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Spectrophotometric Estimation of a few Commercial Drugs using Potassium Permanganate and Amaranth Dye in Acidic Media

P. Adavi Raju*

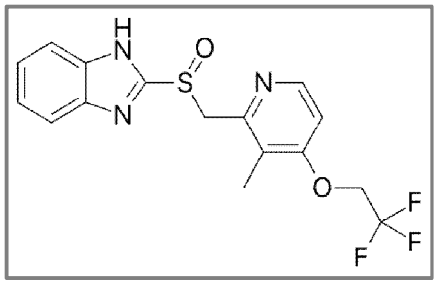
Department of Chemistry, Government Degree and P.G College, Gajwel District, Siddipet-502278, Telangana, **INDIA**

Email:adavirajupagudala@gmail.com

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., Atazanavir sulphate (ATV), Carvedilol (CRV), Dexlansoprazole (DLP) Doxycycline Hyclate (DOX), Omeprazole (OMZ). The method involves the addition of excess $KMnO_4$ of known concentration in the presence of 0.2M H_2SO_4,reactants are allowed to react and the unreacted $KMnO_4$ is estimated by the measurement in the decrease in the absorbance of the Amaranth 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.

Graphical Abstract



Structure of Dexlansoprazole

Keywords: UV-Visible Spectrophotometry, Drugs, $KMnO_4$, Amaranth dye, Quantification, Validation.

INTRODUCTION

Atazanavir sulphate: Atazanavir sulphate (ATV) (fig.1a)is one of the oral antiretroviral protease inhibitors used for treatment for HIV/AIDS. It is chemicallymethyl *N*-[(1*S*)-1-{[(2*S*,3*S*)-3-hydroxy-4-[(2*S*)-2-[(methoxycarbonyl)amino]-3,3-dimethyl-*N*′-{[4-(pyridin-2-yl)phenyl]methyl} butanehydra zido]-1-phenylbutan-2-yl]carbamoyl}-2,2-dimethylpropyl]carbamate. Atazanavir sulphate is official in Indian pharmacopoeia (Figure 1). Atazanavir is an azapeptide HIV-1 protease inhibitor. The compound selectively inhibits the virus specific processing of viral Gag and Gag-pol polyproteins in

HIV-1 infected cells, thus preventing formation of mature virions. only a few methods *viz* HPLC [**1, 2**], UV-Visble Spectrophotometry [**3, 4**], Liquid chromatography [**5**] and using UPLC [**6**] appear in the literature for the determination of Atazanavir sulphate in bulk and pharmaceutical formulations.

Carvedilol: Carvedilol is designated chemically as (±)-1-(carbazol-4- yloxy)-3-[[2-(o-methoxy phenoxy) ethyl] amino]-2- propanol (Figure 2). 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. UV-Visible Spectrophotometry [**7, 8**], HPLC [**9**], UPLC [**10**], GCMS [**11**], Fluorimetry [**12**], Florescence [**13**], appear in the literature for the determination of Carvedilol in bulk and pharmaceutical formulation.

Dexlansoprazole: Dexlansoprazole is a substituted benzimidazole, chemically known as methyl-4-(2, 2, 2-trifluroethoxy)-2pyridyl] methyl] sulfinyl] Benz imidazole (Figure 3). 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 esophagitis. LPZ is also indicated for Zollinger-Ellison Syndrome and acid related Dyspepsia. Methods available include HPLC [**14 ,15**], UV- Visible Spectrophotometer [**16**] NMR [**17**]. LC/MS [**18**], FT-IR [**19**].

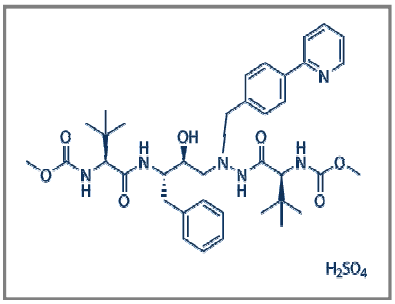


Figure 1. Structure of Atazanavir sulphate

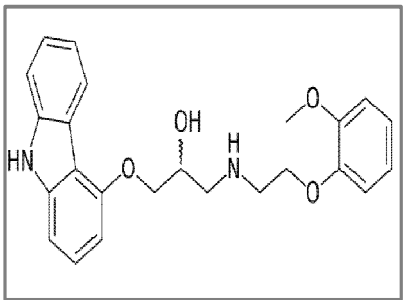


Figure 2. Structure of Carvedilol

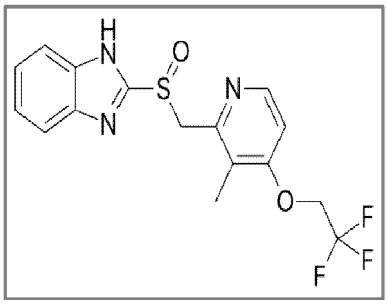


Figure 3. Structure of Dexlansoprazole

Doxycycline hyclate: Doxycycline hyclate molecular mass 512.94 g mol^{-1}. The systematic IUPAC name is Hydrochloride hemi ethanol 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-carboxa-mide (Figure 4). 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 tetracycline's 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, chlamydia, and pelvic inflammatory disease [**20**]. Spectrophotometry Nano precipitation and Spontaneous Emulsification Solvent Diffusion Method [21], Fluorescence Spectroscopic Analysis [**22**]. HPLC [**23**], LC/MS [**24**], UPLC [**25**], Flourimetry [**26**], Mass spectrometry [**27**].

Omeprazole: Omeprazole is chemically 6-methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl) methyl sulfinyl]-1H-benzimidazole (Figure 5). It is an important Benz imidazole derivative which is used in the treatment of gastric and duodenal ulcers, and reflux esophagitis. Its efficacy as an antiulcer and anti-secretory 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 [28], HPLC [29] and UV-Spectrophotometry [30], LC/MS [31], UPLC [32].

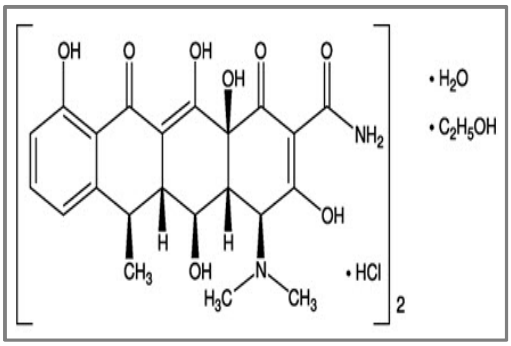


Figure 4. Structure of Doxycyclinehyclate

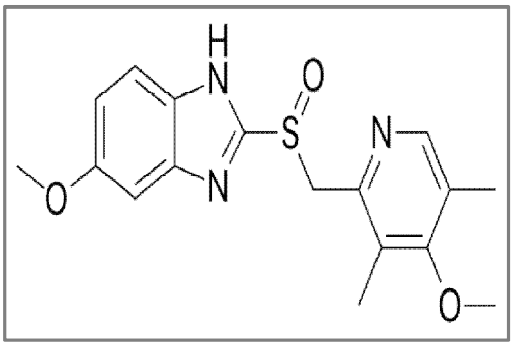


Figure 5. Structure of Omeprazole

Through survey of literature on the above mentioned drugs revealed that quantification based on use of $KMnO_4$ an oxidizing reagent and Amaranth Dye 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 estimation of the above drugs.

MATERIALS AND METHODS

Reagents and standards: The pharmaceutical grade drugs were supplied by Dr.Reddy's laboratory and Arabindo pharmaceutical, Hyderabad. $KMnO_4$, Amaranth Dye 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: A stock solution of Potassium permanganate 7.6 $x10^{-2}$M was prepared and standardized. This solution was further diluted to get 0.145µg mL^{-1} as it is found suitable for oxidation of drugs in acidic medium. Stock solution of $1x10^{-2}$ M of Amaranth dye was prepared by dissolving an appropriate weight of 0.0483gm in 100 mL of double distilled water. Amaranth dye solution was further diluted to get the concentration of 45.48 µg mL^{-1}. Stock solution of both $KMnO_4$ and Amaranth were further diluted to the concentration of 70 µg mL^{-1} respectively. Standard stock solution of drugs was prepared by dissolving accurately weighed 40 mg drug to separate 100 mL volumetric flasks. The stock solutions of ATV, CRV, DLP, DOX, and OMZ were further diluted with the same solvent to obtain working concentrations. Concentrated H_2SO_4 was diluted appropriately with triple distilled water to get 0.2M H_2SO_4 solution.

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 $1mL^{-1}$Sulfuric acid was added. Followed by 1 mL of $KMnO_4$solution (70 µg mL^{-1}). The contents were mixed and the flasks were set aside for 10 min under occasional shaking. Finally, 1mL of Amaranth solution (50 µg mL^{-1}) was added to each flask, diluted to the mark with water and the absorbance of solution was measured at 525 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 6 and 7).

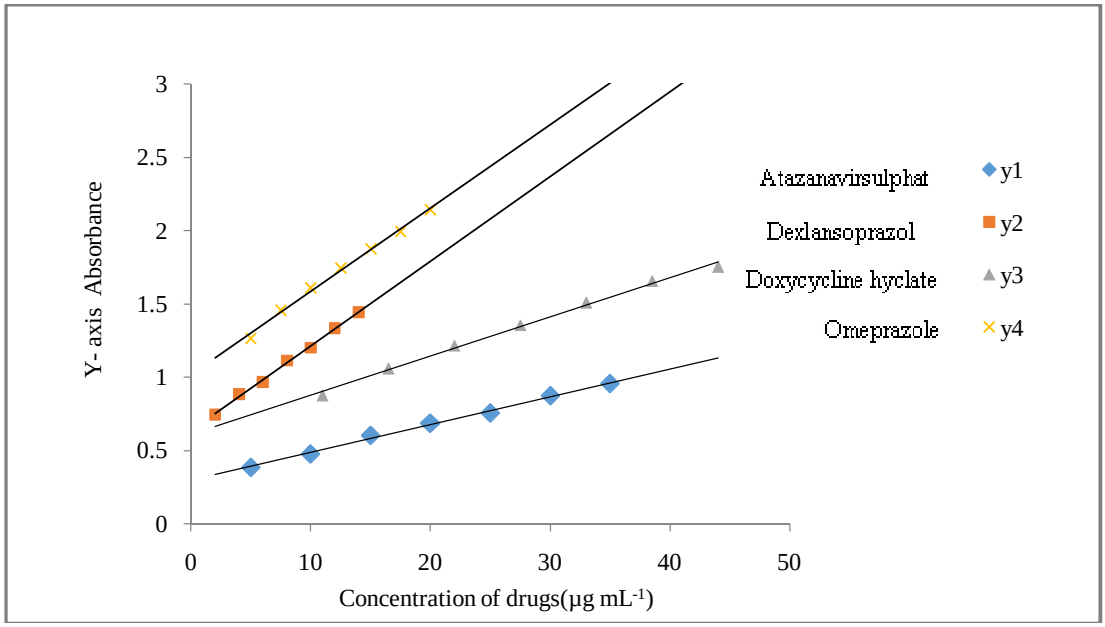


Figure 6. Calibration curves of drugs ATV, DLP, DOX, and OMZ.

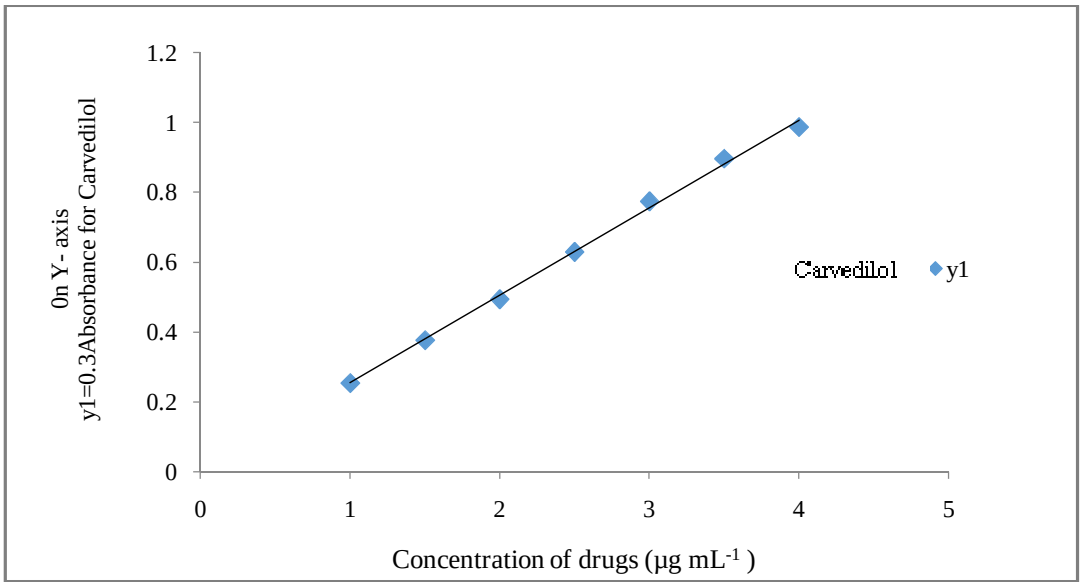


Figure 7. Calibration curve of drug carvedilol.

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: Three tablets of Reyataz-200 mg were weighed and ground in to fine powder. Weight equivalent to 10 mg of Atazanavir sulphate was transferred in 100 mL 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.

Four tablets of(Coreg 100 mg were weight equivalent to 50 mg of Carvedilol was transferred in 100 mL 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-100 mg were weighed and ground in to fine powder. Weight equivalent to 50 mg of Dexlansoprazole was transferred in 100 mL 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.

Three tablets of Doxine-300 mg were weighed and ground in to fine powder. Weight equivalent to 50 mg of Doxycycline hyclate sodium was transferred in 100 mL 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 and five tablets of (Prilosec-100 mg) were and ground in to fine powder. Weight equivalent to 50 mg of Omeprazole was transferred in 100 mL 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.

RESULTS AND DISCUSSION

Effect of acid concentration: The reaction was performed in a series of 10 mL volumetric flasks containing Series of drug samples of 1mL to 7mL. To this series add 1mL of H_2SO_4 followed by 1mL of $KMnO_4$ solution. These flasks were set aside for10 min with occasional shaking; later 1mL of Amaranth dye was added, than completed to 10 mL total volume with water. It was found that the maximum absorbance was obtained at 1.0 mL of 0.2M H_2SO_4. Above this volume, the absorbance decreased. Therefore a volume of 1.0 mL of 0.2M H_2SO_4 was used for all measurements.

Effect of concentration of Drug: Different volumes of drug of random concentration were added to a fixed volume of Amaranth, $KMnO_4$and H_2SO_4. Solutions developed coloration. Absorbance of solutions was measured at 523 nm. Beer's law was obeyed up to certain extent of concentration above which linearity was not observed. This concentration was taken as optimum concentration and stock was prepared (Table 1).

Table 1. Analytical and regression parameters of spectrophotometric methods

Parameter	ATV	CRV	DLP	DOX	OMZ
λ_{max} (nm)	520	520	520	520	520
Beer's Law Limits (**µ**g mL^{-1})	5-35	1.0-4.5	11.0-44.0	2.0-14.0	5.0-20.0
Molar absorptivity (L $mol^{-1}cm^{-1}$)	0.155x 10^6	0.104x 10^7	0.922x 10^5	0.118x 10^7	0.196x 10^6
Sandell sensitivity* (**µ**g cm^{-2})	0.0526	0.004	0.0384	0.0086	0.0178
LOD (**µ**g mL^{-1})	0.6947	0.0673	0.4442	0.1033	0.5008
LOQ (**µ**g mL^{-1})	2.1052	0.204	1.3461	0.313	1.5178
Intercept, (A)	0.012	0.002	0.011	0.007	0.017
Slope, (B)	0.019	0.25	0.026	0.115	0.056
Correlation Coefficient,(R)	0.996	0.997	0.994	0.997	0.995
Standard Deviation of Intercept (Sa)	0.004	0.0051	0.0035	0.0036	0.0085
Standard Deviation of Slope (Sb)	0.0025	0.0151	0.0047	0.008	0.0105
Regression equation,(y)	0.019X	0.25X	0.026X	0.115X	0.056X
Y=bx+a	0.012	0.002	0.011	0.007	0.017

*Limit of determination as the weight in µg mL^{-1} 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 **µ**g mL^{-1}.

Effect of time: A series of drug solution i.e. 1-7 mL of the drug solution was transferred in to separate 10 mL calibrated flasks. To each flask 1mL of 0.2M H_2SO_4 acid solution was added followed by 0.5 mL of $KMnO_4$ solution. The contents were mixed and the flasks were allowed to heat for 10 min. These were cooled and 1mL of Amaranth dye solution was added to each flask. After 5 min all solutions turned black hence the measurements were made immediately after mixing the solutions.

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

Drug	Taken (µg mL^{-1})	Found (µg mL^{-1})	Er (%)	Recovery (%)	RSD (%)	Proposed method mean ± SD
	5.0	4.99	0.40	99.60		99.97
ATV	10.0	10.01	0.33	100.33	0.365	±0.364
	15.0	15.0	0.00	100.00		
	1.0	1.0	0.00	100.00		99.97
CRV	3.0	3.02	0.66	100.66	0.705	±0.704
	4.0	3.97	0.75	99.25		
	12.0	11.98	1.0	99.00		99.66
DLP	20.0	20.01	0.33	100.33	0.665	±0.662
	28.0	27.99	0.33	99.66		
	3.0	3.01	0.33	100.33		99.75
DOX	9.0	8.98	0.57	99.43	0.500	±0.499
	15.0	14.98	0.50	99.50		
	5.0	4.99	1.14	98.86		99.88
OMZ	10.0	10.0	0.00	100.0	0.970	±0.969
	15.0	15.01	0.79	100.79		

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^{-1})	Drug added (µg mL^{-1})	Total found (µg mL^{-1})	Er (%)	Recovery (%)	RSD (%)	Reference method mean ± SD	Proposed method mean± SD	t-test	F-test
	0.50	5.0	5.49	0.67	99.33					
Reyatez	0.50	6.0	6.51	0.4	100.4					
(ATV)	0.50	7.0	7.50	0	100	0.469	99.70	99.93	0.825	0.764
	2.0	0.0	2.01	0.50	100.5		±0.874	±0.469	(2.45)	(4.28)
	4.0	0.0	3.98	0.5	99.5					
	6.0	0.0	5.99	0.17	99.83					
	0.50	1.0	1.51	1.43	101.43					
Coreg	0.50	1.2	1.69	1.11	98.89					
(CRV)	0.50	1.4	1.9	0	100	0.959	99.89	99.91	0.052	0.371
	1.0	0.0	0.99	1	99.00		±0.610	±0.958	(2.45)	(4.28)
	2.0	0.0	2.01	0.5	100.5					
	3.0	0.0	2.99	0.33	99.66					
	0.50	5.2	5.69	1.43	98.57					
Prevacid	0.50	5.4	5.91	1.11	101.11					
(DLP)	0.50	5.6	6.10	0	100	0.888	99.80	99.94	0.225	1.664
	12.5	0.0	12.49	0.4	99.6		±1.290	±0.888	(2.57)	(4.28)
	13.0	0.0	13.02	0.67	100.67					
	13.5	0.0	13.49	0.29	99.71					
	0.50	2.0.	2.49	0.67	99.33					
Doxine	0.50	2.5	3.01	0.5	100.5					
(DOX)	0.50	3.0	3.48	0.8	99.20	0.546	100.06	99.52	1.764	0.25
	1.0	0.0	0.99	1	99		±0.500	±0.544	(2.45)	(4.28)
	3.0	0.0	2.98	0.67	99.33					
	5.0	0.0	4.99	0.2	99.8					
	0.50	3.0	3.47	1.86	99.14					
Prilosec	0.50	4.0	4.51	0.22	100.22					
(OMZ)	0.50	5.0	5.49	0.18	99.82	0.397	99.65	99.60	0.213	0.175
	8.0	0.0	7.99	0.50	99.5		±0.418	±0.395	(2.45)	(4.28)
	9.0	0.0	8.97	0.75	99.25					
	11.0	0.0	10.98	0.33	99.67					

Validation of the proposed methods: Each method developed for quantification of drugs has been validated in terms of precision, accuracy, limit of detection, limit of quantification, linearity, selectivity, robustness and ruggedness. Calibration curves were drawn used to assess the recovery of the drug. To assess the precision, each experiment was repeated at least 3 times and accuracy is estimated in terms of % recovery and % RSD. Excellent percent recovery and RSD being less than 2 for each drug demonstrate accuracy and precision of the methods (Table 2). Further t-test and F-test values have also been calculated using a standard reference method. The t-test and F-test values are less than their permissible range indicating high accuracy of the methods. Limits of Linearity of calibration curves are mentioned in the under the title Beer's law limit. The methods developed have also been applied for the analysis of pharmaceuticals. The recovery experiments performed show high accuracy and precision and the results are compared to the available validated reported methods on each drug. The values % RSD and t- and F- tests are in the permissible range of experimental errors, and show that the methods can be used in both pharmaceutical and drug industries(Table 3).

APPLICATION

The methods are economical compared to other sophisticated analytical instruments, hence can be used for routine analysis of commercially available formulations.

CONCLUSION

The obtained results from the methods for the determination of above mentioned drugs indicate that methods are simple, accurate and precise. 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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