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A UV Spectrophotometric method developed for the simultaneous estimation of Amoxicillin trihydrate and Ranitidine bismuth citrate for *Helicobacter Pylori* infections

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ABSTRACT

The development of Vireodt's method for simultaneous estimation of Amoxicillin trihydrate (AMOX) and Ranitidine Bismuth Citrate (RBC) involves absorbance measurement at 314 nm and 228 nm corresponding to the respective absorption maxima. Both the drugs obey Beer Lambert's law in the range of 5-50 µg/ml. The method developed was validated to determine its accuracy, precision, specificity and ruggedness. The recovery study was carried out by standard addition method. The average percent recovery was found to be 100.36 and 99.6 for Amoxicillin trihydrate and Ranitidine Bismuth Citrate respectively.

Key words: Simultaneous estimation, ranitidine bismuth citrate, amoxicillin trihydrate, percentage recovery.

INTRODUCTION

The significance of the documented association between duodenal ulcers and chronic-active antral inflammation (Schager et al., 1967) was not apparent until Marshall and Warren identified and cultured *Campylobacter* like organisms in the gastric epithelium of patients with gastritis (Marshall and Warren, 1984). Further evidence demonstrated that these bacteria, currently referred to as *Helicobacter pylori (H. pylori)* (Gold et al., 1992) are involved in the pathogenesis of antral gastritis and duodenal ulcers (Blaser, 1990; Goodwin et al., 1989). *H. pylori* appears to be responsible for 95% of gastritis and 65% of gastric ulcers.

The bacterium plays an important role in the pathogenesis of chronic gastritis, peptic ulcer diseases, gastric carcinomas and gastric marginal zone B Cell lymphomas of mucosa-associated lymphoid tissue type (Lind et al., 1996; Pilotto et al., 2000; Maeda et al., 2000). The National Institute of Health Consensus Development Conference has recommended the addition of antimicrobial

agents to antisecretory drugs for the treatment of patients with *H. pylori* associated peptic ulcer diseases (Marais et al., 1999).

Since single therapy is generally inadequate, use of combination therapy of bismuth with other antibiotics is recommended. Dual therapy regimen with antimicrobials or with combination of antimicrobial and antisecretory agents were also attempted (Goodwin et al., 1988).

Double liposomes have the ability to entrap more than one drug of different physicochemical nature in the same system with high loading capacity, ability to offer prolonged release profile, so as to increase half-life of drug, reduce dosing frequency and side effects. For characterization and performance evaluation of double liposome entrapping Amoxicillin trihydrate (AMOX) and Ranitidine Bismuth Citrate (RBC) simultaneous estimation of two drugs was necessary.

RBC, a N-[2-(5-dimethyl amino methyl furan-2 yl methyl-sulfanyl)-ethyl]-N-methyl-2 nitro-1,1 ethane diamine bismuth citrate, is a novel salt of ranitidine with bismuth and citrate. This salt possesses the antisecretory activity of ranitidine, mucosal protective activity and anti *H. pylori* activity of bismuth salts. RBC is a white to off-white amorphous powder. 400mg of RBC is equivalent to ranitidine base 162mg, trivalent bismuth 128mg and citrate 110mg. Highly soluble in water (837g/l at pH 7) and other aqueous solvents and practically in organic solvents, alcohols and fixed oils. The solubility of RBC between pH 4.3 and 3.9 is 100%. A 10% solution in water has a pH of 4.6.

AMOX, (6R)-6-(α-D-4-hydroxy phenyl glycyl amino) penicillianic acid, is a broad spectrum penicillin antibiotic. AMOX is white or almost white crystalline powder. 1.15g of amoxicillin trihydrate are approximately equivalent to 1g amoxycilin. Slightly soluble in water, in methanol and in alcohol. Practically insoluble in tetrachloro methane, chloroform, ether, fixed oils. It dissolves in dilute solutions of acids and alkali hydroxides. A 0.2% solution in water has a pH of 3.5 to 6.0.

Among the various methods available for analysis of amoxicillin trihydrate, microbiological, UV spectrophotometric and fluorimetric methods are superior. Brooks et al. (1981) reported a method of determination of amoxicillin by high performance liquid chromatography with amperometric detection. Good linearity (r=0.9993) was exhibited over the cited range by the amperometric detector. Rao et al. (1982) discussed the colorimetric method for estimation of amoxicillin and the dosage form; measurement was done at 480 nm. Bundgard (1983) reported a new spectrophotometric method for selective determination of amoxicillin in the presence of polymers and other degradation products. The measurement was done at λmax 322 nm. Sane et al. (1983) and Jonkman and Schoenmakes, (1985) have described the determination of amoxicillin in plasma by ion-pair high performance liquid chromatography. Mori et al (1985) reported a fluorimetric method for the determination of amoxicillin with mercurochrome. Fluorimetric determination was made at 535nm.

Analytical methods which have been reported for determination of AMOX include high performance liquid chromatography (HPLC) (Miyazaki et al., 1983; Lebelle et al., 1980), potentiometric method (Mioscu et al., 1988), ultra violet spectrophotometric method (Florey et al., 1994), ion pair performance liquid detecting chromatography (Jonkman and Schoenmakes, 1985), and sequential injection analysis (SQA) (Posamontes and Callao, 2003). Surprisingly no method has been reported for the estimation of RBC in the world literature. UV spectrophotometric method has been developed in our Pharmaceutical Research Laboratory by

scanning RBC (50µg/ml) between 200-400nm and λ_{max} was found to be 314nm, at which further determination was done (Dangi et al., 2004).

A literature survey revealed that no method for simultaneous estimation of AMOX and RBC has been reported elsewhere. The present work is the first report, which illustrates a simple, accurate, economical and reproducible procedure for simultaneous spectrophotometric estimation of both the drugs.

MATERIALS AND METHODS

A Shimadzu 1601 UV/visible spectrophotometer was used for experiments with 10 mm matched quartz cells. Stock solution (100µg/ml) of AMOX and RBC were prepared in acetate buffer (pH 5.0). Working solutions were prepared by appropriate dilutions of stock solution in acetate buffer.

Procedure

For spectroscopic measurement, stock solutions of both the drugs were prepared by accurately weighing 10mg each of AMOX and RBC into 10ml of acetate buffer and diluted separately to 100ml with same to obtain final concentrations of 100µg/ml. The solutions were further diluted to give concentration range of 5-50µg/ml of each drug. Overlain spectra of both the drug solutions were scanned (Fig. 1) and it was observed that AMOX and RBC showed maximum absorbance at 228nm and 314nm, respectively. It was observed that RBC and AMOX showed almost zero absorbance at 228 and 314nm, respectively. Thus, these two wave lengths were employed for the estimation of AMOX and RBC without any interference.

The calibration curves of each drug were plotted at both the λ_{max} i.e. at 228 and 314nm, using concentration range of 5-50µg/ml. Absorptivity of both the drugs were calculated, which showed good linearity.

Table 1. Absorptivity values for AMOX and RBC

Concentration (µg/ml)		Absorptivity at 228nm		Absorptivity at 314nm	
AMOX	RBC	AMOXλ_2E_2	RBCλ_2E_1	AMOXλ_1E_2	RBCλ_1E_1
10	10	0.021	0.0321	0.00048	0.0276
20	20	0.0197	0.0320	0.00047	0.0245
30	30	0.0200	0.0311	0.00048	0.0235
40	40	0.0196	0.0303	0.00048	0.0228
50	50	0.0194	0.0293	0.00048	0.0216
Mean		0.01994	0.03096	0.000478	0.0240
SD		6.30×10^{-4}	1.18×10^{-3}	4.47×10^{-6}	2.27×10^{-3}

SD = Standard Deviation

Table 2. Optical characteristics and regression equation

S. No.	Parameters	Values	
		AMOX	RBC
1	λ_{max} (nm)	228	313.38
2	Beer's law limit (µg/ml)	5 – 50	5 – 50
3	Molar absorptivity	8.40080×10^3	1.3462×10^4
4	Sandell's sensitivity (µg/cm^2/0.001 abs)	0.05009	0.041884
5	Regression equation - Slope - Intercept - Correlation coefficient	 0.01912 0.0203 0.9961	 0.021 0.0579 0.9969

Three mixed standard solutions with concentration 10,20,30µg/ml of AMOX and 15, 25, 35µg/ml of RBC were prepared in acetate buffer. All these standard mixtures were then scanned at 228 and 313.38 nm λ_{max} and absorbance were recorded. The spectral data from these scans were used to determine the concentration of two drugs in sample solutions. The absorptivities for the two drugs are recorded in Table 1. The optical characteristics and regression equation for the calibration curve are presented in Table 2.

Recoveries of the standard mixture solution were analysed by employing simultaneous equations using Cramer's rule and matrices. A set of two simultaneous equations was framed using the absorptivity coefficient values as given below:

$$A_1 = 0.0240\ C_1 + 0.000478\ C_2 \quad \text{(I)}$$
$$A_2 = 0.03096\ C_1 + 0.01994\ C_2 \quad \text{(II)}$$

Where C_1 and C_2 are the concentrations of RBC and AMOX, respectively in µg/ml. A_1 and A_2 are the absorbances of sample solutions measured at 313.38nm and 228nm, respectively, and 0.0240 and 0.000478 are absorptivities at 313.38nm and 0.03096 and 0.01994 are the absorptivities at 228nm of RBC and AMOX, respectively.

By applying Cramer's rule and matrices to equation I and II, concentrations C_1 and C_2 can be calculated by the following equations:

$$C_1 = \frac{A_2\ 0.01994 - A_1\ 0.000478}{0.0004637} \quad \text{(III)}$$

$$C_2 = \frac{A_2\ 0.0240 - A_1\ 0.03096}{0.0004637} \quad \text{(IV)}$$

Table 3. Recovery Studies

Drug in standard mixture solution	Concentration (µg/ml)	Recovery % ± SD (µg/ml)	Coefficient of variance %
AMOX	10	100.6 ± 0.169	0.167
	20	100.8 ± 0.311	0.308
	30	99.68 ± 0.480	0.481
RBC	15	99.2 ± 0.282	0.284
	25	99.0 ± 0.424	0.311
	35	100.6 ± 0.707	0.702

Recovery Studies

To study the accuracy, reproducibility and precision of the proposed method, recovery studies were carried out by taking standard mixture solution of both AMOX and RBC and absorbance was determined at 228 and 313.38nm, respectively. Results of recovery studies were found to be satisfactory and are presented in Table 3.

RESULTS AND DISCUSSION

The proposed method for simultaneous estimation of AMOX and RBC in same dosage form was found to be simple, accurate, economical and rapid. The correlation coefficients of two drugs

were satisfactorily low and recovery of AMOX and RBC was found to be 100.36% and 99.6%, respectively.

The method employs solving of simultaneous equations. Once the equations are determined, analysis required only the measuring of the sample solution at the two wave lengths selected, followed by a few simple calculations.

The proposed method is fast, reliable, accurate and precise, for the determination of AMOX and RBC when they are given in a unit dosage form. Hence, it can be employed for simultaneous estimation of both the drugs in single dosage form.

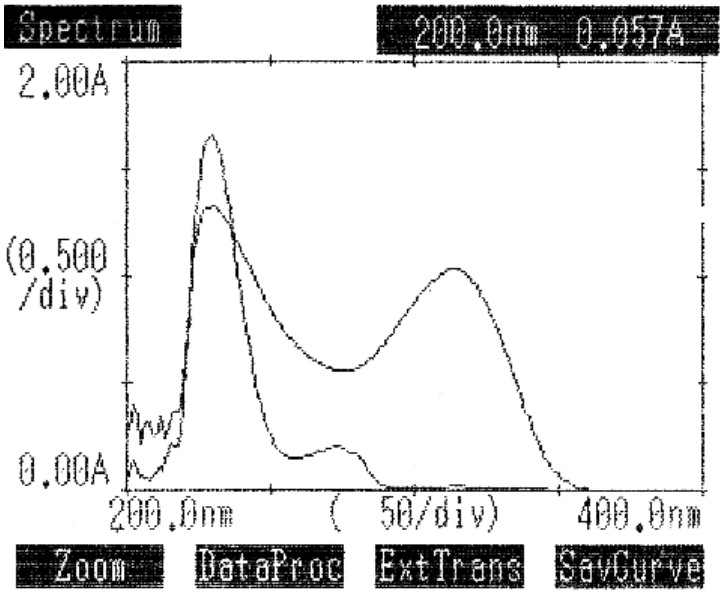

Figure. 1. Overlain spectra of AMOX and RBC

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