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Pharmaceutical Analysis Using N-Bromo Succinamide and Rhodamine-B dye Couple: A Spectrophotometric Study.

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Abstract : Simple, sensitive selective and Precise methods are developed for the UV-Visible Spectrophotometric methods have been developed for the estimation of five drugs *VIZ*., Pantoprazole (PNZ), Doxycycline Hyclate (DOX),Lansoprazole (LPZ), Omeprazole (OME) Carvedilol (CRV).The method involves the addition of excess NBS of known concentration in the prescence of 1*M* HCl, reactants are allowed to react and the unreacted NBS is estimated by the measurment in the decrease in the absorbance of the Rhodamine-B dye (λ_{max}). This method has been applied for the estimation of drugs in their pure form as well as in tablet formulation.The results of analysis have been validated statistically for linearity, accuracy, precision, LOD and LOQ.

KeyWords: UV-Visible Spectrophotometry, Pantoprazole sodium,doxycycline hyclate, Lansoprazole, Omeprazole, Carvedilol, HCl, NBS, Rhodamine-B, Quantification, Validation.

Introduction

Pantoprazole (PNZ),

Pantoprazole is widely used for the treatment of gastric ulcers1, 2 through inhibition of H+, K+,-ATP-ase in gastric parietal cells. This drug is a proton pump inhibitor, used as an anti-ulcerative agent by inhibiting the gastric acid secretion. It is immensely used for the cure of erosion and ulceration of esophagus caused by a gastroesophagal reflux diseas. It is chemically known as sodium 5- (difluoromethoxy) - 2 – [[(3, 4-dimethoxy-2-pyridinyl) methyl] sulfinyl]-1H-benzimidazole sesquihydrate (Fig. 1). Several methods have been reported for the determination of in pharmaceutical formulations and in biological materials including QbD method[1,] UV-Spectrophotometry[2,4], RP-UPLC[3], Photmetric method[5].

Doxycycline Hyclate (DOX)

Doxycycline hyclate (DOX) molecular mass 512.94 g/mol. The systematic IUPAC name is Hydrochloride hemiethanol hemihydrate of (4S,4aR,5S,5aR,6R,12aS)-4- (dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11, 12a-octahydrotetracene-2-carboxamide. DOX is relatively more soluble than doxycycline monohydrate, which is the main reason for its more frequent use in pharmaceuticals. Doxycycline is preferred to other tetracyclines in the treatment of specific infections because of its fairly reliable absorption and its long half-life, which permits less frequent dosage. It is frequently used to treat chronic prostatitis, sinusitis, syphilis, chlamdydia, and pelvic inflammatory diseas. Spectrophotometry[6,8,9], Nanoprecipitation and Spontaneous Emulsification Solvent Diffusion Method[7], Fluorescence Spectroscopic Analysis[10].

Lansoprazole (LPZ)

Lansoprazole(LPZ) is a substituted benzimidazole, chemically known as methyl-4-(2,2,2-trifluroethoxy)-2pyridyl]methyl]sulfinyl]benzimidazole(Fig1). LPZ is a proton pump inhibitor which inhibits the ultimate step in gastric acid secretion. Even the stimulus-independent acid secretion is suppressed. Both basal and stimulus acid is inhibited. Peptic activity is reduced secondary to acid inhibition. LPZ has a greater inhibitory effect on H. pylori than omeprazole, and is thus widely used in the treatment of benign gastric ulcer associated with H. pylori; duodenal ulcer and reflux oesophagatis. LPZ is also indicated for Zollinger-Ellison Syndrome and acid related Dyspepsia. Methods available include HPLC[11,13], RP-HPLC method[12] UV-Spectrophotometer[14] Pharmacetical Analysis method[15].

Omeprazole (OMZ)

Omeprazole is a member of benzimidazole class of drugs. It is an important benzimidazole derivative which is used in the treatment of gastric and duodenal ulcers, and reflux oesophagitis[1]. Its efficacy as an antiulcer and antisecretory agent has been well established. It is a proton pump inhibitor, used in treatment of peptic ulcer disease and NSAID-associated ulceration, in gastro-esophageal reflux disease and the Zollinger-Ellison syndrome. The methods which were reported in the literature for the determination of OMZ include FT-IR spectroscopy[16], HPLC[17] and UV-Spectrophotometry[18-20].

Carvedilol (CRV)

Carvedilol (CRV), Fig. 1, is designated chemically as (±)-1-(carbazol-4- yloxy)-3-[[2-(o-methoxyphenoxy) ethyl] amino] -2- propanol. It is a non-selective β adrenergic antagonist with no intrinsic sympathomimetic activity and is widely used to treat essential hypertension and angina pectoris. Carvedilol is also indicated for the treatment of mild to severe chronic heart failure, Left ventricular dysfunction following myocardial infarction in clinically stable patients. It also has multiple spectra of activities such as antioxidant property, inhibition of smooth muscle proliferation and calcium antagonistic blocking activity. RP HPLC[21], UV- Spectrophotometry[22,24,25], RP-HPLC[23] appear in the literature for the determination of Carvedilol in bulk and pharmaceutical formulation.

Structures of Drugs

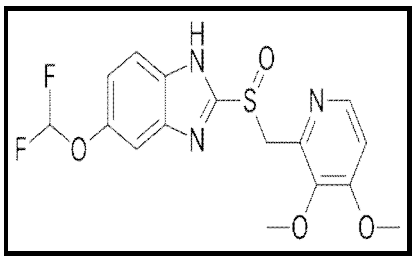

Figure 1(a) Pantoprazole

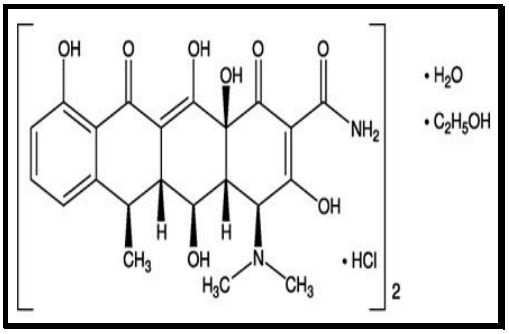

Figure 1(b) Doxycycline Hyclate

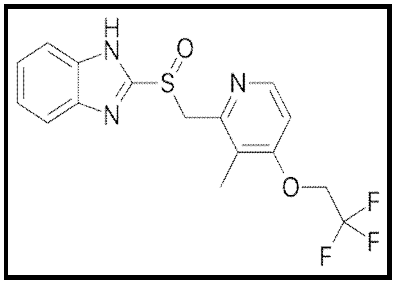

Figure 1(c) Lansoprazole

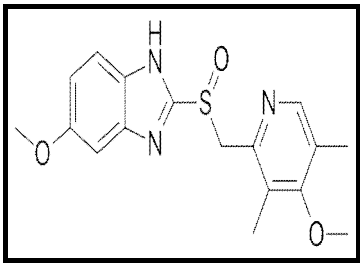

Figure 1(d) Omeprazole

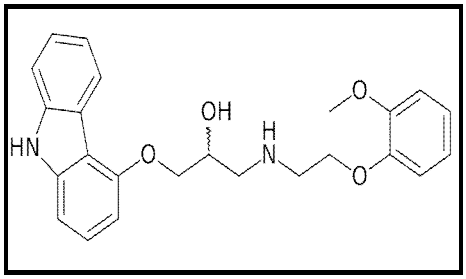

Figure 1(e) Carvedilol

Through survey of literature on the above mentioned drugs revealed that quantification based on use of NBS an oxidizing reagent and Rhodamine-B as analytical reagent have not been yet reported. The present work is an attempt to develop accurate, simple, sensitive, and cost effective method for the analysis of the above drugs.

Experimental

Reagents and standards

The pharmaceutical grade drugs were supplied by Dr.Reddy's laboratory and Arabindo pharmaceutical, Hyderabad.NBS, Rhodamine –B,and HCl were purchased from S.D.fine chem. Pvt.Ltd., Mumbai, India. Whatman filter paper no.42 was used for filtration purpose. All the reagents used were of AR grade and triple distilled water was used throughout the investigation. Tablets were purchased from the Medplus and Appolo medical shops.

Instrumentation and Optical characteristics

All absorbance measurements were recorded on Shimadzu 140 double beam spectrophotometer as well as on Elico 159 single beam and Elico SL-2l0 UV-Visible double spectrophotometers using matched pair of Quartz cells of 10 mm path length. A high precision Analytical Dhona 200 balance was used for weighing the reagents.

Preparation of standard stock solution

N-Bromo succinamide (0.01M) stock solution was prepared by dissolving 0. 1779 gm of sample in 100 ml standard flask with triple distilled water. Rhodamine-B (0.001*M*) solution was prepared by dissolving 50 mg in 100 ml standard flask with triple distilled water.Stock solution of both NBS and Rhodamine-B were further dilueted to the concentration of 70 µg mL^{-1} respectively. Standard stock solution of drugs were prepared by dissoling accurately weighed 40 mg drug to separate 100ml volumetric flasks. The stock solutions of PNZ, DOX, LAN, OMZ and CRV,were further diluted with the same solvent to obtain working concentrations. Concentrated HCl was diluted appropriately with triple distilled water to get 1*M* HCl solution.

Results and Discussion

Assay procedure

A liquots of pure drug solution (1 to 7 mL) were transferred into a series of 10 mL calibrated flask. To each flask, 1mL of $1mL^{-1}$hydrochloric acid was added. Followed by 1mL of NBS solution (70 µg mL^{-1}).The contents were mixed and the flasks were set aside for 10 min under occasional shaking. Finally, 1mL of Rhodamine-B solution (50 µg mL^{-1}) was added to each flask, diluted to the mark with water and the absorbance of solution was measured at 557 nm against a reagent blank after 10 min.

The calibration curve was constructed for all the drugs by plotting the absorbance versus the concentration of drugs. The absorbance data was collected for six replicate and absorbance to concentration ratio called the relative response was determined. The relative responses between 95% to 105% of average only are considered for construction of the calibration curves (figure 2).

Accuracy and Precession studies

Accuracy of the methods developed is determined from the recovery studies on pure drug sample. At least four known concentration of solutions of drugs in Beer's law limit were taken and recovery studies were performed .Excellent recovery showed the validity of the calibration curves for each drug.

Precession of the method is demonstrated by repeating experiment (n=6) and % RSD is worked out % RSD being less than case speaks the high precession of the methods.

Analysis of Pharmaceutical preparation

Five tablets of (Protonix-200mg) were weighed and ground in to fine powder. Weight equivalent to 10mg of Pantoprazole sodium was transferred in 100ml volumetric flask and made up to mark with water. And the solution filtered using a whatman No. 42 filter paper. The resultant of the solution was further diluted to get a required concentration.

Three tablets of (Doxine-300mg) were weighed and ground in to fine powder. Weight equivalent to 10mg of Doxycycline hyclate sodium was transferred in 100ml volumetric flask and made up to mark with water. And the solution filtered using a whatman No. 42 filter paper. The resultant of the solution was further diluted to get a required concentration for the analysis of the drug.

Five tablets of (Prevacid-100mg) were weighed and ground in to fine powder. Weight equivalent to 10mg of Lansoprazole was transferred in 100ml volumetric flask and made up to mark with water. And the solution filtered using a whatman No. 42 filter paper. The resultant of the solution was further diluted to get a required concentration for the analysis of the drug.

Five tablets of (Prilosec-100mg) were and ground in to fine powder. Weight equivalent to 10mg of Omeprazole was transferred in 100ml volumetric flask and made up to mark with water. And the solution filtered using a whatman No. 42 filter paper. The resultant of the solution was further diluted to get a required concentration for the analysis of the drug.

Four tablets of (Coreg 100mg). Weight equivalent to 10mg of Carvedilol was transferred in 100ml volumetric flask and made up to mark with water. And the solution filtered using a whatman No. 42 filter paper. The resultant of the solution was further diluted to get a required concentration for the analysis of the drug.

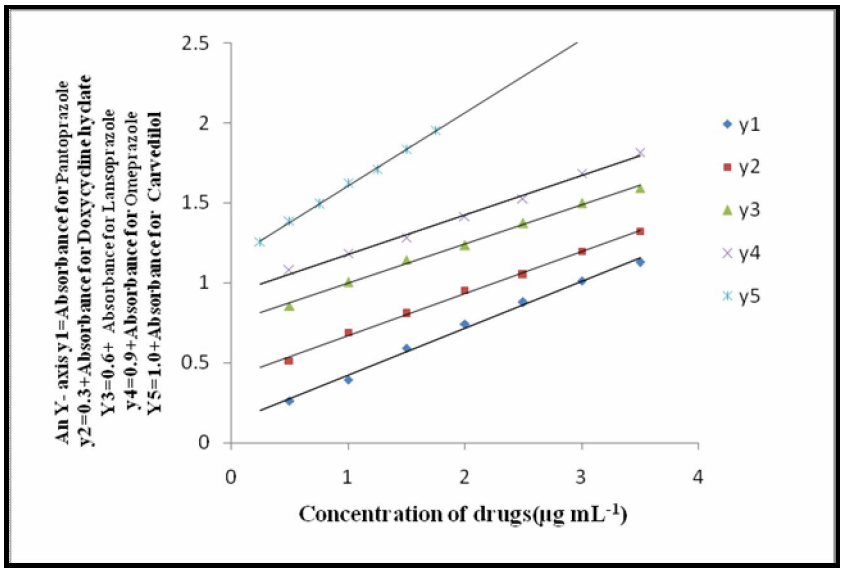

Figure-2 Calibration curves of drugs PNZ, DOX, LAN, OMZ and CRV

Table 1 Analytical and regression parameters of spectrophotometric methods

Parameter	PNZ	DOX	LAN	OMZ	CRV
λ_{max} (nm)	557	557	557	557	557
Beer's Law Limits (µg mL^{-1})	0.5-3.5	0.5-4.0	0.5-4.0	0.5-4.0	0.25-1.75
Molar absorptivity ($L\ mol^{-1}cm^{-1}$)	$0.213x10^5$	$0.434x10^5$	$0.974x10^5$	$0.518x10^5$	$0.414x10^5$
Sandell sensitivity* (µg cm^{-2})	0.0034	0.0037	0.0040	0.0039	0.0022
LOD (µg mL^{-1})	0.0505	0.0700	0.0608	0.0399	0.0443
LOQ (µg mL^{-1})	0.1531	0.2121	0.1844	0.1210	0.1357
Intercept, (A)	0.127	0.104	0.154	0.020	0.159
Slope, (B)	0.294	0.264	0.244	0.256	0.442
Correlation Coefficient, (R)	0.994	0.996	0.996	0.990	0.996
Standard Deviation of Intercept (Sa)	0.0045	0.0056	0.0045	0.0031	0.0060
Standard Deviation of Slope (Sb)	0.0071	0.0086	0.0050	0.0021	0.0045
Regression equation,(y) Y=bx+a	0.294x +0.127	0.264x +0.104	0.244x +0.154	0.256x +0.020	0.442x +0.159

*Limit of determination as the weight in µg / mL of solution, which corresponds to absorbance of A = 0.001 measured in a cuvette of cross –sectional area $1cm^2$ and path length of 1 cm . Y** =a+bX, where Y is the absorbance and x concentration of drugs in µg / mL.

Table 2 Determination of accuracy and precision of the methods on pure drug sample

Drug	Taken (µg/mL)	Found (µg/mL)	Er (%)	Recovery (%)	RSD (%)	Proposed method mean ± SD
PNZ	3.0 3.5 4.0	3.02 3.49 3.99	0.66 0.29 0.25	100.66 99.71 99.75	0.537	100.04 ±0.537
DOX	2.0 4.0 6.0	2.01 3.99 5.99	0.50 0.25 0.17	100.50 99.75 99.83	0.411	100.02 ±0.411
LNZ	2.5 3.0 3.5	2.49 3.01 3.5	0.40 0.33 0.00	99.60 100.33 100.00	0.365	99.97 ±0.364
OMZ	3.5 4.0 5.0	3.48 4.0 5.02	0.57 0.00 0.39	99.43 100.00 100.39	0.482	99.94 ±0.482
CRV	1.0 3.0 4.0	1.0 3.01 3.99	0.00 0.33 0.25	100.00 100.33 99.75	0.290	100.02 ±0.290

Table -3 Results of assay of tablets by the proposed method and statistical evaluation and recovery experiment by standard addition method.

Tablet	Drug in tablet (µg/ml)	Drug added (µg/ml)	Total found (µg/ml)	Er (%)	Recovery (%)	RSD (%)	Reference method mean ± SD	Proposed method mean ± SD	T-test	F-test
	0.50	0.5	0.99	1.00	99.00					
Protonix	0.50	1.0	1.51	0.66	100.66					
	0.50	1.5	1.99	0.50	99.50	0.657	99.15	99.70	1.551	
(PNZ)	2.5	0.0	2.49	0.40	99.60		±0.403	±0.655		2.641
	3.0	0.0	3.01	0.33	100.33					
	3.5	0.0	3.47	0.85	99.15					
	0.50	0.4	0.90	0.00	100.00					
Doxine	0.50	0.8	1.29	0.77	99.23					
	0.50	1.2	1.69	0.59	99.41	0.499		99.62		
(DOX)	1.0	0.0	0.99	1.00	99.00		102.1	±0.497	8.617	0.988
	3.0	0.0	3.01	0.33	100.33					
	4.0	0.0	3.99	0.25	99.75		±0.500			
	0.50	0.3	0.79	1.25	98.75					
Prevacid	0.50	0.6	1.10	0.00	100.00					
	0.50	0.9	1.39	0.71	99.29	0.600		99.68		
(LAN)	2.0	0.0	2.01	0.49	100.49		99.80	±0.598	0.153	0.214
	4.0	0.0	3.99	0.25	99.75		±1.29			
	6.0	0.0	5.99	0.17	99.83					
	0.50	0.2	0.71	1.40	101.40					
Prilosec	0.50	0.4	0.90	0.00	100.00					
	0.50	0.6	1.11	0.90	100.90	0.780		100.19		
(OMZ)	3.0	0.0	2.99	0.33	99.67		99.65	±0.781	1.350	3.490
	3.5	0.0	3.48	0.57	99.43					
	4.0	0.0	3.99	0.25	99.75		±0.418			
	0.50	1.0	1.48	1.33	98.67					
Coreg	0.50	2.0	2.51	0.40	100.40					
	0.50	3.0	3.49	0.29	99.71	0.614	99.89	99.80		
(CRV)	3.5	0.0	3.50	0.00	100.00		±0.61	±0.612	0.208	1.006
	4.0	0.0	4.01	0.25	100.25					
	5.0	0.0	4.99	0.20	99.80					

Analytical Data

A linear correlation was found between absorbance at λmax and concentration of all drugs in the ranges given in table 1. Regression equation analysis of the Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each drug and the values are presented in table 1. The optical characteristics such as Beer's law limits and sandell sensitivity values for both methods are given in table 1. The limits of detection (LOD) and quantization (LOQ) calculated according to ICH guidelines are also presented in table 1 and reveal the very high sensitivity of the methods.

$LOD = 3.3S_a/b$

$LOQ = 10S_a/b$ where S_a = standard deviation of intercept (n=6) , b= slope of Calibration plot

Conclusions

The obtained results from the methods for the determination of above mentioned drugs indicate that methods are simple, accurate and precise. The methods are economical compared to other sophisticated analytical instruments, hence can be used for routine analysis of commercially available formulations. The method is suitable for the determination of these drugs in tablet formation without interference from commonly used recipients. The solvent used for the method are inexpensive and simple to prepare, and could be used in a quality control laboratory for routine drug analysis.

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