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Estimation of Drugs and Pharmaceuticals Using Chloramine-T and Rhodamine-B Dye Couple

Sambashivudu Nasam and G. Venkateshwarlu*

Department of Chemistry, University College of Science, Osmania University, Hyderabad- 500007, **INDIA**

Email: venkateshwarlugoud@yahoo.com

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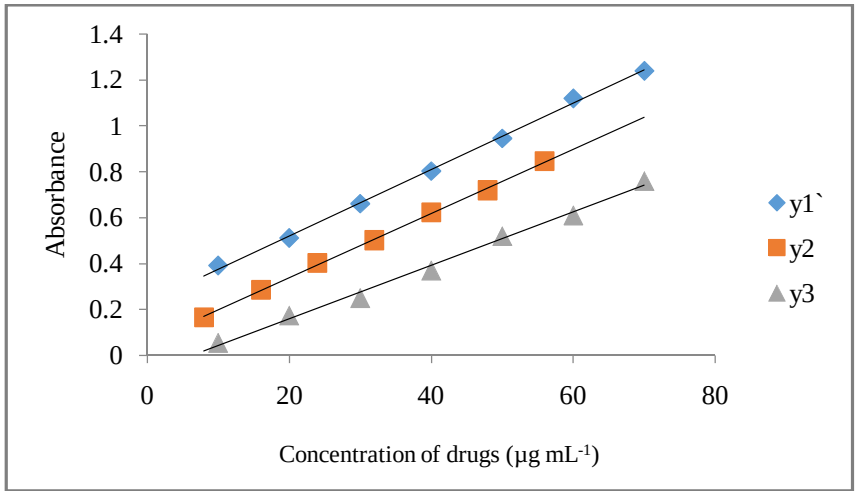
ABSTRACT

A simple, sensitive and selective method has been developed for the spectrophotometric determination of drugs, viz., Naparoxen, Cefdinir, Paroxetine HCl, Ondansetron and Cefedaxitine. The proposed method involve the addition of excess Chloramine-T of known concentration in the presence of acidic medium and the unreacted Chloramine-T is determined by the measurement of the decrease in the absorbance of the dye Rhodamine-B dye. The colored species in acidic medium, reactants are allowed to react and the unreacted Chloramine-T is estimated by the measurement in the decrease in the absorbance of the Rhodamine-B dye (λ_{max} 557 nm). This method has been validated in terms of guidelines of ICH and applied to the quantification of selected drugs in bulk and dosage forms.

Graphical Abstract



Calibration Curves of NAP, CEFD, PAR.

Keywords: Spectrophotometry, Drugs, Chloramine-T, Rhodamine-B, Quantification, Validation.

INTRODUCTION

Naproxen: Naproxen (NAP) [(+)-(S)-2-(6-methoxynaphthalen-2-yl) propionic acid], Due to an aryl acetic structure, naproxen exhibits analgesic and antipyretic properties (Figure 1). Naproxen is used for the reduction of moderate to severe pain, fever, inflammation, rheumatoid arthritis, musculoskeletal disorders and gout. Naproxen has been determined by several analytical methods like: HPLC [**1**], HPTLC [**2**], TLC [**3**], LC-MS/MS [**4, 5**], fluorimetric methods [**6**] and chemiluminescence [**7**]. Although HPLC methods are highly sensitive and specific, they are considered expensive.

Cefdinir: Cefdinir chemically 8-[2-(2-amino-1, 3-thiazol-4-yl)-1-hydroxy-2-nitroso-ethenyl] amino-4-ethenyl7-oxo-2-thia-6-azabicyclo [4.2.0] oct-4-ene-5-carboxylic acid (Figure 2). It is used to treat bacterial infections such as pneumonia, bronchitis, sinusitis, tonsillitis and skin infections. Analysis of cefdinir in bulk, pharmaceutical dosage forms and biological samples has been accomplished by several methods so far, including spectrophotometry [**8, 9**], HPLC [**10, 11**], LC-MS [**12**], electrochemical [**13**] and spectroflourometry [**14**].

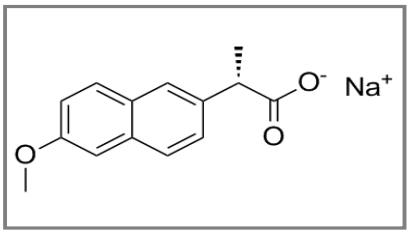


Figure 1. Structure of Naproxen.

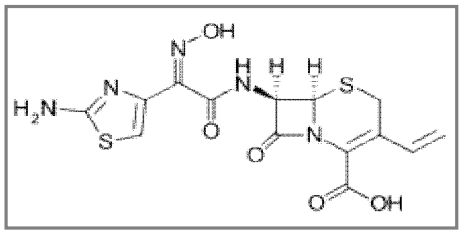


Figure 2. Structure of Cefdinir.

Paroxetine HCl: Paroxetine; (3S, 4R) -3-[(1,3-benzodioxol-5-vloxy)methyl]-4-(4-flurophenyl) piperidine (PRX) is a new generation antidepressant drug (Figure 3). The methods reported for quantitative determination of PRX in tablets and/or biological fluids include RP-HPLC [**15**] and UV [**16**].

Ondansetron HCl: Ondansetron is a 5-HT3 – receptor antagonist used as an antiemetic. Chemically it is 1, 2, 3, 9-tetrahydro-9-methyl-3-[(2-methyl- 1H- imidazol-1-yl)-methyl]-4H-carbazol-4-one (Figure 4). Several methods [**17, 18**] have been reported for the assay of ondansetron.

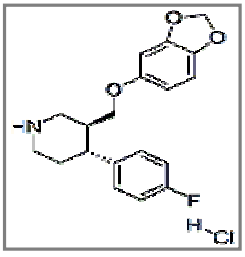


Figure 3. Structure of ParoxetineHC

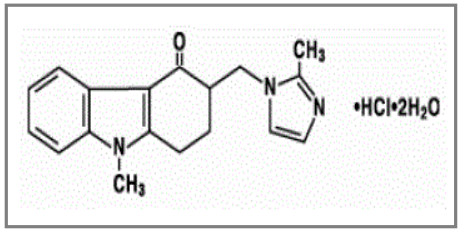


Figure 4. Structure of Ondansetron

Cefpodoxime proxetil: Cefpodoxime, 1-(isopropoxycarbonyloxy) ethyl (6R, 7R)-7-[2-(2-amino-4-thiazolyl)-(Z)-2-(methoxyimino) acetamido]-3-methoxymethyl-3-cephem-4-carboxylate, is an oral third generation cephalosporin antibiotic (Figure 3). It is active against most Gram positive and Gram negative bacteria. It is commonly used to treat acute otitis media, pharyngitis, and sinusitis. The bactericidal activity of Cefpodoxime results from its inhibition of cell wall synthesis. Literature survey reveals that HPTLC, [**19, 20**] HPLC [**21-23**] and Spectrophotometric [**24, 25**] methods has been developed for its estimation in alone or in combination with other drugs.

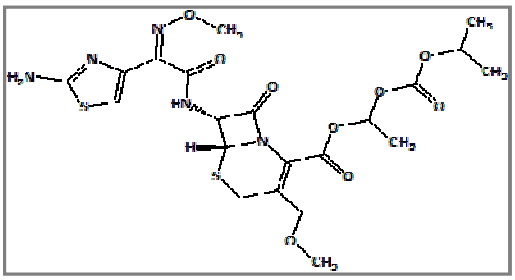


Figure 5. Structure of Cefpodoxime proxetil

MATERIALS AND METHODS

Instrument: The analysis of the drugs were recorded on Shimadzu 140 double beam spectrophotometer as well as on Elico 210 UV- Visible double beam and Elico 159 UV-Visible single beam spectrophotometers using matched pair of Quartz cells of 10 mm path length.

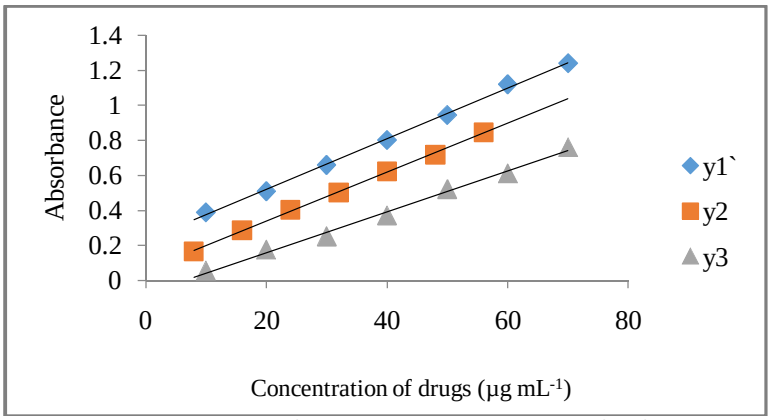
Materials: Chloramine-T solution (0.005 molL^{-1}) was prepared by dissolving0.1408g Chloramine-T in 100 mL standard flask with distilled water. This solution was diluted with water to appropriately to get 45 µg mL^{-1} Chloramine-T for use in spectrophotometric method.

A stock solution of Rhodamine- B was prepared by dissolving0.050g dye in 100mL double distilled water. The dye solution was further diluted to 50 µg mL^{-1} to get working concentration.

The pharmaceutical grade drugs were supplied by hetero drugs Pvt. Limited. Hyderabad. A stock solution of drug was prepared by dissolving accurately weighed 20 mg of pure drug in water and diluting to 100 mL in a volumetric flask with distilled water. The solution was diluted stepwise to get working concentrations.

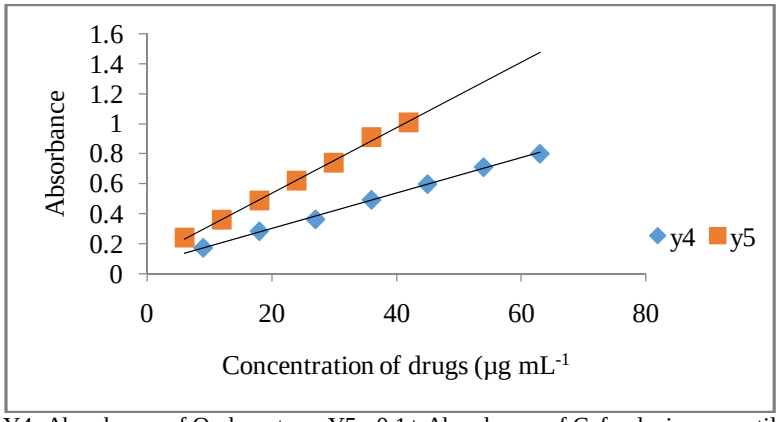
Assay Procedure: Aliquots of pure drug solution (1 to 7 mL) was transferred to a series of 10 mL calibrated flask and to this 1mL of 2 M HCl was added, followed by 1mL of Chloramine-T solution (45µg mL^{-1}) solution . And the solution was shaked for 15 min. The 1mL of 50 µg mL^{-1} of Rhodamine- B solution 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.

Calibration curves (figure 6) were constructed for all the drugs by plotting the absorbance versus the concentration of drugs. The absorbance data was collected for six replicate experiments and absorbance to concentration ratio called the relative response was determined.



Y1=0.2+Absorbance of Naparoxen, Y2=Absorbance of Cefdinir,
Y3= 01-Absorbance of Paroxetine HCl

Figure 6. Calibration Curves of NAP, CEFD, PAR.



Y4=Absorbance of Ondansetron, Y5= 0.1+ Absorbance of Cefpodaximeproxetil

Figure 7. Calibration Curves of OND, CEFP.

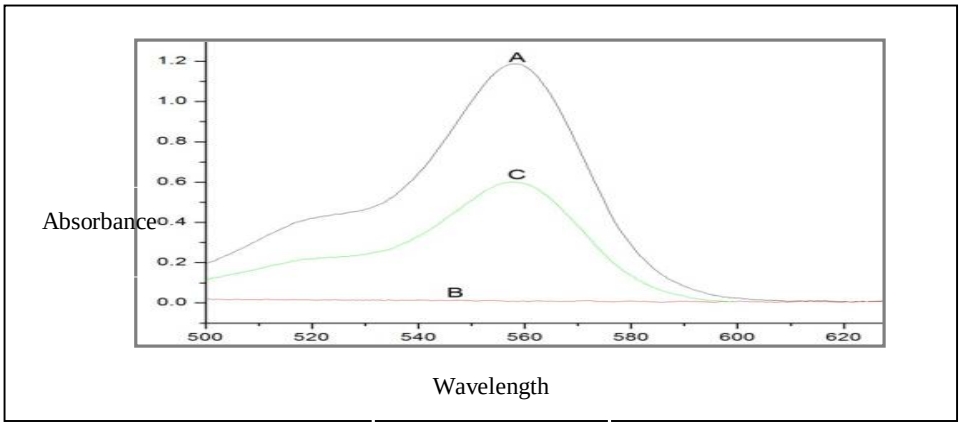


Figure 8. Absorption Spectra of A) Rhodamine-B, B) Neutralization of Chloramine-T with Rhodamine-B, C) Drug and Chloramine-T with Rhodamine-B.

Procedure for Assay of Pure Drug: To test the accuracy and precision of the methods developed pure sample solutions containing drug in the Beer's law limit were chosen. For this study 10, 20, 30 and 40 µg mL^{-1} of NAP; 8, 16, 24 and 32 µg mL^{-1} of CEF; 10, 20, 30 and 40 µg mL^{-1} of PAR; 9, 18, 27 and 36 µg mL^{-1} of OND and 6, 12, 24 and 30 µgmL^{-1} of CEFPO have been taken. The concentration chosen and recovery are tabulated in table 2, for this purpose standard deviation method also adapted.

Procedure for tablets

Naproxen: Five tablets of Naproxen (Napier, 500 mg) each containing 25 mg of NAP were weighed and finely powdered in a mortar. A quantity of powder equivalent to 20 mg of NAP was weighed accurately and dissolved in 100 mL of double distilled water, and sonicated for 20 min. It was used as stock sample solution and was further diluted with the same solvent to get working standard solution.

Cefdinir: Ten tablets of Cefdinir (Zefnir, 300 mg) were weighed and grounded. A quantity equivalent to 10 mg of CEF was transferred into a 100 mL calibrated flask, mixed well and filtered using a filter paper. First 10 mL portion of the filtrate was discarded and a suitable aliquot of the subsequent portion was diluted appropriately to get required concentration and the assay was completed according to the procedure described above.

Paroxetine HCl: Ten tablets of drug (Seroxat, 25 mg) were weighed and grounded and transferred into a 100 mL calibrated flask and added 30 mL of distilled water followed by sanitations for 15 min. The solution was finally made up to 100 mL. It was used as stock sample solution and was further diluted with the distilled water to get working concentration solution for assay.

Ondanesetrone: Ten tablets (Zofran,4 mg) were weighed and grounded. The powder equivalent to10 mg Ondrosertone was stirred well with methanol, sonicated about 30 min. The solutions was filtered through Whitman filter paper in a 100 mLvolumetric standard flask and the residue was washed well with methanol for complete recovery of the drug and methanol was evaporated. The residue was dissolved in 100 mL of distilled water and it was further diluted to get required concentration for the analysis of the drug.

Cefpodoxime proxetil: Ten tablets (Simpod, 200 mg) were powdered and equivalent to about 10 mg of Cefpodoxime proxetil had been taken in to a100 mL of volumetric flask and added about 30 mL of methanol, sonicated for 20 min and filtered through Whitman filter paper No 42. The residue was washed thrice with methanol for complete recovery of drug and methanol was evaporated. The residue was dissolved in 100 mL of distilled water. It was used as stock sample solution. The aliquot portions of this stock solution were further diluted with distilled water to get the final concentration required for the determination of the drug.

RESULTS AND DISCUSSION

Method Development: The ability of Chloramine-T to oxidize drugs, and bleach the color of Rhodamine-B is the basis of the indirect spectrophotometric methods developed here. In these methods, the drugs were reacted with a measured excess of Chloramine-T in acidic medium and the unreacted oxidant was determined by reacting with Rhodamine-B followed by absorbance measurement at 557 nm. The absorbance increased linearly with increasing the concentration of drug. When fixed amount of the dye was added to decreasing amount of oxidant, a concomitant increase in the concentration of dye resulted. This was observed as a proportional increase in absorbance at the respective λ_{max} with increasing concentration of each drug.

Preliminary experiments were conducted to determine the maximum concentrations of Rhodamine-B spectrophotometrically by measuring the absorbance of their acidic solutions at their respective λ_{max} and the upper limits were found to be 5 µg mL^{-1} for Rhodamine-B. Chloramine-T concentration of 4.5µg $mL^{-1}$was found to bleach the redc olor due to5µg mL^{-1} Rhodamine-B. Hence different amounts of drug reacted with 4.5µg mL^{-1} Chloramine-T in these methods before determining the residual Chloramine-T as described under the respective procedure.

Hydrochloric acid was considered to be a convenient medium for these methods. For a quantitative reaction between drug and Chloramine-T, a contact time of 15 min was found sufficient.

Analytical Data: A linear correlation was found between absorbance at λ_{max} and concentration of all drugs in the ranges given in table 1. Regression 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 system and the values are presented in table 1. The optical characteristics such as Beer's law limits and Sandell sensitivity values for these methods are given in table 1. The limits of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines [**11**] are also presented in table 1 and reveal the very high sensitivity of the methods.

$$LOD = 3.3 \times S_a/b, \quad LOQ = 10 \times Sa/b$$

Where S_a= standard deviation of the intercept (n = 6), b = slope of Calibration plot.

Table 1. Analytical and regression parameters of spectrophotometric method

Parameter	NAP	CEFD	PAR	OND	CEFP
λ_{max}, nm	557	557	557	557	557
Beer's law limits µg mL^{-1}	10-70	8-56	10-70	9-63	6-42
Molar absorptivity, L mol^{-1} cm^{-1}	2406.21	8174.895	11918.64	6039.46	13651.91
Sandell sensitivity µg cm^{-2}	0.0833	0.07142	0.0416	0.0769	0.0625
Limit of detection µg mL^{-1}	4.38	1.0628	1.247	0.6716	0.519
Limit of quantification µg mL^{-1}	13.28	3.220	3.780	2.0351	1.572
Intercept, (a)	-0.016	0.058	-0.013	0.054	0.019
Slope, (b)	0.012	0.014	0.024	0.014	0.016
Correlation coefficient, (r)	0.998	0.999	0.997	0.996	0.999
Standard deviation of intercept(Sa)	0.0159	0.0045	0.009074	0.00264	0.0025
Regression equation, Y	0.012X-0.016	0.014X+0.058	0.012X+0.027	0.014X+0.054	0.016X+0.019

Precision and Accuracy: Intra-day precision was assessed from the results of six replicate analyses on pure drug solution. The mean values and relative standard deviation (RSD) values for replicate analyses at three different levels (amounts/concentrations) were calculated. To evaluate the inter-day precision, analysis was performed over a period of five days, preparing all solutions afresh each day.

The accuracy of the methods was determined by calculating the percentage deviation observed in the analysis of pure drug solution and expressed as the relative error. Table 2 summarizes the intra-day precision and accuracy data for the assay of pure drugs solution by the proposed methods.

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

Drug	Taken (µg mL^{-1})	Found (µg mL^{-1})	Error (%)	Recovery (%)	RSD (%)	Proposed method Mean ± SD
	8.0	8.01	-0.125	100.125		100.11
NAP	26	26.09	0.346	100.34	0.1649	±0.1647
	44	44.02	0.045	100.04		
	15	15.02	0.133	100.133		100.07
CEFD	29	29.03	0.103	100.10	0.125	±0.126
	33	33.06	0.181	100.18		
	10	9.99	0.1	99.9		100.027
PAR	25	25.03	-0.12	100.12	0.092	±0.092
	36	36.02	0.055	100.05		
	11	11.06	-0.545	100.54		100.18
OND	17	16.98	0.117	99.88	0.275	±0.275
	38	38.04	-0.105	100.105		
	6	5.96	0.66	99.33		99.95
CEFP	14	14.03	-0.214	100.21	0.414	±0.413
	32	32.04	-0.125	100.125		

Robustness and Ruggedness: To evaluate the robustness of the methods, volume of Sulphuric acid was slightly altered. The reaction time (after adding, time varied was Chloramine-T 15± 2 min) and the time after addition of dye is slightly changed. To check the ruggedness, analysis was performed by three different analysts and on three different spectrophotometers by the same analyst.

Application to Formulations: The proposed methods are applied to the determination of drugs in tablets. The results in table 3 showed that the methods are successful for the determination of drugs and that the excipients in the dosage forms do not interfere. The results are compared to the available validated reported methods on each drug and the results agree well with the claim and also are in agreement with the results obtained by the literature method. Statistical analysis of the results using Student's t-test for accuracy and F-test for precision revealed no significant difference between the proposed methods and the literature method with respect to accuracy and precision.

Table 3. Results of assay of tablets by proposed method and statistical evaluation

Tablet	Drug in tablet (μgmL^{-1})	Drug Found (μgmL^{-1})	Error (%)	Recovery (%)	RSD (%)	Reference method Mean± SD	Proposed method Mean ± SD	t-test	F-test
Napier	12	12.06	0.5	100.5					
(NAP)	18	18.04	0.22	100.22	0.437	100.14±0.321	100.20±0.428	1.49	0.12
	26	25.89	0.42	99.57					
Zefnir ,	14	14.06	0.42	100.42					
(CEFD)	22	21.76	1.09	98.90	0.683	99.91±0.86	100.12±0.125	0.61	0.48
	25	25.06	0.24	100.24					
Seroxat,	10	10.08	0.8	100.8					
(PAR)	15	14.94	0.4	99.6	0.493	100.73 ± 0.25	100.16±0.493	1.60	0.097
	28	28.02	0.0007	100.07					
Zofran,	9	9.01	0.11	100.11					
(OND)	16	16.02	0.12	100.12	0.022	100.9± 0.320	100.1±0.022	2.41	0.001
	32	32.03	0.093	100.09					
Simpod	12	11.98	0.166	99.83					
(CEFP)	16	16.04	0.25	100.25	0.208	100.8±0.301	100.1±0.209	2.10	0.017
	24	24.07	0.29	100.29					

APPLICATION

These are simple, rapid, and cost-effective methods for the determination of drugs have been developed and validated.

CONCLUSION

The proposed methods are more sensitive methods and the methods relyon the use of simple and cheap chemicals and techniques but provide sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC. Thus, they can be used as alternatives for rapid and routine determination of bulk sample and tablets.

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