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.

Keywords: Amoxicillin, bromine cresol, drug sample, spectrophotometric method.

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.

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, HPLC4-7 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 (20), 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.
It can be seen in Figure 2 that the amoxicillin solution has a maximum absorbance at 270 nm.

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.

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.
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 $1 \times 10^{-4}, 2 \times 10^{-4}$ and $3 \times 10^{-4}$ 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.
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.

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 9x10^-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.
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).

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 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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>630</td>
</tr>
<tr>
<td>Linear range ($\mu$g mL$^{-1}$)</td>
<td>1-18</td>
</tr>
<tr>
<td>Regression equation*</td>
<td>$A = 0.00556C$</td>
</tr>
<tr>
<td>correlation coefficient ($r^2$)</td>
<td>0.9976</td>
</tr>
<tr>
<td>slope</td>
<td>0.0551</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0062</td>
</tr>
<tr>
<td>LOD ($\mu$g mL$^{-1}$)</td>
<td>0.05</td>
</tr>
<tr>
<td>LOQ ($\mu$g mL$^{-1}$)</td>
<td>0.15</td>
</tr>
<tr>
<td>molar absorptivity (L.mol$^{-1}$.cm$^{-1}$)</td>
<td>$1.538 \times 10^3$</td>
</tr>
</tbody>
</table>

*(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.](image-url)
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.

<table>
<thead>
<tr>
<th>Sample</th>
<th>declared content</th>
<th>Spectrophotometric method</th>
<th>Standard addition method</th>
<th>$F$ test</th>
<th>$t$ test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Largopen (tablet)</td>
<td>10</td>
<td>8.23±1.12</td>
<td>7.49±0.89</td>
<td>4.82</td>
<td>1.94</td>
</tr>
<tr>
<td>Largopen (powder)</td>
<td>10</td>
<td>7.98±0.75</td>
<td>7.16±0.02</td>
<td>3.97</td>
<td>1.67</td>
</tr>
</tbody>
</table>

*Mean and standard deviation of three replicate samples (GS = $\bar{x} \pm \frac{ts}{\sqrt{N}}$, 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.

<table>
<thead>
<tr>
<th>Amount Added (labelled) (mg)</th>
<th>Amount Found (mg)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>9.72</td>
<td>97.21</td>
</tr>
<tr>
<td>10</td>
<td>9.81</td>
<td>98.10</td>
</tr>
<tr>
<td>10</td>
<td>9.92</td>
<td>99.20</td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>98.16</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Method</th>
<th>Sample</th>
<th>LOD (μg mL⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIA</td>
<td>Pharmaceutical formulations</td>
<td>0.571</td>
<td>(11)</td>
</tr>
<tr>
<td>Voltammetry</td>
<td>Drug and urine</td>
<td>0.50</td>
<td>(13)</td>
</tr>
<tr>
<td>HPLC</td>
<td>Bulk drug</td>
<td>0.347</td>
<td>(7)</td>
</tr>
<tr>
<td>Capillary electrophoresis</td>
<td>Animal plasma samples</td>
<td>0.280</td>
<td>(16)</td>
</tr>
<tr>
<td>Spectroscopy</td>
<td>Pharmaceutical preparations</td>
<td>0.05</td>
<td>This work</td>
</tr>
</tbody>
</table>

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


