A Simple Spectrophotometric Determination of Amoxicillin in Drug Samples

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Abstract: In this study, a fast and simple spectrophotometric method was developed for the determination of amoxicillin in drugs without any extraction steps. The developed method is based on the formation of a colored ion pair complex between bromocresol green and amoxycillin in dimethyl sulfoxide-acetonitrile (50 % v/v) medium. The absorbance of the bluish green complex is measured at a wavelength of 630 nm. The factors that affect the ion pair complex formation, such as time, reagent concentration, etc., have been optimized. Under the optimum conditions, the developed method had an average recovery of 98.16% with a relative standard deviation of 3.62 % and showed a very good linear behavior obeying Lambert-Beer's law in the range 1-18 µg mL^{-1} of amoxycillin concentration. The developed method has been successfully applied to both tablet and powder forms of pharmaceutical preparations. The standard addition method and statistical parameters were applied to test the accuracy of the proposed method, and the obtained results from the two methods showed good agreement with each other.

INTRODUCTION

Amoxicillin is a semi-synthetic antibiotic that belongs to the penicillin family. It is a broad-spectrum antibiotic that is taken with oral route. Amoxicillin is effective against gram-positive and gram-negative microorganisms where it is attached to the cell wall of the bacteria and causes their death. It is found as amoxicillin trihydrate in drugs (1). The structure of amoxicillin is shown in Figure 1.
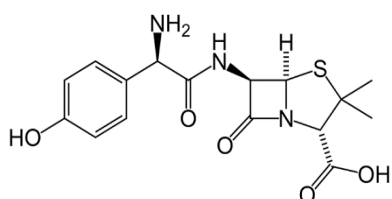
Figure 1: The chemical structure of amoxicillin.

In the last decade, amoxicillin has been mainly used to treat infections of the middle ear (otitis media), tonsils (tonsillitis and tonsillopharyngitis), throat (laryngitis), pharynx (pharyngitis), lungs (bronchitis, pneumonia), and urinary tract (2,3).

In the literature, HPLC[4-7] has been reported as the main used chromatographic technique for the determination of amoxicillin. Potentiometry (8), spectrofluorometry (9), flow injection analysis methods (10,11), thin layer chromatography (12), voltammetry (13), LC-MS (14), capillary electrophoresis (15,16), atomic absorption spectroscopy (17) were among the various methods that have been developed for amoxicillin determination.

Among the mentioned methods, spectrophotometric technique is the most widely used method because it is simple to apply and does not require expensive equipment. Spectrophotometric detection is based

on the formation of a colored (charge-transfer or ion-pair) complex between the drug and a reagent. Color intensity can be then estimated by measuring the absorption intensity by a visible spectrophotometer. The charge-transfer complex is also known as the electron-donor-acceptor (EDA) complex, in which fraction of electronic charge is transferred between the molecular entities. Previous spectrophotometric methods for amoxicillin determination have included the formation of complexes with hematoxylin (18), molybdenum and thiocyanate (19), Folin-Ciocalteu's phenol reagent (20), methylene blue (21), bromocresol green (22), 2,4-dinitrophenylhydrazine (23), diazotized p-aminobenzoic acid and diazotized procaine (24), and 4-bromobenzaldehyde (25). In addition to that, some non-derivative and derivative UV spectrophotometric methods were developed (26-30).

In this work, a UV-Visible spectrophotometric method for the determination of amoxicillin was developed by modifying the method by Keskar and Jugade (22). In the mentioned work, dimethyl sulfoxide (DMSO) was used as a solvent. In this study, by using dimethyl sulfoxide-acetonitrile (DMSO-AcN) mixture (50 % v/v) the absorbance was increased, thus the detection limit was reduced and the linear working range was extended. The developed method was based on the formation of an ion-pair complex between amoxicillin and bromocresol green in dimethyl sulfoxide-acetonitrile (50% v/v). The absorbance was measured at the wavelength 630 nm. The most important advantages of this method are that it did not require pretreatment steps such as extraction and it was performed at room temperature. After the method was optimized with standard solutions, it was successfully applied for the determination of amoxicillin in drug samples.

MATERIAL AND METHODS

Chemicals and Apparatus
All chemicals required for the experiments were obtained from Sigma-Aldrich and Merck companies and were used as purchased without any further purification. Thermo SCIENTIFIC EVOLUTION 220 UV-Visible Spectrophotometer (USA) was used for all the spectrophotometric measurements.

Amoxicillin Solution Preparation
Amoxicillin standard solutions were prepared from amoxicillin trihydrate stored at -20°C. A stock solution of amoxicillin (0.01 M) was prepared by dissolving 0.209 g of amoxicillin trihydrate in the appropriate volume of dimethyl sulfoxide-acetonitrile mixture (50% v/v).

Bromocresol Green Solution Preparation
A stock solution of bromocresol green (0.001 M) was prepared by dissolving 0.0349 g of bromocresol green in the appropriate volume of dimethyl sulfoxide-acetonitrile mixture (50% v/v).

Sample Preparation
LARGOPEN dry powder and LARGOPEN tablets (BILIM PHARMACEUTICALS Beyoğlu ISTANBUL) containing amoxicillin were purchased from the local pharmacy. For the tablets, 5 tablets were accurately weighed to determine the weight of an average tablet where it was found to be 1.3687 g. Then, these five tablets were powdered in a mortar and 0.05 g of this powder was dissolved in 10 mL volumetric flask with dimethyl sulfoxide-acetonitrile mixture (50% v/v) to obtain a homogeneous solution (1 tablet was equivalent to 1176.47 mg amoxicillin trihydrate). For the dry powder, 147.06 mg of the dry powder was directly dissolved in 10 mL volumetric flasks with dimethyl sulfoxide-acetonitrile mixture (50 % v/v) to obtain a homogeneous solution.

Methods
In this work, UV-Visible spectrophotometric method was developed for the determination of amoxicillin. The method involves the formation of an ion-pair complex between amoxicillin and bromocresol green in dimethyl sulfoxide-acetonitrile (50 % v/v) medium. The ion-pair complex shows maximum absorbance at 630 nm and all measurements were taken at this wavelength throughout the study. The optimum conditions for the complex formation were established using standard solutions, then the method was successfully applied for the determination of amoxicillin in drug samples.

RESULTS AND DISCUSSION

The spectrum of Amoxicillin Solution
The spectrum of amoxicillin solution (0.01 M) that was prepared in dimethyl sulfoxide-acetonitrile (50 % v/v) was scanned between 200-750 nm as shown in Figure 2.
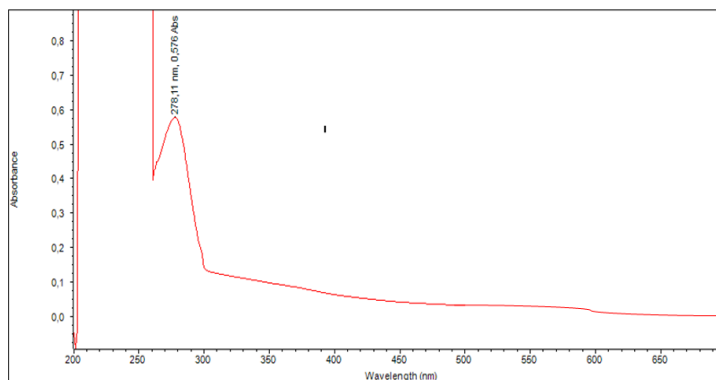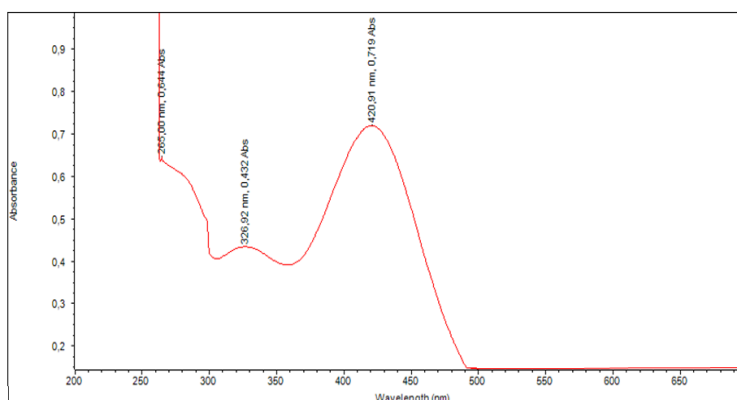
Figure 2: The spectrum of amoxicillin solution (1×10^{-2} M) in the spectral range 200-750 nm.

It can be seen in Figure 2 that the amoxicillin solution has a maximum absorbance at 270 nm.

The Spectrum of Bromocresol Green Solution
The spectrum of the bromocresol green solution (0.001 M) that was prepared in dimethyl sulfoxide-acetonitrile (50 % v/v) was scanned between 200-750 nm as in Figure 3.

Figure 3: The spectrum of bromocresol green solution (1×10^{-3} M) in the spectral range of 200-750 nm.

As can be seen in Figure 3, bromocresol green shows maximum absorbance at 420 nm.
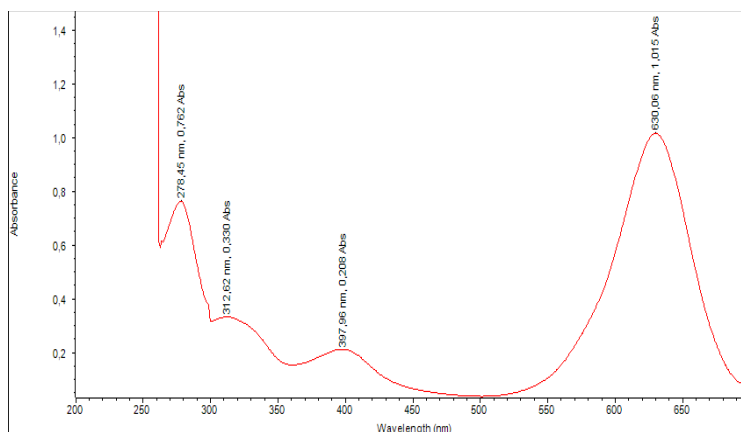
The Spectrum of the Resulting Ion-Pair Complex
When amoxicillin solution (5×10^{-3} M) and bromocresol green solution (5×10^{-3} M) are mixed at room temperature a turquoise ion-pair complex is instantly formed. The spectrum of the formed complex is shown in Figure 4.

As can be seen in Figure 4, the ion-pair complex that formed between amoxicillin and bromocresol green shows maximum absorbance at 630 nm. Neither amoxicillin nor bromocresol green show any absorption at this wavelength. Therefore, the absorbance that is measured at the wavelength 630 nm is only due to the formation of the ion-pair complex.

Optimization of the Experimental Conditions
The effects of various parameters on the absorption intensity of the ion-pair complex that formed from the reaction between amoxicillin and bromocresol green were optimized.

Figure 4: The spectrum of the ion-pair complex that was formed from amoxicillin (5×10^{-3} M) with bromocresol green (5×10^{-3} M).

Effect of Solvents

To investigate the effects of solvents on various variables related to the reaction between amoxicillin and bromocresol green such as solubility, complex formation, and maximum absorbance, absorption intensity was measured using different solvents (dimethyl sulfoxide-acetonitrile mixture, methyl alcohol, ethyl alcohol, acetone, acetonitrile, chloroform, dichloromethane). The obtained data are shown in Figure 5.
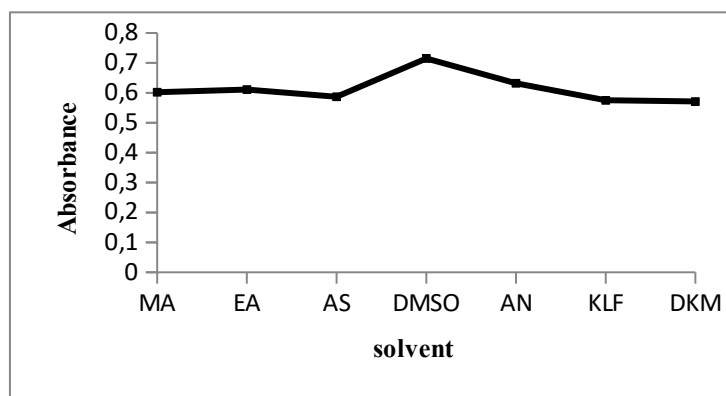

Figure 5: The effect of different solvents on ion-pair complex formation and absorption, (DMSO: dimethyl sulfoxide-acetonitrile, MA: methyl alcohol, EA: ethyl alcohol, AS: acetone, AN: acetonitrile, KLF: chloroform, DKM: dichloromethane)

As can be seen in the figure, among the tested solvents, the highest absorbance value was achieved with dimethyl sulfoxide-acetonitrile mixture, so it was selected for the subsequent steps.

Stoichiometric Ratio

The combining ratio was evaluated by the Continuous Variation Method (Job's method). Amoxicillin and bromocresol green solutions with identical analytical concentrations were mixed in a way that keeps the total volume and the total moles of the two reactants constant but the mole ratio of them varies systematically. The absorbance of each mixture was measured, and then the absorbance was plotted against the mole fraction of one reactant. For this purpose, solutions with the concentrations of 1×10^{-4}, 2×10^{-4} and 3×10^{-4} M were prepared by the appropriate dilution of the amoxicillin and bromocresol green stock solutions that were previously prepared. 10 mixtures with different mole fractions were prepared from these solutions with a total volume of 10 mL, and constant total moles of the two reactants. The absorbance values of these solutions were measured at 630 nm and plotted against the mole fraction of amoxicillin. The resulting graph is shown in Figure 6.
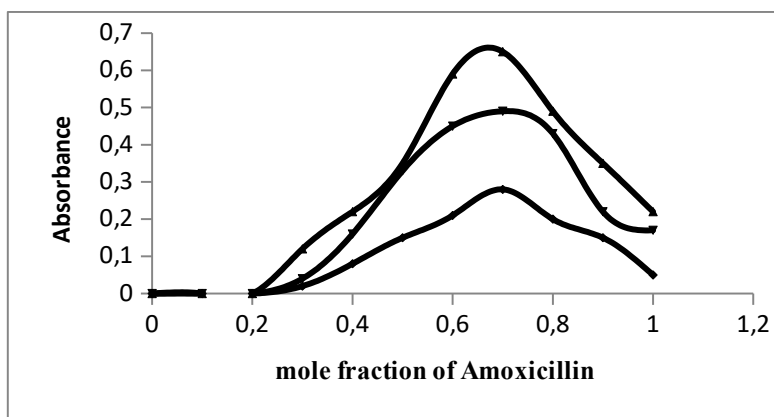
Figure 6: The combining ratio between amoxicillin and bromocresol green according to the continuous variation method (series 1 (diamond): 1×10^{-4} M; series 2 (square): 2×10^{-4} M; series 3 (triangle): 3×10^{-4} M)

As can be seen in Figure 6, the stoichiometric ratio between amoxicillin and bromocresol green is 2:1.

Effect of Reaction Time
When the two solutions of amoxicillin and bromocresol green were mixed a turquoise color immediately developed. The absorbance values of the resulting complex were measured for one hour at an interval of 10 minutes and the results were presented in Figure 7.
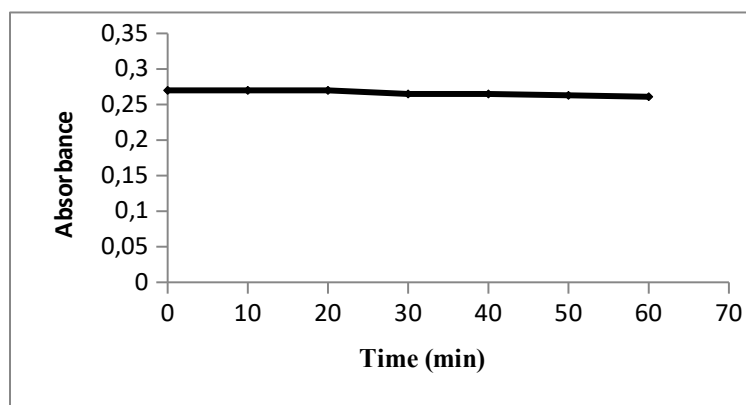

Figure 7: The change in absorbance over time. The complex formed between 2×10^{-5} M amoxicillin and 1×10^{-6} M bromocresol green in DMSO solvent and measured at 630 nm.

As seen in Figure 7, there is no significant change in the absorption of the formed complex at room temperature, and the complex remains stable for at least one hour.

Effect of Bromocresol Concentration
In order to determine the optimum bromocresol green concentration, varying volumes of bromocresol were added to amoxicillin and the absorbance was measured at 630 nm. It was observed that the color intensity increased with the increasing of bromocresol green concentration up to 9×10^{-6} M and decreased after this value. Figure 8 shows the effect of bromocresol green concentration on the absorption intensity of the ion-pair complex.
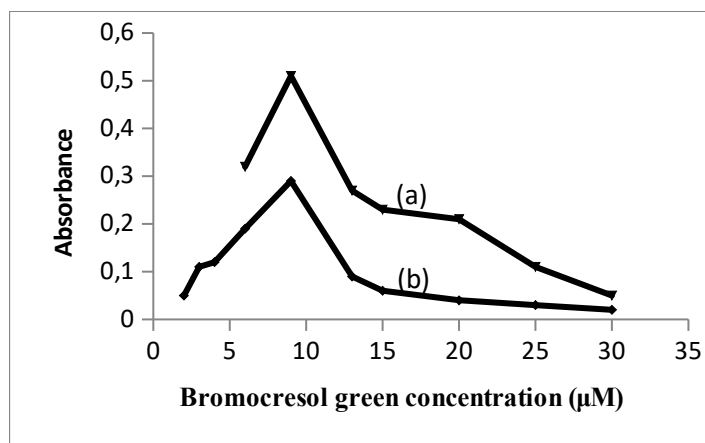
Figure 8: Effect of bromocresol green concentration on the formed complex, (a) 14 µg/mL amoxicillin, (b) 6 µg/mL amoxicillin.

Analytical Characteristics of the Proposed Spectrophotometric Method
After optimizing the experimental conditions of the reaction between amoxicillin and bromocresol green, analytical studies were carried out to determine the range of linear changes between the absorption intensity and amoxicillin concentration.

For this purpose, a series of standard solutions were prepared with different amoxicillin concentrations while the bromocresol green concentration was kept constant. The absorbance of the formed ion-pair complex was measured at 630 nm and a calibration graph was constructed from the obtained data (Figure 9).
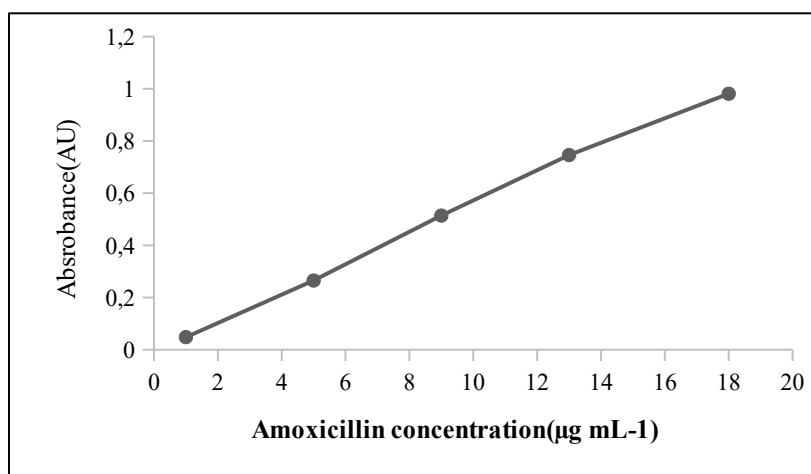
Figure 9: Calibration graph of amoxicillin.

The analytical parameters obtained from the calibration graph are listed in Table 1. The calibration graph of the absorption intensity was plotted against amoxicillin concentration, and the analytical parameters were obtained by the least squares method. Beer's law was obeyed over the concentration range of 1-18 µg mL^{-1} as shown in Figure 9, and the analytical parameters obtained

accordingly were given in Table 1. The regression coefficient of the calibration curve was 0.9976. Limit of detection (LOD) and limit of quantification (LOQ) values were calculated from the equation as LOD=3.3s/m and LOQ=10s/m; where s is the standard deviation of the absorbance values that obtained at the same concentration, and m stands for the slope of the calibration curve (31).

Table 1: Analytical parameters obtained from the calibration graph of amoxicillin determined by the proposed spectrophotometric method.

| Parameters	Values
λ_{max} (nm)	630
Linear range ($\mu g\ mL^{-1}$)	1-18
Regression equation*	A = 0.00556C
correlation coefficient (r^2)	0.9976
slope	0.0551
Intercept	0.0062
LOD ($\mu g\ mL^{-1}$)	0.05
LOQ ($\mu g\ mL^{-1}$)	0.15
molar absorptivity ($L.mol^{-1}.cm^{-1}$)	1.538×10^3

*(A: absorbance, C: concentration in $\mu g\ mL^{-1}$)

Interference Studies

The proposed method was applied on Largopen drugs containing amoxicillin in tablets and powder forms. Drug prospectus stated that there are no excipients in the Largopen tablet, while the powder form contains sucrose as an excipient. Since there is no interaction between sucrose and bromocresol green, interference effects have not been investigated.

Pharmaceutical Application of the Developed Spectrophotometric Method

After the parameters of the developed spectrophotometric method for amoxicillin determination were optimized, it was applied for the analysis of two pharmaceutical preparations (amoxicillin-containing tablet and powder drugs) in order to determine amoxicillin content. Amoxicillin is a broad-spectrum antibiotic that is effective against both gram-positive and gram-negative microorganisms and belongs to the penicillin family. It is used in pharmaceuticals as amoxicillin trihydrate. Some of its trade names are Amoxil manufactured by Glaxosmithkline, Alfoxil manufactured by Actavis, Amoxina manufactured by Mustafa Nevzat and Largopen manufactured by Bilim Pharmaceuticals. In the study, the proposed method was applied successfully on both tablet and powder drugs produced by Bilim Pharmaceuticals. Sample solutions were prepared as mentioned before by dissolving the sample in dimethyl sulfoxide-acetonitrile mixture. Then, bromocresol green solution was added to the prepared solutions and the absorbance of the formed complex was measured at 630 nm. Taking required dilutions into account, amoxicillin concentration in pharmaceuticals was estimated from the calibration curve. Spectra obtained from real sample solutions are shown in Figures 10 and 11.
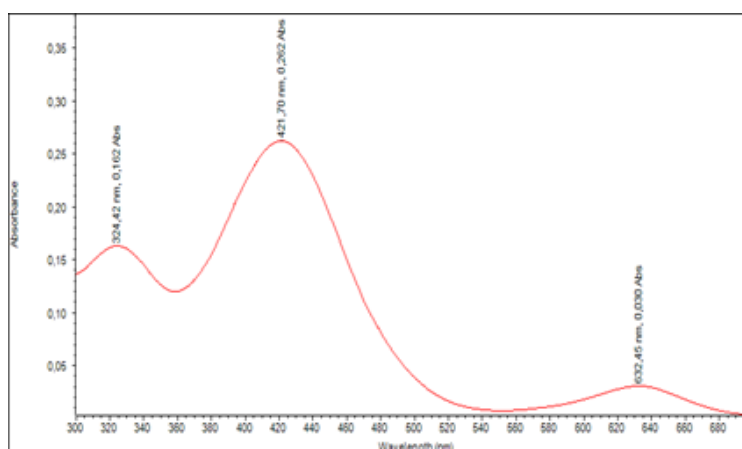
Figure 10: Spectrum obtained from Largopen (tablet) sample solution.
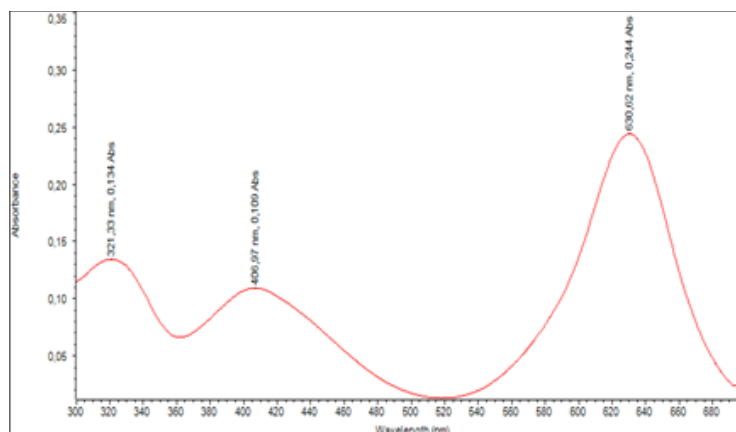
Figure 11: Spectrum obtained from Largopen (powder) sample solution.

On the other hand, the method of standard addition was applied for amoxicillin determination in both drug samples. Increasing concentrations of amoxicillin standard solutions were added to five separate parts of each drug sample, then bromocresol green was added to each of them and the volume was completed to 10 mL with dimethyl sulfoxide-acetonitrile (50% v/v). The absorbance was measured at the wavelength 630 nm. The concentration of amoxicillin in each drug sample was calculated by referring to the calibration curve and the results were compared with each other as shown in Table 2.

Table 2: Amoxicillin concentrations in drug samples and the statistical parameters by spectrophotometric method and standard addition method.

	Amoxicillin (µg/mL)*				
Sample	declared content	Spectrophotometric method	Standard addition method	*F* test	*t* test
Largopen (tablet)	10	8.23±1.12	7.49±0.89	4.82	1.94
Largopen (powder)	10	7.98±0.75	7.16±..02	3.97	1.67

*Mean and standard deviation of three replicates (GS= $x \pm \dfrac{ts}{\sqrt{N}}$, 95% confidence interval)

(95% confidence level *F* = 6.39 and *t* = 2.21)

The content of amoxicillin in each one of the two drug samples was determined by both the developed spectrophotometric method and the standard addition method. Both *t*-test and *F*-test were performed for the developed method. *t* values were found to be 1.94 and 1.67. The results showed that the calculated values of *t* were lower than the critical value (*t* = 2.31, 95% confidence level), so there is no significant difference between the results obtained from the two methods at 95% confidence level. On the other hand, the *F*-test was applied to find out whether there is a significant difference between the precisions of the two methods applied. In all cases, the calculated values of *F* (4.82 and 3.92) were lower than the critical value (*F* = 6.39, 95% confidence level). These results showed that there is no significant difference between the developed spectrophotometric method and the standard addition method and that the developed method can be successfully applied to real samples.

Recovery Studies (Precision and Accuracy)
Recovery studies were carried out to investigate the effects of additives found in the studied drug samples on the developed spectrophotometric method for amoxicillin determination. For this purpose, a known amount of standard amoxicillin was added to the prepared drug samples that also contain a known amount of amoxicillin. Then the ion-pair complex was formed by adding bromocresol green and the absorbance values were measured at 630 nm. Amoxicillin concentrations were calculated using the calibration graph and the results were listed in Table 3.

Table 3: Recovery% and other parameters of drug samples containing (10 mg)* of amoxicillin.

	Amount Added (labelled) (mg)	Amount Found (mg)	Recovery %
Amoxicillin	10	9.72	97.21
	10	9.81	98.10
	10	9.92	99.20
			χ: 98.16
			SD: 0.34
			RSD%: 3.62
			RE%: -6.30

*Average of three replicate (SD: standard deviation; RSD: relative standard deviation; RE: relative error).

As can be seen in the table, the proposed method was successfully applied for the determination of amoxicillin in drug samples where the recovery values found in the range of 97.21-99.20% (average 98.16%) and relative standard deviation was 3.62%.

CONCLUSION AND RECOMMENDATIONS

In this study, a simple, fast, and selective spectrophotometric method that does not require extraction processes was developed for the determination of amoxicillin. The method is based on measuring the absorption intensity of the ion-pair complex formed between amoxicillin and bromocresol green at 630 nm. The developed method was optimized and successfully applied to analyze two different drug samples containing amoxicillin. The results were obtained from both the calibration graph and the standard addition method, and they were given comparatively. The results obtained from the proposed method were also compared using the statistical tests and found to be in good agreement with the other method.

The comparison of the proposed method with the previously some reported methods is presented in Table 4. The proposed method enabled the LOD 0.05 µg mL^{-1} for amoxicillin without complex pre-treatment. The developed method was found to be versatile and have many advantages over the previously reported methods. The method is utilized a single step reaction with no extraction process and no simpler compared to reported methods.

Table 4: Comparison of other methods in the literature.

Method	Sample	LOD (µg mL^{-1})	Reference
FIA	Pharmaceutical formulations	0.571	(11)
Voltammetry	Drug and urine	0.50	(13)
HPLC	Bulk drug	0.347	(7)
Capillary electrophoresis	Animal plasma samples	0.280	(16)
Spectroscopy	Pharmaceutical preparations	0.05	This work

With the developed method, significantly lower limit of detection, wider dynamic range, and higher selectivity were obtained for amoxicillin determination. The method does not require the use of complex equipment and it has the potential to be applied in the routine analysis of amoxicillin in different pharmaceutical formulations.

REFERENCES

1. Zheng F, Wang S, Wen S, Shen M, Zhu M, Shi X. Characterization and antibacterial activity of amoxicillin-loaded electrospun nano-hydroxyapatite/poly(lactic-co-glycolic acid) composite nanofibers. Biomaterials. 2013 Jan;34(4):1402–12. <DOI>.

2. Lim WS, Gander S, Finch RG, McFarlane JT. A novel method for collecting and detecting amoxycillin in urine: a tool for testing antibiotic compliance in the community. Journal of Antimicrobial Chemotherapy. 2000 Nov 1;46(5):835–7. <DOI>.

3. Kaur SP, Rao R, Nanda S. Amoxicillin: a broad spectrum antibiotic. Int J Pharm Pharm Sci. 2011;3(3):30–7.

4. Lee TL, D'Arconte L, Brooks MA. High-Pressure Liquid Chromatographic Determination of Amoxicillin in Urine. Journal of Pharmaceutical Sciences. 1979 Apr;68(4):454–8. <DOI>.

5. De Abreu LP, Ortiz RAM, de Castro SC, Pedrazzoli J. HPLC determination of amoxicillin comparative bioavailability in healthy volunteers after a single dose administration. J Pharm Sci. 2003;6(2):223–30.

6. Douša M, Hosmanová R. Rapid determination of amoxicillin in premixes by HPLC. Journal of Pharmaceutical and Biomedical Analysis. 2005 Feb;37(2):373–7. <DOI>.

7. Basavaiah K, Tharpa K, Prasad NR, Hiriyanna S, Vinay K. Determination of amoxycillin in pharmaceutical forms by visible spectrophotometry and HPLC. 2009; Available from: <URL>.

8. Shoukry MM. Potentiometric studies of binary and ternary complexes of amoxycillin. Talanta. 1992 Dec;39(12):1625–8. <DOI>.

9. El Walily AFM, Abdel-Kader Gazy A, Belal SF, Khamis EF. Selective spectrofluorimetric determination of phenolic β-lactam antibiotics through the formation of their coumarin derivatives. Journal of Pharmaceutical and Biomedical Analysis. 1999 Aug;20(4):643–53. <DOI>.

10. Li Y, Tang Y, Yao H, Fu J. Determination of ampicillin and amoxycillin by flow injection chemiluminescence method based on their enhancing effects on the luminol-periodate reaction. Luminescence. 2003 Nov;18(6):313–7. <DOI>.

11. Xiaofeng X, Zhenghua S. Ultrasensitive determination of amoxicillin using chemiluminescence with flow injection analysis. Journal of Spectroscopy. 2006; 20(1): 37–43. <URL>.

12. Indrayanto G, Sa TK, Widjaja S. Simultaneous Determination of Amoxycillin Trihydrate and Clavulanate Potassium in Pharmaceutical Preparations by Thin-Layer Chromatography/Densitometry. Journal of AOAC INTERNATIONAL. 2000 Nov 1;83(6):1493–6. <DOI>.

13. Fouladgar M, Hadjmohammadi MR, Khalilzadeh MA, Biparva P, Teymoori N, Beitollah H. Voltammetric determination of amoxicillin at the electrochemical sensor ferrocenedicarboxylic acid multi wall carbon nanotubes paste electrode. Int J Electrochem Sci. 2011;6:1355–66. <URL>.

14. Kotte SCB, Tulam VK, Chinta RR, Raghavan S, Dubey P, Murali P. Qualitative analysis of amoxicillin, ampicillin, cephalexin by quadrupole–time of flight (LCMS) using electrospray ionization. International Journal of ChemTech Research. 2012;4:855–61.

15. Pajchel G, Pawłowski K, Tyski S. CE versus LC for simultaneous determination of amoxicillin/clavulanic acid and ampicillin/sulbactam in pharmaceutical formulations for injections. Journal of Pharmaceutical and Biomedical Analysis. 2002 Jun;29(1–2):75–81. <DOI>.

16. Hernández M, Borrull F, Calull M. Determination of amoxicillin in plasma samples by capillary electrophoresis. Journal of Chromatography B: Biomedical Sciences and Applications. 1999 Aug;731(2):309–15. <DOI>.

17. Issa M, Nejem M, Al-Kholy M, El-Abadla S, Helles S, Saleh A. An indirect atomic absorption spectrometric determination of ciprofloxacin, amoxycillin and diclofenac sodium in pharmaceutical formulations. J Serb Chem Soc. 2008;73(5):569–76. <DOI>.

18. Raghavendra G, Suryanarayana R. Spectrophotometric method for the determination of ampicillin and amoxicillin. J Pharm Res. 2010;3:869–72.

19. Mohamed GG. Spectrophotometric determination of ampicillin, dicluxacillin, flucloxacillin and amoxicillin antibiotic drugs: ion-pair formation with molybdenum and thiocyanate. Journal of Pharmaceutical and Biomedical Analysis. 2001 Feb;24(4):561–7. <DOI>.

20. Shafique Ahmad A, Rahman N, Islam F. Spectrophotometric Determination of Ampicillin, Amoxycillin, and Carbenicillin Using Folin-Ciocalteu Phenol Reagent. Journal of Analytical Chemistry. 2004 Feb;59(2):119–23. <DOI>.

21. Prasad B, Gupta S. Extraction spectrophotometric method for the determination of certain β-lactam antibiotics with methylene blue. 2000;62(4):261–6.

22. Keskar MR, Jugade RM. A new spectrophotometric method for determination of amoxicillin using bromocresol green. World Journal of Pharmacy and Pharmaceutical Sciences. 2014;3(2):1340–8.

23. Nagaraja P, Shrestha AK. Spectrophotometric Method for the Determination of Drugs Containing Phenol Group by Using 2, 4- Dinitrophenylhydrazine. E-Journal of Chemistry. 2010;7(2):395–402. <DOI>.

24. Al-Uzri WA. Spectrophotometric determination of amoxicillin in pharmaceutical preparations through diazotization and coupling reaction. IJ S. 2012;53(4):713–23.

25. Abdul-Sattar JA, Atia A-JK, Kadhom SA, Mohammed-Ali LF. Spectrophotometric Determination of Amoxicillin-Application to Capsules. Al-Mustansiriya J Sci. 2009;20(4):35–42.

26. Gujral RS, Haque SM. Simultaneous Determination of Potassium Clavulanate and Amoxicillin Trihydrate in Bulk, Pharmaceutical Formulations and in Human Urine Samples by UV Spectrophotometry. Int J Biomed Sci. 2010 Dec;6(4):335–43. PMID: 23675211.

27. Ünal K, Palabiyik İM, Karacan E, Onur F. Spectrophotometric determination of amoxicillin in pharmaceutical formulations. Turk J Pharm Sci. 2008;5(1):1–16.

28. Tavallali H, Rasti S. Simultaneous determination of amoxicillin and clavulanate potassium by derivative spectrophotometric method. Int J Pharm Tech Res. 2013;5(3):2475–80.

29. Radhi ER, Ali KJ. Spectrophotometric Study for Comparison of Amoxicillin Trihydrate and Levofloxacin Hemihydrate Determination Using Bromocresol Green. International Journal of Pharmaceutical Research. 2019;11(1):1107–13.

30. Hamed AH, Ibrahim SK, DHAHIR S, Mhemeed AH. Indirect Spectrophotometric Determination of Amoxicillin by oxidative with Cerium ammonium nitrate using Arsenazo III as a reagent. Chemical Science. 2019;8(2):289–94.

31. Ermer J, Miller JHM. Method Validation in Pharmaceutical Analysis A Guide to Best Practice [Internet]. 2006 [cited 2022 Mar 3]. ISBN: 978-3-527-31255-9. <URL>.