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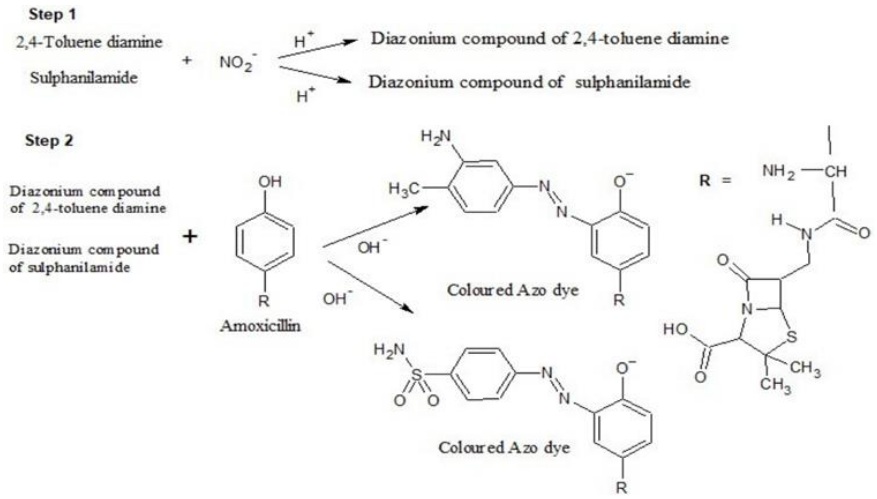
Determination of amoxicillin: A penicillin antibiotic in pharmaceutical dosage samples by spectrophotometric method

Chand **Pasha**[1+]

Abstract

New coupling agents such as 2,4-toluene diamine or sulphanilamide for the determination of amoxicillin spectrophotometrically are described. These methods are straightforward based on the reaction of amoxicillin with diazotized products of 2,4-toluene diamine or sulphanilamide to produce coloured azo dyes with maximum absorption at 462 or 468 nm. Amoxicillin responds linearly from 1.2–24.8 or 1.8–32.0 µg mL^{-1} when coupled with diazotized 2,4-toluene diamine or sulphanilamide. The molar absorptivity and Sandell's sensitivity of amoxicillin with 2,4-toluene diamine or amoxicillin with sulphanilamide azo dyes were 3.307×10^4 or 2.632×10^4 L mol^{-1} cm^{-1} and 1.105×10^{-2} or 1.388×10^{-2} µg cm^{-2}, respectively. The regression equation, correlation coefficient (R^2), detection limit and quantitation limit of amoxicillin with 2,4-toluene diamine or amoxicillin with sulphanilamide were evaluated. The percentage recoveries ranged from 97.00 to 100.50 with a relative standard deviation value was ± 0.98 to ± 1.85%. The method does not need temperature control or solvent extraction and has been applied successfully to determine amoxicillin in pharmaceutical preparation (tablets).



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5. sulphanilamide.

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Highlights

- The 2,4-toluene diamine and sulphanilamide were used for amoxicillin determination.
- The coupling agents with amoxicillin showed high molar absorptivity and sensitivity.
- The method is very simple, sensitive, accurate, and has a high dye stability.
- The method applied to the analysis of amoxicillin in pharmaceutical samples.

[1]Royal Commission Yanbu Colleges and Institutes, Yanbu Industrial College, Yanbu, Kingdom of Saudi Arabia. **+Corresponding author:** Chand Pasha, **Phone:** +966531810596, **Email address:** drcpasha@gmail.com

1. Introduction

Amoxicillin is a β-lactam penicillin antibiotic with the chemical formula $C_6H_{19}N_3O_5S$, that is active against both gram-positive and gram-negative bacteria (Wilson *et al.*, 2004). It is used to treat a variety of bacterial infections caused by susceptible microorganisms, including skin, yeast, urinary tract and middle ear infections, strep throat, and pneumonia. Some of the side effects include nausea and rash, and those who are allergic to penicillin should avoid using it. However, those with kidney issues can use it without risk, and it is safe to use during pregnancy and breastfeeding (Amoxicillin, 2015). According to the most recent report from the European Centre for Disease Prevention and Control (ECDC, 2020), the average consumption of antimicrobial drugs for systemic use in the European Union in 2019 was 18.0 defined as daily doses per 1,000 inhabitants per day. After consumption, antibiotics are excreted as metabolites and unaltered compounds (Boix *et al.*, 2016) together with the high consumption of these compounds, it is not surprising that they reach the aquatic environment through wastewater discharges (Dinh *et al.*, 2011). Thus, the investigation of antibiotic residues in water has become an important topic in environmental science, including the analytical determination at trace levels (ng L^{-1} or µg L^{-1}) (Fonseca *et al.*, 2020; Hernández *et al.*, 2015; Rossmann *et al.*, 2014). The chemical structure of amoxicillin is shown in **Fig. 1**.

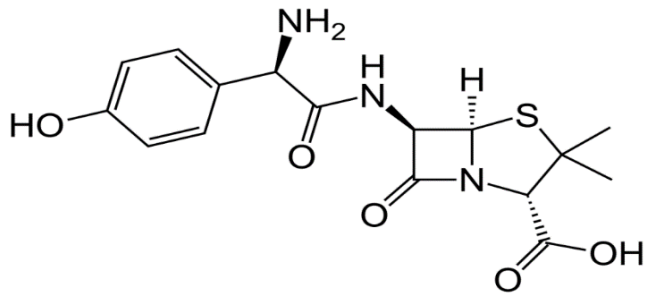

Figure 1. Chemical structure of amoxicillin.

Amoxicillin works by preventing the synthesis of bacterial cell walls (Wilson *et al.*, 2004), and it belongs to the penicillin class of antibiotics, which are broad-spectrum, semisynthetic, acid-stable, orally absorbed antibiotics that inhibit bacterial cell wall synthesis (Blumberg and Strominger, 1974).

According to a review of the literature, there are several methods for determining amoxicillin in pharmaceutical samples, including voltammetry (Fouladgar *et al.*, 2011; Uslu and Biryol, 1999; Santos *et al.*, 2008), fluorimetry (Muñoz de la Peña *et al.*, 2002), colourimetry (Elshafie *et al.*, 1996; Rao and Mohan, 1982), liquid chromatography (Aliev and Babazade, 2011; Fabregat-Safont *et al.*, 2021; Foroutan *et al.*, 2007; Hailekiros *et al.*, 2022; Wen *et al.*, 2008), chemiluminescence (Li *et al.*, 2003; Sun *et al.*, 2005), capillary electrophoresis (Oliva *et al.*, 2011), atomic absorption spectrometry (Mahmoud *et al.*, 2008), UV spectroscopy (Ergin and Yasa, 2022) and spectrophotometry (Ahmed *et al.*, 2004; Al-Abachi *et al.*, 2005; Asan and Seddiq, 2022; Al-Uzri, 2012; Jalal *et al.*, 2023; Othman and Al-Saffar, 2015; Quanmin and Zhanjun, 2006; Singh and Maheshwari, 2010; Ünal *et al.*, 2008). However, some of the methods (Ahmed *et al.*, 2004; Al-Abachi *et al.*, 2005; Foroutan *et al.*, 2007; Fouladgar *et al.*, 2011; Li *et al.*, 2003; Mahmoud *et al.*, 2008; Muñoz de la Peña *et al.*, 2002; Oliva *et al.*, 2011; Quanmin and Zhanjun, 2006; Sun *et al.*, 2005) presented for determining amoxicillin in pharmaceutical dosage were associated with major flaws such as tedious extraction methods, time consumption, lack of sensitivity, heating issues, and cooling effects.

The diazotization reaction of 2,4-toluene diamine or sulphanilamide with sodium nitrite in an acid medium yields diazonium compounds, which are then coupled with amoxicillin in an alkaline medium to yield yellow water-soluble azo dyes. The proposed methods are free of the drawbacks mentioned above and they are risk-free, simple, selective, and precise used for the determination of amoxicillin—a penicillin antibiotic—in pharmaceutical dosage samples by spectrophotometric method.

2. Experimental

2.1. Equipment

A JASCO V-730 spectrophotometer (Serial No. A 023561798) and pH meter (Eutech Instruments pH 510 Serial o. 1398504) were used for spectrometric analysis.

2.2. Chemicals and reagents

Amoxicillin stock solution (1,000 µg mL^{-1}), (Gift sample from Karnataka antibiotics and Pharmaceuticals Limited, Bangalore, India): A 0.104 g of amoxicillin was weighed accurately and dissolved in 5–10 mL of ethanol, shaken thoroughly then the solution is transferred to a 100 mL calibrated flask and filled to the proper level with double-distilled water. By dilution, the working solution was prepared as needed.

A 0.1 mol L^{-1} sodium nitrite solution, 0.5 mol L^{-1} hydrochloric acid solution, 1% of 2,4-toluene diamine or sulphanilamide solutions each and 0.5 mol L^{-1} sodium hydroxide solution were used.

Amoxicillin tablets of different trademarks used:

Vemox 50 (500 mg): Vega Pharma, Panoptic Exim Private Limited, Nagpur, Maharashtra.

Amoxicillin trihydrate (500 mg): Sandmartin Pharmaceuticals Private Limited, New Delhi, India.

EMOX 250 (250 mg): Emkam Pharma private Limited, Meerut, India.

Cipmox-250 (250 mg): Cipla Limited, Mumbai, India.

Amoxirum forte injection (300 mg): 200 mg amoxicillin present with sodium (molecular weight 340.4 mg): Karnataka antibiotics and pharmaceuticals Limited, Bangalore, India.

Amoxicillin tablets solution (1,000 µg mL^{-1})

Amoxicillin tablets/capsules of various brands were obtained from a homegrown dispensary and finely powdered. A precisely weighed quantity of powder (~ 0.25 g) was dissolved in 5–10 mL ethanol, then 80–100 mL distilled water was added, shaken well, filtered into a 250 mL calibrated flask, then the volume was completed to the mark with distilled water and the preparation of amoxicillin solution was continued as described above.

2.3. Procedure for the determination of amoxicillin

In a sequence of 10 mL calibrated flasks, an aliquot of the sample solution containing a known quantity of amoxicillin (µg mL^{-1}) was added. It was then mixed well for 2 min with the addition of 1 mL of a 0.1 mol L^{-1} solution of sodium nitrite and 0.5 mL of a 0.5 mol L^{-1} solution of hydrochloric acid before being

set aside to allow the diazotization reaction to finish. After that, the mixture was thoroughly mixed after being diluted to 10 mL with double-distilled water and added volumes of 1 mL of 1% 2,4-toluene diamine or sulphanilamide and 1.0 mL of 0.5 mol L^{-1} sodium hydroxide solutions. After 5 min the formed coloured azo dyes absorbance was measured at 462 or 468 nm in comparison to the blank reagent.

3. Results and discussion

In the presence of a base, amoxicillin is coupled with the diazonium salt of 2,4-toluene diamine or sulphanilamide to produce a coloured azo dye. The absorption spectra of the azo dye produced by reacting amoxicillin with diazotized 2,4-toluene diamine or sulphanilamide (Fig. 2) had an absorption maximum at 462 nm or 468 nm, respectively.

The plot of absorbance versus concentration of amoxicillin coupled with diazotized 2,4-toluene diamine or sulphanilamide (Fig. 3) demonstrates that the dyes obey Beer's law in the range of 1.2–24.8 μg mL^{-1} of amoxicillin with 2,4-toluene diamine or 1.8–32.0 μg mL^{-1} of amoxicillin with sulphanilamide. Absorption spectra of the azo dye resulted from the reaction of amoxicillin with diazotized sulphanilamide against a reagent blank (2) and a blank reagent against distilled water (3).

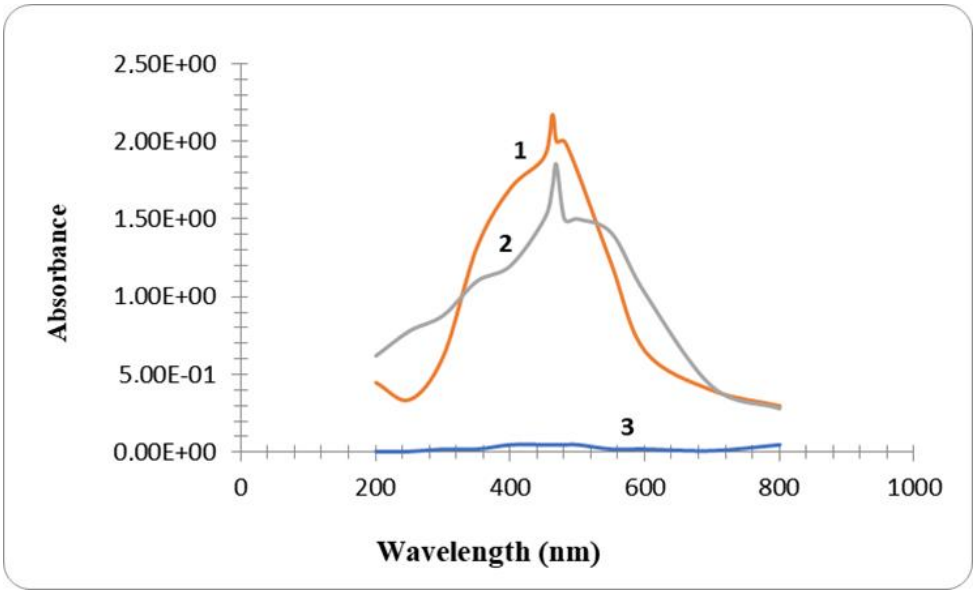

Figure 2. Absorption spectra of the azo dye produced by the reaction of amoxicillin with diazotized 2,4-toluene diamine (1) and sulphanilamide (2) against a blank reagent. The blank reagent spectrum is number (3).

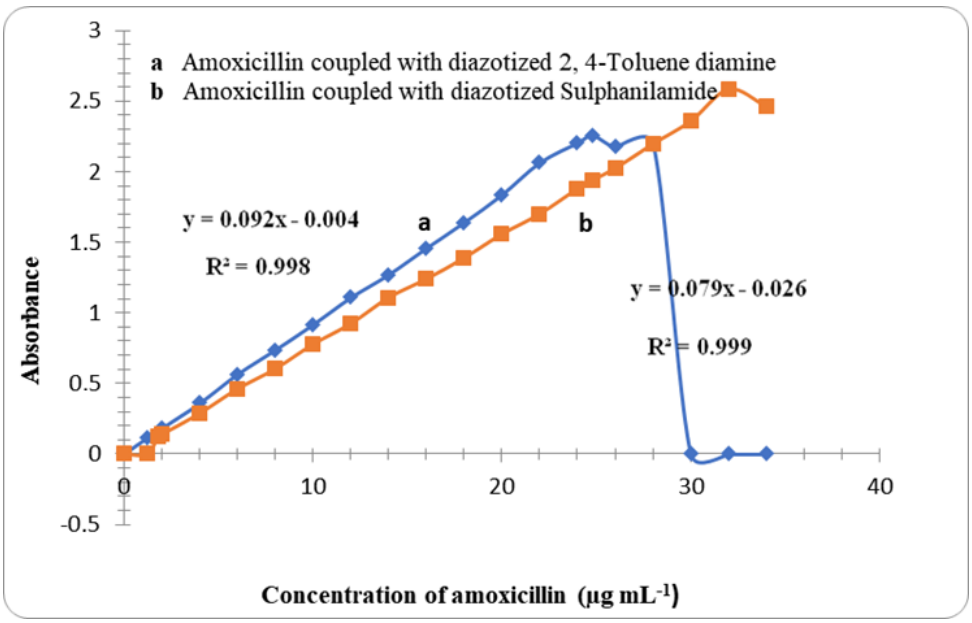

Figure 3. Adherence to Beer's law using amoxicillin coupled with diazotized 2,4-toluene diamine or sulphanilamide.

The reactions for steps 1 and 2 are shown in Fig. 4.

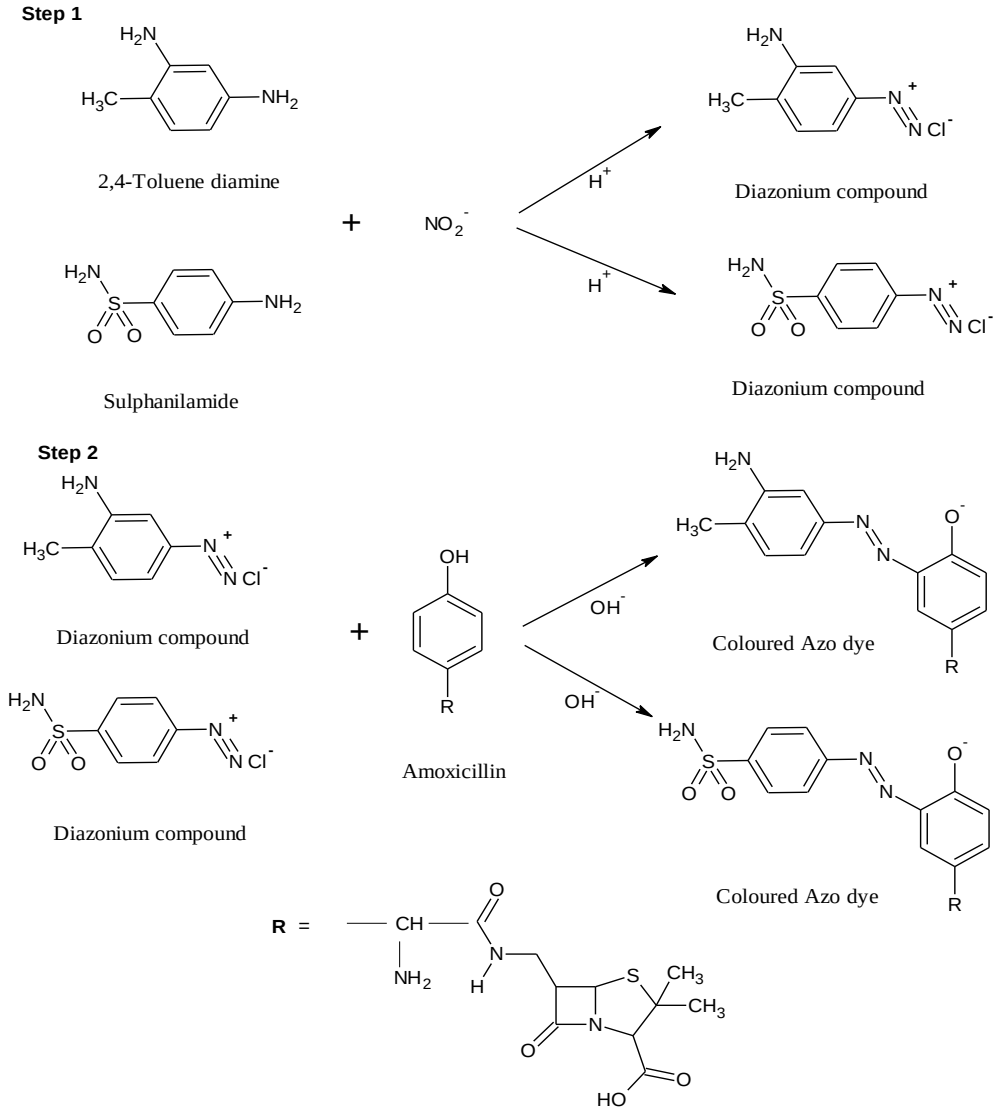

Figure 4. Diazonium salt of 2,4-toluene diamine or sulphanilamide is coupled with amoxicillin to produce coloured azo dyes.

3.1. Effect of temperature, acid and base concentration

The effect of temperature on diazotization reactions, room temperature (25 ± 5 °C) is advised because the loss in colour stability and intensity was seen at low and high temperatures.

The effect of acid on the diazotization reaction of amoxicillin (2 µg mL^{-1}) was examined by adding different acid solutions (0.5 mol L^{-1}), such as HCl, H_2SO_4, CH_3COOH and HNO_3. It was discovered that when amoxicillin was coupled with a diazotized 2,4-toluene diamine or sulphanilamide, CH_3COOH produced low absorbance with low colour stability while HCl produced high absorbance with highest colour stability. Therefore, for the amoxicillin diazotization reaction, 0.5 mL of 0.5 mol L^{-1} HCl was preferred (Table 1).

Table 1. Effect of acid concentration.

0.5 mol L^{-1} acid concentration	Absorbance (A) / mL of acid used					
	2,4-toluene diamine			Sulphanilamide		
	0.25 mL	0.5 mL	0.75 mL	0.25 mL	0.5 mL	0.75 mL
Hydrochloric acid	0.280	0.362	0.348	0.302	0.344	0.340
Sulfuric acid	0.256	0.292	0.306	0.288	0.276	0.287
Acetic acid	0.222	0.246	0.212	0.228	0.244	0.286
Nitric acid	0.248	0.284	0.293	0.232	0.248	0.262

The effect of the base on the diazotization reaction of amoxicillin (2 μg mL^{-1}) was examined by adding different base (0.5 mol L^{-1}) solutions such as NaOH, KOH, NH_4OH and Na_2CO_3. It was discovered that when amoxicillin was coupled with diazotized 2,4-toluene diamine or sulphanilamide, Na_2CO_3 produced low absorbance and NaOH produced high absorbance with highest colour stability. Therefore, for the amoxicillin diazotization reaction 1.0 mL of 0.5 mol L^{-1} NaOH solutions was preferred (Table 2).

3.2. Effect of coupling reagents and nitrite concentration

In the current method, 2,4-toluene diamine or sulphanilamide is used as a coupling agent by adding 0.50 to 2.0 mL of 1% 2,4-toluene diamine or sulphanilamide to a series of nitrite solutions. In an ultimate volume of 10 mL, it was discovered that 1 mL of 2,4-toluene diamine or sulphanilamide (1%) solution produced the brightest and firmest colour (Table 3).

Using the current method with 2 μg mL^{-1} of amoxicillin and adding 1 mL of 0.025-0.150 mol L^{-1} solutions of the nitrite in hydrochloric acid (0.5 mol L^{-1}) to a series of nitrite solutions, the colour reaches its peak intensity when using 1 mL of 0.1 mol L^{-1} sodium nitrite solution. Higher concentrations failed to further increase the absorbance while lower concentrations gave poor results (Table 4).

3.3. Effect of interference

The determination of amoxicillin in the presence of various excipients such as lactose (800 μg mL^{-1}), fructose (1,000 μg mL^{-1}), glucose (1,200 μg mL^{-1}), urea (300 μg mL^{-1}) and starch (600 μg mL^{-1}) did not interfere with the determination of the excipients.

Table 2. Effect of Base concentration.

0.5 mol L^{-1} acid concentration used	Absorbance (A) / mL of Base used					
	2,4-toluene diamine			Sulphanilamide		
	0.5 mL	1.0 mL	1.5 mL	0.5 mL	1.0 mL	1.5 mL
Sodium hydroxide	0.260	0.282	0.266	0.284	0.244	0.264
Potassium hydroxide	0.236	0.262	0.242	0.264	0.224	0.257
Ammonium hydroxide	0.214	0.254	0.204	0.242	0.208	0.246
Sodium carbonate	0.138	0.222	0.188	0.202	0.196	0.220

Table 3. Effect of 2,4-toluene diamine or sulphanilamide solution on absorbance.

1% 2,4-toluene diamine or sulphanilamide solution used (mL)	Absorbance (A) for 2,4-toluene diamine	Absorbance (A) for sulphanilamide
0.50	0.284	0.264
1.00	0.316	0.328
1.50	0.302	0.312
2.00	0.296	0.294

Table 4. Effect of sodium nitrite.

1 mL of $NaNO_2$ solution (mol L^{-1})	Absorbance (A)	
	2,4-toluene diamine	sulphanilamide
0.025	0.164	0.182
0.050	0.188	0.194
0.075	0.192	0.226
0.100	0.288	0.264
0.125	0.248	0.246
0.150	0.240	0.232

3.4. Analytical data

Plotting absorbance versus concentration of amoxicillin resulted in a straight line on the graph. Beer's law is obeyed between the concentrations of 1.2–24.8 μg mL^{-1} of amoxicillin with 2,4-toluene diamine or between the concentrations of 1.8–32.0 μg mL^{-1} with sulphanilamide. The molar absorptivity of the coloured azo dye of amoxicillin coupled with the diazonium salt of 2,4-toluene diamine or sulphanilamide was found to be 3.307×10^4 L mol^{-1} cm^{-1} or 2.632×10^4 L mol^{-1} cm^{-1}, and the Sandell's sensitivity of coloured system with a nitrite-2,4-toluene diamine or nitrite-sulphanilamide were found to be 1.105×10^{-2} μg cm^{-2} or 1.388×10^{-2} μg cm^{-2} with maximum absorption at 462 or 468 nm (Fig. 2 and 3).

The regression equation and correlation coefficient (R^2) of amoxicillin with 2,4-toluene diamine or amoxicillin with sulphanilamide were $y = 0.092x - 0.004$, or $y = 0.079x - 0.026$ and R^2 of 0.998 or 0.999 and have high dye stability (more than 10 h). The detection limit ($D_L = 3.3\sigma/S$) and quantitation limit ($Q_L = 10\sigma/S$) of amoxicillin coupled with diazotized 2,4-toluene diamine or sulphanilamide were found to be 0.351 μg mL^{-1} or 0.420 μg mL^{-1} and 1.065 μg mL^{-1} or 1.274 μg mL^{-1} (where σ = Standard Deviation, [n = 5] and S = slope of the curve). The better optical characteristics and statistical data were obtained under optimum conditions (Table 5).

Table 5. Determination of amoxicillin in various pharmaceutical samples.

Pharmaceutical Samples	Sample taken (µg mL⁻¹)	Using 2,4-toluene diamine		Using sulphanilamide	
		Sample found[a] (µg mL⁻¹) ± %RSD	Rec. (%)	Sample found[a 1] (µg mL⁻¹) ± %RSD	Rec. (%)
Vemox 500 (500 mg/tab)	04.00	3.92 ± 1.43	98.00	3.96 ± 1.62	99.00
	08.00	7.91 ± 1.24	98.87	7.92 ± 1.46	99.00
	12.00	11.94 ± 1.32	99.50	11.90 ± 1.46	99.16
	16.00	15.90 ± 1.20	99.34	15.84 ± 1.28	99.00
	20.00	19.92 ± 1.26	99.60	19.90 ± 1.42	99.50
Amoxicillin Trihydrate (500 mg/tab)	04.00	3.94 ± 1.48	98.50	4.02 ± 1.22	100.5
	08.00	7.90 ± 1.24	98.70	7.98 ± 1.46	99.75
	12.00	11.88 ± 1.44	99.00	11.92 ± 1.32	99.33
	16.00	15.86 ± 1.26	99.12	15.90 ± 1.36	99.37
	20.00	19.92 ± 1.35	99.6	19.82 ± 1.45	99.10
EMOX 250 (250 mg/tab)	04.00	3.90 ± 1.23	97.50	3.94 ± 1.28	98.50
	08.00	7.92 ± 1.44	99.00	7.88 ± 1.32	98.50
	12.00	11.88 ± 1.66	99.00	11.90 ± 1.45	99.17
	16.00	15.92 ± 0.98	99.50	15.88 ± 1.42	99.25
	20.00	19.86 ± 1.46	99.30	19.80 ± 1.26	99.00
Cipmox-250 (250 mg/tab)	04.00	4.00 ± 1.54	100.0	3.94 ± 1.44	98.50
	08.00	7.90 ± 1.22	98.75	7.96 ± 1.28	99.50
	12.00	11.94 ± 1.49	99.50	11.88 ± 1.54	99.00
	16.00	15.94 ± 1.27	99.62	15.86 ± 1.64	99.12
	20.00	19.90 ± 1.33	99.50	19.82 ± 1.24	99.10
Amoxirum forte injection (300 mg/tab)	04.00	3.92 ± 1.42	98.00	3.88 ± 1.26	97.00
	08.00	7.91 ± 1.22	98.87	7.92 ± 1.34	99.00
	12.00	11.94 ± 1.26	99.50	11.84 ± 1.52	98.67
	16.00	15.86 ± 1.34	99.12	15.84 ± 1.26	99.00
	20.00	19.80 ± 1.65	99.00	19.78 ± 1.85	98.90

a. Mean (n=5) ± %RSD {relative standard deviation}.

3.5. Applications

Amoxicillin can be found in a variety of pharmaceutical samples using the provided method, which is straightforward and easy to use. The findings of the recommended methodology closely correspond to the acknowledged content. For all five samples, the percentage recoveries ranged, with a 95% level of confidence, from 97.00 to 100.50 and the relative standard deviation value was ±0.98–±1.85%. The appearance of pharmaceutical samples containing additional ingredients had no negative effects. The outcomes are contrasted with the endorsed spectrophotometric method (Al-Uzri, 2012; Othman and Al-Saffar, 2015). These attest to the fact that the proposed method and the recommended method are not significantly different. To evaluate precision and accuracy, replicate analyses were performed on five different samples that contained amoxicillin at various concentrations (Table 5).

4. Conclusions

New coupling agents such as 2,4-toluene diamine or sulphanilamide, used for the spectrophotometric determination of amoxicillin, are reasonably priced and selective. Compared to some of the reported methods, the procedure is very simple, fast, sensitive, accurate, and has a high dye stability (more than 10 h).

The method need not involve time-consuming separation or solvent extraction procedures and the high accuracy and precision of the proposed methods are highlighted by their low percentage relative standard deviation and percentage recovery values. The proposed methods produce precise, repeatable results that are free from excipient interference and was applied to the analysis of amoxicillin in pharmaceutical samples.

Data availability statement

All data sets were generated or analyzed in the current study.

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Not applicable.

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