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Spectrophotometric Method Development and Validation for Atazanavir Sulphate and Ritonavir In Bulk and Tablet Dosage form using Absorption Ratio Method

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ABSTRACT

A simple, economic, accurate Absorption ratio method was developed for the simultaneous estimation of Atazanavir Sulphate and Ritonavir in bulk and tablet dosage form. 0.1M Hydrochloric acid was used as a diluent. 1% Sodium Lauryl Sulphate is used as surfactant to enhance solubility of drugs in 0.1M hydrochloric acid. The absorptions were observed at 262.8nm and 297nm which were selected based on overlap spectra of Atazanavir Sulphate and Ritonavir. The linearity range was found to be 10-20 µg/ml. The proposed method was validated. The reports was expressed that the proposed method was found to be simple, precise, accurate and rapid for the simultaneous estimation of Atazanavir Sulphate and Ritonavir in bulk and tablet dosage form using absorption ratio method.

Keywords: Atazanavir sulphate, Ritonavir, 0.1M Hydrochloric acid ,1%SLS, Absorption ratio.

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INTRODUCTION

Atazanavir sulphate(ATZ) is chemically (3S,8S,9S,12S) - 3, 12 – Bis (1,1-dimethylethyl) – 8 - hydroxy-4,11-dioxo-9-(phenylmethyl)-6-{[4-(2-pyridinyl)phenyl]methyl{-2,5,6,10,13-penta-azatetra-decanedioic acid dimethyl ester, sulfate (1:1). Its molecular formula and molecular weight are $C_{38}H_{52}N_6O_7$ and 704.8555 respectively and the structural formula is in figure 1. Generally, it is a white to pale yellow crystalline powder. ATZ is slightly soluble in water and soluble in methanol(1). ATZ selectively inhibits the virus-specific processing of viral Gag and Gag-Pol polyproteins in HIV-1 infected cells by binding to the active site of HIV-1 protease, thus preventing the formation of mature virions. Atazanavir is not active against HIV-2 (2). Literature survey revealed that various analytical methods such as UV spectrophotometry (3-6), HPLC (7,8) methods have been reported for estimation of ATZ from its formulations and biological fluids.

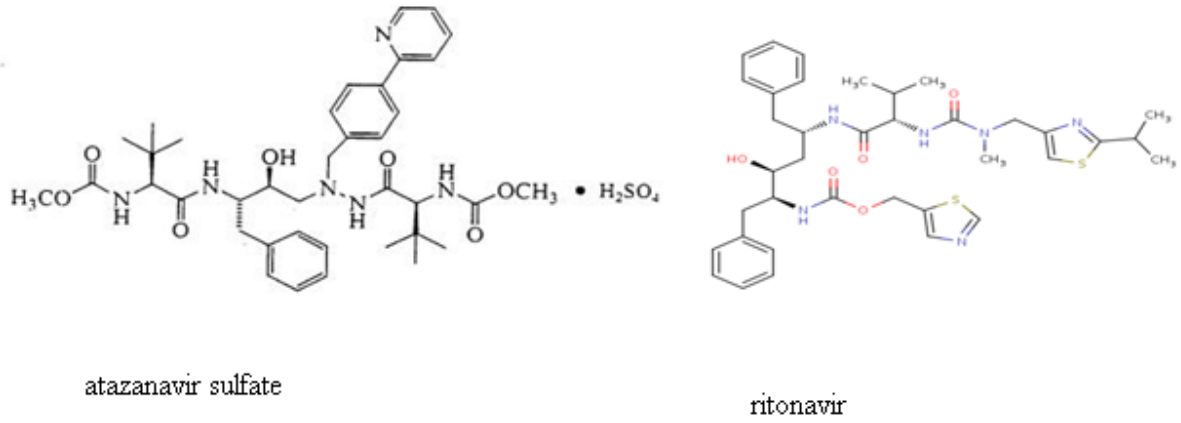

Figure 1: structures of Atazanavir sulphate and Ritonavir:

Ritonavir(RIT) is chemically, 1,3-thiazol-5-ylmethyl N-[(2S,3S,5S)-3-hydroxy-5-[(2S)-3-methyl-2-{[methyl({[2-(propan-2-yl)-1,3-thiazol-4-yl]methyl})carbamoyl]amino}butanamido]-1,6-diphenylhexan-2-yl]carbamate. It is an antiretroviral drug from the protease inhibitor class used to treat HIV infection and AIDS. The molecular formula is $C_{37}H_{48}N_6O_5S_2$ and the structural formula is in figure 1. RIT is a yellow crystalline substance, practically insoluble in water but soluble in ethanol (1,9). It has a molecular weight of 720.95 (2). Detailed survey of literature for RIT revealed several methods that have been reported for the assay of it either alone or in combined form in drug formulations. These analytical techniques include UV-Visible (Vis) spectrophotometry (10-13), HPLC (14-16) and High performance thin layer chromatography (17), LC-MS (18). The scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte to be more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the

development and validation. A survey of literature reveals that very few analytical methods UV Visible spectrophotometry (19), RP-HPLC (20), are available for the drug combination ATZ and RIT, Hence it is proposed to develop new methods for the assay of ATZ and RIT in pharmaceutical dosage forms adapting UV Visible spectrophotometry. The objective of the proposed method was to develop simple and accurate methods for the determination of ATZ and RIT simultaneously using absorption ratio method by UV-Spectrophotometry in pharmaceutical dosage forms.

MATERIALS AND METHODS:

Standards of ATZ and RIT and commercial sample ATZ and RIT tablets were procured from local market and used within their shelf-life period. The Hydrochloric acid from S.D. fine chemical limited, India was of pharmaceutical or analytical grade. Sodium Lauryl Sulphate from Loba cheme laboratory was of pharmaceutical or analytical grade. Quantitative estimation was performed on Labindia UV 3000+ and Elico SL 210 double beam UV visible spectrophotometers with matched 1 cm path-length quartz cells. Absorption spectra was recorded on a fast scan speed, setting slit width to be 1 nm and sampling interval to be auto. Labindia UV Win software was used along with quartz cuvette for the λmax prediction. To develop a suitable and robust absorption ratio method for the determination of ATZ and RIT, different diluents were tried based on the solubility and functional group present in the compound. Finally 0.1M Hydrochloric acid was selected due its positive results. Absorbance were measured at selected λmax (262.8nm and 297nm) based on the overlap spectra of both drug spectrum. The data were collected and analyzed with software in a computer system.

Preparations:

Stock solution of ATZ (200mcg/ml) was prepared by dissolving 10 mg of ATZ in 50 ml of volumetric flask containing 20ml of 0.1M HCl in 1%SLS. The solution was sonicated for about 15 minutes and then made up to volume with mobile phase. From the stock solution, 1ml was pipetted out and transferred into the 10ml volumetric flask to get 20µg/ml concentration. Same procedure followed for RIT standard. The final solutions of both standard drugs solutions were undergone for scanning and overlapped each other. Two wavelengths were selected. Among the two, 297nm is a λmax of ATZ and 262.8 nm is an isobestic point. Then the absorbance was measured at 262.8nm and 297nm and calculated the absorptivity.

Preparation of standard mixture:

From 200µg/ml of ATZ and RIT standard stock solutions, 1ml was pipette out individually and

mixed in 10ml volumetric flask then it was made up to the mark with 0.1M HCl in 1%SLS. Absorbance were measured at selected λmax (262.8nm and 297nm)

Preparation of tablet mixture:

20 tablets were weighed and powdered. The amount of powder equivalent to 15mg of ATZ and 5mg of RIT were weighed and transferred into the 100ml of volumetric flask containing 20 ml of 0.1m HCl in 1%SLS. The solution was sonicated for about 20 minutes and then made up to volume with mobile phase. The solution was filtered. From the filtrate, 2ml was pipetted out and transferred into the 10ml volumetric flask then made up to the mark with 0.1m HCl in 1%SLS. A typical overlap spectrogram of standard ATZ and RIT was shown in figure 2. The amount of drug present in pharmaceutical formulation was calculated through the following formula (21):

Cy=(A1/ax1)-Cx

Cx=((Qm-Qy)/Qx-Qy))(A1/ax1), where, Cy is a concentration of RIT in mixture; Cx is a concentration of ATZ in mixture; Qx(absorption ratio of drug 1)=ax2/ax1; Qy(absorption ratio of drug 2)=ay2-ay1; Qm(absorption ratio of mixture=A2/A1 where A1 is absorption at 262.865nm in mixture; A2 is absorption at 297nm in mixture and a is an absorptivity.

Validation:

The described method has been validated for the assay of ATZ and RIT using following parameters (22). Linearity was studied to find out the relationship of concentration with absorbance. The different concentrations of ATZ and RIT mixtures (10 to 20µg/ml of each drug in the mixture) were taken for linearity. The all solutions were undergone for scanning and measured the absorbance at 262.8nm and 297nm. The calibration graph was constructed by plotting the absorbance versus the final concentration of the drug (µg/mL). Alternatively, the corresponding regression equation was derived. Precision was studied to find out variations in the test methods of mixtures of ATZ and RIT (20µg/ml) on the same day and on different day by using different Instrument (Elico SL210, Labindia UV 3000+) (Ruggedness). The precision of each method was ascertained separately from the absorbance obtained by actual determination of six replicates of a fixed amount of drug (20µg/ml). Precision and Ruggedness were done on the same day and the different day respectively and the %RSD was calculated for each. The accuracy of the method was shown by analyzing the model mixtures contained 12, 15, 18µg/ml of sample solution of mixture of drug 1 and 4, 5 and 6µg/ml of sample solution of mixture of RIT and along with 10µg/ml of bulk standard solutions of ATZ and RIT were taken. After the measurement, the Amount found, Amount added for ATZ and RIT and individual recovery were calculated. LOD and LOQ were calculated based on the calibration curve method.

RESULTS AND DISCUSSION:

Anabsorption ratio method procedure was proposed as a suitable method for the analysis of drug ATZ and RIT in dosage forms. The λmax was found to be 262.8nm and 297nm. The regression equation for the method at 262.8nm was found to be y=0.01x-0.002 (r^2=0.997) where 0.01 is a slope; -0.002 is an intercept; r^2 is correlation coefficient (0.997) and found to be linear over Beer's range 10-20µg/ml respectively. The regression equation for the method at 297nm was found to be y=0.021x-0.010 (r^2=0.9984) where 0.021 is a slope; -0.010 is an intercept; r^2 is correlation coefficient (0.9984) and found to be linear over Beer's range 10-20µg/ml respectively. The linearity graph was shown in figure 3. The percentage of purity of ATZ and drug RIT in tablet dosage form was 102.8849% and 103.709% respectively. The spectrogram of different concentrations of mixtures consist ATZ and RIT was shown in figure 2. The precision of the spectrophotometer system was determined using the %RSD of the absorbance for six replicate injections of the drug. The %RSD was less than 2. Precision data were present in Table 1. In order to verify the accuracy of the described method, recovery studies were carried out by analyzing model mixtures contained 80%, 100% and 120% of sample solution of ATZ and RIT and along with 10µg/ml of bulk standard solution within the linearity ranges. The mean percentage recoveries were found to be 101.3, 101.1 and 98.1%w/w for 80%, 100% and 120% respectively. Accuracy data were present in Table 2. The percent recoveries values indicate less interference from excipients used in formulation. LOD and LOQ values were present in table 3 which summarizes the result of validation parameters.

Table 1: Data for Precision

	Absorbance at 262.8nm	**Absorbance at 297nm**	**Absorption ratio**	**Conc. of Atazanavir sulphate**	**Conc. Ritonavir**
Drug 1 standard	0.263	0.606	2.304183		
Drug 2 standard	0.263	0.22	0.836502		
mixture(m1)	0.489	0.771	1.576687	1.875392	1.84324
mixture(m2)	0.485	0.758	1.562887	1.825371	1.862842
mixture(m3)	0.49	0.761	1.553061	1.819244	1.906992
mixture(m4)	0.491	0.769	1.566191	1.85636	1.87748
mixture(m5)	0.485	0.754	1.554639	1.804645	1.883567
Mixture(m6)	0.481	0.759	1.577963	1.847889	1.809906
Mean	0.486833	0.762	1.565238	1.83815	1.864004
SD	0.003817	0.006633	0.01058	0.026293	0.033975
%RSD	0.783971	0.870505	0.6759	1.430425	1.822677

Table 2: Data for accuracy

	Absorbance at 262.8nm	Absorbance at 297nm	Absorption ratio	Concentration of Atazanavir sulphate	Concentration of Ritonavir	% recovery for Atazanavir sulphate	% recovery for Ritonavir
Accuracy 1 (80%)	0.168	0.3465	2.0624	2.2148	1.437365	100.6731	102.669
Accuracy 2(100%)	0.186	0.394	2.1182	2.5205	1.522887	100.8236	101.5258
Accuracy 3(120%)	0.1985	0.4285	2.1587	2.7429	1.572284	97.9618	98.26778

Table 3: Results summary sheet for the method.

Validation parameter	Atazanavir sulphate	Ritonavir	Acceptance criteria
Linearity range	10-20mcg/ml	10-20mcg/ml	Obeys beer's law
Regression equation	y=0.01x-0.002	y=0.021x-0.010	
R^2	0.997	0.9984	NLT 0.99
slope	0.01	0.021	
intercept	-0.002	-0.01	
% assay	102.8849	103.709	Within 95-105%
Precision(%RSD)	1.430425	1.822677	NMT 2.0
Accuracy	99.8195	100.82086	Closeness to 100%
LOD	1.1354	0.0131	
LOQ	3.4407	0.0397	

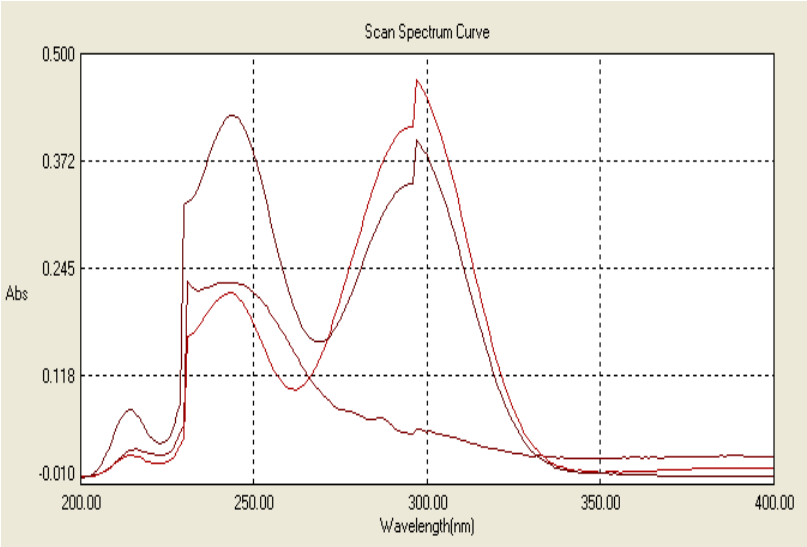

Figure 2: Overlap spectrogram of Atazanavir sulphate and Ritonavir and their mixture

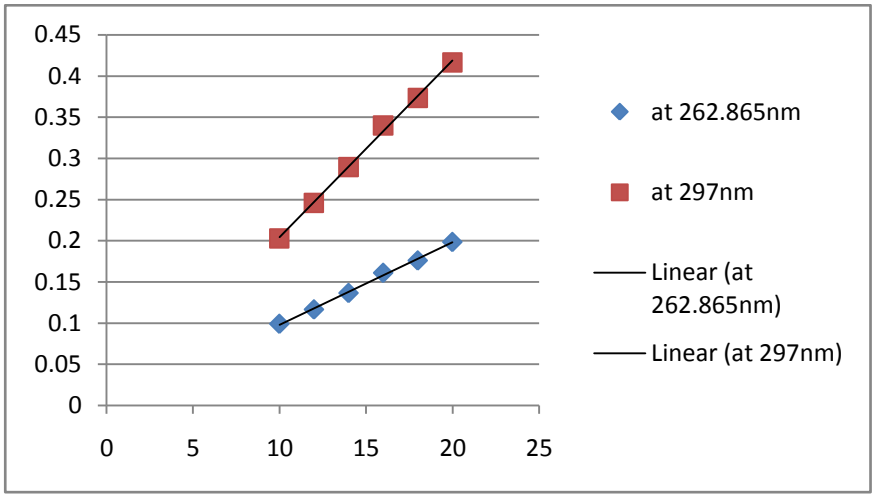

Figure 3: Linearity graph for absorption ratio method for estimation of Atazanavir sulphate and Ritonavir.

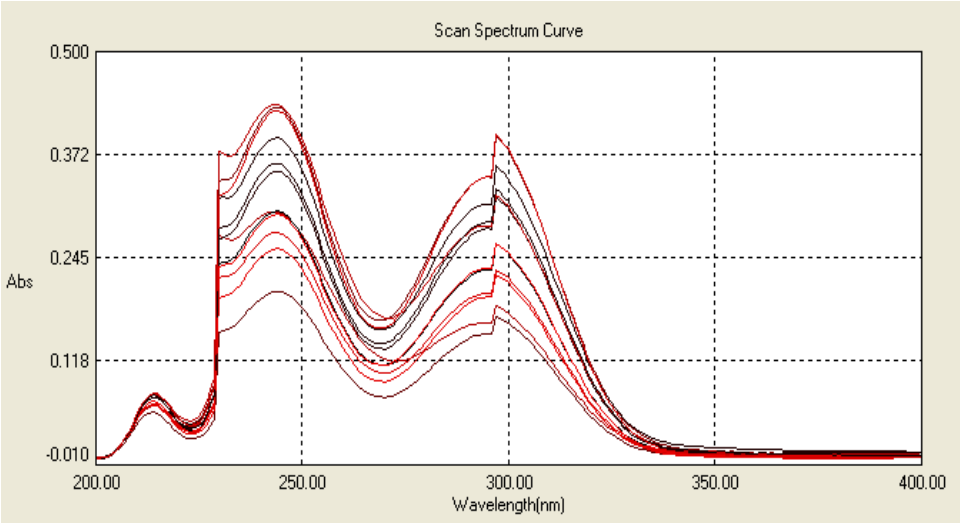

Figure 4: Spectrogram of different concentration of mixtures of Atazanavir sulphate and Ritonavir

CONCLUSION:

The presented method was precise, sensitive and accurate. The advantages of proposed method were its simple procedure for sample preparation. The satisfying recoveries and low coefficient of variation confirmed the suitability of proposed method for the routine analysis of Atazanavir sulphate and Ritonavir in pharmaceuticals.

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