International Journal of Chemistry Research
Vol 1, Issue 1, 2010

Research Article

# DEVELOPMENT OF SIMULTANEOUS SPECTROPHOTOMETRIC METHOD OF AMOXICILLIN TRIHYDRATE AND RANITIDINE BISMUTH CITRATE IN SAME DOSAGES FORM

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***Received: 18 Jun 2010, Revised and Accepted: 28 Jun 2010***

**ABSTRACT**

Amoxicillin trihydrate (AMOX) and Ranitidine bismuth citrate (RBC) are widely used for the treatment of *H.Pylori*. infection. These drugs are aimed for bacterial cure and are recommended for patients with relapsing or complicated duodenal and gastric ulcer. Among the numerous estimation methods for simultaneous estimation viz, derivative spectrophotometery, area calculation, the simultaneous equation method is very simple and does not require complicated mathematical calculations. Being rapid and simple UV spectrophotometery is one of the most extensively used analytical technique for estimation of both the drugs. The absorptivity was found approximately same for all the concentrations and hence both the drugs obeyed Beer's law in 2-20 µg/ ml concentration range. The high values of correlation coefficients ($r^2$) indicated good linearity of calibration curve for both the drugs. Sandell's sensitivity µg/ $cm^2$/0.001/abs unit of RBC and AMOX was found to be sufficient and these shows that very less amount of both drugs can be effectively detected by this method. The recoveries of RBC and AMOX from the standard mixture solution were found to be 100.44% and 99.83% respectively. The recovery results indicated that RBC and AMOX could be quantified by this procedure simultaneously.

**Keywords:** Simultaneous estimation; AMOX and RBC; UV spectrophotometeric method.

## INTRODUCTION

Spectrometry deals with instruments based on the absorption or emission of electromagnetic radiation as a result of its interaction with matter. Absorption spectrometery is the measurement of selected absorption by atoms, molecules or ions of electromagnetic radiation having a definite and narrow wavelength range approximating monochromatic energy[1]. The amount of absorption depends on the wavelength of radiation and the structure of the compound. The absorption of radiation is due to the subtraction of energy from the radition beam when electrons in orbital of lower energy are excited into orbital of higher energy. Since this is in electron transition phenomenon, UV is sometimes called electronic spectroscopy [2]. The technique of UV visible spectrophotometery is one of the most frequently employed in pharmaceutical analysis. It involves the measurement of the amount of ultraviolet (190-380nm) or visible (380-800nm) addition absorbed by a substance in solution instrument which measures the ratio or a function of the ratio of the intensity of two beams of light in UV-visible region[3]. The basis of all spectrophotometric methods for multicomponent sample analysis is the property that all wavelengths (a) The absorbance of a solution is the sum of absorbances of individual components or (b) The measured absorbance is the difference between total absorbance of the solution in the sample cell and that of the solution in the reference (blank) cell. The various spectrophotometric methods which are used for estimation of drug in combine dosage form include simultaneous equation method, absorbance ratio method, geometric correction method, orthogonal polynomial method, difference spectrophotometery, derivative spectrophotometery, and absorption correction method, multicomponent method of analysis and two wavelength quantation method.

### Simultaneous equation method or (Vierodt's method)

If a sample contains two absorbing drugs (X and Y) each of which absorbs at the λmax of the other. It may be possible to determine both drugs by the technique of simultaneous equations (Vierodt's method) provided that certain criteria apply. The information required is the aborptivities of X at and $\lambda_1$ and $\lambda_2$ $ax_1$ and $ax_2$ respectively (a) The aborptivities of Y at and $\lambda_1$ and $\lambda_2$ $ay_1$ and $ay_2$ respectively (b) The absorbances of the diluted sample at $\lambda_1$ and $\lambda_2$, $A_1$ and $A_2$ respectively. Let Cx and Cy be the concentrations of X and Y respectively in the diluted sample. Two equations are constructed based upon the fact that at $\lambda_1$ and $\lambda_2$ the absorbance of the mixture is the sum of the individual absorbance of X and Y.

At $\lambda_1$ $A_1 = a X_1 b Cx + aY_1 b Cy$ ---------- (1)

At $\lambda_2$ $A_2 = a X_2 b Cx + aY_2 b Cy$---------- (2)

For measurements in 1 cm cells b=1

Rearrange eq. (2)

$Cy = A_2 - a X_2 b Cx / aY_2$

Substituting for Cy in eq. (1) and rearranging

$Cx = A_2 aY_1 - A_1 aY_2 / a X_2 aY_1 . a X_1 aY_2$ ----------(3)

$Cy = A_1 a X_2 - A_2 a X_1 / a X_2 aY_1 . a X_1 aY_2$---------- (4)

As an exercise you should drive modified equation containing a symbol (b) for path length for application in situations where $A_1$ and $A_2$ are measured in cells other than 1 cm path length. Criteria for obtaining maximum precision based upon absorbance ratios have been suggested that place limits on the relative concentration of the components of the mixture[4]. The criteria are that the ratios.

$A_2 / A_1 / aX_2 / a X_1$ and $aY_2 / aY_1 / A_2 / A_1$ should lie out side the range 0.1-2.0 for the precise determination of Y and X respectively.These criteria are satisfied only when the $\lambda_{max}$ of two component are reasonably dissimilar an additional criterion is that the two components don't interact chemically thereby negating the initial assumption that the total absorbance is the sum of individual absorbances. The additive of the absorbance should always be confirmed in the development of a new application of this techniques[5-6].

## MATERIALS

The Ranitidine bismuth citrate (RBC) and Amoxicillin trihydrate (AMOX) were generously supplied as a gift sample by M/s Glaxo Smith Kline, Bombay (India) and Torrent Pharmaceuticals, Ahmedabed India respectively. All other chemicals and reagents used were of analytical grade.

### Apparatus and conditions

A double beam Shimadzu UV/Visible 1601 spectrophotometer with data processing capacity was used. Absorption and overlain spectra of both test and standard solutions were recorded over the wavelength range of 200-400nm using 1cm quartz cell at a scaned speed of 100 nm /min and fixed slit width of 3nm.

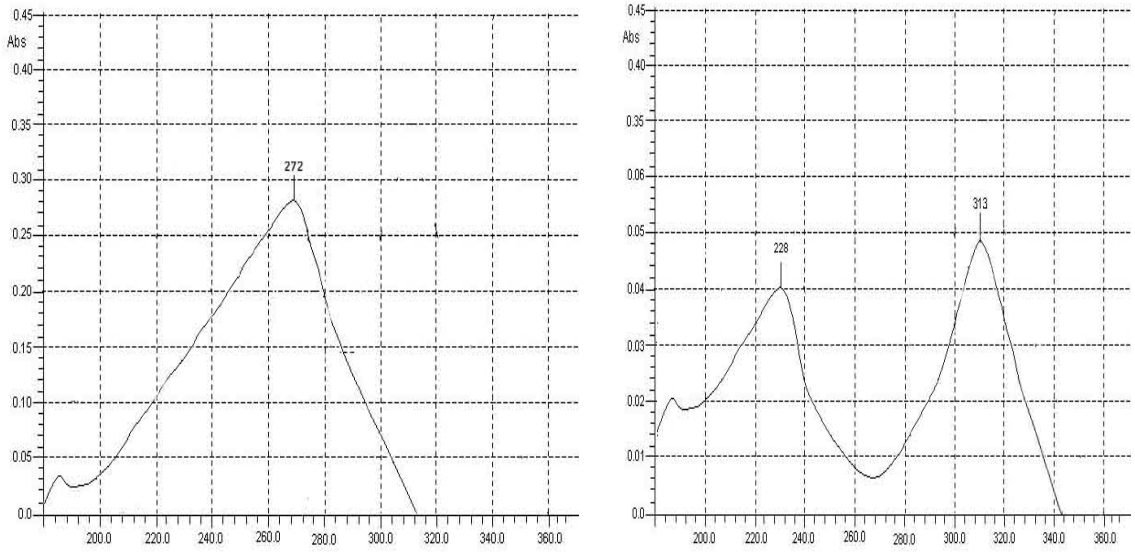
## EXPERIMENTAL

### Standard solutions

A stock solution was prepared by accurately weighing 10mg each of RBC and AMOX into 10ml of acetate buffer (pH 5) and diluted separately to 100 ml with same to obtain a final concentration of 10 µg/ ml. The solutions were further diluted with acetate buffer (pH 5) to reach the concentration range of 2-20 µg/ ml of each drug. Three mixed standard solutions with concentration of 6.0, 8.0, 10.0 µg/ml of AMOX and RBC were prepared in acetate buffer (pH 5) by proper dilution of pre-analyzed stock solutions of both drugs. The solutions were found to be stable for at least 3 days when stored in refrigerator.

### Spectrophotometric measurement

The standard stock solutions of AMOX and RBC at the concentration of 100 µg/ ml were diluted separately to produce solutions at concentration of 10 µg/ ml of each drug. The UV spectra of both drugs were recorded separately against blank. An overlain spectra (Fig 4.1) was recorded and absorption maxima for AMOX and RBC were detected at 272.0 and 313 nm respectively. The calibration curve was recorded for both AMOX and RBC independently at their corresponding wavelengths. The concentration of AMOX and RBC in standard mixture solution was determined by both λmax 272 and 313nm (Table 1 and Table 2).




**AMOX (λmax272)** **RBC (λmax313)**

**Fig. 1: UV absorption maxima of AMOX and RBC in acetate buffer (pH 5)**

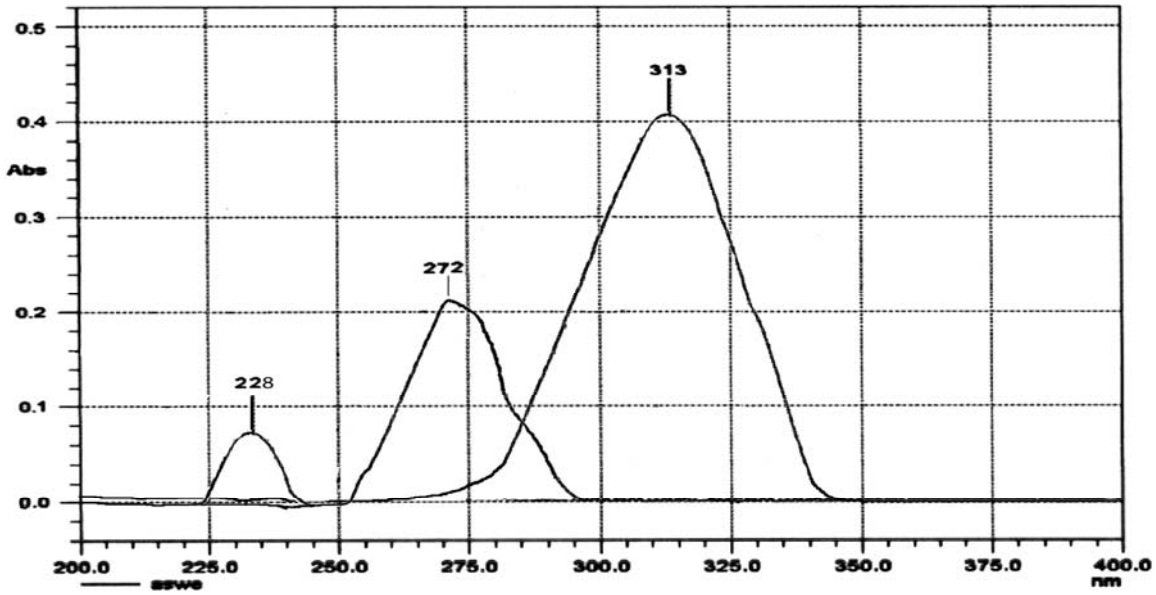



**Fig. 2: Overlain spectra of AMOX and RBC in acetate buffer (pH 5)**

**Table 1: Absorbance and corresponding absorptivity of different RBC at λmax 313 nm and λmax 272 nm**

| S.No. | Concentration (µg/ ml) | Absorbance at (λmax) | | Absorptivity | |
|---|---|---|---|---|---|
| | | 313 | 272 | 313 | 272 |
| 1 | 2 | 0.1030 | 0.0439 | 0.0515 | 0.0219 |
| 2 | 4 | 0.2039 | 0.0924 | 0.0509 | 0.0231 |
| 3 | 6 | 0.2980 | 0.1335 | 0.0496 | 0.0225 |
| 4 | 8 | 0.3905 | 0.1735 | 0.0488 | 0.0216 |
| 5 | 10 | 0.4940 | 0.2261 | 0.0494 | 0.0226 |
| 6 | 12 | 0.5883 | 0.2701 | 0.0490 | 0.0225 |
| 7 | 14 | 0.6931 | 0.3232 | 0.0495 | 0.0230 |
| 8 | 16 | 0.7816 | 0.3636 | 0.0488 | 0.0227 |
| 9 | 18 | 0.8681 | 0.4137 | 0.0482 | 0.0229 |
| 10 | 20 | 0.9661 | 0.4618 | 0.0483 | 0.0230 |

**Table 2: Absorbance and corresponding absorptivity of different AMOX at λmax 272 nm and λmax 313 nm**

| S.No. | Concentration (µg/ ml) | Absorbance At (λmax) | | Absorptivity | |
|---|---|---|---|---|---|
| | | 272 | 313 | 272 | 313 |
| 1 | 2 | 0.0691 | 0.0045 | 0.03095 | 0.00225 |
| 2 | 4 | 0.1098 | 0.0060 | 0.02745 | 0.00150 |
| 3 | 6 | 0.1817 | 0.0117 | 0.03028 | 0.00190 |
| 4 | 8 | 0.2225 | 0.0175 | 0.02780 | 0.00218 |
| 5 | 10 | 0.2914 | 0.0225 | 0.02910 | 0.00225 |
| 6 | 12 | 0.3390 | 0.0241 | 0.02820 | 0.00200 |
| 7 | 14 | 0.3979 | 0.0325 | 0.02840 | 0.00232 |
| 8 | 16 | 0.4505 | 0.0335 | 0.02810 | 0.00209 |
| 9 | 18 | 0.5086 | 0.0400 | 0.02820 | 0.00222 |
| 10 | 20 | 0.5623 | 0.0428 | 0.02811 | 0.00214 |

**Table 3: Absorptivity of RBC and AMOX at two λmax**

| Drugs | Λmax (nm) | Absorptivity (Mean) | SD* |
|---|---|---|---|
| RBC | 313 | 0.04942 | $1.14 \times 10^{-3}$ |
| | 272 | 0.02260 | $4.74 \times 10^{-4}$ |
| AMOX | 313 | 0.002085 | $2.14 \times 10^{-4}$ |
| | 272 | 0.02868 | $1.12 \times 10^{-3}$ |

SD*: Standard deviation of absorptivity

**Determination of analytical parameters**

**Linearity**

Linearity was evaluated by preparing different concentrations in the range of 2-20 µg/ ml for both the drugs and absorbance was measured. Each measurement was carried out in triplicate. The relation ship between the concentration of RBC and AMOX and the variables were measured. The absorption was adjusted by regression and the absorbity coefficients were calculated by using Beer's law.

**Table 4: Statistical data of the calibration curve for the determination of RBC and AMOX by UV-spectrophotometric measurement**

| Drugs | λmax (nm) | Linearity Range (µg/ ml) | Regression equation (Y=mx+c) | $r^2$ | Sa | Sb | SS (0.001ABS Unit/mole) |
|---|---|---|---|---|---|---|---|
| RBC | 313 | 2-20 | Y=0.048x + 0.0107 | 0.9997 | 0.005794 | 0.003647 | 0.020240 |
| AMOX | 272 | 2-20 | Y=0.0279x + 0.0055 | 0.9991 | 0.005338 | 0.003958 | 0.034572 |

**$r^2$ -** Regression correlation coefficient, **Sa-** Standard error of slop, **Sb -**Standard error of intercept, **SS** -Sandell's sensitivity

By applying the cramr's rule and matrices to the above equation concentration of both the sample $C_R$ and $C_A$ may be effectively determined by the following equations.

A set of simultaneous equation were framed as shown below

$A_1 = 0.04942\ C_R + 0.002085\ C_A$ (1)

$A_2 = 0.02260\ C_R + 0.02868\ C_A$ (2)

In these equations $A_1$ and $A_2$ are the absorbance at 313 nm and 272 nm respectively. $C_O$ and $C_A$ are corresponding concentrations of RBC and AMOX in µg/ ml. By applying cramr's rule to equations $C_O$ and $C_A$ were calculated equations are:

$C_R = 0.02868\ A_1 - 0.002085\ A_2/0.0013702$ (3)

$C_A = 0.04942\ A_1 - 0.02260\ A_2/0.0013702$ (4)

**Sensitivity**

Sensitivity of the methods for drugs individually was determined by calculating Sandell's sensitivity **(**µg/ $cm^2$ /0.001/ABS unit) which can be defined as the smallest weight of the substance that can be detected in column of the solution of unit cross section. The weight of the sample can be conveniently expressed in µg and area in $cm^2$ .if the molar extinction coefficient of substance is ε and molecular weight is M then Sandell's sensitivity is given by

$SS = M/\varepsilon$

**Table 5: Sandell's sensitivity of AMOX at 272nm**

| S.No. | Concentration (µg/ ml) | Molar extinction coefficient 0.001abs unit /molecm/dm² | Sandell's sensitivity µg/ cm² /0.001/abs unit |
|---|---|---|---|
| 1 | 2 | $1.44930 \times 10^4$ | 0.0289435 |
| 2 | 4 | $1.15152 \times 10^4$ | 0.0364301 |
| 3 | 6 | $1.27030 \times 10^4$ | 0.0330236 |
| 4 | 8 | $1.16673 \times 10^4$ | 0.0359550 |
| 5 | 10 | $1.22242 \times 10^4$ | 0.0343170 |
| 6 | 12 | $1.18508 \times 10^4$ | 0.0353982 |
| 7 | 14 | $1.19220 \times 10^4$ | 0.0351847 |
| 8 | 16 | $1.1811 \times 10^4$ | 0.0355160 |
| 9 | 18 | $1.85320 \times 10^4$ | 0.0353912 |
| 10 | 20 | $1.17942 \times 10^4$ | 0.0355682 |

**Table 6: Sandell's sensitivity of RBC at 313.0 nm**

| S.No. | Concentration (µg/ ml) | Molar extinction coefficient 0.001abs unit /molecm/dm² | Sandell's sensitivity µg/ cm² /0.001/abs unit |
|---|---|---|---|
| 1 | 2 | $1.778810 \times 10^4$ | 0.0194174 |
| 2 | 4 | $1.760676 \times 10^4$ | 0.0196174 |
| 3 | 6 | $1.715486 \times 10^4$ | 0.0201342 |
| 4 | 8 | $1.685983 \times 10^4$ | 0.0204865 |
| 5 | 10 | $1.706276 \times 10^4$ | 0.0202429 |
| 6 | 12 | $1.693323 \times 10^4$ | 0.0203977 |
| 7 | 14 | $1.709976 \times 10^4$ | 0.0201991 |
| 8 | 16 | $1.687279 \times 10^4$ | 0.0204708 |
| 9 | 18 | $1.665787 \times 10^4$ | 0.0207349 |
| 10 | 20 | $1.668454 \times 10^4$ | 0.0207017 |

**Recovery**

The accuracy of the methods was assessed by taking the known amounts of RBC and AMOX in standard mixture solutions and absorption was determined at λmax of both the drugs. Concentration of both the drugs in the mixture solution was determined by simultaneous equation method. According to this method if sample contain two absorbing drugs X and Y and each of which has absorption maxima at ($\lambda_1 \neq \lambda_2$ ), it may be possible to determine both drugs by simultaneous equation. The following criteria may be applied

The total absorbance of a solution at a given wavelength is sum of absorbance of individual component at the given wavelength.

$A_1 = A^1 X + A^1 Y = a^1 \times b. c \times a^1 y\ b. cy$

$A_2 = A^2 X + A^2 Y = a^1 \times b. c \times a^1 y\ b. cy$

**Table 7: Recovery data of RBC and AMOX in standard mixture solution**

| Drug in standard mixture solution | Concentration (µg/ ml) | Recovery | $C_0V\%$ |
|---|---|---|---|
| RBC | 6.0 | 100.90±0.329 | 0.327 |
| | 12.0 | 100.00±0.378 | 0.376 |
| | 18.0 | 100.44±0.551 | 0.552 |
| AMOX | 6.0 | 99.83±0.434 | 0.434 |
| | 12.0 | 99.25±0.535 | 0.534 |
| | 18.0 | 100.90±0.611 | 0.615 |

* Results are mean of 5 separate measurements, $C_0V\%$ Coefficient of variance/Relative standard deviation.

**RESULTS AND DISCUSSION**

A study of overlain spectra of RBC and AMOX in acetate buffer (pH 5) shows that at 313nm RBC shows maximum absorbance whereas AMOX shows almost zero absorbance. This indicates that there is considerable difference in absorbance peaks of both the drugs and hence there is no interference.

**Linearity**

Table 4 shows the statistical analysis of the experimental data the regression equation from the calibration graphs along with standard error of the slopes and intercepts and regression correlation coefficient for the ultraviolet spectroscopic method.

The absorptivity were found approximately same for all the concentrations hence both drugs obeyed Beer's law in indicated concentration range. The high values of correlation coefficients ($r^2$) also indicate good linearity of calibration curve for both the drugs.

**Sensitivity**

Sandell's sensitivity of RBC and AMOX was found to be sufficient low. Table 5 and 6 which shows that very less amount of both drugs can be effectively detected by this method.

**Recovery**

The recoveries of RBC and AMOX from the standard mixture solution were found to be 100.44% and 99.83% respectively. The recovery results indicated that RBC and AMOX could be quantified by this procedure simultaneously

**CONCLUSION**

The proposed simultaneous equation method is found to be very simple that can be performed by use of any spectrophotometer and dose not requires any costly instrument equipped with special package. It also shows good linearity values and sensitivity. The results demonstrated that simultaneous equation method by

Shimadzu UV/Visible 1601 Spectrophotometer could be useful for technique for determination of RBC and AMOX when they are given in same dosage form.

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