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Development of New Visible Spectrophotometric Assay Methods for Atazanavir in Pure and Dosage Forms

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ABSTRACT

Some new selective accurate and economical spectrophotometric methods for the determination of Atazanavirin pure and dosage forms have been described in the present work. These developed methods have been extended to pharmaceutical formulations as they are simple, economical and sensitive. The present methods involve the formation of highly stable colored species which makes it easier for the determination of Atazanavirin pharmaceutical dosage at the given optimum conditions. The stock solution (1.0 mg/mL) of Atazanavir was prepared by dissolving 100 mg of the drug in 10.0 mL of methanol and made up to 100 mL with distilled water to get a clear solution. Appropriate volume of this stock solution was diluted step wise to get the working standard solutions of 160 µg/mL for Method-M_8;200 µg/mL for Method-M_9& M_{10} and 250 µg/mL for Method- M_{11}respectively. The effect of wide range of excipients and other inactive ingredients usually present in the formulations for the assay of Atazanavir under optimum conditions were investigated. The values obtained by the proposed and reference method for formulations were compared statistically with F and t tests and found not to be different significantly.

Keywords: Atazanavir, UV spectrophotometric Methods, Optical Characteristics, Recovery Studies, Precision.

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INTRODUCTION

Atazanavir Sulfate (ATV) [1] methyl N'-[(1S)-1-{N-[(2S, 3S)-2-hydroxyl-3-[(2S)-2-[(methoxycarbonyl) amino]-3, 3-dimethylbutanamido]-4-phenylbutyl]-N'-{[4-(Pyridin-2-yl)phenyl] methyl} hydrazine carbonyl}-2, 2-dimethyl propyl] carbamate (Figure-1), is an azapeptide HIV-1 protease inhibitor (PI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1).Atazanavir selectively inhibits the virus-specific processing of viral Gag and Gag-poly proteins in HIV-1- infected cells, thus preventing the formation of mature virions. Atazanavir is also sometimes used to prevent infection in healthcare workers or other people who were accidentally exposed to HIV. Keeping in view of the above discussion, in the present study the authors have attempted in developing of few analytical methods for Atazanavir in pure and dosage forms using visible spectrophotometry and High performance liquid chromatography as analytical tools. Basing on the analytical survey till date few HPLC methods [2-10] and no stability-indicating HPLC method was reported for the assay of Atazanavir in pure and dosage forms. The present study is aimed in development and validation of stability indicating method assay of Atazanavir in pure and dosage forms by RP-HPLC as per ICH guidelines. The RP-HPLC method developed by the authors in this present investigation facilitated in studying the stability the Atazanavir related drugs when exposed to various degradation conditions (acidic, basic, heat and oxidative) and would be of great applicable in the analysis of purity and pharmacokinetic studies for quality control and clinical monitoring laboratories. Literature survey reported few spectrophotometric methods [3-7] for the determination of Atazanavir in dosage forms. This prompted the authors to develop new and accurate methods for the determination of Atazanavir in pharmaceutical formulations using UV-visible spectrophotometry utilizing inexpensive analytical reagents that are available in any analytical laboratories. Rao et al. have reported their work on different oxide materials in their earlier studies [11-30].This present paper aimed to develop simple, rapid and sensitive UV-visible spectrophotometric methods for the assaying Atazanavir in pure and formulations of Atazanavir. The proposed methods developed were validated according to the evaluation of the validation parameters.

EXPERIMENTAL

Apparatus

UV-Visible Spectrophotometer: An Elico SL-159 model,2 nm high resolution, double beam, 1cm length quartz coated optics; Wavelength range190-1100nm; High stability,linearity, precision instrument was used for all the spectral measurements.

Precision Balance:0.001 g – 200g readability capacity linearity balance was used in the present assay for weighing the required amount of the drug and the reagents.

Preparation of reagents: Analytical grade chemicals and reagents were used in thepreparation of solutions and all the solutions were prepared in triply distilled water.

Method–M_8 {FC Reagent (2N)}: Folin Cio Calteu reagent used as it is.**Na_2CO_3 solution (10%):** Prepared by dissolving 10 gms of sodium carbonate in 100 mL of distilled water.

Method-M_9 {PHEN (0.2 % W/V)}: Prepared by dissolving 200 mg of o-phenanthroline in 100 mL of 0.1N hydrochloric acid. **Fe (III) solution (1.0 % W/V):** About 100 mg of anhydrous ferric chloride was accurately weighed and dissolved in 100mL of distilled water. 50 mL of above stock solution was further diluted to 100mLwith water. **O-Phosphoric acid solution:** Prepared by mixing 1.3 mL of o-phosphoric acid with 100 mL of distilled water.

Method–M_{10} {2, 2'-Bipyridyl solution (0.01M)}: Aqueous solutions of 2, 2"-Bipyridyl (0.01M), Ferric chloride (0.003 M) and ortho phosphoric acid (0.2 M) were prepared with double distilled water.

Method-M_{11}: Solution of various reagents such as p-chloranilic acid solution (Sd-fine; 0.1 %) were prepared.

Preparation of Stock and Working Standard Solutions: The stock solution (1.0mg/mL) of atazanavir was prepared by dissolving 100mg of the drug in10.0mL of methanol and made up to 100mL with distilled water to get a clear solution. An appropriate volume of this stock solution was diluted step wise to get the working

standardsolutions of **160µg/mL** for **Method-M_8;200µg/mL** for **Method-M_9&M_{10}**and **250µg/mL for Method-M_{11}**respectively.

Procedure for Tablets: About twenty capsules of Atazanavir (ATAZOR-300mg) purchased from local pharmacy were removed pulverized and the powder equivalent to 100mg of Atazanavir was accurately weighed and transferred into a 100mL calibrated flask,60mL of water was added and the content shaken thoroughly for 15-20 min to extract the drug into the liquid phase and later the volume was finally diluted to the mark with double distilled water, mixed well and filtered through What man filter paper No 41. This filtrate was made up to the mark with distilled water in a 100mL volumetric flask. A suitable volume of this filtrate was accurately diluted with distilled water and this solution was used for the determination of Atazanavir as per the proposed procedures described below respectively.

RESULTS & DISCUSSION

Method Development: It involves the optimizationstudies of each proposedmethod that directly influences the color development [optimal conditions]. The optimumconditions for each proposed method were established by varying one variable and observing its effect on the absorbance of the colored products. It involves the careful study of the effect of various parameters which include effect of reagent concentration, order of addition, time, temperature and choice of solvent on color development.

Method–M_8[FC]: The optimum conditions in this method were fixed basing on the study ofthe effects of various parameters, such as volume of 2N FC- reagent, nature and volume of base (NaOH) for maximum color development, keeping time, the order of addition of reagent and the stability of the colored species formed after the final dilution have been studied and the optimum conditions are incorporated in the prescribed procedure.

Method–M_9 (Fe [III]-1, 10-PTL): In order to establish optimum conditions necessary forrapid and quantitative formation of the colored complex with maximum stability and sensitivity, the author had performed controlled experiments by varying one and fixing the other parameters, such as effect of volume of Fe (III) solution, o-Phenanthroline solution and o-Phosphoric acid, temperature, heating time, order of addition of reagents and nature of solvents for final dilution.

Method–M_{10} (Fe[III] - 2,2-Bipyl): The experimental variables for the formation of thestable colored product were optimized. Several experiments were carried out to study the effect of 2,2-Bipyl on the color development. The author has performed controlled experiments by varying one and fixing the other parameters, such as effect of volume of Fe (III) solution, 2,2-Bipyl solution, and o-Phosphoric acid, temperature; heating time, order of addition of reagents and nature of solvents for final dilution.

Method–M_{11} (PCA): The Optimization studies for the color development for the proposedmethod for the assay of Atazanavir and the optimum conditions are incorporated in the prescribed procedure.

Proposed Procedures: After systematic and detailed study of the variousparameters involved (fixation of optimization conditions), the following procedures were proposed for the assay of Atazanavir in pure and pharmaceutical formulations.

Method-M_8 (FC): Into a series of 10mL volumetric flasks, standard solution (160 µg/mL) ofatazanavir in the concentration range of 8.0 – 40.0 µg (0.5-2.5 mL) were transferred. Then 3.0 mL of NaOH solution, 1.0 mL of FC reagent were successively added and kept aside for 5 min. The volume was made up to 10 mL with water. The absorbance was measured at 745 nm against reagent blank. The amount of Atazanavir was deduced from its Beer- Lambert's plot (Figure-2(b)).

Method-M_9 (Fe[III] - 1,10-PTL): Aliquots of standard Atazanavir solution containing 10 to50µg were transferred into a series of 10mL volumetric flasks and 1.0mL of 0.003M ferric chloride was added to each flask. Then 1.0mL of PTL solution was added to all flasks and the volumes in all volumetric flasks were equalized with water. The contents in each flask were gently boiled for 30min and then cooled to room temperature. To the above flasks 2.0mL of OPA was added and the final volume of all volumetric flasks was brought to 10mL with water. The absorbance of the colored product was measured at 510 nm against

corresponding reagent blanks and the amount of Atazanavir was assayed from corresponding calibration graph drawn (Figure 3(b)). **(Fe[III]-2,2'-Bipyridyl):**Aliquots(0.5-2.5mL,200µg/mL)of standard Atazanavir solution were transferred into a series of 10.0mL calibrated tubes and then solutions of $FeCl_3$ (1.0 mL) and 2,2-Bipyridyl (1.0mL) was added successively. The total volume in each test tube was brought up to 4.0mL with distilled water and heated for 10minutes in a boiling water bath at 90°C. After cooling to the room temperature, 2.0mL of O-phosphoric acid was added in each test tube. The absorbance of the orange colored complex was measured after 5min at 495 nm against reagent blank prepared similarly (Figure-4(b)).

Method-M_{11}, PCA: Different aliquots of Atazanavir standard solutions (0.5 -2.5mL;250µg/.mL) were accurately transferred into a series of 10.0mL calibrated tubes and the total volume was adjusted to 3.0mL by adding adequate quantity of acetonitrile. To each tube 2.0mL of 0.1% p-chloranilic acid was added, and the contents were mixed well and kept aside for 10min. The mixture was diluted to the volume with acetonitrile and the absorbance of the colored complex developed in each tube was measured at 520nm against a reagent blank prepared similarly. The concentration of the Atazanavir was read from the standard graph using the Beer's law data (Figure-5(b)).

Method Validation

Spectral Characteristics: The maximum absorption (λ_{max}) of the colored species formed in the above methods was obtained from the absorption spectra obtained by scanning the absorbencies of the colored products formed by spectrophotometer in the wavelength region of 340 to 900nm against similar reagent blank or distilled water. The results were graphically represented in Figure-2(a) to 5(a) for M_8 to M_{11} respectively.

Optical Characteristics: The Beer's law plots of the proposed methods were recorded graphically [Figure-2(b) to 5(b) for $M_8$to M_{11}]. The 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^2) for each proposed method and the values are presented in Table-1(a&b). The analytical parameters such as Beer's law limits, molar absorptivity and Sandell's sensitivity values were calculated for all the proposed methods and their results were summarized in Table-1(a&b). The LOD of all the proposed methods were calculated and the results are listed in the same Table-1(a&b).

Precision: The precision of each proposed methods was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of Atazanavir in total solution. The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated for all the proposed methods The calculated relative standard deviation values were found to be very small below 2% indicating good repeatability and reliability of the proposed methods. The results and their statistical analysis were summarized in Table-1(a&b).

Accuracy: In determining the accuracy of each proposed method a fixed amount of Atazanavir from dosage form was taken and pure standard drug at three different concentrations within Beer's range was added. The total concentration was determined by the proposed method in triplicate and the average percent recovery of the added standard was calculated. The results of the recovery study are tabulated and the results obtained showed excellent mean recovery percent values, close to 100 % which indicate high accuracy of the proposed methods.

Analysis of Formulations: Commercial formulations (tablets) containing Atazanavir were successfully analyzed by the proposed methods. The values obtained by the proposed and reference method [6] for formulations were compared statistically with F and t tests and found not to be different significantly.

Nature of the Colored Species: It is difficult to predict the exact reaction mechanism of colored species formed in the proposed methods. Therefore an attempt have been made by the author to describe the nature of colored species in each of the proposed methods for Atazanavir on the basis of reactive functional moiety (secondary amine group) present in the present studied drug with the reagents used.

Method–M_{10}: The mechanism of the reaction in the proposed methods $M_{10}$is based onthe formation of donor-acceptor (DA) complex through the interaction between tertiary amine moiety of the selected drug [Atazanavir] as n-electron donor and PCA as π- acceptor as shown in the Scheme -1**.**

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CONCLUSION

The proposed visible spectrophotometric methods for the estimation of Atazanavir are possessing reasonable precision, accuracy, simple, sensitive, and can be used as an alternative method to the reported ones for the routine determination of Atazanavir. Moreover, the proposed methods rely on the use of simple and cheap chemicals and are based on well characterized color reactions. The procedures described in this section meet most of the demands of analytical chemists namely selectivity, simplicity, reliability and cost of analysis. The effect of wide range of excipients and other inactive ingredients usually present in the formulations for the assay of Atazanavir under optimum conditions were investigated. The values obtained by the proposed and reference method for formulations were compared statistically with F and t tests and found not to be different significantly. Thus, it can be concluded that visible spectrophotometric methods of Atazanavir are more versatile and easy to apply than other instrumental methods.

Table-1(a): Results of Method Validation of the Proposed Methods for the Determination of Atazanavir

Parameter	M8	M9	M10
λ_{max} (nm)	745	510	495
Beer's law limits (µg/mL)	8.0-40.0	10.0-50.0	10.0-50.0
Molar absorptivity (1 mol^{-1}. cm^{-1})	$1.889x10^3$	$3.143x10^3$	$2.791x10^3$
Sandell's sensitivity (µg.cm^{-2}/0.001 A.U)	0.0597	0.0448	0.0505
Regression equation (Y=a+bc); Slope (b)	0.0160	0.0108	0.0096
Intercept (a)	0.0109	0.0084	0.0111
Correlation coefficient (r)	0.9994	0.9997	0.9995
Relative standard deviation (%)*	0.475	0.986	1.076
% Range of error (confidence limits)			
0.05 level	0.398	0.825	0.900
0.01 level	0.588	1.222	1.332
LOD	0.0234	0.0183	0.0158

* Average of six determinations

Table-1(b): Results of Method Validation of the Proposed Methods for the Determination ofAtazanavir

Parameter	M11
λ_{max} (nm)	520
Beer's law limits (µg/mL)	12.5-62.5
Molar absorptivity (1 mol^{-1}. cm^{-1})	$2.805x10^3$
Sandell's sensitivity (µg.cm^{-2}/0.001 A.U)	0.0628
Regression equation (Y=a+bc); Slope (b)	0.0078
Intercept (a)	0.0087
Correlation coefficient (r)	0.9995
Relative standard deviation (%)*	1.031

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% Range of error (confidence limits)	
0.05 level	0.863
0.01 level	1.277
LOD	0.0255

* Average of six determinations

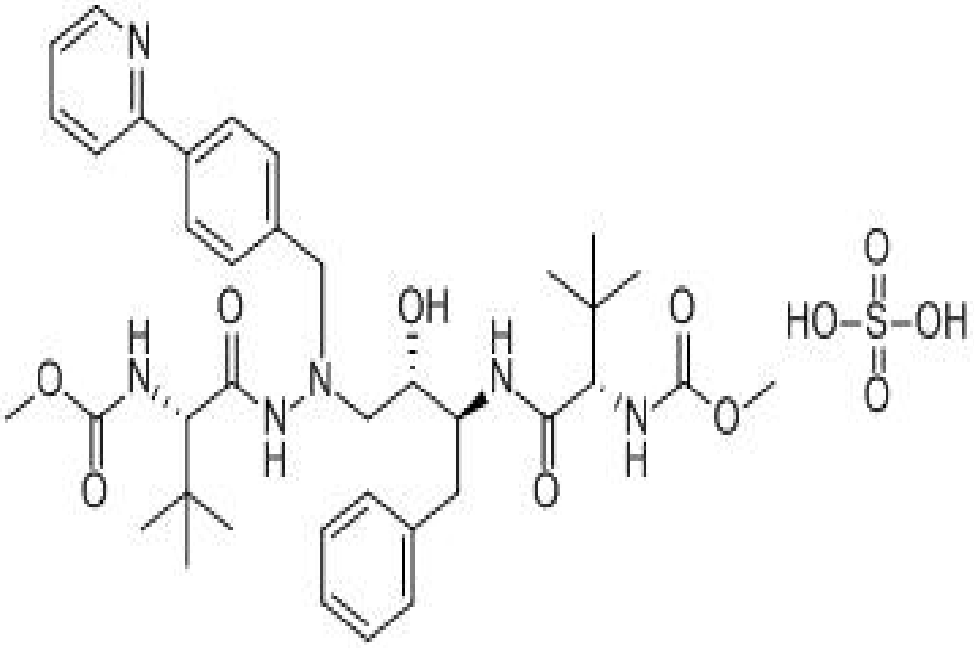

Figure -1 Molecular structure of Atazanavir Sulfate

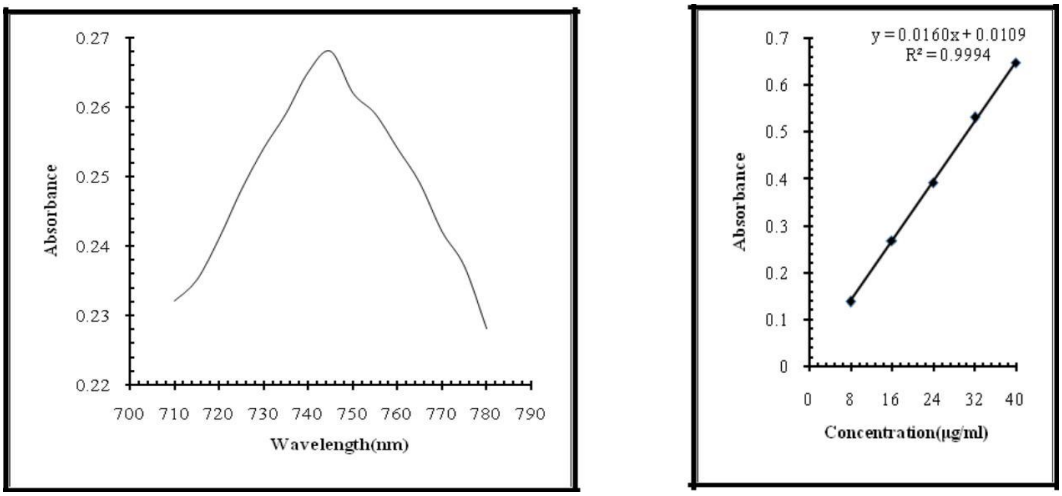

Figure-2(a&b) Absorption spectra and Beer's law plot of Atazanavir for Method-M_8

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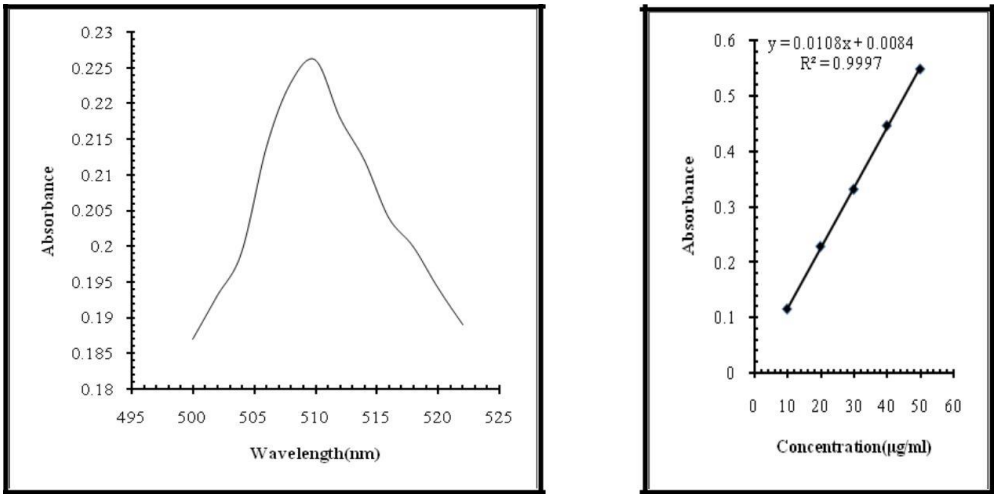

Figure-3(a&b) Absorption spectra and Beer's law plot of Atazanavir for Method-M_9

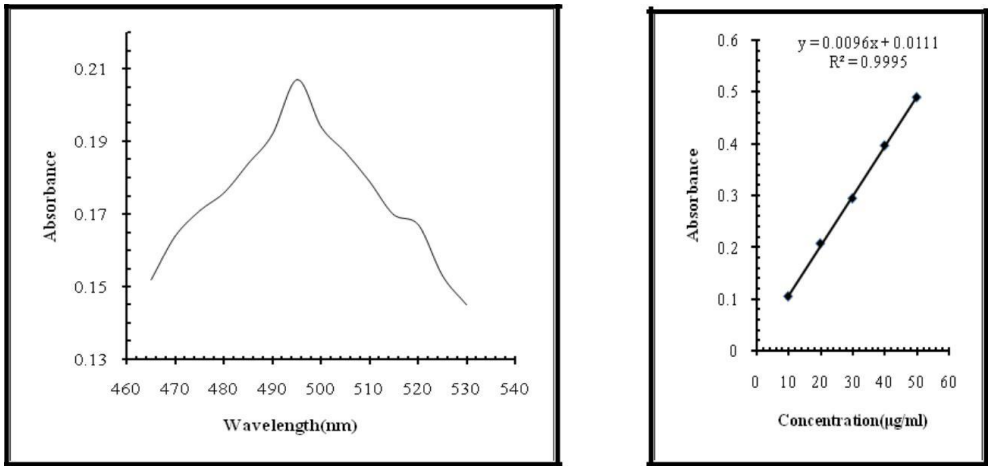

Figure-4(a&b) Absorption spectra and Beer's law plot of Atazanavir for Method-M_{10}

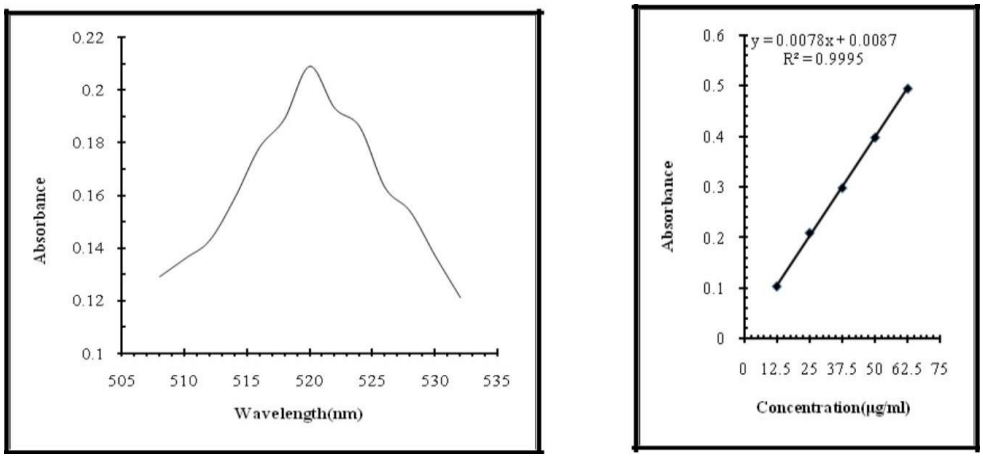

Figure-5(a&b) Absorption spectra and Beer's law plot of Atazanavir for Method-M_{11}

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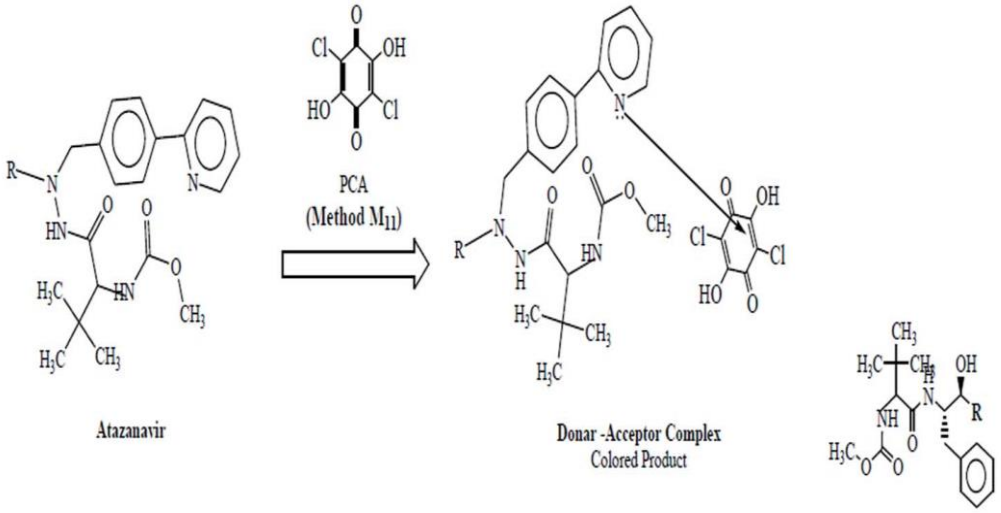

Scheme-1

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