ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF METRONIDAZOLE AND AMOXICILLIN IN SYNTHETIC MIXTURE BY UV-VISIBLE SPECTROSCOPY

PARTH PATEL,* PRIYA VARSHNEY,† MINAL ROHIT‡

*Department of Quality Assurance, Pioneer Pharmacy Degree College, Vadodara 390019. Email: patelparth815@rocketmail.com

Received: 12 Nov 2013, Revised and Accepted: 27 Jan 2014

ABSTRACT

Two simple, accurate, precise, reproducible, requiring no prior separation and economical procedures for simultaneous estimation of metronidazole and amoxicillin in combined dosage form have been developed. First method employs formation and solving of simultaneous equation using 320 nm and 273 nm as two analytical wavelengths for both drugs in distilled water. The second method is a Q value analysis based on measurement of absorptivity at 320 nm and 228 nm (as absorbance point). Metronidazole and amoxicillin at their respective λmax 320 nm and 273 nm and at iso-absorptive point 228 nm shows linearity in a concentration range of 10-30 μg/mL. The results of the analysis have been validated statistically, the relative standard deviation lies in the range of 1.11 – 1.25 for amoxicillin and 1.05 – 1.19 for metronidazole in case of simultaneous equation method and 0.41-1.11 for metronidazole and 0.51-1.23 in the case of Q - analysis method.

KEY WORDS Amoxicillin, Metronidazole, UV spectroscopy, Simultaneous equations, Q – analysis

INTRODUCTION

Amoxicillin (6R) -6-(a-D-4-hydroxy-phenylglycylamino) penicillinate and Metronidazole 2-(2-Methyl-5-nitroimidazol-1-yl) are used clinically as independent nonsurgical periodontal therapies for the treatment of periodontal infections. Amoxicillin trihydrate is the most widely used and prescribed medication for oral-dental infections. Amoxicillin trihydrate is a broad spectrum antibiotic and is active against most periodontal pathogens, particularly facultative and aerobic bacteria. It is also active against bacteria that are responsible for periodontal diseases, namely, Bacteroides gingivitis, and anaerobic bacilli. [1] Metronidazole (MTZ), the most potent tetracycline for collagenase inhibition is effective against a broad spectrum of microorganisms inhibiting both gram positive as well as gram negative organisms, including the beta lactamase producing strains. These two drugs with separate antibacterial spectrum are proposed to be formulated as combination therapy to have a wider antibacterial therapy effective against both aerobic and anaerobic periodontal micro flora and hence apriorional drug delivery device containing both the drugs is being developed in our laboratory. In an attempt to develop a periodontal controlled release drug delivery system of metronidazole and amoxicillin, the primary requirement of suitable assay method for simultaneous estimation of both the drugs is the prerequisite. [2]

The review of literature revealed that the combined dosage form has been estimated by spectrophotometric method by using various solvents in the synthetic mixture but no method is yet reported in distilled water. Thus, this method is cost effective. This paper describes two simple, rapid, accurate, precise and economical methods for simultaneous determination of Metronidazole and Amoxicillin in synthetic mixture.

MATERIALS AND METHODS

Reagents & Instruments

A UV-VIS spectrophotometer Shimadzu UV-1800, single pan electronic balance, was used for the experimental purpose. Double distilled water was used throughout the study. Amoxicillin trihydrate and Metronidazole were obtained as a gift sample from Pioneer Pharmacy Degree College, Vadodara. All the other reagents used were of analytical grade.

Determination of absorption maxima

Accurately weighed 10 mg of MTZ was transferred to a 100 ml volumetric flask and volume was made up with the distilled water to get a solution of concentration 100 μg/mL. 3.0 ml of stock solution was diluted to 10 ml to get a concentration of 30 μg/mL. Solution of AMX was also prepared in a similar way to get a concentration of 30 μg/mL. Both the solutions were scanned in the spectrum mode over the range of 200-400 nm. MTZ showed an absorbance peak at 320 nm, whereas AMX at 273 nm. The overlaid spectra also showed two iso-absorptive points at 228 nm and 235 nm (Fig. 1).

Sample Preparation Method

The sample solution was prepared from standard stock solutions (100 μg/ml) in the ratio of 1:2 (MTZ:AMX). Absorbance of sample solution was simultaneously estimated at 320 nm, 273 nm, and also at its iso-absorptive point 228 nm.

Method I (simultaneous equation method)

Two wavelengths selected for the method are 320 nm and 273 nm that are absorption maxima’s of MTZ and AMX respectively in distilled water. Standard stock solution(s) of 100 μg/ml each of MTZ and AMX were prepared separately in distilled water. The stock solutions of both the drugs were further diluted separately with distilled water to get a series of standard solutions of 10-30 μg/ml concentrations. The absorbances were measured at the selected wavelengths and absorptivities (A 1%, 1 cm) for both the drugs at both wavelengths were determined as mean of three independent determinations. [3-8] Concentrations in the sample were obtained by using the following equations:
Extracted text:

$$C_x = A_1 \cdot \frac{a_2}{a_1} - A_2 \cdot \frac{a_1}{a_2} \cdot ax \cdot ay$$

$$C_y = A_1 \cdot ax \cdot a_2 - A_2 \cdot ay \cdot ax$$

Where, $A_1$ and $A_2$ are absorbances of mixture at 320 nm and 273 nm respectively, $a_x$ and $a_y$ are absorptivities of metronidazole at $\lambda_1$ and $\lambda_2$ respectively and $a_y$ and $a_x$ are absorptivities of amoxicillin at $\lambda_2$ and $\lambda_1$ respectively. $C_x$ and $C_y$ are concentrations of Metronidazole and amoxicillin respectively.

**Method II (absorbance ratio or Q-analysis method)**

From the overlay spectrum of MTZ and AMX, two wavelengths were selected one at 228 nm, the iso-absorptive point for both the drugs and the other at 320 nm, $\lambda_{max}$ of metronidazole. The absorbances of the standard and sample solutions prepared in a similar manner as in the previous method, were measured and the absorptivity values for both drugs at the selected wavelengths are presented in Table I. The method employs $Q$ values and the concentrations of drugs in sample solution were determined by using the following formula, \[9, 10\]

**For Metronidazole at amoxicillin**

$$C_x = \frac{Q_y - Q_x}{Q_x - Q_y}$$

$$C_y = C_x \cdot \frac{a_x}{a_y}$$

Where, $Q_x = \text{Absorbance of sample at 320 nm/ Absorbance of sample at 228 nm}$

$Q_x = \text{Absorptivity of Metronidazole at 320 nm/ Absorptivity of Metronidazole at 228 nm}$

$Q_y = \text{Absorptivity of amoxicillin at 320 nm/ Absorptivity of amoxicillin at 228 nm}$

$A = \text{Absorbance of sample at iso-absorptive point}$

$ax = \text{Absorptivity of Metronidazole at iso-absorptive point}.$

**METHOD VALIDATION**

The described methods have been validated for the assay of both the major components of bulk drug using following ICH parameters. \[10\]

**Linearity**

Linearity was studied by preparing standard solutions at different concentration levels. Calibration curves were prepared using the standard solutions of 10 μg/ml - 30 μg/ml and linear regression analysis was carried out. The regression coefficients are reported in Table I.

**Table 1: Linear regression analysis of calibration curves of MTZ & AMX with both the methods reported here**

<table>
<thead>
<tr>
<th>Concentration (μg/ml)</th>
<th>Method 1</th>
<th></th>
<th>Method 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MTZ</td>
<td>AMX</td>
<td>MTZ</td>
<td>AMX</td>
</tr>
<tr>
<td></td>
<td>320 nm</td>
<td>273 nm</td>
<td>320 nm</td>
<td>Iso-absorptive Point 228 nm</td>
</tr>
<tr>
<td>10</td>
<td>0.575</td>
<td>0.135</td>
<td>0.034</td>
<td>0.006</td>
</tr>
<tr>
<td>15</td>
<td>0.832</td>
<td>0.193</td>
<td>0.051</td>
<td>0.009</td>
</tr>
<tr>
<td>20</td>
<td>1.124</td>
<td>0.259</td>
<td>0.071</td>
<td>0.008</td>
</tr>
<tr>
<td>25</td>
<td>1.430</td>
<td>0.329</td>
<td>0.078</td>
<td>0.005</td>
</tr>
<tr>
<td>30</td>
<td>1.819</td>
<td>0.428</td>
<td>0.102</td>
<td>0.013</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.9939</td>
<td>0.9816</td>
<td>0.9939</td>
<td>0.9816</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0784</td>
<td>0.002</td>
<td>0.0784</td>
<td>0.002</td>
</tr>
<tr>
<td>$A_{10^{th}}$</td>
<td>573.98</td>
<td>133.484</td>
<td>33.74</td>
<td>4.466</td>
</tr>
</tbody>
</table>

**Table 2: Results of precision study (Intra-day and inter-day)**

<table>
<thead>
<tr>
<th>Methods</th>
<th>Inter-Day Precision (n=3)</th>
<th>Intra-Day Precision (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% RSD</td>
<td>% RSD</td>
</tr>
<tr>
<td>SIMULTANEOUS EQUATION</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMX</td>
<td>1.11</td>
<td>MTZ</td>
</tr>
<tr>
<td>Q-ABSORPTION</td>
<td>228 nm</td>
<td>320 nm</td>
</tr>
<tr>
<td>Method - I</td>
<td>0.85</td>
<td>0.41</td>
</tr>
<tr>
<td>Method - II</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Precision**

Precision was studied to find out intra and inter-day variations in the test method of metronidazole and amoxicillin. Calibration curves prepared in medium were run in triplicate in the same day for three
days. %RSD (relative standard deviation) were calculated which should be less than 2%. The results are tabulated in Table II.

Accuracy

To study the accuracy of the proposed methods, recovery studies were carried out using synthetic mixtures of MTZ and AMX in 1:2 ratios to be used in the controlled periodontal drug delivery device. Results of recovery studies were presented in Table III.

### RESULTS AND DISCUSSION

The overlay spectra of MTZ and AMX exhibit λmax at 320 nm and 273 nm for MTZ and AMX respectively which are quite separate from each other. Additionally an iso-absorptive point was observed at 228 nm. Standard calibration curves for MTZ and AMX were linear with correlation coefficients ($r^2$) values in the range of 0.981-0.9993 at all the selected wavelengths. The method was repeated for the same day and % RSD was found to be <1.1% for MTZ and <2% for AMX, similarly the method was repeated for different days and % RSD was found to be <1.10 for MTZ and <1.151 for AMX. The accuracy of the method was confirmed by recovery studies from synthetic mixtures at three different levels of standard additions.

### CONCLUSION

The proposed methods for simultaneous estimation of metronidazole and amoxicillin in combined dosage forms were found to be simple, accurate, precise, economical and rapid. In both the methods percentage recovery was found to be 100% and % RSD found to be less than 2% for both the drugs. Hence, these methods can be employed for routine analysis in quality control laboratory.

### REFERENCES


