DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF AMOXICILLIN AND RANITIDINE IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

The objective of the study was to develop and validate a simple and sensitive RP-HPLC method for simultaneous determination of amoxicillin and ranitidine in pharmaceutical formulations. The chromatographic system consisted of a LC-10 AT VP pump, SPD-10 AVP UV/visible detector. The separation was achieved from Hibar-Purospher star RP-18e (5 µm, 250 x 4.6 mm) column at ambient temperature with a mobile phase consisting of 0.02M phosphate buffer: Acetonitrile (93:7 v/v, pH 3.0) at a flow rate of 1.0 ml/ minute and the retention times were about 8 and 11 minutes for amoxicillin and ranitidine respectively. The peak separation was detected at 230nm. The calibration curve was linear over the concentration range of 0.15µg/mL to 20µg/mL for amoxicillin and 0.039µg/ml to 20µg/ml for ranitidine. The proposed method is accurate, precise, and reproducible within the desired range. The limit of detection was found to be 0.039µg/mL and 0.0195µg/mL and limit of quantification was found to be 0.078µg/mL and 0.039µg/mL for amoxicillin and ranitidine respectively. Therefore this method could be used as a more convenient and efficient option for the analysis of amoxicillin and ranitidine in pharmaceutical dosage form as well as this method can also give help in the pharmacokinetic study of the same drug.

Key Words: Amoxicillin, Ranitidine, simultaneous determination, RP-HPLC method.
INTRODUCTION
Amoxicillin is a semi synthetic penicillin related to ampicillin, and was first described in 1970.\(^1\) Amoxicillin trihydrate is \((2S,5R,6R)-6-[(2R)-2-Amino-2-(4-hydroxyphenyl) acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate. (Figure 1)

![Figure 1: Structure of Amoxicillin trihydrate](image1)

Amoxicillin is bactericidal for both Gram positive and Gram negative bacteria. It is used in the treatment of urinary tract infections, otitis media, respiratory tract infections, enteric infections (Helicobacter pylori infections), bacterial meningitis. It is well absorbed after oral administration.\(^3\) For the quantitative analysis of Amoxicillin Trihydrate several Spectrophotometry\(^4, 5, 6\), HPLC \(^7, 8, 9\), HPLC with photo diode array detection \(^10\) and voltametry\(^11\) methods are available in the literature. But all of these methods are mostly discussed with amoxicillin alone or with other combination of drugs but not with ranitidine.

Ranitidine hydrochloride is a histamine H\(_2\) receptor antagonist. It is \(NN\)-Dimethyl-5-[2-(1-methylamino-2-nitrovinylamino) ethylthiomethyl]furfurylamine (figure 2).

![Figure 2: Chemical Structure of Ranitidine](image2)

Ranitidine inhibits acid production by reversibly competing with histamine for binding to H\(_2\) receptors on the basolateral membrane of parietal cells.\(^12\) It is used where inhibition of gastric acid secretion may be beneficial, as in peptic ulcer disease, including stress ulceration, gastro-oesophageal reflux disease, selected cases of persistent dyspepsia and pathological hypersecretory states such as the Zollinger-Ellison syndrome.\(^13\) Literature survey reveals that Ranitidine hydrochloride has been determined in pharmaceutical formulations by Spectrophotometry\(^14, 15, 16\), cyclic voltametry\(^17\) and polarography\(^18\) and in human plasma by HPLC \(^19, 20\). Although many methods have been reported for determination of amoxicillin and
ranitidine but no HPLC method has been reported for the determination of amoxicillin and ranitidine simultaneously.

Amoxicillin is a medicine which is used to treat bacterial infections caused by susceptible microorganisms. It is usually the drug of choice within the class because it is better absorbed, following oral administration, than other β-lactam antibiotics. The aim of the present study was to develop and validate a simple, rapid, reproducible, accurate and economical method for the determination of amoxicillin and ranitidine simultaneously not only in the pharmaceutical formulations as well as it will help in control and analysis of pharmacokinetics study.

Usually when we are using antibiotics for longer period of time it might cause some ulceration in the GIT. So to reduce these problems the physician prescribed the combination of medicine frequently.

MATERIALS AND METHOD

Instrumentation
An isocratic Shimadzu HPLC system equipped with LC-10 AT VP pump and SPD-10 A UV detector loaded with Shimadzu Class-GC software was used. Purospher star RP-18e (5 µm, 250 × 4.6 mm) column fitted with a 100 µL loop. Mettler electronic balance and Millipore filtration assembly were also used.

Materials and Reagents
Amoxicillin standard and ranitidine hydrochloride were kind gifts from Central Drug laboratory, Karachi, Pakistan and Indus Pharma (Pvt) Limited respectively. Amoxicillin capsules 250 mg (Amoxil®) and ranitidine tablets 300 mg (Zantac®) were purchased from local retail pharmacy. All solvents used were of HPLC grade and all reagents were of Analytical grade. Fresh working solutions were prepared daily.

Chromatographic Condition

Preparation of mobile phase
The mobile phase having 0.02M phosphate buffer: Acetonitrile (93:7) where as the pH was adjusted to 3.0 and the flow rate was established at 1.0 ml/minute. The mobile phase was degassed and filtered through 0.45µm filter. The performance was carried out at ambient temperature and detection was made at 230 nm.
Preparation of standard solutions
Stock solution of amoxicillin (Ref) was prepared by dissolving appropriate amount of amoxicillin trihydrate in mobile phase to obtain final drug concentration of 100µg/mL. Serial dilutions were prepared in mobile phase to obtain a concentration in range of 0.0195µg/mL to 20µg/mL. The dilutions were filtered through 0.45 µm filter and then injected.

Stock solution of ranitidine (ref) was prepared by dissolving appropriate amount of ranitidine in mobile phase to obtain final drug concentration of 100µg/ml. Serial dilutions were prepared in mobile phase to obtain a concentration in range of 0.0195µg/mL to 20µg/mL. The dilutions were filtered through 0.45 µm filter and then injected.

Preparation of sample solutions
Amoxicillin trihydrate contents removed from 20 capsules and weighed portions of powder equivalent to 0.25g of drug was transferred to 50 mL volumetric flask, dissolved in mobile phase, sonicated and filtered. Stock solution was diluted with mobile phase to obtain a concentration of 10µg/ml. Five replicates of each standard and sample solutions were injected under same chromatographic conditions.

Crushed 20 tablets of ranitidine hydrochloride (300mg) and weighed portions of powder equivalent to 0.25g of drug was transferred to 100 mL volumetric flask, dissolved in mobile phase, sonicated and filtered. Stock solution was diluted with mobile phase to obtain a concentration of 10µg/mL. Five replicates of each Standard and sample solutions were injected under same chromatographic conditions.

The amount of amoxicillin and ranitidine present in the working solution were determined by comparing peak area of sample with peak area of standard.

Method Development
The analytical procedures were optimized by varying the pH, composition and flow rate of mobile phase. Finally the best resolution was achieved when mobile phase used was phosphate buffer: Acetonitrile (93:7) whereas pH was adjusted at 3.0 with phosphoric acid with flow rate of 1 ml/minute. The retention time of amoxicillin was found to be 8 minutes. The retention time of ranitidine was found to be 11 minutes.
Method Validation
The proposed method has been developed and validated for the determination of amoxicillin and ranitidine in pharmaceutical formulations. According to International Conference on Harmonization (ICH)^21 guidelines, validation of the method was carried out by using accuracy, linearity, suitability, range, LOD, LOQ, precision, ruggedness and robustness. Accuracy is the closeness between the measured value and true values. Accuracy was determined by replicate analysis of samples containing known amount of analyte. Precision describes the reproducibility of analytical method. Linearity of the method was determined by making several dilutions of amoxicillin and ranitidine in mobile phase. Ruggedness of the method measures the reproducibility of results from laboratory to laboratory and from analyst to analyst.

Specificity differentiates between the active and excipients. Specificity was determined to ensure the separation of amoxicillin and ranitidine with excipients of the formulation. The chromatograms (Fig. 3, 4 and 5) show no interference of amoxicillin and ranitidine with the excipients of formulation.

RESULTS AND DISCUSSION
Initially different tests were performed to select the adequate and optimum conditions for the analysis, such as wavelength, mobile phase and composition, pH of the mobile phase, concentration and range of the solution.

In order to validate the simplicity and efficiency of the proposed method for analysis of the drug in pharmaceutical formulation the following parameters were determined.

Figure 3: Chromatogram of blank

Figure 4: Chromatogram of amoxicillin and ranitidine (Standard)
System Suitability Testing

System suitability testing is used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. Parameters such as theoretical plates, tailing factor, resolution, retention time, and peak area were determined and compared against the specifications. All parameters were found within acceptable range.

Linearity

Linearity of the method was determined by making ten dilutions of amoxicillin in mobile phase from 0.0195µg/mL to 20µg/mL. All dilutions were filtered then injected into HPLC separately. The calibration curve was plotted between peak areas versus known concentrations of amoxicillin. The method shows linearity over a concentration range of 0.078µg/mL to 20µg/mL. The correlation coefficient was found to be $r^2 = 0.999$ (table 1).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Regression Equation</th>
<th>$R^2$</th>
<th>LOD(µg/mL)</th>
<th>LOQ(µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>$Y=15917x-134.1$</td>
<td>0.9999</td>
<td>0.039</td>
<td>0.078</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>$Y=44414x+13816$</td>
<td>0.9992</td>
<td>0.0195</td>
<td>0.039</td>
</tr>
</tbody>
</table>

Linearity as well as the calibration curve for amoxicillin and ranitidine in mobile phase in the range of 0.078 – 20 µg/ml and 0.039 – 20 µg/ml has been determined respectively (Figure 6 and 7).

![Figure 5: Chromatogram of amoxicillin and ranitidine (sample)](image)

![Figure 6: Calibration curve of Amoxicillin](image)
Accuracy was determined by replicate analysis of samples containing known amount of 80 - 120 % of the target amount (Table-2). The percentage recovery was 97.93 – 99.25 % for amoxicillin and 98.08 – 100.01 % for ranitidine accordingly (Table-2).

### Table 2  Accuracy of Amoxicillin and Ranitidine

<table>
<thead>
<tr>
<th>Drug</th>
<th>Sample size</th>
<th>% Conc.</th>
<th>% Recovery</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>3</td>
<td>80%</td>
<td>99.25</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>100%</td>
<td>97.93</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>120%</td>
<td>98.76</td>
<td>0.48</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>3</td>
<td>80%</td>
<td>100.1</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>100%</td>
<td>98.08</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>120%</td>
<td>99.45</td>
<td>1.05</td>
</tr>
</tbody>
</table>

Precision describes the reproducibility of analytical method. Five aliquots of each single concentration were prepared and analyzed on same day and three consecutive days. Precision of the method was determined by intraday and interday studies. The intraday recovery of different concentration from 0.156 – 10 µg/ml is 98.95 – 100.1 % and their RSD is less than 2 that is between 0.021 – 0.207 whereas interday recovery concentration is 98.76 to 100.1 and RSD of different concentration mean value is between 0.12 – 0.435. That shows the results are within the acceptable range (table 3).

### Table 3 Intraday and Interday Precision of method

<table>
<thead>
<tr>
<th>Concentrations (µg/mL)</th>
<th>Intraday</th>
<th>Interday</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RSD%</td>
<td>Recovery%</td>
</tr>
<tr>
<td>10</td>
<td>0.133</td>
<td>100.1</td>
</tr>
<tr>
<td>5</td>
<td>0.207</td>
<td>100.04</td>
</tr>
<tr>
<td>0.625</td>
<td>0.021</td>
<td>98.95</td>
</tr>
<tr>
<td>0.1562</td>
<td>0.028</td>
<td>99.6</td>
</tr>
</tbody>
</table>
Recovery
The recovery of amoxicillin and ranitidine were determined at three different concentrations equivalent to 80, 100 and 120% of active ingredients (table-2).

Robustness
The robustness of method was determined by making small changes in composition, pH and flow rate of mobile phase and detection wavelength. All parameters show no significant effect.

CONCLUSION
Shortly the proposed HPLC method is one of the simple, rapid, reproducible, accurate and economical method for determination of Amoxicillin trihydrate and Ranitidine hydrochloride in pharmaceutical formulations simultaneously. This method can reduce the time for routine quality control analysis of amoxicillin trihydrate and ranitidine hydrochloride in their dosage form as well as it can help in the measurement of drug blood levels in the pharmacokinetic analysis of amoxicillin and ranitidine.

The present work is first reported RP-HPLC method for simultaneous determination of amoxicillin trihydrate and ranitidine hydrochloride in pharmaceutical dosage forms. Literature survey revealed that no RP-HPLC method has been reported for simultaneous determination of amoxicillin trihydrate and ranitidine hydrochloride.

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REFERENCES
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