Simultaneous Estimation of Amoxycillin Trihydrate and Rabeprazole Sodium

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A novel, simple, sensitive, rapid and spectrophotometric method has been developed for simultaneous estimation of amoxycillin trihydrate and rabeprazole sodium. The method involves solving of simultaneous equations based on measurement of absorbances at two wavelengths 247 nm and 292 nm. Both the drugs obey Beer's law in the concentration ranges employed for this method. Results of the method were validated statistically and by recovery studies.

Amoxycillin-trihydrate (AMOX), 6(R)-6-(α-D-(4-hydroxyphenyl)glycyl amino), is a beta lactam antibiotic. Rabeprazole sodium (RAB), 2-[[4-(3-methoxy propoxy)3-methyl-2-pyridinyl]-methyl sulfinyl]-1H-benzimidazole sodium is a newly developed proton pump inhibitor[1] that suppresses gastric acid secretion by specific inhibition of the gastric H^+/K^+ ATPase enzyme system. AMOX and RAB are known to have a synergistic therapeutic effect in peptic ulcer, caused by *Helicobacter pylori (H. pylori)*[2]. *H. pylori* has been implicated in the etiology of chronic gastritis and chronic peptic ulceration with increased risk of gastric adenocarcinoma[3]. Improved eradication rates, clinical efficacy and suppression of resistance have been reported when the drug regimens adopted and advocated as a dual therapy regimen with combination of antimicrobial and antisecretary agents[4]. In our laboratory we have developed a novel dual drug delivery system containing AMOX and RAB in a single dosage form for the treatment of *H. pylori* infection.

Literature survey reveals HPLC and HPTLC methods for analysis of RAB in presence of its degradation products[5]. Other official methods for quantitative estimation of AMOX include spectrophotometric methods[6-8], iodometry[9], potentiometry[10], fluorimetry[11], polarography[12], sequential injection analysis (SIA)[13], isotachophoresis (IT)[14] and HPLC[15, 16] in dosage form.

Although AMOX and RAB are commonly used in dual drug therapy for the treatment of *H. pylori*, yet no method is so far reported for their simultaneous estimation. A successful attempt has been made to estimate two drugs simultaneously by spectrophotometric analysis.

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A Shimadzu UV/Vis spectrophotometer model 1601 (Japan) was employed with spectral bandwidth of 3 nm and wavelength accuracy of ±0.5 nm with automatic wavelength correction with a pair of 10 mm quartz cells. AMOX (Alkem Laboratories, Mumbai), RAB (Dr. Reddy's Laboratories, Hyderabad), sodium hydroxide (CDH, Mumbai) and distilled water were used in the present study.

Stock solutions (100 μg/ml) of AMOX and RAB were prepared by dissolving separately 10 mg of drug in 0.1 N NaOH. The maximum absorbance of AMOX and RAB was obtained at 247 nm (λ_1) and 292 nm (λ_2), respectively. AMOX and RAB showed linearity with absorbances in the range 0-50 μg/ml and 0-20 μg/ml at their respective maxima, which were validated by least square method. Coefficients of correlation were found to be 0.9998 for AMOX and 0.9999 for RAB. For simultaneous estimation of AMOX and RAB, a series of standard solutions in concentration range of 2 to 20 μg/ml, were prepared by diluting appropriate volumes of the standard stock solutions. The scanning solutions of AMOX and RAB was carried out in the range of 200 to 400 nm against sodium hydroxide solution as blank for obtaining the overlain spectra that are used in the analysis (fig. 1). Absorbances and absorptivities of series of standard solutions were recorded at selected wavelengths λ_1 and λ_2.

The observations are presented in Table 1. The optical characteristics and regression values for the calibration curve are presented in Table 2. The method employed

TABLE 1: ABSORPTIVITY VALUES FOR AMOXYCILLIN-TRIHYDRATE AND RABEPRAZOLE-SODIUM

Concentration (μg/ml)		Absorptivity			
		247 nm		292 nm	
AMOX	RAB	AMOX	RAB	AMOX	RAB
2	2	0.0339	0.018	0.0072	0.046
4	4	0.0334	0.016	0.0069	0.045
6	6	0.0335	0.016	0.0068	0.044
8	8	0.0337	0.016	0.0068	0.045
10	10	0.0337	0.016	0.0068	0.044
12	12	0.0330	0.015	0.0068	0.044
14	14	0.0336	0.015	0.0068	0.044
16	16	0.0333	0.015	0.0069	0.044
18	18	0.0321	0.015	0.0068	0.044
20	20	0.0330	0.015	0.0068	0.044
Mean		0.0333	0.0157	0.00686	0.0444
SD		5.2×10^{-4}	9.4×10^{-4}	1.2×10^{-4}	6.99×10^{-4}

SD stands for standard deviation, AMOX is Amoxycillin-trihydrate, RAB = Rabeprazole-sodium

simultaneous equations using Cramer's rule and matrices ($C_1=\lambda_2\varepsilon_2.A\lambda_1-\lambda_1\varepsilon_2.A\lambda_2/\lambda_1\varepsilon_1.\lambda_2\varepsilon_2-\lambda_1\varepsilon_2.\lambda_2\varepsilon_1$ and $C_2=\lambda_1\varepsilon_1.A\lambda_2-\lambda_2\varepsilon_1.A\lambda_1/\lambda_1\varepsilon_1.\lambda_2\varepsilon_2-\lambda_1\varepsilon_2.\lambda_2\varepsilon_1$). A set of two simultaneous equations was framed using the mean of absorptivity values, as given below: $A\lambda_1=0.0333\ C_1+0.0157C_2$(i) and $A\lambda_2=0.00686C_1+0.0444C_2$ (ii), where C_1 and C_2 are the concentrations of AMOX and RAB, respectively in the sample solution (μg/ml). $A\lambda_1$ and $A\lambda_2$ are the absorbances of the sample solution measured at 247 nm and 292 nm, respectively.

By applying the Cramer's rule and matrices to Equation (i) and (ii), concentrations of C_1 and C_2 can be obtained as: $C_1=A_1x0.0444-A_2x0.0157/0.0013708$ (iii) and $C_2=A_2x0.0333-A_1x\ 0.00686/0.0013708$ (iv).

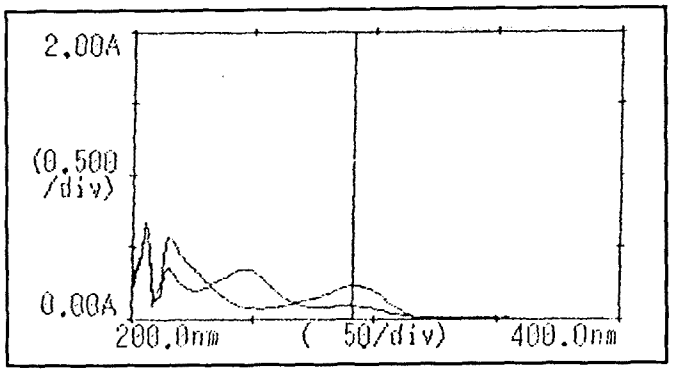

Fig. 1:Overlain spectra of AMOX and RAB

Overlain spectra of amoxycillin trihydrate (AMOX) and rabeprazole sodium (RAB) in 0.1N NaOH

Accuracy of the analysis was determined by performing recovery studies of AMOX and RAB by the proposed method by using different combinations of standard drug

TABLE 2: REGRESSION AND OTPICAL CHARACTERISTICS OF RABEPRAZOLE-SODIUM AND AMOXYCILLIN-TRIHYDRATE

Parameters	RAB	AMOX
λ_{max} (in 0.1 N NaOH)	292 nm	247 nm
Beer's Law range	1–20 μg/ml	1–50 μg/ml
Molar absorptivity (0.001 absorbance unit/mole. cm/dm^3)	17.935×10^3	13.894×10^3
Sandell's sensitivity (μg/cm^2/0.001 absorbance unit)	0.0214	0.0303
Regression values :		
i. Slope	0.428	0.0298
ii. Intercept	0.0281	0.0611
iii. Regression coefficient (r)	0.999	0.999

TABLE 3: RECOVERY STUDIES

Drug in standard mixture solution (μg/ml)		% Recovery ± SD		Coefficient of variance %	
AMOX	RAB	AMOX	RAB	AMOX	RAB
6	4	99.68±0.368	100.5±0.182	0.369	0.180
10	2	99.80±0.169	99.50±0.278	0.167	0.279
8	6	99.72±0.480	100.6±0.424	0.481	0.420

SD stands for standard deviation, the results are mean of three readings (n=3)

solutions of both the drugs. Results of recovery studies were indicating that the method is accurate, reproducible and are presented in Table 3.

The proposed method for simultaneous estimation of AMOX and RAB in combined sample solutions was found to be simple, accurate and reproducible. Once the equations are determined, analysis requires only the measuring of the absorbances of the sample solution at the two wavelengths selected, followed by a few simple calculations. It is a new and novel method and can be employed for routine analysis in quality control R and D laboratories.

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