaction with the dye (after the oxidizing agent reacted with amoxicillin) was heated, a decrease in absorbance was observed with increasing temperature. Therefore, 25°C was selected as the optimal temperature for measurement, as shown in Table (10).

Table (10).
Effect of Temperature on the Stability of the Dye-Bleached Product

Temperature (°C)	Absorbance
20	0.951
25	0.951
30	0.942
35	0.930
40	0.85

The results demonstrate that the colored compound formed after amoxicillin oxidation and subsequent dye bleaching is sensitive to temperature. The observed decrease in absorbance between 30°C and 40°C reflects the thermal instability of the colored product, which may decompose into simpler components with lower light-absorbing capacity. Heating the solution after the reaction does not accelerate the main reaction but compromises the stability of the final colored product, reducing the concentration of the absorbing species and thereby lowering absorbance according to the Beer–Lambert law. Additionally, elevated temperatures

can promote side reactions among remaining solution components and induce structural changes in the colored compound, further diminishing its ability to absorb light efficiently.

Effect of Time on the Stability of the Colored Product After Dye Bleaching

The study of the stability of the colored complex formed after dye bleaching showed that the absorbance reaches a stable value after 1 minute and remains nearly constant for up to one hour. This time period was adopted to ensure the accuracy and reliability of measurements. These results indicate that the colored complex is highly stable over the measured period, which ensures consistent and reliable absorbance readings for analytical purposes.

Table (11). Effect of Time on the Stability of the Colored Complex

Absorbance	Time (min)	Absorbance	Time (min)
0.954	0	0.954	30
0.954	5	0.954	40
0.954	10	0.954	50
0.954	20	0.954	60

Effect of Addition Sequence

The addition sequence O + A + D (Oxidative reagent → Acid → Dye) produced the best results, yielding a clear and stable solution whose color did not change over time. This stability can be explained as follows: adding the oxidizing agent (potassium permanganate) to the acidic medium first ensures optimal conditions for the oxidation process. When the dye (D) is added last, it reacts immediately and completely with the remaining oxidizing agent, resulting in instant and complete bleaching. This rapid and complete reaction prevents any subsequent side reactions that could affect the solution's stability.

In contrast, other sequences such as O + D + A and D + A + O produced unstable solutions that gradually changed color from clear to light purple over time. This behavior is due to the absence of an acidic medium at the ap-

propriate time for oxidation, allowing incomplete or side reactions to occur. In these cases, permanganate ions were not consumed immediately, leading to the appearance of a purple color over time, reflecting solution instability. Based on these results, the sequence O + A + D was adopted for all subsequent analyses to ensure accuracy and reliability of measurements.

Table (12). Effect of Addition Sequence

Addition Order*	Color of Blank	Characteristics
O + A + D	**Clear**	**Stable**
O + D + A	Clear → Purple	Unstable
D + A + O	Clear → Purple	Unstable

***O = Oxidative reagent, A = Acid, D = Dye**

Proposed Method

Graduated volumes of amoxicillin solution (100 µg/mL) within the range of 0.1–3.5 mL were transferred into a series of 10 mL volumetric flasks. To each flask, 1 mL of potassium permanganate solution (0.001 M) was added, and the mixture was left for 13 minutes to complete the oxidation. The solution was then heated to 50 °C to ensure the reaction was complete. After cooling, 0.5 mL of HCl (1 M) was added, followed by 1 mL of methyl red dye(100µg/ml). The flasks were left at room temperature for 6 minutes to allow the color reaction to complete, then the volume was adjusted to the mark with deionized water. Absorbance was measured at 516 nm against a blank prepared in the same manner, except without amoxicillin, and readings were taken at 25 °C.

Calibration Curve

After optimizing the conditions for amoxicillin determination, a standard calibration curve was prepared. Graduated volumes of the standard amoxicillin solution (100 µg/mL) ranging from 0.1–4.5 mL were transferred into a series of 10 mL volumetric flasks. To each flask, 1 mL of potassium perman-

ganate solution (0.001 M) was added, and the mixture was left for 13 minutes to complete the oxidation. The solution was then heated to 50 °C, followed by the addition of 0.5 mL of HCl (1 M) and 1 mL of methyl red solution (100 µg/mL). After 6 minutes, the volume was adjusted to the mark with deionized water. Absorbance was measured at 516 nm against a blank prepared in the same way but without AMOX, with blank readings taken at 25 °C. The results showed a linear response within the range of 1–35 µg/mL of amoxicillin, as illustrated in **Figure 3**. The molar absorptivity (ε) was 26,272.26 $L \cdot mol^{-1} \cdot cm^{-1}$, and Sandell's sensitivity was 0.0139 $\mu g/cm^2$.

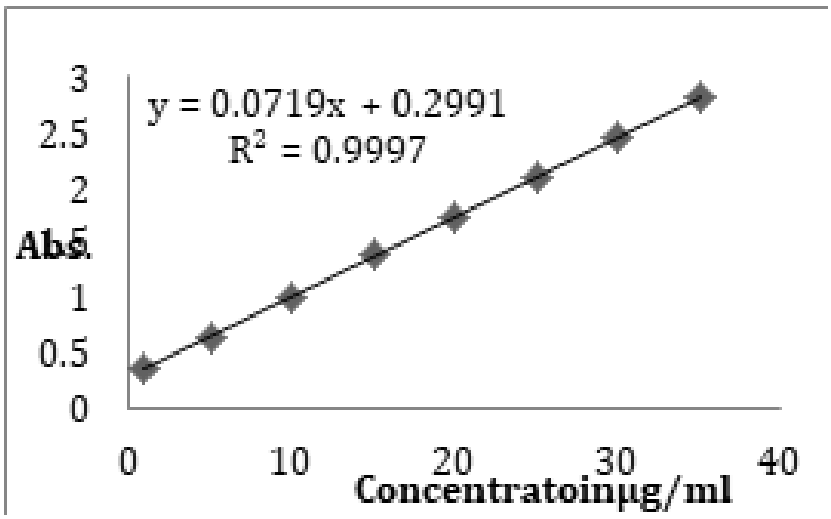

Figure 3 – Calibration curve of amoxicillin by the proposed method

Precision and Accuracy of the Method

A study was conducted to evaluate the precision and accuracy of the proposed method by calculating the percent recovery (Rec%), which reflects the accuracy of the results, and the relative standard deviation (RSD%), which indicates the method's precision. This study was performed using the concentrations of the calibration curve, with seven replicates for each concentration.

The percent recovery ranged from 97.204% to 103.096%, while the RSD% did not exceed 1.5%, indicating that the method possesses good accuracy and precision, as shown in Table 12.

Table 12 – Percent Recovery and Relative Standard Deviation

Taken Conc. (µg/mL)	Found Conc. (µg/mL)	RSD%	Rec%
1	0.972	0.142	97.218
5	4.922	0.082	98.442
10	9.720	0.053	97.204
15	15.454	0.039	103.096
20	20.109	1.475	100.549
25	25.019	0.147	100.077
30	29.887	0.104	99.624
35	34.936	0.150	99.817

*Average of seven readings

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated by measuring absorb the lowest concentration in the curve seven times, using the following equations [16]:

$LOD=3.3\times\delta/slope$….(1)

$LOQ=10\times\delta/slope$….(2)

Where: LOD = limit of detection, LOQ = limit of quantification, δ = standard deviation ,of the blank readings, and slope = slope of the calibration curve.

The calculated values were: LOD = 0.0246 µg/mL, LOQ = 0.0744 µg/mL

The standard deviation of the blank readings was 0.000535, and the slope of the calibration curve was 0.0719.

These values are reasonable, as both LOD and LOQ should be lower than the lowest concentration used in the calibration curve. The purpose of determining LOD and LOQ is to establish the lowest concentration that can be detected and quantified by the method, respectively.

Application of the Proposed Method

The proposed method was successfully applied to pharmaceutical preparations from Kent Pharmaceuticals and Micro Labs Limited using the single standard addition method, the percent recovery method, and the multiple standard additions method. These approaches showed recovery percentages ranging between 99.276% and 104.79%, with relative standard deviations (RSD%) below 1%, as shown in Tables 13–15.

Table 13 – Single Standard Addition Method

	Concentration µg/ml			RSD%	Rec%
	Taken	Added	Found		
Kent pharmaceuticals AMOX	10	5	10.130	0.035	101.307
	10	10	10.360	0.027	103.602
	6	9	6.283	0.038	104.728
MICRO LABSLIMITED	10	٥	10.963	0.038	99.638
	10	10	10.360	0.060	103.602
	10	15	10.422	0.063	104.228

Table 14 – Recovery study Method

	Percentage of added	concentration µg/ml			RSD%	Rec%
		Taken	Added	Found		
Kent pharmaceuticals AMOX	50%	0.5	1	5.130	0.035	102.614
	100%	1	1	10.360	0.027	103.602
	150%	9	6	9.283	0.038	103.152
MICRO LABSLIMITED	50%	5	10	4.963	0.038	99.276
	100%	10	10	10.360	0.060	103.602
	150%	15	10	15.422	0.063	102.818

Table 15 – Multiple Standard Additions Method

Company	Concentration µg\ml		Rec%	RSD%
	taken	Found		
Kent AMOX pharmaceuticals-500	5	4.984	99.693	0.166
MICRO LABSLIMITED-500	5	5.239	104.793	0.155

Study of the Stoichiometric Ratio

To determine the molar ratio of the reaction between potassium permanganate ($KMnO_4$) and methyl red, the Job's method of continuous variations was employed. A series of solutions were prepared in 10 mL volumetric flasks using varying concentrations of the oxidizing agent ($KMnO_4$) in the range of 1×10^{-4} – 9×10^{-4} M, while complementary concentrations of methyl red were prepared to maintain a constant total volume, which was completed to the mark with deionized water.

A blank solution was prepared in the same manner to serve as a reference. The absorbance of each solution was then measured at 516 nm. The results indicated that the molar ratio of the reaction between potassium permanganate and methyl red is **1:1,** as illustrated in the corresponding figure.

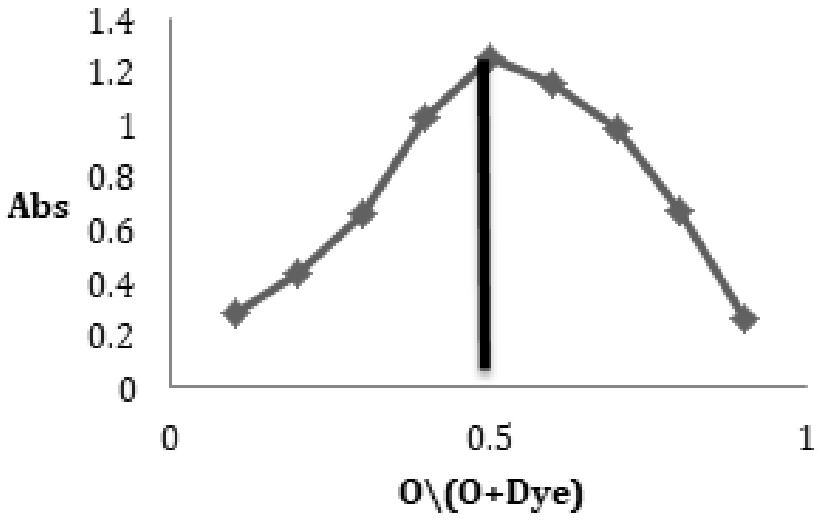

Figure .4 Job's Plot for the Molar Ratio of the Oxidizing Agent to the Dye

The proposed chemical equations for the reactions in the proposed method are as follows:

Half-reaction of Potassium Permanganate Reduction in Acidic Medium:

$$MnO^{4-} + 8\ H^{+} + 5\ e^{-} \rightarrow Mn^{2+} + 4\ H_2O$$

Oxidation of Amoxicillin by Potassium Permanganate in Acidic Medium

$$5\ C_{16}H_{19}N_3O_5S + 94\ MnO_4^{-} + 257\ H^{+} \rightarrow 80\ CO_2 + 176\ H_2O + 15\ NO_3^{-} + 5\ SO_4^{2-} + 94\ Mn^{2+}$$

Consumption of Excess KMnO4 by Methyl Red Dye:

$$5\ C_{15}H_{15}N_3O_2 + 86\ MnO_4^{1-} + 243\ H^{+} \rightarrow 75\ CO_2 + 159\ H_2O + 15\ NO_3^{-} + 86\ Mn^{2+}$$

Comparison of Analytical Characteristics of the Proposed Method

The proposed spectrophotometric method for determining amoxicillin was compared with another spectrophotometric method and an HPLC-UV method, as summarized in Table 16. The proposed method demonstrates superior sensitivity and analytical performance.

Table 16 – Comparison of the Proposed Method with Another Spectrophotometric Method

parameter	Bleaching of dye Method	Verordit [15] method	Verordit [17] method
λ nm	516	468	227
Linearityμg/ml	1-35	1.8-32	1-100
Slope	0.0719	0.079	…
R^2	0.9997	0.999	0.98≤
LODμg/ml	0.0246	0.420	0.5>
LOQ μg/ml	0.0744	1.274	0.5
Ɛ L/mol.cm	26272.26	26320	…
Sand ell's Index mg/cm^2	0.0139	0.0139	…
Rec%	97.20-103.09%	97-100.50%	100.3-105.0%
RSD%	0.037-1.47%	0.98-1.85%	10.8-18.7%

The proposed method has a lower limit of detection (0.0246 μg/mL) and quantification (0.0744 μg/mL) than the reference methods, allowing the detection and measurement of very low concentrations of amoxicillin. It shows excellent linearity over the tested range, with a correlation coefficient of 0.9997, and high molar absorptivity and favorable Sandell's index values, indicating strong sensitivity. Recovery percentages between 97.20 and 103.09% and low relative standard deviations (0.039–1.47%) reflect high accuracy and precision. These results suggest that the proposed dye-bleaching spectrophotometric method is reliable, reproducible, and more sensitive than the other compared methods, making it well-suited for the determination of amoxicillin in pharmaceutical formulations.

Conclusions

A simple, rapid, and sensitive spectrophotometric method was developed for the determination of amoxicillin using methyl red dye through the metachromatic dye-shortening reactions, resulting in a dark pink colored product. The maximum absorbance of this product was measured at 516 nm. The

method demonstrated good accuracy and precision. The proposed method was successfully applied to the determination of amoxicillin in its pharmaceutical capsule formulations. The results showed excellent agreement with the labeled content of the pharmaceutical products and were consistent with those obtained using the single standard addition and percent recovery addition methods.

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