A NEW SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF AMOXICILLIN USING BROMOCRESOL GREEN

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ABSTRACT

Simple and rapid spectrophotometric method has been developed for the determination of amoxicillin in bulk and pharmaceutical formulations. The method is based on the formation of blue ion-pair complex between amoxicillin and bromocresol green in dimethylsulphoxide medium. The studies have been carried out with Spectronic 20D+ (Thermospectronic) visible spectrophotometer. The absorption maxima of the complex was found to be 630 nm. The composition of complex was determined by Job’s method was found to be 2:1 (Drug: Reagent). Different variables affecting the reaction were studied and parameters such as time, reagent concentration etc were optimised. The formed complex was quantified spectrophotometrically at absorption maxima. Concentration of bromocresol green was found to be optimum at 9.6 µM. Under the optimum conditions calibration graph was found to be linear over the range of 1-13 µg/ml with detection limit 0.06µg/ml. Thermodynamic studies were carried out including stability constant (log K=4.97) and Gibb’s free energy change (ΔG = −2.8167 x 10^4 kJ/mol). Validation parameters such as Sandell’s sensitivlty, correlation coefficient, coefficient of determination, standard deviation and relative standard deviation were determined. Recovery studies were carried out. The method was successfully applied for the determination of amoxicillin in pharmaceutical formulations such as tablets, capsules and oral suspension. The proposed method is simple, direct, sensitive and do not require any extraction process. Thus, this method could be readily applicable for the quality control and routine analysis.
Keywords: Amoxicillin, bromocresol green, ion-pair complex, spectrophotometry.

INTRODUCTION

Amoxicillin, semi-synthetic drug belongs to a class of antibiotics called as Penicillin. It is shown to be effective against a wide range of infections caused by wide range of Gram-positive and Gram-negative bacteria in both human and animals. Structure of amoxicillin is as shown in Fig. 1(a). In the past decade, it is used to treat infections of the middle ear (otitis media), tonsils (tonsillitis and tonsil pharyngitis), throat (laryngitis), pharynx (pharyngitis), bronchi (bronchitis), lungs (pneumonia), urinary tract, (UTI), skin etc[1-3].

Several methods have been reported in the literature for the analysis of amoxicillin such as HPLC[4], potentiometry[5], spectrofluorometry[6], flow-injection[7], Thin layer chromatography[8], voltammetry[9] etc.

Bromo cresol green (BCG) as shown in Fig. 1(b) is a sulphonphthalein dye commonly used as indicator and spectrophotometric reagent.

Fig. 1 Structure of (a) amoxicillin, (b) bromocresol green.

Spectrophotometric method is based on the formation of coloured (charge transfer or ion-pair) complex between drug and reagent which can be estimated by visible spectrophotometry. Charge transfer complex is also called as electron donor-acceptor complex in which a fraction of electric charge is transferred between the molecular entities. In ion-pair complex formation, ions of opposite electric charge are produced in the solution due to the electron transfer. Reported spectrophotometric methods of amoxicillin include complex formation with chloranilic acid[4], hematoxyline[10], molybdenum and thiocyanate[11], Folin-Clocaultue phenol reagent[12], methylene blue[13], iodine and wool fast blue[14].

The proposed method is based on formation of ion-pair complex of amoxicillin with bromocresol green. Bromocresol green has been used for the first time with significantly low
detection limit, high sensitivity and wider dynamic range for the determination of amoxicillin. An important feature of the method is that no extraction is required and it is feasible at room temperature.

MATERIALS AND METHODS
Reagents and Chemicals
All chemicals and reagents used were of analytical grade. Amoxicillin was kindly provided by ZIM laboratories. Bromocresol green, dimethyl sulphoxide (DMSO) were obtained from LOBA Chemie. 0.01M solutions of amoxicillin and bromocresol green were prepared in dimethyl sulfoxide as stock solutions. The solutions were further diluted as per requirement.

Apparatus
Spectrophotometric studies were carried out with Spectronic 20D+ (Thermo-Spectronic) visible spectrophotometer. A Mettler balance (Ner-Parma instrument Corp. L.C. =0.01mg) was used for weighing purpose.

Procedure for Calibration Curve
Suitable aliquots of amoxicillin in dimethyl sulphoxide were transferred into series of 10ml of volumetric flasks. Bromocresol green solution was added to make final concentrations of 9.6µM making up the volume with dimethylsulphoxide. The absorbance of blue coloured solution was measured at 630nm against the appropriate reagent blank.

Procedure for dosage forms (tablet, capsules and oral suspension)
Five tablets or capsules were weighed and average weight of one tablet or one capsule was determined. They were powdered and about 0.05g was exactly weighed and shaken with 30ml dimethylsulphoxide for 30 minutes. It was filtered with Watmann filter paper no. 40 and made up 50 ml with dimethylsulphoxide. The same procedure was applied for oral suspension of drug using 1ml suspension. Suitable aliquots were analysed using general procedure describe above. The antibiotic content of tablet, capsule and oral suspension were calculated from calibration curve and also by standard addition method.

RESULTS AND DISUSSION
Absorption Spectra
Amoxicillin form blue coloured ion-pair reaction product with bromocresol green having absorbance maxima 630 nm as shown in Fig. 2. Under the experimental conditions, the
reagent blank showed negligible absorbance at 630 nm. Different experimental parameters affecting the formation and stability of complex were studied to determine the optimum conditions.

![Absorption spectra of amoxicillin (3700µg/ml), bromophenol blue (8µM) and their complex.](image)

**Fig. 2.** Absorption spectra of amoxicillin (3700µg/ml), bromophenol blue (8µM) and their complex.

**Effect of Solvent**
Various solvents like methanol, ethanol, acetone, dichloromethane, dichloroethane, dimethylsulphoxide, chloroform and acetonitrile were studied for solubility, complex formation, to achieve maximum sensitivity and product stability. Dimethylsulphoxide (DMSO) was found to be most suitable solvent for amoxicillin and bromocresol green. Hence it was used as a solvent.

**Stoichiometric Relationship**
Composition of ion-pair complex was established by applying Job’s method of continuous variation as shown in Fig. 3 using equimolar solutions of the drug and the reagent. The results indicated that the complex was formed in the ratio of 2:1 (D: R), the absorbance of the complex was used to calculate stability constant and Gibbs free energy change.
Continuous variation plots for the ion-pair complex of amoxicillin with bromocresol green in DMSO solvent at 630 nm (a) 1x10^{-4}M, (b) 2x10^{-4}M, (c) 3x10^{-4} M.

Effect of Time
Mixture of drug and reagent was prepared; the optimum reaction time was determined by recording the absorbance of the formed complex at different time intervals. The variation has been shown in Fig. 4. It was found that the ion-pair complex was formed instantaneously at room temperature and stable for near about 40 minutes.

Effect of Reagent Concentration
The optimum reagent concentration was determined by adding various volumes of bromocresol green to the drug. The colour intensity was found to be increase with addition of bromocresol green up to 9.6 µM and decreased thereafter. Fig. 5 indicates the effect of concentration of bromocresol green on absorbance. The absorbance was found to be
maximum at bromocresol green concentration of 9.6 μM therefore; this concentration was selected for the preparation of calibration graph.

![Graph showing absorbance against BCG concentration](image)

**Fig. 5 Effect of bromocresol green concentration on ion-pair complex of amoxicillin (a) 15 μg/ml, (b) 7 μg/ml in DMSO solvent at 630 nm.**

Calibration curve for amoxicillin was constructed by plotting absorbance versus concentration in μg/ml. Under optimum conditions, various analytical parameters were obtained and presented in table 1. The value of correlation coefficient indicates good linearity of the present method. High value of molar absorptivity and lower value of Sandell’s sensitivity reflect good and high sensitivity of the method.

**Table 1. Collective data for the Qualitative and Statistical parameters using proposed method.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amoxicillin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>630</td>
</tr>
<tr>
<td>Molar ratio (D:R)</td>
<td>2:1</td>
</tr>
<tr>
<td>Linear range (μg/ml)</td>
<td>1-13</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0339</td>
</tr>
<tr>
<td>Intercept</td>
<td>-0.0066</td>
</tr>
<tr>
<td>Linear regression equation</td>
<td>A=0.0339C -0.0066</td>
</tr>
<tr>
<td>Sandell’s sensitivity (μg/cm²)</td>
<td>0.0294</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9994</td>
</tr>
<tr>
<td>Coefficient of determination($r^2$)</td>
<td>0.9988</td>
</tr>
</tbody>
</table>
Recovery studies were carried out for amoxicillin using calibration curve at three different concentrations. These results are presented in table 2.

### Table 2. Recovery studies of Amoxicillin.

<table>
<thead>
<tr>
<th>S.N</th>
<th>Sample</th>
<th>Method</th>
<th>Taken (µg/ml)</th>
<th>Found (µg/ml)</th>
<th>Recovery (%)</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Amoxicillin</td>
<td>BCG</td>
<td>2.5</td>
<td>2.5</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7.5</td>
<td>7.5</td>
<td>100.00</td>
<td>100.26±0.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12.5</td>
<td>12.6</td>
<td>100.80</td>
<td></td>
</tr>
</tbody>
</table>

*Average of three determinations.*

### APPLICATION

The proposed method has been successfully applied to the determination of amoxicillin in pharmaceutical formulation such as tablet, capsules and oral suspension. Calibration curve as well as standard addition method was adopted for quantitative analysis. The results are presented in table 3.

### Table 3. Analysis of pharmaceutical formulations

<table>
<thead>
<tr>
<th>S. N.</th>
<th>Pharmaceutical Preparationsa</th>
<th>Labelled Amount (mg)</th>
<th>Found Amount (mg)</th>
<th>Calibration curve method b</th>
<th>Standard addition method b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Rexel capsules</td>
<td>250</td>
<td>246.33±1.50</td>
<td>241.66±4.92</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Moxkid tablets</td>
<td>250</td>
<td>251.10±2.13</td>
<td>253.73±3.96</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Moxkid tablets</td>
<td>125</td>
<td>124.03±0.63</td>
<td>126.73±1.01</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Novamax Liquid</td>
<td>125</td>
<td>123.66±1.25</td>
<td>120.86±1.61</td>
<td></td>
</tr>
</tbody>
</table>

*"Ranbaxy laboratories products" , "(Average±SD) of three observations*
The developed method was found to be versatile and have many advantages over the previously reported methods. The proposed method is more sensitive compared to the established method as shown by the molar absorbptivity$^4$. The detection limit is lower than the reported methods$^{4,13}$. The method is utilized a single step reaction with no extraction process and so simpler compared to reported methods$^{11,13}$.

**CONCLUSION**

This method requires only dye and solvents which are comparatively cheaper and readily available. The method is simple as it does not involve adjustment of critical conditions like temperature, pH or tedious sample preparation. This method has many advantages over other analytical methods due to its simplicity, sensitivity, rapidity, low cost instrumentation, accuracy. Due to these advantages this method can be used for quality control and routine analysis.

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**REFERENCES**


