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SPECTROPHOTOMETRIC ESTIMATION OF A FEW COMMERCIAL DRUGS USING N-BROMOSUCCINAMIDE AND RHODAMINE–B COUPLE

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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.*, Atazanavir sulphate (ATV), Rivaroxaban (RXN), Warfarin Sodium (WAR), Rabeprazole sodium (RBZ) Potasium Dobesilate (PD). 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, Drugs, NBS, Rhodamine-B, Quantification, Validation.

INTRODUCTION

Atazanavir sulphate (ATV)

Atazanavir sulphate is one of the oral antiretroviral protease inhibitors used for treatment for HIV/AIDS. It is chemically methyl *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} butanehydrazido]-1-phenylbutan-2-yl] carbamoyl}-2,2-dimethylpropyl] carbamate. Atazanavir sulphate [Fig.1] it is official in Indian pharmacopoeia. Atazanavir is an azapeptide HIV-1 protase 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 Sectrophotometry[3.4], Liquid chromatography[5] and using UPLC[6] appear in the literature for the determination of Atazanavir sulphate in bulk and pharmaceutical formlations.

Rivaroxaban (RXN)

Rivaroxaban is chemically (*S*)-5-chloro-*N*-{[2-oxo-3-[4-(3-oxomorpholin-4-yl) phenyl] oxazolidin-5-yl] methyl} thiophene-2-carboxamide, it is an oral anticoagulant It preventing nonhemorrhagic strokes and embolic events. Rivaroxaban is associated with lower rates of serious and fatal bleeding events than warfarin though rivaroxaban is associated with higher rates of bleeding in the gastrointestinal tract. HPLC method with UV detector[7] HPLC,TLC densitometry, first-derivative and first-derivative ratio spetrophotometry method[8], anti – factor Xa chromogenic assay[9], ultra-performance liquid chromatography – tandem mass spectrometry (UPLC-MS/MS)[10],UV-spetrophotometric method[11] an chiral liquid chromatographic method.[12] The reports includes past quantification references on the drug.

Warfarin Sodium (WAR)

COUMADIN (warfarin sodium) is an anticoagulant that acts by inhibiting vitamin K-dependent coagulation factors. Chemically, it is 3-(α-acetonylbenzyl)-4- hydroxycoumarin and is a racemic mixture of the R- and S-enantiomers. Crystalline warfarin sodium is an isopropanol clathrate. Its empirical formula is $C_{19}H_{15}NaO_4$. HPLC method[13, 14], X-ray difration method[15], spectrophotometric method[16], Fluorescence spetrophotometric analysis[17], Raman spectroscopic method[18] methods for the estimation of drug.

Rabeprazole sodium (RBZ)

Rabeprazole is an antiulcer drug in the class of proton pump inhibitor, used in short-term treatment in healing and symptomatic relief of duodenal ulcer and erosive or ulcerative gastro esophageal reflux disease (GERD). It is chemically as 2-[[[4-(3methoxypropoxy)-3-methyl-2-pyridinyl]-methyl]sulfinyl]-1H–benzimidazole sodium salt, Rabeprazole is in a group of drugs called proton pump inhibitors. Rabeprazole decreases the amount of acid produced in the stomach. it is used to treat symptoms of GERD and other conditions involving excessive stomach acid such as Zollinger-Ellison syndrome. It is also used to promote healing of erosive esophagitis (damage to your esophagus caused by stomach acid). Rabeprazole may also be given with an antibiotic to prevent gastric ulcer caused by infection with helicobacter pylori (H. pylori). It is not for immediate relief of heartburn symptoms. Reverse Phase-HPLC

method[19,20,21], Liquid chromatography/tandem mass spectrometry[22] and UV-Spectrophotometry method.[23,24]

Potassium Dobesilate (PDB)

Potassium dobesilate is chemically Potassium 2, 5-dihydroxybenzenesulfonate, potassium dobesilate exerts anti-tumorigenic effects and may play a useful role in the chemoprevention of skin cancers. Dobesilate is a drug that blocks the activity of FGF, Fibroblast growth factor (FGF) is involved in skin tumorigenesis: it promotes cell viability, induces angiogenesis and stimulates invasiveness. The primary objective was to evaluate the efficacy and tolerability of potassium dobesilate 5% cream in the treatment of actinic keratoses. RP-HPLC method[25, 26], HPLC and UV- spetrophotometry method[27] Spetrophotometric and spectrodensitometric method[28,29],Catalytic reaction mechanism.[30]

STRUCTURES OF DRUGS

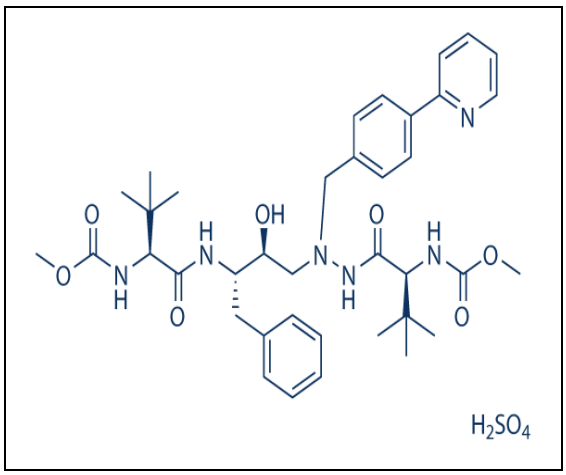

Figure 1(a) Atazanavir sulphate

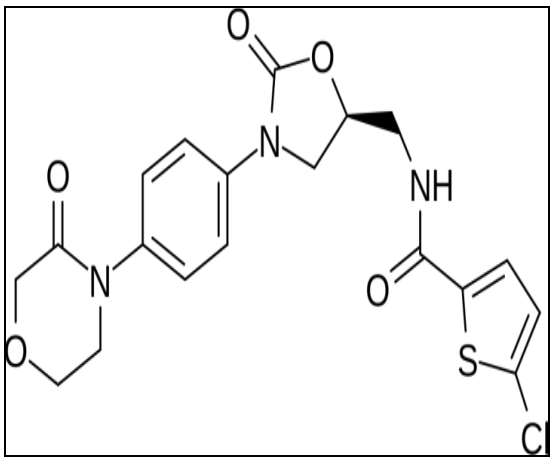

Figure 1(b) Rivaroxaban

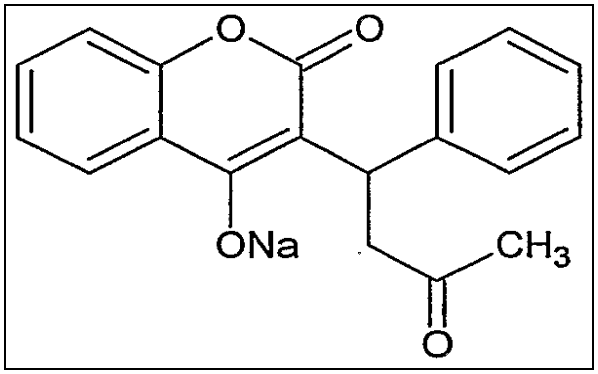

Figure 1(c) Warfarin sodium

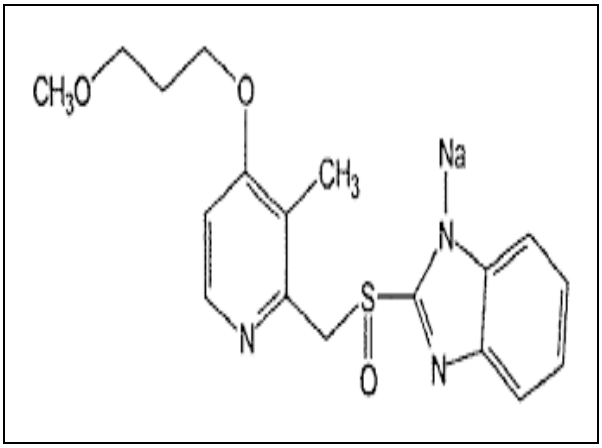

Figure 1(d) Rabeprazole sodium

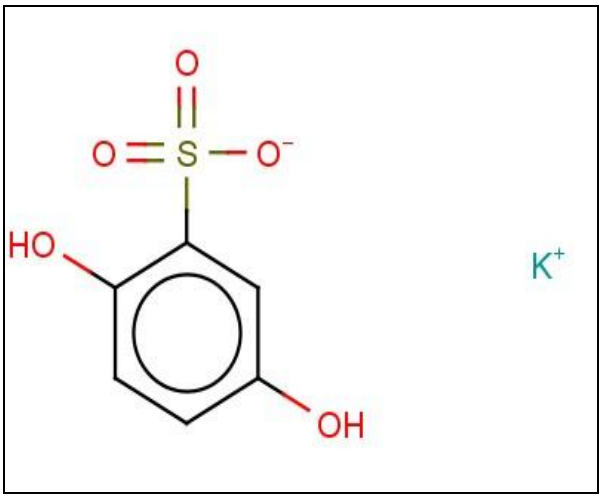

Figure 1(e) Potassium Dobesilate

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 estimation of the above drugs.

METIRIALS AND METHODS

Reagents and standards

The pharmaceutical grade drugs were supplied by Dr. Reddy's laboratory and Arabindo pharmaceutical, Hyderabad. NBS, Rhodamine –B and 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 (0.001M) 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 ATV, RIV, WAR, RBZ and PD 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.

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 occational 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).

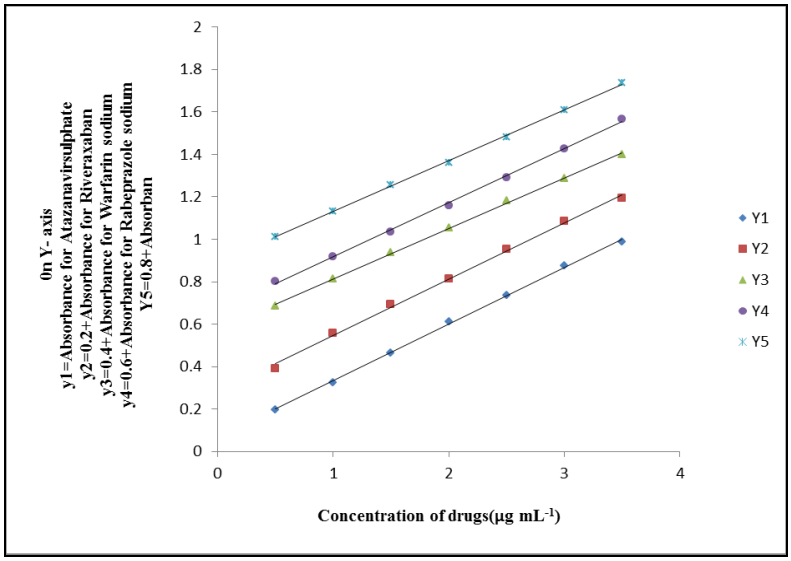

Figure-2 Calibration curves of drugs ATV, RXN, WAR, RBZ and PDB.

TABLE 1 Analytical and regression parameters of spectrophotometric methods

Parameter	**ATV**	**RXN**	**WAR**	**RBZ**	**PDB**
λ_{max} (nm)	557	557	557	557	557
Beer's Law Limits (**µ**g mL^{-1})	0.5-3.5	0.5-4.0	0.5-4.0	0.5-4.0	0.5-4.0
Molar absorptivity (L $mol^{-1}cm^{-1}$)	0.316x 10^5	0.185x 10^5	0.756x 10^5	0.153x 10^5	0.970x 10^5
Sandell sensitivity* (**µ**g cm^{-2})	0.0041	0.0037	0.0042	0.0039	0.0041
LOD (**µ**g mL^{-1})	0.0259	0.1307	0.1392	0.0652	0.0897
LOQ (**µ**g mL^{-1})	0.0786	0.3962	0.4219	0.1976	0.2719
Intercept, (A)	0.065	0.081	0.175	0.063	0.091
Slope, (B)	0.267	0.265	0.237	0.253	0.239
Correlation Coefficient,(R)	0.999	0.997	0.999	0.998	0.999
Standard Deviation of Intercept (Sa)	0.0021	0.0105	0.0100	0.0051	0.0065
Standard Deviation of Slope (Sb)	0.0408	0.0247	0.0030	0.0130	0.0147
Regression equation,(y) Y=bx+a	0.267x +0.065	0.265x +0.081	0.237x +0.175	0.253x +0.063	0.239x +0.091

*Limit of determination as the weight in **µ**g / 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 **µ**g / mL.

RESULTS AND DISCUSTION

Accuracy and Precession studies

Accuracy of the methods developed are 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.

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
ATV	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
RXN	1.0 3.0 4.0	1.0 3.02 3.97	0.00 0.66 0.75	100.00 100.66 99.25	0.705	99.97 ±0.704
WAR	2.0 4.0 6.0	1.98 4.01 5.99	1.0 0.33 0.33	99.00 100.33 99.66	0.665	99.66 ±0.662
RBZ	3.0 3.5 4.0	3.01 3.48 3.98	0.33 0.57 0.50	100.33 99.43 99.50	0.500	99.75 ±0.499
PDB	3.5 4.0 5.0	3.46 4.0 5.04	1.14 0.00 0.79	98.86 100.0 100.79	0.970	99.88 ±0.969

Analysis of Pharmaceutical preparation

Three tablets of (Reyataz-200mg) were weighed and ground in to fine powder. Weight equivalent to 10mg of Atazanavir sulphate 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 (Coumadin-300mg) were weighed and ground in to fine powder. Weight equivalent to 10mg of Warfarin 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 (Xarelto-20mg) were weighed and ground in to fine powder. Weight equivalent to 10mg of Warfarin 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 (Aciphex-20mg) were and ground in to fine powder. Weight equivalent to 10mg of Rabeprazole 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.

Potasim dobesilate 5% cream (Pot.dobesilate-5%- 10mg). Weight equivalent to 10mg of Potassium dobesilate 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.

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
Reyatez (ATV)	0.50 0.50 0.50 2.5 3.0 3.5	0.5 1.0 1.5 0.0 0.0 0.0	0.99 1.51 1.99 2.49 3.01 3.47	1.00 0.66 0.50 0.40 0.33 0.85	99.00 100.66 99.50 99.60 100.33 99.15	0.657	99.346 ±0.874	99.70 ±0.655	0.793	0.561
Xarelto (RXN)	0.50 0.50 0.50 1.0 3.0 4.0	0.4 0.8 1.2 0.0 0.0 0.0	0.90 1.29 1.68 0.99 3.02 3.98	0.00 0.77 1.17 1.00 0.66 0.50	100.00 99.23 98.83 99.00 100.66 99.50	0.687	100.86 ±0.630	99.54 ±0.684	3.477	1.178
Coumarin (WAR)	0.50 0.50 0.50 2.0 4.0 6.0	0.3 0.6 0.9 0.0 0.0 0.0	0.79 1.10 1.39 2.02 3.99 5.98	1.25 0.00 0.71 0.99 0.25 0.33	98.75 100.00 99.29 100.99 99.75 99.66	0.750	99.484 ±0.896	99.74 ±0.748	0.537	0.696
Aciphex (RBZ)	0.50 0.50 0.50 3.0 3.5 4.0	0.2 0.4 0.6 0.0 0.0 0.0	0.71 0.90 1.12 2.99 3.48 3.99	1.40 0.00 1.79 0.33 0.57 0.25	101.40 100.00 101.79 99.66 99.43 99.75	0.998	100.31 ±0.820	100.33 ±1.001	0.037	1.490
Potasium dobesilate	0.50 0.50	1.0 2.0	1.48 2.51	1.33 0.39	98.67 100.39	0.620	99.86 ±0.783	99.71 ±0.618	0.368	0.623

(PD)	0.50 3.5 4.0 5.0	3.0 0.0 0.0 0.0	3.49 3.48 4.01 4.99	0.28 0.57 0.25 0.20	99.72 99.43 100.25 99.80					

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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