INDIRECT SPECTROPHOTOMETRIC DETERMINATION OF AMOXICILLIN TRIHYDRATE IN PURE FORM AND IN PHARMACEUTICAL PREPARATIONS USING CRESOL RED DYE

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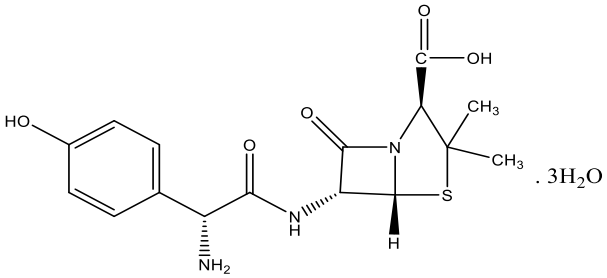
***Abstract**: A rapid, sensitive, and indirect spectrophotometric method was developed for the determination of amoxicillin in pure form and in pharmaceutical preparations. The method is based on the oxidation of the pharmaceutical compound amoxicillin in an acidic medium (hydrochloric acid) with a known excess of the oxidizing agent N-bromosuccinimide (NBS); then the unreacted excess of the oxidizing agent leads to the paling of the color of the cresol red dye, which gives absorption at a wavelength of 442 nm. The method followed Beer's law within a concentration range of 0.25-6.25 µg/ml and with a molar absorptivity of 3.72 × 104 L·mol⁻¹·cm⁻¹. The accuracy of the method reached 101.1%, and the precision of the method (relative standard deviation) was less than 1%. The limit of detection (LOD) value was 0.25 µg/ml, and the limit of quantitation (LOQ) value was 0.82 µg/ml. The method was successfully applied to pharmaceutical preparations of the pharmaceutical compound in the form of tablets and injections.*
***Keywords:** cresol red dye, N-bromosuccinimide, amoxicillin, spectrophotometer.*

Introduction

Amoxicillin, or hydroxyl ampicillin, is a phenolic antibiotic from the beta-lactam group that has significant activity against both Gram-positive and Gram-negative bacteria. This antibiotic is widely used to treat infectious diseases in humans and animals and to promote growth and productivity in agriculture. Amoxicillin has some important advantages over ampicillin [1, 2], including more complete intestinal absorption and little or no effect on food absorption. It is a white crystalline powder that is soluble in water, very slightly soluble in ethanol, and insoluble in oils and fats. However, it is soluble in dilute solutions of hydroxylic acids and bases [3, 4]. It is used in the treatment of acute bacterial sinusitis and community-acquired pneumonia [5]. Amoxicillin trihydrate has the following chemical composition:



Formula [$C_{16}H_{19}N_3O_5S\cdot 3H_2O$] and structure of the amoxicillin; M.wt = 419.46 gm/mole

Various analytical methods have been used to estimate the pharmaceutical compound amoxicillin, including spectroscopic methods based on oxidative coupling reactions [6, 7], diazotization reactions [8-12], and ion association complex reactions [13-15], charge transfer complexes [16], flow injection technique [17-22], and electrical methods [23]. A kinetic method was also used to estimate the drug compound [24], as well as methods of high-performance liquid chromatography [25-30] and atomic absorption and emission spectroscopy [31, 32], and voltammetry [33, 34]. The dye can be used to estimate the drug compound amoxicillin, as is the

case with the use of a number of dyes to estimate a number of drug compounds, including safranin O dye and EBT [35-37].

Experimental part

Apparatus used:

- Shimadzu UV-1800 Double-beam spectrophotometer was used for absorbance measurements with 1 cm matched glass cells.
- Sensitive balance of ADAM type.

Use a water bath to perform the electromagnetic heating process. *Solutions of chemicals used:* the chemicals used were all of a high degree of purity (Tables 1 and 2).

Table 1. Preparing the chemical compounds involved in the experiments

Chemicals	Concentration	Preparation	Final dilution with water
Amoxicillin	(100 µg/ml)	The solution was prepared by dissolving 0.0100 grams in 2 ml of ethanol, then completing the volume to 100 ml of distilled water, then diluting it to 50 µg/mL.	100 mL
Cresol red dye	(200 µg /ml)	The solution was prepared by taking a weight of 0.0200 gm of dye, dissolving it in 10 ml of ethanol, and completing the volume with distilled water to the mark in a 100 ml volumetric bottle	100 mL
N-bromosuccinimide	(1 x 10^{-2}M)	0.1779 gm of N-bromosuccinimide was dissolved in distilled water, then the volume was completed to the mark in a 100 ml volumetric bottle. Other oxidizing agents were also prepared at the same concentration	100 mL
Hydrochloric acid	(1 M)	The acid solution was prepared by taking 8.3 ml of 12.06 M concentrated acid and diluting it to 100 ml with distilled water. The other acids were also prepared at the same concentration	100 mL

Table 2. Preparation of drug solution (Amoxicillin) from the pharmaceutical preparation

Injection 500mg	The content of the injection was dissolved in 2 ml of ethanol and diluted to 100 ml with distilled water to obtain a drug solution with a concentration of 5000 µg/ml, from which 50 µg/ml was prepared
Tablets 1000mg	Amoxicillin tablets were analyzed by weighing five tablets of the pharmaceutical preparation and grinding them well. The equivalent of approximately the weight of one tablet was taken and dissolved in 20 ml of ethanol. Then the solution was filtered, and the filtrate was placed in a volumetric flask with a capacity of 100 ml, and the volume was supplemented with distilled water to the mark to obtain a concentration of 10.000 µg/ml, then diluted to obtain a concentration of 50 µg/ml

The preliminary study. The absorption spectrum of the dye was drawn by taking a concentration of 1 ml of 200 µg/ml, which gives the highest absorption at the wavelength of 434 nm, as in Fig. 1,

then increasing volumes (0.1-3.5 ml) of the dye were taken with 0.5 ml hydrochloric acid and added to volumetric bottles of 10 ml and supplemented with distilled water to the mark. The measurement was made at a wavelength of 434 nm, as shown in Fig. 2.

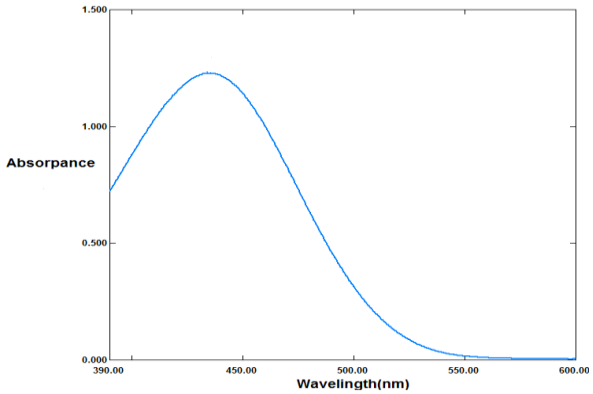


Fig. 1. Absorption spectrum of the dye

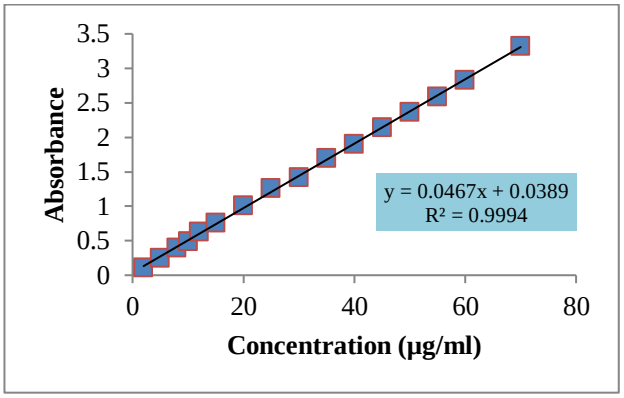


Fig. 2. Standard calibration curve for the dye

A volume of 1 ml, which is equivalent to 20 µg/ml of dye which falls within the standard calibration curve, was taken and used in subsequent studies.

Result and discussion

Effect of different types of oxidizing agents. Different types of oxidizing agents were used, with a volume of 1 ml and a concentration of 1×10^{-2} M. To choose the best oxidizing agent that works to paling the dye at a wavelength of 434 nm, the best oxidizing agent was NBS at a concentration of 1×10^{-2} M. The results were listed in Fig. 3.

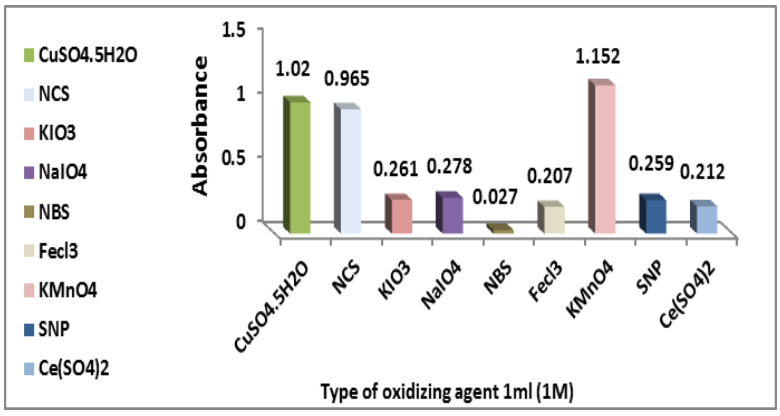


Fig. 3. Effect of oxidizing agent

Effect amounts of the oxidizing agent NBS. Different quantities (0.0-2.0 ml) of NBS were taken at a concentration of 1×10^{-2} M with 1 ml of dye and 0.5 ml of acid. The measurement was made at a wavelength of 434 nm, and it was found that 1 ml of NBS gives the best dye reduction, as in Fig. 4.

Effect of types of acids. Different types of acids were studied with a volume of 0.5 ml and a concentration of 1M to find the best acid that gives the best absorption with 1 ml of the drug at a concentration of 50 µg/ml, 1 ml of the oxidizing agent NBS, and 1 ml of cresol red dye at a concentration of 200 µg/ml. The optimal wavelength was determined to be 442 nm. As shown in Fig. 5, hydrochloric acid is the most effective acidic medium for the reaction.

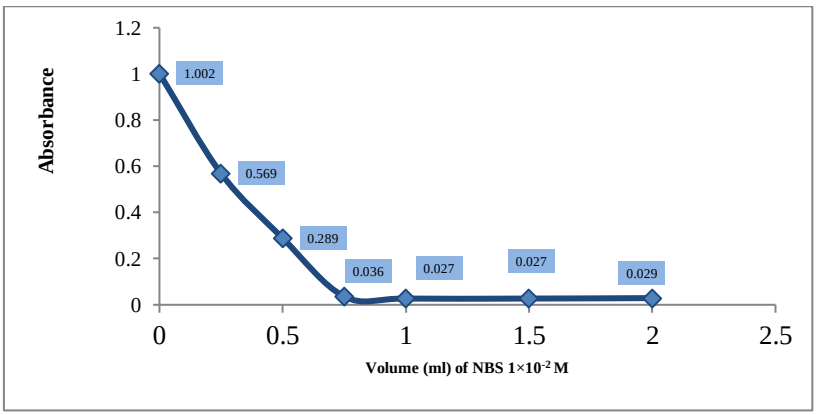


Fig. 4. Effect of different amounts of the oxidizing agent NBS

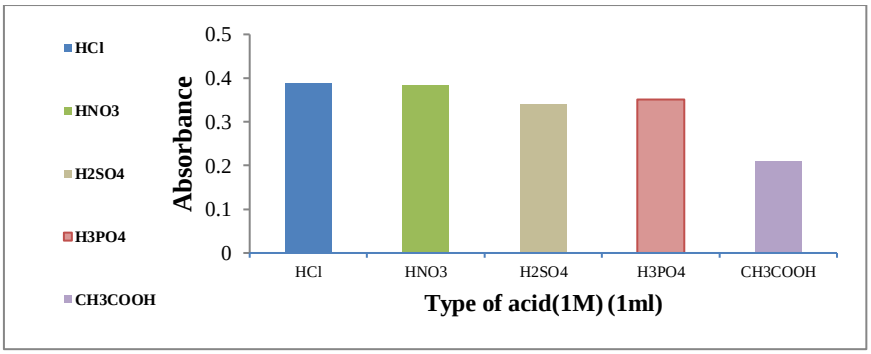


Fig. 5. Effect of acids

Effect of amounts hydrochloric acid at a concentration of 1 M. The effect of different amounts (0.1-1.5 ml) of hydrochloric acid were studied to find the best optimal amount of acid, and the results were listed in Table 3. Through the results, it was shown that 0.5 ml is the optimal amount that gives the best absorption at a wavelength of 442 nm.

Table 3. Effect of different amounts of hydrochloric acid

Volume (ml) of HCl (1M)	0.1	0.25	0.5	0.75	1.0	1.25	1.5
Absorbance	0.185	0.266	0.392	0.314	0.279	0.190	0.043

Effect of oxidation time. Different times (1-15 minutes) were studied to choose the best time for oxidation of the drug compound, which gives the best absorption at a wavelength (442 nm), and the results were listed in Table 4. The table shows that the best oxidation time is 1 minute.

Table 4. The effect of oxidation time on the determination of the drug compound amoxicillin

Time (min)	1	3	5	7	10	15
Absorbance	0.481	0.421	0.392	0.374	0.361	0.353

Study the effect of temperature and stability time. A range of temperatures from room temperature (20°C) to 50°C was investigated in order to determine the optimal conditions for complex formation, including reaction time and stability of the resulting complex. The results were included in Fig. 6. From the results, the figure shows that the best temperature is 20°C, which is the laboratory temperature, with a formation time of 5 min and a setting time of stability 35 min.

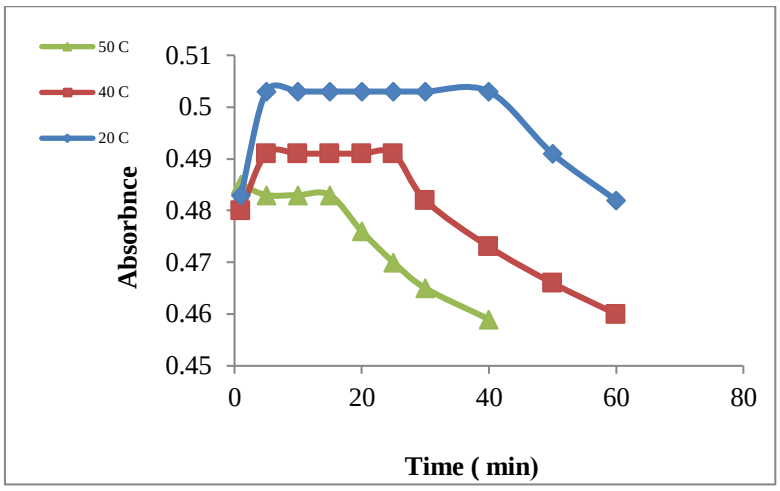


Fig. 6. Effect of temperature on formation and stability time

A summary of the optimal conditions obtained in the determination of the drug compound using cresol red dye is shown in Table 5.

Table 5. Summary of optimal conditions

Experimental Conditions	Result
λ_{max} (nm)	**442**
Hydrochloric acid (1M) (ml)	**0.5**
N-bromosuccinimide (1×10^{-2} M)(ml)	**1**
Cresol red Dye (200 µg\ml) (ml)	**1**
Temperature(C°)	**20**
Development Time (min)	**5**
Stability period (min)	**35**

Final absorption spectrum. After the most optimal conditions for the drug compound were established, its final absorption spectrum was drawn, as shown in Fig. 7.

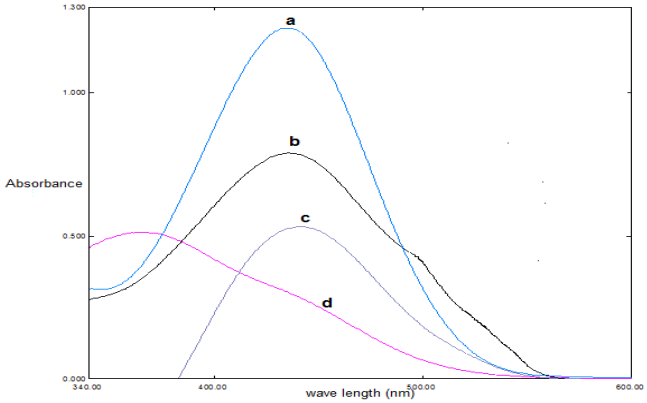


Fig. 7. Final absorption spectrum. (A) absorption spectrum of the dye with acid vs. blank; (B) absorption spectrum of the reaction product vs. water; (C) absorption spectrum of the reaction product vs. blank; (D) blank absorption spectrum vs. water

Standard calibration curve . Increasing amounts of amoxicillin (0.05-1.25 ml) at a concentration of 50 µg/ml were added to a 10 ml volumetric flask and added to the optimal amounts listed in Table 5. Absorption was measured at a wavelength of 442 nm. The standard curve shown in Fig. 8 was obtained, which follows Beer's law within the range of 0.25-6.25 µg/ml.

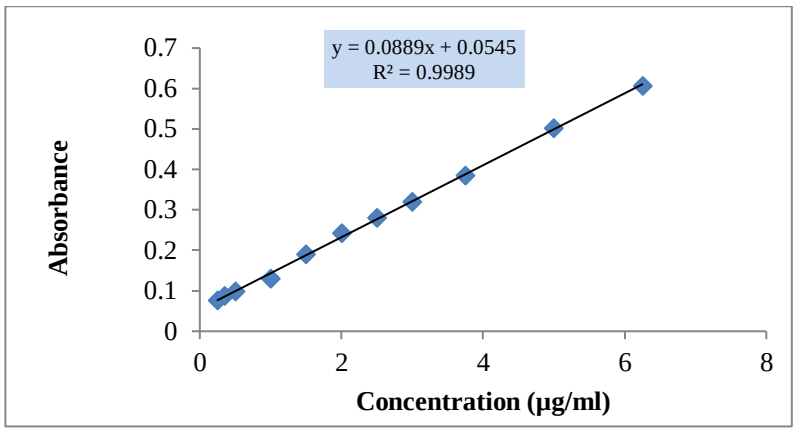


Fig. 8. Standard calibration curve for amoxicillin

Proposed chemical reaction. It is expected that the chemical reaction proceeds through the mechanism shown in Fig. 9, as the drug compound amoxicillin is oxidized by N-bromosuccinimide, and then the remaining oxidizing agent reacts with cresol red dye, resulting in a measurable color change.

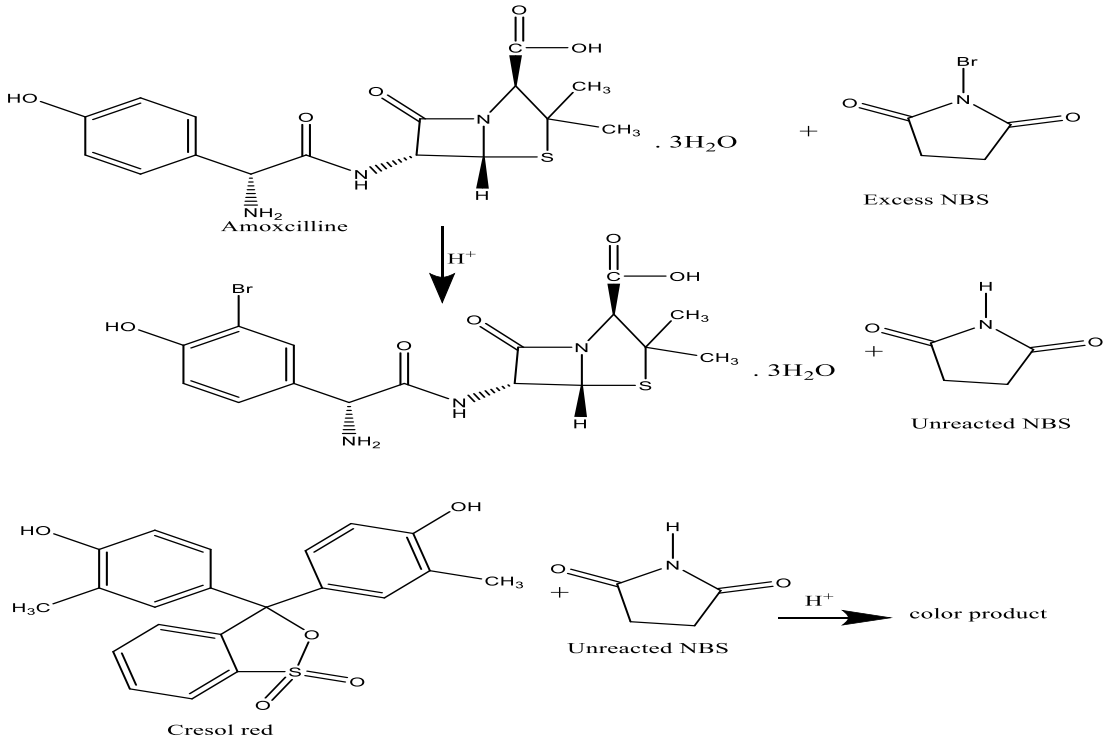


Fig. 9. Mechanism of the proposed chemical reaction for amoxicillin trihydrate

Table 6. Application of the developed method to pharmaceutical preparations

Preparation of Pharmaceuticals	Validated Value, (mg)	Amount Present, (µg/ml)		Drug Content Found, (mg)	Recovery (%)	Average Recovery (%)
		Taken	Found			
Injection NOVATRA PHARMA	500	1	0.99	495	99.00	101.27
		2	2.09	522.5	104.50	
		3	3.01	501.65	100.33	
Tablet	1000	1.5	1.49	993.3	99.33	

BİLİMİLAÇ		2.5	2.53	1.012	101.20	99.97
		5	4.97	994	99.40	

Table 6 shows the extent of the efficiency and success of the developed method in its application to pharmaceutical preparations, where the recovery percentage in the injection ranged between 99% and 104.5%, and in the tablets between 99.33% and 101.2%.

Standard addition method of pharmaceutical preparations. In order to prove the efficiency of the proposed method and its success in determining amoxicillin in its pure form and in its pharmaceutical preparations and that they are free from additional interferences, the standard addition method was applied to two different concentrations of both preparations (injection and tablets), as shown in Fig. 10.

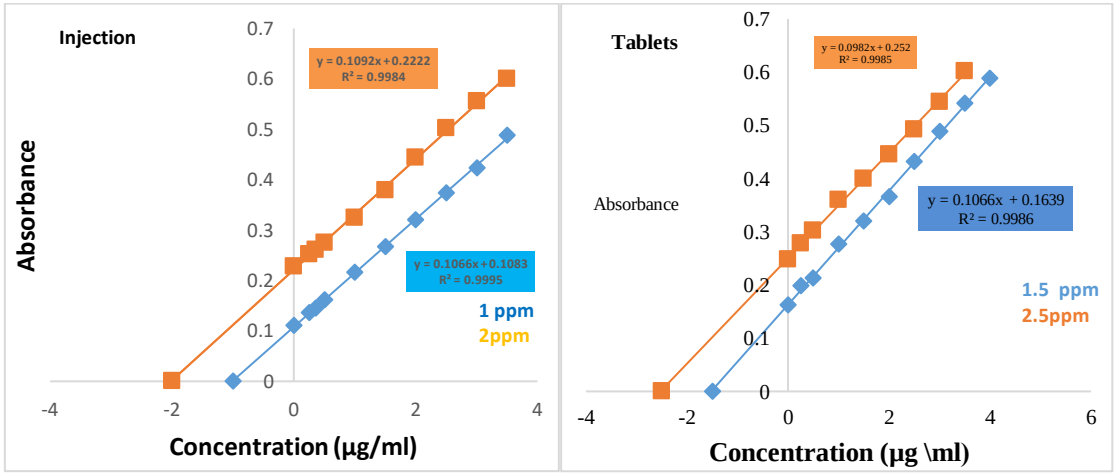


Fig. 10. Standard addition to the pharmaceutical preparation injection and tablets

Table 7. Standard addition method in estimating the drug compound

Pharmaceutical Preparation	Certified Value (mg)	Amount Present (µg/ml)	Recovery (%)	Drug content found (mg)
Amoxicillin Injection	500	1	101.59	507.95
		2	101.73	508.65
Amoxicillin Tablets	1000	1.5	102.50	1.025
		2.5	102.64	1.026

Compare the method with another method. The proposed method for estimating amoxicillin using the cresol red dye was compared to another spectroscopic method, and the results of the comparison were listed in Table 8. The proposed method was characterized as having high sensitivity.

Table 8. Comparison of the current method for estimating amoxicillin with another method

Analytic Parameters	**Current method Dye palace reaction**	**Literature method Diazotization [9]**
λ_{max} (nm)	442	435
Molar absorptivity ($L \cdot mol^{-1} \cdot cm^{-1}$)	3.72×10^4	1.914×10^4
Beer's law Range (µg/ml)	0.25 – 6.25	0.4 –10
Reagent	Cresol red	4- amino benzoic acid

Temperature (C°)	R.T.	R.T
Average of recovery%	101.15	99.811
Relative standard deviation (RSD%)	0.732	1.079

Conclusion

A rapid and sensitive spectrophotometric method was developed for the determination of amoxicillin trihydrate in both its pure form and pharmaceutical formulations, based on a dye decolorization reaction. The method involves the oxidation of amoxicillin in an acidic medium in the presence of N-bromosuccinimide (NBS) as the oxidizing agent. The excess unreacted NBS subsequently reacts with cresol red dye, causing a reduction in its color intensity. The resulting change is measured at a wavelength of 442 nm, with a molar absorptivity of 3.72×10^4 $L \cdot mol^{-1} \cdot cm^{-1}$.

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